Bull. Environ. Contam. Toxicol. (1995) 55:802–809

Environmental Contamination and Toxicology

Analytical Methods for the Determination of Isoproturon and Diflufenican Residues in Runoff and Soil

L. Patty,[1] C. Guyot[2]

[1]Centre National du Machinisme Agricole, du Génie Rural, des Eaux et des Forêts (CEMAGREF), 3 bis quai Chauveau, CP 220, 69336 Lyon Cedex 09, France
[2]Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargoire, 14-20 rue Pierre Baizet, BP 9163, 69263 Lyon Cedex 09, France

Received: 3 January 1995/Accepted: 2 June 1995

The transfer of agrochemical products from treated areas to surface water is mainly under the control of runoff processes during rainfall events. One way of reducing surface water contamination is to limit runoff from treated areas. Grassed buffer strips, studied in the USA since 1965, are effective in erosion (Neibling et al. 1979; Young et al. 1980; Parsons et al. 1991) and nutrient transport limitation (Dillaha et al. 1988, 1989; Vought et al. 1991), but their efficacy in pesticide transfer restriction is still not well documented (Asmussen et al. 1977; Rohde et al. 1980).

A study was conducted in France ("La Jaillière" research farm, Brittany) by Cemagref, I.T.C.F (Institut Technique des Céréales et des Fourrages), Rhône-Poulenc Agrochimie and sponsored by the French Ministry of Agriculture since 1993, to assess the efficacy of grassed buffer strips in reducing the transfer of two herbicides from winter wheat plots to surface water in conditions prone to generate runoff. The development of specific analytical procedures for the determination of herbicide residues in runoff represents an essential step of this experiment. The purpose of the present paper is to describe the various analytical procedures to determine the residues of isoproturon (IPU), a substituted phenylurea, and of diflufenican (DFF), a phenoxynicotinanilide (table 1) in four different types of samples: total runoff, runoff liquid and solid phases after separation by continuous flow centrifugation, and soil. The methods were successfully used in monitoring residues of both herbicides in runoff samples collected at the "La Jaillière" experimental site (I.T.C.F, France) during the 1993 cropping period.

MATERIALS AND METHODS

Isoproturon and diflufenican analytical standards (99.7 %) were obtained from Rhône-Poulenc Agrochimie (Lyon, France). Acetone, dichloromethane, hexane, ethyl acetate, and toluene for residues analysis, and acetonitrile and methanol for HPLC were supplied by SDS and Merck. Sodium sulfate (analytical grade) was heated at 550 °C for 3 hr and stored in a desiccator. Glass wool was used as supplied. Millipore HA 0.45 μm (47 mm) filters and Millipore Millex GV13

Correspondence to: L. Patty

(0.22 μm) filters were supplied by Millipore. Empore C18 disk and Sep-Pak Florisil cartridges were supplied by Varian and Waters, respectively.

Table 1. Physico-chemical properties of isoproturon and diflufenican

	Diflufenican (DFF)	Isoproturon (IPU)
Molecular weight (g)	394.3	206.3
Melting point (°C)	161-162	155-156
Vapor pressure (Pa)*	$4.3\ 10^{-6}$ (25 °C)	$8.1\ 10^{-6}$ (25 °C)
Solubility in water	0.05 mg/L (25 °C)	65 mg/L (22 °C)
Solubility in acetone	100 g/L (20 °C)	
Solubility in dichloromethane		63 g/L (20 °C)
Koc (cm^3/g)	1990	120
Half-life in soil (days)*	175-294 (in field)	12-32 (in field)

* From Rhône-Poulenc Agrochimie

Runoff samples, collected from the experimental site ("La Jaillière", France), were divided into two parts. The first one was extracted as described in method I and the other underwent a continuous flow centrifugation (40000 rpm) so as to separate the liquid and solid phases which were extracted according to methods II and III, respectively.

