Pest Management Science *Pest Manag Sci* **61**:905–916 (2005)
DOI: 10.1002/ps.1108

Environmental fate of herbicides trifluralin, metazachlor, metamitron and sulcotrione compared with that of glyphosate, a substitute broad spectrum herbicide for different glyphosate-resistant crops[†]

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Abstract: The introduction of crops resistant to the broad spectrum herbicide glyphosate, *N*-(phosphonomethyl)glycine, may constitute an answer to increased contamination of the environment by herbicides, since it should reduce the total amount of herbicide needed and the number of active ingredients. However, there are few published data comparing the fate of glyphosate in the environment, particularly in soil, with that of substitute herbicides. The objective of this study is to compare the fate of glyphosate in three soils with that of four herbicides frequently used on crops that might be glyphosate resistant: trifluralin, α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine, and metazachlor, 2-chloro-*N*-(pyrazol-1-ylmethyl)acet-2′,6′-xylidide for oilseed rape, metamitron, 4-amino-4,5-dihydro-3-methyl-6-phenyl-1,2,4-triazin-5-one for sugarbeet and sulcotrione, 2-(2-chloro-4-mesylbenzoyl)cyclohexane-1,3-dione for maize. The distribution of herbicides between the volatilized, mineralized, extractable and non-extractable fractions was studied, along with the formation of their metabolites in laboratory experiments using ^{14}C-labelled herbicides, over a period of 140 days. The main dissipation pathways were mineralization for glyphosate and sulcotrione, volatilization for trifluralin and non-extractable residues formation for metazachlor and metamitron. The five herbicides had low persistence. Glyphosate had the shortest half-life, which varied with soil type, whereas trifluralin had the longest. The half-lives of metazachlor and sulcotrione were comparable, whereas that of metamitron was highly variable. Glyphosate, metazachlor and sulcotrione were degraded into persistent metabolites. Low amounts of trifluralin and metamitron metabolites were observed. At 140 days after herbicide applications, the amounts of glyphosate and its metabolite residues in soils were the lowest in two soils, but not in the third soil, a loamy sand with low pH. The environmental advantage in using glyphosate due to its rapid degradation is counterbalanced by accumulation of aminomethylphosphonic acid specifically in the context of extensive use of glyphosate.

Keywords: glyphosate; herbicide metabolites; herbicide degradation; herbicide half-lives; GM crops

1 INTRODUCTION

The introduction of genetically modified (GM) plants resistant to the broad spectrum herbicide glyphosate, *N*-(phosphonomethyl)glycine, may be considered as a technical answer to crop weed control constraints. It is argued that the substitution of herbicides by glyphosate when introducing GM plants must result in a reduction of the number of different pesticides applied on the crop, and that a decrease of 20–85% in the total amount of herbicides can be expected[1] with a substantial reduction of the pollution load. However, the extensive use of GM plants resistant to glyphosate may increase its transfer to water bodies. This would be enhanced by the development of other agricultural and non-agricultural uses of glyphosate.

Studies on the environmental impact of GM plants are generally focused on gene dispersion, crosses with wild weeds, their toxicity or allergenicity.[2]

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[†] Based on a paper presented at the 4th International Symposium on Environmental Aspects of Pesticide Microbiology: Pesticides, Microbial Functions and Biodiversity in Soil, held at Thessaloniki, Greece on 4–9 September 2004

Contract/grant sponsor: INRA, Organismes génétiquement modifiés et environnement programme
Contract/grant sponsor: CNRS, Impact des biotechnologies dans les agro-écosystèmes programme
Contract/grant sponsor: CETIOM
(*Received 3 September 2004; revised version received 29 March 2005; accepted 7 June 2005*)

Modification of chemical weed control can induce indirect impacts concerning soil and water quality and should be taken into account to complete the overall environment balance of the introduction of GM plants. The substituted herbicides when GM glyphosate resistant plants will be introduced also depend on crop species.

This work is a contribution to the assessment of the environmental balance between GM plant introduction and the resulting modification in herbicide use. This is illustrated with three crops: oilseed rape (*Brassica napus* L), sugarbeet (*Beta vulgaris* L) and maize (*Zea mays* L) from different experimental areas in France (Table 1). All these crops have their GM glyphosate resistant equivalent but presently weed control is made using the broad spectrum herbicides trifluralin, α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine, metazachlor, 2-chloro-*N*-(pyrazol-1-ylmethyl)acet-2′,6′-xylidide, metamitron, 4-amino-4,5-dihydro-3-methyl-6-phenyl-1,2,4-triazin-5-one and sulcotrione, 2-(2-chloro-4-mesylbenzoyl)cyclohexane-1,3-dione (Table 1).

The environmental fate of pesticides is mainly regulated by their behaviour in soils. Following application, most pesticides reach the soil through direct contact and/or after wash off from foliage.[3] In soils, pesticides are affected by various physico-chemical and biological processes conditioning their dissipation and/or accumulation, and their transfer towards other environmental compartments (water, plant, atmosphere). In particular, the understanding and measurement of pesticide retention and degradation are the key steps in risk assessments of their persistence and contribution to pollution.[4]

Few data have been published regarding the degradation of metazachlor and sulcotrione. Degradation is mostly a biological process with half-lives ranging from 4.5 to 77 days for metazachlor and from 1 to 122 days for sulcotrione.[5–9] As a result, these two herbicides may be quite persistent, from 41 to 475 days for trifluralin and from 2.5 days to more than 1 year for metamitron. Their main degradation pathways are volatilization and photodegradation for trifluralin,[10–13] and both biotic and abiotic degradation for metamitron.[6,14–16] In contrast, glyphosate is less persistent with a mainly biological degradation and a DT_{50} range from 3 to 40 days.[17–21] Different experimental conditions of temperature, moisture and soils can explain the wide range of DT_{50} values found for each herbicide.

The objective was to conduct a laboratory study to compare, in the same soils and experimental conditions, the fate of glyphosate with that of trifluralin, metazachlor, metamitron and sulcotrione. Soils used originated from three French experimental areas where GM crops are cultivated and were chosen because of their different pedological properties. During the incubations in the laboratory we tried to separate the different dissipation processes and to characterize the availability of pesticide residues and their persistence in soil. The results were used to evaluate the potential environmental impacts of the different weed-control strategies.

Table 1. Types and amounts of herbicides used in the French experimental areas[a]

	Oilseed rape		Sugarbeet		Maize	
Soil	Herbicide	Rate (kg ha^{-1})	Herbicide	Rate (kg ha^{-1})	Herbicide	Rate (kg ha^{-1})
Non-GM crops weed control						
Châlons	Dimetachlor	1.12	Metamitron	1.54	—[b]	—[b]
	+		+			
	napropamid	1.12	phenmedipham	0.63		
	+		+			
	clomazone	0.18	ethofumesate	0.58		
Dijon	Trifluralin	1.20	Clopyralid	1.91	Alachlor	2.40
	+		+		+	
	metazachlor	0.72	metamitron	1.40	atrazine	1.00
	+		+		+	
	quinmerac	0.18	phenmedipham	0.41	pyridate	0.90
Toulouse	Trifluralin	1.20	—[b]	—[b]	Alachlor	2.40
	+				+	
	napropamid	0.67			atrazine	1.00
					+	
					pyridate	0.90
GM crops weed control						
Châlons						
Dijon	Glyphosate	1.44	Glyphosate	3.06	Glyphosate	2.88
Toulouse						

[a] Data obtained from Centre Technique Interprofessionnel des Oléagineux Métropolitains, France.

