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Bachelorarbeit

„Determination of Chlortoluron in soil samples using High-Performance Liquid Chromatography."

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In der vorliegenden Thesis wurde die Verweildauer des Herbizid-Wirkstoffes Chlortoluron in Bodenproben untersucht. Dazu wurde das Herbizid „CTU 700“ in verschiedenen Aufwandmengen (3, 6 und 12 l/ha) in einem Feldversuch ausgebracht. Im Anschluss wurden über 3,5 Monate Bodenproben gezogen und im Labor mittels Rückstandsanalyse untersucht. Anschließend wurde eine HPLC-Analyse durchgeführt, um eine Aussage darüber treffen zu können, welche Rückstandsmengen die verschiedenen Aufwandmengen im Boden nach diesen 3,5 Monaten verursachen.

In the present study, the retention time of the herbicide active substance Chlortoluron in soil samples was investigated. For this purpose, the herbicide “CTU 700” was applied at different application rates (3, 6 and 12 l/ha) in a field trial. Soil samples were then taken over a period of 3.5 months and analyzed in the laboratory using residue analysis. Subsequently, a HPLC analysis was carried out to investigate the effects of different application rates on the behavior of Chlortoluron in soil over a period of 3.5 months.

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List of Abbreviations

ACN	Acetonitrile
Cl	Chlorine
CH3	Methyl group
e.g	exempli gratia
et al.	et alii
GC	Gas Chromatography
H_2O	Water
HCl	Hydrochloric acid
HPLC	High-Performance Liquid Chromatography
KOC	OC: organic carbon
n	quantity
org.	organic
SPE	Solid Phase Extraction
U/min	Revolutions per minute
UV/VIS	Ultraviolet and Visible Spectroscopy
v/v	Volume per volume

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1. Introduction

The use of Plant protection products is a frequently used working method in agriculture. The aim of their use is to protect crops from harmful organisms such as weeds, pests and fungi. Herbicides, in particular, with their toxic effect on monocotyledonous and dicotyledonous weeds, are used regularly. Domestic sales of herbicides in Germany amounted to 16,850 t in 2022 [1]. This is significantly higher than domestic sales of fungicides (11,529 t) [2] and insecticides (969 t) [3]. For many farmers, the use of herbicides is a reliable way of ensuring the development of their crops and their yields [4]. These act by either being absorbed via the roots and leaves of the weeds or both and inhibiting metabolic processes or destroying tissue [5]. The residence time of herbicides determines how long the active substance remains effective in the plant and also in soil for weeds that germinate later. Although this persistence is beneficial for the further control of weeds, the effect of herbicides also has undesirable effects in soil [6]. Their use can result in the restriction of certain substance conversions. High concentrations of urea herbicides, for example, inhibit the conversion of nitrite to nitrate, which can lead to nitrite accumulation in soil [7]. If these or other Plant protection product components seep into soil, they can be released into the groundwater. According to the Federal Environment Agency, one third of all groundwater bodies in Germany were in poor condition in 2022. The main causes of this are Plant protection products and nitrate [8]. It is also possible that Plant protection products leave residues in plants and animals due to their persistence, which can be detected when they are used as food [9]. In this way, the unwanted chemicals can enter the food chain of humans and animals. In 2021, residues of Plant protection products were found in 65% of the grain sampled in Germany and in 34% of food of animal origin [10]. Plant protection products can also enter our food chain through neighboring surface waters, which are particularly contaminated by precipitation after the application of the product. The water from the streams ends up in rivers and from there in the oceans. The organisms living in the water, such as fish and crabs, which serve as a food source for mankind, absorb the chemical components [11]. However, as long as the Plant protection product residues in food are below 0.01 mg per kg, there is no risk to the consumer, according to the European Parliament [12]. In addition to the aspects mentioned, the retention time of Plant protection products also influences the future cultivation planning of agricultural companies. Certain herbicide substances, for example, present a risk when cultivating subsequent crops, as they can cause damage. [13].

1.1 Research Objectives

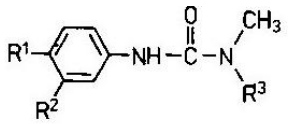
Due to the numerous reasons given above, accurate information regarding the persistence of Plant protection products active substances is important. Therefore, the aim of the present study is to determine the persistence of the herbicide active substance Chlortoluron in soil. This includes the extraction of soil samples taken at regular intervals, followed by analysis using HPLC. Furthermore, the persistence of Chlortoluron with different application rates was tested to make predictions about the influence of higher concentrations on the retention time in soil.

2. Theoretical Background

2.1 Chlortoluron

2.1.1 Description of the active substance

The active substance Chlortoluron is a herbicide from the phenylurea class of substances. It is composed of a chlorinated and methylated phenyl group, which is linked to a dimethylurea. [14]. The composition of Chlortoluron is illustrated in figure 1.



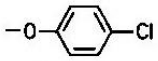
	R^1	R^2	R^3
Fenuron	–H	–H	$-CH_3$
Monuron	–Cl	–H	$-CH_3$
Buturon	–Cl	–H	$-CH(CH_3)-C\equiv CH$
Monolinuron	–Cl	–H	$-OCH_3$
Metobromuron	–Br	–H	$-OCH_3$
Diuron	–Cl	–Cl	$-CH_3$
Neburon	–Cl	–Cl	$-C_4H_9-n$
Linuron	–Cl	–Cl	$-OCH_3$
Chlorbromuron	–Br	–Cl	$-OCH_3$
Isoproturon	$-CH(CH_3)_2$	–H	$-CH_3$
Chloroxuron		–H	$-CH_3$
Fluometuron	–H	$-CF_3$	$-CH_3$
Chlortoluron	$-CH_3$	–Cl	$-CH_3$
Metoxuron	$-OCH_3$	–Cl	$-CH_3$

Figure 1 Composition of Chlortoluron and other phenylurea herbicides [14].

Chlortoluron was introduced in 1969 by the Swiss company “Ciba AG” (now BASF SE) as a selective soil and foliar herbicide [15]. It is used both in fall and in spring on fall seeded cereals. It is effective against a wide range of monocotyledonous and dicotyledonous weeds (figure 2) [15].

Area of application set by the regulatory authority in Germany:

Harmful organism/intended purpose	Cultivated plants/objects
Blackgrass, Loose Silky-bent, Annual Meadow Grass, annual broadleaf weeds (excluding Cleavers, Speedwell species)	Winter triticale
Blackgrass, Loose Silky-bent, Annual Meadow Grass, annual broadleaf weeds (excluding Cleavers, Speedwell species), Loose Silky-bent, annual broadleaf weeds (excluding Cleavers, Speedwell species)	Winter soft wheat, Winter barley
Blackgrass, Loose Silky-bent, Annual Meadow Grass, annual broadleaf weeds (excluding Cleavers, Speedwell species)	Winter soft wheat, Winter rye, Winter barley

Figure 2 Application Areas of the herbicide "CTU 700" [16].

As a phenylurea, Chlortoluron acts as a photosynthesis inhibitor. This means that the active substance blocks the light-dependent electron transport and oxygen evolution during photosynthesis [17]. Inhibiting photosynthesis ultimately results in the weeds being unable to produce energy [18], and thus consequently die. According to the Federal Office of Consumer Protection and Food Safety, the active substance is currently approved in 9 Plant protection products in Germany. These include the products “Carmina 640”, “CTU 700” and “Lentipur 700” [19].

2.1.2 Degradation in soil

According to Thier and Frehse, every soil consists of the same components, but with different compositions. These include an inorganic part consisting of mineral substances and their chemical compounds, a non-living organic part (humus), and the living soil organisms. Additionally, water and gases are also present [14]. Thus, soil is a very complex habitat. Therefore, it is understandable that Plant protection products do not always behave the same way in soil [20]. This even applies to the same product in different types of soil [14]. However, it is a fact that all Plant protection products are adsorbed at varying degrees by soil components [21]. The extent to which an active substance is bound to the surface of soil particles is described by the adsorption coefficient (KOC). A high KOC indicates low mobility of the product in soil. This means that the active substance is strongly bound to the soil particles, making it more difficult for it to be displaced by water. This results in slower degradation [22]. The classification of the adsorption coefficient can be found in table 1.

Table 1 Classification of Plant protection product mobility in soil using the adsorption coefficient (KOC) [22].

Plant protection product mobility	Adsorption coefficient KOC (ml/g)
Low mobility	≥ 1000
Medium mobility	100 to 1000
High mobility	≤ 100

The unit "ml/g" in table 1 refers to the volume of soil solution required to dissolve or to transport a certain amount of the substance in soil [22]. According to Nufarm, the active substance Chlortoluron has a KOC of 108 to 384, indicating that 108 to 384 ml of soil solution is needed to dissolve or transport 1 g of the active substance. Therefore, Chlortoluron is assigned a medium mobility in soil [23]. It is important to note that these values can vary depending on the soil type. For example, adsorption is stronger when the organic matter content is higher [22]. According to the German Agrochemical Industrial Association ("Industrieverband Agrar e.V."), the degradation of Plant protection products in soil occurs through microorganisms or abiotic processes such as hydrolysis. These processes break down the active substances into their components (carbon dioxide, water, etc.) [22]. The degradation of Chlortoluron is mainly microbial, through demethylation and cleavage of the anilide bond [15]. The rate of degradation of a Plant protection product is measured using the half-life period. The designation for this variable is DT50 (abbreviation for dissipation time). This describes how much time is required for the degradation of 50 % of the original amount of the substance [22]. According to Haider, the persistence in soil is highly dependent on climate and soil-related factors. These include the microorganism population and their activity, the presence and absence of oxygen, pH value, temperature and available nutrient sources. These factors vary throughout the year and also influence each other [24]. According to Nufarm, Chlortoluron has a DT50 value of 30 to 40 days and is classified as not easily biodegradable. [23].

2.2 Residue analysis of Plant protection products

There are many different analytical methods described in the literature for residue analysis of Plant protection products. The reason for this is the variety of active substances that are present in different matrices. In principle, however, most residue analysis methods consist of the same steps: extraction, rough separation, purification, enrichment and determination (figure 3) [14], [25].

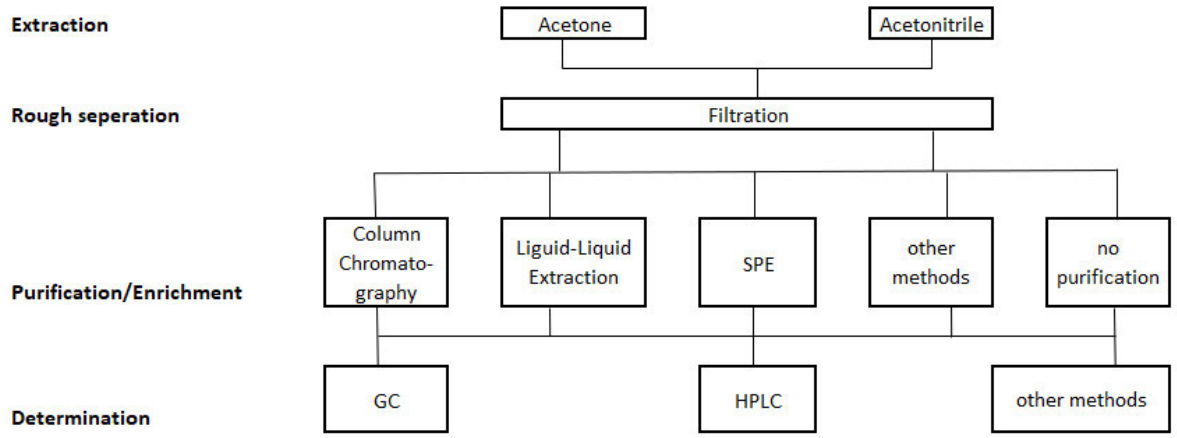


Figure 3 Steps of residue analysis of Plant protection products, modified according to Thier and Frehse [14] and Schwack, Anastassiades and Scherbaum [25].

2.2.1 Soil sampling

Before the soil samples can be analyzed, they must first be provided. To obtain a representative soil sample, according to the Federal Office for the Environment, Forests and Landscape in Bern (Switzerland), several individual samples from an area should be combined into a composite sample. It is assumed that the pollutant content of the composite sample corresponds approximately to the mean value of the pollutant content of the sampled area [26]. According to the Bavarian State Institute for Agriculture, it is also important that these individual samples are evenly distributed over the area. In addition, the resulting composite samples should be stored in a frozen state until they are analyzed [27], as otherwise there is a risk of loss of active substances [28]. Soil sampling should be carried out on arable land at a depth of 20 to 30 cm [29]. Usually, a drill stick (soil sampler), a hammer, a scraper, a container for mixing the samples, sealable bags and a pen for marking the bags are used for this purpose [27]. After taking a soil sample, the drill stick should be cleaned up to avoid contamination [29].

2.2.2 Sample preparation

The preparatory steps of a soil analysis in the laboratory include homogenization, drying and crushing or sieving the sample [30]. Sieving is used to achieve uniform particle sizes, which facilitates the extraction step [31]. The drying process is used to obtain knowledge about the dry matter, which is needed, to calculate nutrient contents or Plant protection product residues in soil [32]. In addition, the drying of soil samples simplifies homogenization and weighing of the individual samples. In this present study, drying of the soil samples was not possible,

because previous tests showed that drying using a drying chamber and freeze-drying, resulted in high losses of the active substance Chlortoluron. As it is rather unusual to not carry out any drying, the common methods are described nevertheless. Drying can be performed using a drying chamber, by freeze drying or air drying. In the drying chamber, soil samples are dried for 24 h at 104°C [30]. Weighing the samples before and after drying determines the dry matter content of the soil [32]. Freeze-drying works by freezing a sample and placing it under vacuum, which results in a change from solid ice to vapor, without it going through a liquid phase. This creates a gentle drying, as no heat is required [30]. Paul et al. prepared soil samples for Chlortoluron residue analysis by crushing the air-dried samples with a mortar and sieving them [33]. Kocarek et al. chose a similar procedure. There, the soil was also dried, ground and then sieved through a 1 mm sieve [34].

2.2.3 Extraction

Once the sample has been successfully prepared and the required amount of soil has been weighed out, extraction takes place. The definition of extraction, according to Gressner and Arndt, is as follows: "Extraction (Latin extrahere = to pull out) refers to a separation process used to dissolve components from a mixture of substances using a suitable extraction agent (solvent), wherein no chemical reaction occurs between the extracted substance and the solvent" [35]. According to Thier and Frehse, the extraction step serves to separate Plant protection product residues from the test material as quantitatively as possible without changing their chemical state. To achieve a high yield, a suitable solvent must first be found and used. The use of polar solvents is typical for the extraction of Plant protection products from soil. The reason for this is that the residues of the active substances are strongly bound to the components of soil and the improved solubility of the substances in the solvent itself [14]. Solvents such as acetone [14], methanol [36], ethyl acetate [37] and water are used most frequently. Due to the strong bond between the active substance and soil, the homogenization of the sample with the solvent must take place over several hours on a shaking machine. Occasionally, this step must also be carried out by extraction in a Soxhlet apparatus or by hot air extraction [14]. For the extraction of the active substance Chlortoluron, Paul et al. used a 30 ml mixture of acetonitrile: 0.1 M HCl (9:1, v/v) as a solvent. This was added to 10 g of soil and 30 g of anhydrous sodium sulfate [33]. Kocarek et al. used a different method. There, 5 g of the dried and sieved soil sample was mixed with 5 ml methanol and then shaken for 15 hours in a shaking apparatus [34]. According to DIN ISO 11264, which deals with the determination of herbicides using HPLC with UV

detection, a mixture of water and acetone is suitable for extraction, which must be shaken together with 50 g of soil for at least 6 hours [38].

