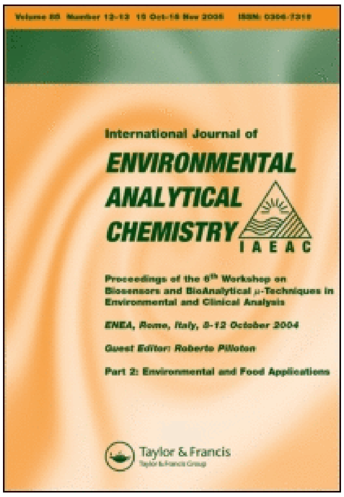

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Detection and Identification of the Herbicide Isoproturon and its Metabolites in Field Samples After a Heavy Rainfall Event

J. Schuelein[ab]; W. E. Glaessgen[c]; N. Hertkorn[a]; P. Schroeder[c]; H. Sandermann Jr[cd]; A. Kettrup[ab]
[a] Institut für Okologische Chemie, Neuherberg, Oberschleiβheim, Germany [b] Lehrstuhl für Okologische Chemie und Umweltanalytik der TU Weihenstephan, Freising, Germany [c] Institut für Biochemische Pflanzenpathologie, Neuherberg, Oberschleiβheim, Germany [d] GSF-Forschungszentrum für Umwelt und Gesundheit GmbH, Freising, Germany

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DETECTION AND IDENTIFICATION OF THE HERBICIDE ISOPROTURON AND ITS METABOLITES IN FIELD SAMPLES AFTER A HEAVY RAINFALL EVENT

J. SCHUELEIN[1,3*], W. E. GLAESSGEN[2], N. HERTKORN[1],
P. SCHROEDER[2], H. SANDERMANN, JR[2] and A. KETTRUP[1,3]

GSF-Forschungszentrum für Umwelt und Gesundheit GmbH; [1]Institut für Ökologische Chemie, Neuherberg, D-85764 Oberschleißheim, Germany; [2]Institut für Biochemische Pflanzenpathologie, Neuherberg, D-85764 Oberschleißheim, Germany; [3]Lehrstuhl für Ökologische Chemie und Umweltanalytik der TU Weihenstephan, Braufakultätsgebäude, D-85350 Freising, Germany

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The occurence of isoproturon [N-(4-isopropylphenyl)-N`, N`-dimethyl urea] and its metabolites was investigated in soil solution, runoff and creek water from a farm in Scheyern (Bavaria, FRG) after a heavy rainfall event following the application in spring 1994. The analytical procedure included enrichment by C 18 solid-phase extraction and reversed-phase HPLC with diode array detection. The major degradation product in most of the samples was identified as 2-hydroxy-isoproturon [N-(4-(2-hydroxyisopropyl)-phenyl)-N`, N`-dimethyl urea] using the authentic compound. The latter has been isolated from wheat cell culture medium and its structure elucidated by NMR and MS. Furthermore, the polar metabolites monodesmethyl-isoproturon [N-(4-Isopropylphenyl)-N`-methyl urea] and 2-hydroxy-monodesmethyl-isoproturon [N-(4-Isopropylphenyl)-N`-methyl urea] and 2-hydroxy-monodesmethyl-isoproturon [N-(4-(2-hydroxyisopropyl)-phenyl)-N`-methyl urea] were identified by comparison of their retention times and DAD-UV spectra with reference compounds.

The detection of isoproturon in soil solution down to 170 cm depth and in creek water in concentrations exceeding 4 µg/l and also of the polar metabolites in concentrations up to 0,9 µg/l indicated the mobility of this phenylurea herbicide and its degradation products. Therefore the metabolites should be considered in studies on the migration of isoproturon and in the ecotoxicological evaluation.

KEY WORDS: Isoproturon, metabolites, mobility, rainfall, soil solution, surface water.