[b] Crop is not cultivated in this area.

2 MATERIALS AND METHODS

2.1 Herbicides

The herbicides used were glyphosate, trifluralin, metazachlor, metamitron and sulcotrione (Table 2). [*Methyl*-^{14}C]glyphosate was purchased from Sigma Chemicals, St Louis, USA (81 MBq mmol^{-1}, 99.2% purity), [U-*ring*-^{14}C]trifluralin from Amersham, Buckinghamshire, UK (2960 MBq mmol^{-1}, 98.6% purity), [U-*phenyl*-^{14}C]metazachlor from BASF, Limburgerhof, Germany (1761 MBq mmol^{-1}, 95.5% purity), [U-*phenyl*-^{14}C]metamitron from International Isotope, Munich, Germany (477 MBq mmol^{-1}, 98.0% purity) and [U-*phenyl*-^{14}C]sulcotrione from Izotop, Budapest, Hungary (720 MBq mmol^{-1}, 91% purity). Water solutions in 0.01 M $CaCl_2$ of labelled herbicides were prepared by isotopic dilution with non-labelled herbicide (analytical standards, >99% purity) at 10.8, 9.1, 8.1, 8.3 mg litre^{-1}, and contained 5.24, 7.72, 7.49 and 6.88 MBq litre^{-1} for ^{14}C-glyphosate, ^{14}C-metazachlor, ^{14}C-metamitron and ^{14}C-sulcotrione respectively. Solutions of ^{14}C-trifluralin were prepared in methanol (8.9 mg litre^{-1} and 7.84 MBq litre^{-1}) because of its low solubility in water.

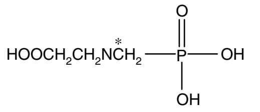
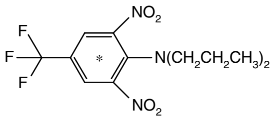
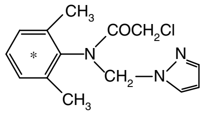
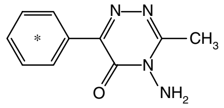
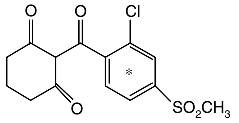
2.2 Soils

Soil samples were taken from the top layer (0–10 cm) of three French experimental sites: Champagne (Châlons), Bourgogne (Dijon) and Midi-Pyrénées (Toulouse), where the impact of GM plants have been studied since 1995. They correspond to different physicochemical properties of soils (Table 3), and different climatic conditions.

2.3 Incubation procedure

Incubations were done with fresh soil samples passed through a 3-mm sieve. Fresh soil equivalent to 10 g of dry soil was placed in 500-ml hermetically stoppered jars. Soil-water content was adjusted to reach field capacity with ^{14}C-herbicides solutions and MilliQ water (Millipore, Molsheim, France) if necessary. The final concentrations of herbicides were 1 mg kg^{-1} dry soil corresponding approximately to application rates of 1 kg ha^{-1}. The applied radioactivity was 0.57, 0.86,

Table 2. Some physicochemical properties[a] of glyphosate, trifluralin, metazachlor, metamitron and sulcotrione

Herbicide	Structural formula	pKa	Molecular mass (g mol^{-1})	Solubility in water (mg litre^{-1})	Vapour pressure (mPa)
Glyphosate		<2 – 2.6 – 5.6 – 10.6[b]	169.1	12 000	Negligible
Trifluralin		—	335.3	0.22	9.5000
Metazachlor		—	277.8	430	0.0490
Metamitron		—	202.2	1700	0.0860
Sulcotrione		3.1[c]	328.8	165[c]	0.0053

[a] Values from Tomlin.[42]
[b] Sprankle *et al.*[17]
[c] Data from Zeneca.

Table 3. Main characteristics of the soils from the Châlons, Dijon and Toulouse experimental sites

Soil site	Clay (g kg^{-1})	Silt (g kg^{-1})	Sand (g kg^{-1})	$CaCO_3$ (g kg^{-1})	pH (water)	Organic carbon (g kg^{-1})	Amorphous Fe (g kg^{-1})	Total Cu (mg kg^{-1})	Total Mn (g kg^{-1})	P_2O_5 (g kg^{-1})
Châlons	93	72	7	819	8.2	20.05	0.68	5.7	0.502	2.72
Dijon	377	296	152	167	8.2	16.28	2.00	16.0	1.819	2.76
Toulouse	235	323	439	3	7.6	9.57	2.08	15.1	0.643	1.31

0.83, 0.81 and 0.68 MBq kg^{-1} dry soil for glyphosate, trifluralin, metazachlor, metamitron and sulcotrione respectively. Each jar contained a vial with 2 ml of 2 M NaOH to trap evolved $^{14}CO_2$ and a vial with 10 ml of water to maintain a constant relative humidity. Glass wool with 1500 µl of paraffin oil was put on the top of incubation devices containing trifluralin, which had a high vapour pressure, to trap volatilized organic compounds while allowing CO_2 to go through. Soil samples were incubated at 28 (± 1) °C in the dark, for 140 days. During incubation, soil water content was adjusted periodically by weighing each jar and adding the required amount of water. Triplicate samples were prepared for each herbicide, soil, and sampling dates (see below). NaOH traps, and glass wool with paraffin, were periodically sampled and replaced.

At 0, 3, 7, 14, 28, 63 and 140 days after beginning incubation, four sequential extractions were done; one extraction with 50 ml of a water solution of $CaCl_2$ 0.01 M for 24 h, followed by three extractions for 24, 24 and 4 h respectively, with 50 ml of NH_4OH (0.54 M) for glyphosate,[22] 50 ml of methanol for trifluralin, metazachlor and metamitron, and 50 ml of a mixture of 10% HCl (0.1 M in water) and 90% acetonitrile (v/v) for sulcotrione. Samples were mechanically shaken at 20 (± 2) °C in the dark and then centrifuged for 15 min at $1800 \times g$. Non-extractable herbicide corresponded to the radioactivity remaining in the soil pellet after the four extractions.

2.4 Chemical analysis

Total radioactivity content of each extract was measured by liquid scintillation counting using a Tri-Carb 2100 TR counter (Packard Instruments, Meriden, CT, USA) with external standardization and Ultima Gold XR (Packard Instruments) as liquid scintillation cocktail. NaOH traps, glass wool with paraffin, and aliquots of 1 ml of each extract were mixed with 10 ml of liquid scintillation cocktail. Radioactivity in the solid samples containing the non-extractable residues was measured by liquid scintillation counting of the $^{14}CO_2$ evolved after combustion in triplicate of 150 mg of ground dry soils using a Sample Oxidizer 307 (Packard Instruments).