2.2.4 Rough Separation

Rough separation is used after the shaking process to separate the soil components and the solvent [25]. A filtration process is suitable for this purpose. By using a filter, e.g. made of paper, which is placed in a funnel, the solid components are separated from the liquid components [39]. The solid components remain in the filter (residue), while the filtrate, consisting of solvent and dissolved substance, collects in a vessel [40]. This process is illustrated in figure 4. In the literature, Paul et al. used a glass wool plug containing 5 g anhydrous sodium sulphate for filtration [33].

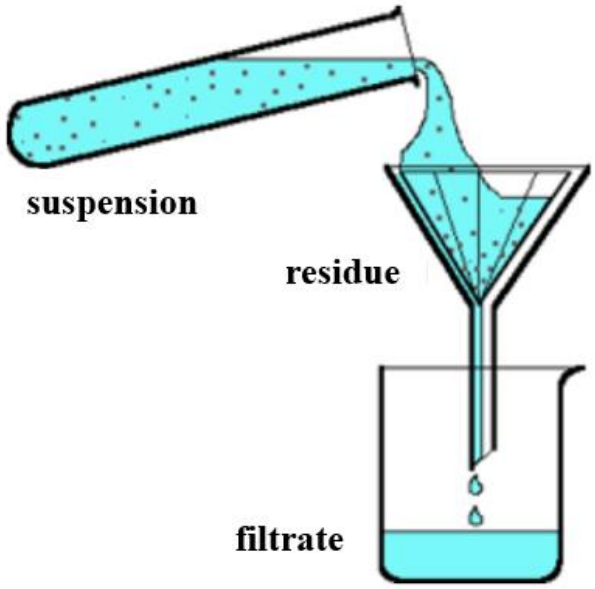


Figure 4 Illustration of a filtering process, modified [40].

2.2.5 Purification and Enrichment

According to Thier and Frehse, the step of purification is important for residue analysis, as the sample contains a high proportion of natural substances of the substrate in addition to the desired active substance. The intensity of the purification depends more on the subsequent determination procedure than on the quantity of accompanying substances. There are a variety of methods for isolating or purifying Plant protection products from a sample. These include Column Chromatography, Gel Chromatography, Liquid-Liquid Partitioning, Solid Phase

Extraction and several others [14]. Paul et al. carried out a Liquid-Liquid extraction for purification using a separatory funnel, in which the filtrate was shaken out twice with 5 ml dichloromethane [33]. Purification according to DIN ISO 11264 was also carried out using this method. After extraction, 30 g sodium chloride and 150 ml dichloromethane or petroleum must be added. The mixture then must be shaken again for 5 minutes. The organic phase is then removed by decanting or using a suction device [38]. The purification step is not important in this study, as the samples were sufficiently purified after filtration. For this reason, the theory of the purification methods is not discussed further at this point.

The following enrichment step serves to increase the concentration of the substance to be analyzed. This is achieved by removing the solvent [41]. The most used method is evaporation of the solvent [42]. A vacuum rotary evaporator was chosen for this study. It works by using a vacuum to lower the boiling point of the solvent, allowing evaporation at lower temperatures. The application of centrifugal forces through a rotational movement and the moderate heating of the samples facilitates the gentle evaporation of the solvent [43]. After successful evaporation of the liquids and any impurities, the concentrated components of the sample remain in the tubes. The next step is redissolution followed by determination. Paul et al. also carried out drying by evaporation to concentrate the sample. Redissolution was then performed in 5 ml HCl (0.1 mol) [33]. In DIN ISO 11264, a rotary evaporator was also used for enrichment. For this purpose, 40% of the sample must be measured after purification and then concentrated to 1 ml at 40 °C. Subsequently, 1 ml of acetonitrile must be used for redissolution, which then has to be homogenized with 1 ml of purified water [38]. To ensure that no solids enter the HPLC analysis, a membrane filter can be used to transfer the samples into the appropriate sample vials after redissolution.

2.2.6 Determination

The next step in residue analysis is the determination of Plant protection products in the prepared samples. In instrumental analysis, chromatography is used as a common method. Chromatography includes three main areas, including Gas Chromatography (GC), Supercritical Fluid Chromatography (SFC) and Liquid Chromatography (LC). A special form of Liquid Chromatography is High-Performance Liquid Chromatography (HPLC) [44]. This is described in more detail below because it was selected as the method for the present study. HPLC is a separation process in which a mixture of substances is separated within a column using a stationary and a mobile phase. The basic principle of HPLC is that the substances to be

separated are distributed between two immiscible phases. The stationary phase is fixed, while the mobile phase moves past the stationary phase in a certain direction together with the substance to be separated. The stationary phase is therefore a solid or gel and the mobile phase, also known as the eluent, is a liquid. Separation takes place due to interactions between the mixtures of substances and the stationary phase. These interactions are based on the different physical and chemical properties of the compounds to be separated. Depending on how strongly a contained compound reacts with the solid, the slower it flows through the column in which the separation takes place. This process is called retention. The time that a substance needs to pass through is therefore the retention time [45]. A detector continuously measures the substances that are leaving the column. The resulting signals are recognized by a connected computer software and evaluated in a chromatogram, also known as an elution curve [46]. A chromatogram represents the dependence of the detector signal on the retention time. Calibration standards can be used to assign the retention time to the individual components based on the chromatograms created. Ideally, these are in the form of a Gaussian distribution, which is also referred to as a peak [44]. Figure 5 shows an example of a separation process in simplified form. The individual colors represent different components that are carried past the stationary phase in the mobile phase and thus separated. The component with the red symbol takes the longest time to pass the column and therefore it has the longest retention time and the last curve on the chromatogram [46].

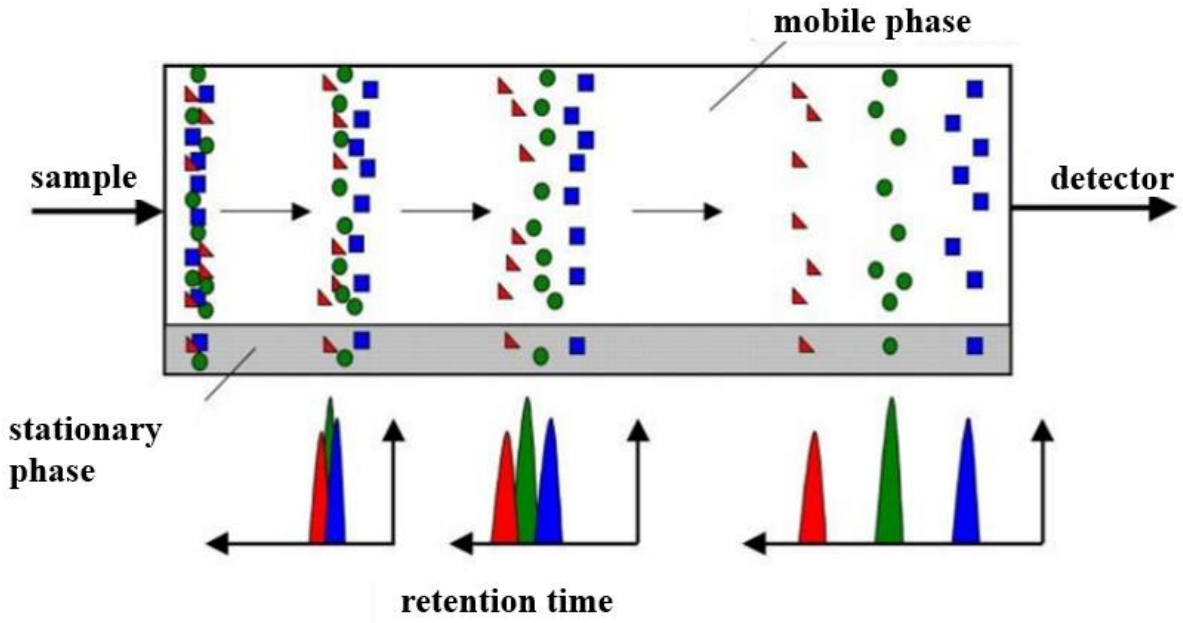


Figure 5 Separation of a mixture of substances consisting of 3 components, with representation of the corresponding chromatograms, modified [46].

The typical HPLC procedure is described below. At the beginning, the eluent is transported from the storage vessel into the degasser. This serves to avoid outgassing in other components of the HPLC, because the air can interfere with detection, due to its different spectral characteristics. From the degasser, the eluent continues to the pump and from there to the 6-port valve (injector). This also takes up the sample, which usually comes from an automatic injector unit and is to be separated. From there, the eluent carries the individual components of the mixture of substances to be separated through the stationary phase (column), driven by pressure. The sample is separated in the column. The downstream UV/VIS detector registers the components present (analytes) based on their different absorption characteristics depending on the wavelength. [46]. A connected computer displays the results using chromatograms [45]. The simplified structure of an HPLC system is shown in figure 6.

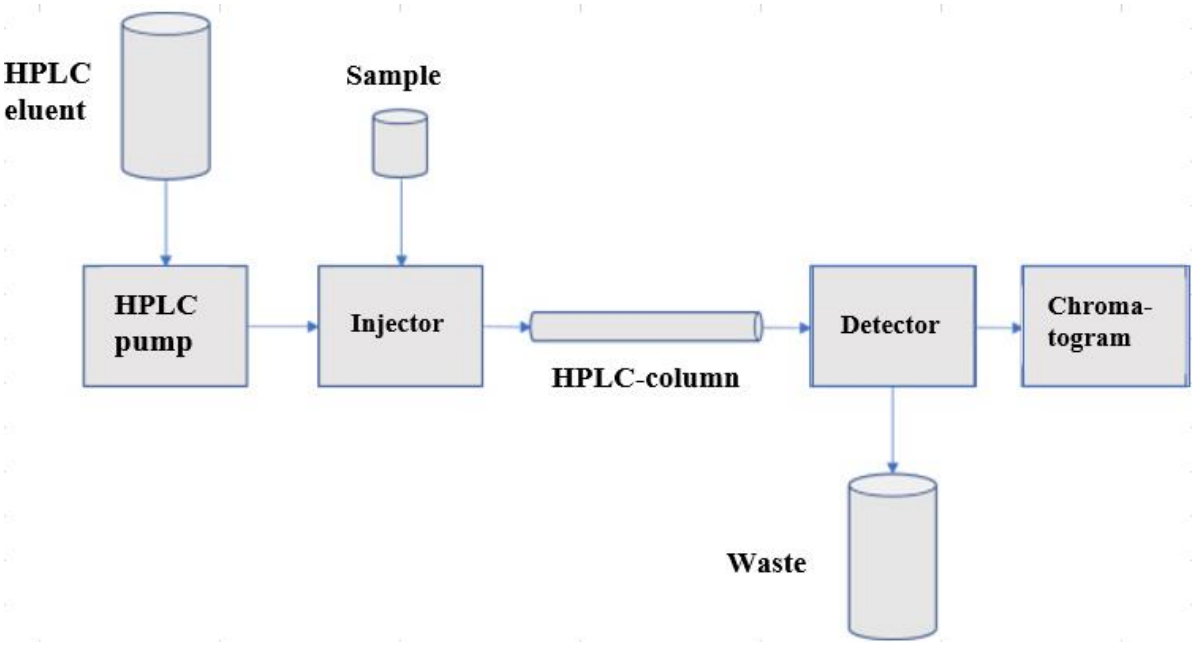


Figure 6 Simplified structure and components of an HPLC, modified [49].

There are a variety of HPLC columns, which differ in material, size and number of separation stages. The various solid-state materials include silica gel, aluminum oxide, magnesium silicate or activated carbon [48]. The length of common columns is 5 to 10 cm and the inner diameter is approximately 2 to 4.6 mm [50]. The number of separation stages of a column determines its separation capacity and therefore the success of the HPLC analysis. Which column is selected for the analysis depends on the properties of the respective analyte [48]. In the literature, a C18 column is frequently used for the HPLC analysis of the herbicide active substance Chlortoluron

[33], [34], [38]. This consists of pure silica gel and is also referred to as the “all-rounder of liquid chromatography” [51]. The designation “C18” comes from the 18 carbon atoms that are coupled to the silane group [48].

3. Material and Method

3.1 Site description

The soil samples required for this study were taken from a field trial located on the test areas of the German Agricultural Society (DLG) in Bernburg, Strenzfeld. According to the DLG, this location has an average annual precipitation of 511 mm and an average annual temperature of 10.1 °C. These arable areas can be assigned the soil type loess-black soil, as well as soil points in the range of 65-94 [52]. This rating is based on a German scale from 0-100, with 100 as best soil in the context of quality, fertility and logistics [53]. Furthermore, the soil can be described as silty loam with the following proportions: 22 % clay, 70 % silt, 8 % sand. The pH value in the trial is 7.5 [52]. This field is particularly well suited for the detection of herbicide residues, as the soil composition prevents rapid leaching of the active ingredients in the event of precipitation.

3.2 Trial description

This field trial is a herbicide trial in winter wheat with 13 variants of 5 replicates each. The winter wheat ("KWS Donovan") was drilled after grain maize on 16.10.2023 with a seed rate of 205 kernels per m² and a row width of 16.7 cm. Field emergence took place on 27.10.2023. The plots are 3 m wide and 15 m long. 6 different herbicides were applied to the 65 plots in total, except for 1 control variant, on 16.11.2023. 5 herbicides were applied in April 2024. The application was carried out using a bicycle sprayer with IDK 90-01 nozzles and a pressure of 2 bar. The test plan is shown in figure 7.

