

# Analysis of nicosulfuron residues in maize field soil by high-performance liquid chromatography

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## RESEARCH ARTICLE

### Abstract

Nicosulfuron is a sulfonylurea herbicide which is applied to control many grasses and broadleaf weed species in maize fields. Carryover or the persistence of nicosulfuron in soil can influence crops in succession even at low concentrations. An experiment was set up to investigate the persistence of nicosulfuron residual used in maize field soil at 80 and 160 active ingredient (ai)/ha application rates at 28 days after sowing to control weeds. The soil was analysed for nicosulfuron residues in surface and subsurface soil by high-performance liquid chromatography (HPLC) using a photo diode array detector. Nicosulfuron residues were dissipated rapidly in the soil surface as compared to subsurface soil. The presence of residues found in the subsurface soil showed the mobility of nicosulfuron into lower layers. At 80 ai/ha, residues were not detected after 60 days. The half-life of nicosulfuron by HPLC was found to be at the range of 14-20 days at different doses and soil depths.

**Keywords:** maize, nicosulfuron, HPLC, residue, half-live

### 1. Introduction

Maize (*Zea mays* L.) is one of the major important cereal crops in Iran. In 2012, the total estimated cultivated area of maize in the world was 178.5 million ha; with a total production of 872 million tons and the global average yield of 4.9 ton/ha (FAOSTAT, 2012). In 2012, the total harvested area of lentil in Iran was 415,000 ha with an average yield of 5.8 ton/ha (FAOSTAT, 2012). The presence of weeds leads to tremendous losses to maize yield (Hartlye and Popay, 1992; Moeching *et al.*, 1999). There is a broad spectrum of grasses and broadleaved weeds that infests maize fields including Jimsonweed (*Datura stramonium* L.), common cocklebur (*Xanthium strumarium* L.) (Karimmojeni *et al.*, 2010); Redroot pigweed (*Amaranthus retroflexus* L.), common lambsquarters (*Chenopodium album* L.), common purslane (*Portulaca oleracea* L.) (Zaremozhadieh and Ghadiri, 2011); velvetleaf (*Abutilon theophrasti* Medik.), Canada thistle (*Cirsium arvense* (L.) Scop.), field bindweed (*Convolvulus arvensis* L.), johnsongrass (*Sorghum halepense* (L.) Pers.), barnyardgrass (*Echinochloa crus-galli* (L.)

Beauv.), purple nutsedge (*Cyperus rotundus* L.), large crabgrass (*Digitaria sanguinalis* (L.) Scop.), and foxtail (*Setaria* spp.) (Mousavi, 2001). Weed control in maize fields in Iran is dependent mainly on chemical methods. A broad range of herbicides with different mechanisms of action has been registered to control weeds in maize in Iran including pre-plant incorporated application of atrazine plus allachlor, and S-ethyl N, N-dipropylthiocarbamate (EPTC), and post-emergence application of (2,4-dichlorophenoxyacetic acid (2,4-D) plus 2-methyl-4-chlorophenoxyacetic acid (MCPA) (Hadizadeh *et al.*, 2006; Mousavi, 2001), nicosulfuron, foramsulfuron, rimsulfuron and nicosulfuron + rimsulfuron (Baghestani *et al.*, 2007). Sulfonylurea herbicides are widely used to control weeds in agricultural systems, especially maize fields. Owing to their high selectivity and low persistence in the environment, they are identified as 'new formulation herbicides' (Sarmah and Sabadie, 2002). Among the sulfonylurea herbicides, nicosulfuron has been registered for weed control in maize. Nicosulfuron [2-[(4,6-dimethoxypyrimidin-2-ylcarbonyl)sulfamoyl]-N,N-dimethylnicotinamide], is a post-emergence herbicide

which is applied at low use rates to control many grasses and broadleaf weed species by maize growers in Iran (Baghestani *et al.*, 2007). This herbicide is rapidly absorbed into the weed leaves and is translocated via the xylem and phloem towards the meristematic zone. It inhibits plant metabolism by inhibiting acetolactate synthase, a crucial enzyme for the biosynthesis of the branched chain amino acids, valine, leucine, and isoleucine, resulting in cessation of cell division and plant growth (Heap, 2000).