HPLC analysis was carried out for all extracts. The three replicates of water–$CaCl_2$ extractions of each sample were pooled to obtain sufficient radioactivity. Glyphosate extracts were concentrated using a rotary evaporator (Büchi, Flawil, Switzerland) under vacuum and then acidified to pH 2 with H_3PO_4. Trifluralin, metazachlor, metamitron and sulcotrione water extracts were concentrated by solid-phase extraction with an Alltech (Deerfield, IL, USA,) C18 cartridge (100 mg) and eluted with methanol. Sulcotrione water extracts were acidified to pH 2 with HCl before concentration on the C18 cartridge. The three successive extracts with non-aqueous solvent were combined, concentrated by evaporation under vacuum, and filtered through a syringe-regenerated cellulose filter (0.45 µm, Alltech). HPLC analyses were performed with a Waters (Milford, MA, USA) chromatography appliance (System controller 610, Autosampler 717) coupled with a radioactive flow detector (Flo-one A-500, Packard–Radiomatic). Glyphosate was analyzed on a Sax Adsorbosphere column (Alltech, 5 µm, 250 × 4.6 mm), and the mobile phase was KH_2PO_4 0.34 g $litre^{-1}$ adjusted to pH 2.1 with H_3PO_4 over 40 min. The extracts of other herbicides were analyzed on a Novapak C18 column (Waters, 5 µm, 250 × 4.6 mm). Reverse-phase chromatography with optimized gradient of water–methanol was used for extract analyses of trifluralin, metazachlor and metamitron. Ion-pair chromatography was used for sulcotrione extracts using optimized gradient of water–methanol with 0.01 M tetra-*n*-butylammonium chloride. In all cases, mobile phase flow was 1.0 ml min^{-1}, and the injected sample volume varied between 100 and 700 µl depending on the radioactivity content and on the solvent nature of each sample.

2.5 Data analysis

Herbicide degradation was described using first-order kinetics:

$$C(t) = C_o \exp(-kt)$$

where $C(t)$ is amount of remaining herbicide (% of initial applied dose) at time t, C_o is initial extractable percentage of herbicide (measured 1 h after soil application), and k the first-order rate constant of degradation (day^{-1}). The values of k were determined by non-linear regression (Marquardt–Levenberg algorithm, SigmaPlot, SPSS Inc, Chicago, IL, USA) and were used to calculate the herbicide degradation half-lives $T_{1/2}$ with the following equation:

$$T_{1/2}(\text{days}) = (1/k)\ln(C_o/50).$$

Metabolite half-lives were estimated using a compartment model (Fig 1) fitted with ModelMaker 4.0 (FamilyGenetix Limited, Oxford, USA). For glyphosate the model assumes that the herbicide may be directly mineralized, degraded into one metabolite and trapped in non-extractable residues (Fig 1a). For the other herbicides, mineralization occurs via metabolite formation and non-extractable residues are built from both the parent and the metabolite (Fig 1b). Each model compartment was defined with equations including concentrations and degradation rate constants k. Degradation rate constants of metabolite compartment were used to determine corresponding metabolite half-life:

$$T_{1/2m} = \ln(2)/k_{\text{metabolite}}.$$

3 RESULTS AND DISCUSSION

The mineralization and residues data for the five pesticides in the three soils were compared (Fig 2). The data are the averages of three replicates which

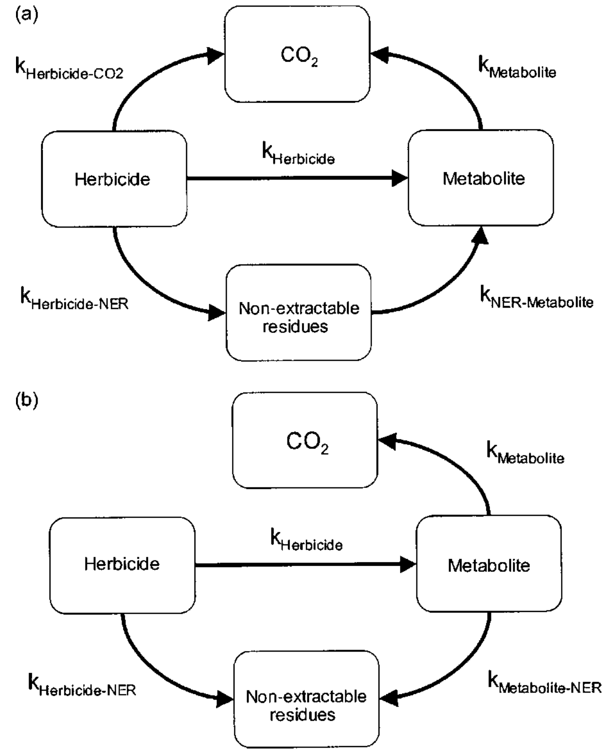


Figure 1. Herbicide degradation, formation of main metabolite and of non-extractable residues models: (a) glyphosate; (b) metazachlor and sulcotrione with: $k_{\text{Herbicide-CO2}}$: Mineralization rate of herbicide; $k_{\text{Herbicide/Metabolite}}$: degradation rate coefficients for herbicide/metabolite; $k_{\text{Herbicide/Metabolite-NER}}$: rate constants of herbicide/metabolite non-extractable residues (NER) formation; $k_{\text{NER-Metabolite}}$: rate constant of metabolite release from NER.

do not differ by more than 5% for glyphosate and metazachlor, and 10% for the three others herbicides. Radioactivity recoveries (in percentage of the initial radioactivity) ranged between 92 and 95% for glyphosate, 96 and 98% for trifluralin, 95 and 98% for metazachlor, 95 and 97% for metamitron and 94 and 96% for sulcotrione.

3.1 Water extractability and pesticide availability

Soil extraction with water solutions ($CaCl_2$ 0.01 M) provided an estimate of the availability of pesticide residues, which could be directly related to the risk of pesticide leaching. Glyphosate and trifluralin were the most sorbed herbicides. Amounts in water extracts were very low and decreased quickly during the first two months of incubation. After 140 days, only the main metabolite of glyphosate, aminomethylphosphonic acid (AMPA), and unidentified trifluralin metabolites were detected.

The availability of metazachlor residues was very high (Fig 2). At the end of incubation, 36% of initial radioactivity was water extractable from Dijon and Toulouse soils, and 27% from Châlons soil. However, metazachlor degraded rapidly to a major unidentified metabolite (M4), and after 140 days only this metabolite was detected in water extracts.

For metamitron, the amounts of water extractable ^{14}C decreased very quickly and only metamitron was detected in the water extracts for the Châlons and Dijon soils. In the Toulouse soil, in addition to metamitron, one unidentified metabolite (less polar than metamitron because it had a greater chromatographic retention time) was detected in water extracts after 140 days of incubation, when it represented 3% of the initial radioactivity.

Most of the sulcotrione residues were water-extractable. They were initially made up of sulcotrione but quickly degraded to acid metabolite 2-chloro-4-methylsulfonylbenzoic acid (CMBA). This metabolite made up 57–66% of the initial radioactivity found in water-extracted residues. Most of its water residues disappeared by the end of incubation in Dijon and Toulouse soils, but 9% remained in Châlons soil.

The proportion of pesticides in water extracts depended primarily on pesticide sorption. However, this proportion was time dependent, in relation to the evolution of sorption interactions and to the pesticide degradation producing metabolites with different sorption characteristics. The amounts of water extractable herbicide may be taken as amounts of readily available herbicide. From these results, the highest risk of short-term leaching pollution mainly involved AMPA, M4, CMBA, and to some extent metazachlor, metamitron and sulcotrione.