INTRODUCTION

Isoproturon [N-(4-isopropylphenyl)-N’,N’-dimethyl urea], a substituted phenyl urea herbicide, is used for pre- and post-emergence control of weeds in wheat. This compound has attained increasing environmental relevance in recent years; in Bavaria it was found as contaminant in several groundwater wells[1], although the GUS (Groundwater Ubiquity Score)-Index, derived from DT50- and K_{OC}-values, classifies isoproturon between “leacher” and “non-leacher”[2]. Local conditions (drainages, macropore- and preferential flow, geomorphology) however, and date and intensity of

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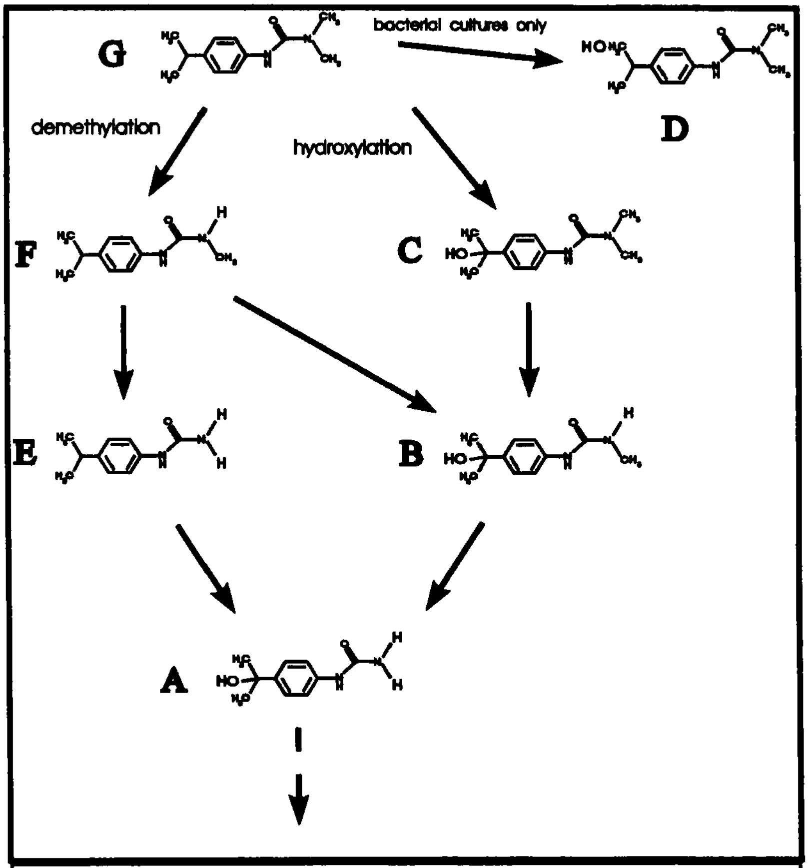
* Correspondence addresses.

precipitations after application can mobilize isoproturon, with the consequence that it contaminates deeper soil layers, ground- and surface water[3–5].

The assessment of the environmental hazard potential of xenobiotics has to take into account the toxicity, mobility and persistence of both the active ingredient and possible metabolites. Therefore, compounds metabolized in soil and plants[6] have to be considered as well as isoproturon (Figure 1).

In order to evaluate the mobility of isoproturon and the significance of its metabolites we investigated different field samples (soil solution, runoff and creek water) obtained during and after a heavy rainfall event in April 1994 a few days after application.



A: **2-hydroxy-didesmethyl-isoproturon** [N-(4-(2-hydroxyisopropyl)-phenyl) urea]
B: **2-hydroxy-monodesmethyl-isoproturon** [N-(4-(2-hydroxyisopropyl)-phenyl)-N'-methyl urea]
C: **2-hydroxy-isoproturon** [N-(4-(2-hydroxyisopropyl)-phenyl)-N',N'-dimethyl urea]
D: **1-hydroxy-isoproturon** [N-(4-(1-hydroxyisopropyl)-phenyl)-N',N'-dimethyl urea]
E: **didesmethyl-isoproturon** [N-(4-isopropylphenyl) urea]
F: **monodesmethyl-isoproturon**[N-(4-isopropylphenyl)-N'-methyl urea]
G: **isoproturon** [N-(4-isopropylphenyl)-N',N'-dimethyl urea]

Figure 1 Pathways for the biodegradation of isoproturon in soil.

EXPERIMENTAL

Sampling methods

Samples were taken from the agricultural area of FAM (Forschungsverbund Agrarökosysteme München) in Scheyern (Figure 2). This site is located 40 km north of Munich, FRG. Its geomorphology is strongly structured.