The length of time when a herbicide remains active in soil is called 'soil persistence'. It is desirable for the chemicals to control weeds during the season of application; it is not desirable for them to persist and affect subsequent crop growth. Herbicides vary in their potential to persist in soil. Nicosulfuron residues have been reported in soil, surface waters, and some crops. Low volatility and photodegradation as well as long persistence of nicosulfuron under certain conditions are of great concerns because low concentration of this herbicide may affect growth of subsequent vegetation (Si *et al.*, 2004). Nicosulfuron is a weak acid with a pKa of 4.3; its water solubility and ionisation increases with increasing pH (Figure 1). Microbial degradation and chemical hydrolysis are the most critical processes determining the fate of nicosulfuron in nature (Berger and Wolfe, 1996). Soil pH, temperature, moisture and organic matter are the main factors affecting sulfonylurea chemical hydrolysis and microbial degradation (Sondhia and Singhai, 2008). Soil pH plays a critical role on the hydrolysis of sulfonylurea in the soil (Hultgren *et al.*, 2002). Numerous analytical methods have developed in recent years to detect the herbicide rates in the soil, both available and unavailable to the plants including high-performance liquid chromatography (HPLC) with either UV or mass spectrometry/MS detection systems (Lian *et al.*, 1996), enzyme-linked immunosorbent assay (ELISA) (Hollaway *et al.*, 1999), capillary electrophoresis (Berger and Wolfe, 1996) and liquid chromatography/mass spectroscopy (Marek and Koskinen, 1996).

Understanding the behaviour of nicosulfuron in the soil using HPLC method can help in making better decisions about accurate application rates and recropping restrictions.

To the best of our knowledge, little or no experiment has been performed to detect nicosulfuron in soil. The objective of this study was to evaluate the persistence of nicosulfuron herbicide applied in maize field soil using HPLC technique.

## 2. Materials and methods

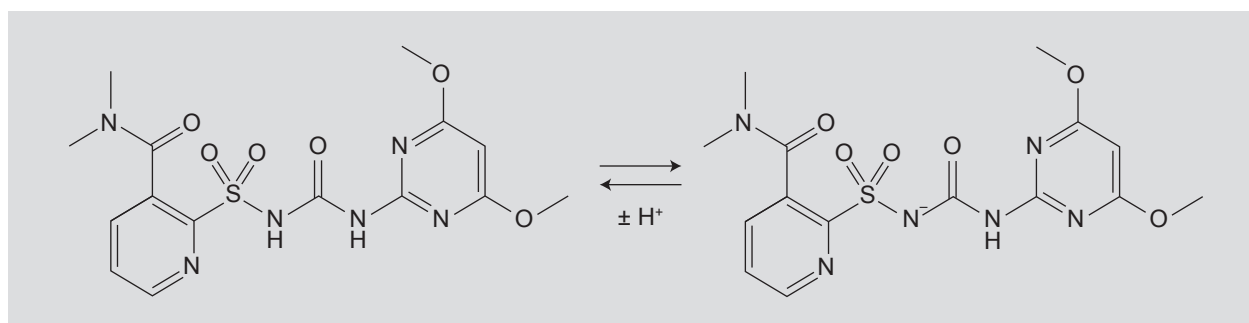
### Field experiments

A field trial was carried out during 2013 at Alashtar County, Lorestan, Iran. The elevation of the experimental area is 1,600 m above sea level. The coordinates of the site are 35°34' N, 22°33' E. The experiment was carried out in a soil characterised as sandy loam. The physicochemical properties of soil are presented in Table 1. The field at the test site had lain fallow in preceding year of study. To prepare the seedbed deep ploughing (20-25 cm) was carried out with a mouldboard plough in autumn followed by disking in the spring. The soil fertility was improved by applying di-ammonium phosphate (18-46-0 N-P-K) and urea at the rate of 250 and 150 kg/ha, respectively, in spring before planting. Moreover, 200 kg/ha N (as urea) was added at the 6-8 leaf growing stage of maize along with irrigation. Maize, variety single cross 704, was planted on 22 June 2013.

**Table 1. Physicochemical properties of the soil of the experimental site at Alashtar, Iran.**

Parameters	0-15 cm	15-30 cm
Clay (%)	35	35
Silt (%)	40	40
Sand (%)	25	25
texture	Clay loam	Clay loam
Organic C (%)	0.8	0.75
pH	7.5	7.5
EC (ds/m) <sup>1</sup>	2.6	2.6
Available N (%)	0.06	0.04
Available P2O5 (mg/kg)	11.4	11.0
Available K (mg/kg)	195	195

<sup>1</sup> EC = soil electrical conductivity in deciSiemens per meter.



