Forschungszentrum Jülich
in der Helmholtz-Gemeinschaft

Institut für Chemie und Dynamik der Geosphäre IV:
Agrosphäre

Studies on the Effect of Soil Moisture and Time of Application on the Distribution of the Herbicide Propoxycarbazone-Sodium (BAY MKH6561) in Plants (Wheat, Blackgrass) and Soil

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Berichte des Forschungszentrums Jülich ; 4163
ISSN 0944-2952
Institut für Chemie und Dynamik der Geosphäre IV: Agrosphäre Jül-4163
D 98 (Diss., Bonn, Univ., 2004)

Zu beziehen durch: Forschungszentrum Jülich GmbH · Zentralbibliothek
D-52425 Jülich · Bundesrepublik Deutschland
☎ 02461 61-5220 · Telefax: 02461 61-6103 · e-mail: zb-publikation@fz-juelich.de

Studies on the Effect of Soil Moisture and Time of Application on the Distribution of the Herbicide Propoxycarbazone-Sodium (BAY MKH6561) in Plants (Wheat, Blackgrass) and Soil

The distribution and concentration of propoxycarbazone-sodium (MKH6561) (a.i.) and its metabolites in plant and soil were investigated. Soil columns and lysimeters were used to examine the dependence of the herbicide action on soil moisture and time of application for crop (wheat) and target grass (ALOMY). Before application of [phenyl-UL-^{14}C] labelled MKH6561, various levels of soil moisture (20 to 60%WHC_{max}) were adjusted in orthic luvisol and gleyic cambisol. After the application at growth stages BBCH22 and 25, different precipitation events were simulated ("normal" precipitation vs. heavy rain). The aim was thus to provide information on plant uptake, translocation in soil and metabolization of a.i. as a function of soil moisture and growth stage. The results will be used to optimise the effectiveness of the compound.

The experiments show higher total MKH6561 content in ALOMY due to the position of the main root horizon ("position selectivity"), compared to the deeper-rooting wheat. Wheat displayed lower percentage content of a.i. but higher content of the major plant metabolite (2-hydroxypropoxy-MKH6561) and thus better tolerance due to faster metabolization. ALOMY showed a similar distribution of both fractions and slowed down "detoxification". On the whole, the concentration [µg/g] of all the fractions considered was higher in ALOMY due to higher total MKH6561 content than in wheat. Due to the increase in mass of the plants and the resulting dilution effect, higher total MKH6561 content was found at the early (BBCH22), and lower content at the later application date (BBCH25), where a reduced effect of the herbicide is to be assumed. The soil moisture is the most important factor responsible for herbicide uptake. Thus, at high soil moisture, the greatest herbicidal effect and, at the same time, the greatest danger to the crop is to be expected. Due to lower pressure head at identical soil moisture on gleyic cambisol in comparison to orthic luvisol, high uptake rates and effectiveness of the herbicide in ALOMY on gleyic cambisol can be estimated.

In the soil, at high moisture, elevated translocation is to be expected. In the subsoil, the concentrations further increase with advancing time after application. Heavy rain led to elevated translocation and plant uptake due to downward water flow and the increase of soil moisture, respectively. In gleyic cambisol, in comparison to orthic luvisol, a higher hydraulic conductivity, lower effective retardation and lower sorptive properties (weak sorption of a.i. to the soil matrix, low K_{OC} values) were identified due to a high sand and coarse pore fraction. Thus, stronger translocation is to be expected in gleyic cambisol but concentrations of >1 µg/kg or contents of >1% of the applied quantity in subsoil layers can be ruled out. Metabolization takes place more slowly than in the plant, forming one major soil metabolite (4-hydroxysaccharin) which accumulates in soil. Degradation in gleyic cambisol was faster than in orthic luvisol.

Untersuchung zum Einfluss von Bodenfeuchte und Behandlungszeitpunkt auf die Verteilung des Herbizids Propoxycarbazone-Sodium (BAY MKH6561) in der Pflanze (Weizen und Ackerfuchsschwanz) und im Boden

Die Verteilung und Konzentration von Propoxycarbazone-Sodium (MKH6561) und seiner Metabolite wurde in Pflanze und Boden untersucht. Durch Versuche mit Bodensäulen und Lysimeterwannen wurden Abhängigkeiten der Herbizidwirkung von der Bodenfeuchte und dem Behandlungszeitpunkt bei Kulturpflanze (Weizen) und Zielgras (ALOMY) aufgeklärt. Vor der Applikation von [phenyl-UL-^{14}C]-markiertem MKH6561 wurden in Parabraunerde und Saurer Braunerde unterschiedliche Bodenfeuchten (20 bis 60%WK_{max}) eingestellt. Nach der Applikation zu zwei verschiedenen Zeitpunkten des vegetativen Wachstums (BBCH22 und 25), erfolgte die Simulation unterschiedlicher Niederschlagsereignisse ("normaler" Niederschlag vs. Starkregen). Ziel ist es, Aussagen über die Wirkstoffaufnahme, Verlagerung im Boden und Metabolisierung in Abhängigkeit von unterschiedlichen Ausgangsvorraussetzungen hinsichtlich Bodenfeuchte und Wachstumsstadien zu liefern. Die Ergebnisse sollen zur Optimierung der Wirkung des Präparates genutzt werden.
Im Zielgras wurden, aufgrund der Lage des Hauptwurzelhorizontes ("Positionsselektivität"), höhere MKH6561-Gesamtgehalte als im tiefer wurzelnden Weizen erreicht. Weizen wies prozentual geringere Wirkstoff- sowie höhere Hauptpflanzenmetabolitgehalte (2-Hydroxypropoxy-MKH6561) und somit bessere Verträglichkeit durch schnelleren Wirkstoffabbau auf. ALOMY zeigte eine anteilsmäßig gleiche Verteilung beider Fraktionen und somit eine verlangsamte "Entgiftung". Insgesamt lagen dort die Gehalte [in µg/g] aller Fraktionen, aufgrund hoher MKH6561-Gesamtgehalte, höher als im Weizen. Durch Massenzuwachs der Pflanzen und dem daraus resultierenden Verdünnungseffekt zeigten sich höhere MKH6561-Gesamtgehalte bei frühem Applikationszeitpunkt (BBCH22) und geringere Gehalte bei späterem (BBCH25). Es ist somit von einer Minderwirkung bei BBCH25 auszugehen. Die Bodenfeuchte ist für die Ausprägung der Herbizidaufnahme maßgeblich verantwortlich. Bei hoher Feuchte ist die beste herbizide Unkrautwirkung sowie gleichzeitig die stärkste Gefährdung der Kultur zu erwarten. Die hohe Wirksamkeit und erhöhte Aufnahmeraten bei ALOMY auf der Sauren Braunerde kann dadurch erklärt werden, dass die Pflanzen - bei gleicher Bodenfeuchte - eine geringere Wasserspannung als auf der Parabraunerde überwinden müssen.
Im Boden sind erhöhte Translokationsraten bei hoher Feuchte zu erwarten. Mit fortschreitendem Probenahmetermin steigen die Gehalte im Unterboden weiter an. Starkregen führt durch abwärts gerichteten Wasserfluss und die Erhöhung der Bodenfeuchte zu hohen Verlagerungsraten und starker Pflanzenaufnahme. Bei der Sauren Braunerde wird im Vergleich zur Parabraunerde, aufgrund eines hohen Sand- und Grobporenanteils, eine höhere hydraulische Leitfähigkeit, geringere effektive Retardation sowie sorptive Eigenschaften (lockere Bindung des Wirkstoffs an die Bodenmatrix, niedrige K_{OC}-Werte) nachgewiesen. Somit ist bei der Sauren Braunerde mit einer stärkeren Verlagerung zu rechnen; Konzentrationen von >1 µg/kg bzw. Gehalte >1% der applizierten Menge können im Unterboden jedoch ausgeschlossen werden. Die Metabolisierung erfolgt langsamer als in der Pflanze. Das Metabolitenspektrum umfasst einen Hauptmetaboliten (4-Hydroxysaccharin), der im Boden angereichert wird. In der Sauren Braunerde wird ein schnellerer Wirkstoffabbau als bei der Parabraunerde nachgewiesen.

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1. Introduction

Modified cultivation methods such as tighter cereal crop rotations with increasing proportions of winter-planted crops (winter wheat) and soil working methods without ploughing, the bringing forward of sowing dates in many agricultural regions and in particular the tendency to apply minimal soil working have increased the occurrence of grass weeds *(AMANN et al. 2000)*. In addition to blackgrass (*Alopecurus myosuroides*) and loose silky-bent (*Apera spica-venti*), couch grass (*Agropyron repens*) and various species of brome (*Bromus spp*.) have long been important grass weeds and thus a problem for cereals *(AMANN et al. 2000)*. They have become a problem due to their vigorous reproduction and strong competition with crops because of their greater mass, thus making harvesting more difficult and affecting the quality of the harvest products, as well as the problems involved in weed control *(KNAUER 1993)*.
Among the grass weeds, blackgrass is the most important one for cereal cultivation in north-west Europe *(BABCZINSKI et al. 2000)*.

Fig. 1: Infestination of blackgrass in winter wheat

A. myosuroides can be controlled by active ingredients from the groups of the urea derivatives i.e. (CTU/IPU), dinitroanilines, thiolcarbamates and aryloxypropionates (FOPs). In recent years the first sulphonylurea agent (flupyrsulfuron) and an oxyacetamide (flurfenacet) have been licensed for the control of blackgrass *(BABCZINSKI et al. 2000)*.
Control is increasingly difficult - especially in the case of the continuous cultivation of winter wheat sown extremely early. The reason is to be found, on the one hand, in the difficulty of chemical control due to environmental restrictions in the application of products containing isoproturon/chlortoluron in many European countries. On the other hand an increasingly reduced effect - due to acquired resistance - is observed in the application of established agents based on urea derivatives or ACCase inhibitors (FOPs) *(FEUCHT et al. 1999)*. With respect to the control of the other grasses mentioned above (brome species and couch grass) no selective herbicides are currently licensed for cereals, except for sulfosulfuron. According to *BABCZINSKI et al. (2000)* few active ingredients are effective for loose silky-bent,

especially in spring. It is therefore important, that new substances should be applied, that have an improved and more selective effect and can be used in the lowest possible dose with good environmental behaviour.

Propoxycarbazone-sodium (MKH6561[1]) is a new active ingredient developed by Bayer CropScience and marketed in Europe under the name of *ATTRIBUT*™ and in the USA/Mexico under the name of *OLYMPUS*™. Chemically, it belongs to a new class of substances, the sulfonylaminocarbonyltriazolinones. Biochemically, however, it is one of the group of acetolactate synthase inhibitors (ALS) that have long been known. With a favourable environmental profile, the herbicide demonstrates effective action against nearly all important monocotyledonous and some dicotyledonous weeds with highly successful control *(WELLMANN et al. 2002)*.

The active ingredient has a similar mode of action and chemical structure to that of the sulfonamides and sulfonylurea compounds, which also inhibit acetolactate synthase. This mode of action, which has been little applied to control grass weeds in cereals so far, makes it possible, for the first time, to control all four grass weeds mentioned above. The mechanism results from the inhibition of cell division; plants display stunting and reduced seed production. Necrosis of the leaves and cessation of growth are observed after a few days of treatment with the result that the plant dies off (dependend on the weather conditions and the growth stage of the plants). Since the plants already stop taking up water and nutrients shortly after application, they represent greatly reduced competition for the cereals *(AMANN et al. 2000)*.

ATTRIBUT™ is used in Europe as an early postemergence herbicide in spring (application rate 42-70 g/ha) in crops of wheat, rye and triticale for controlling the above-mentioned grass weeds. Formulated as 70% water-dispersible granules (70 WG) it is readily water-soluble, mobile in the soil and bioavailable (*AMANN et al. 2000)*.

On the basis of experiments on the action of MKH6561 in the laboratories of Bayer CropScience and from practical experience in its application in the USA and Canada, questions have been raised about its effectiveness. Although it is an extremely active herbicide with good crop tolerance under "normal conditions" concerning soil properties and weather conditions, as well as having an noncritical ecotoxicological profile *(BABCZINSKI et al. 2000)* and environmental behaviour (see Chapter 2.2), under certain conditions reduced effects may result for grass weeds or else damage to crops. MKH6561 is usually tolerated very well by wheat, rye and triticale. Reduced growth has only been observed in a few cases

[1] In the following text the code name MKH6561 is used instead of propoxycarbazone-sodium.

when applied to very "light" and low-sorption soils in combination with unfavourable weather conditions *(FEUCHT et al. 1999)*. Therefore there is, for example, the possibility that different quantities of the active substance may be taken up by the crop and the target grass as a function of soil moisture. This may, in the case of low uptake, lead to insufficient effectiveness for grass weeds and, in case of high uptake rates, may cause damage to crops. Moreover, varying uptake rates of the herbicide from the soil are expected at different growth stages of the plant.

Within the framework of this work, special attention is given to the distribution of MKH6561 in plant and soil. Soil columns and lysimeters were used to examine the dependence of the herbicide action on soil moisture and time of application for the crop and target grass. Spring and winter wheat were chosen as the experimental plants, as well as the grass weed blackgrass.
Before application, various levels of soil moisture were adjusted in the experimental soils (orthic luvisol and gleyic cambisol). After the application of the herbicide at two different stages of the vegetative growth of the crop and target grass, the different precipitation events were simulated ("normal" precipitation vs. heavy rain). The aim is thus to provide information on the behaviour of the active ingredient in plant and soil as a function of different starting conditions with respect to soil moisture and growth stage - as well as under different, in some cases extreme, weather conditions. The results will be used to optimise the effectiveness of the compound.
The key questions of the work can be summarized as follows:

- How do the crop and target grass differ in the uptake, distribution and concentration of the herbicide in the plant, and in the metabolization of the active ingredient?
- What influence does the stage of development of the plant has on the uptake and distribution of the active ingredient?
- How does the initial soil moisture, associated with various precipitation events, influence uptake in the plant and translocation of the herbicide in the soil?
- What influence do the different soil-types have on the uptake of the active ingredient in the plant and the translocation in the soil?

In the following theoretical part (Chapter 2) the physicochemical properties of MKH6561 will be described as well as the environmental behaviour in the compartments soil, water, plant and air.

The empirical part of the study begins with information about the experimental vessels, plants and soils (Chapter 3.1-3.3). This is followed by a description of the determination and adjustment of the soil moisture in the experimental vessels (Chapter 3.4). Chapter 3.5 deals with the radioactivity applied in the experiments; Chapters 3.6 and 3.7 show the experimental setup of the column and lysimeter experiments. In the following Chapters 3.8 and 3.9 the methods for sampling and sample preparation are discussed. Radioactivity recording and active ingredient determination as well as additionally performed investigations (adsorption/desorption characteristics and calculation of pressure head and hydraulic conductivity) are discussed in Chapters 3.10 and 3.11. Chapter 3.12 concerns data processing and statistical methods description.

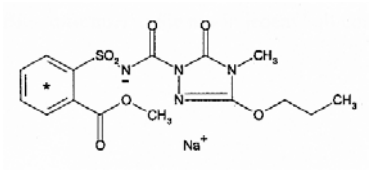
In Chapter 4 the evaluation concept is explained. The statistical evaluation of the data material and the representation of the results of the individual investigation methods start in Chapter 5: the results of the column experiments are presented in Chapter 5 divided into the compartments plant (Chapter 5.1) and soil (Chapter 5.2). Chapter 6 describes the results of the lysimeter experiments according to the same principle, divided into the compartments plant (Chapter 6.1), soil (Chapter 6.2) and percolate (Chapter 6.3). Chapter 7 is devoted to the evaluation of the ad-/desorption study and properties of the experimental soils based on hydraulic functions. The most important results are summarized and interpreted in a discussion with focus on the formulation of the questions underlying this dissertation (Chapter 8) and final conclusions (Chapter 9).

2. The active ingredient MKH6561 and its metabolites

2.1 Description of the Active Ingredient

The chemical and physical properties of the active ingredient MKH6561 are summarized in Table 1.

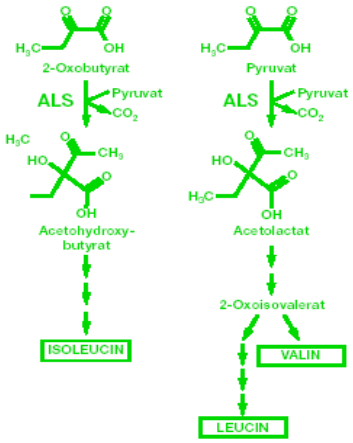
Tab. 1: Physicochemical properties of MKH6561 *(FEUCHT et al. 1999, AMANN et al. 2000)*

Common name:	MKH6561
Commercial name:	*ATTRIBUT*™ (Europe), *OLYMPUS*™ (USA)
Code number:	BAY MKH6561
Chemical class:	Sulfonylaminocarbonyltriazolinone
Chemical name (IUPAC):	Methyl 2-({[(4-methyl-5-oxo-3-propoxy-4,5-dihydro-1H-1,2,4-triazol-1yl)carbonyl]amino}sulfonyl)benzoate sodium salt)
Summation formula:	$C_{15}H_{17}N_4NaO_7S$
Structural formula with labelling position (*) (cf. Chapter 3.5):	
Molecular weight:	420.4 g/mol
Appearence:	colourless, crystalline powder, odourless
Melting point:	230-240 °C
Density:	1.42 g/cm^3 (20 °C)
Vapour pressure (estimated)	< 1 * 10^{-8} Pa (20 °C)
Octanol/water - partition coefficient at 20 °C ($\log P_{ow}$):	-0.30 (pH 4) -1.55 (pH 7) -1.59 (pH 9) (unbuffered): -2.6
Solubility in water (20 °C):	2.9 g/L (pH 4) 42.0 g/L (pH 7-9)
Solubilities in organic solvents (20 °C):	n-heptane, xylene & 2-propanol < 0.1 g/L dichloromethane 1.5 g/L
Dissociation constant:	pK_a = 2.1 (free acid of MKH6561)
Hydrolysis stability (25 °C):	stable at pH 4-9

2.1.1 Mode of Action

MKH6561 inhibits the acetolactate synthase (ALS) which according to *BABCZINSKI et al. (2000)* is present at the start of two synthesis pathways running parallel in chloroplasts to the branched-chain amino acids; on the one hand, leucine and valine, and, on the other hand, isoleucine (Figure 2):

Fig. 2: Biosynthesis of branch-chained amino acids *(BABCZINSKI et al. 2000)*



The inhibition of ALS leads to depletion of these amino acids in the cells of treated weeds/grass weeds thus disturbing the de novo synthesis of proteins and leading to the cessation of cell growth *(BEYER et al. 2000)*.

Mammals do not have any ALS and cannot themselves synthesize the corresponding amino acids required for the biosynthesis of proteins and enzymes; they are dependent on taking them up with their food. This makes ALS inhibitors especially interesting as herbicides. They are plant-specific and therefore have advantageous toxicological properties. All carried out tests showed no toxic effect for man, "non-target organisms" and environment. For example the LD_{50} value[2] for rat (acute oral) was > 5000 mg/kg body weight *(BABCZINSKI et al. 2000)*.

Studies from *BABCZINSKI et al. (2000)* showed that MKH6561 is an effective inhibitor for ALS: a concentration of just 0.0033 mg/L effected a 50% inhibition of the enzyme.

2.1.2 Selectivity

The term selectivity describes the different behaviour of the active ingredient in crop and grass weed. MKH6561 is readily tolerated in wheat, which is not a matter of course for a grass herbicide in postemergence application *(BABCZINSKI et al. 2000)*. Laboratory studies on the biochemical mechanism of selectivity have shown that a different sensitivity of the

target (ALS) for wheat in comparison to the grass weed is probably not the major reason for the selectivity *(BABCZINSKI et al. 2000)*. Whether the ALS is extracted from wheat seedlings or, for example, from blackgrass it is inhibited to approximately the same extent by MKH6561. Nor is there any indication that the uptake of the active ingredient into the grass weed is better or faster in comparison to wheat. The different form of the root system (e.g. spatial distribution in the soil) may contribute to the selectivity (*BABCZINSKI et al. 2000)*. Many indications point to the fact that the rapid metabolization of the active ingredient in wheat (in contrast to the grass weed) is responsible for the selectivity of MKH6561. Wheat is capable of degrading the active ingredient by half within less than one day. Because of the rapid degradation process damage to the crop is prohibited. Basically, just one metabolite can be detected in plants, a hydroxylized form of MKH6561. In contrast to the active ingredient, metabolites of MKH6561 do not display any significant or have very low herbicide activity as 2-hydroxypropoxy-MKH6561 *(PONTZEN 2002)*.

Furthermore MKH6561 is very well tolerated by other cereal species beside wheat: It can be used for all varieties of winter rye and triticale *(BABCZINSKI et al. 2000)*. In barley and oats the active ingredient is only slowly metabolized so that it cannot be used in these crops.

2.1.3 Spectrum of Activity

The spectrum of actitivity of MKH6561 comprises blackgrass, loose silky-bent and various species of brome. In addition to these annual species, the active ingredient also makes it possible to selectively control one of the most important perennial weeds in wheat, couch grass. Due to its systemic properties, the action extends not only to the parts of the plant above ground but also to the underground shoots (rhizomes) of this species since the active ingredient also migrates in the assimilate stream. Furthermore, various broad-leaved weeds (e.g. *Sinapsis arvensis* and *Thlaspi arvense*), mainly from the family Brassicaceae can also be controlled.

For broad-spectrum control, *ATTRIBUT*™ is suitable for mixing and is recommended in combination with a large number of other herbicides that are tailored to the respective weed situation (e.g. *HEROLD*™[3], a flufenacet containing herbicide). However, in the case of a weed density of > 500 plants/m^2 weed control in autumn cannot be dispensed because from this density on irreversible losses of yield are expected *(BABCZINSKI et al. 2000)*. As part of the spraying sequence, MKH6561 represents an active ingredient for follow-up treatment in spring enabling competition from blackgrass to be eliminated at any early point in the

[2] Lethal dose for 50% of the species tested

cultivation of cereals. Since this active ingredient has been little used as yet for the control of grass weeds in wheat it can be proposed as a building block in resistance management. MKH6561 does not have any negative influence on the subsequent crop. Because of all this the main crops can be subsequently cultivated as part of the usual crop rotation *(BABCZINSKI et al. 2000)*.

2.1.4 Application

The application of MKH6561 extends from the start of the vegetation period up to, at maximum, growth stage 32/37 of the crop (BBCH[4]) [= end of shooting] at a water application of 200-300 L/ha. According to *BABCZINSKI et al. (2000)*, 60 g/ha of the 70 WG formulation can be used on sandy and silty soils. Up to 100 g/ha can be used in winter wheat on strongly sorbing soils and at very high densities of black grass. Unfavourable conditions such as strong leaf and soil uptake of the active ingredient combined with a reduced metabolic capacity of stressed cereal crops may lead to paling of the leaves or delayed growth. These symptoms are temporary and in experiments with up to twice the application rate no yield losses resulted.

Even with an applied quantity of 40 g/ha, MKH6561 displays on average better action against blackgrass than isoproturon (IPU), which is the basis of many weed control systems in cereals *(BABCZINSKI et al. 2000)*. Application quantities of 60 and 100 g/ha achieve considerably more reliable and successful control. The application period for MKH6561 differs significantly from that for the aryloxypropionic acids (FOPs). Whereas these leaf-active grass herbicides need adequate temperatures for good leaf action, the optimum date of application for MKH6561 is early postemergence in spring due to the combined leaf and soil action. The date of application is therefore more comparable to application of isoproturon. In contrast to the application of isoproturon blackgrass is reliablely controlled till growthstage BBCH23; in older plants the efficiency of control declines. Due to the residual action, grass weeds emerging after application will also be controlled for a short period of a few weeks.

MKH6561 should not be applied to crops damaged by frost, waterlogging or drought, weakened by deficient nutrition or any other circumstances. According to *WELLMANN et al. (2002)* the control of *A. myosuroides* on sandy, silty and loamy soils is the same at 95-96%. For application on light sandy soils the recommended application quantity should not be exceeded nor an application date chosen after growth stage 29/30 (BBCH) of the crop

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[3] Product of Bayer CropScience

[4] Coding from phenological studies of crops and weeds by the Federal German Biological Agency - chemical industry

(*BABCZINSKI et al. 2000*). On soils with low sorption capacities damage to the crops could occur due to better crop uptake.

2.2 The Environmental Behaviour of MKH6561

Pesticides applied in accordance with good agricultural practice should not have any adverse effects on the environment. In order to assess the influence of a pesticide on the environment - apart from the actual impact on the target organism - it is necessary to investigate the behaviour of the substance in the soil ecosystem, which is the ultimate sink for pesticides, as well as in water and air. In this respect, decisive significance is attached to both abiotic processes such as chemical decomposition (hydrolysis), photochemical degradation, as well as the binding and translocation of the active ingredient and degradation products in the arable soil, and also to biotic processes such as microbial decomposition and, according to *BABCZINSKI et al. (2000),* in the compartments of soil, water and air these processes lead in different ways to the disappearance of an active ingredient from the environment.

2.2.1 Sorption, Mobility and Plant Availability of Herbicides

A wide range of factors play a part in the way in which a herbicide behaves in the environment. The distribution of a substance in the soil and plant, as well as the mobility and sorption in the soil and the uptake and transport in the plant, depend on the chemical and physical properties of the active ingredient itself, on the formulation and application rate as well as on the soil (soil type, content of organic substance, pH value, soil moisture) and environmental conditions (season, precipitation, temperature) and are modified by these factors.

The role of soil moisture and the influence of different growth stages on the distribution of MKH6561 in the soil and plant will be determined from this wide range of factors.

The soil moisture influences both the distribution and availability of the active ingredient in the soil, as well as the uptake and concentration in the plant. With respect to the influence of soil moisture on the adsorption of herbicides at soil components, *HURLE (1982)* comes to the conclusion that in general an increase in adsorption can be expected with decreasing soil water content. In contrast, leaching of the herbicides generally increases, as does the active ingredient uptake in the plants, together with the volume of water applied or the increased soil moisture. Increased water application thus has a significant effect on the movement of herbicides in the soil, on leaching and also on uptake by plants. In this respect,

WALKER (1971) demonstrates an increasing phytotoxicity of various active ingredients with rising water content.

With regard to the different growth stages optimum weed control with standard application quantities of MKH6561 is only achieved in the early, sensitive developmental stages after the two-leaf stage up to the tillering of grasses. In the case of treatment up to developmental stage BBCH23 the average effectiveness was greater than 90% *(AMANN et al. 2000)*. Applications at later dates resulted in lower control success and displayed considerable variations. In the case of grass weeds at more advanced stages of development or if the date of application was too late, as well as in heavy soils with increased sorption, the uptake rates were significantly poorer than at earlier stages of development or in light soils. Greater quantities of herbicide (upper dose range) are then required. The most reliable action against blackgrass is thus achieved if MKH6561 is applied (with a quantity of 42 g/ha) after the cereal crops have turned green when the grass weeds have begun to grow and if the application is completed as early as possible.

Other parameters determining the environmental behaviour and the distribution of herbicides in the soil and plant cannot be ignored. In addition to the variability in the moisture content of the soils, there are also variations in their mineral soil types, percentage of organic substance, pore size distribution and pH value, which have an effect on the sorption, mobility and availability of herbicides to the plant. In general, the sorption and accumulation of organic active ingredients increases in the soil with decreasing pH value and increasing percentage of organic matter, while the mobility and availability to the plant correspondingly decrease *(SCHEFFER et al. 1992)*.

Leaching into deeper soil horizons and the availability of herbicides to plants is predominately determined by the adsorption capacity of certain soil components *(SCHEFFER et al. 1992)*. In general the adsorption increases with increasing active ingredient concentration. At the same time this leads to a reduction of the concentration of the active ingredient in the soil solution. Only those active ingredient fractions are taken up from the soil into the plant that are dissolved in the plant-available water *(cf. WALKER 1972, PESTEMER 1983)*. The adsorbed active ingredient fractions only become available to the plant after desorption *(HURLE 1975)*. With the aid of adsorption isotherms[5], *GRAHAM-BRYCE et al. (1971)* determined in various

[5] Different adsorption isotherms exist (e.g. linear = K_D value, exponential = K_F value); which one is suitable depends on the investigated substance, its concentration range and the soils investigated (cf. Chapter 3.11.1.3).

soils an increasing biological effectiveness (increasing uptake by plants) with decreasing tendency of soil sorption.

Of the mineral soil constituents the clay minerals represent important sorbents due to their surface properties. A correlation with the adsorption of active ingredients has only been reliably demonstrated for cationic active ingredients *(CALVET 1980)*. The humus/organic carbon content in the soils is, in contrast, in a positive relation to the adsorption of many active ingredients due to the strong sorptive properties of the organic substance *(OSGERBY 1970, MERCER et al. 1972, CALVET 1980, SCHEFFER et al. 1992)*.

The soil structure and texture is considered by *HANCE (1983)* to be an important parameter for the adsorption, translocation and plant availability of active ingredients since it determines the soil water characteristics and influences the adsorption via the pore size distribution. In these studies, a rapid movement of water resulted on "light" soils - due to the higher fraction of coarse pores - leading to an increased distribution of active ingredients in the soil. On well-structured soils, a slow movement of water caused increased penetration of the active ingredient into the fine pores of the aggregates, from where - irrespective of the adsorption - it only slowly entered the coarse pores and was thus not taken up in the plants or translocated so strongly. The herbicide fraction in the soil solution of the medium-sized pores is directly available to the plant.

The pH value is of great significance for the behaviour of organic chemicals dissociated in the soil into anions or cations *(cf. LEWANDOWSKI et al. 1997)*. Since in most soils the surface charge is predominately negative *(MÜCKENHAUSEN 1975)*, cationic active ingredients are strongly and anionic active ingredients only weakly sorbed. Like the sulphonylamides and sulphonurea compounds, MKH6561 is a weakly acid herbicide with a low pK_a (negative logarithm of the acid dissociation constant (K_a) of 2.1). The acidity of the herbicide (pK_a) can determine the herbicide adsorption and distribution. If the soil pH > pK_a then most of the herbicide molecules are negatively charged *(cf. FONTAINE et al. 1991)*. These molecules are not adsorbed by clay minerals in the soil because they are also negatively charged due to the high pH value in the soil. In this situation, the herbicides can be taken up by the roots or translocated into deeper soil layers *(cf. HURLE et al. 1980)*.

Not only the soil composition is decisive for adsorption, it also depends on the physico-chemical properties of the active ingredient itself. In contrast to the K_F value[6], which is dependent on the respective soil, the octanol/water distribution coefficient is used to

[6] K_F value = *FREUNDLICH* adsorption isotherm

characterize the absorption capacity. The degree of lipophilicity (log K_{OW}[7]) correlates positively with the adsorption on the organic substance. The greater these hydrophobic properties or the K_{OW} value is, the stronger is the tendency for the substance to become attached to the humic substance of the soil *(cf. LEWANDOWSKI et al. 1997)*. The water solubility of a substance therefore decreases with increasing lipophilicity and rising melting point *(cf. BRIGGS 1981; NICHOLLS 1988)*. This means that active ingredients that are slowly released on the basis of their low solubility in water generally also display high adsorption in soil. However, the water solubility is 2.9 g/L (pH4) and 42.0 g/L (pH 7-9) > 1 g/L, which classifies MKH6561 as readily soluble in water *(BAYER AG 2000)*. Table 1 already showed the effect of the pH value on the octanol/water distribution coefficient and the solubility in water. When the pH value rises (for example from 4 to 7) the water solubility also increases and the lipophilic character of the active ingredient is reduced. The pH value thus has a very strong effect on the soil mobility of the active ingredient. If the soil pH rises, the bound fraction decreases and the substance migrates into the water phase, in which it can be translocated.

The following studies in Chapters 2.2.2-2.2.5 comprise the major experimental findings of Bayer CropScience on active ingredient behaviour in the compartments of plant, animal, soil, water and air. A distinction must be made between degradation studies in the strict sense (e.g. half-life of the disappearance of a component from an environmental compartment) and metabolism studies investigating the degradation path and identifying the major metabolites.

2.2.2 Behaviour in Plants

2.2.2.1 Uptake

There is a systemic uptake of MKH6561 into the plant, which may take place via the root and also, although to a lesser extent, via the leaf *(BABCZINSKI et al. 2000)*. For leaf uptake or the permeation of cutinized tissue layers, the lipophilicity of the active ingredient (measured as the octanol/water distribution coefficient) can be mentioned as an important parameter. Lipophilic compounds usually pass through the membranes more easily then hydrophilic since in the former case the activation energy is sufficient for their entry into the lipoid layers *(cf. SCHILLING 2000)*. However, the active ingredient MKH6561 has low lipophilicity and a low pK_a value *(BABCZINSKI et al. 2000)*. The active ingredient is, moreover, ionized throughout the entire physiologically relevant pH range *(PONTZEN 2002)*. With respect to penetration into the leaf this means that the lipophilic barriers of the cuticula are difficult to

[7] Log K_{OW} = log P_{OW} = logarithm of the octanol/water distribution coefficient

overcome. Laboratory investigates have revealed that about one fifth of the quantity of active ingredient applied penetrates into the leaf. In his study, *PONTZEN (2002)* states that almost 80% of the radioactivity applied with 80% acetonitrile to investigate the active ingredient uptake into the leaf is washed off again after two days. The major fraction of the MKH6561 taken up remains at the application site *(BABCZINSKI et al. 2000)*. Apart from the physico-chemical behaviour of the active ingredient itself, factors such as the application technique, weather conditions and the plant species and the developmental stage of the grass weed have a considerable influence so that the leaf action is subjected to fluctuations.

Since MKH6561 is formulated without any penetration-promoting additives, it has proved beneficial if an additive is added to improve leaf adsorption and uptake *(cf. AMANN et al. 2000)* when the herbicide is not combined with AHL[8] or a compound containing a solvent. Additives such as amine ethoxylates (Frigate™, application rate 0.5 L/ha), alcohol ethoxylates (0.3 L/ha) and vegetable oils (1 L/ha) can be used and there is little difference in their effects. Especially under unfavourable conditions it may be helpful to use an additive. Cold spells or dry weather conditions, e.g. in the case of application in late spring, combined with low atmospheric and soil humidity may lead to a lower root uptake of active ingredient *(FEUCHT et al. 1999)*. Improved leaf uptake can increase the herbicide action. Additives are also useful in very dense stands and if the blackgrass is already well developed. For example, leaf adsorption on species of brome can be increased from 30% to 84% *(AMANN et al. 2000)*. This addition is, however, in general only possible with an application quantity of 60 g/ha. A reduction of the application quantity by the use of additives leads to limited effectiveness due to the reduced soil action. A quantity of 100 g/ha applied at the optimum application date achieves significantly more reliable control of blackgrass at difficult sites than the standard application quantity with or without additives. If leaf uptake is increased, e.g. by strong leaf growth under wet weather conditions at the beginning of shooting, it is no longer possible to add an additive after growth stage 29/30 since this may reduce tolerance in the crop. The same is true of applications at mean daily air temperatures of more than 20 °C and strong solar radiation or strong temperature variations between day and night (more than 15 °C) *(BABCZINSKI et al. 2000)*.

If water conditions are adequate, e.g. at the recommended application dates in spring, root uptake predominates due to the high soil moisture content. In particular after a precipitation event, rapid redistribution may take place in the soil so that increased uptake via the roots becomes possible *(BABCZINSKI et al. 2000)*. Soil action, which is characterized by numerous

[8] Ammonium nitrate urea solution

interactions between the herbicide and the soil components (cf. Chapter 2.2.1), is therefore decisive.

In summary, it can be said that the rate of the action depends on the soil and environmental conditions and increases with rising temperature and moisture content. Under the climatic conditions prevailing in Germany in spring, an early application is therefore recommended.

2.2.2.2 Transport

Differences in the extent of transport within the plant may occur in higher plants *(NEUMANN et al. 1985)*. These differences are due to different rates of uptake and adsorption at cell constituents or due to the metabolism of the applied substances. They result as a function of the morphological and anatomical properties of the plant, the physiology of their transports systems, their metabolism and the respective stage of development. External factors such as air humidity, water supply, temperature and light also have an influence. The type of mobility is specific to the active ingredient and is determined by its chemical/physical properties.

Long-distance transport of the active ingredients can either take place passively with the transpiration flow in the xylem or with the assimilate flow in the phloem or in both transport systems *(cf. JAKOB et al. 1987)*. Xylem-mobile active ingredients are translocated acropetally and primarily accumulate in those parts of the plant with the highest transpiration, usually in the tips and edges of the leaves. The active ingredient concentration is diluted by increasing plant growth and by metabolization *(TISCHNER 1987)*. Phloem-mobile substances are translocated basipetally. The direction of active ingredient transport corresponds to the assimilate transport, which is always directed from the locations of assimilate export ("sources", such as the leaves) to the sites where assimilates are required ("sinks", such as roots, buds and shoot tips). Ambimobile active ingredients, like the herbicide under investigation here, are thus transported both acropetally and basipetally. They can circulate in the plant, be transported to all parts of the plants and display a biological effect there. The herbicide thus reaches the vegetative points (meristems) where ALS inhibition prevents further plant growth.

A translocation of the active ingredient into the roots and other untreated parts of the plant was also demonstrated in studies by *PONTZEN (2002)* through the local application of radiolabelled MKH6561 to leaves of barley seedlings. It became apparent that a large proportion of the radioactive substance remains at the application site. Only somewhat more than 1% of the radioactivity was found in the roots and the remainder of the plant. Very small quantities were excreted via the roots.

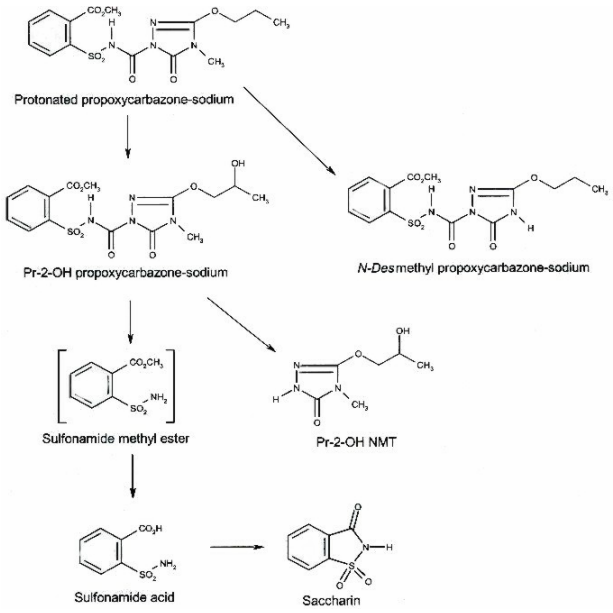
2.2.2.3 Metabolism

As in other environmental compartments, degradation takes places in several steps via metabolites that are degradable to a greater or lesser extent.

In laboratory studies, the metabolism of the active ingredient was investigated after the application of 60 g a.i./ha (2-3 weeks after sowing) in four different matrices of the spring wheat: green material, hay (milk ripeness), straw and grains *(BABCZINSKI et al. 2000)*. The overall residues in the wheat matrix were extremely low: a maximum of 0.4 mg/kg in the green crop and a minimum of 0.005 mg/kg in the grains. The active ingredient was rapidly degraded in wheat. Only slight quantities of the unchanged original components of MKH6561 were found in the green crop (and to a lesser extent in the straw). Detailed studies on degradation with single leaves confirm that the half-life in *A. myosuroides* (22.5 h) is much longer than in wheat (13.8 h), which is one reason for the selective action of MKH6561. Young wheat leaves that are still growing hydroxylate MKH6561 more than twice as fast as fully developed leaves. In contrast, the fully developed leaves of *A. myosuroides* do not display any correlation with age.

The main degradation path in wheat (and also in blackgrass) is the hydroxylation of the propoxy side chain of the molecule with the formation of 2-hydroxypropoxy-MKH6561 *(BABCZINSKI et al. 2000)* as the major metabolite. This hydroxylation as the first degradation step is also known from other ALS inhibitors such as chlorsulfuron, metasulfuron and prosulfuron. The phenyl ring is hydroxylated, the resulting hydroxy metabolites still have a (slight) herbicide effect and are only detoxified in the next step. A cytochrome P_{450} monooxygenase is probably responsible for the hydroxylation of MKH6561 *(PONTZEN 2002)*. Inhibitors of P_{450} enzymes such as 1-aminobenzotriazole or tebuconazole inhibit the metabolization of the herbicide. The opposite effect is achieved with the safener fenchlorazole-ethyl. The hydroxylation of the active ingredient in wheat leaves takes place about three times as fast. Further hydrolysis led, on the one hand, to the formation of 2-hydroxypropoxy-N-methyltriazolinone and, on the other hand, to sulfonamide acid. The latter is in chemical equilibrium with the final metabolite saccharin, traces of which (0.0006 mg/kg) were found in the grains. As a secondary degradation path, a demethylation of the active ingredient has been observed resulting in N-desmethyl-MKH6561 (Figure 3):

Fig. 3: Degradation pathway of MKH6561 in wheat *(BABCZINSKI et al. 2000)*



On the basis of the results for the metabolism in wheat, the active ingredient and 2-hydroxypropoxy-MKH6561 were defined as the relevant residues for establishing the maximum quantity *(BABCZINSKI et al. 2000)*.

After soil application, the metabolism of MKH6561 was examined in the subsequent crops of spring wheat (grain crop), cabbage (leaf crop) and turnips (root crop). In residual quantities of 0.006 to 0.10 mg/kg, 2-hydroxypropoxy-MKH6561 was also already identified as the major metabolite after 30 days of subsequent cultivation. In addition, the uptake of soil metabolites (N-methylpropoxy-triazolinone and saccharin) is of some significance. The former (after hydroxylation) and the latter were found in both a free (0.03 mg/kg) and also a conjugated form (0.05 mg/kg) at a low level. Since no toxicologically relevant metabolites were found in the subsequent crops the active ingredient and the major metabolite were defined as the relevant residues.

Field studies were performed on residues in spring and winter wheat. At the time of harvesting, the residues of MKH6561 and 2-hydroxypropoxy-MKH6561 were below the determination limit for wheat straw and wheat grains in all cases (residue-free situation). On the basis of these residue experiments, an acceptable maximum residue level (MRL) of 0.05 mg/kg was proposed.

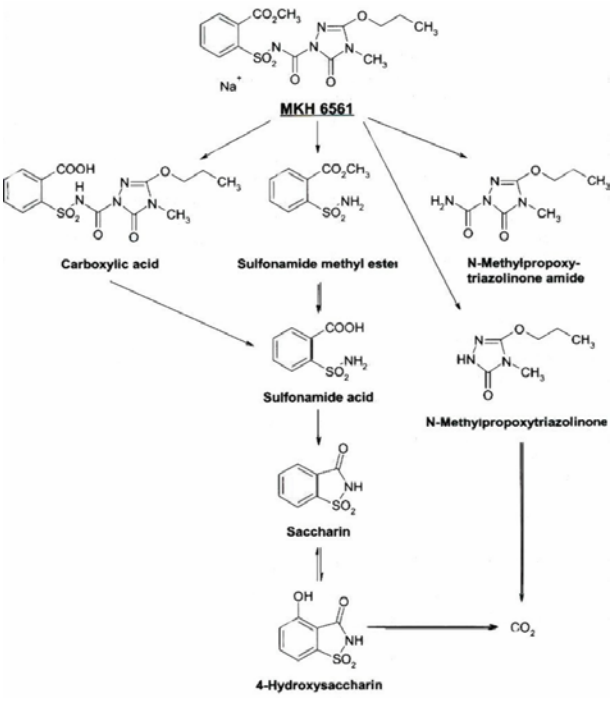
2.2.3 Behaviour in Soil

2.2.3.1 Metabolism

Under laboratory conditions, MKH6561 is degraded by a sequence of different processes such as the splitting of ester and amide bonds, followed by cyclization and oxidation reactions up to the final product of CO_2.

In the first stages the active ingredient is degraded in the soil by splitting of the ester bond to MKH6561-carboxylic acid and/or the triazolinonamide bond so that sulfonamide methyl ester, N-methylpropoxytriazolinone or N-methylpropoxytriazolinone amide result *(BABCZINSKI et al. 2000)*. Sulfonamide methyl ester is further degraded to sulfonamide acid, followed by the formation of saccharin and oxidation to 4-hydroxysaccharin, the major soil metabolite. The final product of mineralization is CO_2, into which N-methylpropoxy-triazolinone is also further degraded (cf. Figure 4).

Fig. 4: Degradation pathway of MKH6561 under aerobic conditions in soil *(BABCZINSKI et al. 2000)*



The above-mentioned metabolites - except for sulfonamide acid - each exceed 10% of the applied radioactivity and in degradation experiments, together with the active ingredient, represent the relevant residues for quantification *(BABCZINSKI et al. 2000)*.

2.2.3.2 Degradation process

The laboratory studies performed by *BABCZINSKI et al. (2000)* on four soils point to a moderate to fast degradation of MKH6561 with an average half-time of approx. 60 days. A

biomineralization of both halves of the molecule to CO_2 was observed in all systems. In addition, separate degradation studies and model calculations were performed with the major metabolites saccharin, 4-hydroxysaccharin, N-methylpropoxytriazolinone, N-methylpropoxy-triazolinonamide and sulfonamide methyl ester. Depending on the soil type and the molecular structure of the metabolites, very different soil half-lives were obtained ranging from nine days for sulfonamide methyl ester and 178 days for 4-hydroxysaccharin *(BABCZINSKI et al. 2000)*.

MKH6561 is degraded up to a certain content under the influence of light. Although this degradation pathway is of no significance for the primary degradation of the initial substance it can make a contribution towards eliminating some residues by mineralization *(BABCZINSKI et al. 2000)*. In studies on the aerobic metabolism, mineralization amounted to 49% after 361 days (phenyl labelled) and 13% after 313 days (triazolinone labelled). MKH6561 was hydrolytically stable for all pH values (pH 5-9) tested *(BABCZINSKI et al. 2000)*. These values show that degradation in soil - as in the case of sulphonylurea herbicides - can be primarily attributed to microbial degradation, and proceeds most rapidly in warm, moist soils with a weak texture and low pH value.

2.2.3.3 Mobility

Apart from its degradation behaviour, above all the mobility of MKH6561 and its metabolites is of decisive significance for its behaviour in soil. Important characteristic properties for mobility are sorption behaviour, which can also influence the degradation rate via the bioavailability, and leaching behaviour in soil columns.

The sorption behaviour of MKH6561 and its major soil metabolites has been studied with various soils in batch equilibrium experiments in the laboratory (*BABCZINSKI et al. 2000)*. In addition to the adsorption isotherms (K_D and K_F values), the K_{OC} values relative to the organic carbon content of the soils were determined. A relatively high potential mobility for the active ingredient and the majority of its soil metabolites results from the findings of these adsorption and desorption studies. In experiments by *FENT et al. (1997)*, the K_D values of the adsorption of MKH6561 for six experimental soils ranged from 0.22 mL/g (loamy sand) to 1.71 mL/g (silty clay). The corresponding K_{OC} values of 12.90 mL/g to 106.20 mL/g were determined. Values of 0.95 mL/g to 2.27 mL/g were calculated for the desorption. This corresponds to K_{OC} values ranging from 38.40 mL/g to 203.60 mL/g. Substances with K_{OC} values from 0.50-50 mL/g and 50-150 mL/g are regarded as having weak absorption and substances with K_{OC} values from 150-500 mL/g as having moderate to elevated sorption *(MCCALL et al. 1980)*.

The values available therefore point to a fairly high mobility of MKH6561. The K_D and K_{OC} values of MKH6561 and some metabolites can be taken from Table 2.

Tab. 2: Ad-/desorption characteristics of MKH6561 and its degradation products

Test substance	Adsorption		Desorption		Mobility class
	K_D (mL/g)	K_{OC} (mL/g)	K_D (mL/g)	K_{OC} (mL/g)	After *MCCALL et al. (1980)*
MKH6561	0.22 - 1.70	12.90 - 106.20	0.95 - 2.27	38.40 - 203.60	high to very high
MKH6561 carboxylic acid	0.10 - 0.61	7.50 - 37.70	0.61 - 1.21	34.90 - 165.40	very high
Saccharin	0.02 - 0.25	4.60 - 15.50	0.09 - 0.64	6.20- 168.40	very high
Sulfonamide acid	0.003 - 0.11	0.10 - 6.80	n.r.	n.r.	very high
N-methylpropoxytriazolinone	0.18 - 1.20	8.90 - 75.70	1.01 - 1.93	42.20 - 271.80	high to very high
4-hydroxysaccharin	7.50 - 46.30	457 - 2873	17.40 - 72.80	810 – 4693	slight to low
N-methylpropoxytriazolinonamide	0.26 - 3.90	10.40 - 552	0.96 - 5.62	38.60 – 910.00	moderate to very high

n.r. = not recorded

The fact that the K_{OC} values of desorption are higher than those of adsorption shows that binding in the soil is in part irreversible. In contrast to the active ingredient, 4-hydroxy-saccharin has low mobility *(HEIN et al. 1999)*. Furthermore, the leaching behaviour of MKH6561 and its soil metabolites were studied with "aged residues" in a leaching experiment in a tipped soil column *(BABCZINSKI et al. 2000)*. The active ingredient and the majority of its soil metabolites demonstrated a high mobility in this experiment.

A three-year lysimeter study (two applications to winter wheat in spring, 70 g/ha, 70 WG) was performed to investigate the leaching behaviour of MKH6561 and its metabolites under practical application conditions. The results show that even under "worst case" conditions (twice the maximum application quantity), a contamination of soil horizons below a soil depth of 1.2 m or contamination of the groundwater can be ruled out: The concentrations of the active ingredient and its metabolites in the percolate were clearly below the critical threshold value of 0.1 µg/L for each sampling. Furthermore, investigations of the soil at the end of the experiment revealed that the active ingredient and its metabolites were almost completely degraded to CO_2 in the course of the experiment.

These laboratory findings have been verified in field studies *(BABCZINSKI et al. 2000)*. They provide the necessary information on the degradation and leaching behaviour of the active ingredient and its metabolites if applied according to good agricultural practice. The field degradation studies performed with a single application of the active ingredient formulated as 70 g/ha were analysed using the residue definition derived from the laboratory studies (active ingredient plus seven metabolites) and the method based on this definition.

The elimination rate (DT_{50}[9]) of MKH6561 total residues amounted to between 12 and 56 days and that of the active ingredient alone between 3 and 21 days. The findings show that both the active ingredient and also its total residues are readily degradable in soil under field conditions.

Model calculations using PELMO to simulate the environmental and leaching behaviour of the active ingredient including its relevant characteristics indicated a soil half-life of approx. 16 days for a single annual application of 70 g/ha to winter wheat in spring in the United Kingdom and Germany and of 42 g/ha in France over a period of ten years *(BABCZINSKI et al. 2000)*. The calculations were performed with the assistance of data from field degradation studies and a K_{OC} value of approx. 46 mL/g. The simulations showed that in no case did MKH6561 exceed a concentration of 0.1 µg/L in the leachate below a depth of one metre.

2.2.4 Behaviour in Water

The photolytic degradation of MKH6561 proceeds relatively slowly in aqueous solution under real environmental conditions (pH value and temperature) and thus makes little contribution to the elimination. Degradation of MKH6561 proceeds mainly by microbial processes both under aerobic and anaerobic conditions.

Studies by *BABCZINSKI et al. (2000)* on the aquatic degradation/metabolism of MKH6561 yielded an average degradation half-life of 101 days in the aerobic system and 22 days in the anaerobic system (in each case in the overall water/sediment system). In the water phase alone, the DT_{50} was on average 12 days.