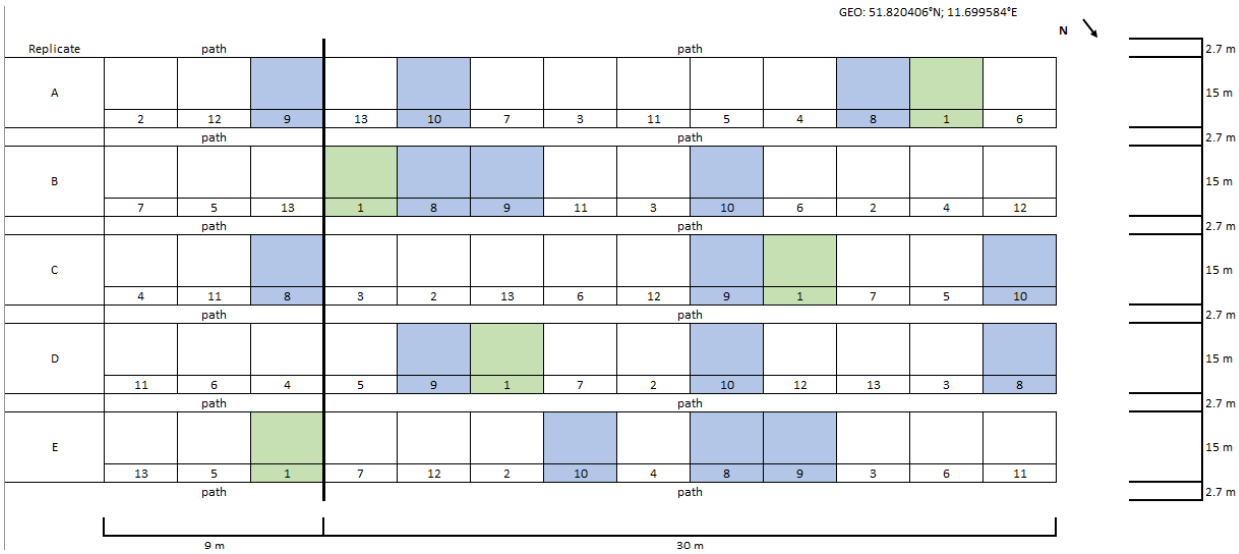


Figure 7 Plan of the field trial, with the relevant plots highlighted in blue and the control variant in green.

The relevant variants for this study are test plots 8, 9 and 10. The herbicides “CTU 700” and “Diflanil 500 SC” were applied to these plots at different application rates on 16.11.2023. CTU 700 contains the active substance Chlortoluron, which is the subject of the present study. The exact application rates are shown in table 2.

Table 2 Application plan.

Variant	Herbicide	Amount/ha	Herbicide	Amount/ha
8	CTU 700	3 l	Diflanil 500 SC	0.2 l
9	CTU 700	6 l	Diflanil 500 SC	0.4 l
10	CTU 700	12 l	Diflanil 500 SC	0.8 l

3.3 Material

The materials used were divided into the areas of chemicals, standard solutions, equipment and consumables and can be found in the following subsections. The results were analyzed using Excel.

3.3.1 Chemicals

- **Acetonitrile**, HPLC-gradient grade (VWR International GmbH).
- **Chlortoluron**, analytical standard (Merck KGaA).
- **Diuron,** analytical standard (Merck KGaA).
- **Double distilled water**.
- **2-Propanol** (S3 Handel und Dienstleistungen UG).

3.3.2 Standard solutions for HPLC determination

- Stock solutions

Stock solution 1 (S1) consists of 1 mg of the Chlortoluron standard and stock solution 2 (S2) of 1 mg of the Diuron standard, filled up to 10 ml propanol. This results in a concentration of 1 mg/ml for each stock solution.

- Working solutions

The working solutions consist of 100 µl of each stock solution filled up to 10 ml acetonitrile/water (50:50, v/v). This results in a concentration of 10 µg/ml for Chlortoluron for working solution 1 (A1) and a concentration of 10 µg/ml for Diuron for working solution 2 (A2).

- Mixed solutions

The mixed solutions, made of S1 and S2, which were used to calibrate the HPLC system are shown in table 3. The 2 stock solutions were used to prepare these 6 standards (M1-M6). Standard M1 consists of 100 µl of S1 and 200 µl of S2, filled up to 10 ml with ACN/H2O (50:50, v/v). This results in a concentration of 10 µg/ml Chlortoluron and 20 µg/ml Diuron. Standard M2 was prepared in the same way, but with a smaller volume of the 2 stock solutions, also filled up to 10 ml ACN/H2O (50:50, v/v). For standard M3, 1 ml of M1 was filled up to 10 ml ACN/H2O (50:50, v/v). To prepare the standards M4 to M6, various aliquots were taken from M3 and filled up to 10 ml with ACN/H2O (50:50, v/v). The exact volumes and concentrations are shown in table 3.

Table 3 Mixed solutions used for calibration in HPLC analysis.

Mixed solutions	M1	M2	M3	M4	M5	M6
Volume	100 µl of S1, 200 µl of S2	50 µl of S1, 100 µl of S2	1 ml of M1	1 ml of M3	0.5 ml of M3	0.1 ml of M3
Concentration Chlortoluron	10 µg/ml	5 µg/ml	1 µg/ml	0.1 µg/ml	0.05 µg/ml	0.01 µg/ml
Concentration Diuron	20 µg/ml	10 µg/ml	2 µg/ml	0.2 µg/ml	0.1 µg/ml	0.02 µg/ml

3.3.3 Equipment and consumables in the laboratory

- **Beakers,** 200 ml, 50 ml.
- **Concentrator,** 5301 (Eppendorf SE).
- **Disposable syringes**, 5 ml (B. Braun SE).
- **Eppendorf Cups**, 1.5 ml (Eppendorf SE).
- **Eppendorf pipettes**, 100 und 1000 µl (Eppendorf SE).
- **Eppendorf tips**, 100 und 1000 µl Volumen (Eppendorf SE).
- **Erlenmeyer flasks**, 100 ml.
- **Filter paper**, 0.17 mm, 185 mm diameter (Macherey-Nagel).
- **Glas funnel**.
- **Halogen moisture analyzer**, HR 73 (Mettler Toledo).

- **HPLC,** 1260 Infinity (Agilent Technologies, Inc.), consisting of:
  - **Analytical Guard Column,** 4.6 x 12.5 mm (Agilent Technologies, Inc.)
  - **Automatic autosampler**, G1329B, 1260 ALS (Agilent Technologies, Inc.).
  - **Detector**, G1365D, 1260 MWD VL (Agilent Technologies, Inc.).
  - **Pump**, G1311B, 1260 Quaternary Pump (Agilent Technologies, Inc.).
  - **Refractive Index Detector,** G1362A, 1260 RID (Agilent Technologies, Inc.).
  - **Separation column**, Polaris 5 C18-A 250 x 4.6 mm (Agilent Technologies, Inc.).
  - **Thermostated column oven**, CTO-10AS VP (Shimadzu Deutschland GmbH).
- **Laboratory shaker**, SM 25 (Edmund Bühler GmbH).
- **Laboratory scale**, LP 62008 (Sartorius AG Göttingen).
- **Measuring cylinder,** 100 ml.
- **Micro-Centrifuge Tubes,** 2.0 ml (VWR International, by Labcon North America)
- **Screw-cap vials**, 100 ml.
- **Short Thread Screw Caps,** ND9, 090300-T (BGB Analytik Vertrieb GmbH).
- **Short Thread Vial,** 32 x 11.6 mm, 0 3 ml (BGB Analytik Vertrieb GmbH).
- **SpeedVac Concentrator,** Concentrator 5301 (Eppendorf SE).
- **SpeedVac Concentrator,** SVC-100H (Savant).
- **Syringe filter,** 0.22 µm (BGB Analytik Vertrieb GmbH).
- **Test sieve** according to DIN-ISO-3310/1. 5 mm (Retsch GmbH).
- **Volumetric flask,** 100 ml.
- **Vortex 2** (IKA-Werke GmbH & CO. KG).

3.3.4 Equipment and consumables in the field

- **Bucket**, volume 10 l.
- **Drill stick**, 30 cm deep.
- **Pen**, permanent marker.
- **Scraper** for the drill stick.
- **Sealable bags**, volume 0.5 l.

3.4 Sampling

The samples were taken in late fall and winter, from November 2023 to March 2024, using a drill stick. For the present study, in addition to the control variant, only the plots in which the agent “CTU 700”, containing the active substance Chlortoluron, was applied were sampled.

The first sampling took place on 16.11.2023 directly after the application, the second on 11.12.2023, the third on 26.01.2024 and the last on 07.03.2024. Four punctures (0-30cm) were made in each plot to obtain a composite sample. The first sampling directly after the herbicide application was carried out at a depth of 15 cm. The samples were then stored in a freezer.

3.5 Soil Sample preparation

In order to establish a suitable preparation method for the residue analysis of soil samples, various publications were used as guidance. These are DIN ISO 11246 from the German Institute for Standardization [38] and publications by Kocarek et al. [34] and Paul et al. [33]. Drying was not carried out in this study, as preliminary tests showed that both drying in a drying chamber and freeze-drying cause high losses of the active substance Chlortoluron. First, the frozen soil samples were defrosted, sieved in a 5 mm sieve and homogenized. Then 5 g of soil was weighed out in 3-fold repetition. Subsequently, 1 ml of Diuron standard (A2), which was mentioned in section 3.3.2, was added to each sample. This has a concentration of 10 µg/ml Diuron in ACN/H20 (50:50, v/v). Diuron serves as an internal standard, as it is a phenylurea with a similar structure to Chlortoluron. This was followed by extraction with 25 ml acetonitrile. The samples were shaken for 15 h at 200 rpm on a laboratory shaker. The samples were then filtered in a funnel using a pleated filter. The filtrate was collected in a beaker. From this, Eppendorf tubes with a volume of 1.5 ml were taken and then dried in a rotary evaporator (SpeedVac Concentrator), to increase the concentration, until there was no solvent left. The samples were then frozen. They were redissolved in 150 µl ACN/H2O (50:50, v/v) and then homogenized with the vortex. The samples from the second soil sampling (11.12.2023) onwards were concentrated twice in the SpeedVac Concentrator, as a low concentration was expected. Therefore, these samples had to be redissolved twice as well.

3.6 HPLC analysis

The standard residues in the samples were determined using HPLC. The conditions under which the HPLC device was operated are based on DIN ISO 11264 [38] and can be found in table 4. The gradient program is described in table 5.

Table 4 HPLC conditions modified according to DIN ISO 11264 [38].

Criteria	Conditions and values
Stationary phase	C 18-column
Mobile phase	ACN
Injektion volume	50 µl
Oven temperature	30°C
Flow rate	1 ml/min
Detection wavelength	211 nm
Eluent A	ACN/H_2O (10:90, v/v)
Eluent B	ACN

Table 5 Gradient program.

Time [min]	Eluent A [%]	Eluent B [%]
0.00	90.0	10.0
18.00	47.0	53.0
18.50	10.0	90.0
19.50	10.0	90.0
20.00	90.0	10.0
26.00	90.0	10.0

3.7 Calculation of the average target concentrations

Based on the exact knowledge of the added quantities of standard, extraction agent and redissolution, the theoretical concentration of the active substances in soil can be calculated. In the following, these are referred to as target concentrations. Those serve as control values for the concentrations measured in the HPLC. In this study the calculation of the target concentrations does not refer to the surface area, but rather to the soil volume. The exact target concentrations for each plot can be found in Appendix 2. To simplify the presentation of the results, an average value for each of the 3 variants was calculated for the target concentrations. The calculations of these average values are as follows:

- Chlortoluron per liter of product: 700 g [15]
- Radius of the drill stick: 0.9 cm
- Chlortoluron application rate for variant 8: 3 l per ha [15]

- Calculation of concentration of Chlortoluron:

$$3 * 700\ g = 2100\ g\ per\ ha$$

- Area per drill stick:

$$\pi * (0.9\ cm)^2 = 2.54\ cm^2$$

- Area for 4 drill stick procedures:

$$2{,}54\ cm^2 * 4 = 10.16\ cm^2$$

- Chlortoluron per 4 drill stick procedures:

$$2100\ g\ per\ ha * 10.16\ cm^2 = 21336\ g$$

- With an average soil sample weight of 154.02 g for 4 samples, the target concentration of Chlortoluron per kg of soil for variant 8 is:

$$\frac{21336\ g}{154.02\ g} = 138\ g\ per\ kg\ soil$$

$$\triangleq 1.38\ \text{mg per kg soil}$$

The average target concentration for variant 8, which received an application rate of 3 l per ha of the herbicide, is 1.38 mg Chlortoluron per kg soil. The same calculation is made for variant 9 and 10, where the application rates are 6 and 12 liters per hectare, with an average soil sample weight of 141.92 g and 159.65 g. This results in a mean target concentration of 3.02 mg Chlortoluron per kg soil for variant 9 and 5.36 mg Chlortoluron per kg soil for variant 10.

3.8 Calculation of the Chlortoluron value

By adding the internal standard Diuron, the measured residue of Chlortoluron could be adjusted by calculation with the residues of Diuron. The recovery rate of the 2 substances was determined in 3 preliminary tests. It was found that the Chlortoluron had an average recovery rate of 13.2% lower than the Diuron. This was taken into account in the calculations using a factor of 1.132. The calculation table for the final value of Chlortoluron can be found in Appendix 2.

4 Results

The results of the residue analysis using HPLC were output by the Agilent ChemStation software in the form of "short reports." These contain, among other things, the chromatograms of the active substances and their concentrations. Such a report can be found in Appendix 1 for illustration purposes. A wavelength of 211 nm was selected for the evaluation of the residues measured in the HPLC and calibrated using the standard solutions described under 3.3.2, as this had the highest intensity for Chlortoluron. The data table for calculating the results of the soil samples from 16.11.2023 can be found in Appendix 2 for illustrative purposes.

Chlortoluron residues in variant 8 over time

The Chlortoluron residues measured in variant 8 for the 4 different soil sample dates are shown in figure 8. It shows the residues in mg per kg soil for the 5 field replicates (A to E). Each column is made up of 3 values that were determined in the laboratory by a 3-fold repetition, and the corresponding standard deviation. The exact numerical values can be found in table 6. Also shown in the diagram is the mean target concentration of 1.38 mg Chlortoluron per kg soil, which should have been detectable in variant 8 on the day of the herbicide application (16.11.2023).

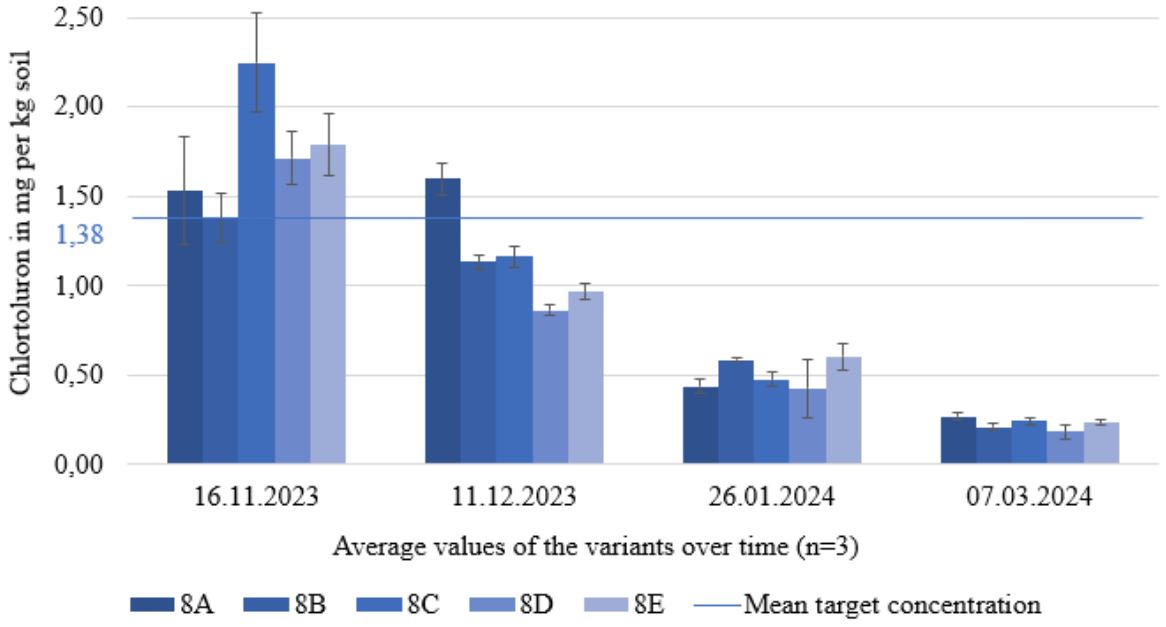


Figure 8 Chlortoluron residues for variant 8 in mg per kg soil ± standard derivation for the 5 field replicates over time.