**Figure 1. Chemical structure of nicosulfuron herbicide (Green and Hale, 2005).**

The experimental design was a randomised complete block with four replications. Nicosulfuron SC 4% g/l (Cruze<sup>®</sup>; Santa Cruz biotechnology, Dallas TX, USA) was applied as a post-emergence herbicide at 2 l/ha (recommended dose) and 4 l/ha (double recommended dose) with a knapsack sprayer using flat fan nozzle at four-leaf stage. Three plots were sprayed with water without any herbicide and considered as control. No other herbicide was applied to control weeds in this experiment. The experimental area was divided into 9 plots of 10×4 m size with a buffer of 1 m between adjacent plots to avoid spray overlap.

### Sampling and storage

Soil samples were collected randomly throughout each plot from 0-15 and 15-30 cm depths using a tube auger 0 (2 h), 3, 7, 15, 30, 60, 90 and 125 days after treatment. Samples were air dried and ground to pass through a 10 mm sieve, mixed thoroughly and 100 g subsamples were taken from each plot for HPLC analysis. Samples were kept in a deep freezer at -20 °C until analysis.

### High-performance liquid chromatography

#### Soil extraction and clean-up

Nicosulfuron from soil samples (2.5 g) was extracted according to a simplified QuEChERS approach (Pinto *et al.*, 2010). The QuEChERS procedure included fewer treatment stages of the sample, while the final procedure is simpler, faster, and cheaper which minimises the errors associated with this step (Pinto *et al.*, 2010). Briefly, 2.5 g of soil sample was weighed in a 15 ml glass centrifuge tube with screw cap, which keeps the tube closed for most of the processes of sample preparation, thus avoiding as much as possible losses of volatile compounds during this stage. 1.5 ml of ultrapure water was added to each tube in order to make pores in the sample more accessible to the extraction solvent and to homogenise water content in different soil samples and the contents were mixed thoroughly for 1 min with a vortex device. 2.5 ml of ethyl acetate (extraction solvent) was added to each tube containing soil and the content was shaken again for 1 min. Then, 1 g of magnesium sulphate was added and the content was shaken for 1 min as quick as possible to prevent formation of MgSO<sub>4</sub> conglomerates. The tube was centrifuged at 5,000 rpm for 7 min.

The supernatant was transferred carefully to another glass centrifuge tube. To remove matrix components in the clean-up step, 3 mg of graphitised carbon black (GCB) and 25 mg of primary secondary amine were added to each tube. Then, the content was shaken for 2 min and centrifuged at 5,000 rpm for 7 min. Finally; the organic portion was kept for quantitative analysis of nicosulfuron-methyl by HPLC.

### Chemicals

Analytical-grade nicosulfuron (99% purity) was supplied by Sigma-Aldrich (Steinheim, Germany). The structure of this herbicide is shown in Figure 1. All solvents including acetonitrile, ethyl acetate and methanol were HPLC grade and purchased from Merck (Darmstadt, Germany). Magnesium sulphate anhydrous, GCB and primary secondary amine were purchased from Sigma-Aldrich.

#### Preparation of standard curve by high-performance liquid chromatography

A stock solution (1000 µg/ml) of nicosulfuron was prepared in acetonitrile and different concentrations (including 0.01, 0.05, 0.1, 0.5, 1.0, 5.0 and 10.0 µg/ml) of nicosulfuron were made by diluting the stock solution. 20 µl of each standard solution was injected into the HPLC and the peak area measured. Each run was repeated three times and the calibration curve was created by drawing the known concentrations of nicosulfuron on the *x*-axis and the average peak area corresponding to each concentration on the *y*-axis (Figure 2).

#### Apparatus conditions

Nicosulfuron was detected with a HPLC equipped with a photodiode array detector, a C18 column (250 mm × 4 mm ID), a mobile phase of acidic water + acetonitrile + *o*-phosphoric acid, 20+80+0.1 (v/v), and a flow rate of 1 ml/min, a UV-detector set at a wavelength of 240 nm. 20 µl was injected for each standard solution and the retention time was 5.9 min. Samples were filtered before injection by a 0.22 µm membrane using a Millipore filtration syringe (Merck Millipore, Billerica, MA, USA).