Four extraction procedures were developed for the different samples: (a) method I: Runoff sample (500 mL) was extracted in a 1-L separatory funnel by three portions of 50 mL of dichloromethane for 15 min under mechanical shaking. The emulsion obtained was settled by a 20 min centrifugation at 3000 rpm. The organic extracts were dried on anhydrous sodium sulfate (preliminary rinsed with dichloromethane) and evaporated to dryness in a rotary evaporator at 40 °C. The residuum was dissolved in 2 mL of toluene with sonication for 1 min; (b) method II: As proposed by Regnault and Simonin (1991), runoff liquid phase sample (150 mL) was filtered on a Millipore HA filter (0.45 μm). An Empore C18 disk was conditioned successively with 10 mL of ethyl acetate, 10 mL of methanol, and 10 mL of ultrapure water (MilliQ). The filtered sample was passed through the Empore disk, and the disk was then dried under reduced pressure for 10 min. The herbicide was eluted with 20 mL of ethyl acetate, and the extract was evaporated to dryness in a rotary evaporator at 40 °C. The residuum was dissolved in 2 mL of toluene by sonication for 1 min; (c) method III: 5 g of moist sample were extracted (as suggested by Ambrus et al. 1981) in a 250 mL Erlenmeyer flask by three portions of 50 mL of acetone for 15 min under magnetic stirring. The organic extracts were passed through a glass wool filter (preliminary rinsed with acetone) and concentrated in a rotary evaporator at 40 °C to a 50 mL final volume. The extract and rinses were transfered into a 1-L separatory funnel. 450 mL of ultrapure water were added. After homogenization, the herbicide was extracted as described in method I; (d) method IV: 100 g of moist sample were extracted in a 500 mL Erlenmeyer flask by two portions of 150 mL of acetone for 15 min under magnetic stirring. Method III was then followed.

The different extracts were purified on Sep-Pak Florisil cartridges (1.70 mL) which were first conditioned with 5 mL of toluene-ethyl acetate mixture (50:50)

and 5 mL of toluene. The different eluates were discarded. The extract was then passed through the cartridge, and the eluate was discarded. Then, the pesticide was eluted with 25 mL of toluene-ethyl acetate mixture (50:50). The eluate was evaporated to dryness in a rotary evaporator at 40 °C. The purified residue was dissolved in 2 mL of hexane for diflufenican. For isoproturon, the purified residue was dissolved in 2 mL of water-acetonitrile mixture (80:20), and before injection, the final extract was filtered through a Millipore Millex GV 13 Filter (0.22 µm).

For the fortification experiment, untreated samples were spiked at different concentrations with organic solutions of the herbicides, according to the following protocol (three replicates for each matrix and each herbicide): (a) runoff: 500 mL of simulated runoff sample (soil reduced to a fine powder and added to drainage water so as to obtain a suspended solids rate of 500 mg/L) were spiked with 2 mL of isoproturon or diflufenican solution; (b) runoff liquid phase: 500 mL of ultrapure water (MilliQ) were spiked with 1 mL of isoproturon or diflufenican solution (three replicates of 150 mL were prepared for each herbicide); (c) runoff solid phase: 5 g of untreated moist soil (2 mm sieved) were spiked with 2 mL of isoproturon or diflufenican solution; (d) soil: 50 and 100 g of untreated moist soil (non-sieved) were, respectively, spiked with 10 mL of isoproturon and diflufenican solution.

The isoproturon spiking solutions (0.5 µg/L to 250 mg/L) were prepared in water-acetonitrile mixture (80:20) for runoff and runoff liquid phase fortifications and in acetone for runoff solid phase and soil fortifications. The diflufenican spiking solutions (0.25 µg/L to 50 mg/L) were prepared in acetone for all fortifications.

Standard solutions were prepared as follows: 10 mg of isoproturon and diflufenican were, respectively, dissolved in 100 mL of acetonitrile and hexane to give a 100 mg/L stock solution. Standard solutions containing 5 to 1250 µg/L of IPU and 5 to 100 µg/L of DFF were prepared by successive dilutions of these two stock solutions.

Diflufenican residues were analysed with a Varian Star 3400 gas chromatograph equipped with a Varian 8200 autosampler and a ^{63}Ni electron-capture detector. The gas flows were: vector gas (N_2): 3 mL/min, make up (N_2): 30 mL/min. The column was a 30 m x 0.32 mm ID nonpolar (J&W, DB5) fused-silica capillary column (film thickness, 0.25 µm) coated with 5 % phenylmethylpolysiloxane. Inlet and detector temperatures were, respectively, 250 and 300 °C. Analysis time was 54 min. Injection was made in splitless mode. The oven temperature program was: initial temperature 150 °C held for 1 min; 150 to 200 °C at 15 °C/min; 200 to 240 °C at 4 °C/min; 240 to 265 °C at 2 °C/min, held for 5 min and 265 to 300 °C at 20 °C/min, held for 10 min. The injection volume was 1 µL, and the retention time of diflufenican was approximately 12 min.