Surface water samples were continuously collected by automatic sampling systems (Model 2900 from ISCO, Lincoln, USA) at several sites along the course of two small creeks, adjacent to field 19 and 20, respectively.

Soil solution samples from 125 cm and 170 cm depth were collected by tensiometer-controlled suction cups (UMS, Munich, FRG), installed horizontally in shaft K2 nearby field 17. During the heavy rainfall additional soil solution passed through drainage pipes installed for protection of the suction pipes in 50 and 90 cm depth of shaft 2.

Runoff was sampled directly by a ladle on field 17 and in containers, which were connected to drainage pipes including the hydrological catchment of field 15 and 17.

Analytical methods

Samples were filtered through glass fibre filters (from Schleicher, Schuell, Dassel, FRG) and then enriched on C18-cartridges (J. T. Baker, Phillipsburg, USA). The analytes were

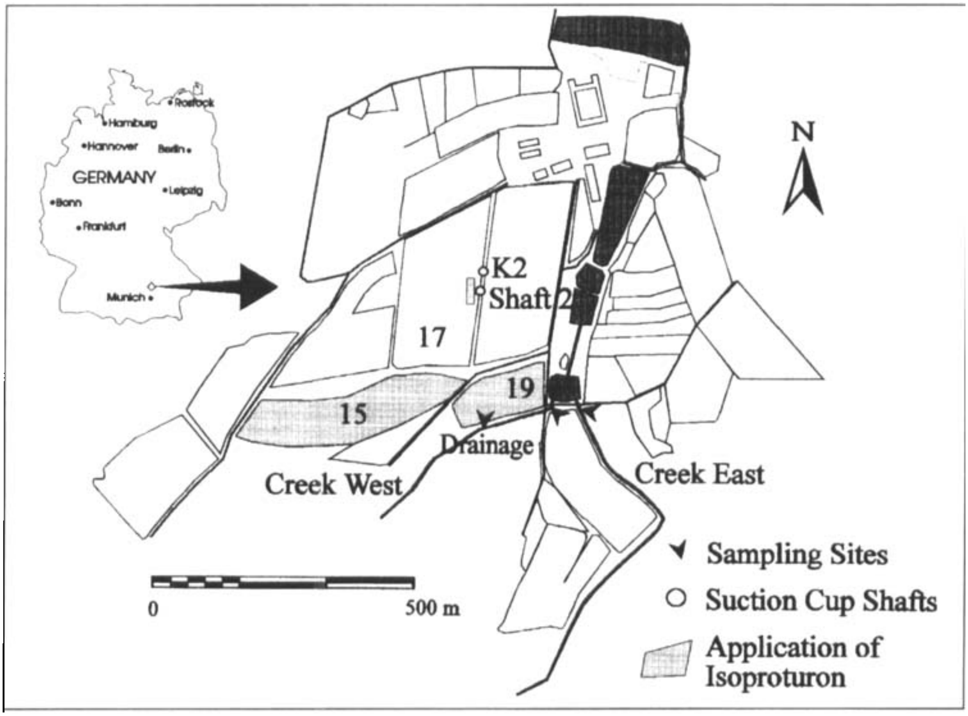


Figure 2 Sampling sites at the agricultural area in Scheyern, FRG.

eluted three times with 1 ml tetrahydrofurane. The solvent was then allowed to evaporate under a stream of nitrogen. The residue was dissolved in 1 ml acetonitrile/water (1:1) and analyzed by Reversed Phase High Pressure Liquid Chromatography (Table 1).

The electrospray mass spectrum of the isolated 2-hydroxy-isoproturon was kindly conducted by Perkin-Elmer (Weiterstadt, FRG).

The proton NMR spectrum was recorded with a Bruker AC 400 spectrometer operating at 400.13 MHz proton frequency.

Identification

Isoproturon and its possible metabolites A, B, D, E, F (Figure 1) were available as standard substances (from Ciba-Geigy, Basel, Switzerland and Dr. Ehrenstorfer, Augsburg, FRG). The metabolite 2-hydroxy-isoproturon (C) was isolated from wheat cell culture medium—after incubation with isoproturon for six days—by using solid-phase extraction and semi-preparative HPLC (Merck system; column: LiChrospher 100 RP-18, 10 μm, 250*10 mm). This compound hasn't been taken into account in degradations studies[7,8] and analytical methods[9] yet.