In studies on the aerobic metabolism, MKH6561 was continuously and intensively metabolized in water-sediment systems. As also in studies on the anaerobic metabolism, MKH6561-carboxylic acid, N-methyl-propoxytriazolinone and sulfonamide acid were found as the major metabolites. The mineralization rate after 100 days was 16% in the case of phenyl labelling and 2% for triazolinone labelling.

2.2.5 Behaviour in Air

In addition to degradation, plant uptake and leaching, pesticides can also disappear by volatilization. On the basis of the vapour pressure, which represents the soil/air transition, and the Henry coefficient, which describes the water/air transition, no significant volatilization of MKH6561 can be expected since due to its low vapour pressure of $< 1 * 10^{-8}$ hPa at 20 °C the substance tends to be translocated with the soil water rather than with the soil air.

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[9] DT_{50} = time required for the degradation of 50% of a chemical compound (active ingredient/metabolites)

In the case of sulphonylurea herbicides, a slight volatilization takes place more rapidly from moist soil than from dry. The influence of soil moisture on volatility can be explained by the fact that herbicide and water molecules compete for free adsorption sites. If sufficient water is present to cover the surfaces of the soil particles, the volatility rate increases.
Moreover, the chemical lifetime of MKH6561 in air of approx. 6.5 h is very short *(HELLPOINTER 1996)*. The active ingredient is rapidly degraded by oxidative processes so that an accumulation due to wet or dry deposition is not to be expected. Only the active ingredient is of relevance for the quantification of the substance in air.
The compartment of air therefore does not play a major part with respect to the elimination of MKH6561 from the environment and for the assessment of its environmental risk.

All the metabolites and the active ingredient are listed in Appendix [A] I together with their different names, abbreviated chemical names and structural formulae.

3. Materials and Methods

The experiments performed in investigating the questions on the effectiveness of MKH6561 (cf. Chapter 1) are discussed in detail in the following Chapter 3 on the basis of the experimental parameters and an extensive description of the methods.

3.1 Experimental Containers

Two different experimental vessels were used (soil columns versus lysimeters) depending on the issue in question. Since both types of vessel display advantages and disadvantages (cf. chapter 8), it was decided not to restrict the investigation to one type of vessel.

3.1.1 Soil columns

16 small columns were used as experimental containers in the column experiments. They are V_2A steel cylinders (25 cm in length with a diameter of 20 cm), which were rammed hydraulically into the "undisturbed" arable soil (orthic luvisol) and filled completely up to the top 2 cm (cf. Figure 5).

Fig. 5: Experimental vessels used in the column experiments

After removal on 06.06.2001, the experimental columns were set up in a growth chamber (*HERAEUS-VÖTSCH*). Each column was placed in a plastic tray, which was half-filled with air-dried medium sand (0.2-1 mm).

3.1.2 Lysimeters

The lysimeter experiments were performed in eight rectangular vessels made of polyethylene (cf. Figure 6).

Fig. 6: Experimental vessels used in the lysimeter experiments

All the lysimeters were divided in half by a polyethylene partition so that access was given to 16 lysimeter(halves) - each 60 cm in length, 80 cm in width, and 50 cm in depth - with a capacity of approx. 240 litres. The outer walls of the lysimeters were insulated with mineral wool (8 cm thick). As protection against moisture, this insulation was surrounded by stainless-steel plate and painted white to prevent strong heating of the soil. There was an outlet at the bottom of each "half lysimeter" through which the percolate flowed into brown glass bottles (2.5 L), which were replaced as required. To stop the outlet from clogging, it was covered by a 10 cm * 10 cm square of metal gauze sealed at the edges with silicon. The lysimeters were constructed with a slight slope by adjusting the height of the "legs" of their supporting structure so that the leachate was better able to run into the outlets. A layer of gravel (2-3 mm) approx. 3 cm in thickness was placed at the bottom of the lysimeters for drainage purposes and plow layer soil (Ap horizon) was then added to a height of approx. 40 cm.
Eight (half) lysimeters were filled with orthic luvisol and another eight with gleyic cambisol.
The soil was mechanically compacted again and remained untouched for several weeks. This prevented later soil subsidence tearing the roots of the plants.

3.2 Experimental Plants

3.2.1 Crop

Spring wheat of the "Triso" variety which had been treated with "Landor™ CT"[10] was used for the column experiments. According to information from the DSV[11] (a German seed improvement company) the percentage of seed germination was about 96% and the thousand-seed grain was 49.4 g.

[10] Product of Bayer CropScience
[11] Deutsche Saatgutveredelung Lippstadt-Bremen GmbH

Winter wheat of the "Drifter" variety was used for the lysimeter experiments. The seed was treated with "Arena^{TM} C" liquid dressing[10]. The percentage of germination was given as > 95% and the thousand-grain weight was 50.3 g.
Before sowing, the caryopses were selected with respect to intactness, uniform size and freedom from atypical discolorations.

3.2.2 Grass weed

The grass weed to be controlled, blackgrass [ALOMY], is a predominantly winter annual variety. The main area of distribution of this variety is Europe, and its major occurrence is in regions characterized by an Atlantic climatic influence.
It is a lime-loving variety which mainly occurs on fresh to moist loamy and clayey soils with a moderate to good supply of humus and nitrogen. However, if there is a continuous supply of water blackgrass may also occur on light soils. Whereas half-bog soils with low pH values are avoided, high stocking densities are especially found in marshes and river plains.
Germination mainly takes place in autumn and spring. It finds favourable conditions for development in winter barley, winter wheat, winter rape and sugar beets *(ARLT 2002)*. Blackgrass requires sufficient soil moisture for germination. As a light germinator, approx. 90% of all plants generally emerge from a depth of up to 2.5 cm - usually at the same time as the crop. If winter cereals are sown early then the blackgrass develops so well that most plants are already have tillers at the start of the winter and thus they are then especially competitive *(ARLT 2002)*. If it has no tillers, *A. myosuroides* is sensitive to intermittent frost in spring.
During shooting and ear emergence, blackgrass develops very rapidly so that the ear is visible about one month before that of winter wheat and at this point it is taller than the wheat. The nutrient competition for nitrogen is of great significance, probably due to the rapid growth in spring. The cereal crop losses primarily result from a reduction of stocking density and number of grains per ear *(BABCZINSKI et al. 2000)*. In the case of winter barley, the grain moisture may also be increased, which reduces the quality and hampers harvesting (combine harvesting). In contrast, blackgrass has usually died off at the time of harvesting winter wheat.

Since the germination ability of the blackgrass seed was not initially known, a germination experiment was performed. 100 seed grains were placed in a plastic tray with the experimental soil (orthic luvisol) and left to germinate. After one to two weeks, the germinated seeds were counted. The percentage of germination was 51% and the thousand-grain weight (approx. 2.2 g) was determined by weighing.

3.3 Experimental Soils

3.3.1 Orthic luvisol

The Ap horizon (plough horizon) of an orthic luvisol of alluvial loess with a high clay and silt fraction was used both for the column and also the lysimeter experiments. The soil was taken from the 7.5 ha plot known as "Im Brühl" from the Merzenhausen district (Rhineland). This soil type is widespread in Germany and is one of the most productive agricultural soils.

3.3.2 Gleyic cambisol

In addition, a sandy soil (gleyic cambisol) from the Kaldenkirchen district with more than 70% sand in the overall profile - also used agriculturally - was included in the experimental programme for the lysimeter experiments. It is especially informative for the issues being dealt with (cf. Chapter 1) to additionally use a more water-permeable soil type with a different chemical and physical composition.

All the chemical and physical characteristics of the soil types studied are compiled in A II; the essential data of the Ap horizons of both soils are listed in Table 3.

Tab. 3: Comparison of selected characteristics of the Ap horizons of both experimental soils (*BURKHARDT 1999, PÜTZ et al. 2001*)

Soil type	**Orthic luvisol**	**Gleyic cambisol**
Sand [%]	6.4	73.3
Silt [%]	78.2	23.1
Clay [%]	15.4	3.6
Total pore volume [%]	46.3	45.2
Coarse pores [%]	12.9	31.9
Medium pores [%]	14.7	8.8
Fine pores [%]	18.7	4.5
pH value [$CaCl_2$]	6.56 [n = 3; s = 0.01; v = 0.15%]	5.65 [n = 3; s = 0.03; v = 0.45%]
pH value [H_2O]	7.10 [n = 3; s = 0.01; v = 0.10%]	7.06 [n = 3; s = 0.01; v = 0.14%]
T value [mval/100g]	11.40	11.00
S value [%]	11.48	2.65
C_{org} [%]	1.05	0.98

s = standard deviation
v [%] = coefficient of variation [%]
n = number of samples

The maximum water-holding capacity and the bulk density can be taken from Table 4d. As discussed in Chapter 2.2.1, on the basis of the characteristic data of the Ap horizons a good sorption but lower mobility and plant availability is assumed for the experiments with orthic luvisol. In the experiments with gleyic cambisol, poorer sorption but greater mobility and plant availability is expected.

3.4 Determination and Adjustment of the Soil Moisture

Immediately after removing the soil columns and filling the lysimeters, the weight of the experimental vessels filled with soil was determined. It was additionally determined how much water had to be added for the later adjustment of the desired soil moisture and to compensate for evaporation losses (also by weighing).

For the column experiments, each column was weighed together with its tray so that the capillary film did not tear. The tare weight, composed of the weight of the tray and medium sand as well as the weight of the empty soil columns, was determined in advance and deducted in the calculation.

For the lysimeter experiments the tare weight of the lysimeters without soil but with the gravel bed was also determined prior to starting the experiments. In addition, the soil material was weighed precisely before being loaded into the containers. A defined quantity of soil - in each case 320 kg - was filled into each half of the lysimeter. Since both halves had to be weighed together, the total quantity of water was distributed between the two halves of a vessel so that it was possible to determine gravimetrically how much water needed to be added.

Since the weight of the soil at a certain moisture level was determined by weighing the vessels, and the actual soil moisture (Chapter 3.4.1) and the maximum water-holding capacity (Chapter 3.4.2) were determined in the laboratory or taken from the literature data, it was possible to calculate the weight to be achieved and the soil moisture to be adjusted. The volume of water thus calculated was added gradually. In the case of excess water (especially for the "dry" variants in the column experiments) and in order to accelerate evaporation, the soil columns or lysimeters were stored in a greenhouse before starting the experiments, until the required quantity of water had evaporated from soil.

The moisture contents were determined as an approximation by taking the mean of several samples obtained at the time of filling the experimental containers. For the column experiments, the samples were taken from around the cylinders that had been pressed into the soil and for the lysimeter experiments the samples were taken while the vessels being filled.

In addition, the assumed weight of the plants sown onto the experimental vessels at the respective stage of application was predicted and included in the calculation (cf. A III & IV).

This procedure was used to determine how much water had to be added to maintain the desired soil moisture as precisely as possible.

3.4.1 Actual soil moisture

Actual soil moisture is referred to as the moisture content of an unprocessed soil in percent (*SCHLICHTING et al. 1995)*. By determining the soil moisture, the dry weight of the soil can be obtained indirectly; it moreover serves as a reference basis for moisture content data.
In the column experiments, the orthic luvisol was sampled by two different methods: on the one hand, by a sampling tube (Chapter 3.4.1.1), which was used to determine the moisture of soils withdrawn undisturbed, and, on the other hand, with a spade from soil pits in the case of disturbed soils (Chapter 3.4.1.2). In this way, it was possible to take a sample across the entire depth studied (approx. 23 cm).
In the lysimeter experiments, the actual moisture was only determined for disturbed soils (Chapter 3.4.1.2), since material from both soils (gleyic cambisol and orthic luvisol) had been filled into the lysimeters.

3.4.1.1 Undisturbed soil

Sampling of the undisturbed soil (column experiments) was performed by 100 cm^3 sampling tubes whose empty weight (tare weight) was determined before sampling. The sampling tubes were driven into the soil in the field. Of the 14 sampling tubes used, seven were driven into the upper region (0-10 cm soil depth) and seven into the lower region (10-20 cm soil depth) of the profile studied. Using a spatula, they were separated about 1 cm below the penetration depth and taken out. The soil projecting above the openings of the sampling tubes was levelled off by a knife so that the volume was completely filled with soil. The openings were sealed with covers.
In the laboratory, the gross fresh weight (FW_G) was determined by weighing. After removing the covers, the weighed sampling tubes were dried at 105 °C for 14 h to constant weight. In order to reweigh the sampling tubes, they were sealed, cooled in a desiccator and the gross dry weight (DW_G) was then determined. The net fresh weight and the net dry weight were determined after substraction of the empty weight. The mean value was taken from the samples in the upper and lower region, respectively. It should be noted that the samples from the upper region were dryer due to solar radiation.

The same sampling tubes were also used to determine the bulk density (ρb); this is defined as weight of the soil solid matter per volume unit - allowing for the soil pore system in natural stratification - after drying at 105 °C. The bulk density is obtained by dividing the weight of the solid matter (net dry weight) by the volume of the sampling ring used.

The bulk density was only additionally determined for the orthic luvisol in the column experiments. Since the gleyic cambisol and orthic luvisol in the lysimeter experiments were heaped soils, no precise determination was possible and no use could be made of literature data. However an estimation of the bulk density was determined. The values given for the lysimeter experiments can first only be estimates which, however, were required for the further course of the experiments (Chapter 3.5). During sampling, however, the bulk density of the individual soil layers investigated was determined by weighing. The values were used for the calculations in Chapter 4.
In the column experiments, uniform values were used for calculation (1.37 mg/L for the two top levels and 1.42 mg/L for the bottom depth level).

3.4.1.2 Disturbed soil

Seven mixed samples were taken in the column and in the lysimeter experiments, respectively. The soil samples from the disturbed soil were packed airtightly in polyethylene bags in order to avoid any moisture loss and kept in cold storage in the laboratory before being processed (on the same day).
In order to determine the net fresh weight (FW_N), 20 g of the soil to be investigated was weighed in a tared weighing bottle. At least two replicates were measured per soil. As already described for the sampling tube method, the soil samples were dried to constant weight in the open weighing bottles at 105 °C and reweighed. Finally the net dry weight (DW_N) was determined.

3.4.1.3 Evaluation

The actual soil moisture (m [%]) for undisturbed and disturbed soils was calculated. The net fresh weight (FW_N) and the net dry weight (DW_N) were set in Equation [1].

$$m\,[\%] = \frac{FW_N - DW_N}{DW_N} * 100 \qquad [1]$$

The results are shown in Tables 4a & b.

3.4.2 Maximum Water-Holding Capacity

The maximum water-holding capacity (WHC_{max}) has to be determined in order to adjust the correct moisture in the soil. The pore volume of the soil is fully saturated with water at this point in time.

The WHC_{max} of the orthic luvisol was taken from literature (*BURKHARDT 1999, PÜTZ et al. 2001*). WHC_{max} of the gleyic cambisol was determined as follows: Five glass funnels were attached to stands and equipped with round filters. The filters were moistened with distilled water until the water dripped off. 100 g of air-dried soil (≤ 2 mm) was added to each glass cylinder. A graduated cylinder was placed beneath each funnel outlet and sealed with Parafilm between the rim of the graduated cylinder and the outlet. A drip funnel was attached above each funnel on the stand so that the outlet remained about 1 cm above the soil surface. 100 mL of distilled water was added to each drip funnel and released drop by drop onto the soil surface to achieve a slow and uniform wetting of the soil. After one hour the amount of water (W_i) in the graduated cylinders was determined.

A mean value was taken from the five batches and subtracted from 100 mL. The resulting value (cf. Equation [2]) corresponds to the quantity of water retained by 100 g of dry soil, i.e. the maximum water-holding capacity (WHC_{max}):

$$WHC_{max} = 100 - \left(\frac{\sum Wi\,[mL]}{5}\right)\,[mL/100g] \qquad [2]$$

The actual soil moisture was calculated according to the method described in Chapter 3.4.1.1 for undisturbed soil and according to Chapter 3.4.1.2 for disturbed soil. The laboratory results for the column experiments are shown in Table 4a:

Tab. 4a: Determination of the actual soil moisture content in the column experiments (orthic luvisol)

Undisturbed soil: sampling by sampling rings	
m [%] (Upper horizon region)	13.54 [n = 7]
s/v [%]	0.64/4.73
m [%] (Lower horizon region)	20.66 [n = 7]
s/v [%]	0.67/3.24
m [%] (Total)	**17.10** [n = 14]

Disturbed soil: sampling by spade	
m [%] (Total)	**18.30** [n = 7]
s/v [%]	0.66/3.61

m [%] = mean value [%]
s = standard deviation
v [%] = coefficient of variation [%]
n = number of samples

The data from the two experimental batches were averaged and 17.7% was determined as the value of the actual soil moisture in the column experiments.

Tables 4b & c show the results of the determination of the actual moisture of orthic luvisol and gleyic cambisol at the beginning of the lysimeter experiments and the determination of the maximum water-holding capacity of gleyic cambisol.

Tab. 4b: Determination of the actual soil moisture in the lysimeter experiments (orthic luvisol and gleyic cambisol)

Disturbed soil: sampling by spade	Orthic luvisol								Gleyic cambisol							
	Lysimeter															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
m [%]	**20.27** [n = 7]		**21.57** [n = 7]		**20.62** [n = 7]		**21.17** [n = 7]		**16.67** [n = 7]		**15.97** [n = 7]		**16.8** [n = 7]		**14.41** [n = 7]	
s/v [%]	0.33/1.10		0.39/1.31		0.15/0.49		0.09/0.28		0.33/1.09		0.08/0.28		0.07/0.23		0.03/0.09	

Tab. 4c: Determination of the maximum water-holding capacity of gleyic cambisol in the lysimeter experiments

Gleyic cambisol (W_i) [mL]	65.7 [n = 5]
s/v [%]	0.8/1.21
WHC_{max} [mL/100g]	**34.3**

The mean values calculated and the values taken from the literature (*) for the actual soil moisture, the bulk density and the maximum water-holding capacity of both soils are summarized in Table 4d.

Tab. 4d: Data on water content and bulk density

	Column experiments	Lysimeter experiments	
Soil type	**Orthic luvisol**	**Gleyic cambisol**	**Orthic luvisol**
Bulk density [g/cm^3]	1.4	1.3	1.3
Soil moisture at start of the experiments [%]	17.7	14.41-16.8	20.27-21.57
WHC_{max} [L/100 g]	45.8*	34.3	45.8*

The values of the bulk density for the lysimeter experiments were values obtained for the disturbed soil after the experiments had been completed. It was not possible to determine the values before starting the experiments since the disturbed soil first had to settle.

3.4.3 Adjusting soil moisture

In the case of the column experiments, the following comments refer to A III and in the case of the lysimeter experiments to A IV. The amount of water to be added or expelled results from the difference in the value of the moisture to be adjusted (20, 40 or 60%WHC_{max}) and the actual moisture content (moisture at start) in percent determined by laboratory methods.

The balance can be expressed in mL/100 g soil (% = mL/100 g). After dividing by 100, the value is obtained in mL/g soil. This result is multiplied by the weight of the theoretically assumed dry soil [g], which is taken as the reference basis for water content data.

The weight of the dry soil can be calculated from the net weight of the soil and the actual soil moisture. The net weight of the soil in the experimental vessels is obtained by subtracting the tare weight from the gross weight (total weight). In this way, the amount of water to be added [mL] was determined relative to the respective experimental vessel (initial value). If this value

is added to the gross value, then this is the gross weight to be adjusted or the weight of the vessel at the desired soil moisture. The final weight to be adjusted (to be achieved by the time of application), is obtained by adding the weight of the plants at the final stage (cf. Chapter 3.4) and the weight of the maximum water fraction adsorbed by the bed of sand, which has to be additionally added (cf. Chapter 3.6.1). In the case of the lysimeter experiments, attention also had to be paid that the amount of water to be added was equally divided between the two halves of the lysimeter. As described, they were weighed together and their joint weight yields the gross weight to be adjusted.

3.5 Applied Radioactivity in the Column and Lysimeter Experiments

In order to detect and balance the active ingredient and its metabolites in plant tissue and in soil it is necessary to use radioactive labelling, especially if very small application quantities are involved (a few grams per hectare). It is thus possible to precisely determine the persistence of the active ingredient and its degradation products as well as the formation of bound residues. This technique allows for an overall mass balance to be drawn up and the conversion products (metabolites) to be traced. Due to the labelling of the phenyl ring (Table 1), this procedure can only identify metabolites whose ^{14}C ring remains intact.

The behaviour of MKH6561 in the environment can be studied by compounds labelled with [phenyl-UL-^{14}C] and [triazolinone-3-^{14}C]. All the investigations within the framework of this work were performed with [phenyl-UL-^{14}C]-labelled MKH6561, provided by Bayer CropScience (Wuppertal Isotope Laboratory). Prior to use, the substance was stored at -20 °C to keep the autoradiolysis as low as possible.

The specific radioactivity of the unformulated active ingredient was 3.82 MBq (103.3 µCi)/mg for the column experiments and 9.71 MBq (262.4 µCi)/mg for the lysimeter experiments. The radiochemical purity was > 98%.

The applied quantity of 70 g/ha was related to the area of the experimental vessels (column experiments 0.0314 m^2 per column, lysimeter experiments 0.48 m^2 per half) and offset against the specific activity of the active ingredient. 0.84 MBq was applied in the column experiments and 32.63 MBq for each half in the lysimeter experiments.

In order to determine whether this content (Bq/mg soil) was above the instrument-specific detection limit of the liquid scintillation counter (LSC) of 0.4 Bq, the volume of the experimental vessels filled with soil was ascertained (soil columns: 7.22 dm^3, lysimeter: 192 dm^3) and multiplied by the bulk density of 1.4 ("undisturbed" orthic luvisol)

and 1.35 ("disturbed" orthic luvisol and gleyic cambisol)[12]. The soil in each experimental column was calculated to weigh 10.11 kg and in each lysimeter half 259.2 kg. In the column experiments 0.098 Bq of radioactivity was calculated per milligram of soil and in the lysimeter experiments 0.13 Bq of radioactivity. This value was multiplied by 300 mg (quantity of sample to be weighed for ^{14}C combustion), which yielded 29 Bq of radioactivity in the soil sample for the column experiments and approx. 38 Bq of radioactivity for the lysimeter experiments. Assuming that the substance is homogeneously distributed, the sample was above the detection limit of 0.4 Bq (cf. Chapter 3.10.1.2), and was thus readily detectable.

Preliminary experiments indicated that 12 mL of water would be sufficient to wet the entire surface of the column experiments and 80 mL of water for the lysimeter experiments. The corresponding amount of radioactivity in 12 mL and 80 mL of application solution, respectively, was therefore applied to each experimental vessel. Since MKH6561 was applied as a 70% water-dispersible granular material, corresponding quantities of the formulation were added before. The actual content of applied radioactivity was calculated from several aliquots (100 µL), which were measured in the LSC and converted to the applied quantity (approx. 12 mL and 80 mL), minus the radioactivity losses which remained as residues in the pipettes or sample vessels.

The following amounts of radioactivity were applied to the experimental vessels (cf. Tables 5a & b):

Tab. 5a: Radioactivity applied in the column experiments

BBCH22	Column	1	2	3	4	5	6	7	8
	MBq	0.9728	0.9730	0.9727	0.9733	0.9722	0.9723	0.9730	0.9710

BBCH25	Column	9	10	11	12	13	14	15	16
	MBq	1.0280	1.1511	1.0294	1.0313	1.0281	1.0310	1.0283	1.0330

Tab. 5b: Radioactivity applied in the lysimeter experiments

	BBCH22				BBCH25			
Lysimeter	1	2	3	4	5	6	7	8
MBq	29.3948	29.4098	29.2950	29.3834	25.0570	25.1445	25.0654	25.1112

Lysimeter	9	10	11	12	13	14	15	16
MBq	29.6710	29.2885	29.4187	29.3982	25.2878	25.2221	25.2313	25.1304

It must be noted that the radioactivity was applied at two different stages in the vegetative growth of plants (BBCH22 and BBCH25) and thus two corresponding application solutions were prepared.

[12] For the disturbed soil, the bulk density was first estimated and then later confirmed in the laboratory.

As follows, the experimental setup of the column experiments and the lysimeter experiments, were presented in detail.

3.6 Column experiments - Small-Pot Experiments with Spring Wheat and Blackgrass

Since different growth stages of the plants should be investigated, two subexperiments were performed with an identical setup. The herbicide was applied to eight soil columns (Subexperiment I) when the (wheat) plants were at growth stage BBCH22. This represents the start of tillering: offshoots become visible in the leaf axils or adjacent to the main shoot; secondary roots and five leaves have been formed (cf. Figure 7a).
Furthermore, the herbicide was applied to the eight soil columns (Subexperiment II) at growth stage BBCH25. This stage represents full tillering. The plant begins to "creep" or leans over to one side; the offshoots have continued to develop (Figure 7b).

Fig. 7a & b: Relevant growth stages of plants for both experiments (BBCH code)

a) BBCH21 start of tillering	**b) BBCH25** main tillering

According to *GEISLER (1988)*, optimum tillering is achieved, on the one hand, by uniform planting of the (wheat) seeds at a depth of 2.5 cm, and, on the other hand, strong tillering can be obtained by regulating the day length in a growth chamber. Both conditions were taken into consideration in the present study so that the length was adjusted to 12 h since under long-day conditions the tillering phase would have been accelerated.

Due to a lack of space, the two subexperiments were staggered and performed under the following controlled conditions in a growth chamber (*HERAEUS-VÖTSCH*):

Light phase:	12 h	20 °C	70% relative humidity
Dark phase:	12 h	15 °C	70% relative humidity

Light sources: HQI-T 1000W/D (^{TM}*OSRAM* "Power Star"), 60 W krypton lamps
Light intensity at plant height: 400 µE/m^2/s

3.6.1 Soil moisture

The moisture content of the soils (orthic luvisol) in the columns was adjusted to 20, 40 or 60% of their respective maximum water-holding capacity (identical procedure in both subexperiments). The scenario under driest conditions was thus close to the permanent wilting point of the plant and the wettest scenario almost achieved field capacity.

The soil moisture of the wettest experimental variant was mainly adjusted via the respective bed of sand which has been adjusted to the maximum water-holding capacity of medium sand before starting the experiments. The water-holding capacity of the sand bed was calculated as described in Chapter 3.4.2. The results of the five batches were averaged (cf. Table 6a) and used to determine the amount of water to be added (Table 6b).

Tab. 6a & b: Determining the water-holding capacity of the sand bed

Sand bed (W_i) [mL]	78.4 [n = 5]
Standard deviation [s]	0.55
Coefficient of variation [v%]	0.70
WHC_{max} [mg/100 g]	**21.6**

Weight sand	**WHC_{max}**	**Water adsorption by the sand bed**	
[g]	[mg/100 g]	[mL/g soil]	[mL/1400 g soil]
1400	21.60	0.216	**302.40**

As can be taken from Table 6b, for a sand weight of 1400 g in the trays approx. 300 mL of water was added to adjust the maximum water-holding capacity of the sand bed.

The maximum water-holding capacity of the sand bed had to be adjusted to 60%WHC_{max} before starting the experiments since the sand itself - although to a limited extent - also adsorbs water. Water added to the already moist sand bed is transferred into the column by capillary forces. Furthermore, sufficient measures were taken to protect against evaporation (aluminium foil covering, cf. Figure 10).

The structure of the soil body was retained as far as possible by water logging from below (twice with 300 mL at the maximum water-holding capacity of the sand bed); heavy surface irrigation would have encouraged puddling of the upper soil region.

However, during the experiment it was not possible to verify exactly how much water was transferred from the sand bed into the column (and vice versa) or had been evaporated. Separate weighing of the two vessels, which would have been necessary for this purpose, was not possible since it had to be ensured that the capillary film between the sand bed and soil column did not tear and that the water gradient in the soil column was maintained. Instead of this the weight of 300 mL of water was added to the gross weight to be adjusted and keeping the sand bed moist. It could therefore be assumed that the sand bed was completely saturated with water (100%WHC_{max}) during the experiments. The water content in the soil columns thus fluctuated around 60%WHC_{max}.

Up to 600 mL water was added via the sand bed. The excess quantity was added from above by means of irrigation heads (cf. Chapter 3.6.4). Irrigation from above and below led to the most homogeneous possible moistening of the entire soil body. Since experimental variants with 20 and 40%WHC_{max} only required a slight addition of water they were irrigated directly from above.

Up to the time of application, the desired moisture content was kept constant. The water losses resulting from transpiration and evaporation were measured gravimetrically (balance: *SARTORIUS GmbH*) and supplemented through irrigation by the addition of precipitation water.

3.6.2 Sowing

The spring wheat and blackgrass were sown on the columns adjusted to the desired soil moisture on 04.09.2001 (Subexperiment I) and on 19.10.2001 (Subexperiment II).

To ensure that each column received the same number of experimental plants, the entire surface of the individual columns were planted in concentric circles using a template (diameter approx. 20 cm) with 19 holes. The distance between the seeds was fixed at approx. 3 cm. Due to the limited space on the column, the precise sowing density according to good agricultural practice was of no significance - in contrast to the lysimeter experiments. 38 grains of spring wheat and 76 grains of blackgrass were sown per column. Two grains of spring wheat and four grains of blackgrass were placed next to each other in each hole in the template. The double or fourfold occupancy ensured germination and uniform emergence of the seed. Surplus seedlings or those that emerged irregularly were removed so that each column had 19 plants.

The sowing depth was kept constant at 2.5 cm (spring wheat) and 0.4 cm (blackgrass). The variability in planting depths was minimized by using a sowing stick to press holes of a corresponding depth into the soil. The seed grains were placed in the holes with tweezers and covered with soil.

The columns were covered with transparent plastic film until the seeds germinated. Air was permitted to enter. The plastic film prevented the soil from drying out. There was no lack of water at any time during germination. On the one hand, the soil surface was sprayed with water and, on the other hand, a single application of 200 mL of water was made by means of the irrigation head for the dry experimental variant. A lack of water during this period would have led to a delay in germination and irregular emergence of the seedlings.

3.6.3 Application

Two different application dates were chosen for the radiolabelled herbicide. The parameter distinguishing the two subexperiments was the growth stage of the wheat plants (start of tillering and main tillering; cf. Table 7).

Tab. 7: Overview of application dates for the column experiments

Subexperiment	**I**	**II**
Sowing	04.09.2001	19.10.2001
Growth stage reached on	BBCH22 25.09.2001	BBCH25 15.11.2001
Days after sowing	21	27

The herbicide was applied in drops. The active ingredient was homogeneously distributed and not in the direct vicinity of the edge regions of the column experiments to prevent active ingredient running down on the inner column surfaces after a possible partial detachment of the soil body from the column. The herbicide was applied directly to the soil, without touching the plants, to ensure an investigation of the effect across the soil.

3.6.4 Irrigation

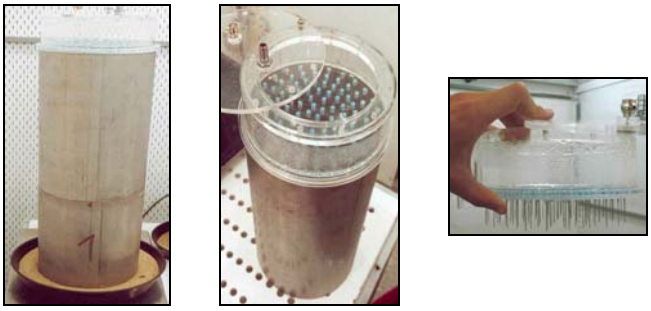
After application, two different precipitation scenarios (A & B) were performed (cf. Table 8):

A) three days without precipitation then two events with "normal" precipitation at an interval of three days;

B) three days without precipitation, then heavy rain followed by a single "normal" precipitation event at an interval of three days.

An irrigation head was mounted on each of the columns. It rested on a steel tube 25 cm in length with a diameter of 20 cm abutting the soil column (cf. Figure 8).

Fig. 8: Irrigation unit for the column experiments



All irrigation heads had 100 uniformly distributed disposable injection needles (*STERICAN*, 0.60 * 30 mm). This enabled a homogeneous and slow application without puddling the topsoil. In order to reduce evaporation losses each irrigation head had a closable lid. Normal

and heavy rain was distinguished by applying different amounts of water through the irrigation heads: A rain event with "normal precipitation" was defined as 7 mm (7 L/m^2). Relative to the surface of the columns (0.0314 m^2), this yields 0.219 L per column, which was achieved by adding 220 mL of water. A heavy rain event was defined as 16 mm (16 L/m^2). Relative to the individual soil column this yielded 0.502 L so that 500 mL of water was added. Table 8 shows details of the different irrigation scenarios:

Tab. 8: Irrigation scenarios in the column experiments

Scenario	Precipitation event	Date	Quantity [mL]	Days after sowing	Columns
A	3 days without precipitation				1-6; 9-14
	normal precipitation	28.09.2001 01.10.2001	220 each	24 27	1-6
	normal precipitation	17.11.2001 20.11.2001	220 each	24 27	9-14
B	3 days without precipitation				7, 8; 15, 16
	heavy rain normal precipitation	28.09.2001 01.10.2001	500 220	24 27	7, 8
	heavy rain normal precipitation	17.11.2001 20.11.2001	500 220	24 27	15, 16

The overall setup of the column experiments with the different levels of soil moisture, amounts of precipitation and growth stages is summarized in Figures 9 & 10.

Fig. 9: Schematic of the experimental setup for the column experiments

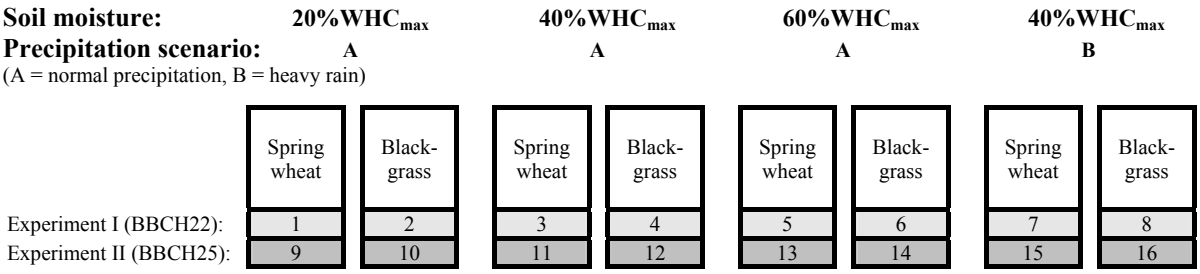


Fig. 10: Experimental setup for the column experiments - illustrated by the example of Subexperiment I (BBCH22)

3.7 Lysimeter Experiments with Winter Wheat and Blackgrass

On the basis of the results from the column experiments, outdoor lysimeter experiments with winter wheat were performed to investigate the same issues but under conditions in accordance with agricultural practice.

3.7.1 Soil Moisture

The lysimeter experiments were performed with two moisture variants (45 and 60%WHC_{max}), in each case with the same soil moisture level in one lysimeter (two lysimeter halves). The different levels of soil moisture after sowing were adjusted and maintained throughout the entire experiments until application of the herbicide by regular, selective irrigation to compensate for the water losses arising from evapotranspiration. Furthermore, for the moister experimental variant, the natural precipitations were included until the soil moisture had been adjusted. The experimental vessels for the drier variant were protected against natural precipitation. If a rain event was expected and also overnight, lysimeters were placed under a canopy with a roof made of plastic sheets (permeable to UV light) and irrigated in a controlled manner with rainwater. After the soil moisture had been adjusted, the lysimeters for the moister variant were also placed under this canopy and subjected to controlled conditions.
The lysimeters were weighed twice a week on an underfloor balance (*TOLEDO*, type 8134) in order to gravimetrically record the water losses and to adjust and maintain the soil moisture.

3.7.2 Sowing

Winter wheat and blackgrass were sown on the lysimeters on 12.12.2001, about five weeks after the orthic luvisol had been filled in and about seven weeks after the gleyic cambisol (cf. Chapter 3.1.2). The long period between filling the soil into the lysimeter and sowing was intended for setting of the soil. Sowing and emergence at a relatively late date in the year were possible since it was not the goal of the experiments to achieve the maximum yield. The lysimeters were first placed in a greenhouse where the temperatures were higher than in the field until the seedlings had caught up with the growth of plants in the field.
The plant density was fixed at approx. 400 winter wheat and approx. 500 blackgrass seeds per square metre, according to agricultural practice. The percentage of germination was calculated in order to take into account any seeds that were not capable of germination. Furthermore, the density was converted to the surface area of the lysimeter halves (0.48 m^2).
192 grains of winter wheat were thus planted at the corresponding depth in six rows (with 32 grains each) per vessel and pressed into the soil. 470 grains of blackgrass (weighed via the

thousandgrain weight; approx. 1.04 g) were distributed across the surface as homogeneously as possible. The raw spacing between the rows was approx. 10 cm for winter wheat with a planting interval of approx. 2.5 cm. The planting depth for winter wheat was approx. 2.5 cm and approx. 0.4 cm for blackgrass.

3.7.3 Application

The radiolabelled herbicide was applied to plants with the target stage of BBCH22 at the end of February 2002 and to plants with the target stage of BBCH25 at the beginning of March 2002 (cf. Table 9).

Tab. 9: Overview of application data for the lysimeter experiments

Growth stage	BBCH22	BBCH25
Sowing	12.12.2001	
Stage reached on	28.02.2002	8.03.2002
Days after sowing	79	87

The herbicide was applied a few millimetres away from each side of the lysimeters in drops by distributing it homogeneously using a 5 mL pipette. Since two different soils were used in the experimental programme with different chemical and physical properties (cf. Chapter 3.3) the plants growing on them reached the required application stage at slightly different dates. However, the herbicide was applied as soon as the majority of plants had reached the required stage.

3.7.4 Irrigation

As for the column experiments, precipitation scenario A (cf. Chapter 3.6.4) was selected and all the lysimeter halves were homogeneously irrigated with collected precipitation water using a commercial gardener's watering can with a fine rose. After application, no irrigation was performed for three days, followed by two "normal precipitation events" at an interval of three days. Converted to the area of each lysimeter half (0.48 m^2) this was approx. 3 litres per rain event (cf. Table 10).

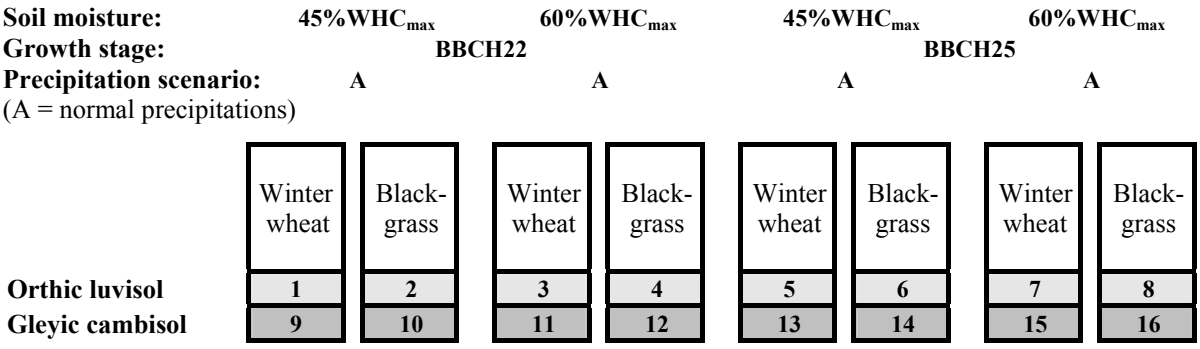
Tab. 10: Irrigation scenario in the lysimeter experiments

Scenario	Precipitation event	Date	Amount [mL]	Days after sowing	Lysimeter
A	3 days without precipitation				1-4 & 9-12 (BBCH22)
	normal precipitation	04.03.02	3000	83	
		07.03.02	3000	86	
A	3 days without precipitation				5-8 & 13-16 (BBCH25)
	normal precipitation	12.03.02	3000	91	
		15.03.02	3000	94	

In contrast to the column experiments, the leachate drained off and was collected.

The experimental setup of the lysimeter experiments is shown in Figure 11.

Fig. 11: Schematic of the experimental setup for the lysimeter experiments



3.8 Sampling the Plant and Soil Material

Since sampling and sample preparation was performed similarly for the column and lysimeter experiments, the procedures are summarized in Table 11. In the case of the column experiments samples were taken on two dates at intervals of one week and on three dates for the lysimeter experiments.

Tab. 11: Sampling the plant material after application of MKH6561

	Column experiments		Lysimeter experiments		
Stage BBCH22 (application)	**Sampling date**		**Sampling date**		
	1	**2**	**1**	**2**	**3**
Sampling date	04.10.2001	11.10.2001	11.03.2002	18.03.2002	25.03.2002
Days after application	9	16	9	16	23
Growth stage reached	BBCH27	BBCH29	BBCH27	BBCH29	BBCH31

Stage BBCH25 (application)	**Sampling date**		**Sampling date**		
	1	**2**	**1**	**2**	**3**
Sampling date	23.11.2001	30.11.2001	19.03.2002	26.03.2002	02.04.2002
Days after application	8	15	9	16	23
Growth stage reached	BBCH31	BBCH33	BBCH31	BBCH33	BBCH35

In the column experiments, six plants per vessel were removed on the first sampling date and divided into the fractions of leaf & shoot and roots. Mixed samples were prepared from this sample material - subdivided into the fractions to be investigated. The shoot with the leaves was cut off above the hypocotyl (transition zone between root and shoot). The associated roots were taken out together with the surrounding soil by a cartridge corer (*HUMAX*). A probe with a diameter of 35 mm and a length of 30 cm was used to sample the entire height of the column - without compressing the soil body.

After sampling, the cohering roots to be identified were carefully freed from soil using a spatula and rinsed in distilled water; an aliquot of the washing solution of both the root and leaf material was measured by LSC. This was followed by further washing procedures until no ^{14}C radioactivity was measurable in the washing water. The soil material adhering to the roots and remaining in the corer from all six samples - which were distributed over the entire surface of the column – were separated into the individual depth levels to be studied.

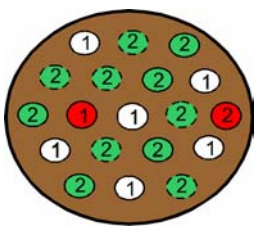
The soil material from all the sample cores from one column was combined for each individual depth and mixed thoroughly. The resulting 23-cm-deep holes were filled with sand using the polyethylene cartridges of the corer with the same circumference and length.

The plant samples were first weighed and their fresh weight determined. They were then wrapped in aluminium foil, shock frozen in liquid nitrogen and then sealed in polyethylene. The samples were stored at -20 °C before further processing.

In addition, one plant per sampling date was freshly harvested from each vessel for macroautoradiographic investigations (cf. Chapter 3.10.5) and rinsed under running distilled water. The parts of the plants below ground were also sampled by the corer.

This procedure was part of sampling in all experimental scenarios. The completion of the column experiments one week later was at the same time as the second sampling date: All remaining plants were harvested, fractionated into leaf and root mass and combined into mixed samples. The procedure at the second sampling date corresponded to that of the first one. It merely differed in the number of plants to be processed. Altogether, 64 different plant samples were obtained from the column experiments for further processing. At the two sampling dates, six and approx. eleven plants, respectively, were taken from 16 soil columns, divided into the two fractions to be investigated. Furthermore, at both sampling dates 16 plants (i.e. one per column and sampling date) were analysed using macroautoradiography. The way in which the sample material was obtained in the column experiments is shown schematically in Figure 12.

Fig. 12: Sampling in the column experiments (schematic and in the photograph)



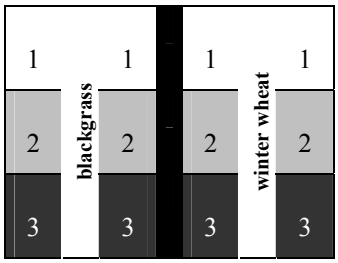
white 1 = **1st sampling date:**
6 plants & soil material (from 6 cores)

green 2 = **2nd sampling date:**
approx. 11 plants & soil material (from 4 cores)

red 1, 2 = **1st & 2nd sampling date:**
plants for macroautoradiographic investigation
(dotted circles = only plants sampled)

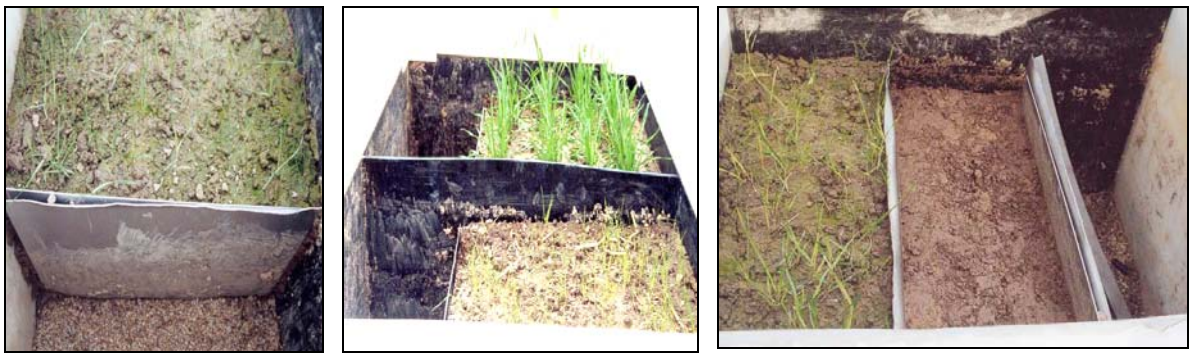
In the lysimeter experiments, sampling and preparation of the samples and separation into the required fractions was performed in an identical manner to that of the column experiments and can be taken from the preceding description. In contrast to the column experiments, each half of the lysimeter was divided into three by plates driven into the soil. Each third was used for a different sampling date; the partitions were only driven into the soil immediately before sampling. The soil material was carefully removed in layers using a large spatula according to the fractions to be studied (cf. Figures 13 & 14).

Fig. 13: Sampling in the lysimeter experiments (schematic)



1 = **1st sampling date:** at least 15 plants & soil material
2 = **2nd sampling date:** at least 15 plants & soil material
3 = **3rd sampling date:** at least 15 plants & soil material

Fig. 14: Sampling in the lysimeter experiments



In order to keep the plates stable - they were intended to prevent the sample material from slipping - the soil material that had been removed was replaced by sand. At least 15 plants were taken from each third of the total of 16 lysimeter halves for the study and mixed samples prepared.

During sampling, as in the column experiments, the plant material was separated into the two fractions to be studied (shoot & leaves, roots) so that 96 different plant samples were available for further processing. In addition, two plants were removed from each lysimeter half at every sampling date for macroautoradiographic studies, which yielded a sample volume of 96 plants. The roots of the plants used for macroautoradiography were carefully released.

In the column experiments, the soil material was investigated at three different depths and in the lysimeter experiments at four depths:

- column experiments: 0-5 cm, 5-10 cm and 10-23 cm
- lysimeter experiments: 0-5 cm, 5-10 cm, 10-20 cm and 20-40 cm

The different thickness of the layers is based on the assumption that in the deeper soil region much smaller quantities of the substance to be investigated will be detectable.

The plant roots were recovered by the Humax corer so that in the column experiments at the first sampling date six samples of the fractions per column were available. All six samples from a vessel and the corresponding depths were fractionated after the polyethylene cartridges had been opened and after the plant roots had been extracted and combined with the other sample material obtained at this sampling date from the same column in the corresponding fractions.

The soil material of all plants used for macroautoradiography was assigned to the corresponding soil fractions. In order to prevent mixing of soil material from various layers during sampling, the top two centimetres of soil material was removed separately and the Humax corer driven into the soil at this point. After fractionation, the top two centimetres were assigned to the corresponding fraction (0-5 cm).

After the column experiments had been completed, the soil samples from the second sampling date were obtained, also in the form of mixed samples subdivided into fractions. Since it cannot be ruled out that due to the first sampling some of the sample material at the edge fell into the resulting holes, at the "final sampling" sample material was obtained again by the Humax corer. However, it was only possible to make approx. four cuts per column. After each cut, the resulting holes were refilled by polyethylene cartridges filled with sand.

In the lysimeter experiments, soil samples were obtained for each lysimeter half (16 in total) by digging out the entire layer and separating it according to depth levels (four) and sampling date (three), and then combining identical fractions and mixing them thoroughly.

In this way, 192 different soil samples were available for further processing in the laboratory in the case of the lysimeter experiments and 96 for the column experiments. The sample material was stored at -20 °C in a refrigerated container until it was processed.

The actual soil moisture content (cf. Chapter 3.4.1) was determined for each individual soil sample during sampling so that the soil moisture content in the individual soil layers studied

was precisely determined at the date of sampling. Soil moisture content was determined at three times and a mean value was calculated.

In the lysimeter experiments, each layer was weighed separately. The weight of the dry soil was calculated from the mass [kg] including soil moisture, and the bulk density (ρb) was obtained by dividing it by the volume of each layer.

In the column experiments, investigation of the sand bed below each of the 16 columns allowed to check how much radiolabelled herbicide had percolated through the soil body. At the end of the experiments, a mixed sample was measured to obtain a mean value.

In addition, the quantity of percolate collected was determined in the lysimeter experiments. Furthermore, the total ^{14}C radioactivity was determined 2-3 days after irrigation events.

Moreover, the washing solution of each experimental vessel of the plant samples combined into a mixed sample (leaves or roots) was collected in the column and lysimeter experiments and the total ^{14}C radioactivity in the washing solution determined. This was used to identify the fraction of pesticide that was not taken up by the plant but remained adhering externally. For example, in the case of the leaves active ingredient fractions might remain adhering to the leaves due to soil contact (binding processes with the cuticle) or could even be taken up. A contamination of the samples could result, i.e. it would no longer be possible to unambiguously determine the active ingredient uptake via the roots.

It was possible to collect sufficient sample material by the procedure described so that later analyses were performed in triplicate.

Table 12 summarizes the experimental variables in the column and lysimeter experiments.

Tab. 12: Overview of experimental variables in column vs lysimeter experiments

Column experiments		**Lysimeter experiments**
16 columns	⇦ **experimental vessels** ⇨	8 lysimeters
orthic luvisol	⇦ **soil type** ⇨	orthic luvisol/gleyic cambisol
spring wheat/ALOMY	⇦ **experimental plants** ⇨	winter wheat/ALOMY
climate chamber	⇦ **experimental conditions** ⇨	field
20, 40, 60%WHC_{max}/heavy rain	⇦ **soil moisture** ⇨	45 and 60%WHC_{max}
BBCH22 and 25	⇦ **application date** ⇨	BBCH22 and 25
2	⇦ **number of sampling dates** ⇨	3
0-5, 5-10, 10-23	⇦ **depth levels [cm] in soil** ⇨	0-5, 5-10, 10-20, 20-40
sand bed	⇦ **other compartments** ⇨	percolate

3.9 Preparation and Homogenization of the Sample Material

All the soil samples were air-dried and sieved to < 2 mm. The samples were additionally homogenized in order to determine the total ^{14}C radioactivity (planetary ball mill, *RETSCH*) (7 min at 200 rpm).

The plant samples were dried for approx. five days on precooled plates in a freeze drying apparatus (STERIS^{TM}, Lyorac GT2), their dry weight was determined and they were subsequently homogenized. Larger plant parts were preground in an electric coffee grinder (Aromatic KSM2, *BRAUN*) and then ground in a vibratory mill - as were the smaller samples without any pregrinding (*RETSCH*, type *MM*) (4 min at 70 rpm).

3.10 Recording the Radioactivity and Determining the Active Ingredient

The studies described in this chapter will provide information about the ^{14}C radioactivity content taken up into the plant and distributed within the plant and also remaining in the topsoil and in part translocated.