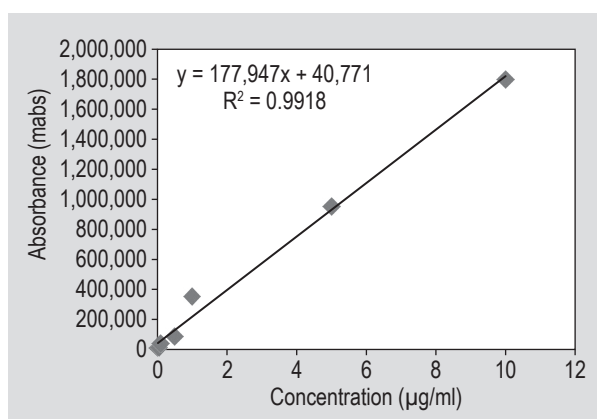


Figure 2. Calibration curve of nicosulfuron at concentrations 0.01-10 µg/ml.

### Statistical analysis

Dissipation data of nicosulfuron were fitted to Equation 1 (SigmaPlot Version 12, Systat Software Inc., San Jose, CA, USA):

$$f = \frac{a}{1 + \exp(-(x-x_0)/b)} \quad (1)$$

Where the parameter *a* is maximum dissipation of herbicide, *b* is the slope of the curve around the  $X_0$  and  $X_0$  donates time required for 50% dissipation. Calibration curve of nicosulfuron was plotted by software Excel 2007 (Microsoft, Redmond, WA, USA).

## 3. Results and discussions

### Calibration curve

Different concentrations of nicosulfuron (0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10 µg/ml), prepared from the standard solutions of nicosulfuron, were injected into HPLC and the calibration curve was drawn based on concentration of nicosulfuron versus corresponding peak. The calibration curve was linear with a best-fit regression equation of  $y = 177,947x + 40,771$  and  $R^2=0.9918$  (Figure 2).

### Recovery and limit of quantification

The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. It was determined based on a signal-to-noise approach: determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of

analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal-to-noise ratio is 10:1. The accuracy and precision of the method was established by recovery tests by extraction and analysis of five replicates at three different levels (0.01, 0.05, 0.1 mg/kg). The average recoveries of nicosulfuron in soil were varied from 78 to 85%. The limit of quantification of nicosulfuron from soil was detected to be 1 µg/kg.

### Persistence of nicosulfuron in maize field soil

The results of extracted herbicide concentration in the soil for different rates of nicosulfuron from 0-15 cm soil depth are shown in Table 2. Results of the nicosulfuron dissipation in 0-15 cm soil depth of maize field displayed that initial concentration of nicosulfuron residues in the surface soil depth (0-15 cm) were 42.9 and 85.2 µg/kg at the recommended rate of application (2 l/ha) and double recommended dose (4 l/ha), respectively. The residues of nicosulfuron in the surface soil dissipated to 36.8 µg/kg at recommended dose and 71.1 µg/kg at double dose on day 3. On day 0, 15.2 and 22.5 µg/kg residues were found in the subsurface soil at recommended and double doses, respectively, which decreased to 13.8 and 19.6 µg/kg at by day 3.

After 7 days, the residues were 30.2 µg/kg at recommended dose and 59.0 µg/kg at double dose in the surface soil which became 21.0 µg/kg and 42.6 µg/kg, respectively, at the recommended and double doses. At the recommended and double rates of application the dissipation of nicosulfuron were 50.9 and 50.1% after 15 day in in the surface soil (0-15 cm) which increased up to 74.8 and 78% by the 30<sup>th</sup> day. Residues in the subsurface soil (15-30 cm) on 15<sup>th</sup> day were

**Table 2. Determination of nicosulfuron residues at 0-15 cm depth (subsurface) in field soil at Alashtar, Iran by high-performance liquid chromatography.**

Time (days)	2 l/ha nicosulfuron application		4 l/ha nicosulfuron application	
	Residue remaining ± SD (µg/kg) <sup>1</sup>	% dissipation	Residue remaining ± SD (µg/kg) <sup>1</sup>	% dissipation
0	42.9±0.035	0	85.2±0.020	0
3	36.8±0.04	14.2	71.1±0.035	16.5
7	30.2±0.025	29.4	59.0±0.05	30.7
15	21.0±0.03	50.9	40.6±0.038	52.3
30	10.8±0.045	74.7	18.7±0.025	78.1
60	4.1±0.2	90.4	7.4±0.048	91.3
90	BDL <sup>2</sup>		BDL	
125	BDL		BDL	

<sup>1</sup> Average of three replicates; SD = standard deviation.