3.2 Degradation kinetics and pesticide persistence in soils

Comprehensive extraction with adapted solvents is necessary to evaluate the total amount of herbicide residues in soils. Potentially available residues extracted with water formed a variable part of the total residue. The identification of parent herbicides and their metabolites is necessary to derive the best estimate of the half-life ($T_{1/2}$), a parameter that is classically used for risk assessment. First-order kinetics correctly described herbicide degradation except for metamitron in the Toulouse soil, which was better described with second-order kinetics (Fig 3 and Table 4). Metabolite half-lives of glyphosate, metazachlor and sulcotrione were estimated from a mechanistic model (Fig 1a, b).

Glyphosate was only detected in ammonia extracts. $T_{1/2}$ decreased as follows across the three soils: Toulouse > Dijon > Châlons (Table 4). These values are consistent with those reported by Rueppel *et al*,[18] but are generally very small compared with those reported elsewhere in the literature[23–25] because of optimum conditions of soil temperature and moisture in our study. Glyphosate was more persistent in the Toulouse soil where adsorption was highest, and less persistent in Châlons soil where adsorption was weakest (Table 5).[17,24,26] In particular, glyphosate degradation decreases when soil pH is low, and when soil phosphate content is low.[26] AMPA was more persistent than glyphosate (Table 4), with soil $T_{1/2}$ of the same order as glyphosate. These results are consistent with those reported in the literature, showing high persistence of AMPA.[18,23,25,27]

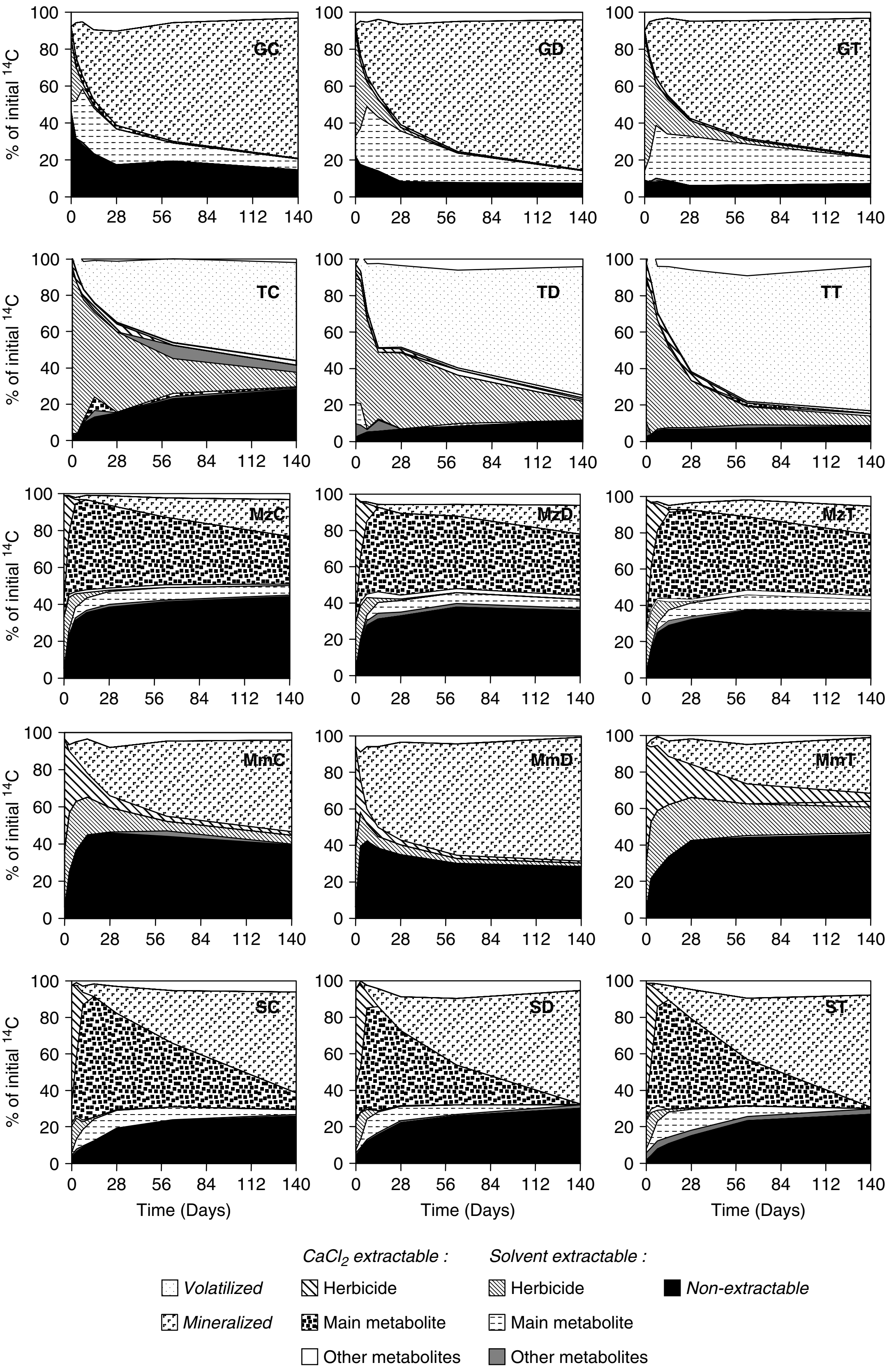


Figure 2. Fate of glyphosate (G), trifluralin (T), metazachlor (Mz), metamitron (Mm) and sulcotrione (S) in Châlons (C), Dijon (D) and Toulouse (T) soils. Distribution of the initial radioactivity between the different analyzed fractions and between active ingredients and their metabolites.

Trifluralin $T_{1/2}$ decreased as follows: Châlons > Dijon > Toulouse (Table 4). The values of $T_{1/2}$ were smaller than those reported in the literature because of the significant volatilization observed in our study.[12,13,28,29] Trifluralin was more persistent in soils with high soil organic carbon content (Table 3), where adsorption was more important (Table 5).[30,31]

The $T_{1/2}$ of metazachlor was small and decreased as follows: Toulouse > Dijon > Châlons (Table 4). They are consistent with those reported by Beulke and Malkomes.[9] Contrary to their observations, degradation was more important in soils with high organic carbon content. The half-life of M4 was large compared with those of metazachlor (Table 4), but varied similarly.

The $T_{1/2}$ of metamitron was highly variable and decreased as follows: Toulouse > Châlons > Dijon (Table 4). The values are consistent with those of Vink and Van der Zee,[14] Vischetti *et al*,[15,32] and Van der Paas *et al*.[16] The degradation rate of metamitron increases significantly with number of applications[16,33] which may explain the very low metamitron $T_{1/2}$ value in Dijon soil compared to the two other soils.