The total ^{14}C radioactivity in the plant (leaf and shoot, roots) and soil samples was determined by combusting (*PACKARD*, Oxidizer 306) and detection by LSC. The total ^{14}C radioactivity of the percolate (lysimeter experiments) and the washing solution for the plant parts was measured directly by LSC. In the column experiments the total ^{14}C radioactivity of mixed samples of the sand bed was determined by combusting. The fractions of active ingredient and metabolites and their concentrations were balanced in the case of plant material after extraction and in the case of soil material after desorption. With respect to the metabolites, it must be noted that a separation was made in the present study. Since not all the metabolites occurring were available as reference substances, only those metabolites were considered in detail for which corresponding reference substances were available. The remaining metabolites were combined as the "total fraction of metabolites not characterized in detail".

The ^{14}C radioactivity in the desorption and extraction solutions was determined directly (without further processing). By using thin-layer and high-performance liquid chromatography, a separation was first made into parent compounds and metabolites, and the active ingredient fractions were determined. The active ingredient fractions in the plant and soil material were then determinated and the concentrations calculated.

3.10.1 Combusting and radioactivity measurements

As already mentioned, the sample material was available in both liquid and solid form. Since quantitative ^{14}C radioactivity measurement by LSC can only be performed on liquid sample material, in a first step the solid samples had to be correspondingly processed. This will be described in more detail in the following.

3.10.1.1 Solid sample substance

In order to determine the total ^{14}C radioactivity, solid samples (plant and soil samples) were combusted, the $^{14}CO_2$ was adsorbed in 10 mL of Carbosorb™ (*PACKARD*) and mixed with 10 mL of Permafluor-Scintillator™ *(PACKARD*). The ^{14}C radioactivity was quantified by LSC (cf. Chapter 3.10.1.2).

Soil and sand samples as well as desorption residues were ground and then combusted in three aliquots of 500 mg each. Plant samples were combusted in three aliquots of 300 mg each. The sample material was pressed into pellets (hand-operated press, *PARR INSTRUMENTS*). Per pellet, the sample material was weighed in approx. 300 mg cellulose tissue (*KIMBERLY-CLARK CORP.*). Soil and sand samples (500 mg) were additionally mixed with approx. 300 mg of crystallized cellulose (per pellet) to improve the combustion properties. In order to verify the instrument functions, additional standards of known ^{14}C radioactivity were combusted and the recovery rate determined. The recovery rate at the Oxidizer was at least 96%.

3.10.1.2 Liquid sample substance

The ^{14}C measurement for all sample solutions was performed in a liquid scintillation counter (LSC, *CANBERRA*, TRI-CARB™ 2500TR) with quench correction by means of sample-specific calibration series and external standardization.

For the measurement, the untreated sample is mixed with a scintillation cocktail typically composed of an aromatic organic solvent, a scintillation substance and suitable solubilizers. In the oxidizer, pretreated solid samples were automatically mixed with these substances (cf. Chapter 3.10.1.1). Ultima Gold™ or Instant Scintillation Gel™ (*PACKARD)* served as scintillators for aqueous samples.

The radioactivity in the percolate (lysimeter experiments) was measured directly in the LSC after scintillator had been added. In each case, three aliquots (of 5 mL each) were measured in the same quantity (5 mL) of Ultima Gold™ in the LSC.

The detection limit of the LSC is defined in the sense of a discrimination limit as the smallest quantifiable amount of radioactivity that can be differentiated from a zero value in a statistically significant manner. On the basis of the double zero value, this amount was 0.4 Bq for all samples measured in the LSC. The following detection limits were thus identified for the active substance equivalents determined (Table 13).

Tab. 13: Detection limits of MKH6561 for column and lysimeter experiments

	Column experiments		**Lysimeter experiments**	
	Plant material	Soil material	Plant material	Soil material
Weighed quantity [g]	0.1	0.5	0.1	0.5
Specific radioactivity [MBq/mg]	3.82		9.71	
Detection limit active ingredient equivalents [µg/g]	**0.0011**	**0.0002**	**0.0004**	**0.0001**

It should be noted here that if values are below the detection limit (a few values from the bottom layers of the soil material), this will be indicated in the corresponding chapters. All measured ^{14}C contents (active substance equivalents) of the plant material were above the detection limit.

3.10.2 Extraction of plant samples

Two parallel samples of each ground plant sample (approx. 0.09 g each) were mixed with 1.6 mL of acetonitrile-water (80:20, v/v) and shaken for 1.5 h (*Unimax 1010, HEIDOLPH* at 500 rpm). The samples were then centrifuged for 15 min in a table centrifuge (*Mikro-Rapid, HETTICH*) at 4000 rpm. The clear supernatant was decanted. The extraction was repeated three times, the supernatants were combined and air-dried with N_2 at 50 °C. The samples were then dissolved in 2 mL of methanol and shaken for 1.5 h (*Unimax 1010, HEIDOLPH*, at 500 rpm). Any residues were dissolved in an ultrasonic bath. Assuming that no losses occurred during the entire process, an aliquot of 50 µL each was taken on two occasions from the resulting methanol solution and measured. A mean was obtained from these values and the extractable contents thus determined. The results, furthermore, provide indications of how the chromatographic separation method should be performed, which will be described in more detail in Chapter 3.10.4.

The (non extractable) residues of the plant material remaining in the centrifuge tubes were transferred onto filter paper, dried at 30 °C, pressed to pellets, combusted and measured by LSC for balancing.

3.10.3 Desorption of soil samples

To describe the potential bioavailability of the active ingredient and its metabolites, a desorption was performed for all soil samples (in two steps) using aqueous solution with a low salt concentration. Since the salt content in soil solution is comparable to the desorption solution, no matrix-destructive effects on the soil structure are to be expected. Furthermore, the desorption steps are closely related to processes occurring during drying and remoistening events in nature.

Two samples of 10 g each of air-dried, sieved fine soil (< 2 mm) were weighed into 150 mL centrifuge tubes and in a first desorption step, after the addition of 50 mL of 0.01 M $CaCl_2$ solution, they were desorbed for 4 h at 160 rpm in a horizontal shaker (*BÜHLER, type A2)*. The sample quantity of 10 g is intended to ensure that measurable desorbable fractions were also present in the bottom soil layers investigated.

The samples were then centrifuged for 15 min at 2000 rpm *(BECKMANN, type GPKR)*. From the clear supernatant, 5 mL was pipetted three times for radioactivity measurement and 25 mL for active ingredient analysis. The entire solution quantity withdrawn was replaced in the second desorption step by 40 mL of fresh 0.01 M $CaCl_2$ solution and the sample was treated again in the manner described above. In contrast to the first desorption step in addition to the three times 5 mL, 30 mL of desorption solution was additionally pipetted for active ingredient analysis.

After the addition of Ultima Gold^{TM}, the solutions from the first and second desorption step were measured separately and the ^{14}C radioactivity contents determined after desorbing the solutions once or twice, respectively. The pipetted solutions for active ingredient analysis from both desorption steps were combined for each of the two parallel samples and concentrated to 0.5-5 mL in the vacuum rotary evaporator at 40 °C in order to subsequently determine the active ingredient and metabolite fraction. After each step, samples of the distillate from the receiver of the rotary evaporator were examined for ^{14}C radioactivity. The receiver contained pure water thus ensuring that no losses of radioactivity occurred in the sample liquid. As in extraction, the respective mean value was then determined from the two parallel samples.

Due to the larger sample volume, in the lysimeter experiments the desorption solutions were concentrated by means of a *TURBO VAP*^{TM} *(ZYMARK)* at 40 °C.

The desorbed soil was dried at 30 °C together with the 5 mL of desorption solution remaining after pipetting, ground (high speed planetary ball mill, *RETSCH*) and combusted for balancing

(cf. Chapter 3.10.1). The remaining 5 mL of desorption solution was subtracted from the nondesorbed fractions in a later balancing process.

The detected ^{14}C contents of the residues are composed of three fractions: first of all the incompletely desorbed fraction, secondly the fraction that could (possibly) have been extracted by a different/stronger extractant and finally the non-extractable, bound residues. These residues formed by irreversible incorporation in clay minerals or cavities of the organic soil structure and also by chemical reactions with the molecules of the humic substance remain in the matrix in the form of the active ingredient or its metabolites and cannot be extracted without using matrix-modifying extraction methods. A characterization of the individual "residue fractions" was of no relevance for the questions under investigation so that they were not further studied here and only the overall content was determined.

3.10.4 Chromatographic separation methods

Two qualitative analytical methods were used for the chromatographic separation of plant extracts and desorption solutions from the soil as well as standard solutions: thin-layer chromatography (TLC) and high-pressure liquid chromatography (HPLC). The aim is to provide information concerning the percentage distribution of the ^{14}C-labelled active ingredient and its metabolites and to assure the results by application of two different methods.

As a preparation for the chromatographic separation, the extracts and concentrated desorption solutions (cf. Chapter 3.10.2 and 3.10.3) were centrifuged for 15 minutes at 12,000 rpm (*Mikro-Rapid, HETTICH)*. The resulting clear supernatant was applied onto TLC plates or injected into the HPLC device, as is described in more detail in the following.

3.10.4.1 Radio thin-layer chromatography (radio-TLC)

Aliquots of the sample solutions from the clear supernatant were applied onto silica gel plates (silica gel $60F_{254}$, layer thickness 0.25 mm, 200 * 200 mm; *MERCK*). Before use, the TLC plates were conditioned for at least 4 h at 105 °C in order to remove any moisture adhering to the separating layer. The volume of the solutions was related to the ^{14}C activity of the solutions and amounted to 50-100 µL for the soil solutions and plant extracts; 50 µL of the available reference substances was additionally applied in each case. This was done with the aid of the *LINOMAT IV (CAMAG)* in 1 cm-wide bands adjacent to each other in the concentration zone with the distances from the starting spots (relative to their centre) being 2 cm. The inactive standards [propoxycarbazone-sodium (active ingredient), 2-hydroxy-

propoxy-MKH6561 (major plant metabolite) and 4-hydroxysaccharin (major soil metabolite)] were taken up by dissolving 5 mg of substance in the same medium in which the samples were dissolved. For the soil samples this was a 0.01 M $CaCl_2$ solution and methanol was used as a solvent for the plant extracts. The reference substances were regularly prepared freshly and stored at 4 °C. The standards were applied at the starting line of the plate both separately and also overlapping the sample substances by 0.5 cm and were developed with the sample mixture (cochromatography).

As soon as all samples had been applied and had dried, each TLC plate was developed separately. The mobile phase system A was used by default for the extraction and the desorption solutions of the plant and soil material both for the column and also for the lysimeter experiments. System B was used as a control and back-up for the bands.

Composition of the mobile phase systems

A (TLC1): chloroform/methanol/glacial acetic acid	(90:10:1)
B (TLC2): dichloromethane/methanol/water/25% ammonia solution	(75:30:3:1.5)

The plates were developed in closed glass vessels with filter paper inserts to condition the atmosphere in the chamber.

The mobile phase, which was always freshly prepared, ascended upwards due to the capillary force and entrained the individual substances of the mixture. Depending on the affinity to the stationary (carrier) and mobile phase, the individual substances migrated upwards with the solvent at different rates (cf. *LEWANDOWSKI et al. 1997)*. The individual components of the mixture reached different heights on the carrier and were thus separated from each other. The carrier was removed from the elution chamber before the mobile phase had reached a predefined "finishing line". After drying, the TLC plates were examined under UV light (Fluotest Universal, *ORIGINAL HANAU*). The bands of the inactive reference substance spots were localized and labelled with radioactive ink. The TLC plates were exposed on image plates and deposited in a safe. After a period of time, the length of which depended on the radioactivity applied (24 h for > 16 Bq per spot), the image plates were read out with the aid of the *BAS Reader*™ *(RAYTEST, Isotopenmessgeräte GmbH)*.

The intensity and separation of the parent compounds and metabolites and the distribution of the radioactive bands of the individual tracks was examined using a image plate scanner from the BAS series from Fuji Photo Film [*FUJI BAS 1000 (RAYTEST, Isotopenmess-geräte GmbH)*]. The agreement of the radioactive bands of the test substance and the bands of

the reference compounds labelled with radioactive ink provides unambiguous identification of the radioactive parent compounds and the metabolites in the sample material.

With the aid of the *TINA*^{TM} *Version 2.09* PC evaluation software (*RAYTEST, Isotopenmessgeräte GmbH),* the stored grey stages were converted into the manufacturer-defined unit PSL. After subtracting the background, the surface integral of the peaks of the radioactive substance spots were quantified. The peaks were assigned to MKH6561 and the two known metabolites, and the percentage distribution of the active ingredient and the metabolites determined in the next evaluation step.

Each sample was only applied once. However since two parallel samples were available (see Chapter 3.10.3), a mean value was taken for further calculations. The metabolites and the parent substance were additionally identified by comparing the Rf values of the sample substance spots with those of the inactive substance spots. The Rf value of a substance is the ratio of the distance travelled by the substance to that of the mobile phase (mobile phase front) and is a characteristic parameter for each substance. High Rf values indicate high mobility and show that the substance is not too closely bound to the soil. In preliminary experiments, the inactive standards were additionally applied individually and together so that the migration distances and Rf values were known. The following Rf values of the active ingredient and the possible metabolites are shown in Table 14.

Tab. 14: Range of Rf values for the active ingredient and metabolites using the mobile phase mixtures

A = chloroform/methanol/glacial acetic acid (90:10:1)
B = dichloromethane/methanol/water/25% ammonia solution (75:30:3:1.5)

Solvent mixture	MKH6561	4-hydroxysaccharin	2-hydroxypropoxy-MKH6561
A (TLC1)	0.39-0.41	0.03-0.04	0.10-0.13
B (TLC2)	0.81-0.86	0.55-0.62	0.66-0.69

Attention must be paid to the following aspects in performing and evaluating thin-layer chromatography.

Optimum thin-layer chromatography can only be ensured if in 50 up to a maximum of 100 µL of the solutions evaporated to 0.5-4 mL approx. 16 Bq can be found as a guide value for an exposure time of 24 h. If quantities smaller than 16 Bq were to be applied onto the plate and it was not possible to concentrate the samples any further, which was above all the case for the bottom soil layers in the experimental vessels and for plant samples in the column experiments, the plates were exposed for a longer period. However, the longer exposure (up to 10 days) may lead to an "overdevelopment" of the image plate, e.g. at the active ingredient band, which makes further investigations and interpretation more difficult. The active

ingredient band is most intensive for the desorption solutions of the soil material. The metabolites occurring in weaker bands may, moreover, be obscured by the background radiation (depending on the shielding). Due to the low radioactivity in some cases in the present study, values < 16 Bq also had to be taken into consideration. According to *SOP 07/08-1 (1994)*[13], it is not meaningful to work with minimal contents of as little as 1.6 Bq in 100 µL sample liquid if precise information is required. These radioactivity contents were defined as the lowest "evaluation limit" for a maximum exposure time of up to 10 d. Furthermore, the following must be taken into consideration.

Since in particular the matrix effects influence measuring accuracy, it was decided for the following qualitative analysis of the TLC plates in the present work that only those signals of each fraction (active ingredient and metabolites) would be regarded as reliably determinable whose mean signal yield per millimetre of track (PSL-Bkg) was twice as high as that of the background (peak/noise ratio of 2:1).

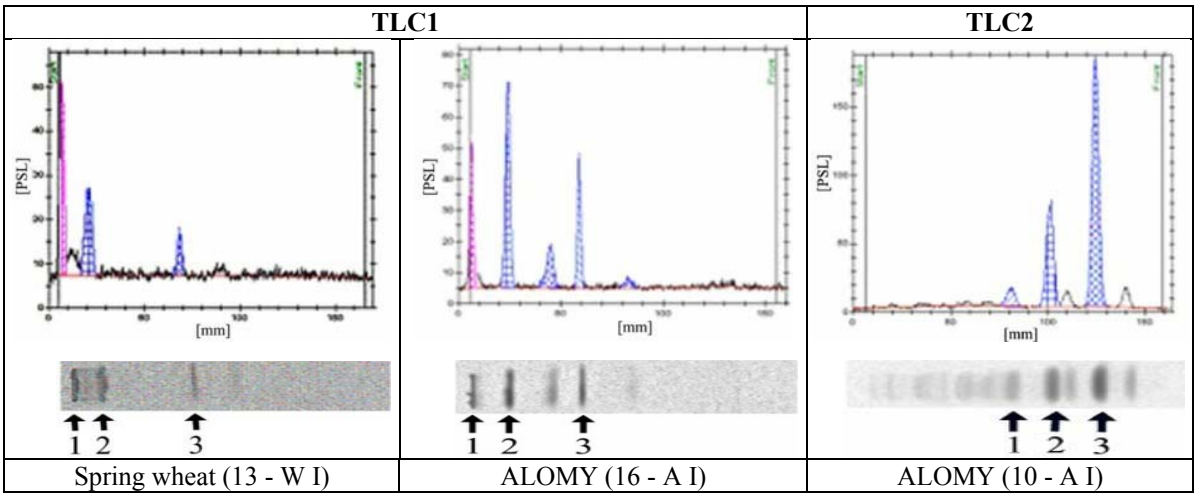
Attention was paid in all experiments that all substances applied to one plate had roughly the same radioactivity contents.

The separation of active ingredient and metabolites performed by chromatographic methods was successful, as shown by singular signals in all TLC chromatograms and well identified bands of the active ingredient and the metabolites.

The standard mobile phase (TLC1) and the reference mobile phase (TLC2) applied can therefore be used for plant (Fig. 15a) and soil (Fig. 15b) samples.

[13] SOP = standard operation procedure, Research Centre Jülich (Agrosphere)

Fig. 15a: Comparison of TLC chromatograms using the standard (TLC1*) and the reference (TLC2**) mobile phase system (plant/leaf)

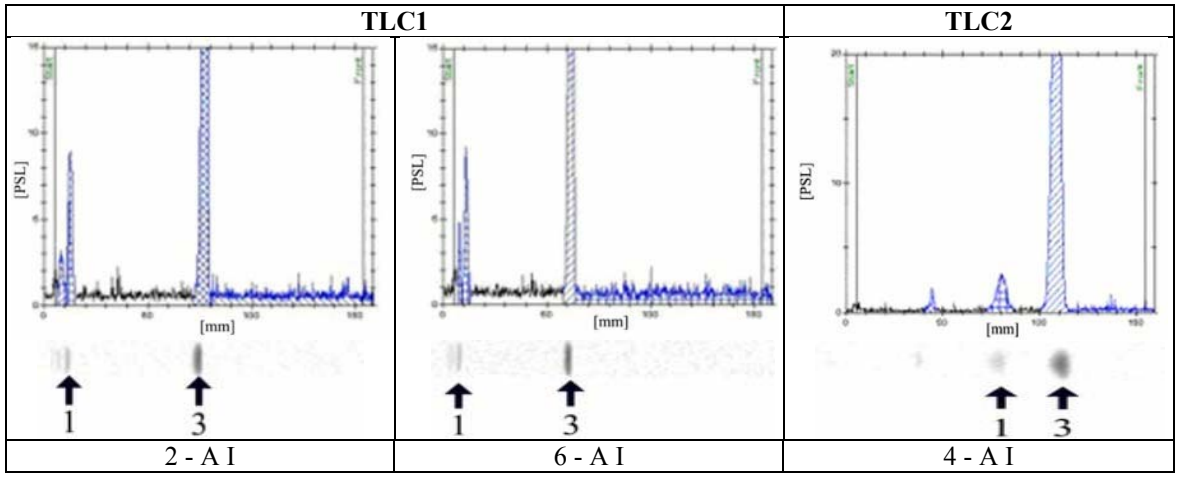


* applied for column and lysimeter experiments
** applied for lysimeter experiments

1 = 4-hydroxysaccharin
2 = 2-hydroxypropoxy-MKH6561
3 = propoxycarbazone-sodium

Legend for Fig. 15a:
13 - W I = column 13 (60%WHC_{max}/BBCH25), sampling date I
16 - A I = column 16 (40%WHC_{max}/BBCH25), sampling date I
10 - A I = lysimeter 10 (20%WHC_{max}/BBCH22), sampling date I

Fig. 15b: Comparison of TLC chromatograms using the standard (TLC1*) and the reference (TLC2**) mobile phase system (topsoil, 0-5cm)



* applied for column and lysimeter experiments
** applied for column and lysimeter experiments

1 = 4-hydroxysaccharin
2 = 2-hydroxypropoxy-MKH6561 (not detected)
3 = propoxycarbazone-sodium

Legend for Fig. 15b:
2 - A I = column 2 (20%WHC_{max}/BBCH22), sampling date I
6 - A I = column 6 (60%WHC_{max}/BBCH22), sampling date I
4 - A I = column 4 (40%WHC_{max}/BBCH22), sampling date I

(The marked area next to active ingredient peak defines the background and is subtracted; the marked peak next to the concentration zone can be assigned to the ^{14}C radioactivity remaining at the starting spot which is not separated in active ingredient or metabolites)

Besides propoxycarbazone-sodium [1], 2-hydroxypropoxy-MKH6561 [2] and 4-hydroxy-saccharin [3], further peaks were detected in some cases (in part not marked in the figures). However, a characterisation of these peaks was not possible since no reference substance was available.

Before evaluation of all chromatograms in Chapters 5 and 6, a few remarks concerning distinctive features of the pictured chromatograms should be made without further interpretation.

In a comparison of the TLC1 chromatograms of spring wheat and blackgrass (Fig. 15a), the active ingredient band [3] of ALOMY is blackened more intensively than for spring wheat; the band of the major plant metabolite formed in the plant [2] is in this case similarly pronounced for both blackgrass and spring wheat.

The TLC1 chromatograms of the soil material (Fig 15b) showed no visual differences with respect to the distribution of active ingredient and 4-hydroxysaccharin at the top soil layer investigated at 20 and 60%WHC_{max}.

The bands of the two different mobile phase systems (TLC1 & 2) show different migration distances due to the mobile phase composition and the resulting Rf values. Depending on the TLC plate, the migration distances - when using the same mobile phase system - may slightly vary.

3.10.4.2 High-performance liquid chromatography (HPLC)

Another separation method is high-performance liquid chromatography. This method can be used to unambiguously identify the active ingredient and the metabolites and also their percentage distribution in the sample.

Aliquots of the extracts were sampled by an automatic sample dispenser (automatic injection device GINA 50, *GYNKOTEK*) and send through the separation column under high pressure (Lichrospher 60 RP-Select B^{TM}, 5 µm, 250 * 4 mm); substances with a large number of polar OH groups (in this case the metabolites) were first routed to the detector after chromatographic separation. The polarity of the mobile phase was gradually modified by means of a gradient programme. The aqueous solution became increasingly nonpolar after the addition of acetonitrile (flow 0.7 mL/min., 5 min. 0.2 wt% aqueous H_3PO_4, then in 30 min. with acetonitrile). Table 15 shows the gradient used.

Tab. 15: HPLC gradient for separating MKH6561 and metabolites

Time [min]	Gradient [H_2O (H_3PO_4 0.2%)/ACN]
0	70/30%
1	70/30%
10	68/32%
15	64/36%
17	57/43%
21	52/48%
23	25/75%
29	10/90%
32	70/30%
40	70/30%

In this way, the more strongly nonpolar active ingredient was passed to the detector.

Detection was first performed in a UV detector (UVD 160, *GYNKOTEK)* at a wavelength of 210 nm and then by a radioactivity detector (type LB506C, *BERTHOLD*) with a solid scintillator (yttrium glass). The height of the peaks and the area under a peak is a measure of the quantity of sample injected into the device. A peak corresponds to a detected substance and is superimposed on the peak of the radiodetector, which is not usually visible.

Before each day of measurements, the instrument was recalibrated and the time window determined for the inactive reference substances whose UV signals were to be clearly seen. The corresponding time windows and retention times in the UV for each metabolite and the active ingredient are shown in Table 16.

Tab. 16: Retention times [min] for the HPLC separation of MKH6561 and the metabolites present as reference substances

Substance	4-hydroxysaccharin	2-hydroxypropoxy-MKH6561	MKH6561
Samples in $CaCl_2$ (soil)	4-6	12-15.0	26.5-29.0
Samples in MeOH (plant)	4-6	12-14.5	26.5-29.0

Qualitative information (percentage distribution) concerning the sample composition can be determined via the computer program *(WinFlow™ Version 1.3, Radiochromatography Software)* if there are sufficient ^{14}C contents in the sample material. However, such an evaluation of the HPLC chromatograms was only possible for a few samples and only unambiguous if not only background noise but also clear peaks could be seen in the ^{14}C channel of the detector. It was only possible to evaluate the topsoil layer (0-5 cm) with the PC program since sufficient ^{14}C radioactivity was present in the samples here. Since the chromatograms were only qualitatively and not quantitively evaluated via a calibration function, an absolute detection limit of 15 Bq can be given here for the entire metabolite spectrum. This can be explained by the low specific radioactivity of the active ingredient and by the low active ingredient concentrations in the samples to be investigated.

In the other cases, the HPLC facility merely served to separate the sample liquid, which was additionally collected in its separate fractions (active ingredients or metabolite) and measured with the much more sensitive LSC after the addition of a scintillator (Instant Scintillation Gel^{TM}) with the blank value being subtracted. Such a procedure became necessary due to the extremely low radioactivity contents. Low contents which were not visible in the ^{14}C channel or chromatograms that were difficult to evaluate can be reliably determined by this method. It is also possible to calculate the percentage distribution since the radioactivity contents of the injected extracts or the previously concentrated desorption solutions were determined in advance. The maximum quantity of 150 µL was always injected.

The total radioactivity contents (in 50 µL) were determined in the concentrated desorption solutions with the addition of 5 mL of Ultima Gold^{TM} scintillator. Aliquots of organic phases (extracts of the leaf and root material dissolved in methanol) were measured with the 5 mL Toluol^{TM} scintillator (*PACKARD*).

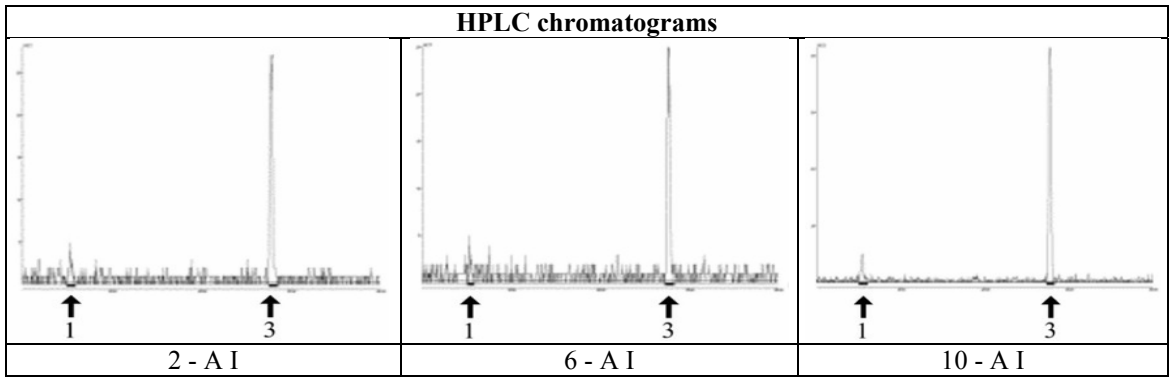
Other instrument specifications are listed in Table 17.

Tab. 17: Specifications of HPLC used for measuring and analysing MKH6561

Detection	at 210 nm with UV detector (UVD 160 Gynkotek)/radioactivity detector (type LB 506C Berthold)
Separation column	Lichrospher 60 RP-Select B^{TM}, 5 µm, 250 * 4 mm
Eluent	acetonitrile and water
Flux	0.7 mL/min
Injection volume	150 µL
Pump	Yasco PU 1580 HPLC
Autosampler	Dionex Gina 50
Column oven	Dionex STH
Fraction collector	Dionex SF2120

Figure 16 shows HPLC chromatograms of the soil material of the column experiments (as an example for all experiments). The soil moisture extremes of 20 and 60%WHC$_{max}$ were taken into account at the first sampling date for application stages BBCH22 (and BBCH25). Moreover, only the depth level of 0-5 cm is included, due to high ^{14}C radioactivity in the top layer (limitation: sensitivity of the detector).

Fig. 16: Comparison of HPLC chromatograms of the desorption solutions (topsoil, 0-5cm)



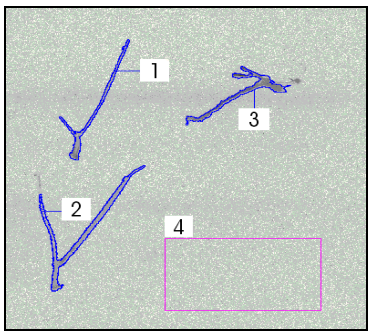
Legend for Fig. 16:
2 - A I = column 2 (20%WHC_{max}/BBCH22), sampling date I
6 - A I = column 6 (60%WHC_{max}/BBCH22), sampling date I
10 - A I = column 10 (20%WHC_{max}/BBCH25), sampling date I

No visual differences between the experimental parameters can be observed due to the parameter values of 4-hydroxysaccharin [1] and MKH6561 [3]. The evaluation of all compiled HPLC chromatograms will be discussed in the further course of the study.

3.10.5 Macroautoradiography

The spatial distribution of the radioactivity of the herbicide (active ingredient and metabolites) in the entire plant (wheat and blackgrass) can be qualitatively analysed on an image plate (FUJI BAS 1000), where the radioactive radiation causes blackening of the plate.
Immediately after harvesting, the experimental plants were fixed in DIN A5 cassettes lined with aluminium foil by means of double-sided adhesive tape. Before exposure, the plants were then smoothly covered by 2 µm thick plastic film (Hostaphan RE 2.5; *HOECHST*) to avoid chemographic artefacts. Finally, they were covered by an image plate. The cassettes were wrapped in a lead apron and stored at -20 °C. After approx. 72 h, the image plates were removed from the cassettes, the *BAS Reader*™ program used to read the radiation deposited on them and the image plates further evaluated by the computer program (*TINA*™ evaluation software). With the aid of this program it is possible to obtain values going beyond a mere consideration of the image so that the different blackening intensities of the plants from different macroautoradiographies can be compared with each other. In order to perform such an evaluation, the blackened areas of each plant were first labelled with the aid of the evaluation software (cf. Figure 17).

Fig. 17: Evaluation procedure in the macroautoradiographic studies



Legend for Fig. 17:

[1] & [2] leaf

[3] root

[4] background

Within the same plant, the material was grouped into leaf [1] & [2] and root [3] material and the entire leaf material combined for evaluation purposes. The blackening intensity was calculated minus a defined background value [4] and related to an exposure period of 24 h as well as to the quantity of active ingredient applied in each case (cf. Table 5a & b)

3.11 Ad-/Desorption Characteristics and Calculation of Pressure Head and Hydraulic Conductivity

On the one hand the aim is to characterize the two soil types studied in more detail. On the other hand, these studies served to support the argumentation that the active ingredient MKH6561 behaves differently in different soil types (orthic luvisol/gleyic cambisol). The aim of Chapter 3.11.1 is to study adsorption/desorption behaviour. Chapter 3.11.2 gives attention to the pH-values and organic carbon contents (C_{org}) and Chapter 3.11.3 is concerned with the theory of water movement and mass transport based on hydraulic functions.

The data evaluation/discussion of the results will be presented in the empirical section of this study in Chapter 7.

3.11.1 Adsorption/desorption study

In addition to results from the leaching experiments, the adsorption/desorption studies of the radiolabelled herbicide were intended to provide information on its translocation behaviour and uptake in the plants. The change in the concentration of a substance in the soil solution due to sorption phenomena is an important mechanism which influences the bioavailability and degradation of a substance decisively.

The study was designed on the basis of the *OECD GUIDELINE FOR THE TESTING OF CHEMICALS (2000)*, Part 106 "Adsorption - Desorption Using a Batch Equilibrium Method", which is discussed in more detail in the following.

3.11.1.1 Quantities of active ingredient and radioactivity used

The experiments were performed with [phenyl-UL-^{14}C]-labelled MKH6561.

In a first step, a stock solution was prepared, for which the radiolabelled active ingredient of low concentration from the preceeding lysimeter experiments was mixed with inactive MKH6561 in order to raise the active ingredient concentration to 1.43 g/L. The ^{14}C radioactivity of this stock solution was 16.03 MBq according to an ^{14}C labelled share of approx. 2% (cf. specific ^{14}C radioactivity in Chapter 3.5).

In a further step, six different concentrations of the active ingredient were prepared with 0.01 M $CaCl_2$ solution in environmental concentration ranges clearly below the maximal water solubility of 42 g/L by a dilution series:

A **1 g/L** MKH6561	B **0.5 g/L** MKH6561	C **0.1 g/L** MKH6561	D **0.05 g/L** MKH6561	E **0.01 g/L** MKH6561	F **0.001 g/L** MKH6561
decreasing MKH6561-concentration →					

In order to verify whether the desired concentrations had actually been reached, the ^{14}C radioactivity was measured in individual measuring flasks. The actual active ingredient concentration was calculated on the basis of these values. The results can be taken from Table 18.

Tab. 18: Comparison of desired and actual active ingredient content

	A	B	C	D	E	F
Desired active ingredient concentration [g/L]	**1**	**0.5**	**0.1**	**0.05**	**0.01**	**0.001**
Measured radioactivity [MBq]	11.079	5.535	1.110	0.566	0.113	0.011
Actual active ingredient concentration [g/L]	0.989	0.496	0.099	0.051	0.010	0.001
pH value [$CaCl_2$]	7.01	6.87	6.35	6.25	6.34	6.34

The application solutions were stored in the dark at 4 °C.

3.11.1.2 Preliminary experiments

As a pre-treatment, the soils were air-dried and sieved to < 2 mm. All the centrifuge tubes filled with soil were first equilibrated with 0.01 M $CaCl_2$ solution for 24 h (*BÜHLER* horizontal shaker, *type A2* at 160 rpm) and subsequently centrifuged (*BECKMANN* centrifuge, *type GPKR*). Preliminary experiments and adsorption/desorption study were performed with these equilibrated samples. All studies were performed at room temperature (20 °C) in the dark.

Preliminary experiments will be described in detail in the following:

1) Determining the soil/solution ratio

A high fraction of the active ingredient adsorbed at the soil at equilibrium ensures optimum conditions for adsorption/desorptions studies. The soil/solution ratio must therefore be determined in advance.

The soil/solution ratio should be adjusted in such a way that after an equilibration period of 24 h at the latest approx. 20-80% of the applied active ingredient is adsorbed at the soil. Furthermore the soil/solution ratio should be as wide as possible in the range between 1:5 and 1:20.

In duplicate, the soil quantities of both experimental soils of 2.5, 10 and 25 g were mixed with 50 mL of the application solution with an active ingredient concentration of 0.0001 g/L. The ratios of soil to solution were thus 1/20, 1/5 and 1/2. After being shaken for 24 h on a horizontal shaker (*BÜHLER*, *type A2)* at 160 rpm, the samples were centrifuged for 15 min (*BECKMANN, type GPKR* at 2000 rpm). Aliquots of 100 µL were measured in the LSC. The potentially adsorbed fraction was calculated indirectly by measuring the contents in the solutions after substraction from the total contents. Subsequently, the ^{14}C radioactivity content in the air-dried soil residue was also determined by combusting for balancing purposes in order to ensure that the concentration decrease in the equilibrium solution was actually due to adsorption of the active ingredient to the soil and not, for example, due to volatilization. The contents measured in the desorption residues should agree with the potential (calculated) values.

Furthermore, it is to be expected that with a narrower soil/solution ratio the adsorption of a chemical will increase. The level is determined by the active ingredient properties and its concentration in the solution.

2) Determining the time to equilibration

This experiment was intended to determine the shaking times necessary for equilibration.

In a triplicate, 10 g soil of both soils was mixed with 50 mL of the application solution of the highest concentration (1 g/L) and shaken for a total of 30 h. After shaking for 4, 8, 22, 26 and 30 h, centrifugation was performed for 15 min and the ^{14}C radioactivity of clear supernatant was measured. Two control samples (soil without active ingredient in $CaCl_2$ solution) were sampled after 22 h and 30 h. After termination of the preliminary study aliquots were taken for thin-layer chromatographic analysis so that the stability of the test substance was checked.

3.11.1.3 Adsorption experiments

The experimental conditions corresponded to the requirements determined (cf. Chapter 7.1.1): shaking time 22 h, soil/solution ratio 1:5 for 50 mL application solution, samples at different concentrations in duplicate, horizontal shaker with 160 rpm at 20 °C in a darkened room. In addition, for both soil types a sample with $CaCl_2$ solution was treated in the same way as the samples treated with application solution as control.

After shaking, the samples were centrifuged at 2000 rpm and two aliquots (1 mL) of the clear supernatant were taken for ^{14}C measurements. The adsorbed fraction was calculated from the decrease in concentration of the active ingredient in solution. Subsequently, the pH value was determined and is especially important for substances which are present in the form of ions. Finally, each solution was analysed by thin-layer chromatography.

3.11.1.4 Desorption experiments

Desorption studies show whether a substance is reversibly or irreversibly bound to the soil matrix. In general, it is assumed that the adsorption and desorption kinetics are identical.

At the end of the adsorption experiments, the clear supernatants were decanted and replaced by 50 mL of 0.01 M $CaCl_2$ solution. The samples were shaken for 22 h and centrifuged; two aliquots each (1 mL) were taken to determine the radioactivity and the supernatants were decanted. The desorption cycle was repeated for five times. After the 5^{th} run, the content in the solution was lower than the detection limit of the LSC.

The fraction still adsorbed were calculated from the measured concentration of the active ingredient in the supernatant. This provided the starting situation for the following desorption steps. According to the adsorption experiments the pH values of the first and fifth desorption solutions were measured and thin-layer chromatographic analysis was performed. Finally, the ^{14}C radioactivity in the desorbed soil was determined to draw up a radioactivity balance.

3.11.1.5 Evaluation

The ^{14}C concentrations in the adsorption and desorption equilibrium solutions were used to calculate the *FREUNDLICH* coefficients (K_F and n values) and *FREUNDLICH* adsorption isotherms in both soils for MKH6561. The calculations were based on *FREUNDLICH* adsorption and desorption equations (Equation [3]) and their linear forms (Equation [4]) *(OECD GUIDELINE FOR THE TESTING OF CHEMICALS 2000)*.

$$C^{ads/des}{}_s\,(eq) = K^{ads/des}{}_F * C^{ads/des}{}_{aq}\,(eq)^{1/n}\ [\mu g/g] \qquad [3]$$

$$\log C^{ads/des}{}_s\,(eq) = \log K^{ads/des}{}_F + 1/n * \log C^{ads/des}{}_{aq}\,(eq) \qquad [4]$$

Where $K^{ads/des}{}_F$ is the *FREUNDLICH* adsorption/desorption coefficient [$\mu g^{1-1/n} * g^{-1} * mL^{1/n}$], $C^{ads/des}{}_s$ (eq) is the concentration of active ingredient [µg/g] adsorbed to the soil at adsorption/desorption equilibrium, $C^{ads/des}{}_{aq}$ (eq) is the concentration in the aqueous phase [µg/mL] at adsorption/desorption equilibrium and 1/n is the unitless *FREUNDLICH* adsorption/desorption exponent.

In addition the distribution coefficients (K_D and K_{OC} values) were calculated for all concentrations (Equations [5] & [6]).

$$K_D = \frac{\mu g\ adsorbed\ \ active\ ingredient\ /\ g\ soil}{active\ ingredient\ \ concentrat\ ion\ of\ \ the\ equilibriu\ m\ solution\ \left[\mu g\ /\ mL\right]}\ [\text{mL/g}] \qquad [5]$$

$$K_{OC} = \frac{K_D\ ^*\ 100}{\%C_{org}}\ [\text{mL/g}] \qquad [6]$$

Furthermore, the percentage adsorption and desorption rates for the corresponding soil were calculated; in the desorption experiments, for each desorption step as well as averaged over all the steps (total desorption rate). The percentages give an impression of the amounts adsorbed, i.e. the quantitative relations between adsorbent and active ingredient at each concentration.

3.11.2 pH value and organic carbon content

3.11.2.1 pH value

The pH value of the two experimental soils was determined in a slurry of 10 g of air-dried fine soil and 25 mL of 0.01 M $CaCl_2$ solution (*SCHLICHTING et al. 1995).* After the solution had been added, the vessel with the sample was placed on a horizontal shaker (*BÜHLER*, *type A2*) for 20 minutes in order to obtain an optimum mixture. Measurements were made by a pH meter (*MP 300, METTLER TOLEDO)*.

3.11.2.2 Organic carbon content (C_{org})

The organic carbon content (C_{org}) in the soil is of major significance for the translocation of xenobiotics in the soil. The C_{org} content was determined for the orthic luvisol and gleyic cambisol by an reduction analyse device (*VARION EL, ELEMENTAR)*. This analysis was

performed by the Central Department of Analytical Chemistry (ZCH) at Research Centre Jülich. The organic carbon content (C_{org}) was calculated from these results (*cf. BKA 1994*).

3.11.3 Water movement and mass transport in soil

The description of water movement and mass transport in soil serves to investigate the active ingredient behaviour at different levels of soil moisture (column experiments: 20, 40 and 60%WHC_{max}; lysimeter experiments 45 and 60%WHC_{max}) taking into consideration the soil types used (orthic luvisol and gleyic cambisol).

3.11.3.1 Movement of water in soil

Water movement in soil is driven by a potential gradient and can be described by Darcy's law. The relation between water content θ [$L^3 L^{-3}$] and pressure head h [L] follows a soil-dependent characteristic curve and is termed water retention curve[14]. The course of the curve depends on the soil texture and structure so that different water contents were found in different soils at the same water tension. In the present study, retention and hydraulic conductivity functions were determined for the two soil types (orthic luvisol and gleyic cambisol).

3.11.3.2 Relation between pressure head and water content (soil water retention θ(h))

Before beginning the experiments, individual retention points for drainage at the different pressure heads were established by means of undisturbed 100 cm^3 sampling tube samples for both soil types. A curve fit was performed from the experimentally determined points (best possible approximation to all points) and θ_s, θ_r, α, n and m were determined (cf. Table 19).

Tab. 19: *MUALEM & VAN GENUCHTEN* parameters for the experimental soils *(VAN GENUCHTEN 1980)*

	θ_s [$cm^3 cm^{-3}$]	θ_r [$cm^3 cm^{-3}$]	α [1/cm]	n [-]	m [-]	l [-]
Orthic luvisol	0.390	0.000	0.0337	1.360	0.266	0.5
Gleyic cambisol	0.452	0.045	0.0162	1.957	0.489	0.5

θ_s denotes the saturated and θ_r the residual vol. water content; α is the reciprocal value of bubbling pressure and l, m and n are shape parameters of the *VAN GENUCHTEN* function.

[14] In the water retention curve, the pF value (decadic logarithm of the water tension h) is plotted against the water content (θ).

It was possible to describe the precise curve behaviour by means of Equation [7] *(VAN GENUCHTEN 1980)*:

$$\theta(h) = \theta_r + (\theta_s - \theta_r) * (1 + (\alpha|h|)^n)^{-m} \qquad [7]$$

$$m = 1\text{-}1/n$$

The water contents for the given water tensions θ(h) can be specified for both soils.

3.11.3.3 Hydraulic conductivity K(h)

Draining a soil reduces its hydraulic conductivity K [L * T^{-1}]. Consequently, dry soil results in low water conductivities.

The hydraulic conductivity of a soil as a function of water content K(h) [cm * d^{-1}] was calculated according to Equations [8] and [9] (*VAN GENUCHTEN 1980)*.

Equation [8] was used to determine K(h) and modified in part (Equation [10]). The experimentally determined saturated water conductivity Ks [cm * d^{-1}] is 84.1 cm * d^{-1} for orthic luvisol and 4752.0 cm * d^{-1} for gleyic cambisol and is included in Equation [10]. S_e is the effective water saturation in soil.

$$K(h) = \left[\frac{\left[1 - (\alpha|h|)^{mn} \left[1 + (\alpha|h|)^n \right]^{-m} \right]^2}{\left[1 + (\alpha|h|)^n \right]^{m\lambda}} \right] * K_s \qquad \lambda = 0.5 \qquad [8]$$

$$|h| = \left(\left(\frac{\theta - \theta_r}{\theta_s - \theta_r} \right)^{-\frac{1}{m}} - 1 \right)^{\frac{1}{n}} * \frac{1}{\alpha} \qquad [9]$$

$$K_\theta = K_s * S_e^{\frac{1}{2}} * \left(1 - \left(1 - S_e^{\frac{1}{m}} \right)^m \right)^2 \qquad S_e = \frac{\theta - \theta_r}{\theta_s - \theta_r} \qquad [10]$$

3.11.3.4 Mass transport in soil

Mass transport in soil is examined in order to explore the distribution, translocation and residence time of a substance in the soil. Knowledge of the influence of boundary conditions (e.g. infiltration intensity, water contents) on translocation, adsorption/desorption and transformation of applied substances allows to estimate or prevent undesirable mass transport.

In order to determine the effective retardation (delay in mass transport) it is necessary to know the bulk density (ρb) [g/cm^3] and the K_D value [cm^3/g]. Since both values are soil-specific, they were determined in the laboratory. In addition, it must be noted here that the bulk density of the heaped soil (lysimeter experiments) varies and differs from the bulk density of the

undisturbed soil. This will also be discussed in the evaluation (Chapter 7.3.3), as well as various K_D values.

The retardation (R_{eff}) was calculated as (Equation [11]):

$$R_{eff} = \frac{1 + bulk\ density\ (\rho b) * K_D\ value}{\theta} \qquad [11]$$

3.12 Data Processing and Statistics

The statistical evaluation of the findings of the present study was performed with the PC software SPSS for Windows, Version 6.0.1 and Excel 2000 on a PC with an MS Windows 2000 operating system.

3.12.1 Descriptive statistics

For a statistical consideration of the data obtained arithmetic mean and the standard deviation (s) were calculated. In addition, minimum and maximum values were determined and the variation coefficient (v [%]) calculated.

3.12.2 Correlation analysis

The strength of the relation between two or more variables is expressed by a measure given in the form of a correlation coefficient [r] *(BORTZ 1999)*.

With a few exceptions, in the present study the correlations between two or more variables were investigated by means of the product-moment correlation coefficient according to *PEARSON* [15].

The closer the correlation coefficient approaches 0 the smaller is the relation between the measured values. A value close to 1 illustrates a strong positive relation, a value close to -1 a strong negative (opposite) relationship.

An additional value is given in the correlation analysis which indicates whether the relation found also holds in the parent population, i.e. whether r is significantly different from zero in the parent population. The error probability [p] specifies the probability with which the dependence between the variables X and Y is also actually present in the parent population.

[15] It did not prove necessary to rank the data and use these results to make a correlation analysis (distribution-independent *SPEARMAN* rank correlation coefficient) especially since experimental data were available and *PEARSON's* product moment correlation coefficient was used for the metric data.
The only exception was the non-metric data from macroautoradiography so that it is easier to obtain qualitative than quantitative statements.

The value of the correlation coefficient [r] determined in the random sample is significantly different from zero at a basic significance level of $1-\alpha = 0.95$ if the error probability $p \leq \alpha = 0.05$ (5%). The conventional asterisk symbols are used for the results (cf. Table 20).

Tab. 20: Assessing the correlation coefficient and valid significance level for the statistical calculations (*BÜHL et al. 1997)*

Value [r]	Interpretation
r < 0.5	low correlation
0.5 < r < 0.7	medium correlation
0.7 < r < 0.9	high correlation
r > 0.9	very high correlation

***	**most highly significant**	$p \leq 0.001$
**	**highly significant**	$p \leq 0.01$
*	**significant**	$p \leq 0.05$
ns	**not significant**	$p > 0.05$

According to *BORTZ et al. (1995)*, in order to ensure the validity of the calculated significances, the scope of the random samples to be correlated is decisive. Overviews of the optimum sample size can be taken from literature on research methodology and evaluation (e.g. *BORTZ et al. 1995*) and will not be discussed in detail. In the present study it must be noted that due to a very small n (in part n = 1) the sample sizes are not sufficient to obtain meaningful results. The background to the in part very small sample sizes is that due to the issue to be examined ('How can the different active ingredient contents in the plant and soil material be explained?'), there are many factors of influence (crop and target grass, growth stage of the plant, soil type, soil moisture, time of application, date of sampling I-III and normal precipitation vs. heavy rainfall). This leads to a multifactoral experimental design, which separates the individual measured values into, in part, very small units. Furthermore, attention must be given to the way in which these factors influence each other and which (unknown) interactions result. In order to obtain meaningful results on the influence of individual parameters and the different subaspects of the interaction of soil/plant, a much larger experimental design would therefore be necessary.

Altogether, for the above-mentioned reasons a correlation-specific evaluation had to be dispensed with in many cases. In its place, particular attention was paid to the descriptive statistics.

Due to the methodological relation between correlation and regression analysis, the statements made above also refer to the regression, which will be explained in the following.

3.12.3 Regression analysis

The regression analysis determines the type of relationship between the different variables (*BAHRENBERG et al. 1990)*. The extent to which a target variable Y (dependent variable) is explained by an initial variable X (independent variable) is verified. That is to say Y is

inferred from X. This requires a semantic separation between the two variables. A straight line Y = a + bX (ordinate section: a; slope: b) must be sought which best describes this dependence (linear regression analysis). The straight line optimally represents the scatter diagram of the pairs of values when the sum of the distances of the points from this straight line is smallest (cf. *BAHRENBERG et al. 1990)*.

The measure of the quality of the relation between X and Y is known as the coefficient of determination (B). It is defined as the square of the correlation coefficient [$B = r^2$].
As a quotient of the "explained" scatter to the overall scatter, the coefficient of determination expresses how great the scatter of the theoretical Y values is in relation to the overall scatter of the observed Y values [x% of the scatter of the interesting variables Y is statistically explained by the variation of the X values].
Since the overall scatter is composed of explained and unexplained scatter, it is greater than the explained scatter. As a consequence, the quotient is always ≤ 1. The percentage is obtained by multiplying this quotient by 100.

4. Concept for Evaluating the Data Material

In following chapter the concept underlying the evaluation of the data material is described. This concept forms the basis of the presentation and interpretation of results in Chapters 5 and 6. For reasons of clarity, the concept is presented separately, since it is identical to both the column and the lysimeter experiments. Moreover, it should be noted that the structure of Chapters 5 and 6 resembles this chapter.

First, the determination of the total ^{14}C radioactivity and the MKH6561 equivalents resulting for the experiments (column and lysimeter experiments) in the plant and soil material is described. Subsequently, the determination of the extractable and desorbable contents as well as the distribution of the active ingredient and the metabolites occurring in the extracts and desorption solutions is explained. Finally, the establishment of the overall balance is discussed which, based on the total ^{14}C contents, allows statements to be made concerning the concentration of active ingredient, identifiable metabolites and not characterized radioactivity of the extractable and desorbable fractions. Moreover, the non-extractable and non-desorbable residues are taken into account and the non-recorded contents are discussed.

As a summary and for brief information, each chapter contains an overview in the form of a condensed presentation of the respective results discussed.

4.1 Total ^{14}C Radioactivity

The determination of the total ^{14}C radioactivity shows how much radioactivity appears in the plant parts and soil layers or possibly in the sand bed (column experiments) or percolate (lysimeter experiments).