It is evident that the target concentration of 1.38 mg Chlortoluron per kg of soil could be detected in all 5 field repetitions. When looking at those measured residues, the diagram shows that the values on 16.11.2023 fluctuate strongly between the field repetitions (from 1.38 to 2.25 mg per kg). This date also presents the largest standard deviations of the laboratory replicates: 0.14 to 0.30 mg per kg (table 6). The fluctuations between the field replicates become smaller on the later soil sampling dates. This is particularly noticeable from 26.01.2024 onwards. The Chlortoluron residues there are between 0.43 to 0.58 mg per kg soil. The standard deviations of the 3 laboratory replicates are also getting smaller over time, with the exception of plot 8D on 26.01.2024. The figure also shows that the level of residues measured in one plot compared to the other plots does not remain stable over time. Plot 8A, for example, had the highest residues on 16.11.2023, but the samples from 26.01.2024 show that plot 8D had the highest Chlortoluron content. The situation is similar with the other plots. The columns also illustrate that for variant 8 the Chlortoluron residues in the soil decrease over time (figure 8).

Table 6 Chlortoluron residues in mg per kg soil for variant 8 for the 5 field replicates with the standard derivation of the 3-fold laboratory repetition, over time.

	Chlortoluron in mg per kg soil ± standard derivation for each replicate				
Date of soil sampling	8A	8B	8C	8D	8E
16.11.2023	1.53 ± 0.30	1.38 ± 0.14	2.25 ± 0.28	1.71 ± 0.15	1.79 ± 0.17
11.12.2023	1.60 ± 0.09	1.13 ± 0.04	1.16 ± 0.06	0.86 ± 0.03	0.97 ± 0.05
26.01.2024	0.44 ± 0.04	0.58 ± 0.01	0.48 ± 0.04	0.43 ± 0.17	0.60 ± 0.08
07.03.2024	0.27 ± 0.02	0.21 ± 0.02	0.24 ± 0.02	0.18 ± 0.04	0.24 ± 0.02

Chlortoluron residues in variant 9 over time

The Chlortoluron residues detected in variant 9 across 4 distinct soil sampling dates are illustrated in figure 9. This figure presents the residues in mg per kg of soil for the 5 field replicates (A to E). Each column includes 3 values obtained through laboratory analysis via a 3-fold repetition, along with their corresponding standard deviations. The data underlying the diagram is provided in table 7. Furthermore, the diagram indicates the target concentration of 3.02 mg Chlortoluron per kg soil, which was expected to be measurable in variant 9 on the day of herbicide application (16.11.2023).

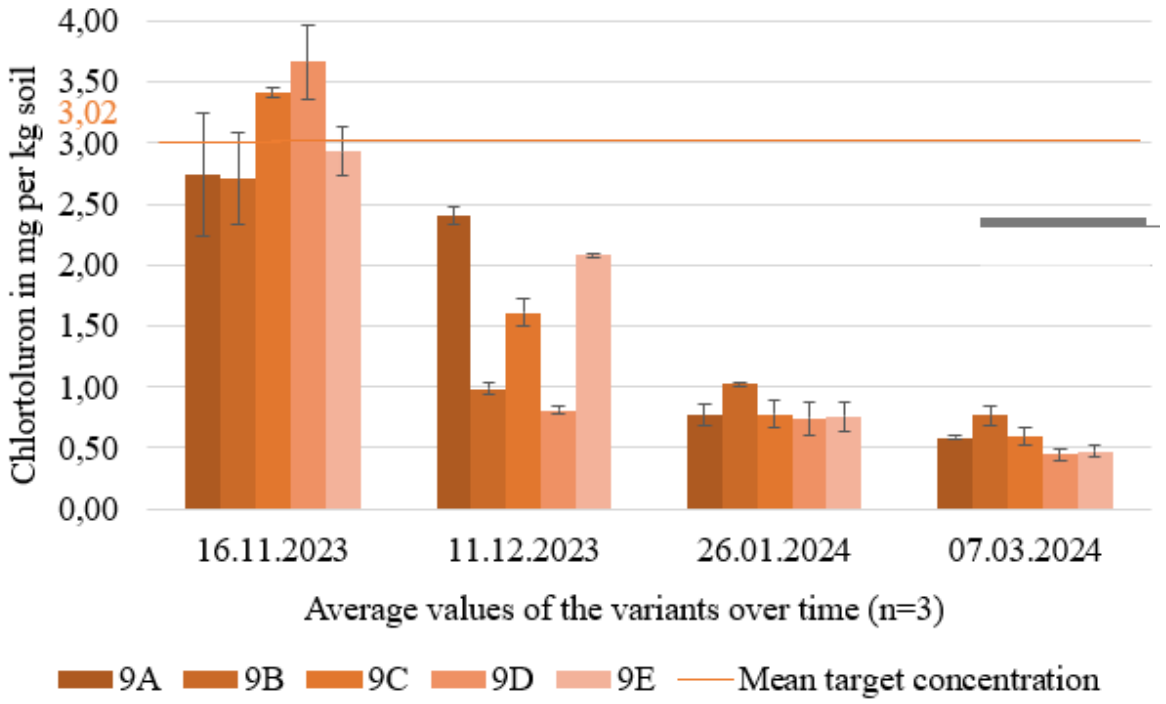


Figure 9 Chlortoluron residues for variant 9 in mg per kg soil ± standard derivation for the 5 field replicates over time.

The target concentration was only reached twice on 16.11.2023, in plot 9C and 9D. The residue measurements of the soil samples from 16.11.2023 show values in plots A, B and E that are roughly at the same level (2.74, 2.71, 2.93 g per kg of soil). Plots C and D, on the other hand, show slightly higher Chlortoluron residues in the soil at 3.41 and 3.67 mg per kg of soil. The standard deviations for the values of this soil sampling date are, with the exception of 9C (0.04 mg per kg soil), quite high compared to the standard deviations of the other soil sampling dates (0.20 to 0.51 mg per kg soil). The soil samples from 11.12.2023 show a very high fluctuation of the residues with 0.99 to 2.40 mg per kg of soil. The standard deviations range from 0.02 to 0.11 mg per kg of soil. The Chlortoluron residues of the soil samples from 26.01.2024 show a fairly uniform level for variant 9. The values here vary between 0.74 and 1.02 mg per kg soil, with the two plots 9A, 9C, 9D and 9E achieving values of 0.74 to 0.78 mg Chlortoluron per kg. The standard deviations here are between 0.01 and 0.14 mg Chlortoluron per kg of soil. The soil samples taken on 07.03.2024 also show Chlortoluron residues that do not fluctuate too much. Here, the values are between 0.45 and 0.76 mg Chlortoluron per kg soil with standard deviations of 0.02 to 0.07 mg per kg soil. In general, it is noticeable that the trend in variant 9 is for the Chlortoluron residue in the soil to decrease over time. It is also worth mentioning that when looking at the general loss of active substances over time, the Chlortoluron residues in plots 9B and 9D show similar residues on 11.12.2023 and 16/01/2024. For example, the

residues in plot 9B stabilize at around 1 mg Chlortoluron per and in plot 9D at 0.80 mg Chlortoluron per kg of soil. The other plots have a higher gradation between the individual soil sampling dates (table 7). Similar to variant 8, the ratio of the measured values in the plots changes over time. For example, plot 9D had the highest residue of Chlortoluron on 16.11.2023, but 3.5 weeks later it had the lowest residue of all replicates (table 7).

Table 7 Chlortoluron residues in mg per kg soil for variant 9 for the 5 field replicates with the standard derivation of the 3-fold laboratory repetition, over time.

	Chlortoluron in mg per kg soil ± standard derivation for each replica				
Date of soil sampling	9A	9B	9C	9D	9E
16.11.2023	2.74 ± 0.51	2.71 ± 0.37	3.41 ± 0.04	3.67 ± 0.31	2.93 ± 0.20
11.12.2023	2.40 ± 0.07	0.99 ± 0.04	1.61 ± 0.11	0.81 ± 0.03	2.08 ± 0.02
26.01.2024	0.77 ± 0.09	1.02 ± 0.01	0.78 ± 0.11	0.74 ± 0.14	0.76 ± 0.12
07.03.2024	0.58 ± 0.02	0.76 ± 0.07	0.59 ± 0.07	0.45 ± 0.05	0.47 ± 0.05

Chlortoluron residues in variant 10 over time

The Chlortoluron residues detected in variant 10 across 4 distinct soil sampling dates are illustrated in figure 10. This figure presents the residues in mg per kg of soil for the 5 field replicates (A to E). Each column includes 3 values obtained through laboratory analysis via a 3-fold repetition, along with their corresponding standard deviations. The data underlying the diagram is provided in table 8.

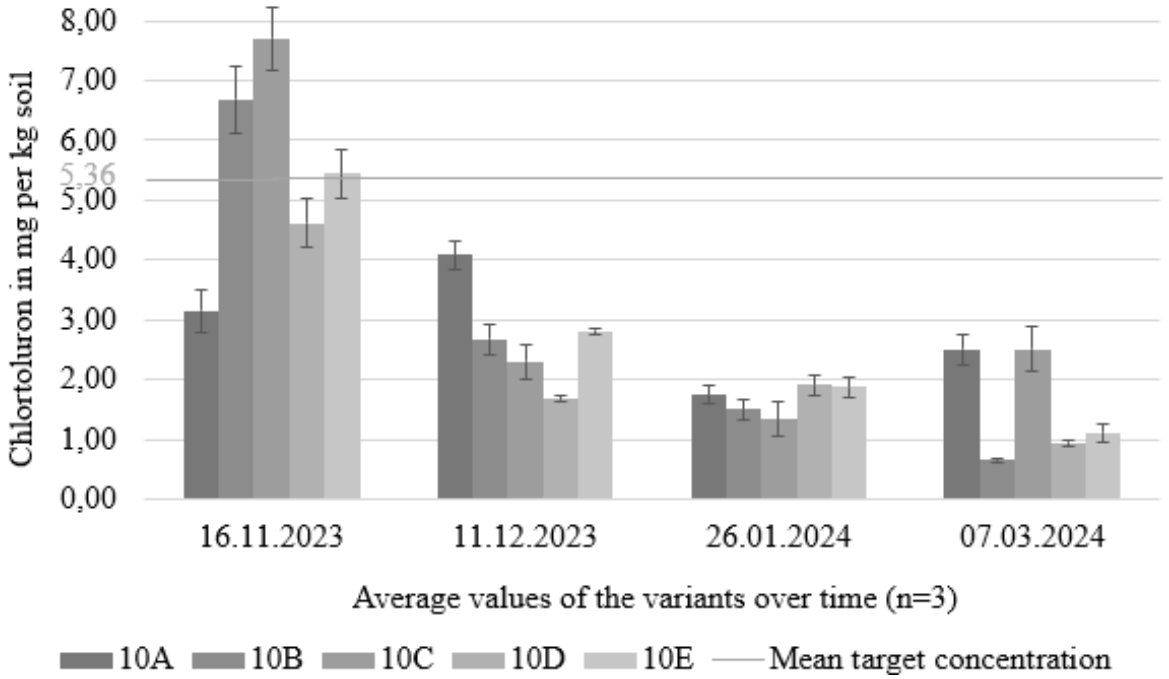


Figure 10 Chlortoluron residues for variant 10 in mg per kg soil ± standard derivation for the 5 field replicates over time.

The mean target concentration of 5.36 mg Chlortoluron per kg of soil was detected in 3 out of the 5 field replicates on 16.11.2023. The Chlortoluron content in the soil on 16.11.2023 on this date varies greatly between the individual field replicates. Values of 3.14 to 7.70 mg Chlortoluron per kg soil were obtained here, with high standard deviations of 0.35 to 0.57 mg per kg soil. 3.5 weeks after the herbicide application, on 11.12.2023, Chlortoluron residues are still varying in the range of 1.67 to 4.08 mg Chlortoluron per kg soil. The corresponding standard deviations are a lot smaller here compared to the first soil sampling date (0.04 to 0.28 mg per kg of soil). The residues measured in the soil samples of 26.01.2024 are approximately at the same level in all field replicates (1.34 mg to 1.91 mg Chlortoluron per kg soil with standard deviations of 0.16 to 0.29 mg per kg soil). The soil samples taken on 07.03.2024 show fluctuating Chlortoluron residues. While the values in plots 10B, 10D and 10E are at approximately the same level (0.64 to 1.10 mg per kg of soil), the values in the A and C replicates are approximately almost 3 times higher than the mean value of the other 3 plots. The corresponding standard deviations range between 0.05 and 0.36 mg per kg of soil (table 8). The A and C replicates show with the residue of 2.51 mg per kg soil even higher Chlortoluron residues than at the previous soil sampling date (26.01.2024). When looking at the residues for variant 10 over time, it is noticeable that the Chlortoluron content in soil decreases over the months, except for some plots already mentioned in the current text. With this variant, it is also

noticeable that the amount of residues measured in one plot does not remain stable in relation to the other plots. On 16.11.2023, for example, the A replicate achieved the lowest residue of all replicates with 3.14 mg Chlortoluron per kg soil. When looking at this plot over time, it is noticeable that this ranking does not continue. 26 days after achieving the lowest residue, the same plot reached the highest residue (table 8).

Table 8 Chlortoluron residues in mg per kg soil for variant 10 for the 5 field replicates with the standard derivation of the 3-fold laboratory repetition, over time.