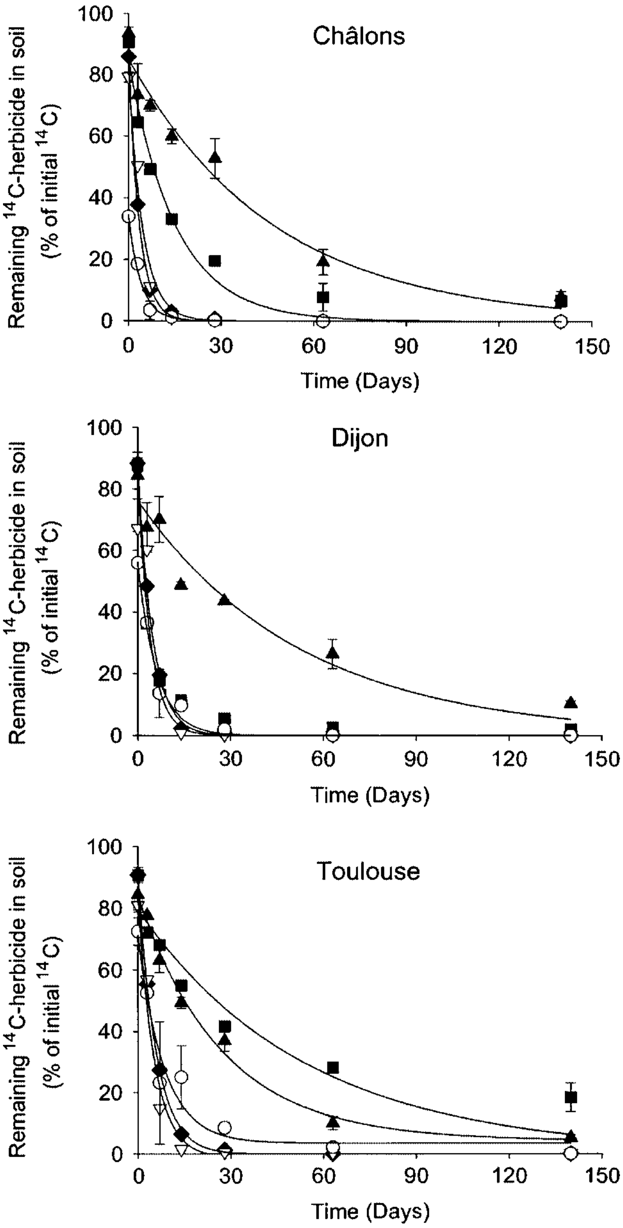


Figure 3. Degradation kinetics of glyphosate (○), trifluralin (▲), metazachlor (◆), metamitron (■) and sulcotrione (▽) in Châlons, Dijon and Toulouse soils (lines are adjustment with first-order kinetics).

Table 4. Herbicides and their main metabolites degradation rates k and half-lives $T_{1/2}$, with initial amounts of herbicide Co in Châlons, Dijon and Toulouse soils

Herbicide/ metabolite	Châlons				Dijon				Toulouse			
	k(±SD) (day^{-1})	C_o(±SD) (% initial ^{14}C)	$T_{1/2}$ (day)	r^2	k(±SD) (day^{-1})	C_o(±SD) (% initial ^{14}C)	$T_{1/2}$ (day)	r^2	k(±SD) (day^{-1})	C_o(±SD) (% initial ^{14}C)	$T_{1/2}$ (day)	r^2
Glyphosate	0.260 (±0.002)	34.6 (±1.0)	<<1	0.98	0.142 (±0.005)	55.9 (±0.8)	0.8	0.96	0.094 (±0.004)	71.5 (±1.3)	3.7	0.99
AMPA	0.026 (±0.016)	—	25	0.59	0.020 (±0.007)	—	34	0.84	0.009 (±0.002)	—	75	0.93
Trifluralin	0.021 (±0.002)	86.0 (±2.6)	25.2	0.95	0.018 (±0.002)	78.4 (±2.9)	24.2	0.92	0.037 (±0.002)	85.0 (±2.2)	14.2	0.97
Metazachlor	0.290 (±0.005)	87.0 (±0.6)	1.9	0.99	0.216 (±0.003)	89.5 (±0.6)	2.7	0.99	0.174 (±0.002)	91.8 (±0.5)	3.5	0.99
M4	0.003 (±0.000)	—	218	0.98	0.002 (±0.000)	—	309	0.97	0.002 (±0.000)	—	326	0.98
Metamitron	0.066 (±0.005)	85.5 (±2.9)	8.1	0.96	0.239 (±0.019)	87.2 (±2.8)	2.3	0.97	0.018 (±0.018)	79.8 (±3.1)	25.4	0.89
Sulcotrione	0.228 (±0.015)	81.8 (±2.3)	2.1	0.98	0.178 (±0.023)	72.2 (±4.0)	2.0	0.93	0.203 (±0.015)	84.0 (±2.6)	2.5	0.97
CMBA	0.009 (±0.001)	—	71	0.97	0.014 (±0.003)	—	46	0.91	0.012 (±0.001)	—	55	0.96

Table 5. Herbicides adsorption coefficients K_f (±SD) in Châlons, Dijon and Toulouse soils [a]

Soil Herbicide	Châlons	Dijon	Toulouse
Glyphosate	34.8 (±0.6)	41.9 (±0.5)	276 (±13)
Trifluralin	33.1 (±5.6)	27.9 (±2.7)	20.6 (±2.2)
Metazachlor	1.73 (±0.05)	1.29 (±0.06)	1.26 (±0.04)
Metamitron	2.12 (±0.22)	1.56 (±0.08)	1.45 (±0.09)
Sulcotrione	0.40 (±0.01)	0.51 (±0.01)	1.66 (±0.08)

[a] From Mamy and Barriuso.[44]

This phenomenon seems to be predominant compared to the influence of pedological characteristics like soils texture or organic carbon content.[6,14] In fact, Cox *et al*[34] reported that metamitron $T_{1/2}$ does not depend on soil type. However, the more significant metamitron degradation in Châlons soil than Toulouse soil may be related to the finer texture of Châlons soil,[6] as we have indicated previously, or to soil organic carbon content.

The $T_{1/2}$ of sulcotrione was small and homogeneous in the three soils (Table 4). These values are consistent with those reported by Baer and Calvet[8] obtained under the same temperature and moisture conditions. Sulcotrione dissipation was more rapid in soils with alkaline pH (Châlons and Dijon)[7] where its adsorption was weaker (Table 5), and in soils containing more organic carbon. The $T_{1/2}$ of CMBA was higher than those of sulcotrione (Table 4), and shows differences between the three soils due to its acidic character. CMBA was more persistent in Châlons soil with alkaline pH and high soil organic carbon content.

Glyphosate was the quickest degraded herbicide in the Châlons and Dijon soils. In the Toulouse soil, it was more persistent than sulcotrione and metazachlor. Trifluralin and metamitron were the most persistent herbicides in the three soils. The degradation of glyphosate, trifluralin and sulcotrione depended on the strength of their soil adsorption. Conversely, no relationship between retention and degradation was apparent for metazachlor and metamitron. Glyphosate, metazachlor and sulcotrione metabolites were more persistent than their parent herbicides. Metazachlor metabolite was the most persistent and AMPA was more persistent than CMBA in Toulouse soil but less persistent in the two others soils.