The LSC measurement results [Bq] are related to the weighed quantity [g] and offset against the recovery rate of the ^{14}C oxidizer (recovery (rec) > 96%). This value (averaged from three parallel measurements) gives the total ^{14}C radioactivity [Bq/g soil or plant] (Equation [12]).

$$\text{total } ^{14}\text{C radioactivity [Bq/g]} = \frac{\text{LSC measured value [Bq]}}{\text{weighed quantity [g]} * \text{recovery [\%]}} \qquad [12]$$

The active ingredient equivalents [µg/g soil or plant] are calculated on the basis of Equation [13] as the quotient of radioactivity in the fraction [Bq/g] and the specific radioactivity of the substance used [kBq/mg].

$$\text{active ingredient equivalents [}\mu\text{g/g]} = \frac{\text{radioactivity in the fraction [Bq/g]}}{\text{specific radioactivity [kBq/mg]}} \qquad [13]$$

The active ingredient equivalents indicate the maximum possible quantity of the active ingredient. In interpreting the uptake rates in leaves and roots and comparing the plants with each other, the active ingredient equivalents [µg/g] are divided by the applied radioactivity [kBq] * 1000 and are therefore uniformly related to the same potentially applied amount of 1000 kBq (cf. Equation [14]).

Active ingredient equivalents [Bq/g] per 1000 kBq =

$$\frac{\text{active ingredient equivalents } [\mu g/g]}{\text{applied radioactivity [kBq]} * 1000} \quad [14]$$

In this way, it is possible to compare the MKH6561 equivalents in the plants as a function of the experimental variables of soil moisture and growth stages. The distribution of the ^{14}C radioactivity is balanced in all examined compartments as follows:
In the soil material the ^{14}C radioactivity [kBq] of all soil layers is calculated by multiplying the total ^{14}C radioactivity [Bq/g] by the volume [L] and the bulk density [g/mL] (Equation [15]). In the plant material the ^{14}C radioactivity [kBq] of the total plant mass is calculated by multiplying the total ^{14}C radioactivity [Bq/g] by the dry weight [g] and division by 1000 (for conversion to kBq) (Equation [16]).

Soil layer [kBq] = total ^{14}C radioactivity [Bq/g] * soil volume [L] * bulk density [g/L] [15]

Total plant mass [kBq] = total ^{14}C radioactivity [Bq/g] * dry weight [g] / 1000 [16]

The summed-up contents of the individual soil layers [kBq] and the considerably smaller contents of the plant material [kBq] in comparison to the soil layers should approximately reach the calculated, applied contents so that complete balancing is performed. The contents are moreover expressed in percent of applied ^{14}C radioactivity by division of the applied radioactivity [kBq] (Equation [17]).

$$\text{percentage content [\%]} = \frac{\text{kBq (layer)}}{\text{applied radioactivity [kBq]}} \quad [17]$$

The percentage contents serve to compare the ^{14}C radioactivity distribution in the different depth levels of the soil and to compare the two sampling dates on the same column.
Due to the extrapolation of individual random sample contents to the entire column/lysimeter, 100% of the applied ^{14}C radioactivity is not always recovered; moreover, some contents above 100% are calculated. For a comparison of the overall balance of the columns the measured values are therefore always corrected to 100%.

It should be noted that in the lysimeter experiments the applied contents per lysimeter third are included in the calculations. However, each third does not exactly contain one third of the total radioactivity per lysimeter, because the surfaces are of different size since the steel plates are installed in slightly differently[16]. The applied quantity is always related to these surfaces after having subtracted the radioactivity losses caused by the percolate from the total contents applied.

4.2 Desorption/Extraction and Active Ingredient Analysis

Since the active ingredient equivalents determined can either be the active ingredient or metabolites, precise statements on their distribution are only possible after the metabolites have been identified and the percentage distribution of active ingredient and metabolites has been determined. Moreover, their concentrations after extraction (plant material) and desorption (soil material) are determined by suitable analysis methods (cf. Chapter 3.10). As mentioned in this chapter, the metabolites are divided into those with and those without reference substance (combined in a residue fraction). In addition, contents of the non-desorbable/non-extractable residues are determined. The percentage of the single fractions (plant and soil samples %) are determined by division of the total ^{14}C radioactivity [Bq/g] * 100 (Equation [18]).

$$\text{desorbable and extractable fractions, residues, non-recorded contents [\%]} = \frac{\text{desorbable and extractable fractions, residues, non-recorded contents [Bq/g]}}{\text{total } ^{14}C \text{ radioactivity [Bq/g]} * 100} \quad [18]$$

From these results a balance of the total ^{14}C radioactivity can be drawn up (cf. Equation [19]).

$$\text{total } ^{14}C \text{ contents [100\%]} = \text{desorbable and extractable fractions [\%]} + \text{residues [\%]} + \text{non-recorded contents [\%]} \quad [19]$$

With the methods described in Chapter 3.10.4 it is possible to obtain information about the distribution of the individual fractions in the extracts and desorption solutions.

The interpretation of the percentage values performed later in this study refers to the sum of active ingredient and all occurring metabolites and to the total radioactivity in the extracts/desorption solutions. For a comparison of active ingredient and the two metabolites

[16] Note: The differences are inaccuracies in the millimetre range, but relative to the total soil volume per lysimeter third their influence is not insignificant.

present as the reference substance, their sum is taken as the reference value in a separate evaluation.

The interpretation of the percentage distribution and characterization of active ingredient and metabolites in the extracts of the plant material and in the desorption solutions of the soil material and the drawing up of the figures are exclusively based on HPLC values. The results from thin-layer chromatography only served to back up the HPLC methods. Correlations of quotients formed between active ingredient and the major degradation product (2-hydroxypropoxy-MKH6561 in plant or 4-hydroxysaccharin in soil matrizes) of up to four seperation methods applied, confirm the legitimation of this procedure.

For the plant material, quotients resulting from two thin-layer chromatography (TLC1 & 2) are correlated with those determined by $HPLC_{cuts}$ (active ingredient compounds are separated with the aid of HPLC and individually measured by LSC) to ensure that the values obtained by the respective methods are comparable (Table 21a).

Tab. 21a: Leaf and root: Quality of separation methods applied - correlation of quotients (active ingredient and 2-hydroxypropoxy-MKH6561)

	Plant							
	Column experiments				**Lysimeter experiments**			
	leaf		**root**		**leaf**		**root**	
Separation method	Wheat $HPLC_{cuts}$	ALOMY $HPLC_{cuts}$	Wheat $HPLC_{cuts}$	ALOMY $HPLC_{cuts}$	Wheat $HPLC_{cuts}$	ALOMY $HPLC_{cuts}$	Wheat $HPLC_{cuts}$	ALOMY $HPLC_{cuts}$
TLC1	**r = 0.96****	**r = 0.97****	**r = 0.93****	**r = 0.92****	**r = 0.97****	**r = 0.98****	**r = 0.97****	**r = 0.98****
	r = 0.98** R^2 = 0.96 y = 1.0876x - 0.0119		**r = 0.94**** R^2 = 0.88 y = 0.8092x + 0.2826		**r = 0.98**** R^2 = 0.96 y = 1.1965x + 0.1136		**r = 0.98**** R^2 = 0.96 y = 1.1624x + 0.005	
TLC2	n.a.				**r = 0.98**** R^2 = 0.96 y = 1.1826x - 0.0683			

n.a. = not applicable

The correlation coefficients of the quotients range from r = 0.92** to r = 0.98** (always n > 26) and illustrate very high correlation of the three methods according to combined leaf and root material and separate correlation of the two plant species (wheat and blackgrass).

Since the TLC2 mobile phase was only applied exemplary in the lysimeter experiments for plant material, correspondingly few values were obtained for this reference mobile phase (n = 15). Consequently, the correlation of TLC2/$HPLC_{cuts}$ was performed for the combined leaf and root material.

For soil material, a comparison of the quotients of MKH6561 and 4-hydroxysaccharin obtained by HPLC methods and radio thin-layer chromatography was performed (Table 21b).

Tab. 21b: Soil: Quality of separation methods applied - correlation of quotients (active ingredient and 4-hydroxysaccharin)

	Soil					
	Column experiments				**Lysimeter experiments**	
Separation method	**topsoil** TLC1	**subsoil** TLC1	**topsoil** $HPLC_{cuts}$	**subsoil** $HPLC_{cuts}$	**topsoil** TLC1	**subsoil** TLC1
HPLC chromatograms	**r = 0.84*** R^2 = 0.72 y = 0.829x + 0.731		**r = 0.81*** R^2 = 0.65 y = 0.8544x + 2.8748		**r = 0.86**** R^2 = 0.74 y = 0.9092x - 0.6637	n.a
$HPLC_{cuts}$	**r = 0.91**** R^2 = 0.83 y = 0.7108x + 0.7914		n.a		n.a	**r = 0.77**** R^2 = 0.58 y = 0.602x + 0.8531
TLC2	**r = 0.91**** R^2 = 0.83 y = 0.8763x + 0.2017		**r = 0.90**** R^2 = 0.81 y = 0.6057x + 6.4317		**r = 0.88**** R^2 = 0.77 y = 0.9448x + 1.0078	

n.a. = not applicable

The quotients of TLC1 & 2, $HPLC_{cuts}$ and evaluations of HPLC chromatograms were related to each other. Since it is found in evaluating the column experiments that the two HPLC methods provided nearly the same results, a collection and measurement of the individual fractions ($HPLC_{cuts}$) is omitted for the samples of the top soil layer (0-5 cm) in the lysimeter experiments and HPLC chromatograms have been evaluated. This is possible due to high ^{14}C radioactivity contents applied and the ^{14}C labelling of the application substance (in the case of low ^{14}C radioactivity contents an evaluation of chromatograms is problematic due to rising evaluation errors). Depth levels below 5 cm have been investigated exclusively by $HPLC_{cuts}$. The results are assured by evaluating TLC plates.

The number of values included in the calculations differed depending on the combination of the separation methods used (always n > 35). For TLC2, as an exception, only exemplary chromatograms were produced (n = 12). HPLC chromatograms could only be evaluated for the topsoil layer (0-5 cm) and in exceptional cases (high radioactivity contents) for the 5-10 cm layer. The correlation coefficients range from r = 0.77* to 0.91** for all four methods applied, according to a combined top- and subsoil and a separate correlation additionally performed in the lysimeter experiments.

On the whole, good agreement of all quotients was found between the different seperation methods indicated by well correlating quotients. One mobile phase system can be reproduced very well by the respective other mobile phase system. High to very high correlations of the quotients reveal statistical backup of the quoted data. Slight variations were due to once-only measurement of each sample and definition of background values for weak radioactive samples (chromatogram evaluation).

As can be taken from Figure 21b, the correlation of TLC1/HPLC$_{cuts}$ for samples from the subsoil (5-10 cm, 10-20 cm and 20-40 cm) can "only" be described as high according to Table 20 and is due to the fact that some values are near the detection limit.

In a further step, the actual active ingredient and metabolite contents in the extraction and desorption solutions are calculated from the initially determined percentage distributions of active ingredient and metabolites. For this purpose, the percentage distribution of the known total ^{14}C radioactivity (active ingredient equivalents) in the extracts/desorption solutions is converted into quantitative values [µg/g].
However, the distribution of active ingredient and metabolites generally only relates to the extractable/desorbable fraction. No statements can be made on non-extractable and non-desorbable fractions, for which the percentage distribution may be different.

5. Evaluation of Column Experiments

In the following description of the results of the column experiments, the plant analyses (Chapter 5.1) and the soil analyses (Chapter 5.2) will be presented.

5.1 Plant analyses

5.1.1 Macroautoradiographic investigations - Images and distribution of radioactivity in the plant

The distribution of radiocarbon in the plant at the two sampling dates are shown by presenting the macroautoradiographs of the experimental plants (Figures 18a & b). The two application dates and the corresponding minimum and maximum soil moisture (20 and 60%WHC_{max}) are compared.

Fig. 18a: Macroautoradiographs of spring wheat

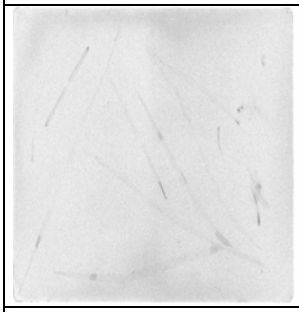
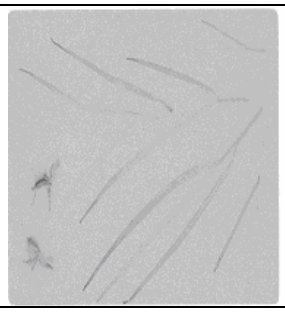
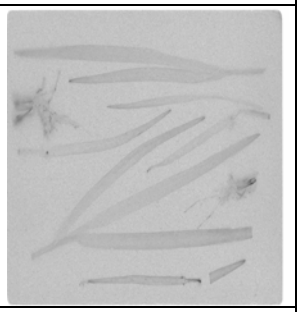
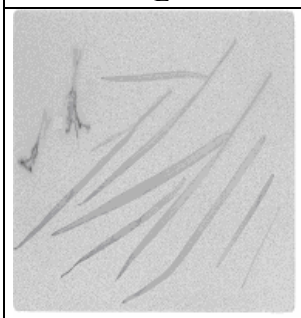
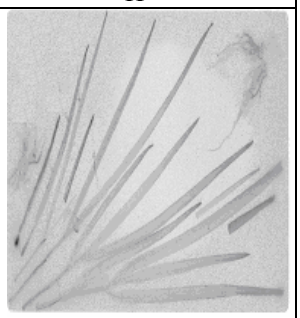
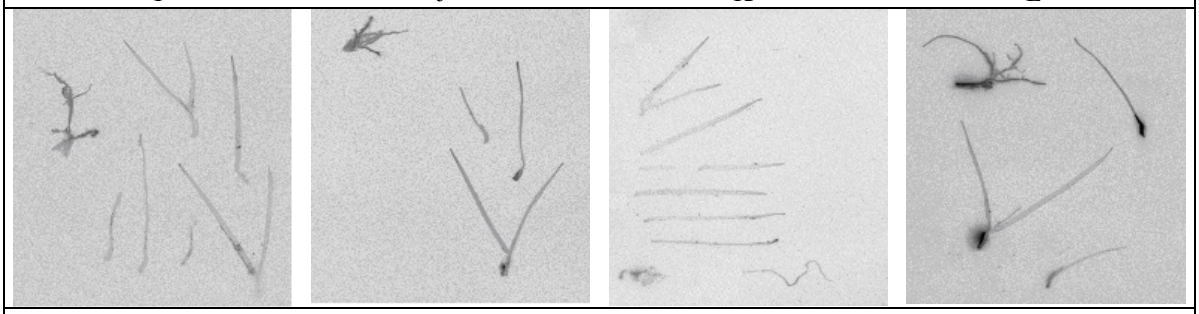
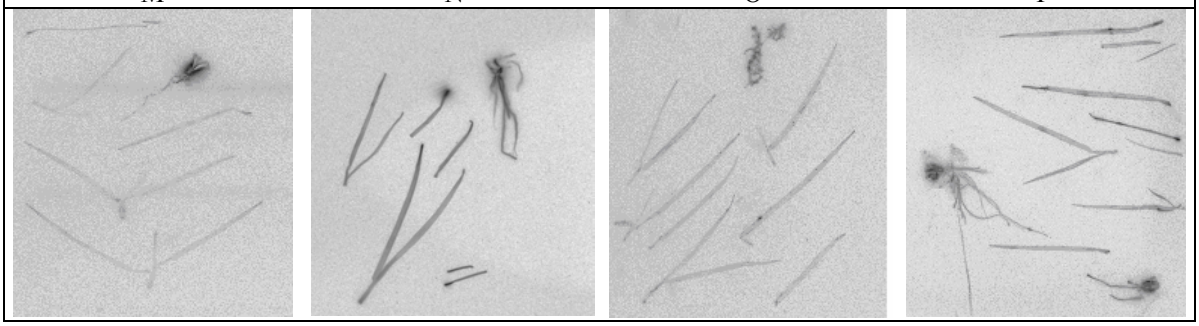
Sampling date I		Sampling date II	
20%WHC_{max}	60%WHC_{max}	20%WHC_{max}	60%$WHCK_{max}$
Application stage BBCH22			
A	B	C	D
			
Application stage BBCH25			
E	F	G	H
			

Fig. 18b: Macroautoradiographs of blackgrass

Sampling date I		Sampling date II	
20%WHC$_{max}$	60%WHC$_{max}$	20%WHC$_{max}$	60%WHC$_{max}$
Application stage BBCH22			
I	J	K	L
			
Application stage BBCH25			
M	N	O	P
			

As can be seen from the figures, blackgrass and spring wheat differ considerably with respect to the distribution of the radioactively labelled active ingredient inside the plant and the blackening intensities of the individual plant parts.

In the case of blackgrass, the active ingredient is distributed very homogeneously in the plant in all experimental variants (macroautoradiographs I-P), indicating a good herbicidal effect. There are almost no differences in the spatial distribution.

In the case of spring wheat, in contrast, some leaf tips and the front third of the leaves are blackened more intensively than the basal leaf sections (macroautoradiographs E-H), which indicates translocation of the radioactive substance and the metabolites with the transpiration flow.

Moreover, especially the older leaves are blackened more intensively than the younger ones due to longer uptake and higher bioavailability of the active ingredient (macroautoradiograph H).

The macroautoradiographs of blackgrass display (especially at application stage BBCH22) stronger blackening than spring wheat at the same stage (macroautoradiographs I-L vs. A-D). *A. myosuroides* has probably taken up more active ingredient, but it has a much smaller leaf

area, so that this statement must be verified by converting the blackening to the area (cf. Chapter 5.1.2.3).

The different sampling dates do not reveal any correlation with time.

In contrast, the soil moisture has a more significant effect. Both plant species show a weaker intensity of blackening at application stage BBCH22 with 20%WHC_{max} than on extremely moist soils with 60%WHC_{max} (macroautoradiographs A, C, I and K vs. B, D, J and L), since active ingredient uptake is probably lower in dry soils. At stage BBCH25, in contrast, the blackening of spring wheat (macroautoradiographs E-H) is almost the same for all soil moistures and is, moreover, higher than for the entire BBCH22 stage (macroautoradiographs A-D). *A. myosuroides* also shows less blackening at stage BBCH25 with 20%WHC_{max} than with 60%WHC_{max} (macroautoradiographs M and O vs. N and P) and appears similarly strongly blackened at both application dates (macroautoradiographs I-L vs. M-P).

It should be noted that due to the separation into two subexperiments slightly more active ingredient was applied at stage BBCH25 (cf. Table 5a), which contributes to stronger blackening at this stage (macroautoradiographs E-H and M-P). However, this factor was considered in the further analysis (Chapter 5.1.2.3).

5.1.2 Total ^{14}C radioactivity of the plant material

The total ^{14}C radioactivity of the plant material was determined using the methodology described in Chapter 3.10.1. In the following, the results will be described and discussed.

Since in the column experiments the content of total ^{14}C radioactivity in the plants is < 1% and thus extremely low in comparison to the soil layers, the % fractions are not discussed within the framework of a ^{14}C overall balance (including soil material). Instead, the active ingredient equivalents [µg/g] contained in the leaves and roots (Figures 19 & 20) will be interpreted. For a comparison of the columns, the representation was related to 1000 kBq of potentially applied substance. A tabular compilation of the MKH6561 active ingredient contents is shown in A V. The experimental parameter "heavy rain" shown in the figures is neglected. However, this parameter was considered in the interpretation of the active ingredient translocation in the soil (Chapter 5.2).

In the following, heavy rain occupies intermediate moisture (between 40 and 60%WHC_{max}) in the investigations of the plant material due to the increase in soil moisture at 40%WHC_{max} caused by such rain.

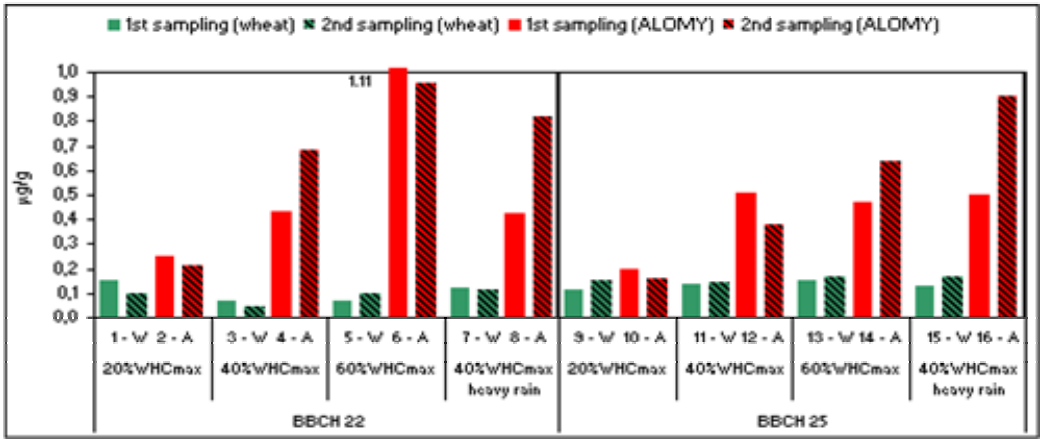
5.1.2.1 Active ingredient equivalents contained in leaf material

This section deals with the description and discussion of the active ingredient equivalents contained in leaf material. In the relevant graphical representations (and also for later figures of the same type) the ordinate section is always structured as follows:

The division into the two different application dates, BBCH22 (columns 1-8) and BBCH25 (columns 9-16), is followed by the specification of the different soil moisture (%WHC_{max}). Each of the 16 soil columns and the experimental plants to be allocated to these experimental variants are numbered and coloured in the bar chart: wheat (W) is shown in green and blackgrass (A) in red. For the first sampling date no shading was chosen for the bars and for the second a dark shading.

First of all, the results at application date BBCH22 are considered. For application at the beginning of tillering, the active ingredient equivalents relative to 1000 kBq of applied substance are lower in the leaf material of spring wheat than in blackgrass (cf. Figure 19).

Fig. 19: Active ingredient equivalents [µg/g] (relative to 1000 kBq of applied substance) contained in spring wheat and ALOMY as a function of application date, soil moisture and sampling date in leaves



The active ingredient equivalents contained in the spring wheat leaves are independent of soil moisture (no trends can be recognized). The active ingredient equivalents contained in the driest variant (20%WHC_{max}) of the spring wheat are comparatively high; this may be caused by active ingredient components externally adhering to the leaf material. Another possible explanation of a comparatively high uptake of herbicides despite a relatively dry soil may be a rain event within 0-5 days after application. This is sufficient for heavy active ingredient uptake. *UPCHURCH (1966)* specifies that the rain event causes a smaller effect at a later point in time. The concentrations in the leaves of spring wheat are slightly lower for the other soil moistures examined. The second highest equivalent contents in column 7 (heavy rain

event) are due to comparatively much MKH6561 being translocated to deeper soil layers, so that the "position selectivity" (cf. Chapter 2.1.2) - which in the case of normal precipitation is responsible for a lower uptake of herbicide into the crop plant - does not take effect. Due to increased displacement into deeper soil layers, the active ingredient became increasingly available for spring wheat and could be taken up accordingly.
For blackgrass in contrast to spring wheat at BBCH22 a continuous increase from dry to moist experimental variants was observed (cf. correlation between water tension in soil and water uptake in the plant in Chapter 7.3.1.1); the active ingredient equivalents are in all cases higher than in spring wheat. Maximum values of 1.11 µg/g are reached at a maximum water holding capacity of 60% (column 6). As a consequence of the high water saturation, a strong desorption of MKH6561 can be assumed here, so that with increased water consumption by the plants an elevated herbicide uptake and translocation of MKH6561 or its metabolites is likely.

The following paragraph is devoted to the description and interpretation of the results of application at growth stage BBCH25. In this case, too, the active ingredient contents of ALOMY are generally higher than for spring wheat. Possible explanations can be found in Chapter 5.2.2. In the case of spring wheat, the active ingredient equivalents are almost on the same level across all experimental parameters and are, moreover, clearly higher than for application at the beginning of tillering. This is indicative of comparatively high uptake rates at a later application date (the active ingredient equivalents continue to grow despite the dilution effect due to fresh weight increase). In the leaf material of blackgrass the active ingredient distribution across the different soil moistures proceeds similarly to the earlier application stage after treatment at the time of main tillering. However, it is on the same, in total lower, concentration level. This is attributed to the stronger increase in fresh weight of the plant biomass.

In comparison to sampling dates I and II it may be pointed out for both plant species and application dates that no significant effect can be observed with respect to the value changes from sampling date I to date II; i.e. the values at date II are either higher or lower than those at date I. Possible explanations of higher values at the second sampling date are as follows: on the one hand, higher active ingredient equivalents contained in the plants at the second sampling date may be attributed to longer period of water and thus herbicide uptake. Moreover, increased soil moisture presumably leads to increased illuviation of the herbicide

into deeper regions of the soil and thus also into the deeper-seated root zone of the wheat, so that its uptake is enhanced.

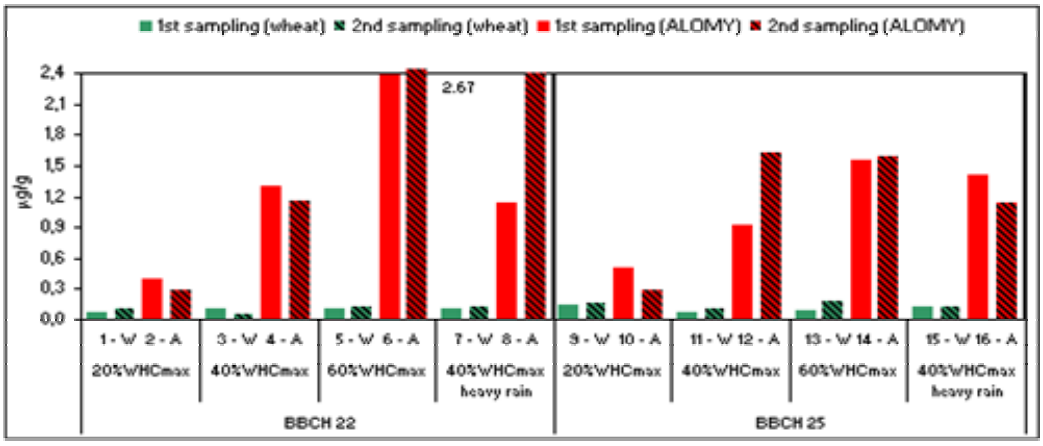
Apart from root uptake, $^{14}CO_2$ formed due to degradation processes in the soil can be directly taken up during photosynthesis and incorporated into photosynthesis products. This can be explained by increasing active ingredient equivalents at the second sampling date due to the prolonged possibility of degradation. Moreover, this causes an increase of ^{14}C radioactivity in those plants which can take up active ingredient by the roots to a comparatively less extent (e.g. limited by dryness). This uptake possibility is attributed to the column experiments performed in a closed room (growth chamber). In the lysimeter experiments (Chapter 6.1.2.1) performed outdoors the uptake of $^{14}CO_2$ is probably lower.

Lower active ingredient equivalents at the second sampling date may be explained as follows: for example, the grass weed affected stops taking up water and nutrients and thus also herbicide uptake shortly after treatment (cf. Chapter 1). Moreover, water uptake is presumably limited by the dryness of the soil (above all at 20%WHC_{max}). Besides incorporation into the plant matrix, part of the total ^{14}C radioactivity might have been metabolized to $^{14}CO_2$ due to progressing degradation and could have left the plant via atmospheric emissions. Root exudates are also possible in this connection. As is known from the macroautoradiographic investigations (cf. Chapter 5.1.1), the highest ^{14}C radioactivity contents are found in older leaves. These may fall off later in the experiments, so that the high active ingredient equivalents contained in them may not have been included.

5.1.2.2 Active ingredient equivalents contained in root material

In the following description and discussion of the results of the investigation of the active ingredient equivalents contained in the root material, the differences between the two plant species are as pronounced as for the leaf material (cf. Chapter 5.1.2.1), as can be taken from Figure 20.

Fig. 20: Active ingredient equivalents [µg/g] (relative to 1000 kBq of applied substance) contained in spring wheat and ALOMY as a function of application date, soil moisture and sampling date in roots



The active ingredient equivalents are in all cases considerably higher for *A. myosuroides* than for spring wheat, where the values are on a constant low level across all experimental parameters. The active ingredient equivalents increase for blackgrass with rising soil moisture. Maximum values of 2.67 µg/g of active ingredient equivalents are observed for the wettest variant (60%WHC_{max}) at stage BBCH22 (column 6). Differences between the two different application dates can only be observed for ALOMY, where the active ingredient equivalents at stage BBCH22 are generally higher than at stage BBCH25. Explanations can be found in Chapter 5.1.2.1. A comparison of sampling dates I and II shows findings (and corresponding interpretations) similar to those for the leaf material (Chapter 5.1.2.1).

The much higher active ingredient equivalents contained in the leaves and roots of blackgrass in contrast to spring wheat are also attributed to the different, specific rooting of the soil for both plant species. The roots of blackgrass only penetrate the top 5 cm of the soil. Consequently, higher fractions of the substance applied to the soil are taken up (cf. Chapter 5.2.2). The lower column region contains lower herbicide concentrations, so that spring wheat, whose roots penetrate the column in the entire profile depth, takes up considerably less active ingredient. Spring wheat, moreover, penetrates into regions with lower active ingredient concentration with increasing root growth. Blackgrass, in contrast, spreads in the layer most strongly affected by the substance with increasing root growth.
In total, a high correlation was observed between the quantities of radiocarbon in the leaves and the roots (r = 0.91**). This implies that in the case of high contents in the roots there are also high contents in the leaves. A separate consideration of the two plant species reveals the following values: spring wheat: $r = 0.44^{ns}$; ALOMY: r = 0.81**. Whereas in the case of

ALOMY an approximately similarly pronounced correlation was observed, the correlation for spring wheat was on a lower level. Consequently, no correlation between root and leaf content was detected for wheat.
Furthermore, it can be stated that (at least for ALOMY) the contents in the roots are elevated in comparison to those in the leaves. This is probably attributable to the fact that the substance is taken up by the roots in the case of soil application and, moreover, can be transported back to the roots through the phloem. Moreover, soil particles still adhering to the root epidermis (in spite of washing) may exert an influence. It can never be completely ruled out that the values may thus be distorted.

It might be concluded from these (preliminary) results that the application of MKH6561 should be completed before the end of stage BBCH22: At a later application date the crop - due to higher uptake rates and thus high active ingredient quantities in the leaves - could be damaged, if the total radioactivity is present in the form of parent substance. As the following investigations shows (cf. Chapter 5.1.4), the share of the parent substance in total radioactivity is very low at both application stages. This means that no damage to the crop plants is to be expected as a function of the application stage. Irrespective of this, the effectiveness of the herbicide for ALOMY must be considered. As already described in Chapter 2.1.4, a stronger uncertainty of the herbicide effect is to be expected after stage BBCH22; this is indicated by the measured, comparatively low concentrations at stage BBCH25.
As a further conclusion, soil moisture has a considerable influence on the uptake of the herbicide into the target grass at the earlier application date. High concentration in the leaf material of blackgrass, especially at high soil moisture, indicate a good herbicidal effect of the substance.

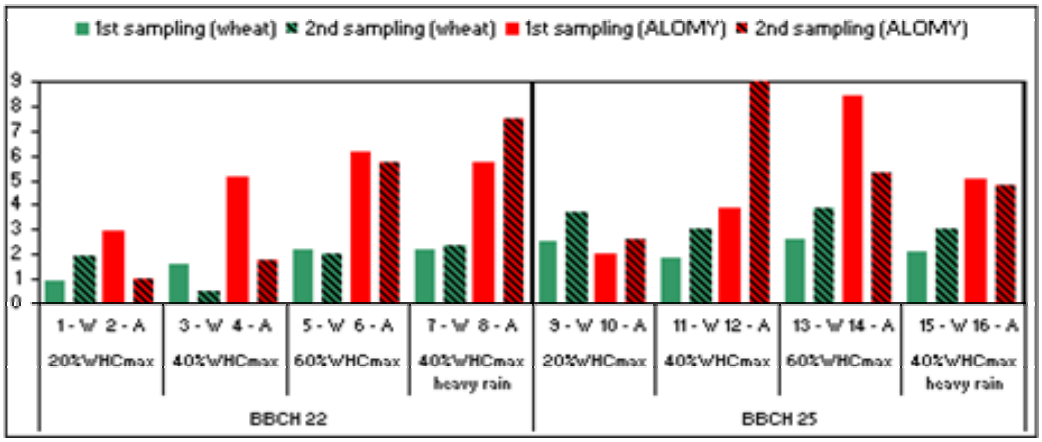
5.1.2.3 Excursion: Additional evaluation of the macroautoradiographic investigations

In addition to the results already discussed, a quantitative determination of the contents from the macroautoradiographic investigations was performed.
The blackening intensities shown in Figures 21 & 22 for leaf and root material show the background-corrected intensity of blackening (PSL[17]-Bkg[18]) per mm^2 of leaf area relative to 24 h of exposure. The blackening intensity is shown as a function of the investigation parameters.

[17] Photon-stimulated luminescence
[18] Background

The evaluation of the macroautoradiographs of the leaf material is shown in Figure 21.

Fig. 21: Blackening intensity [(PSL-Bkg) per mm^2 of leaf area relative to 24 h of exposure] as a function of application date, soil moisture, experimental plant and sampling date in leaves



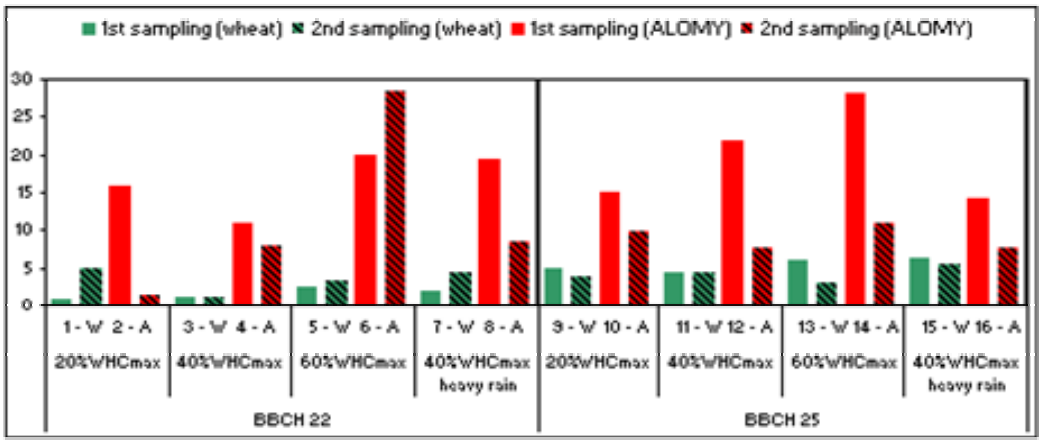
Considering stage BBCH22, lower values are found for spring wheat than for ALOMY at the same soil moisture. This means that probably less of the radiolabelled substance has been taken up by the wheat plant, resulting in less blackening. As a trend, more substance is taken up by both plant species with increasing soil moisture; this increase is more pronounced for ALOMY than for spring wheat.

At stage BBCH25, there is almost no increase with rising soil moisture for spring wheat; the values vary and are on a higher level than at the BBCH22 stage. The values for ALOMY rise with increasing soil moisture. In comparison to stage BBCH22, no significant trend was observed at this stage depending on soil moisture.

Single values deviating from these trends (for example, comparatively high values for wheat of the driest experimental variant at the second sampling date at BBCH22) reflect the variability of the plants on the experimental columns.

Figure 22 shows the evaluation of the macroautoradiographs of the root material.

Fig. 22: Blackening intensity [(PSL-Bkg) per mm^2 of root area relative to 24 h of exposure] as a function of application date, soil moisture, experimental plant and sampling date in roots



As in the case of the leaf material, the values of *A. myosuroides* are, in principle, also on a higher level for the root material in comparison to spring wheat.

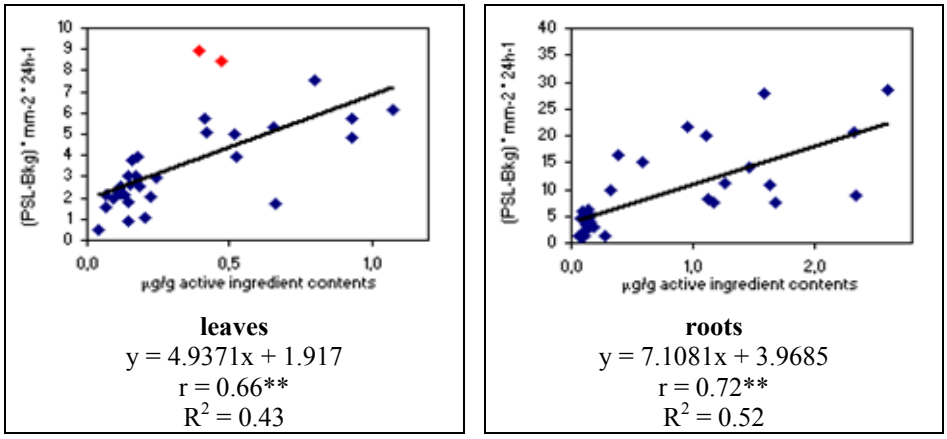
For stage BBCH22, spring wheat shows a slight increase in blackening intensities with rising soil moisture at the first sampling date. Furthermore, varying higher contents are perceivable at the second sampling date. The contents for blackgrass vary at the first sampling date and continuously rise at the second.

At stage BBCH25 the difference in soil moisture seems to be less effective: The contents in spring wheat are on a similar level across all soil moistures at both sampling dates. *A. myosuroides* shows rising blackening intensities at the first sampling date with increasing soil moisture and varying intensities at the second.

It must be noted, that due to its methodological restrictions this procedure can only be used for the purpose of obtaining a first insight into the contents to be expected. Due to the tendentious agreements (also in the interpretation), however, these results should not be ignored. Among the methodological problems is the sample size of n = 1. Due to the great sample volume, for instance, no replicates were made - a simultaneous processing of the samples would be necessary, but was not possible due to the lack of resources. Another reason is the limited number of plants on the column - it was necessary to have sufficient plant material for all investigations to be carried out. In considering the result, therefore, it must be taken into account that the natural variability of the plants is not compensated for by averaging. Furthermore, a comparable leaf thickness of wheat and blackgrass is assumed. However, since there were only slight differences, this assumption is justifiable.

The tendentious agreement of the results from the macroautoradiographic investigations and the contents determined by measured values is additionally supported if both methods are correlated with each other (cf. Figure 23).

Fig. 23: Correlation of active ingredient equivalents [µg/g] and blackening intensities (macroautoradiography) in leaves and roots
outliers are marked in red



The correlation coefficient shown in Figure 23 is the *SPEARMAN* rank correlation coefficient. The fact that only a medium correlation (leaves r = 0.66**, roots r = 0.72**) can be detected is probably due to the use of only one plant for macroautoradiography (column experiments) in each case so that the sample is too small. It should be furthermore noted that there is a significantly higher correlation in the case of leaf material if the two values marked in red are regarded as outliers and not included in the correlation (r = 0.78**).

When considered separately, the leaf material correlates at r = 0.68** and the root material at $r = 0.29^{ns}$ for spring wheat. Blackgrass shows $r = 0.40^{ns}$ for both the leaf material and the root material. Thus, reliable correlations have only been obtained for the leaf material of spring wheat.

On the whole, it must be stated for the relevance of macroautoradiography with respect to the correlation-specific evaluation that due to predominantly low correlations a precise statement on the active ingredient contents (also with a view to the initially mentioned restrictions of macroautoradiography) can rather be made by measuring the total ^{14}C contents.

5.1.3 Extraction of plant material

In the following, the results of the extraction described in Chapter 3.10.2 are presented. The percentage distributions of the extractable active ingredient fractions and of the non-extractable residues in the leaf and root material are shown (cf. Figures 24a & b).

The extractable and non-extractable fractions should add up to 100% of the measured total ^{14}C radioactivity. Due to slight inaccuracies in processing the sample material, this is seldom the case. The reasons will be discussed in more detail in connection with the explanation of the percentage distribution of desorbable and non-desorbable fractions in soil material (Chapter 5.2.3).

Fig. 24a: Distribution of extractable fractions and non-extractable residues in percentage (additive) in leaves
total ^{14}C radioactivity = 100%

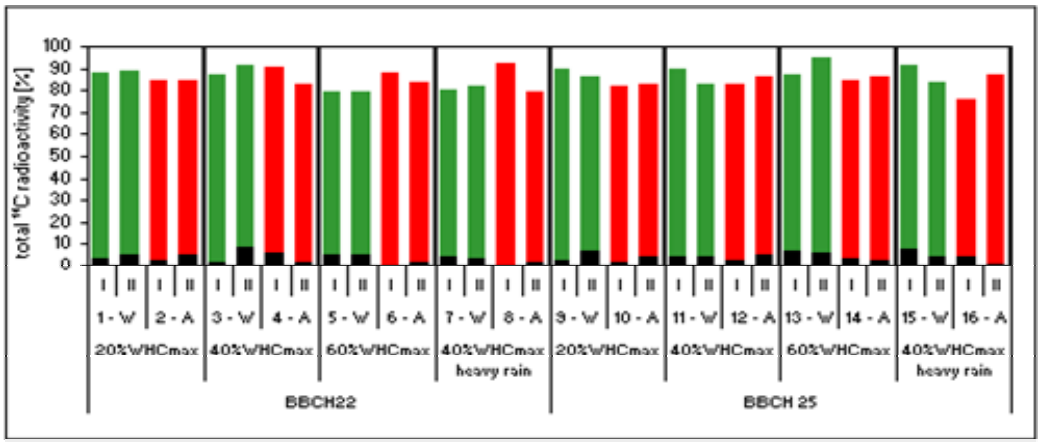
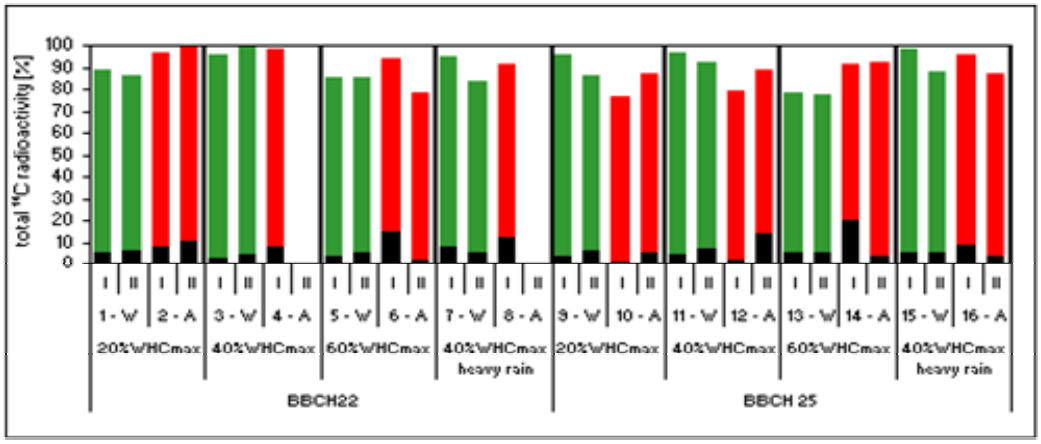


Fig. 24b: Distribution of extractable fractions and non-extractable residues in percentage (additive) in roots
total ^{14}C radioactivity = 100%



Legend for Fig. 24a & b:

Extractable residues		Non-extractable residues
Spring wheat (W)	Blackgrass (A)	Residues
I/II = Sampling date 1/2		

(for column 4 - A II and 8 - A II there was not enough root material available for extraction)

As can be taken from the Figures, the fractions extractable with the solvent mixture used amount to > 80%.

For the non-extractable residues the percentage is mostly below 5% and is elevated for the majority of experimental columns at sampling date II compared to sampling date I. This is

due to the fact that in parallel to metabolization (cf. also Chapter 5.1.4) non-extractable residues are formed in the plant, so that this fraction increases with progressing sampling date. As a consequence, the extractable fractions decrease with progressing time (Figures 24a & b).

5.1.4 Active ingredient and metabolite distribution in plant material

As already mentioned, the discussion of the metabolite spectrum is based on the active ingredient (MKH6561), the major plant metabolite (2-hydroxypropoxy-MKH6561) and the major soil metabolite (4-hydroxysaccharin), since only these were available as reference substances and could thus also be reliably determined by the methods described. Chromatograms which show the active ingredient and metabolite distribution in plant are given in Chapter 3.10.4.1.

The qualitative analysis of the TLC plates revealed that in total, however, at least five different metabolites can be detected (in part only in traces) in the plant material (incl. 4-hydroxysaccharin, which is exclusively formed in soil and is later taken up into the plant).

5.1.4.1 Leaf material

In the following, the results for the leaf material will be discussed. Figures 25a & b show the percentage distribution of active ingredient, 2-hydroxypropoxy-MKH6561 and 4-hydroxy-saccharin across all experimental parameters from combined results of the $HPLC_{cuts}$. Information concerning the undetermined ^{14}C radioactivity occurring in the extracts is given in Figure 25a. Figure 25b shows the values for better comparability of the distribution of the three metabolites with each other (per sample) extrapolated to 100%. In A VI the results of HPLC and TLC (leaf and root material) are shown in tabular form.

Fig. 25a: Distribution of active ingredient, 4-hydroxysaccharin and 2-hydroxypropoxy-MKH6561 in percentage in leaves
extracted radioactivity = 100%
determined by $HPLC_{cuts}$

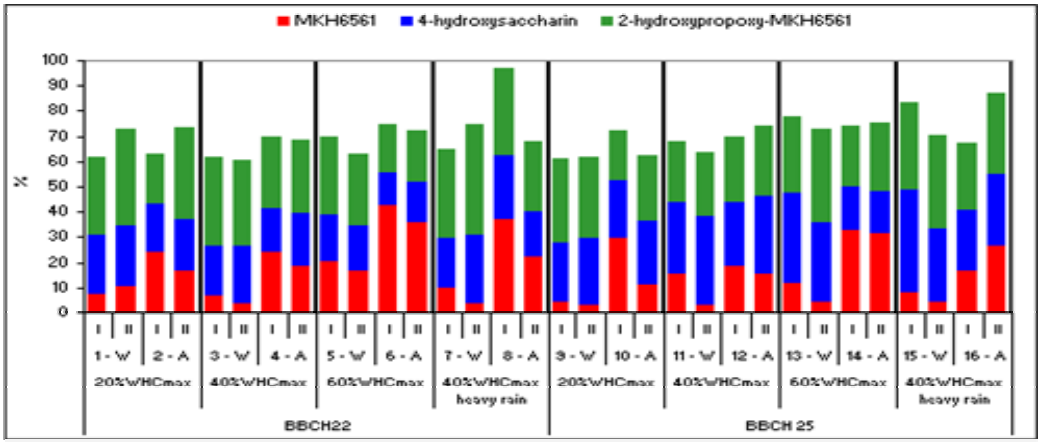
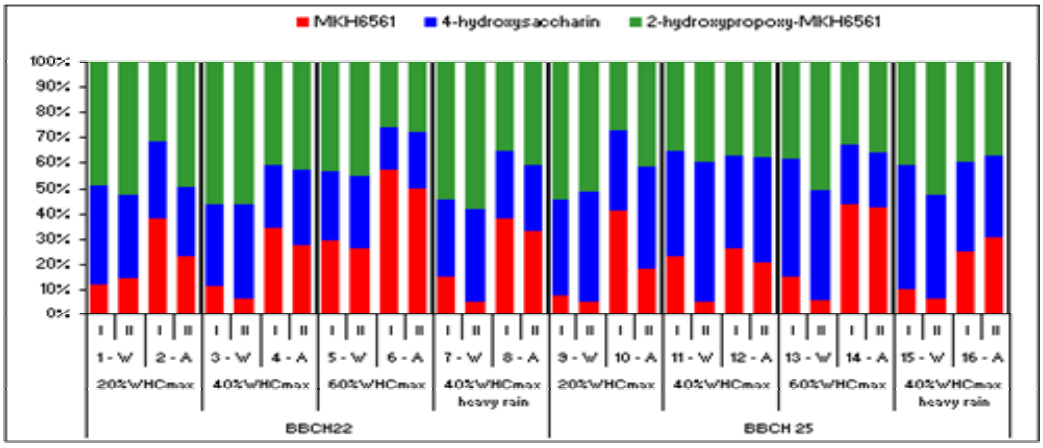


Fig. 25b: Distribution of active ingredient, 4-hydroxysaccharin and 2-hydroxypropoxy-MKH6561 in percentage (extrapolated to 100%) in leaves
extracted radioactivity = 100%
determined by $HPLC_{cuts}$



Within the sample material of spring wheat the percentage distribution of active ingredient and metabolites is characterized by low active ingredient contents (shown in red). Comparatively high contents of 4-hydroxysaccharin (shown in blue) and 2-hydroxypropoxy-MKH6561 (shown in green) were measured.

Within the samples of blackgrass similarly high percentage distributions of the three fractions are predominantly found.

In comparing the two plant species the following is to be stated:

The percentage contents of the active ingredient are comparatively lower in wheat than in ALOMY. The percentage contents of the major soil metabolite and the major plant metabolite, in contrast, are more pronounced in wheat. Furthermore, Figure 25a permits the additional statement that the undetermined ^{14}C radioactivity in the extracts is comparatively higher in wheat.

These differences are attributed to the fact that blackgrass due to its root formation takes up more active ingredient in the top soil layers (position selectivity) and that, in addition, a lower degradation of MKH6561 takes place. In spring wheat, whose roots penetrate much deeper into the soil, a more intensive metabolism takes place. The ratio of major soil metabolite to active ingredient rises with increasing depth in the subsoil, which will be shown in more detail in Chapter 5.2.4. Due to the deeper rooting of the wheat plant it also takes up water and active ingredient fractions there. Event though in principle significantly lower active ingredient equivalents are present in the subsoil region, wheat can take up more 4-hydroxysaccharin than ALOMY due to this deeper rooting (resulting in higher concentrations in the leaves).

In a comparison of sampling dates I and II it should be noted that the absolute quantity of extractable radioactivity hardly changes (cf. Chapter 5.1.3). However, the distributions of active ingredient, 2-hydroxypropoxy-MKH6561 and 4-hydroxysaccharin vary at the two sampling dates. The percentage values of MKH6561 mostly drop from sampling date I to sampling date II.

This illustrates stronger degradation and intensive metabolism of the active ingredient to the weakly active 2-hydroxypropoxy-MKH6561 and to other metabolites with prolonged time after application. This applies to both wheat and blackgrass. The percentage contents of 2-hydroxypropoxy-MKH6561 in most cases rise accordingly. The percentage contents of 4-hydroxysaccharin also rises from the first to the second sampling date due to increased uptake with soil water.

Referring to the fraction of unknown ^{14}C radioactivity it is to be stated that 2-hydroxy-propoxy-MKH6561 is the first degradation step *(BABCZINSKI et al. 2000)*; further degradation products result from this major plant metabolite as metabolization progresses, so that a corresponding reduction of its concentration should take place. In spring wheat, this degradation is presumably further advanced (higher fraction of unknown metabolites, Figure 25a). Blackgrass degrades the active ingredient more slowly, so that high active ingredient and high 2-hydroxypropoxy-MKH6561 contents are present at both sampling dates and the unknown metabolite fraction is correspondingly smaller. In addition, it may be stated that both the active ingredient and the major plant metabolite have an herbicidal effect, so that in total a slowed-down "detoxification" is to be detected for ALOMY.

In comparing the application dates (BBCH22 and 25) the percentage contents of the active ingredient are lower in blackgrass and spring wheat with advanced application date (BBCH25), which illustrates a stronger inactivation of the active ingredient in blackgrass. For spring wheat, in contrast, this points to a better tolerance.