Isoproturon residues were analysed with a Kontron high performance liquid chromatograph. The equipment included two pumps (Kontron 422S), an autosampler (Kontron 465) with a 200 µL injection loop, a spectrophotometric UV detector (Kontron 430), a pre-column and a column Lichrospher 60 RP

Select B (250 x 4 mm, silica-C8, 5 µm). The isocratic chromatographic separation was performed using different mobile phases: acetonitrile-water mixture (42:58) for runoff extracts and runoff liquid phase extracts and acetonitrile-water mixture (30:70) for soil and runoff solid phase extracts. The flow rate was 1 mL/min and the injection volume was 80 µL. In the chromatographic conditions described, the retention times of isoproturon were approximately 10 min and 27 min.

RESULTS AND DISCUSSION

The analytical methods were applied to the determination of residues of both herbicides in runoff, runoff liquid and solid phases and soil. Calibration curves were prepared by plotting peak areas versus standard solution concentrations. Linear regression coefficients of the calibration curves were used to calculate the residue concentrations in the samples. The linear correlation coefficient was 0.999 for IPU and 0.99 for DFF.

Recovery rates were calculated at different fortification levels (chromatograms of spiked samples are presented in figures 1 and 2). Recoveries of both herbicides ranged between 61 and 108 % (tables 2 and 3). The relative deviation (Rd) varied from 1 to 27 % for isoproturon and from 0.5 to 23 % for diflufenican. Due to the inconstancy of recovery rates observed during method development, three successive extractions had to be made. Mean recovery rates of isoproturon from runoff (method I), runoff liquid phase (method II), runoff solid phase (method III) and soil (method IV) were 87, 88, 87, and 83 %, respectively. For DFF the results were 94, 87, 96, and 84%, respectively (table 4).

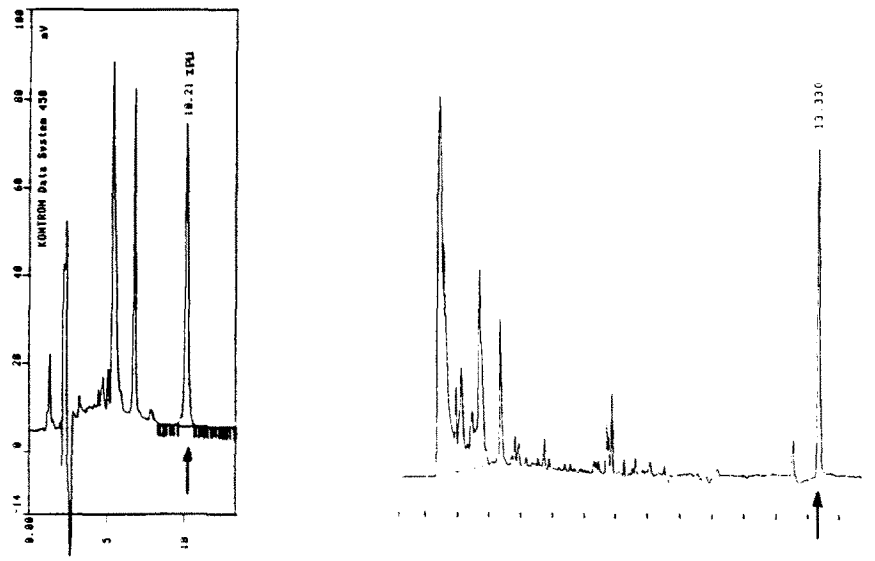
C18 cartridges were also used for the extraction of runoff liquid phase. The mean recovery rate of IPU was 83 %. The results being consistent with those obtained with Empore C18 disks, the latter were preferably used because of their easy use.

Detection and quantitation limits are presented in table 4 for the different methods and for each herbicide. The detection limit represents the minimal detectable amount of herbicide. The quantitation limit represents the minimal amount of product determined in the experimental conditions described. The calculated limits were obtained with the following formula:

DL (Detection Limit) = Average of 3 blank analysis + 3 standard deviations

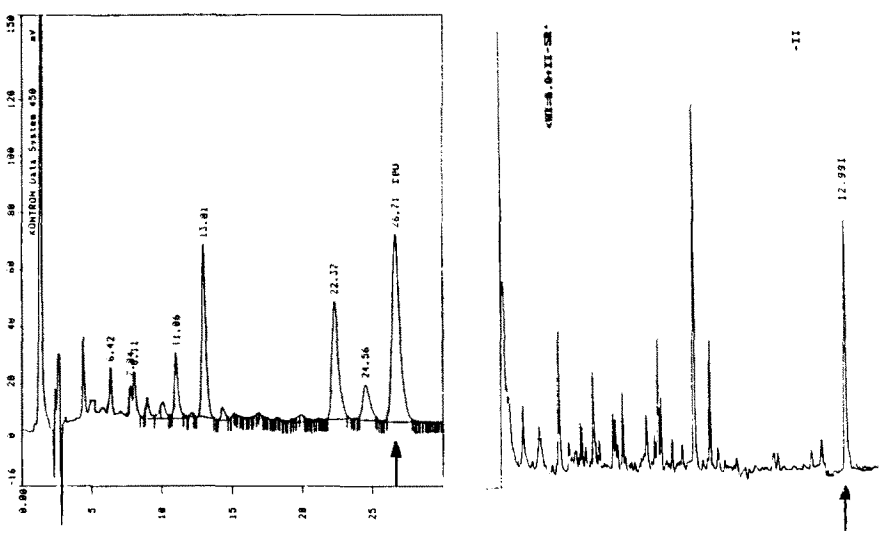
QL (Quantitation Limit) = 2 x DL.

The observed limits were obtained by injection of different samples spiked at low levels. The difficulties encountered in integrating and resolving the peaks at low concentration levels partly explains the differences obtained between calculated and observed limits. The observed limits were used exclusively in the study because they provided reliable results. These limits ranged between 0.01 to 0.1 µg/L and 0.1 to 30 µg/kg. The highest values (1, 6, 12 and 30 µg/kg) were obtained with soil and runoff solid phase samples where a large interference of natural products was observed.



(a) (b)

Figure 1. Typical chromatograms of spiked samples: (a) untreated runoff spiked with IPU at 1 µg/L, (b) untreated runoff spiked with DFF at 1 µg/L



(c) (d)

Figure 2. Typical chromatograms of spiked samples: (c) soil spiked with IPU at 30.1 µg/kg (dry), (d) soil spiked with DFF at 12.05 µg/kg (dry)

Table 2. Recovery rates (mean of 3 determinations) of isoproturon for various spiked samples: runoff, runoff liquid and solid phases and soil

Method I	Spiking level (µg/L)					
(runoff)	500	100	10	1	0.1	
mean (%)	87	92	94	90	69	
σ	13	16	4	1	19	
Rd (%)	15	17	4	1	27	
Method II	Spiking level (µg/L)					
(runoff liquid phase)	555.5	138.9	55.5	27.8	5.5	2.78
mean (%)	78	99	88	85	90	86
σ	5	2	4	4	3	8
Rd (%)	6	2	4	5	3	9
Method III	Spiking level (µg/kg dry)					
(runoff solid phase)	1205	602.4	120.5	30.1		
mean (%)	91	96	93	68		
σ	3	3	5	5		
Rd (%)	3	3	5	7		
Method IV	Spiking level (µg/kg dry)					
(soil)	1205	602.4	120.5	30.1	6.02	
mean (%)	76	85	85	95	72	
σ	1	2	3	4	5	
Rd (%)	1	2	3	4	7	

Rd: Relative deviation; σ: standard deviation

Table 3. Recovery rates (mean of 3 determinations) of diflufenican for various spiked samples: runoff, runoff liquid and solid phases and soil

Method I	Spiking level (µg/L)				
(runoff)	100	50	10	1	0.1
mean (%)	77	82	97	106	108
σ	8	3	6	0.8	10
Rd (%)	10	4	6	0.7	9
Method II	Spiking level (µg/L)				
(runoff liquid phase)	100	100	10	1	0.1
mean (%)	64	86	96	86	104
σ	0.8	12	6	5	15
Rd (%)	1	14	6	6	14
Method III	Spiking level (µg/kg dry)				
(runoff solid phase)	120.5	60.2	12.05		
mean (%)	97	91	100		
σ	0.5	7	14		
Rd (%)	0.5	8	14		
Method IV	Spiking level (µg/kg dry)				
(soil)	120.5	60.2	12.05	1.205	
mean (%)	73	97	107	61	
σ	4	4	6	14	
Rd (%)	5	4	6	23	

Rd: Relative deviation; σ: standard deviation

Table 4. Mean recovery rates and validity limits of each method (µg/L of liquid sample and µg/kg of dry solid sample)