3.3 Identification of pesticide dissipation mechanism

Of the different dissipation mechanisms, volatilization can be considered negligible for glyphosate, metazachlor, metamitron and sulcotrione because of their low vapour pressures (Table 2). In comparison, the literature reports a large number of examples of trifluralin volatilization.[13,35] In this study, experimental conditions (high temperature, soil water content, and non-incorporation into the soil of the applied trifluralin) probably contributed to enhanced volatilization.[28,30,36] The total volatilized radioactivity (in % of the initial radioactivity) at 140 days after treatment decreased as follows: Toulouse (79) > Dijon (70) > Châlons (54). Volatilization decreases when soil organic carbon content increases (Table 3 and Fig 2) because it enhances trifluralin sorption and hence reduces its volatilization.[30,31]

The mineralization of herbicides depended on their chemical nature, and decreased as follows: glyphosate > sulcotrione, metamitron > metazachlor > trifluralin (Fig 2). Mineralization was the main dissipation mechanism for glyphosate. More than 74% of the initial radioactivity was recovered as $^{14}CO_2$. These values are consistent with others reported in the literature.[17,19,20] Mineralization kinetics did not show a lag phase, implying that no adaptation of microorganisms was necessary. The mineralization pattern suggests that glyphosate was co-metabolized.[17–19,21,27] Glyphosate mineralization directly depends on its sorption on soils.[17,19,24,27] Particularly, when soil pH and phosphate content decreased and when soil copper and iron contents increased, glyphosate sorption increased and its mineralization decreased.[19,37] The fact that maximum glyphosate mineralization occurred in the Dijon soil, despite its high iron and copper contents (Table 3), may be due to its high manganese content, which is a cofactor of glyphosate microbial conversion to CO_2.[19]

Trifluralin mineralization was less than 2.5% of the initial radioactivity at 140 days. This low mineralization is consistent with results reported in the literature, showing that biological degradation is not the main dissipation mechanism for trifluralin.[10,11,13]

The mineralization of metazachlor did not show a lag phase and its rate was constant during all incubations. The maximum percentage of mineralization varied from 15 to 20% of the initial radioactivity in the three soils. To our knowledge, no other result about metazachlor mineralization in soils has been published.

The maximum percentages of metamitron mineralization depended on soil type, and decreased as follows (in % of initial radioactivity): Dijon (67) > Châlons (49) > Toulouse (30). There was no relationship between metamitron sorption and mineralization (Table 5 and Fig 2). High mineralization rate in Dijon soil resulted probably from accelerated degradation as a result of repeated metamitron sprayings at this site.

The mineralization of sulcotrione was high and comparable with that of glyphosate. It decreased as follows: Toulouse (62.3%) > Dijon (62.2%) > Châlons (55%). These values are consistent with those of Cherrier *et al*.[38]

3.4 Formation of non-extractable (bound) residues

The formation of non-extractable (bound) residues (NER) resulted in a decrease in herbicide availability. However, herbicide residues are still in the soil and a part of these NER can correspond to the trapping or chemical stabilisation of herbicide residues associated with soil constituents. The environmental impact of NER depended of the reversibility of their formation mechanism interactions. NER made an important contribution to the apparent dissipation of both metazachlor and metamitron, where they represented up to 45% of initial radioactivity. Conversely, their contribution was low for glyphosate dissipation ($<15\%$ of initial radioactivity) (Fig 2).

Non-extractable ^{14}C from ^{14}C-glyphosate was detected at the onset of herbicide application. In the Châlons and Dijon soils, this fraction decreased during the incubation, but in the Toulouse soil it remained constant at about 7% of the initial radioactivity. These values are consistent with those of Smith and Aubin[20] and Von Wirén-Lehr *et al*.[21] Glyphosate NER increased with soil organic carbon content (Fig 2 and Table 3), which is a key factor involved in formation of NER for most pesticides.[39]

For trifluralin, amounts of NER increased during 140 days and were 30, 12 and 9% in Châlons, Dijon and Toulouse soils respectively. These values are consistent with those of Laabs *et al*.[13] Amounts of NER also increased with soil organic carbon content (Fig 2 and Table 3).

Metazachlor NER formation was a kinetic process that can be described by the compartment model of Fig 1b. These NERs were 44, 36 and 35% in Châlons, Toulouse and Dijon soils respectively at 140 days. The comparison of the rate constants between the compartments allowed estimation that metazachlor itself directly contributed to the formation of NER and not metabolite M4 as shown by the ratio of their respective rate constants for NER formation: $k_{\mathrm{metabolite}}/k_{\mathrm{herbicide}}$. Contribution of M4 to NER formation was very low; 1 and 0.3% in Chalons and Toulouse soils respectively and even negligible in Dijon soil. Similar to glyphosate and trifluralin, the amounts of NER were largest in the Châlons soil, which has the highest organic carbon content.

For metamitron, NER amounts decreased in the following order: Toulouse (45%) > Dijon (40%) > Châlons (28%). They were formed very rapidly in the Châlons and Dijon soils (<14 days), and declined slightly with time afterwards. This shows that the formation of NER was not an irreversible process.

For sulcotrione, NER were rapidly formed (28 days) and then increased slowly until day 140. At day 140, these residues represented 30, 27 and 26% in Dijon, Toulouse and Châlons soils respectively. From the $k_{\mathrm{metabolite}}/k_{\mathrm{herbicide}}$ ratio for NER formation the contribution of the metabolite to NER formation ranged between 10 and 18.4% for the different soils (Table 6).

The amounts of NER were low for glyphosate and trifluralin in the Dijon and Toulouse soils. In contrast, they were very high for metazachlor and metamitron, and less for sulcotrione. The formation of NER generally depends on organic carbon content of the soils. It is frequently considered as a dissipation process contributing to possible non-permanent removal of herbicide.[40] This is shown by the decrease in amounts of NER for glyphosate, metamitron and metazachlor. For most herbicides, there is competition between mineralization and NER formation (Fig 2) explained by herbicide stabilisation with reduction of availability to degrading microorganisms. Bound residues could contribute to delayed contamination and to maintain the potential for a gradual release of chemicals at very low concentrations.

4 CONCLUSION

The assessment of pesticide impact on the environment is focused on pesticide behaviour in soil,

Table 6. Rate constants of the non extractable residues formed from glyphosate, metazachlor, sulcotrione and of their metabolites

Herbicide	Soil	Rate constants of herbicide and metabolite non extractable residue (NER) formation (±SD) (day^{-1})		Ratio $k_{\mathrm{metabolite}}/k_{\mathrm{herbicide}}$ (%)
Glyphosate	Châlons	Glyphosate-NER	0.164 (±0.081)	
		AMPA-NER	0.005 (±0.006)	3.4
	Dijon	Glyphosate-NER	0.059 (±0.02)	
		AMPA-NER	0.008 (±0.008)	14.5
	Toulouse	Glyphosate-NER	0.070 (±0.011)	
		AMPA-NER	0.003 (±0.006)	16.3
Metazachlor	Châlons	Metazachlor-NER	0.119 (±0.010)	
		M4-NER	0.001 (±0.000)	1.0
	Dijon	Metazachlor-NER	0.085 (±0.007)	
		M4-NER	0	—
	Toulouse	Metazachlor-NER	0.065 (±0.004)	
		M4-NER	0.000 (±0.000)	—
Sulcotrione	Châlons	Sulcotrione-NER	0.030 (±0.008)	
		CMBA-NER	0.003 (±0.000)	10.0
	Dijon	Sulcotrione-NER	0.034 (±0.013)	
		CMBA-NER	0.004 (±0.002)	11.9
	Toulouse	Sulcotrione-NER	0.021 (±0.008)	
		CMBA-NER	0.004 (±0.001)	18.4