With respect to soil moisture, the highest percentage fraction of MKH6561 is apparent at 60%WHC_{max} at both application dates (for both wheat and blackgrass). This could be an indication that the effectiveness of the herbicide at 60%WHC_{max} may also simultaneously cause the strongest hazard to spring wheat. This is due to high uptake rates exceeding the metabolization capacity of the plant and thus reducing the inactivation of the active ingredient. However, the active ingredient equivalents (cf. also Chapter 5.1.2.1) do not confirm this assumption. For wheat they are too low to exert a damaging influence according to the known active ingredient and metabolite distribution. Further results on this topic will be

given in Chapter 5.1.5, where the contents of active ingredient and metabolites are summarized.

A further aspect to be noted with respect to the influence of soil moisture is as follows: The above mentioned high influence of soil moisture on active ingredient uptake (especially for ALOMY) may also cause increased metabolization activity. At the same time, however, the plant species may also be decisive for the intensity (or weakness) of the metabolization processes. It is therefore not possible to unambiguously define a cause-effect relationship, but interactions of different influential factors must rather be assumed.

5.1.4.2 Root material

Figures 26a and b show the percentage distribution of active ingredient, 4-hydroxysaccharin and 2-hydroxypropoxy-MKH6561 in the extraction solutions of the root material. This is based on the same methodological procedure as already applied for the leaf material (cf. Chapter 5.1.4.1).

Fig. 26a: Distribution of active ingredient, 4-hydroxysaccharin and 2-hydroxypropoxy-MKH6561 in percentage in roots
extracted radioactivity = 100%
determined by $HPLC_{cuts}$

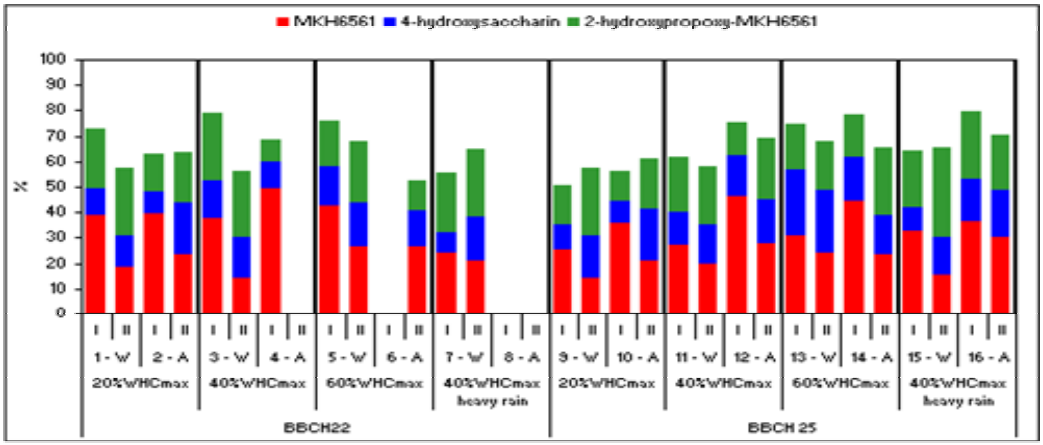
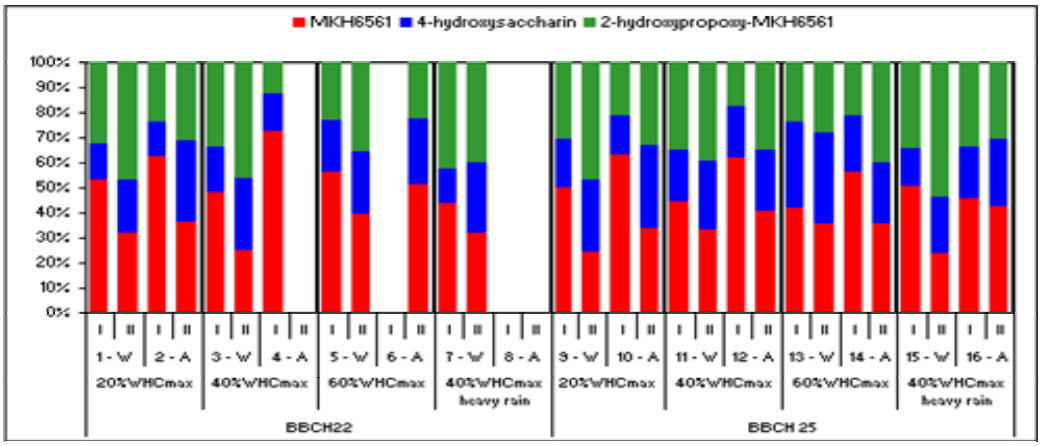


Fig. 26b: Distribution of active ingredient, 4-hydroxysaccharin and 2-hydroxypropoxy-MKH6561 in percentage (extrapolated to 100%) in roots
extracted radioactivity = 100%
determined by $HPLC_{cuts}$



(for columns 4 - A II, 6 - A I and 8 - A I and II there was either not enough root material available for evaluation or the ^{14}C radioactivity was too low to enable meaningful statements to be made)

In spring wheat, higher active ingredient contents were found in comparison to the detected metabolites that occur. This also applies to blackgrass. One reason for this could be that the metabolization process in the root material has not yet progressed so far. It is also likely that, in addition, MKH6561 was adsorbed on the root epidermis due to the close contact with the soil material and that residues were left despite the washing process.

In a comparison of the two plant species, the differences with respect to the percentage active ingredient fraction are rather small; these contents are slightly higher in the root material of blackgrass than in wheat. No differences were observed for 4-hydroxysaccharin; in contrast, the contents for 2-hydroxypropoxy-MKH6561 are higher in wheat than in blackgrass. The fractions of unknown radioactivity are at least at stage BBCH25 higher in wheat than in blackgrass (stage BBCH22 can only be evaluated to a limited extent due to a lack of data). Even though the percentage contents of the active ingredient in the root material are on a higher level, they are on the whole comparable to those from the leaf and are therefore to be interpreted correspondingly.

In a comparison of the two sampling dates, a clear drop in active ingredient contents from sampling date I to II is found across all experimental columns. However, the contents of 4-hydroxysaccharin and 2-hydroxypropoxy-MKH6561 are higher at the second date than at the first. These results correspond to those of the leaf material. With respect to the application dates, the active ingredient contents (just as in the leaf) tend to be lower at BBCH25 than at BBCH22.

Concerning the influence of soil moisture it is to be stated that the highest active ingredient contents are reached at 60%WHC_{max} (BBCH22); at stage BBCH25, in contrast, the active ingredient contents are about equally high for all soil moistures. The soil moisture thus only seems to have a minor influence on the percentage distribution of active ingredient and metabolites in the root material.

5.1.4.3 Active ingredient degradation in the plant: quotient formation of active ingredient and major plant metabolite

In order to obtain information on the degradation of the substance in the plant as a function of the parameters to be investigated in this study (soil moisture and application date), the quotient was formed between the active ingredient and 2-hydroxypropoxy-MKH6561. The interpretation of the quotients (leaf and root material) was performed using $HPLC_{cuts}$. Subsequently, a correlation with thin layer chromatographic investigations was calculated (Chapter 4.2).

As can be seen from Figures 27a & b, the quotients are predominantly on a higher level for blackgrass than for spring wheat. Moreover, the quotient for ALOMY increases in the leaf with rising soil moisture (rising MKH6561 and decreasing major plant metabolite fractions). In spring wheat, the highest quotients are in the leaf and root material mostly at 60%WHC_{max}.

Fig. 27a: Quotient of MKH6561 and 2-hydroxypropoxy-MKH6561 in leaves determined by $HPLC_{cuts}$

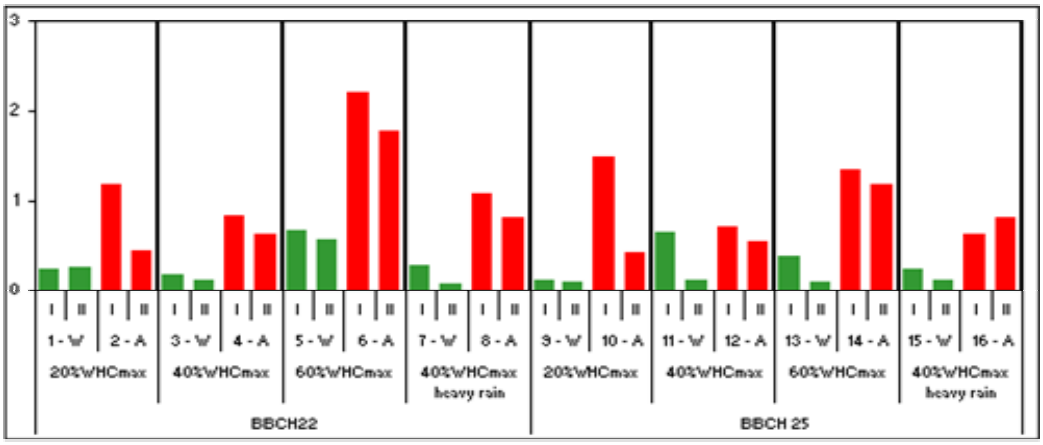
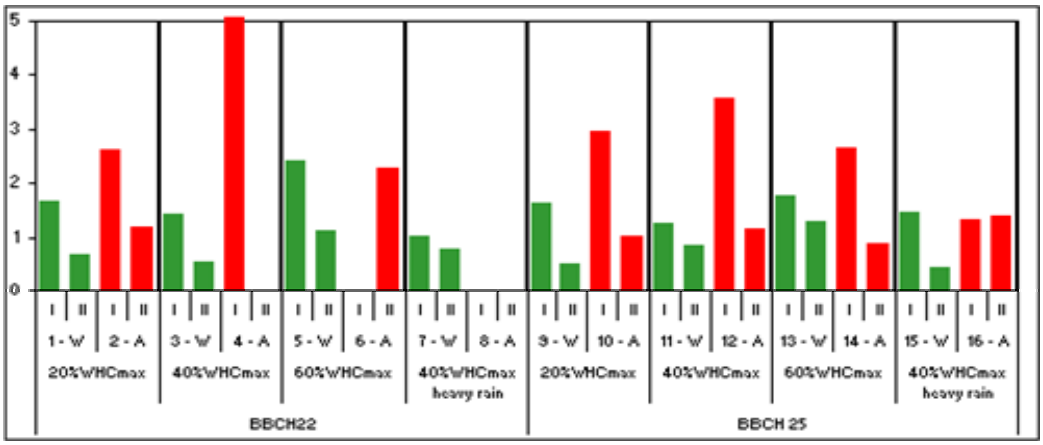


Fig. 27b: Quotient of MKH6561 and 2-hydroxypropoxy-MKH6561 in roots determined by $HPLC_{cuts}$



Legend for Fig. 27a & b:

Spring wheat (W)	Blackgrass (A)
I/II = Sampling date 1/2	

Due to the rising uptake of MKH6561 the plants can possibly reduce the active ingredient contents in an increasingly poorer manner as a consequence of the high volume. In order to avoid damage to the spring wheat, however, a degradation of the subsequently supplied MKH6561 contents is necessary. On the basis of the preceding results, damage to spring wheat therefore appears most probable at a soil moisture of 60%WHC_{max}.

In a comparison of the sampling dates, a decrease of the quotients from the first to the second date is observable in nearly all experimental variants in both the root and leaf material. The lower quotients at the second sampling date may be explained by enhanced active ingredient degradation over the period of investigation.

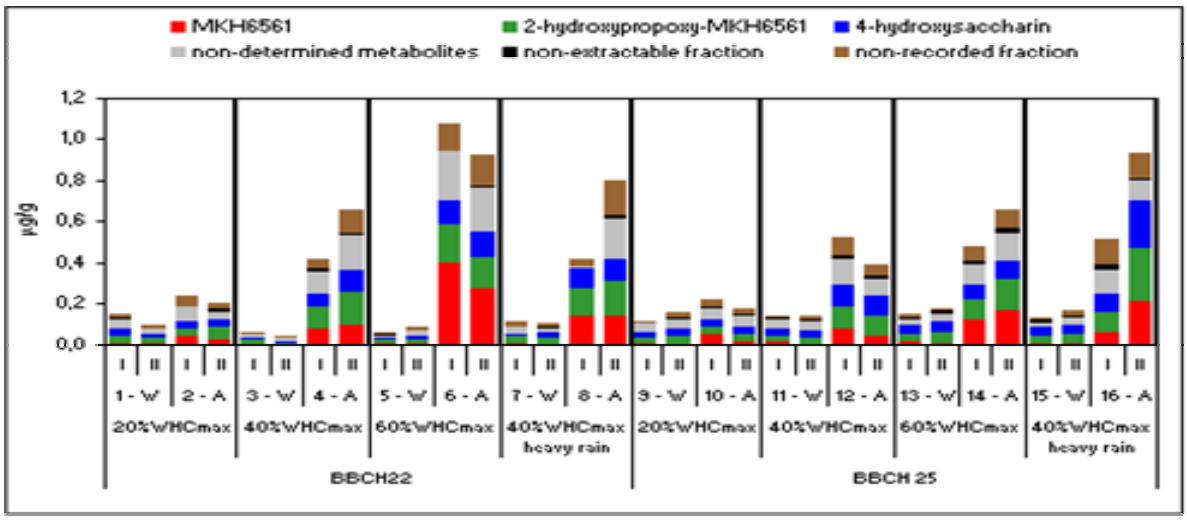
5.1.5 Determination of the concentration of the individual fractions in µg/g in plants

The values previously given exclusively in percent are represented in µg/g after conversion including the total ^{14}C contents (Figures 28 & 29). The values are additionally given in tabular form in A VII.

Figure 28 shows all the fractions investigated in the leaf: concentration of MKH6561 (red) and the metabolites considered [2-hydroxypropoxy-MKH6561 (green) as well as 4-hydroxy-saccharin (blue)] are shown. Added to this is the concentration of non-determined metabolites/the active ingredient equivalents not determined more precisely in the extracts (grey). The latter is ^{14}C radioactivity not distributed to the metabolites (and active ingredient) present as reference substances. In contrast to the previously specified contents determined by $HPLC_{cuts}$, the fractions not extractable by acetonitrile/water (black) were determined by combustion. The non-recorded contents (brown) show the fraction that is

missing to reach the total ^{14}C contents. This fraction can be neither allocated to the extractable nor to the non-extractable fraction. This may also be attributed to metabolization to $^{14}CO_2$.

Fig. 28: Concentration [µg/g] of parent substance and metabolites (2-hydroxypropoxy-MKH6561, 4-hydroxysaccharin and non-determined metabolites) as well as the non-extracted and non-recorded fractions in leaves



First, it may be stated that all examined fractions of the wheat basically display lower concentration than blackgrass. This corresponds to the fact that the active ingredient equivalents contained in wheat are in total less pronounced.

In a comparison of the application stages, wheat hardly displays any active ingredient in the leaves at both stages or the active ingredient fractions were almost completely metabolized to 2-hydroxypropoxy-MKH6561. The thesis already set up in Chapter 5.1.2.2 that damage to wheat may occur at BBCH25 (due to elevated contents in the leaves) must therefore be rejected.

In contrast, high MKH6561 contents occur in ALOMY (increasing with soil moisture). Blackgrass, however, can also degrade the active ingredient, as illustrated by comparatively high concentration of 2-hydroxypropoxy-MKH6561. The influence of soil moisture is also perceivable for 2-hydroxypropoxy-MKH6561: the concentration of this metabolite are higher at high soil moisture than at low moisture levels. This also applies to 4-hydroxysaccharin and the metabolite fraction not characterized more precisely.

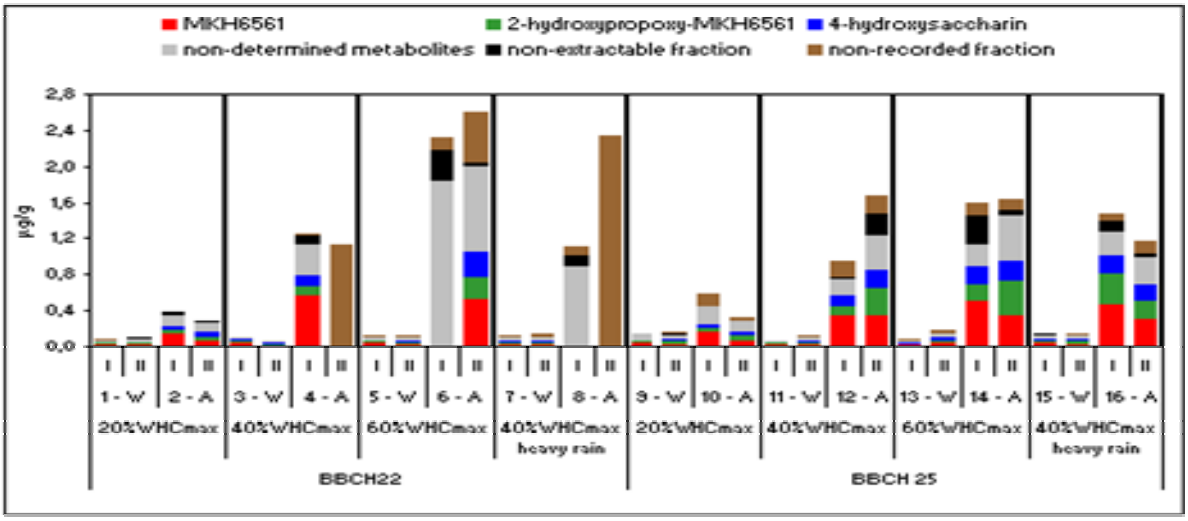
No statements can be made on the non-extractable fractions due to the very low values in this figure. In total, MKH6561 hardly reveals the tendency towards formation of non-extractable residues in the leaf material.

With respect to the non-recorded concentration it may be stated that these are basically higher in ALOMY and rise with increasing soil moisture. It should be noted that this may also be a consequence of the higher total contents.

In a comparison of the sampling dates, reduced active ingredient and rising metabolite concentration can be seen in the majority of cases for both plant species from the first to the second date. This illustrates the metabolization process progressing with time.

The concentrations of the individual fractions in the root material (Figure 29) are roughly comparable to those in the leaves.

Fig. 29: Concentration [µg/g] of parent substance and metabolites (2-hydroxypropoxy-MKH6561, 4-hydroxysaccharin and non-determined metabolites) as well as non-extracted and non-recorded fractions in roots



The total ^{14}C contents in the root material are higher for ALOMY than for wheat and are basically on a higher level than in the leaf material. This causes higher active ingredient and metabolite contents in the roots. As already discussed in Chapter 5.1.4.2, the MKH6561 percentage contents are elevated in the root material of ALOMY. Moreover, they rise with increasing soil moisture.

With respect to the other fractions examined, statements similar to those for the leaf material can be made: concentration of 2-hydroxypropoxy-MKH6561, 4-hydroxysaccharin and the non-determined metabolite fraction increasing with soil moisture are found. As in the leaf material, the non-extractable fraction is very small and the non-recorded fraction rises with increasing soil moisture. Due to the similarity to the leaf material, a further interpretation can be omitted here (cf. statements on leaf analyses).

For the results obtained in comparing the application stages, it is difficult to make any concrete statements due to the lack of data for ALOMY (in columns 4 - A II; 6 - A I and 8 - A I & II no differentiating fractionation could be performed). For wheat reference is made here to A VII - as can be seen from this table, no regularities can be derived for any of the fractions.

In a comparison of the sampling dates, the active ingredient contents of the two plant species in most cases decrease towards the second date; the fractions of the degradation products increase correspondingly. This also corresponds to the findings in leaf material.

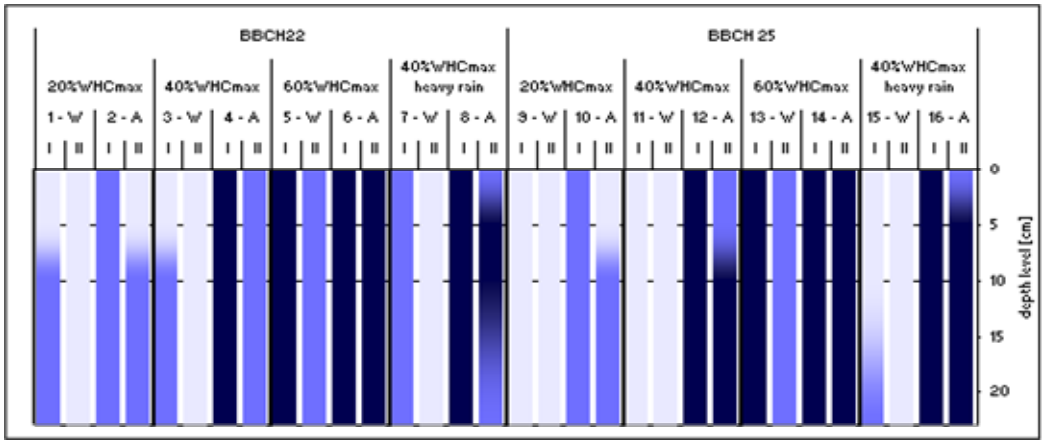
5.2 Soil analyses

First, the soil moisture conditions at the date of sampling are described (Chapter 5.2.1). This is followed by a discussion of the total ^{14}C radioactivity (Chapter 5.2.2). Furthermore, the determination of desorbable and non-desorbable fractions (Chapter 5.2.3) as well as the active ingredient and metabolite determination (Chapter 5.2.4) are dealt with. Finally, the determination of the concentration of the fractions investigated [µg/g] is illustrated (Chapter 5.2.5).

5.2.1 Soil moisture content

The soil moisture conditions at the dates of sampling will first be described. In order to enable a comparison with the soil moistures adjusted in advance (20, 40 and 60%WHC_{max}), the actual moisture of the soil samples is expressed in %WHC_{max}. Figure 30 shows a summary of the results for the depth levels investigated. For illustrative reasons, three levels (0-20, 20-40 and 40-60) were allocated to the %WHC_{max} values. A VIII gives a survey of the numerical values (plus the actual soil moisture) in tabular form.

Fig. 30: Soil moisture (%WHC$_{max}$) of the depth levels investigated



Legend for Fig. 30:

0 - 20 (%WHC$_{max}$)	20 - 40 (%WHC$_{max}$)	40 - 60 (%WHC$_{max}$)

As shown in the figure, the soil moisture clearly increases in the experimental columns in some cases from the upper (0-5 cm) to the lower (5-10 cm and 10-23 cm) layers. Moreover, the values are below the moisture adjusted in advance. Both findings can be attributed to the high root density of the plants investigated and evaporation processes in the upper root zone. The experimental columns with blackgrass all display a considerably higher soil moisture, i.e. lower water uptake and still great quantities of active ingredient in solution which can be taken up. In contrast, spring wheat extracts more water from the soil due to the intensive root penetration of the entire column and the strong transpirational pull in the growth chamber due to the larger leaf mass. The moisture contents at the second sampling date are in most cases below those of the first sampling date. This corresponds to the processes already mentioned (water extraction).

5.2.2 Total ^{14}C radioactivity of the soil material

In the following, the results of the analysis of the total ^{14}C radioactivity of the soil material are discussed. On the one hand, the values are shown as percentages of the radioactivity applied (Figure 31a) and extrapolated to 100% for better comparison of the columns (Figure 31b). On the other hand, they are shown (after conversion into active substance equivalents) in µg/g (Figure 32). A tabular representation is chosen in A IX.

Fig. 31a: Distribution of total ^{14}C radioactivity in percentage per soil layer
applied radioactivity = 100%

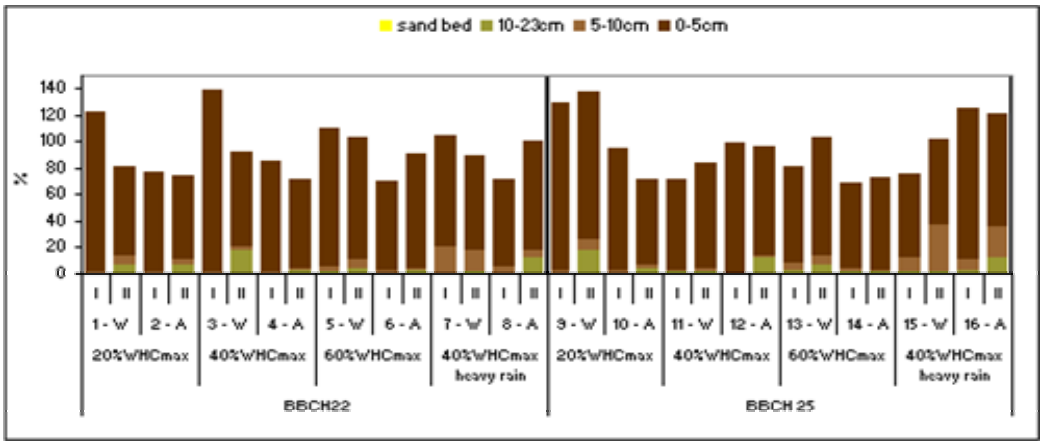
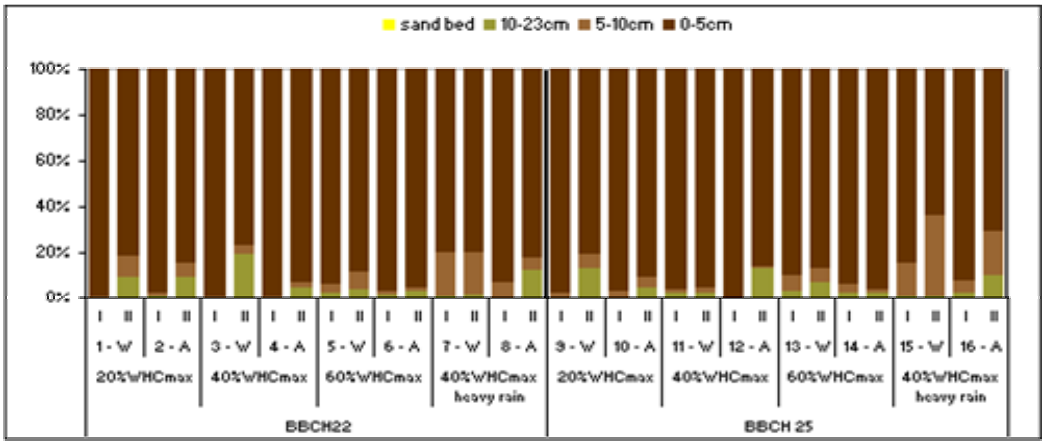


Fig. 31b: Distribution of total ^{14}C radioactivity in percentage (extrapolated to 100%) per soil layer
applied radioactivity = 100%



In the following, the values will first be described using Figures 31a & b. Possible explanations for the individual phenomena will then be given in connection with the discussion of various possible translocation processes.

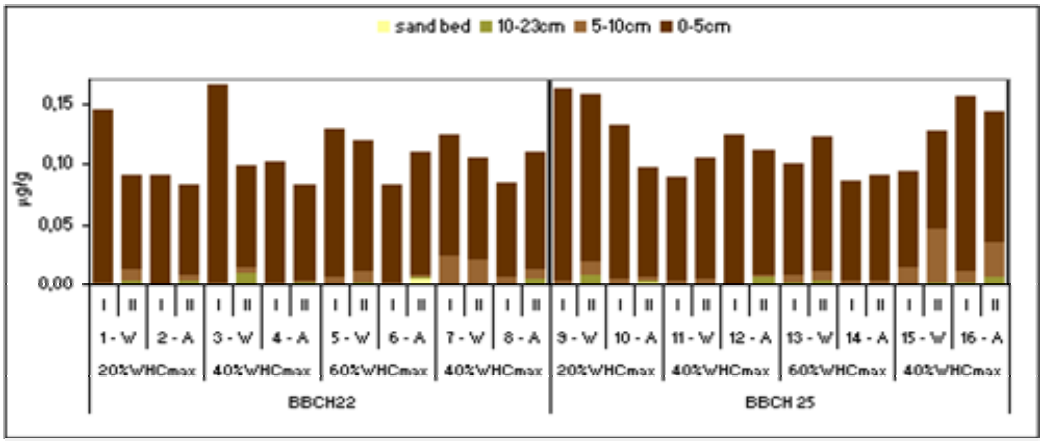
Figures 31a and b show a strongly differentiated distribution of the total ^{14}C radioactivity in the individual soil layers. The highest ^{14}C contents are found in the upper column region (0-5 cm), where the substance was applied. ^{14}C contents are also detectable below 5 cm profile depth, but in smaller fractions.

A comparison of the application dates BBCH22 and 25 does not show any differences with respect to the ^{14}C contents at the depth levels investigated. It is striking to note that hardly any ^{14}C radioactivity is found in the central/lower region of the soil column at the first sampling date (in contrast to the second) across all experimental parameters (in some cases near the limit of detection and high coefficients of variation). The second sampling shows considerably higher translocated contents of total ^{14}C radioactivity. With respect to the first

sampling date, above all, a weak translocation of the substance is to be noted for the particularly moist variants (e.g. 5 - W I and 7 - W I). At the second date, however, no rule-relatedness concerning soil moisture is observed. It may furthermore be stated that the strongest translocation takes place on the columns with heavy rain event. Finally, higher translocated contents are noticeable in wheat in comparison to blackgrass.

In analysing the results, it should furthermore be noted that the bulk density was only determined approximately (cf. Chapter 3.4.1.1). However, slight differences in bulk density which may vary within a small space have an influence on the percentage values per layer. Moreover, the bottom layer (13 cm) is much thicker than the upper ones (5 cm each). Figure 32 can be used for relativizing the different layer thicknesses. This figure shows the active ingredient equivalents in the soil layers examined in µg/g.

Fig. 32: Distribution of active substance equivalents [µg/g] per soil layer



Some of the values (cf. Figure 32) of the first sampling date for the bottom soil layer (10-23 cm) are below the detection limit of 0.0002 µg/g (columns 1, 3, 4, 10 and 12). These columns display a relatively low soil moisture.

The results show that binding processes and translocation processes take place. The processes involved in the mobility of herbicides in soil were explained in the introductory chapters (cf. Chapter 2.2.1).

The active ingredient equivalents in the top soil layer examined decrease towards the second sampling date presumably due to increasing mineralization to $^{14}CO_2$ in the majority of cases. Photodegradation in addition to degradation by microorganisms plays a minor role *(BRUMHARD et al. 1999a)*. It can be stated that radioactivity losses may be attributed mostly to mineralization since volatilization of the active ingredient and metabolites can only contribute marginally to losses (cf. Chapter 2.2.5). An effect due to a slightly inhomogeneous

application of the active ingredient would also be possible, which affects point sampling. However, an unequal distribution of the herbicide on the column can also contribute to higher active ingredient equivalents at the second sampling date. On the whole, decreasing and varying recovery rates and incomplete radioactivity balances are encountered in balancing. Collecting organic volatiles as well as $^{14}CO_2$ by means of suitable "traps" in a closed system would have reduced the non-explainable fraction, but was not representable due to plant cover on the column.

The higher values in the deeper soil layers at the second sampling date are indicative of translocation processes with time. Moreover, it is known from experiments on mobility (cf. Chapter 2.2.3.3) that there is a leaching potential for the active ingredient and the majority of its metabolites. However, the one-week interval in the present experimental design (without additional spray irrigation) between the sampling dates is too short to assume translocation caused by "natural" processes (diffusive and advective transport). This aspect will be discussed in detail in the following.

The high translocation increasingly affects the experimental columns with wheat, whose deep-reaching roots possibly play a particular role here: ^{14}C-MKH6561 or the metabolites could also have been translocated in the basipetal direction after uptake in the upper root zone and could thus have passed into deeper soil regions. It would also be possible that the pull of the plant roots causes a water movement into the main root horizon of the respective plant species, so that at least slight active ingredient fractions accumulate in the corresponding depth (wheat has deeper roots than ALOMY). Moreover, "entrainment effects" are possible with increasing root growth into the depth. Furthermore, due to the deep-growing roots, channels may be produced after their die-off, in which translocation processes proceed faster than in the soil itself. Other types of macropore flow (preferential flow) could also be responsible for the transport of the substance into the subsoil in the case of the particularly moist experimental variants. Due to preferential flow, the contact time between the substance to be translocated and the soil matrix is reduced and the filter and buffer effect of the soil is limited *(PÄTZOLD 1998)*. Both, the unchanged active ingredient which is only weakly adsorbed and the major soil metabolite which is normally strongly adsorbed can be translocated. In general, the translocation by macropore flow takes place immediately after application through the coarse pores. On the assumption that this translocation process has an heavy influence in the column experiment performed, however, a translocation would already be detectable at the first sampling date. This is obviously not the predominat case in the

column experiments but can contribute to herbicide dislocation in the lysimeter experiments. The results of the column experiments rather seem to indicate a slow penetration of the substance through the soil. In this connection, in addition to mass flow, transport processes due to diffusion are also conceivable, but such a translocation from higher to lower concentrations only takes place over short distances.

It should be furthermore emphasized that it cannot be completely ruled out that soil particles are dumped from the top downwards by the first sampling operation. The herbicide adsorbed on these particles could also be recorded at the second sampling in the subsoil due to the limited space on the column, so that increased contents result, which no longer correspond to the contents produced by "normal" translocation processes. In contrast to the column experiments, a different soil sampling technique was used in the lysimeter experiments (cf. Chapter 3.8), in which a dumping of soil particles is less probable. The results of the lysimeter experiments should therefore also be taken into account for a general statement concerning the translocation tendency of MKH6561 (cf. Chapter 6.2.2). It will be shown by further investigations (cf. Chapter 5.2.4) whether only active ingredient and single metabolites formed later are mobile.

An important factor of influence is soil moisture: The slightly pronounced translocation of the herbicide in dry soil is presumably triggered exclusively by the two rain events (irrespective of the soil moisture adjusted before). The "drying" of the topsoil has also an effect so far as even for the moist experimental variants a comparatively dry topsoil region is found (cf. also A VIII), so that a process counteracting the translocation of the ^{14}C contents (strong soil adsorption at dry conditions) is likely. The infiltration of water via the fine pores is slowly and dissolved matter has more time to diffuse in immobile spheres. Moreover, heavy rain has a great influence: for this experimental variant, the translocation due to more intensive irrigation is most pronounced (fast infiltration via preferential flow). Further information concerning a translocation to be expected for different soil moistures is given via the evaluation of the hydraulic functions and description of hydraulic conductivity (Chapter 7.3.2) and of effective retardation (Chapter 7.3.3). At heavy rainfall events and irrigation on very moist soils, the capacity of the topsoil to retain water is exceeded and the macropores which are relevant for transport processes can become waterpathways.

The active ingredient equivalents in the sand bed will not be further discussed here, since all the values are close to or below the detection limit of 0.0002 µg/g. The concentration for column 6 (Sampling II) is an exception: it amounts to 0.0044 µg/g and is thus above the

detection limit. In this case, a slight contamination of the sand bed may be assumed due to a dumping of topsoil material with adsorbed active ingredient components caused by sampling.

5.2.3 Desorption of soil material

In the following, the desorbable and non-desorbable fractions [%] of the active ingredient and its metabolites in the soil material will be discussed in more detail.

As shown in Figures 33a & b, 70-90% of the measured total ^{14}C radioactivity can be desorbed.

Fig. 33a: Distribution of the desorbable fractions and non-desorbable residues in percentage in soil (application stage BBCH22)
total ^{14}C radioactivity = 100%

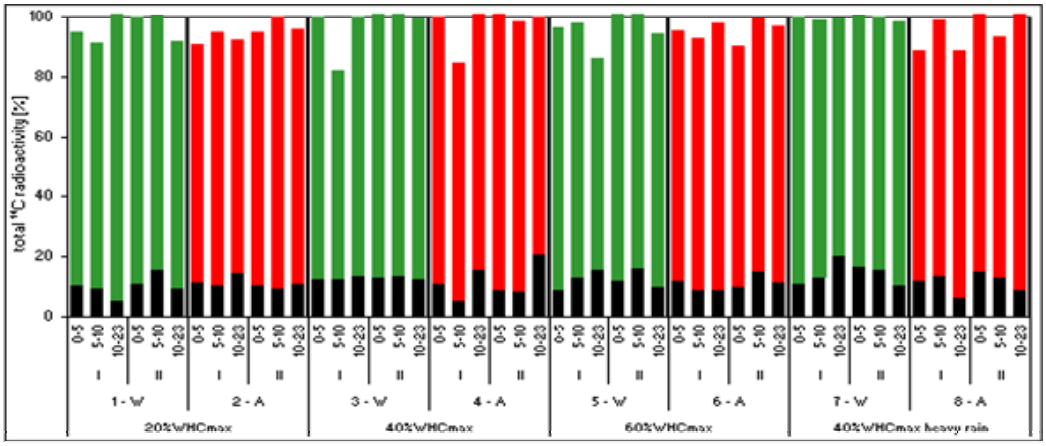
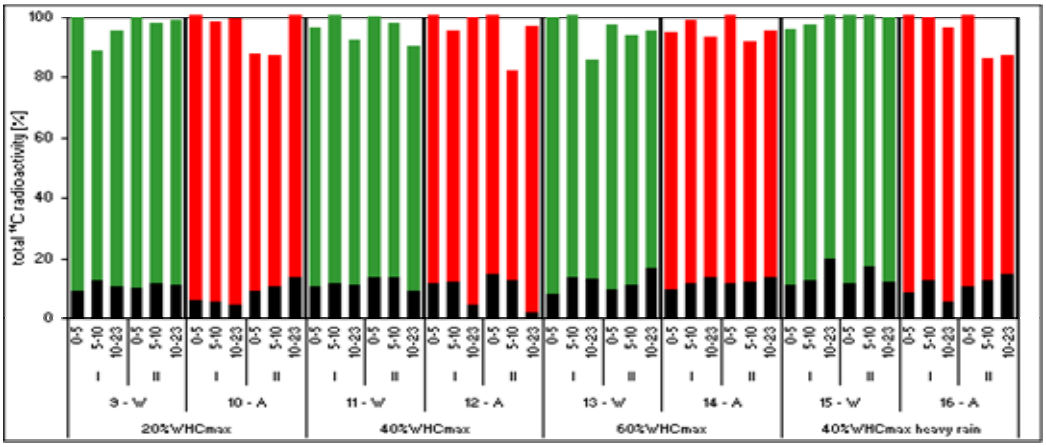


Fig. 33b: Distribution of the desorbable fractions and non-desorbable residues in percentage in soil (application stage BBCH25)
total ^{14}C radioactivity = 100%



Legend for Fig. 33a & b:

Desorbable fraction		Non-desorbable fraction
Spring wheat (W)	Blackgrass (A)	Residues
I/II = Sampling date 1/2		

As can be seen in the figures, no differences are observed for the individual experimental parameters (application date, soil moisture, plant species, sampling date and depth level) with

respect to desorbability. The desorbable contents of the first and second desorption step were measured separately (cf. Chapter 3.10.3). The following can be stated (not discernable in Figures 33a & b): In the first desorption step, in general, more than 90% of the total desorbable fraction (active ingredient and metabolites) were desorbed, so that only small contents were available for desorption in the second step.
Comparatively little ^{14}C radioactivity was therefore found in the soil residues after two desorption steps. An increased formation of residues with progressing time and a dependence on the experimental parameters was not observed.

As already illustrated in the case of the extracts and residues in the plant material, the desorbable fractions and the residues in the soil mostly do not add up to 100% (cf. Chapter 5.1.3), but range between 85 and 105%. No definite cause can be determined here for either the plant or the soil material and different explanation approaches are possible.
Above all, even slight errors in processing the sample material may cause an incomplete radioactivity balance. They were likely to occur during the multistage analysis process and can have negative effects on subsequent steps (e.g. ^{14}C oxidizer, LSC) (increasing error frequency due to several measurement steps). Moreover, losses of the active ingredient due to metabolization and evolution of $^{14}CO_2$ may result from the air drying and storage of the soil samples and the "heat" produced during grinding the solid sample material. Another possibility is a slight adsorption on the inner test vessel walls (plastic cover and glass vessels) during sample processing.
Generally, it may be stated that the liability to error in measuring very small contents as are dominant in the subsoil is comparatively high.

5.2.4 Active ingredient and metabolite distribution in soil material

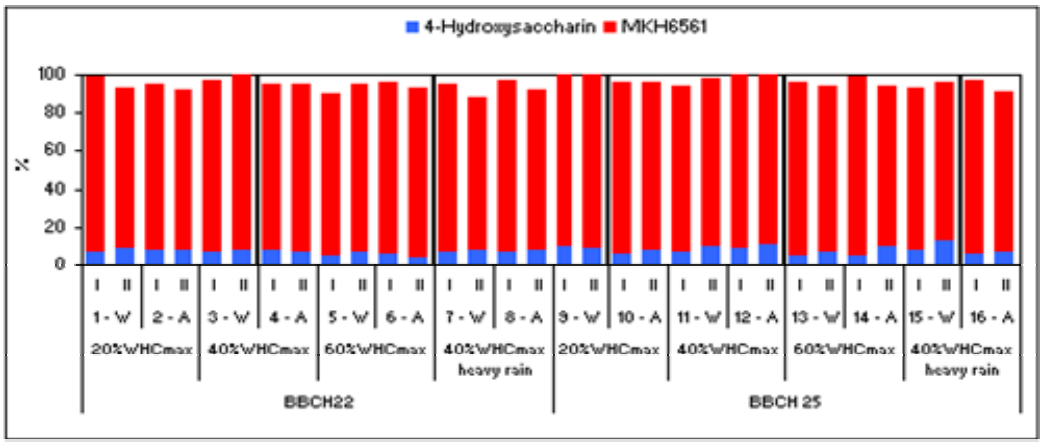
5.2.4.1 Metabolite spectrum

In discussing the metabolite spectrum of the soil material, the focus is on the active ingredient and the metabolite 4-hydroxysaccharin. This is due to the fact that the other metabolites (e.g. 2-hydroxypropoxy-MKH6561) were present only in traces that they could not be unambiguously identified. Chromatograms which releval the metabolite spectrum in soil are reported in Chapter 3.10.4.1 (TLC) and in Chapter 3.10.4.2 (HPLC).
Statements concerning the percentage fractions recovered in the desorption solutions on the basis of $HPLC_{cuts}$ can be made by means of Figure 34 and A X. Active ingredient and to a less

extent (5-12% of desorbed radioactivity) the major soil metabolite are almost exclusively found. This underlines a good stability of the substance in soil.

Figure 34 enables a direct comparison of the percentage distribution of MKH6561 and 4-hydroxysaccharin in the topsoil for all experimental parameters.

Fig. 34: Distribution of MKH6561 and 4-hydroxysaccharin in percentage in topsoil (0-5 cm)
desorbed radioactivity = 100%
determined by $HPLC_{cuts}$



As can be taken from the figure, the metabolization in the topsoil proceeds independently of soil moisture and occurs to a comparable extent across all experimental parameters. The plant species (crop/grass weed) on the column and the application stage do apparently not have an effect on metabolization. It should be noted that in most cases higher contents of the major soil metabolite are found at the second sampling date (7-12%) in comparison to the first sampling date (5-9%), so that a progressive metabolization of the active ingredient with time is recorded.

A graphical representation of the percentage distribution of MKH6561 and 4-hydroxy-saccharin in the subsoil is omitted here. In A X, however, these results are presented in tabular form.

Since the radioactivity contents in the desorption solutions do not significantly exceed the detection limit, it is difficult to draw concrete conclusions. This applies, in particular, to the TLC values of depth level 10-23 cm, since the radioactivity of the samples hardly exceeds the double zero value (background) there (Chapter 3.10.4.1). Moreover, especially in the case of values close to the detection limit, slight measuring inaccuracies are not compensated by averaging since each parallel sample is only measured once (cf. Chapter 3.10.3).

Column 5 (sampling date I) permits the following description as an example (where the trends shown are comparable to those of the other columns):

The detection by means of HPLC and the independent evaluations of the TLC plates have shown that at depth level 0-5 cm 84.91% (TLC = 88.93%) of the applied radioactivity is to be characterized as MKH6561, i.e. the signals in HPLC and the Rf values on the TLC plates are in agreement with those of the inactive standard (cf. Chapter 3.10.4.1). Analogously, 5.19% (TLC = 3.91%) of the applied radioactivity is to be characterized as 4-hydroxysaccharin. At depth level 5-10 cm, 84.19% (TLC = 86.04%) accounted for MKH6561 and 8.46% (TLC = 7.55%) for 4-hydroxysaccharin and at depth level 10-23 cm 76.74% was MKH6561 and 17.1% 4-hydroxysaccharin. A thin-layer chromatographic investigation was not performed in the bottom layer due to insufficient contents.

On the whole, the above results demonstrate at least an increase of the major soil metabolite with increasing depth.

5.2.4.2 Active ingredient degradation in soil: quotient formation of active ingredient and major soil metabolite

In order to verify the statements already made with respect to active ingredient uptake and degradation, in a further evaluation step quotients of the active ingredient and 4-hydroxysaccharin fractions detected in the desorption solutions of the soil material were calculated. For quotients becoming smaller the fraction of 4-hydroxysaccharin increases in percent and the MKH6561 fraction decreases; in analogy, for a rising quotient the fraction of 4-hydroxysaccharin is lower and the MKH6561 fraction higher. In Figures 35a & b quotients are formed for the results of $HPLC_{cuts}$. In Chapter 4.2, these values were compared to radio thin-layer chromatography using the two mobile phase systems (TLC1 and 2) and quotients obtained by evaluation of HPLC chromatograms.

Fig. 35a: Quotient of MKH6561 and 4-hydroxysaccharin in soil (application stage BBCH22)
determined by HPLC methods ($HPLC_{cuts}$)

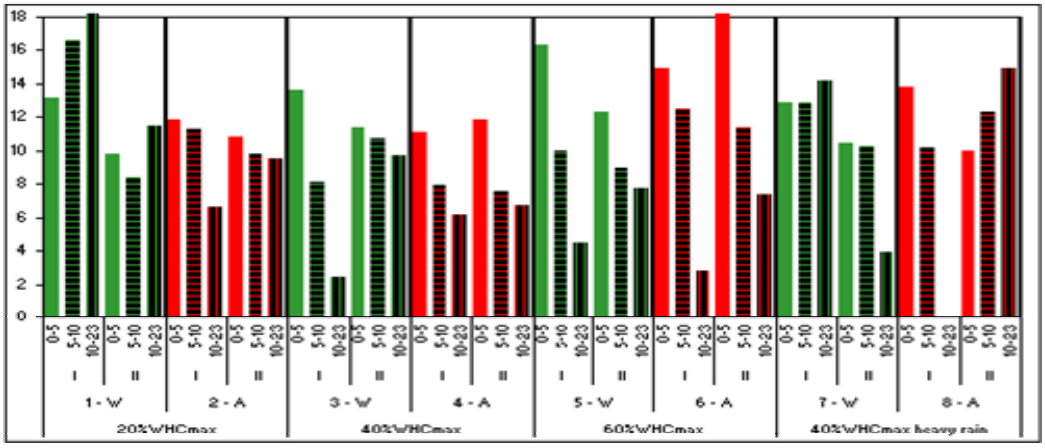
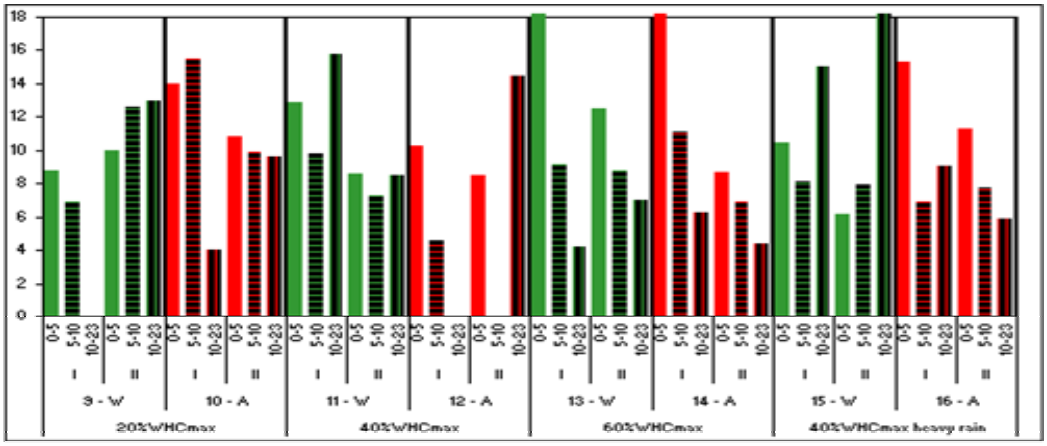


Fig. 35b: Quotient of MKH6561 and 4-hydroxysaccharin in soil (application stage BBCH25)
determined by HPLC methods ($HPLC_{cuts}$)



Legend for Fig. 35a & b:

Spring wheat (W)	Blackgrass (A)
I/II = Sampling date 1/2	

(columns 8 - A I (10-23 cm), 9 - W I (10-23 cm), 12 - A I (10-23 cm) and 12 - A II (5-10 cm) represent missing data due to insufficient radioactivity contents in the desorption solutions; no graphical representation of these values was possible)

The figures show in the majority of cases the highest quotients for the top layer (0-5 cm). The finding that the majority of quotients decrease with increasing profile depth shows that comparatively more soil metabolite was to be found in the bottom profile region (except for the driest experimental variant, as will be described later). During degradation at the upper depth level, mobile "intermediate products" are presumably formed *(BABCZINSKI et al. 2000)*, which are translocated downwards. Finally, 4-hydroxysaccharin is formed, which can be designated as "low mobile" (cf. *HEIN et al. 1999*) and which thus remains at the corresponding soil depth. In most cases, the strongly mobile parent substance itself is probably translocated. Subsequent MKH6561 supply into the depth could also be caused by macropore flow in the soil body. In the deeper soil layers, the active ingredient is degraded well in the case of sufficient oxygen and not excessively high moisture content. An alternative explanation approach would be that the major soil metabolite is translocated into deeper soil layers due to very high soil moisture. This would also explain the relatively low quotients in the central and deep soil layer under the 60%WHC_{max} experimental condition. However, it cannot be stated here which of the possible explanations is applicable.

In a comparison of the different soil moistures, the quotients in the topsoil are the highest for 60%WHC_{max}. Contrary to the expectation that in the experimental variant with 20%WHC_{max} metabolization is not very pronounced due to the lack of water, the water supply in the topsoil seems to be sufficient for metabolization, presumably supported by the two rain events. Rapid degradation can also take place in dry soil, if that soil was moist a comparatively short time

before *(HURLE et al. 1976)*; this prerequisite was given in the present study due to the rain events simulated at the beginning of the experiments. It is moreover conceivable that especially in the case of the dry experimental variant 4-hydroxysaccharin is particularly poorly translocated and "accumulates" in the topsoil. In contrast to the parent substance, the major soil metabolite has very high half-lives (DT_{50}), which supports its "persistence" in the soil. The poor translocation and degradation conditions moreover also explain the comparatively high quotients found here in the central and lower depth level.

In considering the different sampling dates it becomes apparent that the quotients at 0-5 cm depth decrease from the first to the second date. This indicates an increased degradation of MKH6561 to 4-hydroxysaccharin. With respect to the two bottom soil layers no regularities concerning the variation of the quotients can be observed. It must rather be assumed that, depending on the individual case and/or in interaction, many different factors exert an influence (degradation and translocation processes).

5.2.5 Determination of the concentration of the individual fractions in µg/g in soil

The values of all fractions in the soil so far specified are exclusively in percent. In Figures 36a & b, they are shown in µg/g, including the total ^{14}C contents (cf. also Chapter 4.2). A tabular list of the contents of parent substance and metabolites and of the non-desorbable and non-recorded fractions is given in A XI.