	Chlortoluron in mg per kg soil ± standard derivation for each replica				
Date of soil sampling	10A	10B	10C	10D	10E
16.11.2023	3.14 ± 0.35	6.68 ± 0.57	7.70 ± 0.52	4.62 ± 0.40	5.44 ± 0.40
11.12.2023	4.08 ± 0.23	2.68 ± 0.25	2.29 ± 0.28	1.67 ± 0.05	2.81 ± 0.04
26.01.2024	1.75 ± 0.16	1.50 ± 0.16	1.34 ± 0.29	1.91 ± 0.17	1.87 ± 0.18
07.03.2024	2.51 ± 0.25	0.64 ± 0.05	2.51 ± 0.36	0.95 ± 0.05	1.10± 0.14

5 Discussion

Chlortoluron residues in the control variant

In the soil samples taken directly after the herbicide application on 16.11.2023, low Chlortoluron residues were also detected in the control variant. As shown in figure 11, the measured values are below 0.08 mg Chlortoluron per kg of soil. The standard deviation for plot 1E is comparatively so high, because of an outlier value. This can be seen in Appendix 2. Reasons for outlier values such as this may be due to insufficient homogenization of the soil samples, so that 1 of the 3 laboratory replicates contained more Chlortoluron. The measured values in all 5 field replicates should theoretically be 0, as no herbicide was applied in the control variant. The fact that small amounts of Chlortoluron were detected could be due to active substance residues in soil. 2 years previously, this field had been planted with spelt, which could also have been treated with the herbicide "CTU 700" or another herbicide, which contains the same active substance. Another reason could be that the field sprayer used for fertilizing and applying fungicides could have contained some residues of the active substance Chlortoluron. These could have been spread while fertilizing or spraying fungicides. Or the person pushing the bicycle sprayer could have spread the product on the other plots with the soles of their shoes by running after it. Contamination of the samples during soil sampling or laboratory work can be excluded, as the plots of the control variant were always examined first. Therefore, no carryover of the active substance is possible by the used equipment.

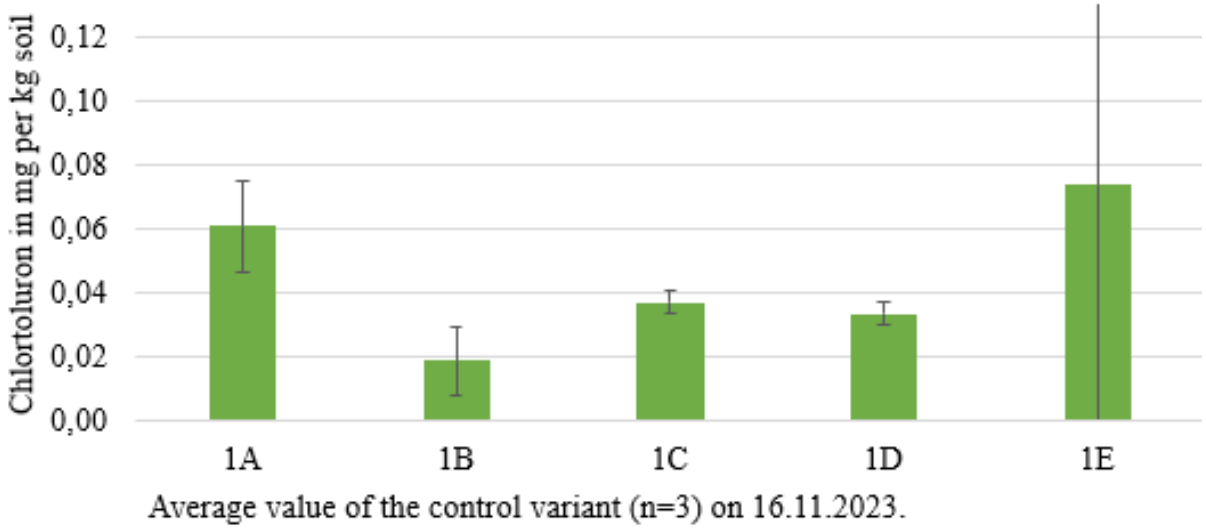


Figure 11 Chlortoluron residues in the control variant in mg per kg soil ± standard derivation of the 3-fold laboratory repetition, on 16.11.2023.

Chlortoluron residues in variant 8 over time

The degradation of Chlortoluron over a period of 3.5 months is shown for variant 8 in figure 12 and table 9. In these the mean values of the 5 field replicates with their standard deviations are represented, as well as the mean target concentration of 1.38 mg Chlortoluron per kg soil, which should have been detectable in the soil on the day of herbicide application.

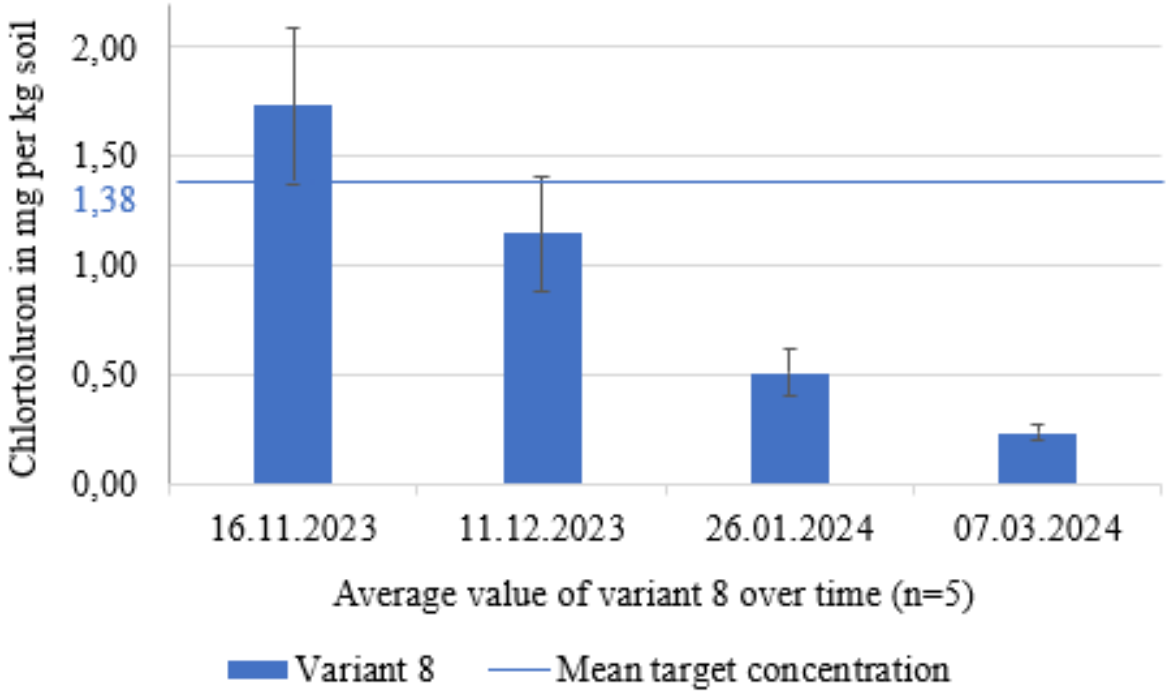


Figure 12 Average Chlortoluron residues of the 5 field replicas in variant 8 in mg per kg soil ± standard derivation over time.

When looking at the residues on the day of application (16.11.2023), it is noticeable that the mean target concentration was achieved and even exceeded by 0.35 mg per kg soil by the average Chlortoluron residues. However, when looking at the calculation table (Appendix 2), it becomes clear that the target concentration could only be achieved by calculating using the correction factor. In this table the extraction losses of the internal standard (Diuron) for each sample can be seen. These amount to 40% on average. Only with using the correction factor of 1.132, the Chlortoluron values of the individual samples could reach and even exceed their target concentrations. However, the correction factor is only an average value that was determined from previous studies. It is therefore possible that this may have caused Chlortoluron values that are higher than the target concentration of some samples. A solution for this calculation should be found for future investigations to avoid these high values. However, when looking at Appendix 2, there are some samples that did not reach the target

concentration for Chlortoluron. This could be due to the fact that the calculation is based on the surface area of the drill stick and the soil mass, among other things. However, the mass of soil extracted per 4 punctures with the drill stick varied considerably in some cases, so that the calculation using this procedure may not be entirely optimal. On the other hand, it is possible that the missing active substance was lost during the residue analysis. This could perhaps be due to the extraction or the solvent with which the active substances were extracted. Since Chlortoluron and Diuron are both very non-polar, it is possible that the acetonitrile/water mixture (50:50, v/v) is too polar for 100% extraction. However, various solvents were also tested in preliminary investigations, including diethyl ether, which is less polar. Nonetheless the result of these tests showed that acetonitrile/water (50:50, v/v) achieved the highest Chlortoluron residues. Further studies could therefore investigate whether the volume ratio of the solvents should be adjusted. For example, less water and more acetonitrile or more diethyl ether. This needs to be found out in further investigations. Another reason for the Chlortoluron residues, that were below or above the target concentrations, could be an uneven distribution of the herbicide. Since the herbicide was applied with a bicycle sprayer, it is possible, that the person pushing the sprayer could have caused operating errors (uneven driving speed, etc.), which can result in an uneven distribution of the herbicide. This could lead to lower or higher residues in some parts of the plot. Also shown in figure 12 is the fact that the Chlortoluron content in soil decreases over time at a single application rate of 3 l/ha and approaches zero. The standard deviations of the individual field repetitions also decrease over time. On 16.11.2023, the standard deviation is still comparatively highest at 0.36 mg per kg of soil, followed by 0.27 mg per kg of soil on 11.12.2023 (table 9). There could be several reasons why the Chlortoluron residues on these dates vary so greatly between the field replicates. On the one hand, these mean values are based on the values of the 3-fold laboratory replicate, which also showed high standard deviations, as described in the results section. These may have been caused by insufficient homogenization of the soil samples due to their high moisture content, as no drying was possible. That could lead to a different Chlortoluron content in each of the 3 weights of soil, that were taken for extraction. Other reasons for the deviations between the 5 field replicates could possibly be attributed to the herbicide application. As the “CTU 700” was not applied with a field sprayer, but with a bicycle sprayer, the error caused by human operation should not be underestimated. In particular, an uneven distribution due to an uneven driving speed or the sprayer boom being held at an angle. It is also possible for the herbicide to stick to the boots of the person pushing the bicycle sprayer. This can lead to an uneven distribution of the herbicide on the ground. In addition, the plants may have shielded the soil to varying

degrees, which can also result in varying amounts of Chlortoluron in soil. However, blocked sprayer nozzles can also cause an uneven spray pattern. The fact that the Chlortoluron residue of a plot, as described in the results section, does not remain constantly at the highest or lowest value over time can be explained by the fact that new soil samples were taken at each date. This means that not the same location in the plot was sampled. This can lead to different measurements in soil due to soil differences, the proportion of organic matter or uneven herbicide application. The fact that, as described above, the deviations between the field repetitions become smaller over the 3.5-month period is presumably due to the degradation of Chlortoluron in soil. As can be seen in table 9, the content in the soil approaches zero as time progresses (on 26.01.2024: 0.51 mg per kg of soil and on 07.03.2024: 0.23 mg per kg of soil). At an application rate of 3 l/ha, the active substance residues will therefore settle at a level of just above zero or zero, which also explains the low standard deviations (table 9).

Table 9 Average value of Chlortoluron of the field replicas in variant 8 in mg per kg soil ± standard derivation over time (n=5).

Date of soil sampling	Average value of Chlortoluron of the field replicas in mg per kg soil ± standard derivation
16.11.2023	1.73 ± 0.36
11.12.2023	1.15 ± 0.27
26.01.2024	0.51 ± 0.11
07.03.2024	0.23 ± 0.04

Chlortoluron residues in variant 9 over time

The mean Chlortoluron residues of variant 9 with the associated standard deviation and the expected mean target concentration of 3.02 mg Chlortoluron per kg of soil are shown in figure 13. The pure numerical values can be found in table 10. The graph shows that when 6 l/ha (twice the recommended amount) was applied, the mean target concentration was reached and exceeded. The average Chlortoluron residue achieved 3.09 mg per kg soil after the herbicide application. The reasons for this are again the calculation of the Chlortoluron value using the correction factor or uneven herbicide distribution. That only 2 out of 5 field replicates could reach the mean target concentration is again due to possible losses of active substance during the residue analysis, a not fully developed calculation of the target value and uneven herbicide application.

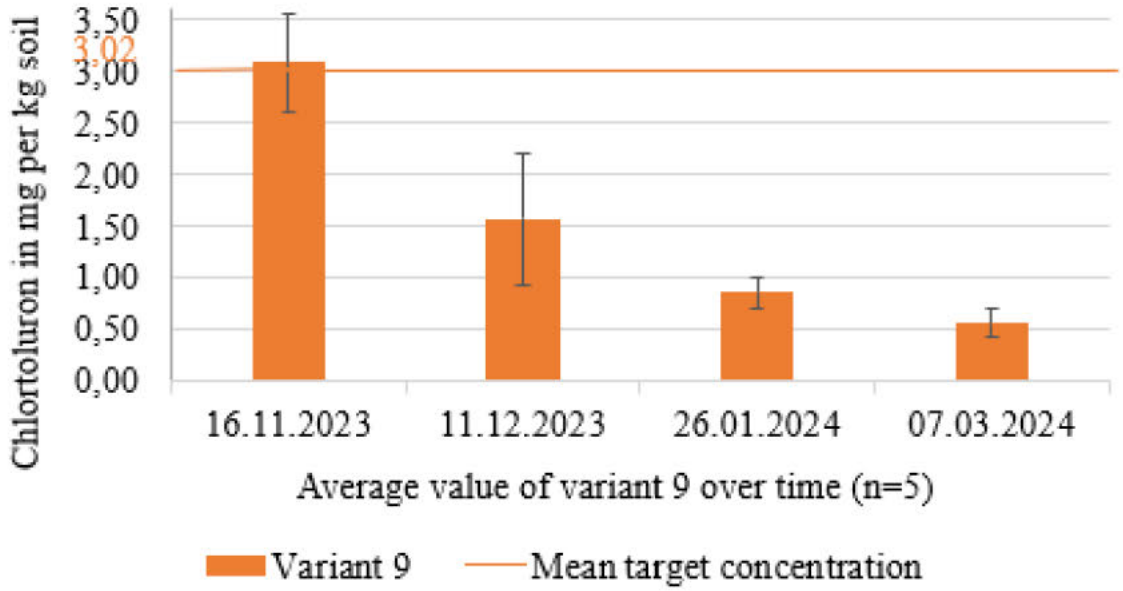


Figure 13 Average Chlortoluron residues of the 5 field replicas in variant 9 in mg per kg soil ± standard derivation over time.

It can be seen that both the Chlortoluron content in soil and the standard deviations decrease over a period of 3.5 months. On the first 2 dates, the field replicates show comparatively high standard deviations. The reason for this could again be uneven herbicide application on the plots and insufficient homogenization of the soil samples in the laboratory. On the last soil sampling date on 07.03.2024, there was still an average of 0.57 mg Chlortoluron in 1 kilogram of soil. The fact that the standard deviations become smaller over time is probably also due to the fact that the active substance levels out during degradation (table 10).

Table 10 Average value of Chlortoluron of the field replicas in variant 9 in mg per kg soil ± standard derivation over time (n=5).