<sup>2</sup> BDL = below detectable level (1 µg/kg).

detected to be 7.5 µg/kg at recommended dose and 12.5 µg/kg at double dose, decreased to 4.5 and 7.5 µg/kg after 30 days with 70.4 and 66.6% dissipation (Table 3).

On day 60, residues in the surface soil were 4.1 and 7.4 µg/kg at the recommended and double doses, respectively. No residues of nicosulfuron were found in the surface soil (0-15 cm) on days 90 and 125 at recommended dose and double rate of application. At day 60 after application, nicosulfuron residues were found to be below detectable level at recommended rate, however, 3.0 µg/kg residues were detected at double rate in the subsurface soil depth (15-30 cm) (Table 2 and 3).

As observed, the rate of herbicide dissipation was higher in the surface soil depth (0-30 cm) compared to subsurface soil (15-30 cm) (Figure 3).

According to sigmoidal model parameters (Table 4),  $X_0$  values, which is the half-life of nicosulfuron herbicide and estimated from the dissipation time curves, were different between the soil depths and herbicide doses (Table 4). Lower  $X_0$  values indicate longer persistence of herbicide. Accordingly, soil persistence of nicosulfuron was longer in the subsurface soil depth, so that the half-lives of nicosulfuron at recommended and double dose were found to be 14.76 and 20.03 in 0-15 soil depth and 15.85 and 18.60

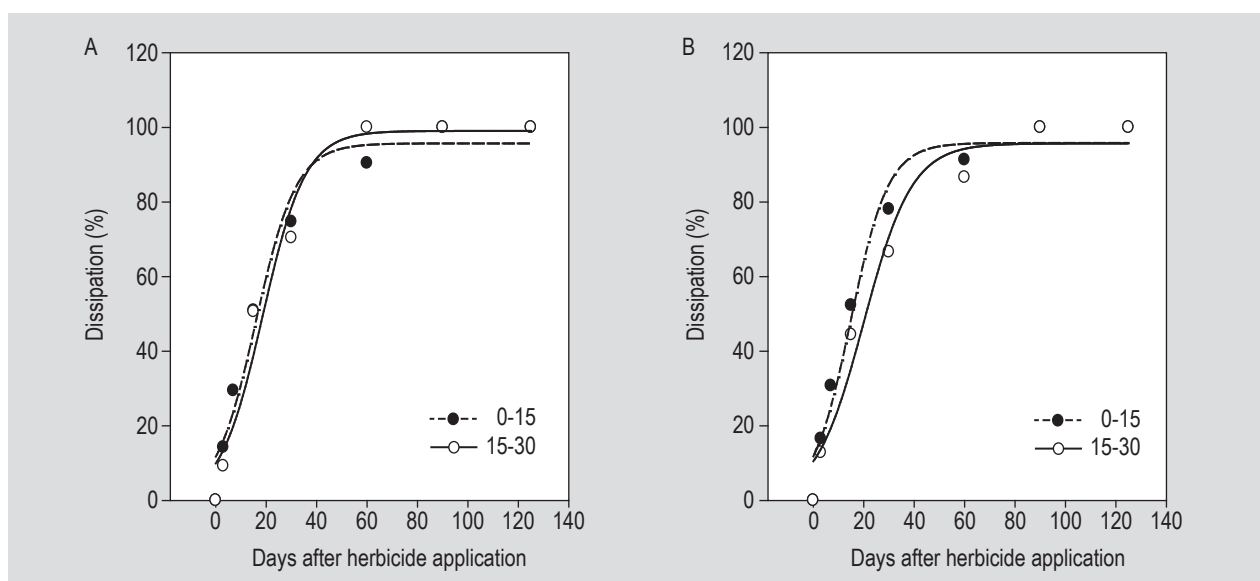
**Table 3. Determination of nicosulfuron residues at 15-30 cm depth (subsurface) in field soil at Alashtar, Iran by high-performance liquid chromatography.**

Time (days)	2 l/ha nicosulfuron application		4 l/ha nicosulfuron application	
	Residue remaining ± SD (µg/kg) <sup>1</sup>	% dissipation	Residue remaining ± SD (µg/kg) <sup>1</sup>	% dissipation
0	15.2±0.035	0	22.5±0.045	0
3	13.8±0.025	9.2	19.6±0.035	12.8
7	NA <sup>2</sup>		NA	
15	7.5±0.045	50.6	12.5±0.028	44.4
30	4.5±0.045	70.4	7.5±0.028	66.6
60	BDL <sup>3</sup>		3.00±0.05	86.6
90	BDL		BDL	
125	BDL		BDL	

<sup>1</sup> Average of three replicates; SD = standard deviation.