Figures 36a and b show the desorbable fractions of active ingredient (in red), 4-hydroxy-saccharin (blue) and other non-determined metabolites/non-identified radioactivity (grey) as well as the non-desorbable fraction (black) and the non-recorded fraction (brown) at the depth levels 0-5 cm, 5-10 cm and 10-23 cm. It should be added that in some cases the values were so low that a graphical representation was omitted (e.g. column 1, depth level 10-23 cm etc.).

Fig. 36a: Concentration [µg/g] of parent substance and metabolites (4-hydroxysaccharin and non-determined metabolites) and of the non-desorbable and non-recorded fractions in soil (application stage BBCH22)

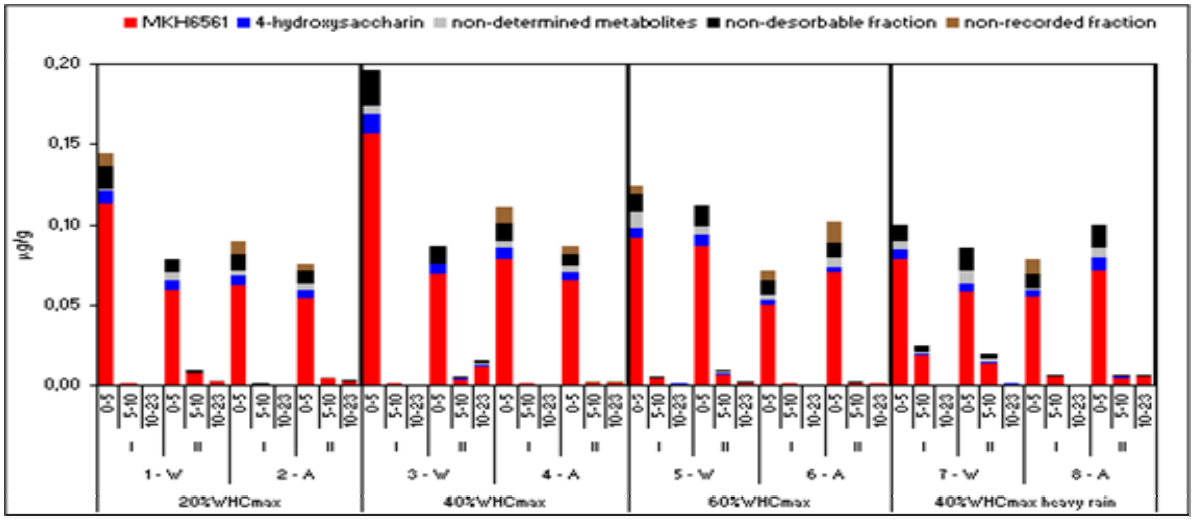
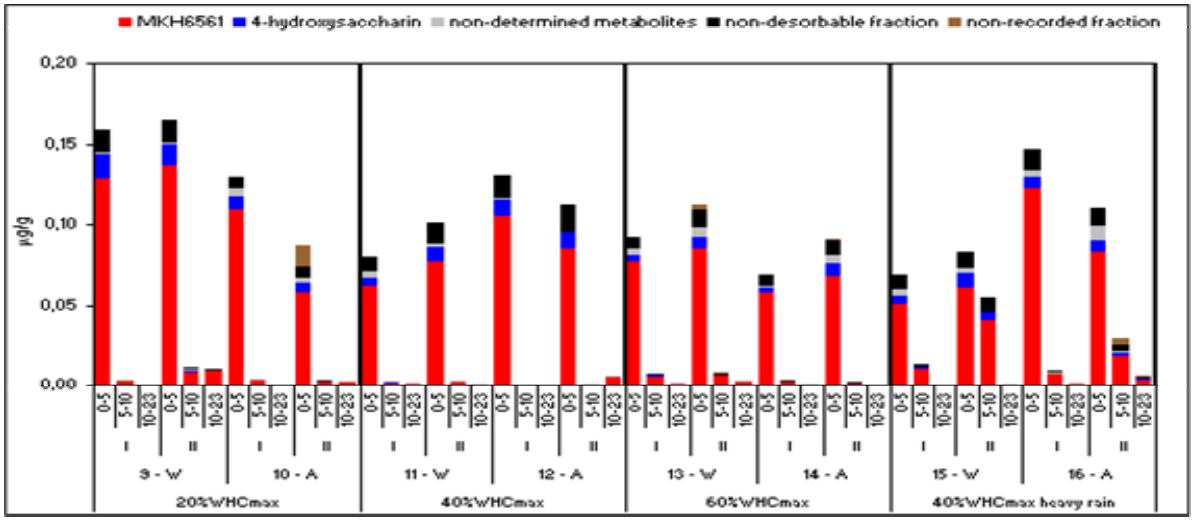


Fig. 36b: Concentration [µg/g] of parent substance and metabolites (4-hydroxysaccharin and non-determined metabolites) and of the non-desorbable and non-recorded fractions in soil (application stage BBCH25)



The figures show good stability of the active ingredient in the soil. All the other fractions are represented to a much lesser extent. Furthermore, the highest total contents are found in the top 5 cm of the soil profile. Only in the case of the heavy rain variants is comparatively more ^{14}C radioactivity contained in the two bottom depth levels. Against this background, the question arises as to how high the relevance of the interpretations and explanation approaches already made in the preceding chapters concerning the contents of the individual fractions in the bottom soil layers is to be assessed. Even though translocation and degradation processes take place in the bottom layers, it is found here that the top soil layer is of greater significance for interpretation due to higher contents.

6. Evaluation of Lysimeter Experiments

The following chapter is devoted to the evaluation of the lysimeter experiments. Reference should be made once again to the summary of the experimental parameters of the lysimeter experiments in Table 12.

Following the structure of Chapter 4, the results of the plant (Chapter 6.1) and subsequently those of the soil analyses (Chapter 6.2) are presented.

6.1 Plant analyses

6.1.1 Macroautoradiographic investigations - Images and distribution of radioactivity in the plant

The macroautoradiographs of winter wheat and blackgrass of the first and third sampling date are shown as examples at application dates BBCH22 and 25 for both soil types (Fig. 37a & b).

Fig. 37a: Macroautoradiographs of winter wheat and blackgrass (orthic luvisol)

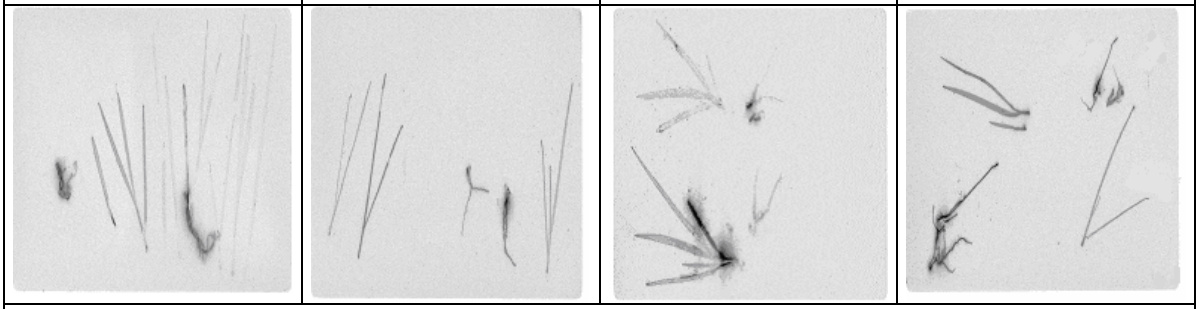
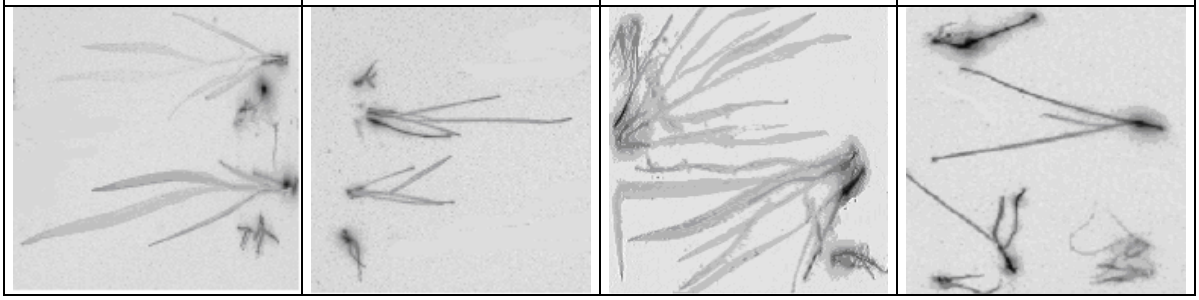
winter wheat	**blackgrass**	**winter wheat**	**blackgrass**
45%WHC$_{max}$		60%WHC$_{max}$	
BBCH22 / sampling date I			
A	B	C	D
			
BBCH22 / sampling date III			
E	F	G	H
			

Fig. 37a: Contd.

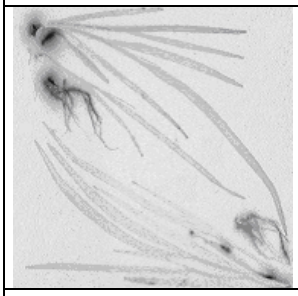
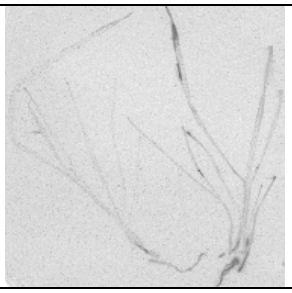
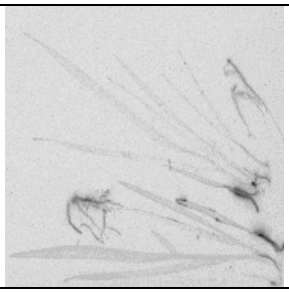
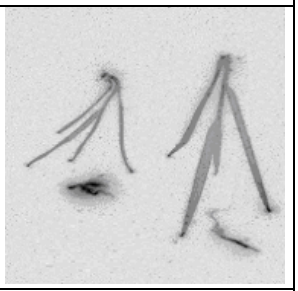
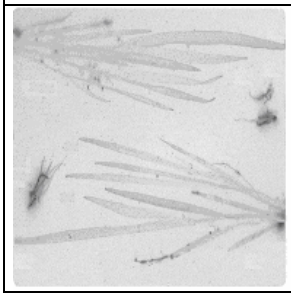
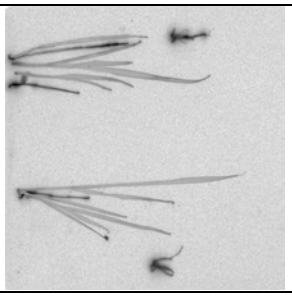
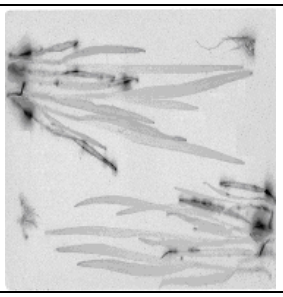
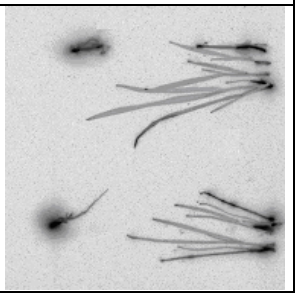
winter wheat	**blackgrass**	**winter wheat**	**blackgrass**
45%WHC$_{max}$		60%WHC$_{max}$	
BBCH25 / sampling date I			
I	J	K	L
			
BBCH25 / sampling date III			
M	N	O	P
			

Fig. 37b: Macroautoradiographs of winter wheat and blackgrass (gleyic cambisol)

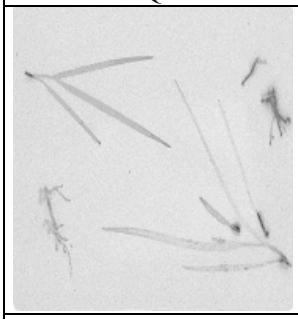
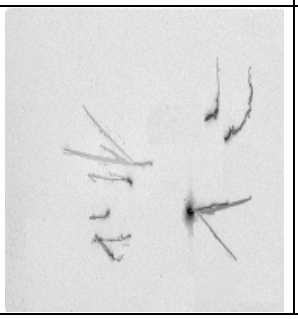
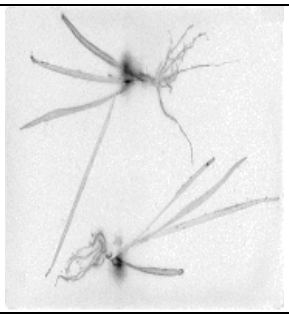
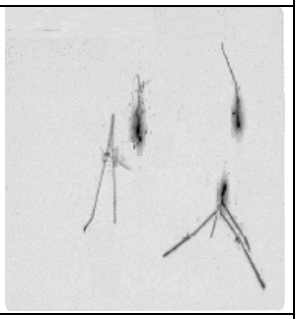
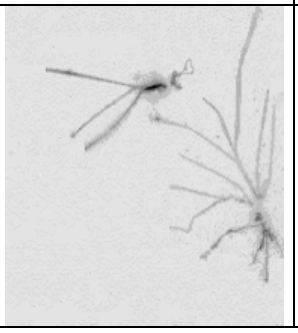
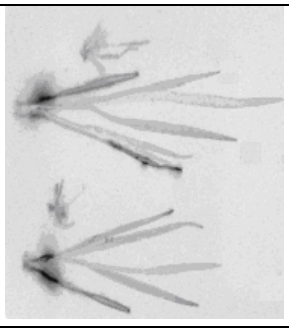
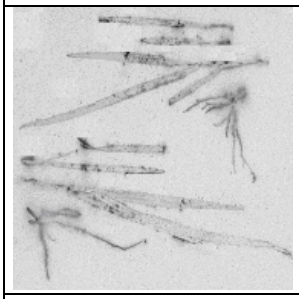
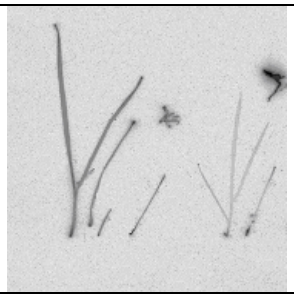
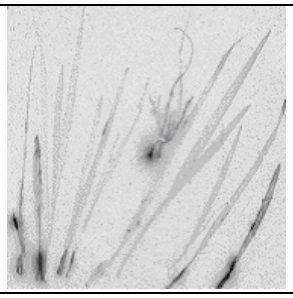
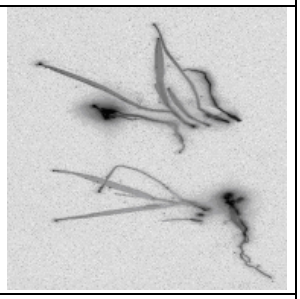
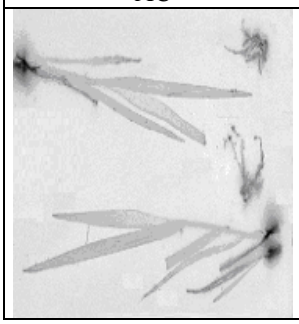
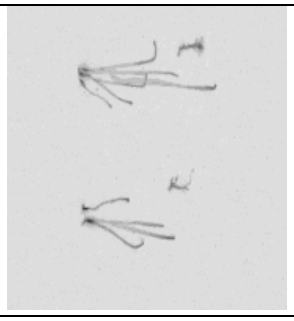
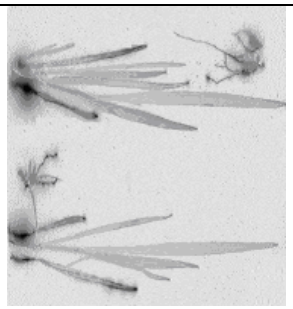
winter wheat	**blackgrass**	**winter wheat**	**blackgrass**
45%WHC$_{max}$		60%WHC$_{max}$	
BBCH22 / sampling date I			
Q	R	S	T
			
BBCH22 / sampling date III			
U	V	W	X
			

Fig. 37b: Contd.

winter wheat	**blackgrass**	**winter wheat**	**blackgrass**
45%WHC$_{max}$		60%WHC$_{max}$	
BBCH25 / sampling date I			
Y	Z	AA	AB
			
BBCH22 / sampling date III			
AC	AD	AE	AF
			

In a comparison of Figures 37a & b the following should be noted: In general, the blackening intensity of the sample material in a lysimeter is similarly pronounced across all plants at one sampling date. In the individual case, however, a high variability is found (ex.: macroautoradiographs A and E). Furthermore, the blackening intensity homogeneously distributed within even-aged leaves of a plant suggests a homogeneous distribution of the active ingredient. Older leaves are often blackened more intensively than younger ones (ex.: macroautoradiograph C). In comparing the two plant species, the macroautoradiographs of blackgrass are more intensively blackened than those of winter wheat under the same experimental conditions (ex.: macroautoradiographs C and D; X and W). In considering the soil types, a darker coloration of the plant material is found on gleyic cambisol in comparison to orthic luvisol (ex.: macroautoradiographs AC and M; Z and J). The macroautoradiographs are generally more intensively blackened at 60%WHC$_{max}$ than at 45%WHC$_{max}$ (ex.: macroautoradiographs L and J; X and V). With respect to the application dates no regularity can be derived in comparing the blackening intensities. Concerning the sampling dates, more intensive blackening always occurs at the third date.

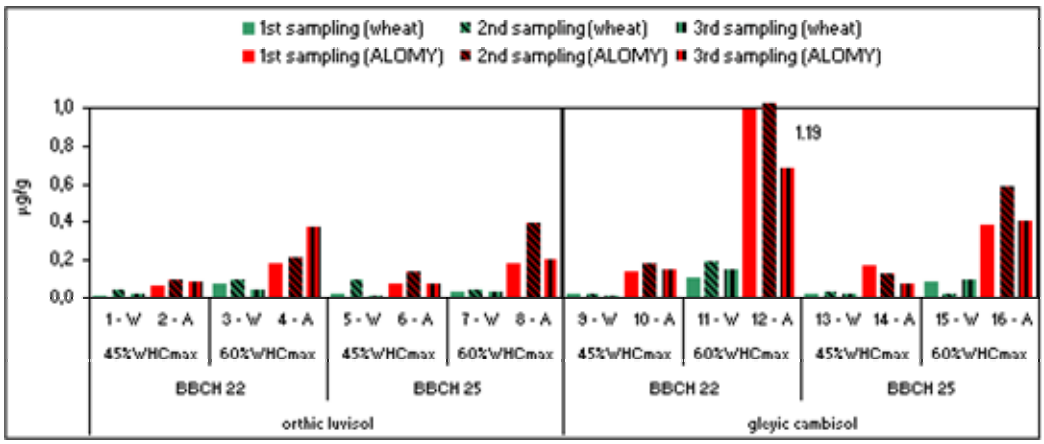
6.1.2 Total ^{14}C radioactivity of the plant material

As already mentioned in the column experiments, the percentage fractions of applied radioactivity passed into the plant are not discussed within the scope of an overall consideration (including soil material) since they are negligible. Less than 1% of the radioactivity applied onto the soil was taken up into the plant. In the following, the active ingredient equivalents in leaves and roots (separately in Chapters 6.1.2.1 and 6.1.2.2) will be discussed (cf. also A XII).

6.1.2.1 Active ingredient equivalents of the leaf material

Figure 38 shows the active ingredient equivalents (relative to 1000 kBq of applied substance) in the leaf material of winter wheat (shown in green) and blackgrass (red) for the two soil types used, the application dates and the respective soil moistures. The different shading of the bars characterizes sampling dates I-III.

Fig. 38: Active ingredient equivalents [µg/g] (relative to 1000 kBq of applied substance) of winter wheat and ALOMY as a function of soil type, application date, soil moisture and sampling date in leaves



Possible explanations for different active ingredient equivalents under the different experimental conditions were already presented in more detail for the column experiments (Chapter 5.1.2.1). Another detailed discussion can thus be omitted here, if the results are similar. Separate explanations/interpretations are given in the case of deviations or changed experimental design.

As shown in the figure, the active ingredient equivalents in the leaves of blackgrass are significantly increased compared to those in winter wheat.

A comparison of the two soil types shows higher contents in the leaf material of gleyic cambisol for blackgrass and winter wheat. Desorption experiments (Chapter 6.2.3) showed that the differences in desorbability between the two soil types are too small to explain different uptake rates into the plant and different translocation rates in the soil (cf. Chapter 6.2.2). Slightly higher desorbable fractions were only determined for the gleyic cambisol. A high active ingredient uptake into the plant could only have been explained by significantly higher desorbable fractions in the soil. These findings coincide with slightly different C_{org} contents and the T values (total sorption capacity of the soil) of both soils (cf. Table 3). Tillage and agricultural use have levelled off the characteristics formed in the course of pedogenesis and the two soils are very similar with respect to their soil-chemical parameters. However, the intention of the desorption experiments was not to determine different desorption rates of the two soils: Two desorption steps were carried out and a comparatively large quantity of solution was applied onto a small amount of soil. Thus, no equilibrium could be achieved between the active ingredient components adsorbed on the soil and those available in solution phase, as is indispensable for adsorption/desorption studies. Instead, it was attempted to dissolve the maximum possible amount (active ingredient and metabolites) from the exchanger bodies, so that similarly high desorbable contents were determined. The influence of the soil type on active ingredient uptake into the plant can only be specified satisfactorily after evaluating the additionally performed adsorption/desorption study (cf. Chapter 3.11.1) and after the evaluation in Chapter 7.1 as well as the description of water movement and substance transport (Chapter 3.11.3) with a detailed evaluation in Chapter 7.3. In the course of these investigations, clear differences were found between the two soil types used: different K_D values resulted from adsorption/desorption studies for the two soils. In comparison to orthic luvisol, gleyic cambisol displayed lower sorptive properties and thus higher mobile active ingredient fractions that can be taken up into the plant. The low sorption capacity of gleyic cambisol is thus also decisively responsible for damage to crop plants. The evaluation of the hydraulic functions (water movement and substance transport in soil) can reliably explain the higher plant uptake in gleyic cambisol due to lower water tension under given experimental conditions. One aspect is the predominance (in contrast to orthic luvisol) of considerably more coarse pores, in which soil water is readily available for plant roots, and a high sand fraction (cf. Table 3). In orthic luvisol, in contrast, water and the plant-available active ingredient fraction can diffuse into abundantly available fine pores and thus escapes uptake.

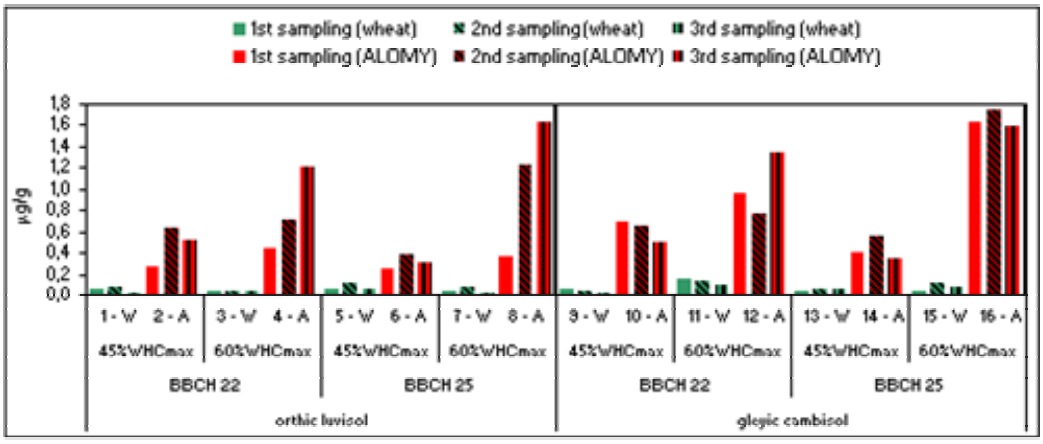
Slight deviations from the pore size distribution shown in literature are due to the fact that the soil is not naturally stored, but heaped. Nevertheless, the coarse pore fraction of orthic luvisol has probably decreased presumably due to the destruction of secondary pores such as fissures, earthworm and root ducts, which account for part of the coarse pore fraction (heaping, settling and recompaction of the soil). The result is probably hardly affected with respect to the main statement to be made regarding the comparison of the two soils investigated.
Another possible explanation for the higher active ingredient uptake in gleyic cambisol is that a larger soil volume (0-10 cm) comes into contact with active ingredient. In orthic luvisol, in contrast, only the top 5 cm contain active ingredient in most cases. This means that in gleyic cambisol more root mass has the possibility of taking up active ingredient.

Moreover, soil moisture seems to have a great influence on the uptake and active ingredient equivalents in the leaf: Both plant species (especially blackgrass) show clearly increased active ingredient equivalents at 60%WHC_{max} than at 45%WHC_{max}. These observations also confirm the results of the calculations based on hydraulic functions and description of water uptake (with dissolved active ingredient components) into the plant due to lower water tension at 60%WHC_{max} (cf. Chapter 7.3.1.2). The application stage also has an influence on the active ingredient equivalents in the leaf: for ALOMY they are higher at BBCH22 than at BBCH25; presumably, the mass increase of the leaves after application at a later date leads to a dilution effect and a reduced effect for AMOMY is to be expected in case of application at the time of main tillering. In the leaf material of winter wheat, this trend can only be recognized at 60%WHC_{max} (not valid for winter wheat at 45%WHC_{max}). In considering the sampling dates I-III no regularities can be identified.
On the whole, it is found that - with two exceptions - all the essential statements concerning the influence of the individual experimental variables are in agreement with those of the column experiments and could thus be confirmed. One exception is an increase of the total contents in the leaf material with increasing soil moisture, which is also pronounced in wheat in the lysimeter experiments; the other exception concerns the observation that in the column experiments the active ingredient equivalents of wheat at stage BBCH25 are in all cases higher than at BBCH22. In the lysimeter experiments, in contrast, this is not always the case. However, further interpretations of these differences are not possible within the scope of the present study due to low case numbers.

6.1.2.2 Active ingredient equivalents of the root material

The active ingredient equivalents in the root (cf. Figure 39) are comparable to those in the leaf with respect to the following parameters: similarly pronounced relations of the concentration is found in a comparison of the plant species (higher concentration in ALOMY in contrast to wheat), soil moistures (active ingredient equivalents in ALOMY and wheat rise with increasing soil moisture) and soil types (highest concentration in plants derived from gleyic cambisol). Furthermore, low concentration is dominant here for winter wheat, too, which change from the first to the third sampling date without any clear trend.

Fig. 39: Active ingredient equivalents [µg/g] (relative to 1000 kBq of applied substance) of winter wheat and ALOMY as a function of soil type, application date, soil moisture and sampling date in roots



Further deviating results are to be described as follows: ALOMY shows a rising trend for the concentration on orthic luvisol from the first to the third sampling date. It remains unclarified, however, whether this trend (which is not clear in all cases) is indeed indicative of a regularity.

With respect to the application dates, no regularity can be derived for wheat under any experimental condition. For blackgrass, in contrast, the active ingredient equivalents of the respective columns decrease in a comparison of BBCH22 and 25 (soil moisture 45%WHC_{max}). At 60%WHC_{max}, in contrast, the active ingredient equivalents increase. This applies to both orthic luvisol and gleyic cambisol. These findings can be explained, on the one hand, by a mass increase of the plant material (in the case of decreasing concentration) and, on the other hand, by enhanced active ingredient uptake due to high soil moisture.

On the whole, considerably higher concentration is measured in the roots compared to the leaves for ALOMY.

As in the column experiments, the active ingredient equivalents of leaf and root material were correlated. The total correlation (ALOMY and winter wheat) is r = 0.62**. In a correlation performed separately according to plant species, a coefficient of r = 0.61** is obtained for ALOMY and of r = 0.70** for winter wheat. The correlation is medium in all cases; the herbicide is almost uniformly distributed within the plant and only slight contaminations due to contents adsorbed at the epidermis are expected.

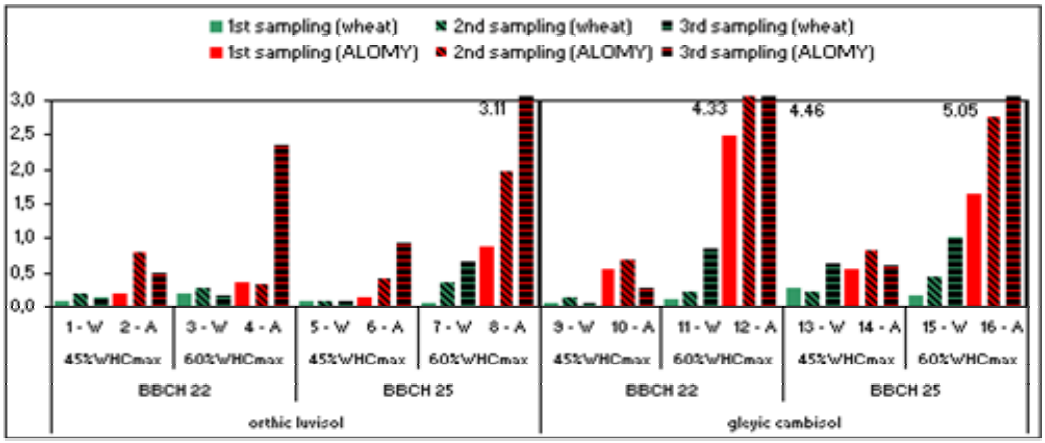
In comparing the active ingredient equivalents of column and lysimeter experiments (root material) it can be stated that no differences concerning the effect of soil moisture were found. Regarding the date of application, the findings for ALOMY are not consistent (lysimeter experiments) and therefore no standard valuation can be performed. Furthermore the values at different sampling dates are inconsistent (column experiments); only for ALOMY an increasing trend, proceeding with time, can be derived (lysimeter experiments).

6.1.2.3 Excursion: Additional evaluation of the macroautoradiographic investigations

As for the column experiments, in this chapter, the blackening intensities of the leaf and root material are determined using the methodology already described (cf. Chapter 5.1.2.3) and interpreted with the same methodological restrictions [here: n = 2] as specified there.

Figure 40 first shows the values for the leaf material.

Fig. 40: Blackening intensity [(PSL-Bkg) per mm^2 of leaf area relative to 24 h of exposure] as a function of soil type, application date, soil moisture, experimental plant and sampling date in leaves

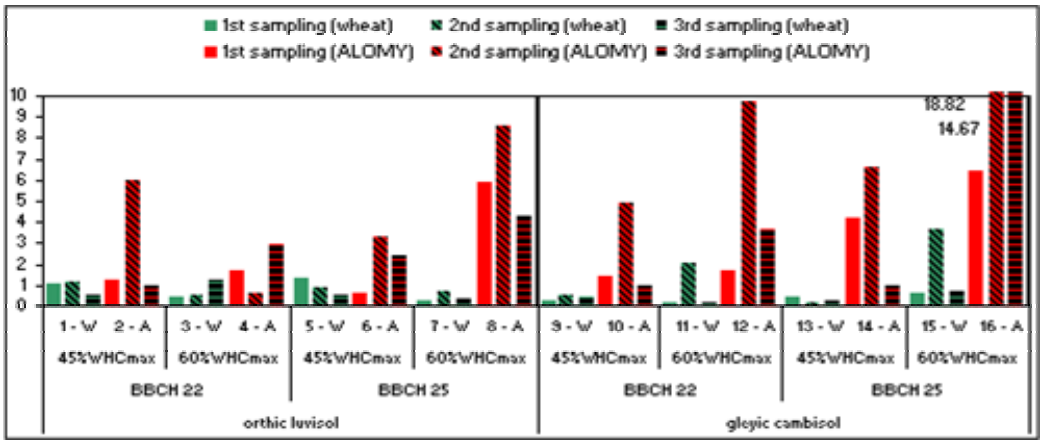


As can be seen from the figure, the leaf material of blackgrass shows a substantially higher blackening intensity than that of winter wheat. There is a dependence on soil moisture (irrespective of the plant species), which is shown by higher blackening intensities at higher soil moisture. The highest values are reached at 60%WHC_{max}, especially in the case of

blackgrass on lysimeter halves 8, 12 and 16. The soil type also has a strong influence on the blackening intensities of the plants: active ingredient uptake seems to be considerably higher in plants on gleyic cambisol than on orthic luvisol. Moreover, predominantly higher values are found in the plant material at application stage BBCH25 than at stage BBCH22. This applies to both wheat and blackgrass. A comparison of the three sampling dates shows that, as a trend, more active ingredient is taken up into the plant (wheat and ALOMY) with progressing time, since sufficient water supply is always ensured due to high soil moisture. Wheat, in addition, showed impairments of growth and plant health (leaf chloroses, mildew and aphids) at the third sampling date at 60%WHC_{max} above all on gleyic cambisol. This was presumably due to a comparatively high uptake and poor degradation of the herbicide in the plant.

Figure 41 shows the results of the macroautoradiographic investigation for the root material.

Fig. 41: Blackening intensity [(PSL-Bkg) per mm^2 of root area relative to 24 h of exposure] as a function of soil type, application date, soil moisture, experimental plant and sampling date in roots

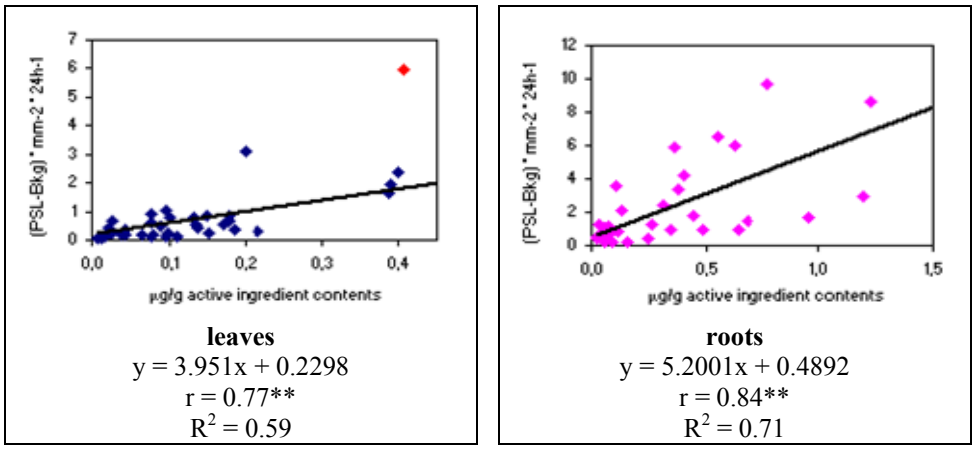


Results differ from those of the leaf analyses. On the whole, the blackening intensities are on a higher level. Deviations from the results of the leaf material are found with respect to the influence of soil moisture for orthic luvisol, where in most cases no increase in blackening intensity is identified with rising soil moisture. A difference is also found with respect to the soil type. In the case of wheat, the values for gleyic cambisol are in most cases not clearly higher than for orthic luvisol. Moreover, the blackening intensities for wheat on orthic luvisol are not higher at application date BBCH25 than at BBCH22. In a comparison of the sampling dates no uniform trend concerning a change in values from the first to the third date can be observed for the roots.

Finally, reference should be made once again to the already mentioned methodical restrictions in evaluating the macroautoradiographs (cf. Chapter 5.1.2.3).

The blackening intensities to assess the connectivity of the results are correlated with the active ingredient equivalents (cf. Figure 42).

Fig. 42: Correlation of active ingredient equivalents [µg/g] and blackening intensities (macroautoradiography) in leaves and roots
outliers are marked in red



High correlations of $r = 0.77^{**}$ (leaves) and $r = 0.84^{**}$ (roots) are achieved across both plant species. In the case of leaf material it should be mentioned that the correlation would even experience a rise to r = 0.86** without the outlier marked in red. If wheat and ALOMY are considered separately, the following coefficients result: leaf material of wheat $r = 0.45^{ns}$, root material $r = 0.38^{ns}$; for blackgrass: r = 0.71** (leaves) and r = 0.76** (roots). With the aid of these results it can be shown, that the values of macroautoradiography can be used to obtain a first insight into the active ingredient concentration to be expected - at least in the case of ALOMY (high correlations). On the other hand, the correlations for winter wheat are too weak to furnish reliable results - here, the methodological problem that low contents are "susceptible" to result-distorting value changes seems to have an effect (e.g. due to active ingredient components adsorbed on the root epidermis). Since, moreover, only two experimental plants were subjected to macroautoradiography, this may have an even stronger effect and be reflected in low correlations.

6.1.3 Extraction of plant material

Figures 43a & b show the distributions of the extractable fractions and non-extractable residues in percent (relative to the total ^{14}C contents) in the leaf and root material.

Fig. 43a: Distribution of extractable fractions and non-extractable residues in percentage (additive) in leaves
total ^{14}C radioactivity = 100%

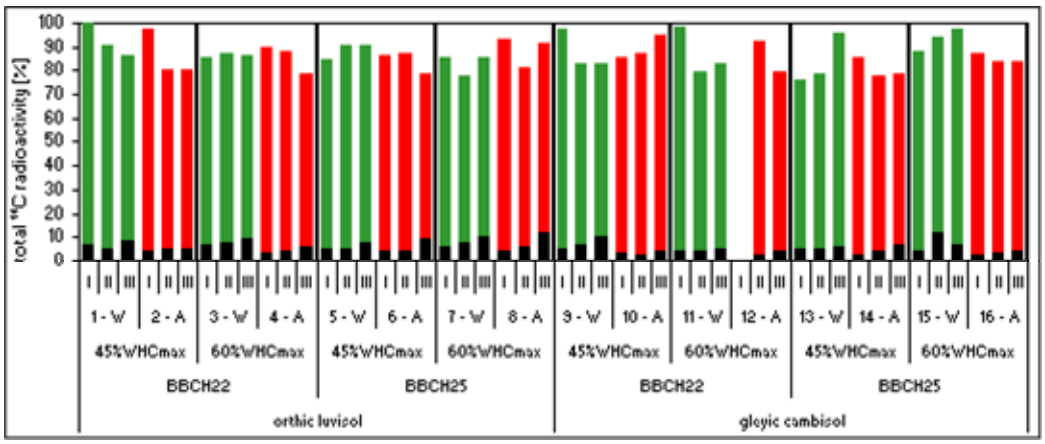
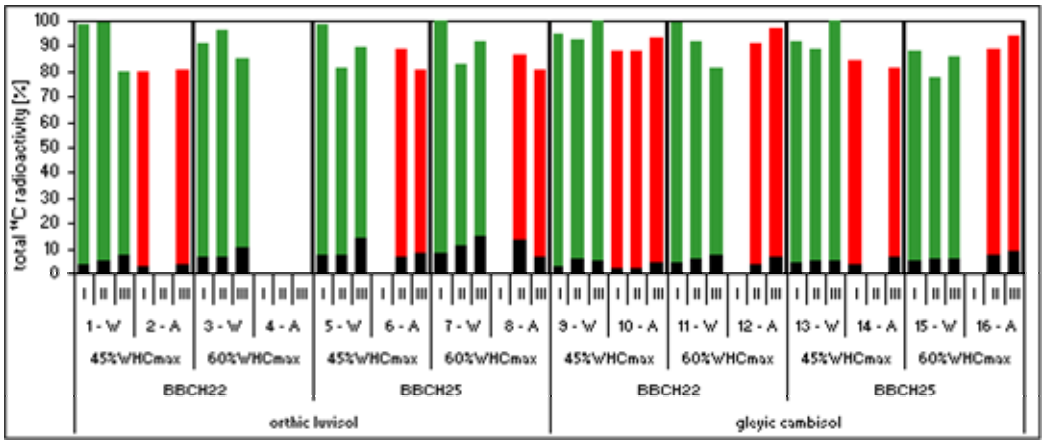


Fig. 43b: Distribution of extractable fractions and non-extractable residues in percentage (additive) in roots
total ^{14}C radioactivity = 100%



Legend for Fig. 43a & b:

Extractable residues		Non-extractable residues
Winter wheat (W)	Blackgrass (A)	Residues
I/II/III = Sampling date 1/2/3		

(in the case of 12 - A I (leaf) as well as 2 - A II, 4 - A I-III, 6 - A I, 8 - A I, 12 - A I, 14 - A II and 16 - A I (root) insufficient plant material was available for extraction)

As mentioned already in the column experiments (cf. Chapter 5.1.3) the extractable fractions in the leaf and root material are in the range of 80% in all cases. No influence of the experimental parameters was observed concerning an increase or decrease of the values.

However, in the majority of cases an increase in the percentage contents of the non-extractable residues is found with progressing sampling date. This trend also became apparent in the column experiments. Possible explanations for the increase of non-determinable fractions were already given in Chapter 5.2.3 and are not separately presented here.

6.1.4 Active ingredient and metabolite distribution in plant material

The percentage distribution of the active ingredient and metabolites found in the leaf are shown in Chapter 6.1.4.1 and in the root in Chapter 6.1.4.2 using $HPLC_{cuts}$. Exemplary TLC chromatograms can be found in Chapter 3.10.4.1.

As for the column experiments, on the one hand, the undetermined ^{14}C radioactivity in the extracts is included in the representation and, on the other hand, the distribution of the fractions (active ingredient and two metabolites) is extrapolated to 100% for better comparability. The allocation of colours to the fractions examined also corresponds to that in the column experiments. The percentage contents are shown in tabular form in A XIII.

6.1.4.1 Leaf Material

As can be seen from Figures 44a & b, the percentage distribution across all experimental parameters is relatively uniform.

Fig. 44a: Distribution of active ingredient, 4-hydroxysaccharin and 2-hydroxypropoxy-MKH6561 in percentage in leaves
extracted radioactivity = 100%
determined by $HPLC_{cuts}$

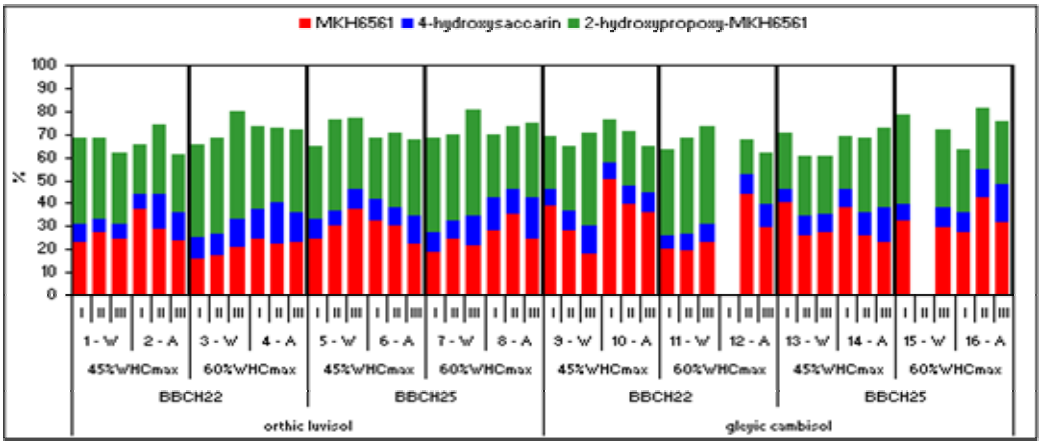
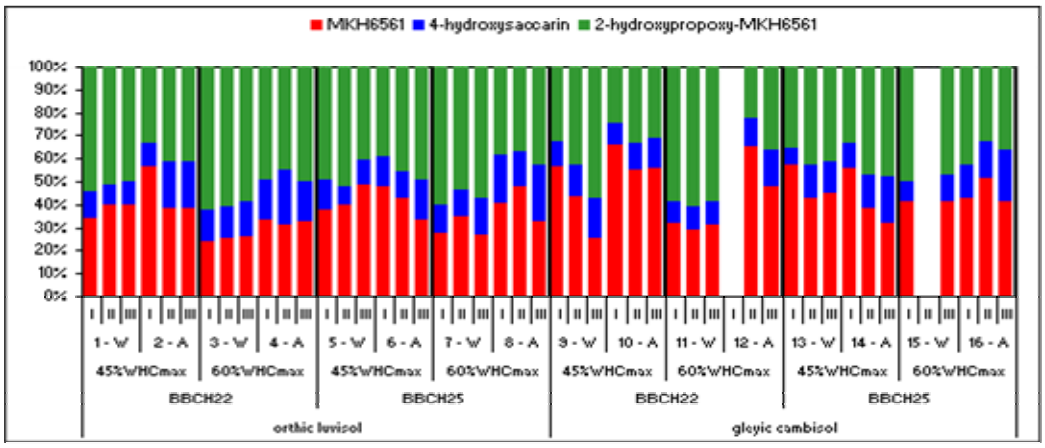


Fig. 44b: Distribution of active ingredient, 4-hydroxysaccharin and 2-hydroxy-propoxy-MKH6561 in percentage (extrapolated to 100%) in leaves
extracted radioactivity = 100%
determined by $HPLC_{cuts}$



(12 - A I and 15 - W I are missing data due to insufficient sample material)

In the extracts of both plant species, the percentage contents of active ingredient and 2-hydroxypropoxy-MKH6561 account for the largest fractions. The percentage contents of 4-hydroxysaccharin and the metabolite fraction not characterized (Figure 44a) are lower.

In a detailed comparison of the two plant species, in most cases the percentage contents of the active ingredient are higher in blackgrass than in winter wheat. The percentage contents of 2-hydroxypropoxy-MKH6561, in contrast, are higher in winter wheat compared to blackgrass, since the latter is able to degrade the active ingredient more rapidly *(BABCZINSKI et al. 2000)*. No differences between the two plant species are apparent in considering 4-hydroxysaccharin and the undetermined metabolite fraction.

A comparison of sampling dates I-III does not show any changes in the distribution of MKH6561 in most cases. Two overlaying processes seem to play a role: as already shown for the column experiments, progressing active ingredient degradation with time and a constant high uptake of unchanged active ingredient (irrespective of plant species). In contrast, the percentage contents of 2-hydroxypropoxy-MKH6561 and 4-hydroxysaccharin rise with time, which indicates increasing active ingredient degradation and rising uptake rates of the major soil metabolite not formed in the plant.

No rule-relatedness can be derived with respect to application dates BBCH22 and 25.

In a comparison of the two soil types, differences are identified concerning the active ingredient fractions. Presumably, higher fractions are contained in plants on gleyic cambisol due to the high subsequent delivery and uptake rate of unchanged active ingredient (cf. Chapter 6.1.2.1) than in plants on orthic luvisol.

Due to the constantly high soil moisture, enhanced by the two rain events performed and the resulting high subsequent delivery of herbicide into the plant, hardly any differences are to be observed between 45 and 60%WHC_{max} concerning the fractions of active ingredient in the extracts. Comparably high MKH6561 contents were observed in the column experiments at 60%WHC_{max} (cf. Chapter 5.1.4.1). The relations of the active ingredient contents are different between the two plant species and closer in the lysimeter experiments; thus, for example, physiological differences between spring and winter wheat or differences caused by the experimental setup may have an influence on the values.

The differences in the two experimental variants, also considering increased uptake rates at high soil moisture, can only be clarified in detail after conversion to the total ^{14}C contents in Chapters 5.1.5 and 6.1.5.

6.1.4.2 Root material

As can be seen from Figures 45a & b, very high active ingredient fractions are found in the roots for both plant species.

Fig. 45a: Distribution of active ingredient, 4-hydroxysaccharin and 2-hydroxypropoxy-MKH6561 in percentage in roots
extracted radioactivity = 100%
determined by $HPLC_{cuts}$

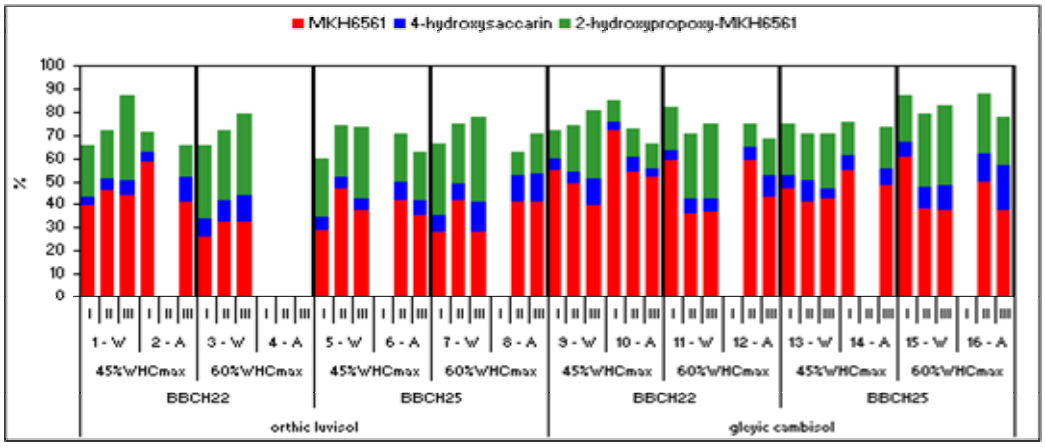
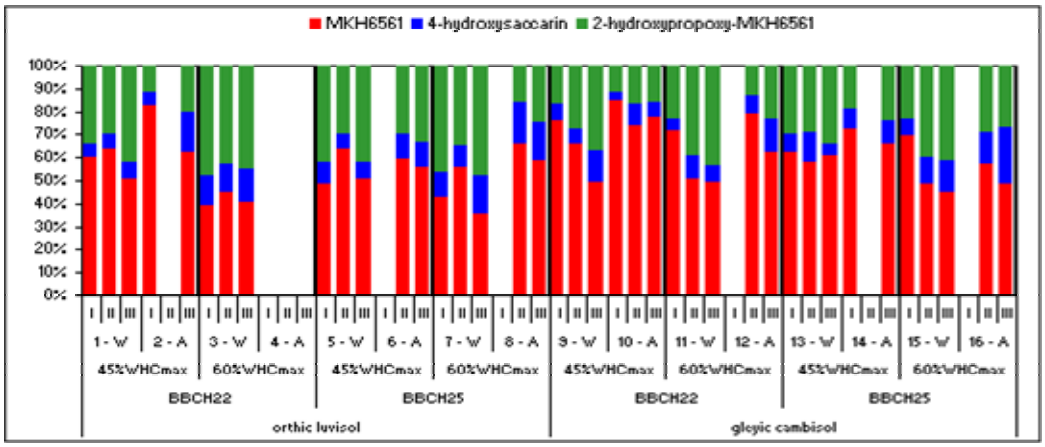


Fig. 45b: Distribution of active ingredient, 4-hydroxysaccharin and 2-hydroxy-propoxy-MKH6561 in percentage (extrapolated to 100%) in roots
extracted radioactivity = 100%
determined by $HPLC_{cuts}$



(Due to an insufficient quantity of root material, no values could be determined for lysimeters 2 - A II, 4 - A I-III, 12 - A I, 14 - A II and 16 - A I)

The percentage contents of the other fractions examined are correspondingly lower.

In winter wheat, however, the percentage contents of the active ingredient are slightly lower than in blackgrass. On the other hand, the contents of 2-hydroxypropoxy-MKH6561 are higher than in ALOMY; this is attributable to a faster active ingredient degradation of the crop. No differences are found for 4-hydroxysaccharin and the unknown ^{14}C radioactivity.

In comparison to the leaf material, the active ingredient contents in the root material are clearly higher on the whole. This is due to presumably the active ingredient components left (despite washing) on the root epidermis.

With respect to the different experimental parameters, as a trend, decreasing active ingredient contents from sampling date I to III were observed; this illustrates progressing degradation with time.

With respect to the other experimental parameters (application date and soil moisture) no rule-relatedness can be observed. Only the active ingredient contents on gleyic cambisol display higher values, as in the leaves, and are to be interpreted accordingly (high subsequent delivery rate of unchanged MKH6561).

6.1.4.3 Active ingredient degradation in the plant: quotient formation of active ingredient and major plant metabolite

The formation of quotients of active ingredient and 2-hydroxypropoxy-MKH6561 was performed for all the separation methods used (Chapter 3.10.4). The quotients were

interpreted using $HPLC_{cuts}$ (Figures 46a & b) and correlated with thin-layer chromatographic methods (Chapter 4.2).