Date of soil sampling	Average value of Chlortoluron of the field replicas in mg per kg soil ± standard derivation
16.11.2023	3.09 ± 0.48
11.12.2023	1.58 ± 0.64
26.01.2024	0.86 ± 0.15
07.03.2024	0.57 ± 0.13

Chlortoluron residues in variant 10 over time

The mean Chlortoluron residues in the soil and their standard deviations for variant 10 over time are shown in figure 14 and table 11. The residues measured on the day of application could exceed the mean target concentration of 5.36 mg Chlortoluron per kg soil. On this date the average value measured is 5.50 mg per kg soil and therefore 0.14 mg higher as expected. The same reasons for exceeding the mean target concentration apply here as for variant 8 and 9. The starting value of the plots treated with 12 l herbicide per hectare on 16.11.2023 is 5.50 mg Chlortoluron per kg soil. After 3.5 months, this drops to an average value of 1.51 mg per kg of soil. Likewise to variants 8 and 9, the Chlortoluron residues show higher standard deviations on the first 2 soil sampling dates, which then become less after approx. 40 days, on 26.01.2024.

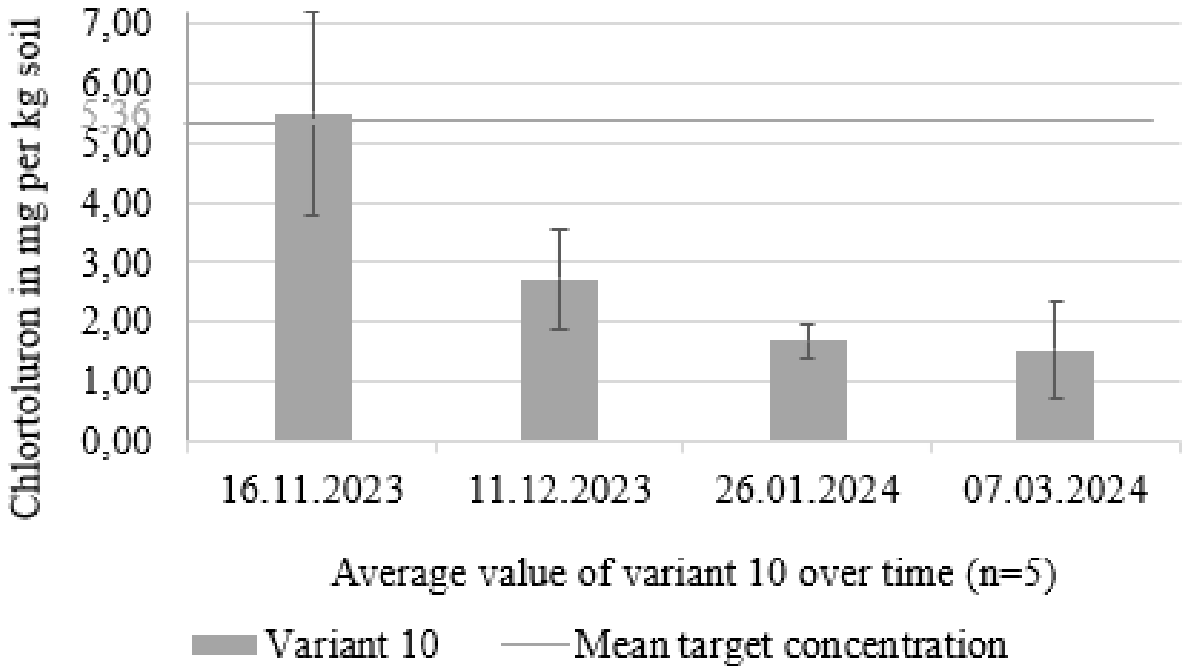


Figure 14 Average Chlortoluron residues of the 5 field replicas in variant 10 in mg per kg soil ± standard derivation over time.

However, in variant 10 the standard deviation of the measurements increases again on 07.03.2024. This is due to possible outlier values. There, 2 of the 5 field repetitions (10A, 10C) had achieved comparatively extremely high residues. As these values were even higher than the values obtained from the same plots on 26.01.2024, there is probably a human-induced error here. The soil samples on 07.03.2024 were taken by a different person than the soil samples taken on the other dates, which could have resulted in Chlortoluron residues in the samples from the A and C repetition. Despite these outliers, the trend for variant 10 is that the active

substance decreases over time in the soil and levels off at 1.51 mg Chlortoluron per kg of soil after 3.5 months.

Table 11 Average value of Chlortoluron of the field replicas in variant 10 in mg per kg soil ± standard derivation over time (n=5).

Date of soil sampling	Average value of Chlortoluron of the field replicas in mg per kg soil ± standard derivation
16.11.2023	5.50 ± 1.71
11.12.2023	2.71 ± 0.85
26.01.2024	1.68 ± 0.29
07.03.2024	1.51 ± 0.82

Chlortoluron residues of the three variants over time in comparison

When comparing the Chlortoluron residues of the 3 variants over time, the 3 different application rates of the herbicide are reflected in the soil samples examined (figure 15).

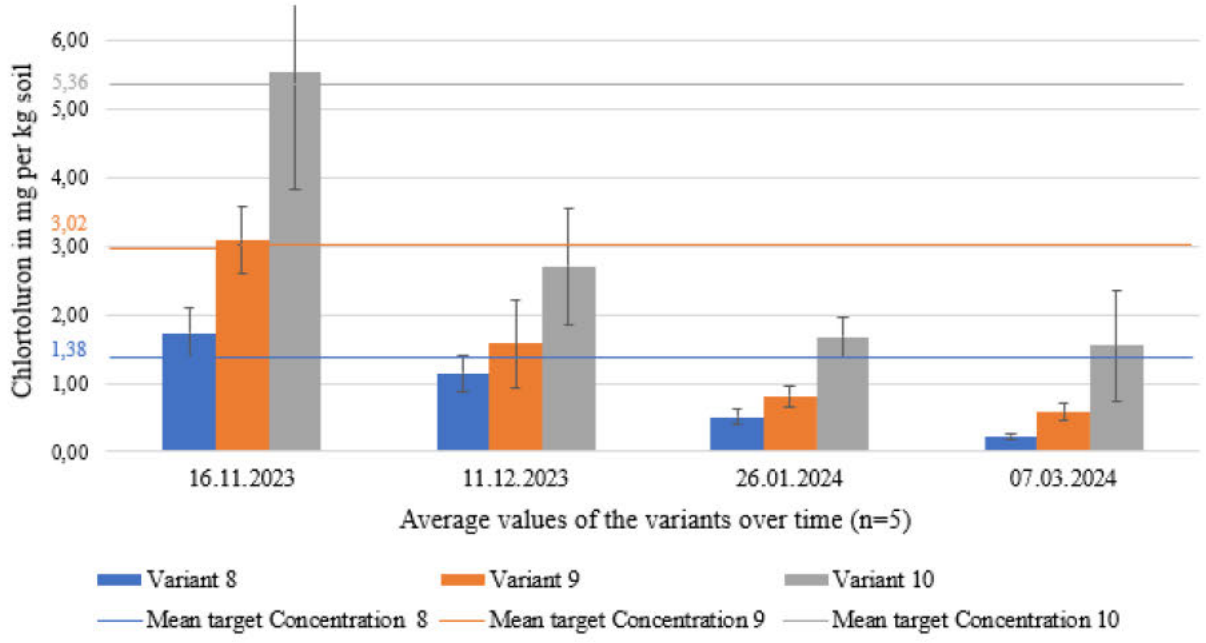


Figure 15 Average Chlortoluron residues of the 5 field replicas in all 3 variants in mg per kg soil ± standard derivation over time.

In the soil samples taken on 16.11.2023 directly after application, the gradation of the application rates can be clearly seen. In variant 8, 3 l/ha "CTU 700" was applied, in variant 9 6 l/ha and in variant 10 12 l/ha. This ratio (1:2:4) can be seen in the measured Chlortoluron residues. In variant 9, at 3.09 mg per kg of soil, approximately twice as much active substance

was measured as in variant 8 (1.73 mg per kg of soil). At 5.50 mg per kg of soil, variant 10 had approx. 4 times as much residue as variant 8 with 1.73 mg per kg of soil. This fact indicates that the chosen method for residue analysis is definitely suitable. The fact that the ratio of residues between the variants (1:2:4) in the subsequent soil samples is no longer as accurate as on the first soil sample date is presumably due to degradation in soil. Since this is strongly influenced by a variety of reasons, such as precipitation, temperatures, microorganism activity and soil type, it is logical that Chlortoluron in soil degrades to a greater or lesser extent over time. According to the weather service, a summed-up precipitation of 234 mm fell between the date of the first and last soil sampling [52], which could favor leaching into deeper soil layers than the 30 cm sampling depth. In combination with the KOC of Chlortoluron, which was described in the theoretical background of this study, this is a possible explanation. Chlortoluron has a KOC of 108 to 384, indicating that 108 to 384 ml of soil solution is needed to dissolve or transport 1 g of the active substance. The amount of precipitation could therefore result in the Chlortoluron residues to partly leach into deeper soil layers. However, over the period of 3.5 months, the trend remains visible that a higher application rate also leaves higher residues in soil. This is particularly noticeable when comparing the Chlortoluron residue of variant 10 on 07.03.2024 (1.51 mg per kg soil) with the Chlortoluron residue of variant 8 on 11.12.2023 (1.15 mg per kg soil). Here it can be seen that more Chlortoluron was detected in the soil after 3.5 months with 4 times the application rate of “CTU 700” than after 26 days with a single application rate. It can therefore be concluded that the more herbicide is sprayed, the more residues can be detected in soil. Furthermore, higher concentrations (application rate of 12 l/ha) remain in the soil for a longer period of time, while lower application rates of 3 or 6 l/ha show after 3.5 months residues tending towards 0 and just above. Here, further tests could be carried out by taking additional soil samples to check how long it takes for the Chlortoluron to be completely degraded at the application rates.

A comparison of the half-life period (DT50) of “CTU 700” (30 to 40 days) with the measured Chlortoluron residues shows that when applying 3 l/ha 66.18 % of the substance was still detectable in the soil after 26 days (table 12). As no measurements were taken after 40 days, but only after 70 days due to frequent snowfall and precipitation, no statement can be made as to whether only half of the herbicide was still detectable in the soil after 40 days. However, when looking at variants 9 and 10, where twice and 4 times the amount of herbicide was sprayed, it is noticeable that the DT50 of 30 days is the correct. At 6 l/ha (variant 9), 51.10 % of the active substance was still detected in the soil after 26 days, on 11.12.2023. At an

application rate of 12 l/ha, 49.02 % of the Chlortoluron was still in soil at the same time of measurement (table 12).

Table 12 Average Chlortoluron residues for the three variants over time in %, with the residues after 26 days highlighted in yellow (n=5).

Application rate	Residues in %			
	16.11.2023	**11.12.2023**	**26.01.2024**	**07.03.2024**
3 l/ha	100.00	66.18	29.19	13.23
6 l/ha	100.00	51.10	26.32	18.48
12 l/ha	100.00	49.02	30.33	27.96

The fact that comparatively more residue was detected in soil after 26 days with a single application (variant 8) than with the higher application rates can have various causes. Degradation in the soil can be influenced by the microorganism activity, the presence and absence of oxygen in the soil, pH values, temperature and available nutrient sources. For example, a higher proportion of organic material will lead to slower degradation of herbicides in soil. Since maize was grown on this field in the previous year and its harvest residues were incorporated into the soil, it is possible that some areas may contain higher harvest residues, which favored the slower degradation of Chlortoluron in variant 8. This partly uneven distribution of crop residues could be observed during soil sampling. However, another reason could again be uneven herbicide application in combination with sampling at different locations in the plots. When looking at the Chlortoluron residues as a percentage, it is also noticeable that the distance between the individual application rates increases over time. On 11.12.2023, variant 8 had the highest residues, followed by variant 9 and lastly variant 10. Over time, however, the reduction in residues after 40 days (26.01.2024) is approaching the same level (approx. 30%). 3.5 months after the application on 07.03.2024 it shows again that higher application rates leave higher residues in soil. This may be due to the change in weather. As described in the theoretical background of this study, the degradation of Chlortoluron is mainly microbial. Since microbial activity is for example influenced by moisture and temperature in soil, it can be assumed that the activity is limited in winter, because of damp and cold soil. This would explain, why the degradation of Chlortoluron is stuck on one level in January, but in March the gradation of the application rates in soil samples can be seen again, due to more active microorganism.

6 Conclusion

The present results allow the following conclusions to be drawn:

- The recommended application rate of 3 l/ha achieves a residue of 0.23 ± 0.04 mg Chlortoluron per kg soil after 3.5 months.
- Higher application rates of 6 and 12 l/ha lead to higher residues in soil 3.5 months after application (0.57 ± 0.13 and 1.51 ± 0.82 mg Chlortoluron per kg soil).
- From the results it can be deduced that higher application rates achieve higher concentrations in soil and therefore remain longer in soil. The effects of higher concentrations in soil and organisms and how long it takes for the different application rates to degrade fully could be investigated in further studies.
- The ratio of the application rates to each other (1:2:4) is detectable in the soil samples taken on the day of application. This ratio is lost over time, due to degradation of the active substance. However, the trend that higher application rates produce higher residues in the soil remains visible.
- The DT50 of 30 to 40 days specified by the "CTU 700" is correct for double and quadruple application rates. With a single application rate of 3 l/ha, however, approx. 66 % of the active substance is still detectable in the soil after 26 days. In order to obtain a more precise statement about the accuracy of the DT50, soil samples should be taken after 30 and 40 days in further investigations. This was not possible in this study due to snow and precipitation.
- With the selected method, only 60 % of the target concentration of the internal standard (Diuron) could be detected. This may be due to losses that occurred during the residue analysis or uneven herbicide application. With using the established correction factor for calculating the final Chlortoluron value, the corresponding target concentration could be exceeded in 30 out of 45 samples. Further tests could be carried out, to adjust the correction factor, the extraction method and the calculation of the target concentration of Chlortoluron, to achieve residues that do not exceed the target concentration, but are also not too far below.
- For similar or further studies, it is advisable not to apply the Plant protection products with a bicycle sprayer, but with a motorized field sprayer that is either operated by a tractor or is self-propelled. The reason for this is the uneven distribution of the sprayer, which could have led to the high standard derivations between the field replicas and therefore influences the results.

7 Summary

The use of herbicides is a frequently used method of weed control in modern agriculture. Precise knowledge of the persistence of the active substances used is important both for their long-term effect and for their impact on the environment. The aim of the study was therefore to use a prior established method for extracting different application rates of the phenylurea Chlortoluron from soil samples, with subsequent HPLC analysis. For this purpose, the herbicide "CTU 700" was applied in a field trial at the recommended application rate of 3 liters per hectare, but also at 6 and 12 liters per hectare. The soil samples, which were 30 cm deep, were taken at 4 different times over a period of 3.5 months, starting with the day of application in November and ending in March, to make a statement about the degradation of the active substance. In order to make a reliable statement, each variant was repeated 5 times in the field and 4 soil samples were taken per plot. For the residue analysis in the laboratory, each sample was repeated 3 times. The results of this study show that the residues of applying 3 l/ha amount to 0.23 mg Chlortoluron per kg soil after 3.5 months. The higher the application rates, i.e. 6 and 12 l/ha, the higher the residues in soil (0.57 and 1.51 mg per kg of soil). This means that the more herbicide is applied, the longer the active substance remains in soil. The effects of this increased amount of active substance in soil and how long it takes to be fully degraded could be investigated in further studies. The choice of method for residue analysis was appropriate as the ratio of the application rates (1:2:4) was visible in the soil samples on the day of application. However, individual parameters, such as the volume ratio of the solvent, could be tested in further studies, as the extraction losses of the internal standard were quite high with 40 %. The calculation of the target concentration for Chlortoluron and the inclusion of a correction factor should also be adjusted, as these resulted in Chlortoluron residues, that were higher than theoretically possible. In addition, the use of a bicycle sprayer probably led to an uneven distribution of the herbicide, which may have also influenced the results. Despite the potential for improvement, the chosen method managed to show the trend based on the pure HPLC values, and is therefore suitable.