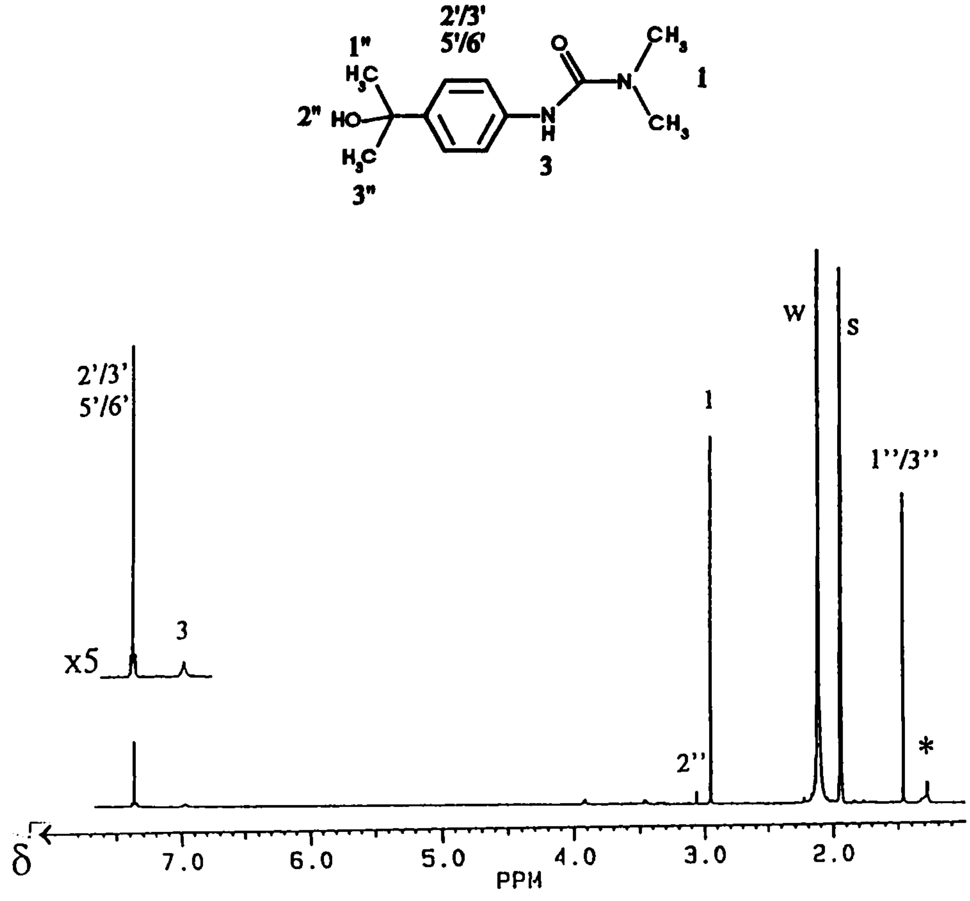
The structure of 2-hydroxy-isoproturon (C) was elucidated with ^{1}H-NMR and MS. This compound shows two pairs of methyl singlet resonances at 2.94 and 1.46 ppm, respectively, one proton at N3, and a singlet corresponding to a single proton resonating at 3.03 ppm (Figure 3). This resonance did not show chemical exchange on the NMR time scale and remained persistent on prolonged storage in CD_3CN at room temperature. Therefore the singlet at 3.03 ppm was attributed to a tertiary hydroxy function bound to the central C atom of an isopropyl moiety.

Table 1 Recoveries, relative standard deviations and chromatographic conditions for analyzing isoproturon and its metabolites.

	Rec. (%)	*RSD (%)*
Isoproturon	98	2,5
Monodesmethylisoproturon	94	7,5
Didesmethylisoproturon	87	8,4
2-Hydroxyisoproturon	82	3,0
2-Hydroxy-monodesmethylisoproturon	20	7,8

HPLC-system with diode-array-detection (190-367 nm) by Varian GmbH (Darmstadt, FRG)
Column: LiChrospher 100 RP-18, 5μm, 250*4 mm, (Merck, Darmstadt, FRG)
Flow rate: 1 ml/min
Mobile phase: acetonitrile (A)/aqueous potassium dihydrogen phosphate buffer 1 mM, pH = 6,5 (B)
Gradient program:

Time (min)	*A (%)*	*B (%)*
0	5	95
15	35	65
30	60	40
35	100	0
45	100	0



7.34	(4H,	AA'BB',	H_{ar})
6.95	(1H,	s (br),	NH)
3.03	(1H,	s,	OH)
2.94	(6H,	s,	NCH_3)
2.10	(water)		
1.46	(6H,	s,	CH_3)

Figure 3 ^{1}H-NMR-spectrum and chemical shifts δ [ppm] in CD_3CN of 2-hydroxy-iso proturon, recorded with a Bruker AC 400 spectrometer operating at 400, 13 MHz proton frequency.

The electrospray mass spectrum of the isolated compound was recorded by Perkin-Elmer and showed a protonated molecular ion at m/z 223 ($[M + H]^+$) in the positive mode indicating a molecular mass of 222.

Field samples were analyzed by above mentioned HPLC-method (Figure 4). Isoproturon and its metabolites were verified by using retention times and UV-spectra (Figure 5).

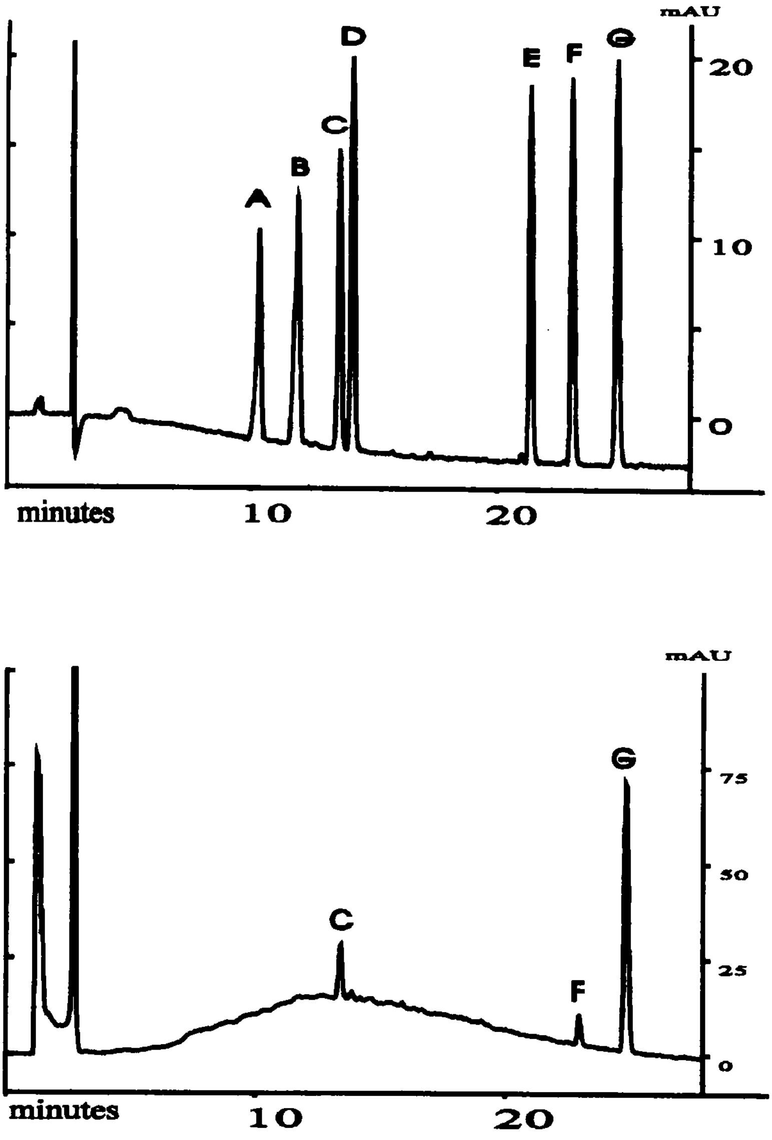
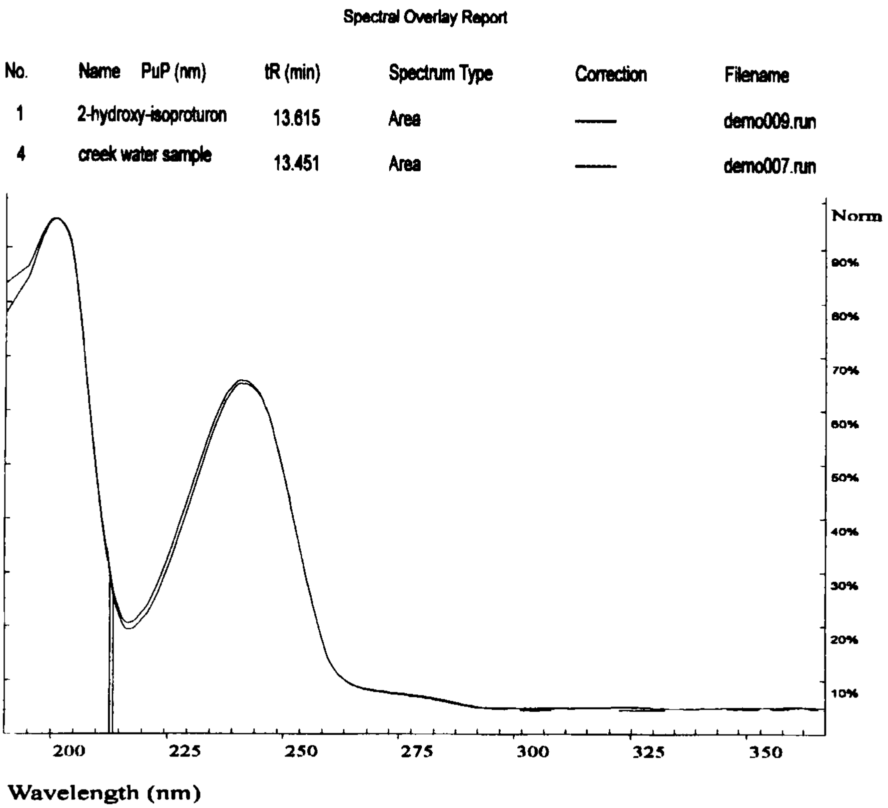