Fig. 46a: Quotient of MKH6561 and 2-hydroxypropoxy-MKH6561 in leaves determined by $HPLC_{cuts}$

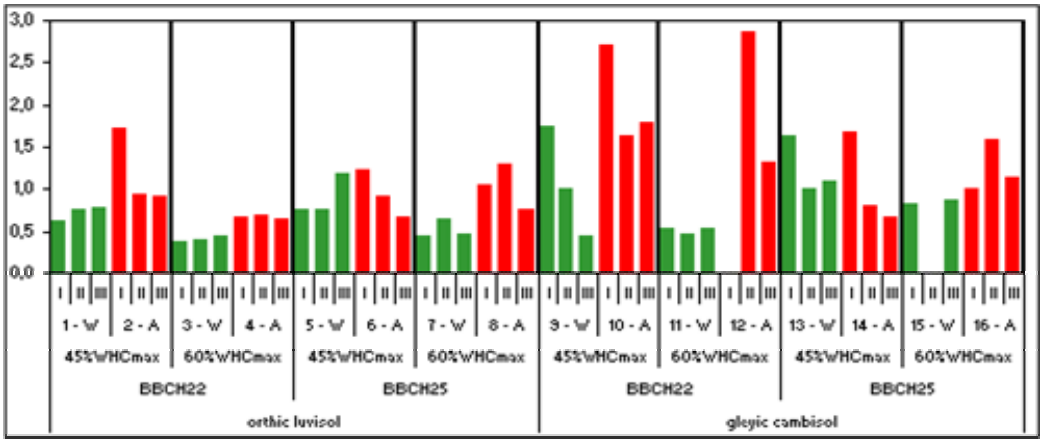
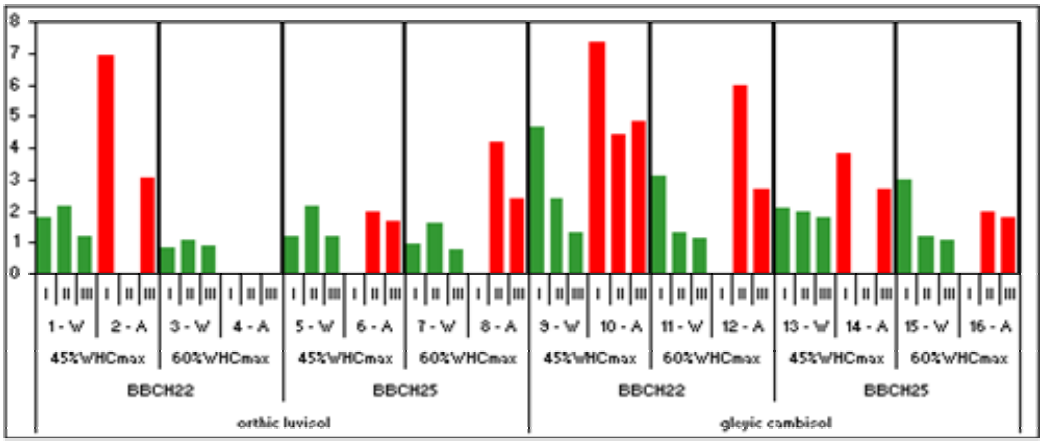


Fig. 46b: Quotient of MKH6561 and 2-hydroxypropoxy-MKH6561 in roots determined by $HPLC_{cuts}$



Legend for Fig. 46a & b:

Winter wheat (W)	Blackgrass (A)
I/II/III = Sampling date 1/2/3	

(Due to the small amount of dry substance for blackgrass, the extraction and thus the determination of the active ingredient components is sometimes incomplete. This applies to 12 - A I and 15 - W II for leaf material and to 2 - A II, 4 - A I-III, 6 - A I, 8 - A I, 12 - A I, 14 - A II and 16 - A I for root material)

The large number of missing data has to be taken into account when evaluating Figure 46b. Generelly, the figures show lower quotients for winter wheat than for blackgrass. This points to the better metabolization capacity of the active ingredient in wheat in contrast to ALOMY. In contrast to the observations in the column experiments at 60%WHC_{max}, a comparison of the quotients of different soil moisture shows lower quotients than at 45%WHC_{max}, indicating an increasing active ingredient degradation due to rising soil moisture.

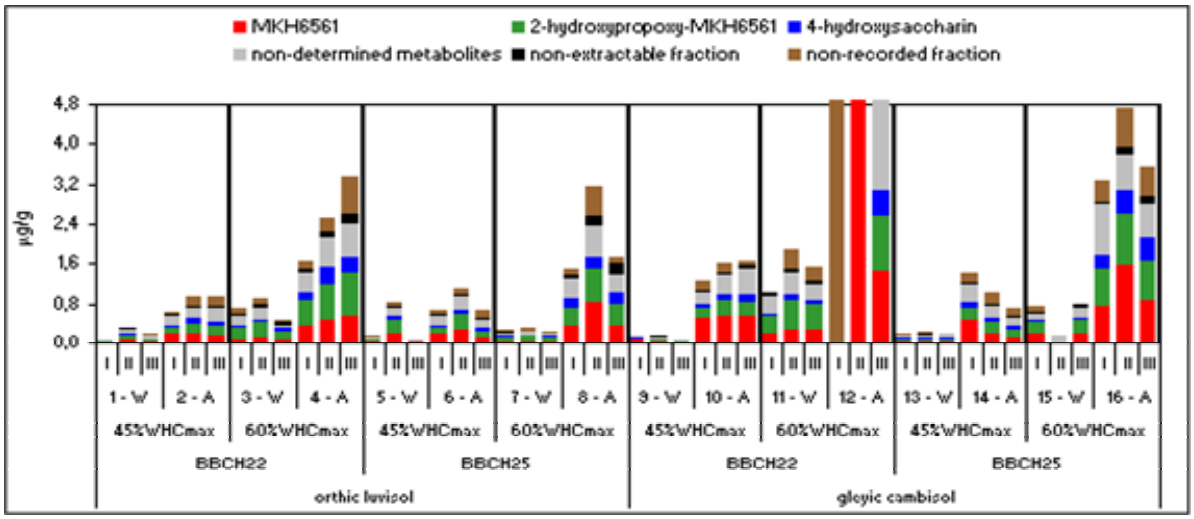
In their majority, the quotients decrease from the first to the third sampling date, which documents increasing degradation with time. Moreover, the quotients are elevated for gleyic cambisol, since larger amounts of unchanged active ingredient were taken up into the plant.

6.1.5 Determination of the concentration of the individual fractions in µg/g in plants

In Figures 47 & 48, the values of all fractions previously given in percent are represented in µg/g after conversion including the total ^{14}C contents. The structure of the figures and the colour allocations of the individual fractions are identical to the column experiments (cf. Chapter 5.1.5). A XIV additionally shows a tabular representation of these results.

The fractions determined in the leaf will be discussed first (Figure 47).

Fig. 47: Concentration [µg/g] of parent substance and metabolites (2-hydroxypropoxy-MKH6561, 4-hydroxysaccharin and non-determined metabolites) and of the non-extractable and non-recorded fractions in leaves



Generelly, the concentration of the fractions examined [µg/g] are increased in blackgrass compared to winter wheat due to significantly higher total ^{14}C contents and high uptake rates.

In a comparison of the application stages, blackgrass mostly displays higher active ingredient concentration and higher concentration of 2-hydroxypropoxy-MKH6561 at the early stage than at the later one. No rule-relatedness can be indicated for winter wheat.

Due to the already mentioned high total ^{14}C contents, rising contents are found for all fractions examined with increasing soil moisture. As for the column experiments (Chapter 5.1.5), no statements can be made on the non-extractable fraction due to low values in the figure.

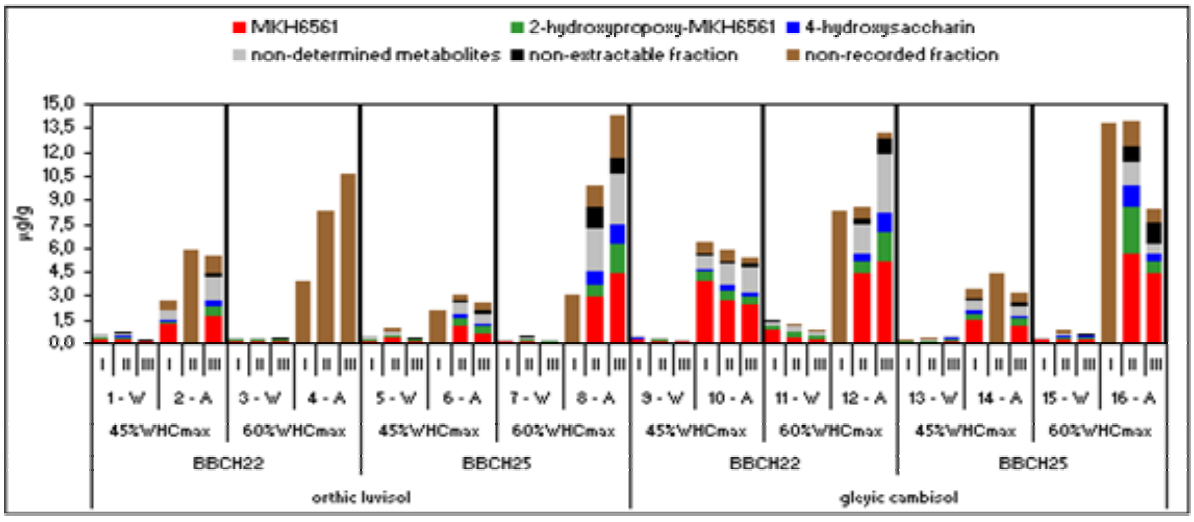
In a comparison of the sampling dates (I-III) in the majority of cases a decrease in active ingredient and an increase in metabolite concentration becomes apparent for both plant species. In individual cases, rising active ingredient concentration is also shown. The contents

of 4-hydroxysaccharin rise with prolonged time after application from the first to the third sampling date.

In considering the concentration in the plants on both soil types, higher active ingredient concentration is identified on gleyic cambisol than on orthic luvisol. This also applies to the metabolites investigated in the majority of cases.

The concentration of the individual fractions [µg/g] in the root material is discussed as follows (Figure 48). It should be noted that for some samples only the total ^{14}C contents were determined and no extraction carried out (cf. Chapter 6.1.3). Consequently, the non-recorded fraction (shown in brown) is, in some cases, the result of exhaustive combustion (ex.: 4 - A I-III). However, in other cases (ex.: 8 - A II-III), the non-recorded fraction refers to the "gap" between the total ^{14}C contents and identified residues.

Fig. 48: Concentration [µg/g] of parent substance and metabolites (2-hydroxypropoxy-MKH6561, 4-hydroxysaccharin and non-determined metabolites) and of the non-extractable and non-recorded fractions in roots



For the major part, the concentration of the individual fractions in the root material is comparable to those of the leaf material. Due to the higher total ^{14}C fractions, however, they are all on a higher level. Agreements relate to higher concentration of all fractions in blackgrass in comparison to winter wheat, higher concentration at the early application stage in contrast to the later one (with a few exceptions for ALOMY at 60%WHC_{max}) and to rising concentration of all fractions examined with increasing soil moisture (at least for ALOMY). Comparable to the leaf material is also the decrease in active ingredient and an increase in the metabolite fraction from sampling dates I-III (in the majority of cases) and higher active

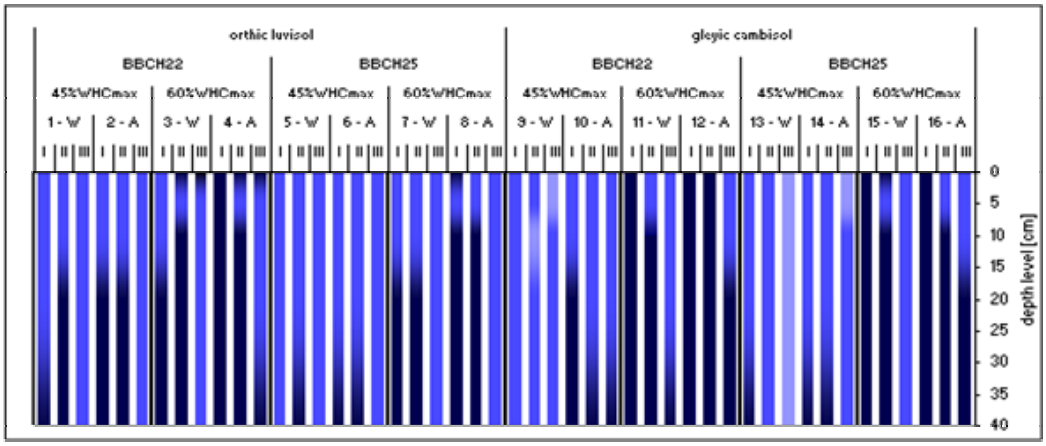
ingredient and metabolite concentration in plants on gleyic cambisol in comparison to orthic luvisol.

6.2 Soil analyses

6.2.1 Soil moisture content

The soil moisture in the lysimeter experiments is shown in Figure 49 in %WHC_{max} and in A XV plus the actual soil moisture at the four depth levels examined. For representation purposes, the soil moistures are divided into three stages (30-40, 40-50 and 50-60% WHC_{max}).

Fig. 49: Soil moisture (%WHC_{max}) of the depth levels investigated



Legend for Fig. 49: 30 - 40 (%WHC_{max}) | 40 - 50 (%WHC_{max}) | 50 - 60 (%WHC_{max})

It should be noted that for both soil types the different soil moistures (45 and 60%WHC_{max}) were maintained by spray irrigation events. On the one hand, the soil moisture was increased by spray irrigation in the lysimeters with 45%WHC_{max}. On the other hand, the soil moisture in the lysimeters with 60%WHC_{max} could only be slightly increased and water not retained by the soil matrix against gravity flew off the lysimeter outlets as percolate (see Figure 56).

Gleyic cambisol is drier at the respective sampling dates than orthic luvisol, since the sandy soil becomes dry more rapidly and the plants can extract more water from the soil.

In considering the depth profile, the soil moisture in the upper layers investigated (0-5 cm and 5-10 cm) is mostly less pronounced in comparison to the bottom layers (10-20 cm and 20-40 cm).

In comparing the two plant species, a slightly reduced soil moisture was found for wheat, since wheat penetrates the soil more intensively and deeply (rooting) and also transpires more due to the greater leaf mass.

Moreover, in most cases a decrease in soil moisture is identified from sampling date I to III, since in the further course of the experiments no water was added and water was increasingly extracted from the soil due to evaporation and uptake into the growing plants.

6.2.2 Total ^{14}C radioactivity of the soil material

The distribution of the total ^{14}C radioactivity within the soil profile is shown in Figures 50a & b in percent of the applied ^{14}C radioactivity and in Figure 51 in µg/g. The contents are shown in tabular form in A XVI.

Fig. 50a: Distribution of total ^{14}C radioactivity in percentage per soil layer
applied radioactivity = 100%

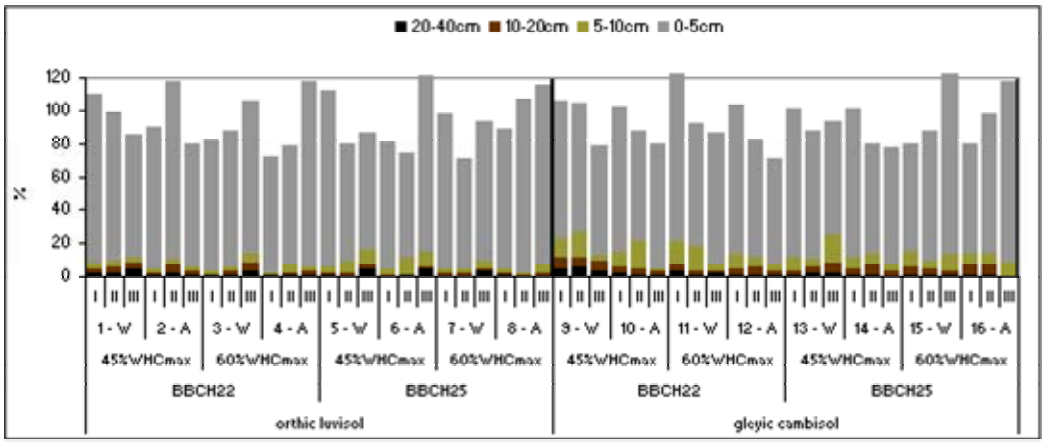
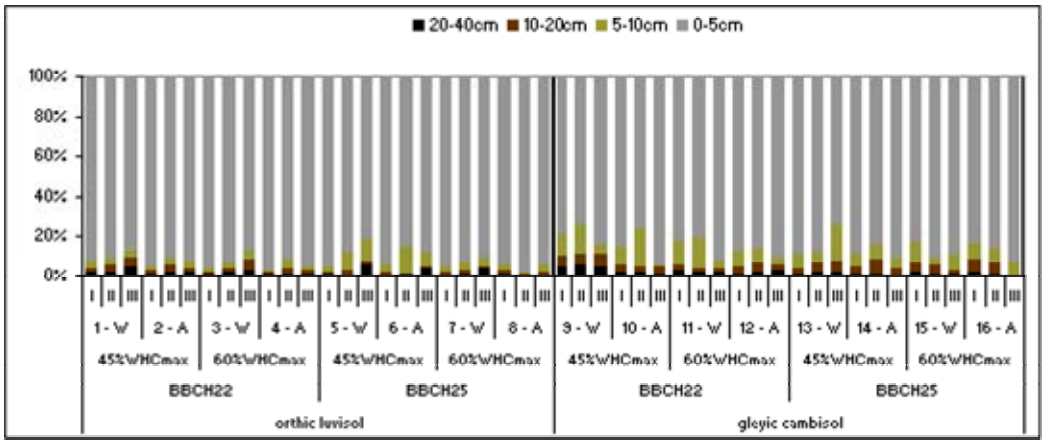


Fig. 50b: Distribution of total ^{14}C radioactivity in percentage (extrapolated to 100%) per soil layer
applied radioactivity = 100%



As can be seen from Figures 50a & b, the highest total ^{14}C radioactivity contents are present in the 0-5 cm layer; the percentage contents of the bottom layers are much lower. Translocation takes place corresponding to the column experiments by mass flow, diffusion and macropore flow; these "natural" processes were already illustrated in Chapter 5.2.2. It should be noted that the density was gravimetrically determined for each soil layer

(lysimeter/sampling date). However, even slight differences in soil density and weighing errors have a strong influence on the total ^{14}C contents per layer, particularly in the case of low ^{14}C contents in the subsoil. The variations in soil density within the profile may be attributed to recompaction of the soil, slightly inhomogeneous surfaces (elevated and lowered regions) and resultant layers not dug up exact to the millimetre.

In comparing the application dates and concerning the influence of the two different plant species on translocation processes, no difference becomes apparent with respect to ^{14}C radioactivity in the individual soil layers.

In considering the percentage contents in the subsoil of the two soil types, higher (translocated) contents are found for gleyic cambisol than for orthic luvisol. This distribution is due to higher mobile active ingredient fractions in gleyic cambisol. The influence of the soil type with respect to the mobility and active ingredient translocation in the soil will be illustrated in more detail in the following. It should be noted that comparable processes are found to have influence on active ingredient uptake (cf. Chapter 6.1.2.1). Reasons can also be found for differently mobile contents which, on the one hand, are taken up into the plant and, on the other hand, can be translocated in the soil.

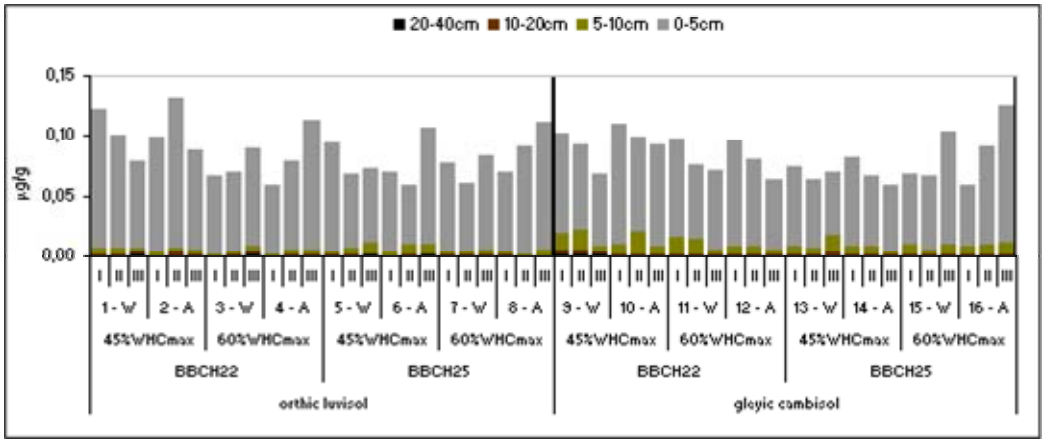
In order to obtain well-founded information concerning the sorptive properties of both soil types, an adsorption/desorption study was performed with MKH6561, which revealed low K_D values of the gleyic cambisol in comparison to the orthic luvisol (cf. Chapter 7.1). Thus, the substance is weakly sorbed to sandy soil, so that during subsequent dilution of the soil solution (e.g. caused by a rain event) the substance desorbs again and can be translocated with the percolate. Studies performed by *FENT et al. (1997)* have confirmed the differences in sorption behaviour (cf. Table 2).

In order to obtain information on the hydraulic conductivity and effective retardation (delay in material translocation), which besides the sorptive properties are decisively responsible for the translocation of substances in soil, these properties were calculated by evaluations of the hydraulic functions (cf. Chapter 7.3). Accordingly, gleyic cambisol is characterized by higher hydraulic conductivity and lower effective retardation. The higher sand and coarse pore fraction thus causes a much stronger translocation of the herbicide in the soil. Orthic luvisol, in contrast, has a lower hydraulic conductivity and a higher effective retardation. Moreover, in recompacting the silty-clayey soil material, the pores were presumably filled up more strongly, which additionally reduces a translocation in the soil (and also plant uptake).

These findings reliably explain the elevated translocation in gleyic cambisol.

Figure 51 excludes the different layer thicknesses by showing the active ingredient equivalents in µg/g. This representation shows that mainly an input into the depth level of 5-10 cm took place and hardly any active ingredient equivalents [µg/g] were determined at the depth levels 10-20 cm and 20-40 cm.

Fig. 51: Distribution of active ingredient equivalents [µg/g] per soil layer



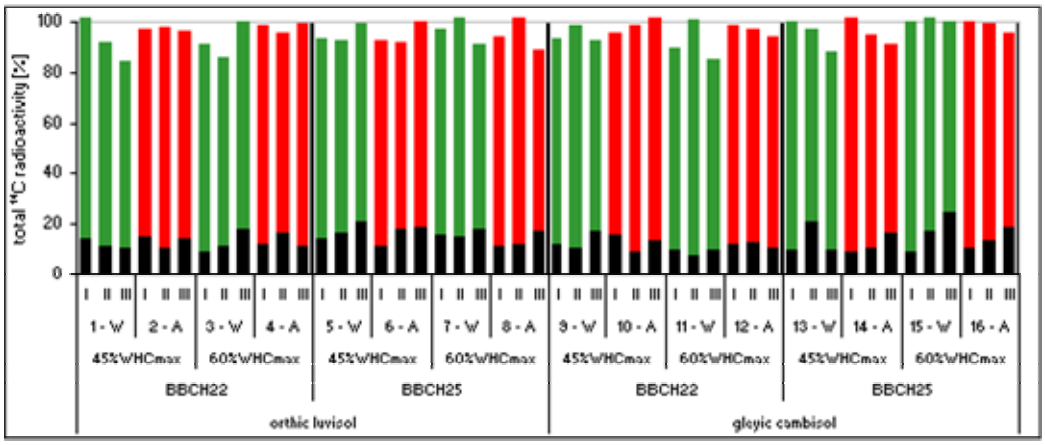
Differences concerning the influence of soil moisture (45 and 60%WHC_{max}) on herbicide translocation cannot be identified, although different hydraulic conductivity and effective retardation (Chapters 7.3.2.2 and 7.3.3.2) were determined for the different soil moistures. Since both soils are very moist, the soil moisture is completely sufficient for (maximum) translocation.

In comparing the radioactivity distribution at the different depth levels as a function of the individual sampling dates, it was found that an increase in total ^{14}C radioactivity takes place with depth in the course of time (from sampling date I to III) in orthic luvisol (lysimeters 1-8). For gleyic cambisol, in contrast, constant active ingredient equivalents were determined in most cases. This suggests that in gleyic cambisol the translocatable contents (active ingredient/metabolites) have already been translocated at the first sampling date via macropores. In orthic luvisol, the translocation of the maximum possible contents took place much more slowly due to a higher fraction of medium and fine pores and good sorptive properties. It can be furthermore stated that the contents in the bottom soil layers examined are lower in comparison to the column experiments (orthic luvisol). This confirms the thesis set up in Chapter 5.2.2 that comparatively high contents in the subsoil (at the second sampling date) were caused to a great extent by the sampling technique (carry-over, dumping of topsoil material with adsorbed herbicide) applied in the column experiments and prevented in the lysimeter experiments.

6.2.3 Desorption of soil material

The percentage distribution of the desorbable fraction and of the non-desorbable residues of the active ingredient and its metabolites in the topsoil (0-5 cm) is shown in Figure 52.

Fig. 52: Distribution of desorbable fractions and non-desorbable residues in percentage in topsoil (0-5 cm)
total ^{14}C radioactivity = 100%



Legend for Fig. 52:

Desorbable fraction		Non-desorbable fraction
Spring wheat (W)	Blackgrass (A)	Residues
I/II/III = Sampling date 1/2/3		

With respect to high desorbable (potentially plant-available) percentage contents of total ^{14}C activity (75-90%) and the lower residue fractions (10-20%) the findings in Chapter 5.2.3 (column experiments) are confirmed and interpreted accordingly.

The desorbable fractions in the subsoil are not dealt with in this chapter. As in the topsoil, no influence of the experimental parameters on the desorbable and non-desorbable fractions can be observed, so that no new findings would be derived from a representation of results. However, the data collected are included in the representation of the contents of each individual fraction examined [µg/g] in Chapter 6.2.5.

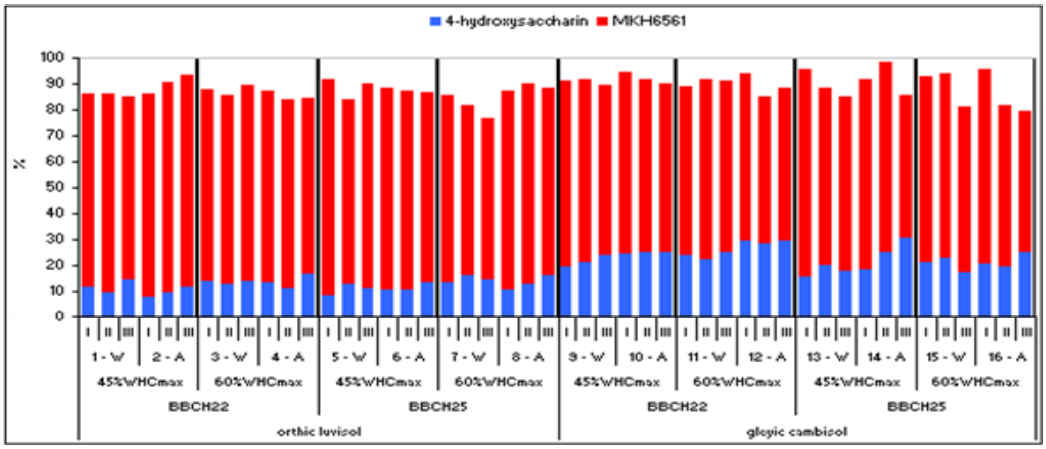
6.2.4 Active ingredient and metabolite distribution in soil material

6.2.4.1 Metabolite spectrum

The metabolite spectrum in the desorption solutions of the soil material was examined by TLC and HPLC methods. Exemplary chromatograms, presented with the data material of the column experiments, can be found in Chapters 3.10.4.1 (TLC) and 3.10.4.2 (HPLC).

In the lysimeter experiments, the percentage distribution of active ingredient (shown in red) and 4-hydroxysaccharin (blue) in the desorption solutions of the topsoil (0-5 cm) is shown in Figure 53. The values were determined by evaluating HPLC chromatograms.

Fig. 53: Distribution of MKH6561 and 4-hydroxysaccharin in percentage in topsoil (0-5 cm)
desorbed radioactivity = 100%
determined by HPLC methods (chromatogram evaluation)



As can be seen from the figure, the active ingredient fraction accounts for the largest percentage fraction in the desorption solutions of the topsoil material; the fraction of the major soil metabolite is lower.

Striking to note is the rapidly beginning metabolization of the active ingredient as well as differences between the two soil types: in orthic luvisol, approx. 10% of the radioactivity is present as 4-hydroxysaccharin in the desorption solutions at the first sampling date; it amounts to approx. 20% in gleyic cambisol.

Metabolization processes take place faster and more intensively in gleyic cambisol than in orthic luvisol. Reasons may be the better aeration of gleyic cambisol with otherwise similar soil-chemical parameters (pH value, C_{org}, etc.). The clay- and silt-containing orthic luvisol, in contrast, possibly has "anaerobic zones" at relatively high soil moistures throughout the experiments. Since active ingredient degradation to 4-hydroxysaccharin decisively proceeds via microbial degradation processes and hydrolysis only plays a minor role (*BRUMHARD et al. 1999b*), this is reflected in low 4-hydroxysaccharin contents. Moreover, 4-hydroxy-saccharin is only produced by oxidation from its "precursor substance".

The experimental variables of plant species, soil moisture and application stage only seem to play a minor role with respect to metabolization.

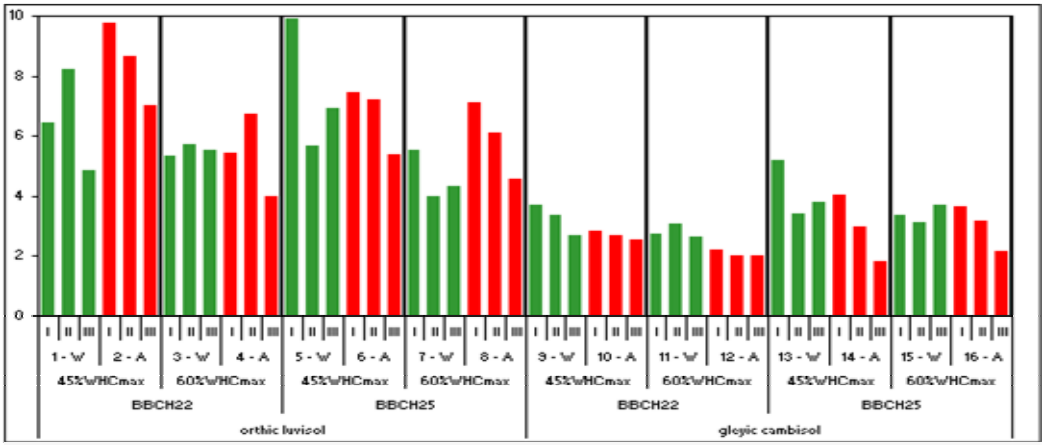
In a comparison of the sampling dates (I-III) in most cases an increase in the percentage contents of 4-hydroxysaccharin can be observed with progressing sampling date. A stronger increase is found if the period from application up to first sampling date is referred to. Upon soil contact of the active ingredient, a rapid degradation by microorganisms seems to set in, which is slowed down in the course of time.

A XVII shows in tabular form the percentage distribution of active ingredient and 4-hydroxy-saccharin for all experimental parameters and soil depths. In the majority of cases, an increase in the percentage contents of the major soil metabolite with increasing soil depth is to be observed. Possible explanations for the translocation of 4-hydroxysaccharin (but also of parent substance) were given in connection with the evaluation of the column experiments and will not be addressed here (Chapter 5.2.4.2). Furthermore, it should be borne in mind that above all at the bottom depth level investigated (20-40 cm) contents near the detection limit are frequently encountered, so that the informative value of the percentage contents in the subsoil region is low.

6.2.4.2 Active ingredient degradation in the soil: quotient formation of active ingredient and major soil metabolite

Quotients of active ingredient and 4-hydroxysaccharin in the desorption solutions were formed for the depth level of 0-5 cm (Figure 54). HPLC chromatograms, $HPLC_{cuts}$ and TLC1 & 2 were compared in Chapter 4.2.

Fig. 54: Quotient of MKH6561 and 4-hydroxysaccharin in topsoil (0-5 cm) determined by HPLC methods (chromatogram evaluation)



Legend for Fig. 54:

Winter wheat (W)	Blackgrass (A)
I/II/III = Sampling date 1/2/3	

As already mentioned in the column experiments, low quotients indicate an elevated percentage predominance of 4-hydroxysaccharin, comparatively faster active ingredient degradation and metabolite accumulation.

In a comparison of the two soil types, the quotients in the topsoil of orthic luvisol are higher than in gleyic cambisol and decrease in their majority with time (from the first to the third sampling date).

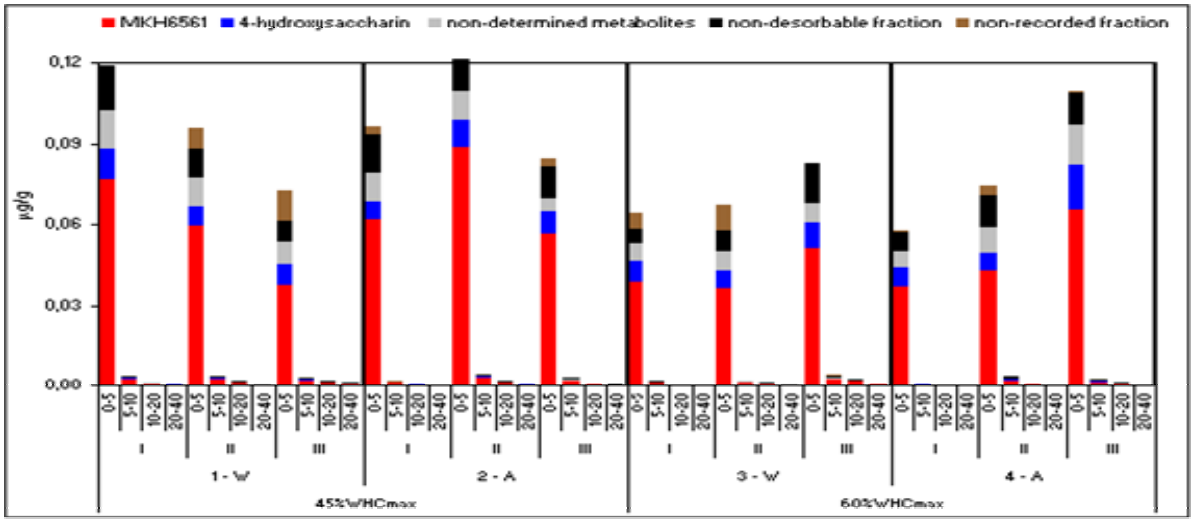
The influence of soil moisture is also apparent for both soil types: the quotients are higher at 45%WHC_{max} than at 60%WHC_{max}. *LYNCH (1995)* gives a range of 40-50%WHC_{max} for good microbial activity in soil and rapid degradation occurs under these optimum moisture conditions. This finding is in contrast to the quotients determined for the column experiments (Chapter 5.2.4), where the highest quotients were found at 60%WHC_{max}. One reason may be that water was logged in the column experiments (longer "anaerobic phases"), whereas in the lysimeter experiments the percolate could flow out from the bottom of the lysimeter so as to always ensure aerobic conditions. However, this cannot be finally clarified. Another explanation is the "overinterpretation" of the quotients formed. In considering the metabolite spectrum, no differences in the contents of 4-hydroxysaccharin are found for either the column or the lysimeter experiments as a function of soil moisture, so that different active ingredient contents are present in a comparison of the two experiments. Minor differences of MKH6561 already provide trends in one or the other direction for quotient formation. The very similar values should thus not be overinterpreted.

Quotients for the entire subsoil are not specified, since the topsoil is more important for interpretation. However, in the majority of cases, a decrease of the quotients with increasing soil depths has been demonstrated, a trend already indicated by the rising percentage fractions of 4-hydroxysaccharin with depth (cf. A XVII). In other cases an increase of quotients has been observed in the subsoil at the second sampling date due to translocation of parent substance and low degradation.

6.2.5 Determination of the concentration of the individual fractions in µg/g in soil

Figures 55a-d show in an overall approach the concentration [µg/g] of active ingredient (shown in red), 4-hydroxysaccharin (blue) and non-identified radioactivity (grey) in the desorption solutions as well as the non-desorbable (black) and the non-recorded contents (brown) at all soil depths examined (0-5 cm, 5-10 cm, 10-20 cm and 20-40 cm) (cf. also tabular representation in A XVIII).

Fig. 55a & b: Concentration [µg/g] of parent substance and metabolites (4-hydroxysaccharin and non-determined metabolites) and of the non-desorbable and non-recorded fractions in orthic luvisol
a: application stage BBCH22



b: application stage BBCH25

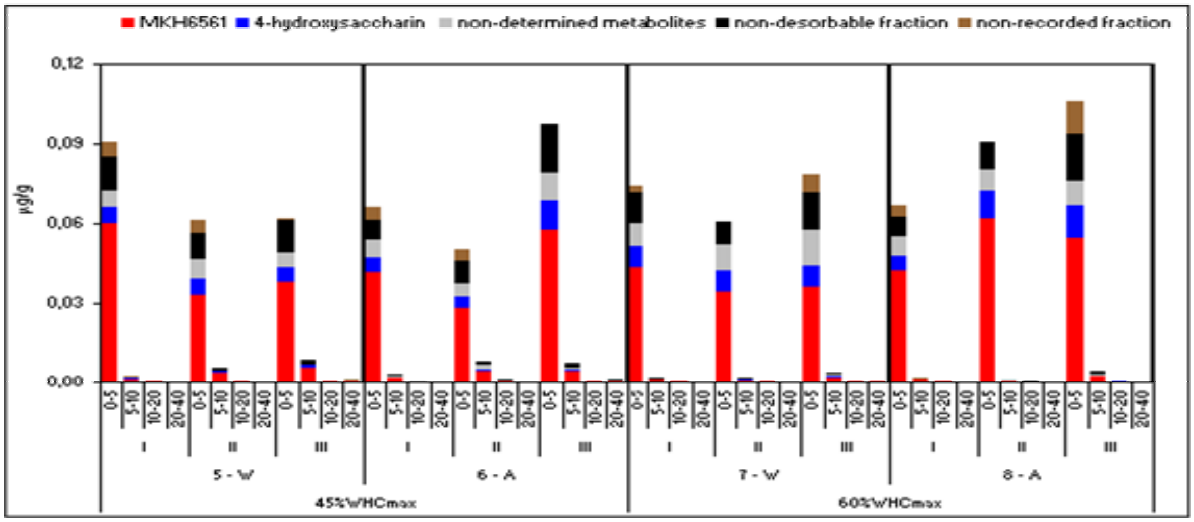
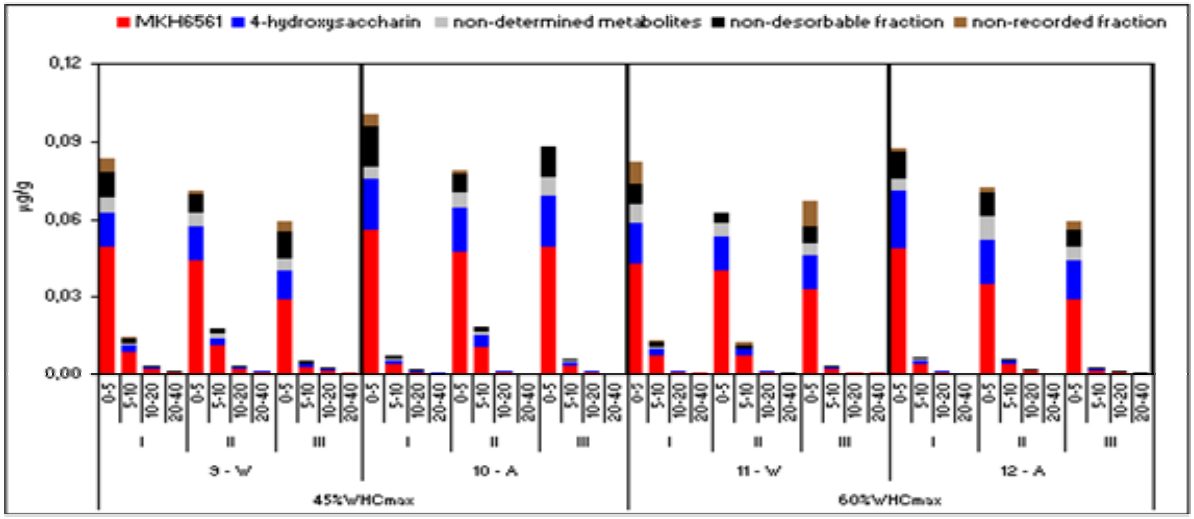
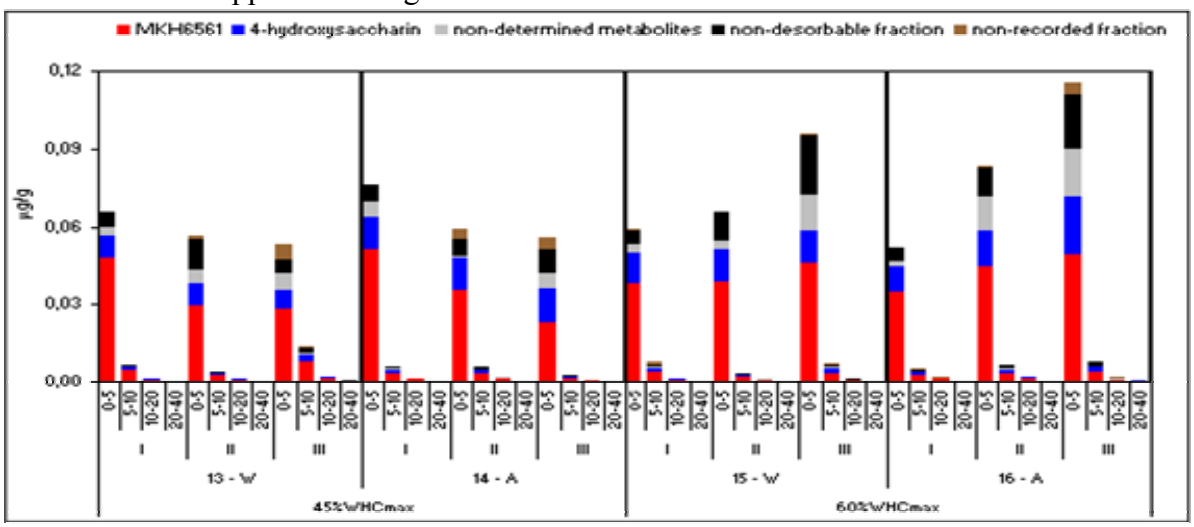


Fig. 55c & d: Concentration [µg/g] of parent substance and metabolites (4-hydroxysaccharin and non-determined metabolites) and of the non-desorbable and non-recorded fractions in gleyic cambisol
c: application stage BBCH22



d: application stage BBCH25



The concentration of all fractions is the highest both in orthic luvisol (Figures 55a & b) and in gleyic cambisol (Figures 55c & d) at the depth level 0-5 cm. The unchanged active ingredient is the dominant fraction. Differences between the two soil types are found in considering the metabolite fraction and the non-desorbable fraction: In the case of gleyic cambisol, the concentration of 4-hydroxysaccharin is higher due to intensive active ingredient degradation, whereas the non-desorbable fraction is mostly more pronounced in orthic luvisol.

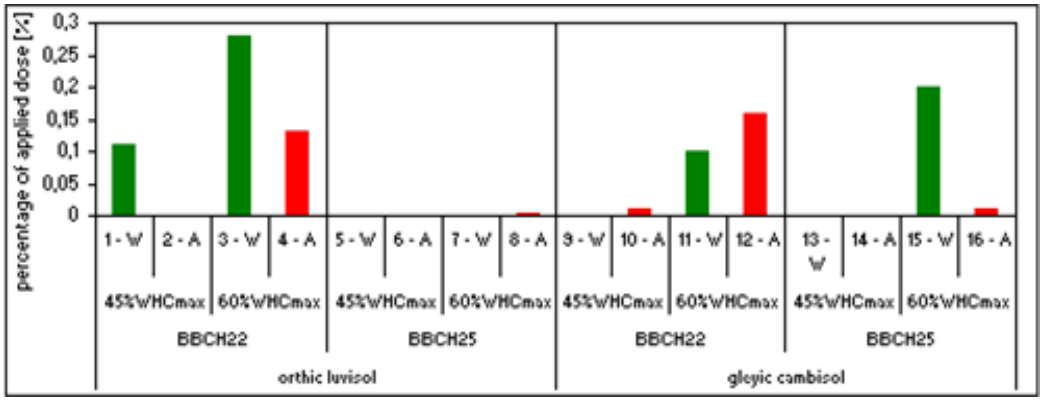
Below a soil depth of 5 cm different concentration is found for both soil types. For gleyic cambisol considerably higher concentration of all fractions examined and of the total contents were measured than for orthic luvisol in the subsoil and thus a higher active ingredient translocation (above all into the depth level of 5-10 cm) determined. With increasing depth in the subsoil the total contents and the contents of the examined fractions (active ingredient and

metabolites) steadily further decrease and cannot be interpreted any more in the bottom soil layer (20-40 cm). For both soil types the percentage distributions of the examined fractions in the subsoil have changed (higher percentage fraction of the metabolite fraction, but active ingredient still predominant; cf. A XVII). However, this increase is of hardly any relevance due to low total contents.

6.3 Investigation of the percolate

This chapter summarizes the results on quantification of ^{14}C labelled compounds in the percolate throughout the experimental period. Main focus was laid on balancing the contents that are extracted from the soil-plant system by percolate runoff (cf. Figure 56).

Fig. 56: ^{14}C contents of the percolate in percentage (lysimeter experiments)
applied radioactivity = 100%



In the percolate and thus presumably also in deeper soil layers ^{14}C radioactivity is already identified in small quantities two days after the first spray irrigation event. In this chapter, however, only those ^{14}C contents of the total volumes in the percolate will be discussed which have percolated through the soil body and have run off from the outlets of the lysimeters. In a comparison with the requirements to be met by pesticides in registration-relevant studies (cf. *LYNCH 1995*) the values in the percolate are below 5% measured against the total ^{14}C contents applied (here up to 0.3% of the applied radioactivity). No characterization of the constituents (active ingredient and metabolites) was performed.

Except for the soil moisture and the soil type, all the other experimental parameters do not seem to exert any influence on the total radioactivity contents in the percolate. Comparatively high contents are reached in the percolate (e.g. lysimeters 3 and 4 (orthic luvisol) and 11 and 12 (gleyic cambisol)) at high soil moisture (60%WHC_{max}). The relatively low concentration in the soil material of the subsoil layers (cf. Chapter 6.2.2) suggest that the herbicide

translocation in orthic luvisol has in part been caused by macropore flow and is thus locally limited to macropores. In gleyic cambisol, a slightly higher percolate discharge can be observed due to higher hydraulic conductivity and higher ^{14}C contents in the subsoil region or due to a further advancing of the translocation front, indicating that translocation by macropore flow is not the only process to be taken into consinderation. The processes of translocation and the factors of influence were described in detail in the corresponding soil chapters (cf. Chapter 6.2.2).

7. Ad-/Desorption Characteristics and Hydraulic Properties

As already explained in Chapter 3.11, the results of the studies performed in addition to the column and lysimeter experiments will be presented in the following.

7.1 Adsorption/desorption study

The results of the adsorption/desorption study will be discussed in this chapter. More precise information about the methodological procedure can be found in 3.11.1 and the following chapters.

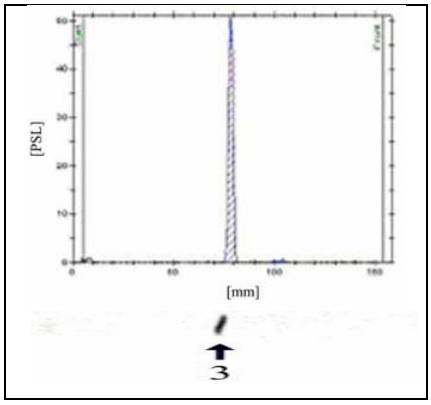
7.1.1 Preliminary experiments

An evaluation of the results of the preliminary experiments necessary for the adsorption/desorption study is given below:

1) Verification of the radiochemical purity of the active ingredient

The radiochemical purity was determined to be 99.58% by thin-layer chromatography (Figure 57).

Fig. 57: Verifying the radiochemical purity of the stock solution*



Legend for Fig. 57:

* stock solution (active ingredient concentration 1.43 g/L) mobile phase system A (TLC1)

3 = propoxycarbazone-sodium

No degradation of the active ingredient has thus been found in the stock solution (storage at -20 °C).

2) Stability of the active ingredient in aqueous solution

The stability check based on hydrolytic degradation studies (30 d) at < 25 °C, performed by Bayer CropScience; the active ingredient fraction in aqueous solution may be regarded as stable *(BRUMHARD et al. 1999b)*; i.e. the fraction of unchanged initial active ingredient was > 90%. For this reason, no further stability check was performed.

3) Soil/solution ratio

The results show an increase of the very low adsorbed percentage contents at rising soil/solution ratio. The calculated contents (indirect determination by measuring the ^{14}C contents in the desorption solutions) were in good agreement with the ^{14}C contents in the desorption solutions determined by combusting. Orthic luvisol displayed higher adsorbed contents and thus a higher affinity for MKH6561 than gleyic cambisol (cf. Table 22).

Tab. 22: Adsorption of MKH6561 as a function of soil/solution ratio
active ingredient concentration 0.0001 g/L
applied radioactivity = 100%
calculated by means of the ^{14}C contents in the desorption solutions by double determination

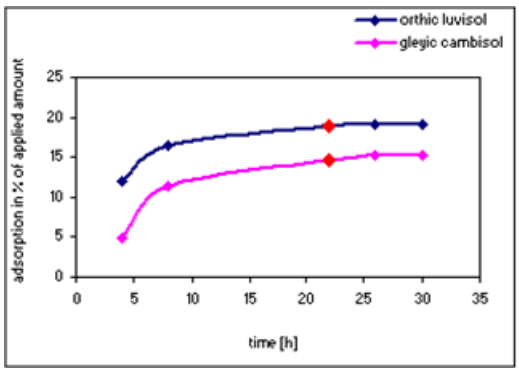
Soil	Soil/solution ratio		
	1:2	1:5	1:20
	adsorbed active ingredient fraction [%]		
Orthic luvisol	28.52 (s = 0.14)	15.56 (s = 0.47)	7.01 (s = 0.34)
Gleyic cambisol	19.31 (s = 0.34)	10.50 (s = 0.11)	5.08 (s = 0.37)

The required conditions (cf. Chapter 3.11.1.2) were approximately fulfilled with a soil/solution ratio of 1:5. The same conditions were chosen for both soil types to ensure comparability.