Method	Herbicide	Mean recovery rates	Detection limits (µg/L)		Quantitation limits (µg/L)	
		(%)	calculated	observed	calculated	observed
I	IPU	87 (σ: 16, Rd: 18)	0.004	0.05	0.008	0.1
	DFF	94 (σ: 14, Rd: 15)	0.004	0.01	0.008	0.02
II	IPU	88 (σ: 8, Rd: 9)	0.005	0.05	0.01	0.1
	DFF	87 (σ: 16, Rd: 18)	0.01	0.05	0.02	0.1
Method	Herbicide	Mean recovery rates	Detection limits (µg/kg)		Quantitation limits (µg/kg)	
		(%)	calculated	observed	calculated	observed
III	IPU	87 (σ: 12, Rd: 14)	0.6	6	1.2	30
	DFF	96 (σ: 10, Rd: 10)	1.4	1	2.8	12
IV	IPU	83 (σ: 9, Rd: 11)	0.13	0.1	0.26	6
	DFF	84 (σ: 20, Rd: 24)	0.7	0.1	1.4	1

Rd : Relative deviation (%); σ: standard deviation

The methods were successfully used in a preliminary study in which both herbicides residues were monitored in runoff samples. Samples were collected at the experimental site after application of Quartz GT (IPU 500 g/L and DFF 62.5 g/L) on January 15, 1993 on three winter wheat plots (25 x 5 m). Runoff samples from each wheat plot: B0, B6 and B12, were collected after filtration through a 0-, 5.7- and 11.1-m wide grassed buffer strip, respectively. The average slope was approximately 7 %.

The results for the first 1993 runoff event are presented in table 5. The different concentrations varied between 0.2 µg/L and 2440 µg/kg according to the herbicide, the matrix and the sample considered. It appeared that a specific analytical method had to be developed for each herbicide and each matrix.

These preliminary results clearly show that grassed buffer strips are efficient with respect to the limitation of herbicides transfer by runoff. Actually, isoproturon and diflufenican concentrations in the different matrixes were reduced by more than 90 % in the 5.7-m grassed buffer strip. In agreement with its weak adsorption capacity, isoproturon was primarily detected in the runoff liquid phase. Conversely, due to its high affinity for soil (see table I), diflufenican was mainly found in runoff solid phase.

More comprehensive results are necessary to confirm these preliminary observations (paper in preparation).

Table 5. Concentration of isoproturon (IPU) and diflufenican (DFF) in the first runoff sample (April 15, 1993) collected at "La Jaillière" (I.T.C.F, France) experimental site (results corrected using recovery rates)

Wheat plot	Runoff Volume (L)	Concentration in runoff (µg/L)		Concentration in runoff liquid phase (µg/L)		Concentration in runoff solid phase (µg/kg dry)	
		IPU	DFF	IPU	DFF	IPU	DFF
B0	19.2	59	17	110	1	307	2440
B6	12.2	2.2	1.8	2.3	< 0.1	< 30	130
B12	8.2	0.6	0.2	< 0.1	0.4	< 30	38

REFERENCES

Ambrus A, Lantos J, Visi E, Csatlos I, Sarvari L (1981) General method for determination of pesticide residues in samples of plant origin, soil and water. I: extraction and clean up. J Assoc Off Anal Chem 64: 733-742

Asmussen L E, White A W, Hauser E W, Sheridan J M (1977) Reduction of 2,4 D load in surface runoff down a grassed waterway. J Environ Qual 6: 159-162

Dillaha T A, Sherrard J H, Lee D, Mostaghimi S, Shanholtz V O (1988) Evaluation of vegetative filter strips as a best management practice for feed lots. J Water Pollut Control Fed 60: 1231-1238

Dillaha T A, Reneau R B, Mostaghimi S, Lee D (1989) Vegetative filter strips for Agricultural nonpoint source pollution control. Am Soc Agric Eng 32: 513-519

Neibling W H, Alberts E E (1979) Composition and yield of soil particles transported through sod strips. 1979 Summer meeting of the Am Soc Agric Eng Paper n° 79-2065: p 12

Parsons J E, Daniels R B, Gilliam J W, Dillaha T A (1991) The effect of vegetation filter strips on sediment and nutrient removal from agricultural runoff. Proceedings for the Environmentally Sound Agriculture Conference (USA) 1: 324-332

Regnault L, Simonin B (1991) Methode de dosage des résidus d'isoproturon dans l'eau potable. Rapport confidentiel n° 9115643, Rhône-Poulenc Agro (Lyon, France): p 34

Rohde W A, Asmussen L E, Hauser E W, Wauchope R D, Allison H D (1980) Trifluralin movement in runoff from a small agricultural watershed. J Environ Qual 9: 37-42

Vought L B M, Lacoursiere J O, Voelz N J (1991) Stream in the agricultural landscape. VATTEN 47: 321-328

Young R A, Huntrods T, Anderson W (1980) Effectiveness of vegetated buffer strips in controlling pollution from feedlot runoff. J Environ Qual 9: 483-487