Selbstständigkeitserklärung

Ich versichere, dass ich die vorliegende Arbeit selbstständig verfasst, in gleicher oder ähnlicher Fassung noch nicht in einem anderen Studiengang als Prüfungsleistung vorgelegt und keine anderen als die angegebenen Hilfsmittel und Quellen (einschließlich der angegebenen oder beschriebenen Software) benutzt habe.

Bernburg, den

M. Lambert .....

Melanie Lambert

8 References

[1] **Statista GmbH.** 2024. Inlandsabsatz von Herbiziden in Deutschland in den Jahren 1977 bis 2022.

https://de.statista.com/statistik/daten/studie/154717/umfrage/entwicklung-des-absatzes-von-herbiziden-in-deutschland/ , Access 28.12.2023.

[2] **Statista GmbH.** 2024. Inlandsabsatz von Fungiziden in Deutschland in den Jahren 1977 bis 2022.

https://de.statista.com/statistik/daten/studie/154729/umfrage/entwicklung-des-absatzes-von-fungiziden-in-deutschland/, Access 28.12.2023.

[3] **Statista GmbH.** 2024. Inlandsabsatz von Insektiziden, Akariziden und Synergisten in Deutschland in den Jahren 1977 bis 2022.

https://de.statista.com/statistik/daten/studie/154721/umfrage/entwicklung-des-absatzes-von-insektiziden-in-deutschland/, Access: 28.12.2023.

[4] **Oerke, E.-C.** 2006. Crop losses to pests. Journal of Agricultural Science 144, 31-43. Cambridge University Press.

https://www.nap-pflanzenschutz.de/fileadmin/SITE_MASTER/content/IPS/Integrierter_Pflanzenschutz/BZL_Heft_IntegrierterPflanzenschutz_Mai2018.pdf, Access: 28.12.2023.

[5] **Bayer CropScience Deutschland GmbH.** Herbizide.

https://agrar.bayer.de/Pflanzenschutz/Pflanzenschutzmittel/Herbizide/Wirkung%20und%20Funktion, Access: 18.06.2024.

[6] **Umweltbundesamt.** 2015. Brühl, C.A., Alscher, A., Hahn, M., Berger, G., Bethwell, C., Graef, F., Schmidt, T., Weber, B.: Protection of Biodiversity in the Risk Assessment and Risk Management of Plant protection products (Plant protection products & Biocides) with a Focus on Arthropods, Soil Organisms and Amphibians.

https://www.umweltbundesamt.de/publikationen/daten-zur-umwelt-2017-1, Access: 03.01.2024.

[7] **Haider, K**. 1996. Biochemie des Bodens, Ferdinand Enke Verlag, Stuttgart.

[8] **Umweltbundesamt**. 2022. Chemischer Zustand des Grundwassers.

https://www.umweltbundesamt.de/themen/wasser/grundwasser/zustand-des-grundwassers/chemischer-zustand-des-grundwassers, Access: 03.01.2024.

[9] **Bundesamt für Verbraucherschutz und Lebensmittelsicherheit**. 2019. Verbleib von Pflanzenschutzmitteln in der Umwelt.

psm_Folienserie_Naturhaushalt_Modul3_Verbleib.pptx (live.com), Access: 31.03.2023.

[10] **Statista GmbH**. 2021. Anteil der Proben mit Pflanzenschutzmittelrückständen in ausgewählten Lebensmittelgruppen in Deutschland im Jahr 2021.

https://de.statista.com/statistik/daten/studie/1343400/umfrage/pflanzenschutzmittelrueckstaende-lebensmitteln-produktgruppen/, Access: 03.01.2024.

[11] **Heinrich Böll Stiftung**. 2022. Pestizide im Wasser: Da schwimmt was mit.

https://www.boell.de/de/2022/01/12/pestizide-im-wasser-da-schwimmt-was-mit, Access: 03.01.2024.

[12] **Europäischen Parlaments und der Rat**. 23. Februar 2005. Verordnung (EG) Nr. 396/2005 über Höchstgehalte an Pestizidrückständen in oder auf Lebens- und Futtermitteln pflanzlichen und tierischen Ursprungs und zur Änderung der Richtlinie 91/414/EWG des Rates, ABl. L 70 vom 16.3.2005, S. 1.

[13] **Bayerische Landesanstalt für Landwirtschaft**. 2022. Nachbaurisiko bzw. Ausschluss von Nachbaukulturen beim Herbizideinsatz. https://www.lfl.bayern.de/mam/cms07/ips/dateien/herbizid_nachbaurisiko_22.pdf, Access: 02.01.2024.

[14] **Thier, H.-P., Frehse, H**. 1986. Rückstandsanalytik von Pflanzenschutzmitteln, Georg Thieme Verlag.

[15] **RÖMPP-Redaktion. Lindemann M.** 2015. Chlortoluron, RD-03-01596 in Böckler F., Dill B., Eisenbrand G., Faupel F., Fugmann B., Gamse T., Heretsch P., Matissek R., Pohnert G., Rühling A., Schmidt S., Sprenger G., RÖMPP [Online], Stuttgart, Georg Thieme Verlag.

https://roempp.thieme.de/lexicon/RD-03-01596 , Access: 03.01.2024.

[16] **Dehner Agrar**. 2021. Datenblatt „CTU 700“.

https://www.dehner-agrar.de/app/uploads/sites/2/2021/01/GA_CTU_700.pdf, Access: 03.01.2024.

[17] **Draber, W., Kluth, J.F., Tietjen, K., Trebst, A.** 1991. Herbizide in der Photosyntheseforschung.

https://onlinelibrary.wiley.com/doi/abs/10.1002/ange.19911031210?casa_token=DK9haQYUnIwAAAAA:ctmy-huAWfgR8Pq7A8U73Ho9ThxfBHuADKpLorqKvOQP8Vp3bd_HsHodjzqDVmBgqfyLGzJJ-fXoY7m5, Access: 03.01.2024.

[18] **Häder, D.-P**. 1991. Photosynthese. Georg Thieme Verlag, Stuttgart, New York 1999, ISBN 3-13-115021-1.

[19] **Bundesamt für Verbraucherschutz und Lebensmittelsicherheit**. 2023. Liste der zugelassenen Pflanzenschutzmittel in Deutschland.

https://www.bvl.bund.de/SharedDocs/Downloads/04_Pflanzenschutzmittel/psm_uebersichtsliste.pdf?__blob=publicationFile&v=24, Access: 03.01.2024.

[20] **Graham-Bryce, I.J**.1981.The Behaviour of Plant protection products in Soil, in The Chemistry of Soil Processes (Greenland, D.J., Hayes, M.H.B., Herausg.), John Willey and Sons Ltd., New York, S. 621-670.

[21] **Adams, Jr., R.S**.1973.Factors Influencing Soil Adsorption and Bioactivity of Plant protection products, Residue Rev. 47, 1-54.

[22] **Industrieverband Agrar e.V.** Drainageabfluss und Versickerung. Best Management Praktiken zur Verringerung der Einträge von Pflanzenschutzmitteln durch Drainageabfluss und Versickerung in Gewässern. https://www.iva.de/sites/default/files/benutzer/%25uid/publikationen/04ansicht_rz_book_dt_240420_web.pdf, Access: 18.05.2024.

[23] **Nufarm Deutschland GmbH**. 2022. Sicherheitsdatenblatt “Lentipur 700“.

https://www.raiffeisen.com/agrar_sdb/33637/f3eead1b1098e65a3f1a46e31a742025?variante=, Access: 18.05.2024.

[24] **Haider, K.** 1996. Biochemie des Bodens, Ferdinand Enke Verlag, Stuttgart.

[25] **Schwack, W., Anastassiades, M., Scherbaum, E.** 2003. Multimethoden versus Wirkstoffvielfalt. Rückstandsanalytik von Pflanzenschutzmitteln. Aus Chem. Unserer Zeit, 2003, 37, 324 – 335. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

https://onlinelibrary.wiley.com/doi/pdf/10.1002/ciuz.200300298, Access: 09.06.2024.

[26] **Hämmann, M., Desaules, A.** 2003. Probenahme und Probenvorbereitung für Schadstoffuntersuchungen in Böden, Herausgegeben vom Bundesamt für Umwelt, Wald und Landschaft, Bern.

[27] **Bayerische Landesanstalt für Landwirtschaft**. 2019. Institut für Ökologischen Landbau, Bodenkultur und Ressourcenschutz: Anleitung zum Ziehen von Nmin-Probe.

https://www.lfl.bayern.de/mam/cms07/iab/dateien/anleitung_ziehen_von_nmin_bodenproben_.pdf, Access: 12.01.2024.

[28] **Drescher, N., Weinmann, W.D**.1979. Entnahme und Vorbereitung von Bodenproben, DFG-Methodensammlung, Abschnitt IX.

[29] **Kape. H.-E., von Wulffen, U., Roschke, M.** 2009. Hinweise zur Probenahme von Boden, Pflanzen und Düngemitteln. Herausgegeben von der Landesanstalt für Landwirtschaft, Forsten und Gartenbau des Landes Sachsen-Anhalt.

https://llg.sachsen-anhalt.de/fileadmin/Bibliothek/Politik_und_Verwaltung/MLU/LLFG/Dokumente/04_themen/pfl_ernaehr_duengung/pn_broschuere_lsa_09.pdf, Access: 09.06.2024.

[30] **Barrenstein, A., Bartels, U., Böhlefeld, T., Conrady-Pigorsch, R., May, M., Müller, I., Raudschus, M., Schäfer, K**. 2006. Vorbereitung von Feststoffproben für die chemische Untersuchung. Merkblatt 54. Herausgegeben von dem Landesumweltamt Nordrhein-Westfalen, Essen.

https://www.lanuv.nrw.de/fileadmin/lanuv/abfall/pdf/merk54.pdf, Access: 09.06.2024.

[31] **Mettler Toledo**. Siebanalyse. Digitalisieren und automatisieren Sie Arbeitsabläufe zur Bestimmung der Korngrößenverteilung.

https://www.mt.com/de/de/home/applications/Laboratory_weighing/sieve-analysis.html, Access: 09.06.2024.

[32] **Labor Datenbank**. 2024. Bsp: Berechnung der Trockensubstanz und Glühverlust.

https://labordatenbank.de/manual_page/75, Access: 09.06.2024.

[33] **Paul, G.U., Marcellin, Y.K., Soumahoro, S.N., Albert, T**. 2022. Health Risks of Triazine and Phenylurea Herbicides in Sediments of M'Badon Bay, Abidjan (Cote d'Ivoire). Chemistry Africa 5, 1139–1151.

https://link.springer.com/article/10.1007/s42250-022-00394-5#citeas, Access: 09.06.2024.

[34] **Kocarek, M., Kodesova, R., Kozak, J., Drabek, O.** 2010. Field Study of Chlortoluron Transport and its Prediction by the BPS Mathematical Model.

https://www.researchgate.net/publication/241700169_Field_Study_of_Chlortoluron_Transport_and_its_Prediction_by_the_BPS_Mathematical_Model, Access: 14.01.2024.

[35] **Gressner, A., M., Arndt, T**. 2019. Lexikon der Medizinischen Laboratoriumsdiagnostik, 3. Auflage, Springer Verlag.

[36] **Dao, T.H., Lavy, T.L., Dragun J**.1983.Rationale of the Solvent Selection for Soil Exraction of Plant protection product Residues, Residue Rev. 87, 91-104.

[37] **Smith, A.E., Milward, L.J**.1983. Comparison of Solvent Systems for the Extraction of Diclofop Acid, Picloram, Simazine, and Triallate from Weathered Field Soils, J. Agric. Food Chem. 31, 633-637.

[38] **Deutsches Institut für Normung e.V.** 2005. Bodenbeschaffenheit - Bestimmung von Herbiziden - Hochleistungsflüssigkeitschromatographie mit UV-Detektion (ISO 11264:2205).

[39] **Spektrum.** Lexikon der Chemie: Filter.

https://www.spektrum.de/lexikon/chemie/filter/3327, Access: 09.06.2024.

[40] **Thomas Seilnacht**. Filtrieren.

https://www.seilnacht.com/versuche/filtrier.html, Access: 09.06.2024.

[41] **Hoffmann, M., Gärtner, H.** 2010. Chemie Formeln, Gesetze und Fachbegriffe. Compact Verlag. ISBN 978-3-8174-7892-7, S. 454.

[42] **Gressner, A.M., Arndt, T.** 2005. Springer Lexikon. Klinische Chemie. Medizinische Labordiagnostik von A bis Z. Springer-Verlag. ISBN 978-3-540-23660-3, S. 360.

[43] **Kintek Solution.** 2024. Was Ist Das Funktionsprinzip Eines Rotationsverdampfers?

https://de.kindle-tech.com/faqs/what-is-the-functional-principle-of-a-rotary-evaporator, Access: 09.06.2024.

[44] **Cammann, K**. 2001. Instrumentelle analytische Chemie: Verfahren, Anwendungen und Qualitätssicherung. Spektrum Akademischer Verlag GmbH, Heidelberg, Berlin. ISBN 3-8274-0057-0.

[45] **Antec Scientific**. Was ist HPLC?

https://antecscientific.com/de/produkte/techniken/was-ist-hplc/# , Access: 09.06.2024.

[46] **Bundesanstalt für Materialforschung und -prüfung.** Chromatographische Verfahren.

https://netzwerke.bam.de/Netzwerke/Content/DE/Downloads/Nike/chromatographie.pdf?__blob=publicationFile, Access: 09.06.2024.

[47] **Rotard, W**. Technische Universität Berlin, Vorlesung Umweltchemie. Zu finden unter:

Bundesanstalt für Materialforschung und -prüfung: Chromatographische Verfahren.

https://netzwerke.bam.de/Netzwerke/Content/DE/Downloads/Nike/chromatographie.pdf?__blob=publicationFile, Access: 09.06.2024.

[48] **Hug, H**. 2015. Instrumentelle Analytik. Theorie und Praxis. Verlag Europa-Lehrmittel, Nourney, Vollmer GmbH & Co. KG. ISBN 978-3-8085-7219-0.

[49] **Reichelt Chemietechnik GmbH + Co.** HPLC – Eine Methode für die biochemische Forschung.

https://www.rct-online.de/magazin/hplc-biochemische-forschung/, Access: 09.06.2024.