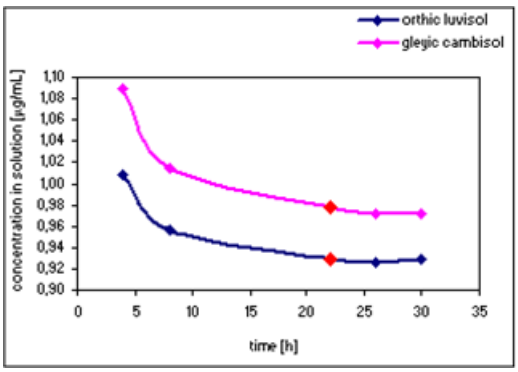
4) Time to establish equilibrium

The results of this preliminary experiment are summarized in Figures 58a & b, which show the increase in adsorption (Figure 58a) and the decrease in active ingredient concentration in solution (Figure 58b) as a function of time:

Fig. 58a & b: Time to establish equilibrium for orthic luvisol and gleyic cambisol
equilibrium marked in red



a) adsorption increase



b) decrease in active ingredient concentration

Irrespective of the soil type, equilibrium was established at the latest after t = 22 h (study period 30 h), i.e. in the following hours the equilibrium concentration did not change by more than 5%. The quantitative adsorption processes are almost completed at this time upon

reaching a plateau (equilibrium). Thus, a shaking duration of 22 h was chosen for the adsorption/desorption experiments with MKH6561.

At the end of the experiment, 94.4% of the radioactivity in the desorption solutions of orthic luvisol and 93.7% of the radioactivity in the desorption solutions of gleyic cambisol were identified as MKH6561, so that no significant degradation of the compound was detected.

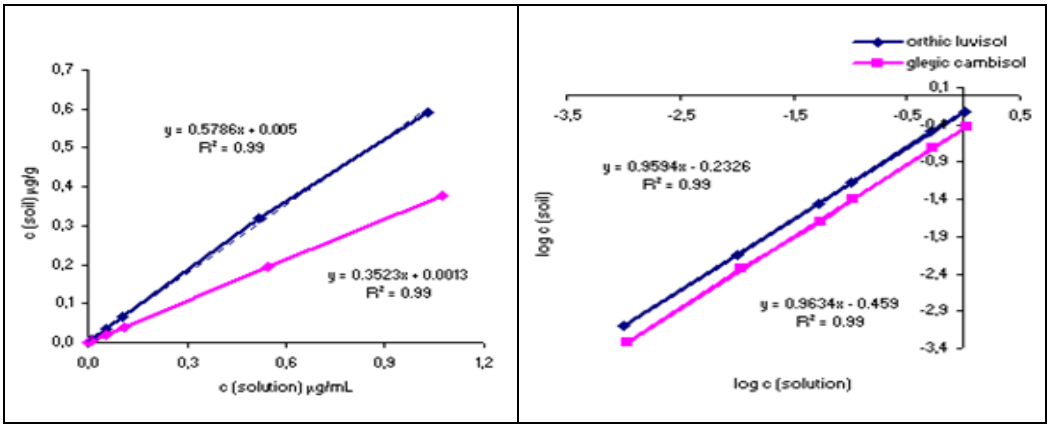
7.1.2 Results of the adsorption/desorption study

7.1.2.1 Adsorption measurements

The adsorption behaviour of MKH6561 can be described in the chosen concentration range examined (0.001-1 g/L) for both soil types (orthic luvisol and gleyic cambisol) using the *FREUNDLICH* equation (Equation [3]).

The six different concentrations in solution and the calculated soil concentrations were plotted against each other for both soil types and slight deviation from linearity was identified (cf. Figure 59).

Fig. 59: Adsorption isotherms* for MKH6561 in orthic luvisol and gleyic cambisol



* scale of the figure on the right is logarithmic

In order to describe the adsorption conditions by means of the *FREUNDLICH* equation (linear form, Equation [4]) the following parameters were derived from the logarithmic plot (Table 23):

Tab. 23: Parameters of the *FREUNDLICH* adsorption equation for orthic luvisol and gleyic cambisol

	orthic luvisol	gleyic cambisol
K_F $[\mu g^{1-1/n} * mL^{1/n} * g^{-1}]^*$	0.59	0.35
1/n $[-]^*$	0.96	0.96
R^2	0.99	0.99

* *FREUNDLICH* coefficients

The K_F value is a measure of the intensity of adsorption and is higher for orthic luvisol ($K_F = 0.59$) than for gleyic cambisol ($K_F = 0.35$).

Both *FREUNDLICH* isotherms are different in their slopes (identical in logarithmic plotting with a very good approximation ($R^2 > 0.99$) to the *FREUNDLICH* equation). The distance between the two straight lines shows differences in the sorption capacity of the two soils. For the same concentration in the solution [µg/mL] different contents are thus to be expected on the exchanger bodies (soil): Higher contents in orthic luvisol and lower contents in gleyic cambisol have been found. The different contents of MKH6561 may be explained by different soil types, i.e. the higher contents of orthic luvisol can be explained by the dominant silt and clay fraction with good sorptive properties due to larger mineral surfaces. Flat slopes indicate slow increase in adsorption with rising solution concentration; saturation phenomena do not occur, since no strong flattening of the isotherms was observed.

The percentage adsorption rates in comparison to the relative desorption rates (the desorption conditions are discussed in Chapter 7.1.2.2) for each individual concentration level are given as a measure of the levels of adsorption (Table 24).

Tab. 24: Ad-/desorption behaviour of MKH6561 in different concentrations and soil types

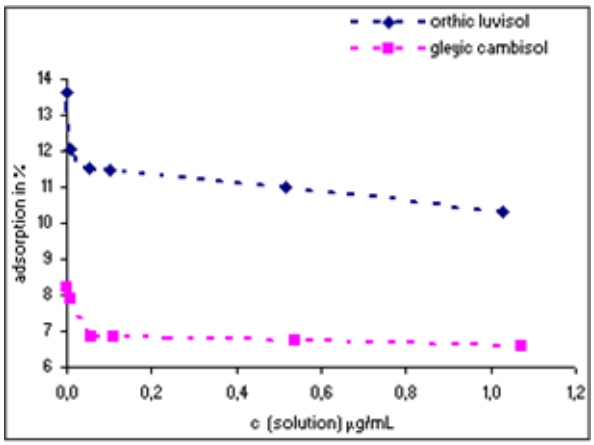
Soil type	concentration	adsorbed ^{14}C activity*	desorbed ^{14}C activity** (desorption cycle 1-5)						^{14}C activity in soil residue***
	[g/L]	[%]	1 [%]	2 [%]	3 [%]	4 [%]	5 [%]	total [%]	[%]
Orthic luvisol	**1**	**10.33**	**85.93**	**11.56**	**1.12**	**0.53**	**0.29**	**99.44**	**0.19**
	[s]	0.00	0.01	0.00	0.00	0.01	0.00	0.02	0.00
	0.5	**11.02**	**85.08**	**11.50**	**1.56**	**0.51**	**0.27**	**98.92**	**0.15**
	[s]	0.53	4.00	0.51	0.07	0.02	0.01	4.62	0.04
	0.1	**11.46**	**83.74**	**10.14**	**1.94**	**0.55**	**0.34**	**96.71**	**0.19**
	[s]	0.02	0.17	0.02	0.00	0.12	0.02	0.33	0.00
	0.05	**11.54**	**82.72**	**10.85**	**2.51**	**0.66**	**0.33**	**97.07**	**0.26**
	[s]	0.05	0.00	0.01	0.07	0.09	0.02	0.18	0.01
	0.01	**12.05**	**82.10**	**11.50**	**2.52**	**0.61**	**0.27**	**97.00**	**0.30**
	[s]	0.84	3.90	0.96	0.17	0.01	0.02	5.06	0.18
	0.001	**13.63**	**78.70**	**11.93**	**3.41**	**0.49**	**0.30**	**94.83**	**1.33**
	[s]	0.21	1.64	0.19	0.15	0.05	0.00	2.03	1.30
Gleyic cambisol	**1**	**6.57**	**90.31**	**8.17**	**0.82**	**0.40**	**0.18**	**99.89**	**0.10**
	[s]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
	0.5	**6.75**	**90.76**	**7.67**	**0.78**	**0.42**	**0.21**	**99.84**	**0.10**
	[s]	0.03	0.00	0.06	0.01	0.01	0.00	0.08	0.00
	0.1	**6.86**	**90.78**	**7.45**	**0.76**	**0.66**	**0.34**	**99.99**	**0.12**
	[s]	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02
	0.05	**6.84**	**91.16**	**6.93**	**0.72**	**0.39**	**0.22**	**99.41**	**0.15**
	[s]	0.03	0.00	1.79	0.00	0.03	0.01	1.84	0.04
	0.01	**7.89**	**89.11**	**8.10**	**1.49**	**0.53**	**0.24**	**99.46**	**0.17**
	[s]	0.00	0.00	0.03	0.01	0.00	0.00	0.05	0.04
	0.001	**8.19**	**84.55**	**7.71**	**5.80**	**0.58**	**0.28**	**98.92**	**0.20**
	[s]	0.00	0.00	0.03	0.01	0.05	0.00	0.09	0.24

* applied radioactivity = 100%
** adsorbed radioactivity = 100%
*** determined by combusting

Table 24 shows low adsorbed fractions, which are lower in gleyic cambisol (6.57-8.19%) than in orthic luvisol (10.33-13.63%). An increase in adsorption is generally observed from the highest to the lowest concentration.

The correlation between the slope of the regression straight lines (Figure 59) and relative adsorption can be well established: since the slope is in all cases smaller than 1 ($1/n < 1$), the adsorption rates [%] decrease with rising concentration in the soil solution (Figure 60).

Fig. 60: Adsorbed MKH6561 as a function of concentration in the soil solution



The distribution coefficients determined for each concentration level of adsorption (K_D and K_{OC} values) were averaged for the entire concentration range (six concentration levels) and are shown in Table 25 for the two soils. These values are used to determine the effective retardation (cf. Chapter 7.3.3) and enable a comparison with values determined by Bayer CropScience.

Tab. 25: Distribution coefficients of MKH6561 in both experimental soils
concentration range 0.001-1 g/L; average from six concentrations

Soil type	adsorption	
	K_D (mL/g)	K_{OC} (mL/g)
Orthic luvisol	0.67 (s = 0.07)	62.99 (s = 6.90)
Gleyic cambisol	0.38 (s = 0.04)	40.18 (s = 4.17)

The results are comparable to the values determined by Bayer CropScience [K_D values between 0.22 mL/g (loamy sand) and 1.71 mL/g (silty-loamy clay); cf. Chapter 2.2.3.3]. The substance can therefore be regarded as weakly sorbed and after *MCCALL et al. (1980)* as highly mobile.

Comparatively lower K_D values in gleyic cambisol are in comparison with orthic luvisol coupled with a lower adsorption capacity and affinity of the soil for MKH6561.

7.1.2.2 Desorption measurements

The extent to which MKH6561 can be desorbed was determined in five successive desorption cycles.

Whereas a relative decrease in adsorption was recorded with increasing herbicide concentration (Table 24 and Figure 60), no similar correlation was observed for the desorption rates measured as a function of radioactivity adsorbed before.

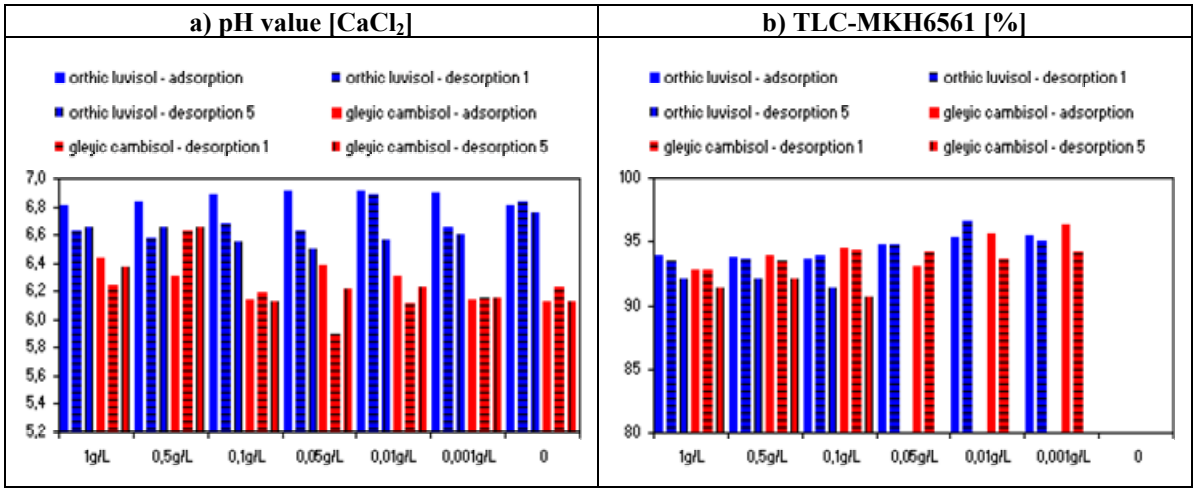
It can be taken from Table 24 that the desorbed fraction clearly decreased with increasing number of desorption steps. Fractions of 85.93-78.70% are directly desorbed during the first desorption step in orthic luvisol. Corresponding studies in gleyic cambisol revealed fractions of 90.31-84.55% to be desorbed. The contents are very low in the other desorption steps and reached values below 0.5% of the absolute desorbable amount in the fifth step.

The very low ^{14}C residues determined by combusting for control purposes reached a level of 0.1-1.33% of the radioactivity applied. An almost complete reversibility of the adsorption of MKH6561 has thus been confirmed. Adsorbed MKH6561 can be completely dissolved in the soil solution by the exchanger bodies with a dilute salt solution. Due to the in total low adsorbed and highly redesorbable fractions, the desorption isotherms for MKH6561 in orthic luvisol and gleyic cambisol were not represented. It should be noted in this connection that according to *LYNCH (1995)* a desorption study is not necessary within the framework of registration-relevant studies, if the adsorption is < 25% of the applied substance.

7.1.2.3. pH values and active ingredient purity

The pH values and percentage fractions of unchanged active ingredient in the adsorption and desorption solutions of the two soil types are shown in Figure 61a & b.

Fig. 61a & b: pH values and active ingredient purity during the adsorption/desorption study



For all six different active ingredient concentrations the pH value and the percentage content of MKH6561 were determined after the adsorption step and after the first and fifth desorption step.

The pH values undergo changes during the adsorption/desorption study. In orthic luvisol, decreasing values are found for the concentration range investigated during adsorption and in most cases decreasing values from the first to the fifth desorption step. It is assumed that more and more acid cations are dissolved from the exchanger sites and brought into solution in the course of time, which reduces the pH value of the solutions. In gleyic cambisol, this trend is not observable for each concentration. In a comparison of the pH values of the two experimental soils, the values of orthic luvisol are much higher than those of gleyic cambisol, as was already shown in Chapter 3.3.2. It should be furthermore noted that the pH values have never dropped below the pK_a value of 2.1, so that the dissociated form of the active ingredient is always present. Thus, in no case do the adsorption/desorption conditions change during the experiment due to a change from the dissociated to the undissociated form.

As expected, no appreciable decrease in active ingredient purity compared to the initial situation (stock solution) was observed in any of the equilibrium solutions after the adsorption step. However, a slight decrease is observable for both soil types and the MKH6561 percentage contents are still above 90% in the 5^{th} desorption step.

Since only very little ^{14}C radioactivity can in part be identified in the last desorption step, no statement can be made here even after rotating for some of the samples (concentration range < 0.05 g/L) concerning their MKH6561 percentage distribution.

7.2 pH value and organic carbon content (C_{org}) of the experimental soils

The reaction state of the Ap horizon of the gleyic cambisol can be designated as "medium-acid" and that of the orthic luvisol as "very weakly alkaline" *(BKA 1994)*. The measurement results (mean values from three replicates each) were already shown in Table 3 for the characterization of both soil types.

The C_{org} contents of the Ap horizons of both experimental soils are very similar and were presented in comparing selected soil characteristics in Table 3.

7.3 Hydraulic properties of the experimental soils

In the following, the evaluations of the hydraulic functions revealing water and solute transport in the soil will be described. All calculations were performed according to the equations presented in Chapter 3.11.3.

In order to describe the relation between water tension and water content, the water contents (θ) were plotted against the pressure heads (h) as retention functions (cf. Figures 62a & 63a). In order to describe the hydraulic conductivity, the decimal logarithm of K (cm * d^{-1}) was plotted against the decimal logarithm of h [cm] (cf. Figures 62b & 63b).

Since the soil moisture is given in %WHC_{max} in the present study, θ(h) (calculated from *VAN GENUCHTEN* function) had to be converted at a pF value of 0.6 (to be allocated to 100%WHC_{max}) to 20, 40 and 60%WHC_{max} (column experiments) as well as 45 and 60%WHC_{max} (lysimeter experiments). By rearranging the *VAN GENUCHTEN* function (Equation [7] in [9], cf. Chapter 3.11.3.3) it was possible to calculate h for given θ. The values determined for both soils at the different soil moistures are shown in Tables 26a & b.

Tab. 26a & b: Calculation of pressure head (h) and hydraulic conductivity (K(h)) from WHC_{max}

	a) column experiments					
	Fraction WHC_{max}	**θ** [cm^3cm^{-3}]	**h** [cm]	**log10 (h)** [cm]	**K(h)** [$cm*d^{-1}$]	**log10 K(h)** [cm]
Orthic luvisol	0.2	0.08	**2705.44**	**3.43**	**1.21E-05**	**-4.92**
	0.4	0.15	**386.87**	**2.59**	**3.29E-03**	**-2.48**
	0.6	0.23	**115.18**	**2.06**	**9.37E-02**	**-1.03**

	b) lysimeter experiments					
	Fraction WHC_{max}	**θ** [cm^3cm^{-3}]	**h** [cm]	**log10 (h)** [cm]	**K(h)** [$cm*d^{-1}$]	**log10 K(h)** [cm]
Orthic luvisol	0.45	0.17	**275.46**	**2.44**	**8.60E-03**	**-2.07**
	0.6	0.23	**115.18**	**2.06**	**9.37E-02**	**-1.03**
Gleyic cambisol	0.45	0.20	**153.23**	**2.19**	**1.60E+01**	**1.20**
	0.6	0.27	**95.33**	**1.98**	**9.02E+01**	**1.96**

The values shown in Tables 26a & b can be entered into the functions shown in Figures 62a & 63a in order to describe the relation between pressure head (h) and water content (θ); the values determined for describing the hydraulic conductivity were entered in Figures 62b & 63b.

Fig. 62a & b: Retention and hydraulic conductivity function after *MUALEM & VAN GENUCHTEN (VAN GENUCHTEN 1980)* for 20, 40 and 60%WHC_{max} (column experiments)

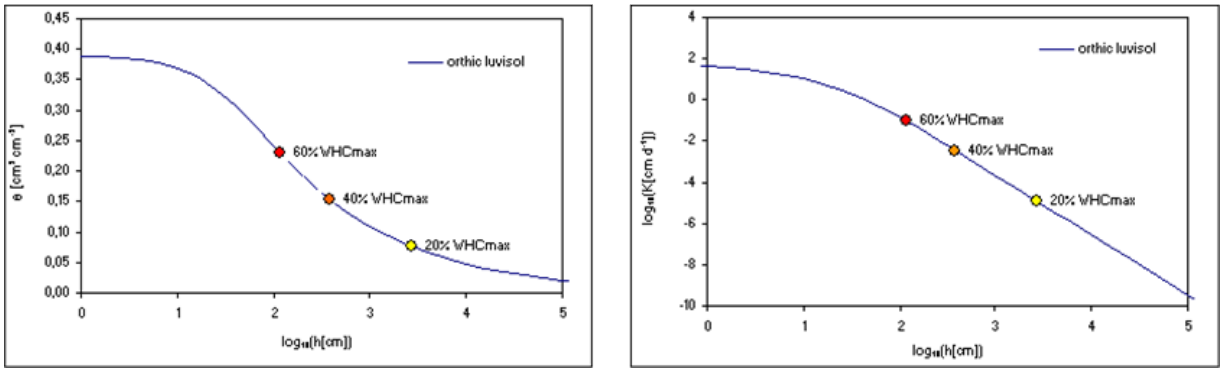
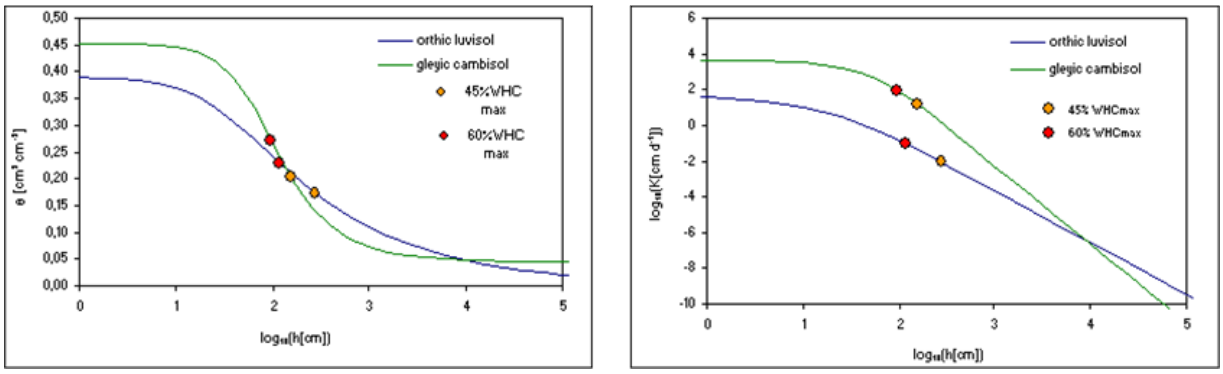


Fig. 63a & b: Retention and hydraulic conductivity function after *MUALEM & VAN GENUCHTEN (VAN GENUCHTEN 1980)* for 45 and 60%WHC_{max} (lysimeter experiments)



The values shown relate to the decimal logarithm of h and K(h) in the figures. The curves originate from a curve fit from experimentally determined retention points for drainage at different pressure heads (cf. Chapter 3.11.3).

7.3.1 Pressure head (h) and water content (θ)

The results described as follows show the correlation between pressure head in the soil and water uptake into the plant for both the column and the lysimeter experiments. The evaluation relates here to Tables 26a & b as well as to Figures 62a and 63a.

A low pressure head (h) in the soil results in a high water and substance uptake into the plant.

7.3.1.1 Column experiments

The figures show that plants on undisturbed orthic luvisol have to overcome higher pressures at 20%WHC_{max} (h = 2705.44) than at 40 and 60%WHC_{max} (h = 386.87 and 115.18, respectively); this leads to comparatively low active ingredient uptake into the plant. For the questions underlying the present study this means that rising active ingredient contents are

expected with rising soil moisture. As shown in Chapter 5.1.2, this increasingly also applies to blackgrass.

7.3.1.2 Lysimeter experiments

As can be seen from the figures, plants must overcome a higher matrix potential at lower water contents (45%WHC_{max}) than at higher water contents (60%WHC_{max}) on both orthic luvisol and gleyic cambisol. This results in lower plant uptake at 45%WHC_{max}. The plants must also overcome a higher tension for water uptake on orthic luvisol than on gleyic cambisol. This is also a reason for the higher effectiveness of the substance on gleyic cambisol. It should be furthermore noted that the differences in pressure head between the two soil types are higher at 45%WHC_{max} (orthic luvisol and gleyic cambisol: h = 275.46 and 153.23, respectively) than at 60%WHC_{max} (orthic luvisol and gleyic cambisol: h = 115.18 and 95.33, respectively). It may thus be stated that different uptake rates of active ingredient into the plant are to be expected for MKH6561 application on different soil types at different soil moistures. In particular, the highest active ingredient uptake is expected for gleyic cambisol in combination with the highest water content (60%WHC_{max}). This was confirmed in Chapter 6.1.2. More detailed interpretations concerning plant uptake can also be found there.

7.3.2 Hydraulic conductivity K(h)

The evaluation of the hydraulic conductivity K(h) [cm/d] in the soil types and for the soil moistures examined relates to Tables 26a & b as well as to Figures 62b & 63b.

With respect to active ingredient translocation and the ^{14}C contents at the individual depth levels examined, Chapter 5.2.2 (column experiments) and Chapter 6.2.2 (lysimeter experiments) provide detailed information.

7.3.2.1 Column experiments

As can be seen from the figures, the log10 K(h) values rise from -4.92 (20%WHC_{max}) through -2.48 (40%WHC_{max}) to -1.03 (60%WHC_{max}) in the column experiments with orthic luvisol; the hydraulic conductivity increases continuously. Thus, a higher translocation of the active ingredient is to be assumed for rising hydraulic conductivity and soil moisture. However, these expectations are not unambiguously confirmed within the framework of the present study (cf. Chapter 5.2.2): the values determined experimentally only show a slightly higher translocation at 60%WHC_{max} and no sufficiently great differences, as calculated. This may be

due to the fact that no higher translocation took place on account of the boundary conditions of the experiments and the active ingredient properties.

7.3.2.2 Lysimeter experiments

The figures show that a stronger translocation is to be assumed due to higher hydraulic conductivity (by several decades) for gleyic cambisol compared to orthic luvisol. However, a lower mass flow of the soil solution as in orthic luvisol also implies active ingredient quantities to be taken up into the plant more slowly and at lower content due to higher sorption capacities (cf. also Chapter 6.1.2). These statements refer to moist soils only. At increased drainage of the coarse pores which contribute primarily to solute transport, the hydraulic conductivity curve of the gleyic cambisol falls below the curve of the orthic luvisol (cf. Figure 63b). According to *SCHEFFER et al. (1992)* pF values > 4 can be expected in the field frequently.

In comparing the two soil moistures, log10 K(h) is higher at 60%WHC_{max} (orthic luvisol: 9.37 E-02; gleyic cambisol: 9.02 E+01) than at 45%WHC_{max} (orthic luvisol: 8.60 E-03; gleyic cambisol: 1.60 E+01). It is thus found that especially at high soil moisture a stronger translocation of the active ingredient is to be expected for both soils. In the present study, however, these expectations were not unambiguously met for the relevant questions (cf. Chapter 6.2.2). An essential reason may be that it was not possible to keep the moisture constant throughout the experimental period in the column and lysimeter experiments. In addition to that, soils with predominat secondary pores can only provide approximated results *(SCHEFFER et al. 1992)*.

7.3.3 Effective retardation (R_{eff})

Tables 27a & b show parameters to describe the retardation in substance translocation for the column and lysimeter experiments.

Tab. 27a & b: Parameters for describing the effective retardation of the column and lysimeter experiments

	a) column experiments				
	WHC_{max}	**θ** [cm^3cm^{-3}]	**ρ b** [g/cm^3]	**$K_{D\,1}$** [mL/g]	**$R_{eff\,1}$**
Orthic luvisol	0.20	0.08	1.57	0.67	**14.7**
	0.40	0.15	1.57	0.67	**7.9**
	0.60	0.23	1.57	0.67	**5.6**

	b) lysimeter experiments						
	WHC_{max}	**θ** [cm^3cm^{-3}]	**ρ b** [g/cm^3]	**$K_{D\,1}$** [mL/g]	**$R_{eff\,1}$**	**$K_{D\,2}$** [mL/g]	**$R_{eff\,2}$**
Orthic luvisol	0.45	0.17	1.57	0.67	**7.1**	*(0.38)*	***(4.5)***
	0.60	0.23	1.57	0.67	**5.6**	*(0.38)*	***(3.6)***
Gleyic cambisol	0.45	0.20	1.41	n.a.		0.38	**3.6**
	0.60	0.27	1.41			0.38	**3.0**

n.a. = not applicable

For the calculation of effective retardation the K_D values determined in Chapter 7.1 for the two soil types used were applied (orthic luvisol: K_{D1} value; gleyic cambisol: K_{D2} value).
The K_{D2} value is thus only of relevance for the lysimeter experiments. The K_D values were used in addition to the bulk density of the respective soil type to calculate the respective R_{eff} values (orthic luvisol: R_{eff1} and gleyic cambisol: R_{eff2}). For a comparison of the two soil types, in addition, the K_{D2} value was also used for orthic luvisol and served as an example to calculate the effective retardation of orthic luvisol and gleyic cambisol (R_{eff2}). In this way, the differences between the two soil types assuming identical adsorption/desorption behaviour were to be pointed out.

7.3.3.1 Column experiments

Since the column experiments were only carried out with orthic luvisol and only the K_{D1} value was used, the results are to be interpreted by means of the R_{eff1} values.
The effective retardation (R_{eff1}) decreases according to Table 27a with rising soil moisture (R_{eff1} at 20%WHC_{max} = 14.7, 40%WHC_{max} = 7.9 and 60%WHC_{max} = 5.6). This is indicative of strong retardation, as expected, which decreases, however, with increasing soil moisture. Elevated substance translocation is thus to be expected with increasing soil moisture.

7.3.3.2 Lysimeter experiments

In the lysimeter experiments, the effective retardation shown in Table 27b on the assumption of identical K_D values (R_{eff2}) is higher at 45%WHC_{max} than at 60%WHC_{max} and higher in orthic luvisol (45%WHC_{max} = 4.5; 60%WHC_{max} = 3.6) than in gleyic cambisol (45%WHC_{max} = 3.6; 60%WHC_{max} = 3.0).

Using the K_{D1} values determined for orthic luvisol makes the differences with respect to effective retardation (R_{eff1}) even more apparent (45%WHC_{max} = 7.1; 60%WHC_{max} = 5.6), so that orthic luvisol exhibits strongly retarded substance translocation in contrast to gleyic cambisol.

Since heaped soil is used in the lysimeter experiments, differences from undisturbed soil are to be expected with respect to bulk density. This has thus impacts on the calculation of effective retardation: for the calculations, the bulk densities of undisturbed soils were used, since the bulk densities determined during sampling were heterogeneous. For orthic luvisol, therefore, a higher soil density than for gleyic cambisol was included in the calculations (higher soil densities in clayey-silty orthic luvisol in comparison to the rather sandy gleyic cambisol were reliably identified). Even on the assumption of identical bulk densities for both soils, however, the effective retardation would still be higher in orthic luvisol than in gleyic cambisol.

8. Discussion

In this chapter, the results of the column and lysimeter experiments described in the preceding sections are summarized and discussed in relation to the key questions underlying this study. In addition to that, questions concerning design and performance of the experiments are discussed. It should be noted that the results of the column experiments in many cases agree with those of the lysimeter experiments; differences are pointed out separately.

First of all, the results of the plant analyses will be discussed. Concerning the question of whether differences in crop (wheat) and target grass (ALOMY) are found with respect to the uptake, distribution and concentration of MKH6561 in the plant and to the metabolization of the active ingredient, the following can be stated:

In introductory macroautoradiographic investigations, different distributions and concentrations of MKH6561 in the plant had already been identified, so that differences in the herbicidal effect are to be expected for both plant species. Macroautoradiographs of ALOMY have shown, in contrast to wheat, a more homogeneous distribution of the active ingredient in the plant. More intensive blackening on bioimager plates throughout the exposure of ALOMY indicates a good herbicidal effect and increased active ingredient contents in comparison to wheat.

These results were confirmed by the determination of the active ingredient equivalents (total MKH6561). In the plant material of blackgrass, significantly higher total MKH6561 contents of 0.16-1.11 µg/g (leaf) and 0.29-2.67 µg/g (root) (column experiments) and of 0.06-1.19 µg/g (leaf) and 0.25-1.74 µg/g (root) (lysimeter experiments) were measured due to the position of the main root horizon in the top 5 cm (highest herbicide concentration due to application onto the soil surface) compared to the deeper-rooting wheat. In the latter, total MKH6561 contents of 0.04-0.17 µg/g (leaf) and 0.06-0.18 µg/g (root) were determined in the column experiments and of 0.008-0.19 µg/g (leaf) and 0.02-0.16 µg/g (root) in the lysimeter experiments. This "position selectivity" was responsible for decisively higher uptake rates of the herbicide in the target grass. Wheat in most cases displayed lower percentage contents of active ingredient and higher percentage contents of 2-hydroxypropoxy-MKH6561 and thus better herbicide tolerance due to a more intensive metabolization (degradation of the active ingredient). Blackgrass, in contrast, showed a similar percentage distribution of both fractions. Laboratory trials conducted by *PONTZEN (2002)* showed comparable results due to faster metabolization in wheat in comparison to blackgrass. However, high degradation rates of the active ingredient, indicated by in part very high major plant metabolite percentage fractions,

were also detected in ALOMY. On the whole, the concentration [µg/g] of all the fractions considered was higher in ALOMY than in wheat due to higher total MKH6561 (relative to the applied radioactivity).

In the following, the influence of the growth stage of the plants at the time of application on the MKH6561 contents of the experimental plants will be discussed. Due to mass increase of the plant material and the resulting dilution effect, high total contents of the herbicide per gram of plant material were found for the early application date (BBCH22) in both experiments and low contents at the later application date (BBCH25). However, slightly elevated total MKH6561 contents were also found in the leaf material of spring wheat (column experiments) at the later application date. Nevertheless, damage to the crop is not to be expected here due to low active ingredient (a.i.) contents. On the whole, concerning the influence of application dates BBCH22 and 25 on the distribution of active ingredient and metabolites, it may be stated that the active ingredient contents were lower at the advanced application date (BBCH25) for both plant species. Thus, a stronger inactivation due to metabolization of the active ingredient was observed in ALOMY and a better tolerance in spring wheat in comparison to a younger plant (BBCH22). In contrast, a good herbicidal effect is to be expected at an early application date. Regarding the percentage distribution of active ingredient and metabolites, however, the growth stages of the plants in the lysimeter experiments had no effect and thus no similarities between the two experimental approaches had been found. Wheat studies, conducted by *PONTZEN (2002)*, revealed an increasing amount of undegraded MKH6561 with increasing plant age (7 to 21 d) after incubation of secondary leaves. However, this amount slightly decreased, when applied to 28 d old plants and nearly no differences between 21 d and 28 d old plants (corresponding to growth stages BBCH22 and BBCH25, respectively) have been observed. These results can explain why no influence of time of application at the lysimeter experiments, regarding the distribution of active ingredient and metabolites, has been found. In contrast to these findings, an distinct influence of the application date on the total ^{14}C-contents in the plant is given. The (in most cases) lower total MKH6561 contents at the later sampling date in both experimental plants (for both the column and the lysimeter experiments) cause lower active ingredient concentration [µg/g] at stage BBCH25.

Furthermore, the influence of soil moisture on herbicide uptake into the plant was studied. Due to the low pressure head in moist soil, plants can easily take up water and substances. The corresponding expectation that the uptake of MKH6561 also increases with rising soil moisture, was confirmed by a calculations based on hydraulic functions. Calculations revealed

that highest herbicide concentrations in the plant (wheat and ALOMY) were to be expected at 60%WHC_{max}. Predictions based on evaluation of the hydraulic functions were confimed by determination of the total ^{14}C contents in ALOMY. In contrast, the influence of soil moisture on total uptake is slightly lower in wheat (no soil moisture dependence was observed in the column experiments with wheat). This deviation may be explained, for example, by active ingredient constituents externally adhering to the leaf material and by soil moisture differences inside the columns. Concerning the distribution of active ingredient and metabolite contents as a function of soil moisture, the highest percentage active ingredient contents were determined, in most cases for both plant species in the column experiments at 60%WHC_{max}. Due to elevated uptake, plants were increasingly less able to metabolize the active ingredient contents. This is also illustrated by rising quotients of MKH6561 and 2-hydroxypropoxy-MKH6561 with soil moisture. Elevated absolute concentration [µg/g] is attributed to the rise in total MKH6561 which results from a rise in soil moisture. The diverging metabolization at different soil moistures cannot be unambiguously confirmed in the lysimeter experiments; this is presumably due to the fact, that the two soils used do not differ enough concerning the adjusted soil moisture. The influence of soil moisture on metabolization is comparatively strong since both soils are very moist. Moreover, different uptake rates are achieved at the same soil moisture under the influence of different soil types. For water uptake, plants on orthic luvisol must overcome a higher pressure head at identical soil moisture than on gleyic cambisol. This also explains the very high effectiveness of the herbicide in ALOMY on gleyic cambisol. Consequently damage to crop plants on gleyic cambisol is likely. In summary, it was found that the soil moisture was the most important factor responsible for herbicide uptake in both soil types and that high uptake rates are to be expected at high soil moisture.

In the following, the results of the soil analyses will be described. The evaluation of the active ingredient equivalents, at different soil depths, shows the highest herbicide concentration in the topsoil, caused by the application of the substance onto the soil surface. A certain translocation potential was observed against the background of detectable contents in the subsoil (depth level 5-10 cm; in deeper layers low contents at or below the detection limit) and the detection of active ingredient equivalents in the percolate.

In the column and lysimeter experiments (orthic luvisol), total MKH6561 contents of generally < 3% of the applied radioactivity are found at the lower depth levels (soil moisture: 20-45%WHC_{max}) at the first sampling date. More intensive translocation (up to 5.52% of the

applied radioactivity at 5-10 cm soil depth) was only found in the column experiments at the first sampling date, and at particularly high soil moisture (60%WHC_{max}). The concentration in the subsoil generally rise with advancing sampling date. In the lysimeter experiments, the two soil moistures adjusted are too similar to indicate dependencies. Moreover, a heavy rainfall event simulated in the column experiments led to comparatively high translocation rates (5.30-20.32% of the applied radioactivity) and plant uptake due to downward water flow and the increase in soil moisture. Since the soil moisture in the column and lysimeter experiments was only kept constant until the beginning of application, and a drying of individual soil layers was observed during sampling, the influence of soil moisture on translocation processes detected in the experiments is lower than expected, particularly since the evaluation of the hydraulic functions (water and mass transport) shows a more pronounced increase in hydraulic conductivity with rising soil moisture for both experimental soils. On the whole, it may be stated that elevated translocation in the soil (diffusive and advective transport with water in addition to "entrainment effects" with increasing root growth into the depth) and elevated uptake rates into the plant are to be expected at high soil moisture.

Another parameter investigated was the influence of the soil type on translocation processes. Differences in sorption behaviour and hydraulic conductivity of the two experimental soils are attributable to different soil physicochemical properties (above all with respect to particle and pore size distribution).

In gleyic cambisol, in comparison to orthic luvisol, a higher hydraulic conductivity, lower effective retardation and low sorptive properties (weak sorption of the active ingredient to the soil matrix, indicated by low K_F, K_D and K_{OC} values) were identified due to a high sand and coarse pore fraction. Thus, comparatively stronger herbicide translocation is to be expected in gleyic cambisol than in orthic luvisol.

Only predictions can be made concerning the process of translocation into deeper soil layers (below 40 cm soil depth) - processes such as mass flow, diffusion and macropore influence are involved to a different extent.

Clearly detectable differences in herbicide translocation in orthic luvisol and gleyic cambisol relate to the top 20 cm profile depth. Below this region, a similar low concentration occurs in both soil types. This also corresponds to the results of the field studies by Bayer CropScience. In these studies, concentrations of 1 µg/kg or contents of > 1% of the applied quantity could be ruled out in deeper soil layers *(FRITZ et al. 1999)*. The same order of magnitude was reached in the subsoil in the present experiments.

Concerning the transport processes and transport conditions below 40 cm as well as a hazard to groundwater, only predictions based on the adsorption/desorption measurements and the leaching tests can be made. The cut-off values for elevated mobility in the soil and a possible hazard to groundwater (*ANONYMOUS 2000*) show that the critical values are exceeded: water solubility in the alkaline range (pH 7-9) is 42 g/L and thus > 30 mg/L (cut-off value for elevated water solubility). Moreover, the averaged K_D values (K_D = 0.53 mL/g; n = 2) and the respective K_{OC} values (K_{OC} = 52 mL/g; n = 2) of both experimental soils reached the same orders of magnitude as the specified cut-off values of K_D < 0.75 mg/L and K_{OC} < 50 mg/L. However, in further lysimeter studies (*FRITZ et al. 1999*), which were performed for a more precise estimation of the leaching potential, a contamination of soil below 1.20 m or of groundwater could be ruled out (no increase above the critical value in the percolate of 0.1 µg/L). No groundwater hazard must therefore be expected, which is due to rapid active ingredient degradation (cf. the following sections). After spring application, the water movement in the following summer is not permanently directed downwards due to low precipitation and increased evapotranspiration, so that only very little MKH6561 reaches a threshold of 1.2 m.

A further aspect of the present study was the investigate of the metabolism of the active ingredient MKH6561 in soil. Metabolization takes place much more slowly than in the plant, forming a smaller number of metabolites. The active ingredient and metabolite spectrum in the desorption solutions of the soil material basically consists of unchanged MKH6561 and to a lesser extent of 4-hydroxysaccharin. Other degradation products were neglectible. According to studies on aerobic metabolism by *HELFRICH et al. (1999)* and *RIPPERGER et al. (1999*) a rapid degradation of MKH6561 was detected in the laboratory for various soils (DT_{50} = 22.8-88.5 d). In studies by *BLUMHORST et al. (1999)* 4-hydroxysaccharin content reached > 10% of the applied substance. Separate degradation studies showed DT_{50} values of 178.4-953.8 d for this metabolite. In contrast to the active ingredient, a residue problem due to accumulation of the major soil metabolite in the soil could therefore not be ruled out on the basis of laboratory studies. However, the field studies performed by Bayer CropScience showed a rapid degradation of total residues (MKH6561 and metabolites) with DT_{50} values of 15-56 d. A DT_{50} value of < 30 d indicates low persistence and good degradation behaviour (*HURLE 1982)*. In the case of DT_{50} values > 90 d, the persistence "allowed" in EU Directive 91/414/EEC would be exceeded and registration prohibited if active ingredient/metabolites are toxicologically relevant.

In both the column and the lysimeter experiments from the present study, the soil-borne metabolite (4-hydroxysaccharin) was also recovered from the plant material. It is thus taken up into the plant and accumulates due to elevated uptake as a result of increasing soil moisture and time after application (from sampling dates I to III). In the topsoil, comparatively high 4-hydroxysaccharin contents confirm a time dependence of degradation in final sampling (sampling dates II to III). In some cases, a percentage increase of 4-hydroxysaccharin was detected in the subsoil. However, because the radioactivity content in the desorption solutions is generally at or below the detection limit, a comprehensive evaluation of metabolization to 4-hydroxysaccharin is not possible. According to investigations by *HEIN et al. (1999)*, 4-hydroxysaccharin is classified as a substance of very low mobility (K_D values for adsorption: 7.5-46.3 mL/g), in contrast to the active ingredient itself (K_D values for adsorption: 0.22-1.71 mL/g). For this reason, a translocation in the form of intermediate products and at particularly high soil moisture is to be assumed. Accumulation takes place due to the already mentioned "persistence".

In comparing both soil types, metabolization in sandy soil was much faster (higher 4-hydroxysaccharin contents) than in loamy-silty soil (major soil metabolite fraction in gleyic cambisol twice as high as in orthic luvisol). This may be attributed to better degradation conditions in gleyic cambisol (higher microbial activity which is responsible for degradation due to stronger aeration). In contrast, degradation via photolysis and hydrolysis was not observed. Photolysis, which can contribute to the degradation of MKH6561 *(BRUMHARD et al. 1999a)*, would have revealed, if relevant, differences in total MKH6561 in the topsoil at the two different application dates (weak photolysis and higher total MKH6561 left due to higher degree of soil coverage of the plants at BBCH25).

It was not the main goal of this work to elucidate how active ingredient degradation proceeds in deeper soil layers (below the Ap horizon) down to the aquifer. It should be noted here that the microorganism density generally decreases with increasing soil depth, so that biological degradation is also increasingly restricted (*HOCK et al. 1995)*.

Anaerobic degradation studies in soil were not available, since no anaerobic conditions are to be expected due to spring application. The influence of "anaerobic zones" postulated in Chapter 6.2.4.1 on a low degradation of the active ingredient to 4-hydroxysaccharin is to be assumed, but cannot be confirmed by studies.

Non-desorbable residues, in the soil, were found in the range of 10-15% of the total ^{14}C radioactivity recovered. Indications for a possible accumulation potential according to EU Directive 91/414/EEC (non-extractable fraction > 70% of the applied radioactivity in

100 days; cf. *LYNCH 1995)* cannot be derived, since with 0.01 M of $CaCl_2$ solution only the plant-available fraction was desorbed and no comprehensive (solvent) extraction of the soil material was performed. Moreover, the investigation period was too short to be able to make a relevant statement.

After discussing the results of the present study in the preceding sections, the difficulties/problems encountered during the experiments will now be dealt with.
Soil columns have the advantage that due to their small size they are mobile and can thus be flexibly handled. Moreover, many different experimental variants can be installed in parallel and "undisturbed" arable soil can be used as soil material. Limited space on the column and the resulting small number of experimental plants makes an up-scaling to field conditions difficult. Thus, for example, only one plant per column could be withdrawn for macro-auto-radiographic investigations and 4-6 plants each for determining the active ingredient equivalents and the active ingredient and metabolite distribution. Moreover, the sampling technique applied is to be critically questioned (cf. Chapter 5.2.2) and the specified trans-location should be verified against the background of edge effects and the dumping of topsoil material with adsorbed herbicide (largely excluded in the lysimeter experiments by digging off each individual soil layer). In the column experiments, moreover, there was a closer root/soil ratio in the steel cylinder, so that higher uptake rates than in the lysimeter experiments were observed when considering the active ingredient equivalents relative to 1000 kBq of applied substance.
Lysimeters have the advantage that due to their size the natural (field) conditions can be reproduced more realistically (e.g. by placing the seed in rows). Moreover, the experiments did not take place in a growth chamber, but outdoors. Under controlled conditions (growth chamber) optimum conditions prevail concerning temperature and light, which may cause optimum plant growth due to elevated transpiration and uptake rates. The plant is more variable in the field (*GROSSE-BRAUCKMANN 1977)*, so that lower uptake rates are to be expected (provided that the same scenario is applicable). Another advantage is that more sample material per condition was available for the investigations. In addition, the possibility of percolate runoff enabled a better reproduction of the natural situation (descending water movement) than through the soil moisture backed up from below without runoff possibility (column experiments). The advantage over adjusting the moisture by spray irrigation from above was already pointed out in Chapter 3.6.1. Of disadvantage was that the soil was filled into the lysimeters and was thus not "naturally" bulked (provided that an Ap horizon can be

naturally stored at all). Of disadvantage is also that the lysimeters due to their size and weight (up to half a ton) are comparatively difficult to handle. Moreover, comparatively few experimental variables can be simulated in parallel for lack of space.
On the whole, better control of parameter adjustment is contrary to reference to practice in small column experiments. Vice versa, lysimeters are closer to practice but defined conditions are more difficult to establish. However, the results of the two experiments show good agreement in many important aspects. In order to compensate the advantages and disadvantages of the two methods (practicability and reproducibility of experiments and transferability to the field situation) both scales were chosen.

A further point of discussion is the adjustment of soil moisture. As already mentioned in the preceding chapters, the soil moisture was only kept constant during the experiments until application, since maintenance throughout the experiments was not possible due to the withdrawal of soil by sampling. The moisture contents determined in the soil were subject to changes due to various factors. Apart from evaporation, the soil depth (moisture lower in the upper region than in the lower one), sampling date (moisture content decreasing with progressing time), plant species (dryer in the case of wheat due to higher transpiration rates) and soil type also affected the water conditions. The soil moisture was only determined at the respective sampling dates and not recorded throughout the experiments. This resulted in differences between translocation rates determined experimentally and those described by calculations based on hydraulic functions for different soil moistures. It would have been possible to use TDR probes[19] to solve this problem, but this proved impracticable at least for the column experiments, since undisturbed soil was to be used and the vessels were too small.

[19] time-domain reflectometry

9. Conclusions

In this final chapter, overall conclusions are drawn concerning the influence of the experimental conditions on the distribution and concentration of MKH6561 in plant and soil.

- **Uptake, distribution and metabolization (Wheat vs. ALOMY):** The distribution of MKH6561 in the plant differs greatly for crop and grass weed. Wheat displayed better herbicide tolerance due to lower uptake rates and faster active ingredient degradation to the major plant metabolite (2-hydroxypropoxy-MKH6561) and up to five further metabolites. ALOMY, in comparison, showed higher uptake rates and a reduced "detoxification". Thus, effective herbicidal action against the grass weed and good crop tolerance are to be expected under normal conditions (soil properties and weather conditions).

- **Time of application:** Application of MKH6561 to plants, at main tillering (BBCH25), leads to a reduction in the effect of the herbicide in ALOMY and a greater tolerance in wheat. This is due to the mass increase of the plants and increased metabolization and inactivation of the active ingredient in comparison to application at start of tillering (BBCH22). The dilution-effect caused by the increase in fresh weight of the plant biomass is offset by increased uptake rates of MKH6561.

- **Soil moisture:** Elevated mobile fractions of total MKH6561 are to be expected at high soil moisture (with transfer to the field situation in rainy autumn) due to increasing desorption:

  The highest uptake rates of total MKH6561 and highest active ingredient content are to be expected in both plant species and soils due to low pressure head at high soil moisture (60%WHC_{max}). Thus, the best herbicidal effect on grass weed and, at the same time, the greatest danger to the crop is to be expected at elevated moisture. However, for harmful effect in wheat the concentration of the active ingredient in the experiments is too low. In the case of low uptake rates, at low soil moisture (20%WHC_{max}), this may lead to insufficient effectiveness for grass weeds where root uptake is negligible. Under very dry soil conditions uptake via penetration into the leaf becomes predominant. Increasing translocation in soil due to high hydraulic conductivity and low effective retardation is to be expected at high moisture.

- **Precipitation events:** Heavy rainfall causes increased downward mass flow; therefore high translocation rates are to be expected. Moreover an increase in soil moisture due to heavy rainfall leads to elevated uptake rates of the herbicide into the plant.

- **Soil type:** The different physicochemical properties of the two soils investigated affect greatly the behaviour of MKH6561 in plant and soil. Higher uptake in plant and stronger translocation rates in soil are to be expected in "light" low sorptive soils (gleyic cambisol) in comparison to "heavy" high sorptive soils (orthic luvisol).

  The cut-off values for elevated mobility in the soil are exceeded and the results of the leaching and adsorption/desorption studies reveal a leaching potential for both soil types. Further, in low sorptive soils minor concentrations of the active ingredient and metabolites in the subsoil (10-40 cm soil depth) cannot be ruled out. However, a hazard to groundwater is not to be expected due to low precipitation and increased evapotranspiration after spring application and metabolization of the active ingredient. The active ingredient and metabolite spectrum in soil basically consists of unchanged MKH6561 and, to a lesser extent, the major soil metabolite (4-hydroxysaccharin). For the period under investigation, comparatively good stability and the long-term effect of MKH6561 in soil was thus shown. Under less aerobic conditions (high moisture and stagnating soil water due to high clay fraction), metabolization is reduced and high concentrations of undegraded MKH6561 and metabolites occur. With respect to comprehensive active ingredient degradation, however, the short experimental period (max. 23 d from application to sampling) did not permit any conclusions to be drawn. Metabolisation is increased in gleyic cambisol due to better degradation conditions for aerobic microorganisms (better aeration).

- **Sampling date:** In plants, MKH6561 is continuously taken up at adequate soil moistures. Due to the degradation of the active ingredient, the percentage concentration of 2-hydroxypropoxy-MKH6561 increases with time. The soil-borne metabolite 4-hydroxysaccharin is taken up into the plant and concentrates as a result of time after application. In soil, a higher translocated concentration of total MKH6561 is expected at the latter sampling dates in comparison to the first; in orthic luvisol the concentration of total MKH6561 in the subsoil increases more slowly due to stronger sorption. In the topsoil, high concentration of 4-hydroxysaccharin is expected, at a later sampling date, due to active ingredient translocation or degradation and metabolite accumulation. In the subsoil, the concentration of 4-hydroxysaccharin increases due to translocation processes at high soil moisture, good degradation conditions (optimum oxygen and moisture content) and accumulation due to "persistence" of the major soil metabolite.

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Appendix

Forschungszentrum Jülich
in der Helmholtz-Gemeinschaft

Jül-4163
Februar 2005
ISSN 0944-2952