Figure 4 a, b HPLC-chromatogram at 244 nm of a standard mixture of isoproturon and metabolites (a) and of a creek water sample collected during a heavy rainfall event (b). Letters above the peaks correspond with those given in Figure 1.



Spectral Overlay Report

No.	Name PuP (nm)	tR (min)	Spectrum Type	Correction	Filename
1	2-hydroxy-isoproturon	13.615	Area	——	demo009.run
4	creek water sample	13.451	Area	——	demo007.run

Figure 5 UV-spectrum of 2-hydroxy-isoproturon isolated from wheat and peak C of a creek water sample.

RESULTS

Temporal patterns of isoproturon in field samples

Isoproturon was applied on fields 15 and 19 the 28 th March 1994 and on a 750 m^2 part of field 17 the 5th April (Figure 2).

In April 12./13.th 1994 heavy precipitations occurred (Figure 6 a) and massive runoff, interflow and fast leaching in the vadose zone was observed. As a result of these unfavourable weather conditions after pesticide application, creek water adjacent to the agricultural areas was contaminated by runoff, interflow and drain flow (Figure 6 b).

The peak period of isoproturon concentration was only short and the amounts were decreasing quickly the precipitations had stopped. Mainly runoff was responsible for this contamination. After ending of rainfall contamination was also caused by interflow and drainflow. High pesticide concentrations in drain flow were reported previously[10] and this fact could be confirmed in our study by measuring isoproturon concentrations up to 18 µg/l in the creek west drainage. The drain flow was small comparing to the whole creek water flow however, and therefore the isoproturon concentration was watered down quickly.