[50] **Shimadzu Deutschland GmbH.** Partikelgrößenbestimmung von HPLC-Säulenmaterial.

https://shimadzu-laborwelt.de/application/partikelgroessenbestimmung-von-hplc-saeulenmaterial/, Access: 09.06.2024.

[51] **Brodacz, W**. 2018. C18-Phasen in der HPLC: Arbeitspferde zwischen Dauergalopp und Kollaps.

https://www.labo.de/chromatographie/c18-phasen-in-der-hplc------arbeitspferde-zwischen-dauergalopp-und-kollaps.htm, Access: 09.06.2024.

[52] **DLG e.V.** Standort/Betrieb.

https://www.dlg-ipz.de/de/ueber-uns/standort, Access: 09.06.2024.

[53] **Bundesministerium für Ernährung und Landwirtschaft**. 2019. 100er Boden – bestbewerteter Boden in Deutschland.

https://www.bmel.de/DE/themen/landwirtschaft/pflanzenbau/bodenschutz/boden100er.html , Access: 07.02.205.

[54] **WetterKontor GmbH**. Wetterrückblick Bernburg an der Saale (Anhalt).

https://www.wetterkontor.de/de/wetter/deutschland/rueckblick.asp?id=N548&datum0=23.02.2024&datum1=07.03.2024&jr=2024&mo=10&datum=14.03.2024&t=2&part=2 , Access: 25.11.2024.

9 Appendix

Appendix 1: Short report of the HPLC of sample 8A.1 from the 16.11.2023.

Short Report (ESTD)

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Data file:	C:\Chem32\1\Data\BA_Lambert 2024-11-08 11-17-49\016-1901.D
Sample name:	8A.1
Description:	

Instrument:	HPLC_02	Location:	16
Injection date:	11/8/2024 7:40:58 PM	Injection:	1 of 1
Acq. method:	BA_Lambert.M	Injection volume:	50.000
Analysis method:	BA_Lambert.M	Acq. operator:	SYSTEM
Last changed:	11/8/2024 11:15:04 AM		

Sample amount:	0.000	Sample type:	Sample
Multiplier:	1	Dilution:	1
Calib. data modified:	11/5/2024 1:24:43 PM	Lims ID:	

Column name:
Serial #:

Sample related custom fields:

Compound related custom fields:

Name
Chlortoluron
Diuron

Short_ESTD.rdl [Rev. 1] Printed: 11/11/2024 10:28:30 AM Page 1 of 3

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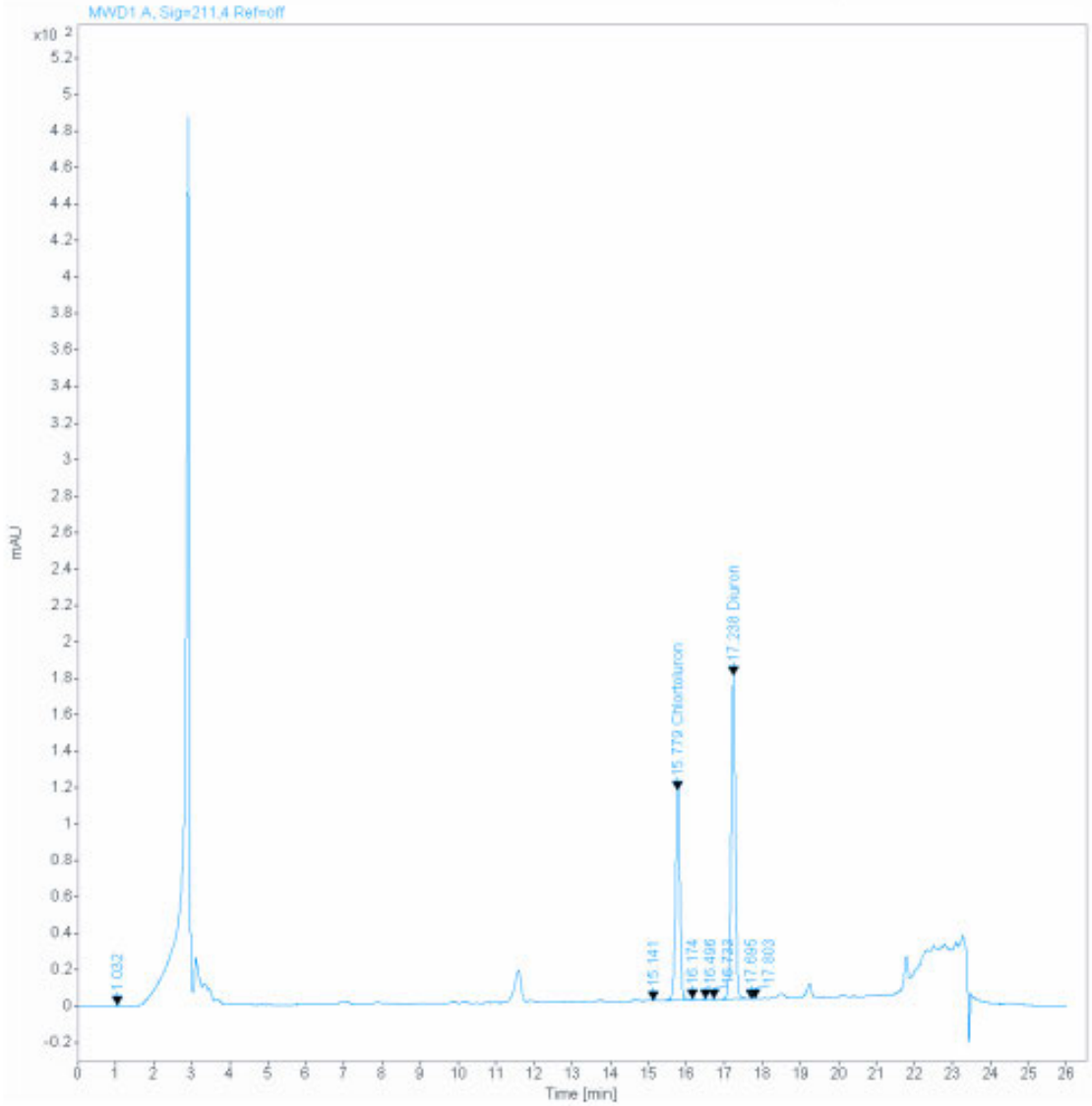

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Percent report based on Area

Signal: MWD1 A, Sig=211,4 Ref=off

Name	RT [min]	RF	Area	Amount [µg/ml]	Group
Chlortoluron	15.779	0.00230	921.1277	2.11623	
Diuron	17.238	0.00277	1369.7999	3.79208	
			Sum	5.9083	

Group Summary:

Group	Area	Amount	Unit

Summed Peaks:

Name	Start Time [min]	End Time [min]	Total Area	Amount

Appendix 2: Table with data based on soil samples from 16.11.2023. The final Chlortoluron residue is highlighted in green.

Sample number	Initial weight in g	Dry matter (DM) in %	Dry Soil mass in g (80.63 % DM)	HPLC-residues		Calculated on 1 g soil (100% DM) (incl. factor 10 for concentration and factor 25 for extraction volume)		Correction of chlortoluron with internal standard diuron and the factor 1.132		Extraction loss of Diuron in %	Target values chlortoluron µg/g soil (100% dry matter)	Deviation of chlortoluron from the target value in %
				Chlortoluron in µg/ml	Diuron in µg/ml	Chlortoluron in µg/g soil	Diuron in µg/g soil	Chlortoluron in µg/g soil	Target concentration of Diuron in µg/g soil			
1A.1	5,01	80,63	160,32	0,13	3,70	0,05	1,53	0,08	2,48	38,33	0,00	
1A.2	5,00	80,63	160,32	0,09	3,56	0,04	1,47	0,05	2,48	40,67	0,00	
1A.3	5,01	80,63	160,32	0,09	4,00	0,04	1,65	0,05	2,48	33,33	0,00	
1B.1	5,02	80,63	150,31	0,04	3,70	0,02	1,52	0,02	2,47	38,33	0,00	
1B.2	5,01	80,63	150,31	0,01	3,40	0,00	1,40	0,01	2,48	43,33	0,00	
1B.3	5,04	80,63	150,31	0,04	2,90	0,02	1,19	0,03	2,46	51,67	0,00	
1C.1	4,99	80,63	136,34	0,06	3,80	0,02	1,57	0,04	2,49	36,67	0,00	
1C.2	5,01	80,63	136,34	0,06	3,80	0,02	1,57	0,04	2,48	36,67	0,00	
1C.3	5,01	80,63	136,34	0,07	3,74	0,03	1,54	0,04	2,48	37,67	0,00	
1D.1	4,99	80,63	151,16	0,06	3,68	0,02	1,52	0,04	2,49	38,67	0,00	
1D.2	5,04	80,63	151,16	0,06	3,70	0,02	1,52	0,04	2,46	38,33	0,00	
1D.3	5,01	80,63	151,16	0,05	3,70	0,02	1,53	0,03	2,48	38,33	0,00	
1E.1	5,00	80,63	125,73	0,03	3,78	0,01	1,56	0,02	2,48	37,00	0,00	
1E.2	5,00	80,63	125,73	0,32	3,82	0,13	1,58	0,19	2,48	36,33	0,00	
1E.3	5,04	80,63	125,73	0,03	3,71	0,01	1,52	0,02	2,46	38,17	0,00	
8A.1	4,99	80,63	159,52	2,12	3,79	0,88	1,57	1,24	2,49	36,83	1,34	7,13
8A.2	5,04	80,63	159,52	2,52	3,61	1,03	1,48	1,50	2,46	39,83	1,34	-11,92
8A.3	5,02	80,63	159,52	3,13	3,72	1,29	1,53	1,84	2,47	38,00	1,34	-37,57
8B.1	5,02	80,63	158,73	2,61	3,80	1,07	1,56	1,52	2,47	36,67	1,35	-12,94
8B.2	5,00	80,63	158,73	2,32	3,80	0,96	1,57	1,36	2,48	36,67	1,35	-0,79
8B.3	5,03	80,63	158,73	2,15	3,79	0,88	1,56	1,25	2,47	36,83	1,35	7,03
8C.1	5,01	80,63	143,10	3,40	3,74	1,40	1,54	2,00	2,48	37,67	1,49	-33,96
8C.2	5,00	80,63	143,10	4,31	3,73	1,78	1,54	2,55	2,48	37,83	1,49	-70,38
8C.3	5,01	80,63	143,10	3,63	3,54	1,50	1,46	2,19	2,48	41,00	1,49	-46,81
8D.1	5,01	80,63	154,57	2,64	3,67	1,09	1,51	1,57	2,48	38,83	1,38	-13,40
8D.2	5,02	80,63	154,57	3,03	3,36	1,25	1,38	1,87	2,47	44,00	1,38	-35,17
8D.3	5,04	80,63	154,57	2,93	3,79	1,20	1,55	1,70	2,46	36,83	1,38	-23,14
8E.1	5,03	80,63	154,16	2,99	3,78	1,23	1,55	1,74	2,47	37,00	1,39	-25,74
8E.2	4,99	80,63	154,16	3,29	3,61	1,36	1,50	1,98	2,49	39,83	1,39	-42,62
8E.3	5,01	80,63	154,16	2,75	3,64	1,13	1,50	1,64	2,48	39,33	1,39	-18,27
9A.1	5,01	80,63	153,95	3,84	3,68	1,58	1,52	2,28	2,48	38,67	2,78	17,97
9A.2	5,02	80,63	153,95	4,58	3,85	1,89	1,59	2,65	2,47	35,83	2,78	4,53
9A.3	5,03	80,63	153,95	5,64	3,79	2,32	1,56	3,28	2,47	36,83	2,78	-18,27
9B.1	5,02	80,63	134,27	5,06	3,70	2,08	1,52	2,99	2,47	38,33	3,18	6,16
9B.2	5,03	80,63	134,27	3,88	3,72	1,59	1,53	2,28	2,47	38,00	3,18	28,37
9B.3	5,02	80,63	134,27	4,85	3,74	2,00	1,54	2,85	2,47	37,67	3,18	10,53
9C.1	5,01	80,63	135,90	5,87	3,90	2,42	1,61	3,38	2,48	35,00	3,15	-7,50
9C.2	5,02	80,63	135,90	6,04	3,93	2,49	1,62	3,46	2,47	34,50	3,15	-9,95
9C.3	5,00	80,63	135,90	5,84	3,85	2,41	1,59	3,39	2,48	35,83	3,15	-7,89
9D.1	5,03	80,63	137,21	5,66	3,68	2,33	1,51	3,34	2,47	38,67	3,12	-7,33
9D.2	4,99	80,63	137,21	6,79	3,85	2,81	1,59	3,95	2,49	35,83	3,12	-26,90
9D.3	5,03	80,63	137,21	5,05	1,85	2,08	0,76	3,70	2,47	69,17	3,12	-18,76
9E.1	5,02	80,63	148,27	5,06	3,34	2,08	1,38	3,13	2,47	44,33	2,88	-8,53
9E.2	5,04	80,63	148,27	5,14	3,94	2,11	1,62	2,93	2,46	34,33	2,88	-1,53
9E.3	5,04	80,63	148,27	4,60	3,62	1,89	1,48	2,73	2,46	39,67	2,88	5,18
10A.1	5,03	80,63	167,46	5,48	3,14	2,25	1,29	3,47	2,47	47,67	5,11	32,09
10A.2	5,02	80,63	167,46	3,71	1,51	1,53	0,62	2,82	2,47	74,83	5,11	44,73
10A.3	5,01	80,63	167,46	3,84	1,51	1,58	0,62	2,93	2,48	74,83	5,11	42,68
10B.1	5,01	80,63	152,66	12,35	4,02	5,10	1,66	7,00	2,48	33,00	5,60	-24,97
10B.2	4,99	80,63	152,66	10,08	3,66	4,18	1,52	6,02	2,49	39,00	5,60	-7,48
10B.3	5,04	80,63	152,66	12,10	3,81	4,96	1,56	7,01	2,46	36,50	5,60	-25,23
10C.1	5,01	80,63	156,46	13,99	3,78	5,77	1,56	8,19	2,48	37,00	5,46	-49,87
10C.2	5,04	80,63	156,46	10,62	2,59	4,36	1,06	7,16	2,46	56,83	5,46	-30,99
10C.3	5,01	80,63	156,46	13,42	3,87	5,54	1,60	7,76	2,48	35,50	5,46	-42,05
10D.1	5,04	80,63	154,11	8,42	3,58	3,45	1,47	5,03	2,46	40,33	5,55	9,33
10D.2	5,03	80,63	154,11	5,63	1,62	2,31	0,67	4,23	2,47	73,00	5,55	23,84
10D.3	5,01	80,63	154,11	7,93	3,84	3,27	1,58	4,61	2,48	36,00	5,55	16,99
10E.1	5,01	80,63	167,54	9,89	4,00	4,08	1,65	5,62	2,48	33,33	5,10	-10,13
10E.2	5,03	80,63	167,54	9,88	3,85	4,06	1,58	5,71	2,47	35,83	5,10	-11,84
10E.3	4,99	80,63	167,54	8,06	3,39	3,34	1,40	4,98	2,49	43,50	5,10	2,36