water and air, and on quantification of pesticide amounts in each compartment.[41] The main dissipation pathways of herbicides were mineralization for glyphosate and sulcotrione, volatilization for trifluralin and non-extractable residues formation for metazachlor and metamitron. The high mineralization rates of glyphosate and sulcotrione lead to their rapid disappearance. However, the high amounts of volatilized trifluralin observed probably resulted in atmospheric contamination. Lastly, the high amounts of metazachlor and metamitron as non-extractable residues lead to soil contamination, which may represent an additional and delayed risk of environmental pollution, particularly into water. Pesticide availability, as estimated from the quantification of extractable herbicide amounts, is directly related to pesticide leaching risk. Metabolites M4 and CMBA, as well as metamitron, metazachlor and sulcotrione were highly water-extractable and therefore readily available for leaching or runoff with risks of surface or ground water pollution.[40] Trifluralin, metamitron, AMPA, M4 and CMBA, being non-aqueous solvent-extractable, are less available and susceptible to later release in the environment. Herbicide persistence in soils was estimated from their degradation half-lives. The five herbicides had a low persistence, particularly glyphosate, but in soils where glyphosate adsorption is high (low pH and phosphate content), it is more persistent than sulcotrione and metazachlor. Glyphosate, trifluralin and sulcotrione degradation depend on strength of their adsorption on soils but no relationship appeared between metazachlor and metamitron adsorption and degradation. Glyphosate, metazachlor and sulcotrione metabolites were persistent and may accumulate in soil following several applications leading to an increase in environment contamination risks. Furthermore, herbicide degradation was studied under optimum temperature and moisture conditions for microbial degradation, and persistence will increase with lower temperature and moisture.

In the context of chemical weed control based on GM crops, we simulated the fate of the five herbicides investigated here based on the doses usually applied. Figure 4 shows amounts of remaining herbicides and metabolites in soils (extractable and non-extractable) after 5 months of incubation. The amounts of glyphosate residues in soils depended on soils type and on weed control management

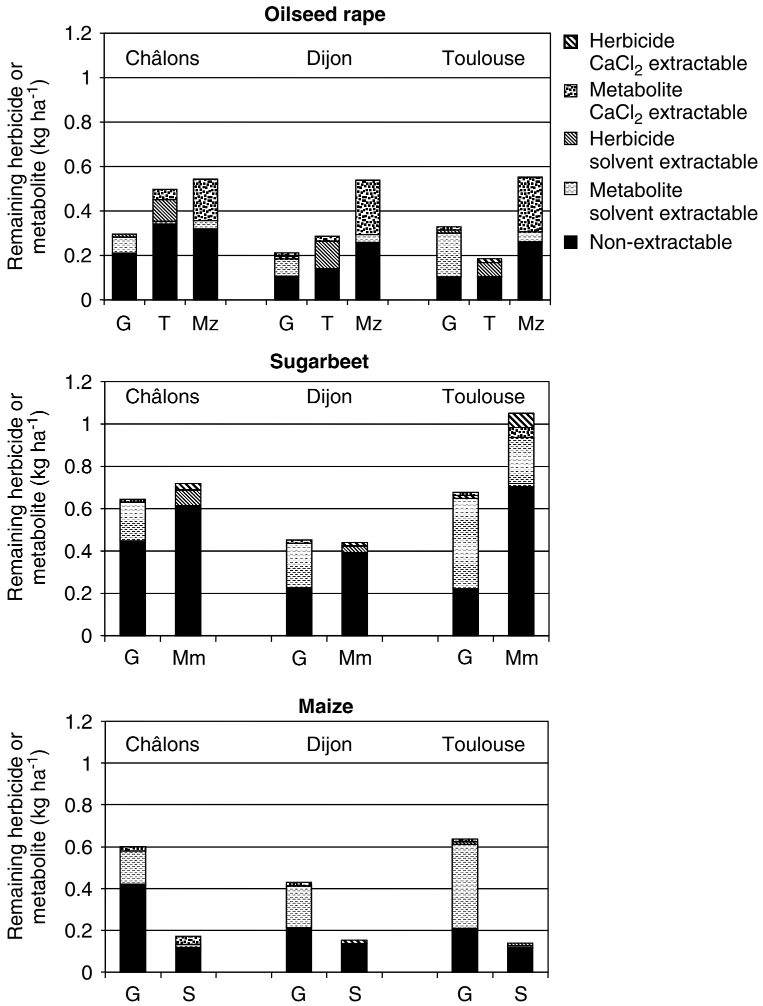


Figure 4. Remaining herbicides (G = glyphosate, T = trifluralin, Mz = metazachlor, Mm = metamitron, S = sulcotrione) and metabolites in rape, sugarbeet and maize in Châlons, Dijon and Toulouse soils after 140 days. Amounts applied are taken from Table 1 (if no value, maximum amounts are used).

within the crop. Particularly, for oilseed rape and sugarbeet, glyphosate may provide a favourable answer to environmental contamination. Soil residues are generally lower than trifluralin, metazachlor and metamitron residues, implying a decrease of potential contamination of soil and consequently water and air. However, in the case of maize, glyphosate may lead to higher soil contamination because of very low sulcotrione doses and high glyphosate doses. Nevertheless, weed control management in non-GM crops usually involves a combination of several herbicides (Table 1) resulting in a higher total dose applied compared to glyphosate-based weed control. Among the five herbicides studied, glyphosate seems to be the least potentially polluting herbicide with respect to soil, water and air. However, the benefits of using glyphosate resistant crops will depend on soils type (glyphosate is more persistent in soil with low pH, low phosphate content and high amorphous iron content), way and level of substituted herbicides degradation, and on the application rate. Furthermore, glyphosate metabolite AMPA is more persistent and in fact it is increasingly detected in water bodies in France.[43] This finding should be related to the important and increasing use of glyphosate.

ACKNOWLEDGEMENTS

The authors acknowledge financial support from the INRA (Institut National de la Recherche Agronomique) programme 'Organismes génétiquement modifiés et environnement' and the CNRS (Centre National de la Recherche Scientifique) programme 'Impact des biotechnologies dans les agro-écosystèmes'. Laure Mamy was supported by a fellowship from INRA and CETIOM (Centre Technique Interprofessionnel des Oléagineux Métropolitains). The authors are grateful to BASF for supplying metazachlor and to Laboratoire Sols et Environnement (ENSAIA-INPL/INRA) for supplying sulcotrione.

REFERENCES

1 Messéan A, Impacts des plantes transgéniques pour l'agriculture. *CR Acad Agric Fr* **86**:87–96 (2000).

2 Gray A, Genetically modified crops: broader environmental issues. *J Comm Biotech* **10**:234–240 (2004).

3 Torstensson L, Behaviour of glyphosate in soils and its degradation, in *The Herbicide Glyphosate*, ed by Grossbard E, and Atkinson D, Butterworth, London, pp 137–150 (1985).

4 Barriuso E, *Estimation des risques environnementaux des pesticides*, Institut National de la Recherche Agronomique, Paris 123 pp (2004).

5 Walker A and Brown PA, The relative persistence in soil of five acetanilide herbicides. *Bull Environ Contam Toxicol* **34**:143–149 (1985).

6 Allen R and Walker A, The influence of soil properties on the rates of degradation of metamitron, metazachlor and metribuzin. *Pestic Sci* **18**:95–111 (1987).