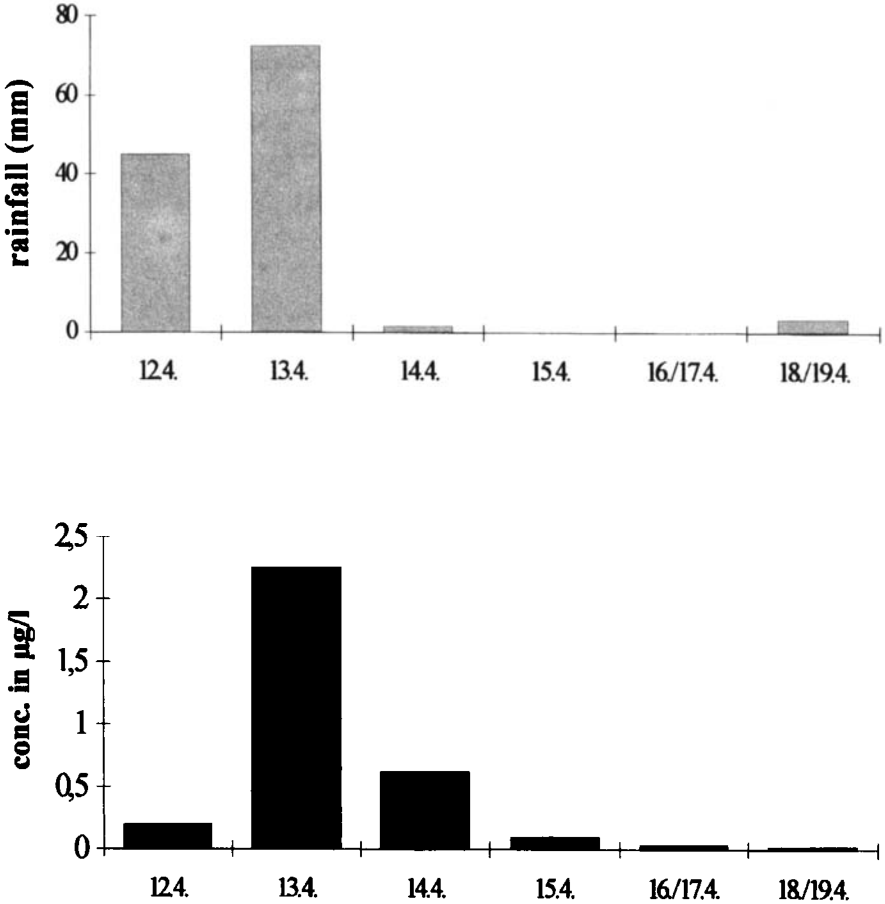


Figure 6 a, b Rainfall pattern (a) and concentrations of isoproturon at creek west mouth (b) during and after the heavy rainfall event in April 1994.

Heavy rainfall caused massive water-infiltration into soil; the drenched soil had high water capacity and therefore transport of dissolved and colloidal bound pesticide in deeper soil layers occured quickly. Isoproturon was detected down to 170 cm depth, even though soil solution was extracted below a part of field 17 (shaft K2, Figure 2), where no isoproturon had been applied. This compound was transported laterally by runoff and interflow over a distance of ten meters and infiltrated into deeper soil.

The time course of isoproturon concentration in soil solution in 125 cm depth (Figure 7) shows the slow dissappearance of this pesticide in subsoil, where minor microbiological degradation occurs.

Isoproturon metabolites in field samples

In some samples concentrations were high enough to determine the isoproturon metabolites too (Table 2). The major compound quantified in soil solution and creek