<sup>2</sup> NA = not analysed.

<sup>3</sup> BDL = below detectable level (1 µg/kg).



**Figure 3. Dissipation patterns of nicosulfuron at two depths at recommended dose (A) and double dose (B) by three parameters sigmoidal model.**

**Table 4.** The three-parameter sigmoidal model to determine dissipation time of nicosulfuron herbicide at surface and subsurface soils at Alashtar, Iran.<sup>1,2</sup>

Parameters	Surface soil (0-15 cm)		Subsurface soil (15-30 cm)	
	2 l/ha	4 l/ha	2 l/ha	4 l/ha
Maximum dissipation of herbicide (a)	95.84±4.26	95.71±5.23	95.72±4.56	99.06±5.26
The slope of the curve around the X <sub>0</sub> (b)	7.52±7.52	9.57±2.45	8.05±1.80	8.47±2.16
Time required for 50% dissipation (X <sub>0</sub> )	14.76±14.76	20.03±3.02	15.85±2.24	18.60±2.74
R <sup>2</sup>	0.98	0.98	0.98	0.98
Root mean square error	0.96	0.95	0.96	0.95
P	0.0001	0.0009	0.0001	0.0008

<sup>1</sup>  $f = \frac{a}{1 + \exp(-(x-x_0)/b)}$ ; R<sup>2</sup>=0.98.

<sup>2</sup> Values ± standard error.

days in 15-30 cm soil depth (Table 4). This difference could be attributed to higher organic matter and consequently increased microbial activity in the surface soil than 15-30 cm soil depth. Previous studies have shown that herbicides phytotoxicity and persistence in the soil was affected by the soil organic matter content and soil texture (Hance, 1984; Kookana *et al.*, 1998; Rahman and James, 2002; Rahman and Matthews, 1979). However, the soil behaviour of certain herbicides, for example those belonging to triazine and sulfonylurea groups, is also influenced markedly by soil pH levels (Brown, 1990; Kookana *et al.*, 1998).

#### 4. Conclusions

The present study revealed that residues of nicosulfuron could not be detected by HPLC in the surface (0-15 cm) and subsurface soil on days 90 and 125. According to Martins and Mermoud (1999) the degradation and movement of sulfonylurea herbicides are closely related to soil solution pH. Soil pH is found to be the key characteristic affecting the degradation rate of sulfonylurea herbicides. Degradation of sulfonylurea herbicides in acidic soils is faster due to enhanced chemical hydrolysis occurring at low pH levels (Beyer *et al.*, 1988). Regarding that the pH of the experimental field soil was 7.5, this may be the reason that nicosulfuron was not found on 90 and 125 days at recommended and double rates.

It has been demonstrated that the half-life of sulfonylurea herbicides in different soils noticeably depended on pH, temperature, texture, moisture and organic matter contents of the soil (Blacklow and Pheloung, 1991; Sarmah *et al.*, 1999). In the present study, the half-lives of nicosulfuron ranged from 14-20 days at different doses and soil depths.

Detection of traces of nicosulfuron herbicide by HPLC in the subsurface soil indicates its mobility into lower layers. Presence of sulfosulfuron residues in the subsurface soil under a wheat cropping system has been reported by Sondhia and Singhai (2008).

Injury potential from carryover of nicosulfuron depends on the availability of the herbicide to plants at phytotoxic levels, the sufficient persistence of herbicide in soil, and the susceptibility of rotational crops to the residual concentration of a herbicide remaining in the soil one year after herbicide application. Residues of nicosulfuron were detected up to 60 days in this study, indicating there is minimal risk of carry-over to subsequent rotational crops when the planting occurred after 60 days after nicosulfuron application. Further studies need to be carried out to for a better understanding of the behaviour of nicosulfuron in different soils and climates. Furthermore, similar research is needed to determine the residues of other herbicides that are applied to weed control in maize fields.

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