7 Rouchaud J, Neus O, Callens D and Beulke R, Sulcotrione soil persistence and mobility in summer maize and winter wheat crops. *Weed Res* **38**:361–371 (1998).

8 Baer U and Calvet R, Fate of soil applied herbicides: experimental data and prediction of dissipation kinetics. *J Environ Qual* **28**:1765–1777 (1999).

9 Beulke S and Malkomes HP, Effects of the herbicides metazachlor and dinoterb on the soil microflora and the degradation and sorption of metazachlor under different environmental conditions. *Biol Fertil Soils* **33**:467–471 (2001).

10 Messersmith CG, Burnside OC and Lavy TL, Biological and non-biological dissipation of trifluralin from soil. *Weed Sci* **19**:285–290 (1971).

11 Savage KE, Nitralin and trifluralin persistence in soil. *Weed Sci* **21**:285–288 (1973).

12 Jolley AV and Johnstone PK, Degradation of trifluralin in three Victorian soils under field and laboratory conditions. *Austr J Exp Agric* **34**:57–65 (1994).

13 Laabs V, Amelung W, Fent G, Zech W and Kubiak R, Fate of 14C-labeled soybean and corn pesticides in tropical soils of Brazil under laboratory conditions. *J Agric Food Chem* **50**:4619–4627 (2002).

14 Vink JPM and Van der Zee SEATM, Some physicochemical and environmental factors affecting transformation rates and sorption of the herbicide metamitron in soil. *Pestic Sci* **46**:113–119 (1996).

15 Vischetti C, Marucchini C, Leita L, Ceccon P and Giovanardi R, Soil behaviour of metamitron in laboratory and lysimeter studies. *Agronomie* **17**:367–373 (1997).

16 Van der Pas LJT, Matser AM, Boesten JJTI and Leistra M, Behaviour of metamitron and hydroxy-chlorothalonil in low-humic sandy soils. *Pestic Sci* **55**:923–934 (1999).

17 Sprankle P, Meggit WF and Penner D, Adsorption, mobility and microbial degradation of glyphosate in the soil. *Weed Sci* **23**:229–234 (1975).

18 Rueppel M, Brightwell BB, Schaefer J and Marvel JT, Metabolism and degradation of glyphosate in soil and water. *J Agric Food Chem* **25**:517–528 (1977).

19 Moshier LJ and Penner D, Factors influencing microbial degradation of ^{14}C-glyphosate to $^{14}CO_2$ in soil. *Weed Sci* **26**:686–691 (1978).

20 Smith AE and Aubin AJ, Degradation of 14C-glyphosate in Saskatchewan soils. *Bull Environ Contam Toxicol* **50**:499–505 (1993).

21 Von Wirén-Lehr S, Komoßa D, Gläßgen WE, Sandermann H Jr and Scheunert I, Mineralization of [^{14}C]Glyphosate and its plant-associated residues in arable soils originating from different farming systems. *Pestic Sci* **51**:436–442 (1997).

22 Aubin JA and Smith AE, Extraction of [14C]Glyphosate from Saskatchewan soils. *J Agric Food Chem* **40**:1163–1165 (1992).

23 Roy ND, Konar SK, Banerjee S, Charles DA, Thompson DG and Prasad R, Persistence, movement and degradation of glyphosate in selected Canadian boreal forest soils. *J Agric Food Chem* **37**:437–440 (1989).

24 Eberbach PL, Applying non-steady-state compartmental analysis to investigate the simultaneous degradation of soluble and sorbed glyphosate (*N*-(phosphonomethyl)glycine) in four soils. *Pestic Sci* **52**:229–240 (1998).

25 Grunewald K, Schmidt W, Unger C and Hanschmann G, Behavior of glyphosate and aminomethylphosphonic acid (AMPA) in soils and water of reservoir Radeburg II catchment (Saxony/Germany). *J Plant Nutr Soil Sci* **164**:65–70 (2001).

26 Sprankle P, Meggit WF and Penner D, Rapid inactivation of glyphosate in the soil. *Weed Sci* **23**:224–228 (1975).

27 Nomura NS and Hilton HW, The adsorption and degradation of glyphosate in five Hawaïan sugarcane soils. *Weed Res* **17**:113–121 (1977).

28 Savage KE and Barrentine WL, Trifluralin persistence as affected by depth of soil incorporation. *Weed Sci* **17**:349–352 (1969).

29 Zimdahl RL and Gwynn SM, Soil degradation of three dinitroanilines. *Weed Sci* **25**:247–251 (1977).

30 Hollist RL and Foy CL, Trifluralin interactions with soil constituents. *Weed Sci* **19**:11–16 (1971).

31 Tavares MCH and Rezende de O MO, Effect of humic acid on the sorption of trifluralin by soils. *J Environ Sci Health* **36**:749–767 (1998).

32 Vischetti C, Marucchini C, Casucci C, Leita L and Porzi G, Transformation of metamitron in a sandy clay loam soil. *Agronomie* **19**:477–481 (1999).

33 Walker A and Welch SJ, Further studies of the enhanced biodegradation of some soil-applied herbicides. *Weed Res* **32**:19–27 (1992).

34 Cox L, Walker A, Hermosin MC and Cornejo J, Measurement and simulation of the movement of thiazasulfuron, clopyralid and metamitron in soil columns. *Weed Res* **36**:419–429 (1996).

35 Glotfelty DE, Taylor AW, Turner BC and Zoller WH, Volatilization of surface- applied pesticides from fallow soil. *J Agric Food Chem* **32**:638–643 (1984).

36 Bardsley CE, Savage KE and Walker JC, Trifluralin behavior in soil. II. Volatilization as influenced by concentration, time, soil moisture content and placement. *Agron J* **60**:89–92 (1968).

37 Morillo E, Undabeytia T, Maqueda C and Ramos A, Glyphosate adsorption on soils of different characteristics. Influence of copper addition. *Chemosphere* **40**:103–107 (2000).

38 Cherrier R, Perrin-Ganier C and Schiavon M, Degradation of sulcotrione in a brown soil amended with various organic matters. *Agronomie* **24**:29–33 (2004).

39 Loiseau L and Barriuso E, Characterization of the atrazine's bound (nonextractable) residues using fractionation techniques for soil organic matter. *Environ Sci Technol* **36**:683–689 (2002).

40 Barriuso E, Calvet R, Schiavon M and Soulas G, Les pesticides et les polluants organiques des sols. Transformations et dissipation. *Etude et Gestion des sols* **3**,**4**:279–295 Numéro spécial (1996).

41 Gaillardon P, Evaluation du comportement environnemental des pesticides dans le cadre des procédures d'homologation, in *Estimation des risques environnementaux des pesticides*, E Barriuso Coord., Institut National de la Recherche Agronomique, Paris pp 25–40 (2004).

42 Tomlin CDS (ed), *The Pesticide Manual.* 12th edn. BCPC, Farnham, Surrey, UK (2000).

43 IFEN (Institut Français de l'Environnement), Pesticides in water, Fifth annual report, Data 2001, Institut Français de l'Environnement, Orléans, Vol 37, 27 pp (2003).

44 Mamy L and Barriuso E, Glyphosate adsorption in soils compared to herbicides replaced with the introduction of glyphosate resistant crops. *Chemosphere* DOI: 10.1016/j.chemosphere.2005.04.051(2005).