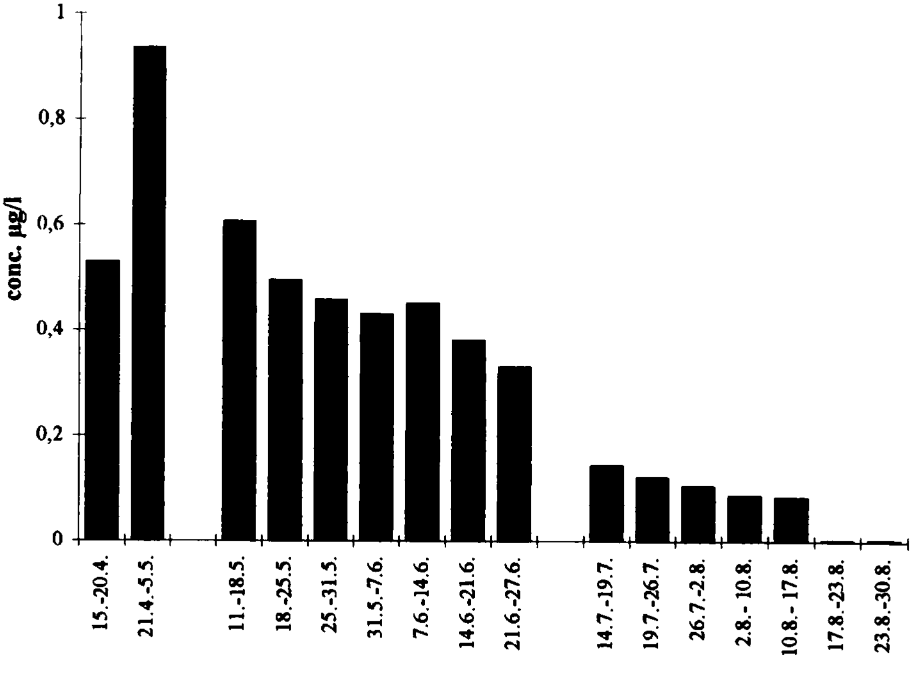


Figure 7 Concentrations of isoproturon in soil solution (125 cm depth) of shaft K2 after the heavy rainfall event at 12./13. April 1994.

Table 2 Concentrations in µg/l of isoproturon (G), monodesmethyl-isoproturon (F), 2-hydroxy-isoproturon (C) and 2-hydroxy-monodesmethyl-isoproturon (B) in different field samples (sampling sites see Figure 2).

Soil solution (shaft 2)				
	G	*F*	*C*	*B*
14.4. field 17 depth: 50 cm	18, 58	0, 62	2, 18	0, 16
14.4 field 17 depth: 90 cm	17, 20	0, 57	2, 09	0, 11
Creek water				
	G	*F*	*C*	*B*
12.4. creek east mouth	0, 32	0, 04	0, 07	0, 00
13.4. creek west drainage	18, 35	1, 54	3, 89	0, 53
13.4. creek west mouth	4, 17	0, 38	0, 89	< DL
13.4. creek east mouth	2, 79	0, 28	0, 42	< DL
14.4. creek east mouth	0, 70	0, 09	0, 12	0, 00
15.4. creek east mouth	0, 22	0, 03	0, 04	0, 00
Runoff				
	G	*F*	*C*	*B*
13.4.94 field 17*	31, 54	1, 18	0, 45	< DL
13.4.94 field 17**	0, 86	0, 03	0, 04	0, 00
13.4.94 field 15**	11, 10	1, 39	1, 27	0, 24

* = sampling at two points by a ladle
** = whole catchment area

water was 2-hydroxy-isoproturon (C). Only in two runoff samples monodesmethyl-isoproturon (F) was predominant. 2-hydroxy-monodesmethyl-isoproturon (B) was quantified in a few samples and didesmethyl-isoproturon (E) in only one sample. 2-hydroxy-didesmethyl-isoproturon (A) and 1-hydroxy-isoproturon (D) have not yet been detected in field samples.

The predominant occurrence of 2-hydroxy-isoproturon (C) in these samples may be an indication of two facts:

1. Hydroxylation is an important step in metabolisation of isoproturon.
2. Higher mobility of 2-hydroxy-isoproturon in comparison to monodesmethyl-isoproturon can be presumed.

The first assumption must be researched by further investigations, including photochemical, hydrolytical and microbiological degradation studies. The second can already be assessed, because 2-hydroxy-isoproturon was mobilized vertically into subsoil and laterally into creek water in higher amounts than monodesmethyl-isoproturon.

Running studies in 1995, that also include analysis of soil samples (0–5 cm) and groundwater samples, are confirming this hypothesis. The major metabolite in soil samples is monodesmethyl-isoproturon (F); therefore a higher adsorbance of the demethylated compound in comparison to 2-hydroxy-isoproturon seems to be likely. The mobility of 2-hydroxy-isoproturon is also indicated by detection of high amounts in samples from two wells (depth. three and five meters) nearby an applicated field. The concentrations were up to seven-fold higher than these of monodesmethyl-isoproturon (F).

CONCLUSIONS

The results reported in the previous sections provide relevant information on the fate of isoproturon outdoors. It has been recognized that heavy rainfall can cause short term contamination of surface water with isoproturon and long term contamination of deeper soil layers. The metabolite 2-hydroxy-isoproturon was identified as the main metabolite in most of the field samples. There are indications for a high mobility of this metabolite in soil. Polar metabolites should, therefore, be considered in further studies on mobility and persistence of phenylurea herbicides and in ecotoxicological evaluations.

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