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# Soil Dissipation of the Post-Emergence Herbicide Sulcotrione in Maize Crops Treated with Organic Fertilizers

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Sulcotrione (2-[2-chloro-4-(methylsulfonyl)benzoyl]-l,3-cyclohexanedione) is a new herbicide applied at the rate of 450 g ha-l post-emergence in maize crops at the maize 5 to 6 leaves stage. It is efficiently used against a broad range of broadleaf weeds (Solanum nigrum, Chenopodium album...) and some annual grasses in corn (Wilson and Foy, 1990). It is absorbed by the leaves and also by the roots. At our knowledge, nothing so far has been published about the method of analysis of sulcotrione in soil, nor about the sulcotrione biodegradation in soil. Using 14C-sulcotrione, and measuring in the extracts the 14C by liquid scintillation, Wilson and Foy (1992) observed that the adsorption of sulcotrione onto soil is greater when the organic matter concentration in soil is greater. The soil clay content and pH had no significant influence on sulcotrione adsorption. We previously observed that the atrazine and metolachlor soil biodegradations in corn crops were slowed down by the recent organic fertilizer treatments (Rouchaud et al., 1994). The assumption was made that this effect was due to the increase of the adsorption of the herbicides on the soil organic matter (Honnay,l993); in the adsorbed state, the herbicides could be in some way protected against the soil microbial activity which metabolizes them. In the present work, we studied sulcotrione soil biodegradation in maize crops recently treated or not with cow manure, pig slurry or green manure.

## MATERIALS AND METHODS

A 32x36 m field at Melle, Belgium (clay 7%, silt 38%, sand 55%, organic matter 2.2%, pH 6.7, sandy loam) was tilled on 2-9-1993 (days-month-year), and subdivided into 6x8 m plots. Four plots were sown at 17 kg seed ha-l with yellow mustard (cv. Emergo) as a green manure treatment on 2-9-1993. The green manure, which had reached a height of 45-50 cm, was soil incorporated by tilling on 4-3-1994. On 12-11-1993, either cow manure or pig slurry at 50 tons ha-l each were applied to four other replicate plots. Similarly, on 4-3-1994, either cow manure or pig slurry were applied to other four plots not yet treated with organic fertili-

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zers. The plots treated with cow manure or pig slurry were immediately rotary-tilled to 15 cm depth after treatment. Four control plots were not treated with organic fertilizers. There were thus 4 replicate plots for each organic fertilizer treatments and control. Only one organic fertilizer treatment had been applied, and only once, onto each plot except for the control plots which were left untreated. On 11-5-1994, the entire field was rotary-tilled to 15 cm depth, and maize (cv. Aviso) was sown. On 17-6-1994, sulcotrione was applied at 450 g ha-l by spraying the emulsion of Mikado (30 g% sulcotrione, Zeneca) in water (750 1 ha-l), the maize plants having 5 to 6 leaves. At intervals after sulcotrione application, samples were taken separately (and analyzed separately) in the 0-10 cm superficial soil layer of each of the four replicate plots of each of the organic fertilizers treatments and control (Table 1). In addition, on 15-7-1994 and 11-8-1994 single samples were taken separately from the 10-20 cm soil layer in each of the four replicate plots; each soil sample was analyzed separately, one analysis being thus made with the soil of each replicate plot. For each soil sample, 15 cores (2.5 cm diameter) were taken from each replicate plot at random points, the cores from each replicate plot were bulked together and then stored at -25°C until analyzed.

The same maize trial was made at Cortil-Noirmont, Belgium (clay 12%, silt 82%, sand 6%, organic matter 1.9%, pH 6.7, silt loam). No green manure however was assayed. One of the cow manure or pig slurry were applied only on 14-3-1994. Maize was sown on 29-4-1994, and sulcotrione was applied on 7-6-1994 at the maize 5 to 6 leaves stage. At intervals after sulcotrione application, samples were taken for analysis from the 0-10 cm superficial soil layer (Table 2). In addition, on 8-7-1994 and 8-8-1994 samples were taken from the l0-20 cm soil layer. At harvest, maize foliage and grain samples were taken at random in each of the field plots of crops at Melle and Cortil-Noirmont.

Thin-layer chromatography (TLC) was made by using silica gel 60F254 20x20 cm, 0.2 mm thick plates. The sample was applied as a band. Standards were applied on another part of the TLC plate, next to the band of the sample solution.

Gas-liquid chromatography (GLC) conditions were the following. Electron capture detection. Injection and detection at 270°C. Glass column 1.80 m x 2 mm i.d. containing 5% SE 30 on Gas-Chrom Q 80-100 mesh. Nitrogen carrier gas was at 50 ml min-1. The isomers of unmethylated sulcotrione were not separated on the TLC plate, but recovered as a mixture in the broad TLC band at Rf= 0.15-0.45 of the soil analysis procedure. On the other hand, unmethylated sulcotrione gave 3 GLC separated isomers. The first isomer had a retention time of 1.3 min with column oven at 230°C. The second and third isomers had retention times of 2.5 and 3.6 min, respectively, with column oven at 250°C. GC-MS indicated the same molecular ion for each of the 3 isomers of sulcotrione, and similar mass fragmentation patterns. The relative amounts of the 3 isomers changed according to their concentration in the sample, and the previous treatments of sulcotrione made during the analytical procedure (ethyl acetate extraction from 0.24 N HCl in water, TLC on silica gel). Previously untreated sulcotrione mainly (~80%) gave the second isomer, for which are given the IR, lH-NMR and MS spectra. At the soil residue level however, and after the analytical procedure, the first isomer was the main one (~80%). The standard for analysis of the mixture of isomers was obtained by making a recovery of sulcotrione from HCl 0.24 N at the level corresponding to 1 mg kg-l soil (i.e. 0.1 mg in 100 g soil), and further proceeding as in the soil analysis procedure, with successive ethyl acetate extraction and TLC on silica gel. For the measurement of the sulcotrione soil concentrations, the sum of the GLC peaks was made for the 3 sulcotrione isomers.

Methylation of sulcotrione allowed its GLC detection with a sensitivity about 4 times greater than unmethylated sulcotrione. Moreover, it confirmed the soil residue results obtained with unmethylated sulcotrione. In the soil analysis procedure, the isomers of methylated sulcotrione were not separated by TLC before GLC analysis. The TLC of their mixture however was made in separated experiment on silica gel plate with ether as eluent; the first, second and third isomers of methylated sulcotrione were at Rf=0.76, 0.23 and 0, respectively. GLC of methylated sulcotrione separated 3 isomers. The first isomer had a retention time of 1.8 min with column oven at 175°C, and 0.3 min at 250°C. The second and third isomers had retention times of 2.3 and 3.4 min, respectively, at 250°C. GC-MS indicated the same molecular ion for each of the 3 isomers of methylated sulcotrione, and similar fragmentation patterns. As with unmethylated sulcotrione, the relative amounts of the 3 isomers of methylated sulcotrione changed according to their total concentration in the sample, and the previous treatments of sulcotrione. Previously untreated sulcotrione gave by methylation mainly (~80%) the second isomer, for which are given the IR, lH-NMR and MS spectra. At the soil residue level however, and after the analytical procedure, the first isomer of methylated sulcotrione was the main one (~80%). The standard for analysis of the mixture of isomers of methylated sulcotrione was obtained by making a recovery of sulcotrione from HCl 0.24 N at the level corresponding to 1 mg kg-l soil, and further proceeding as in the soil analysis procedure, with successive ethyl acetate extraction, TLC on silica gel and methylation. For the measurement of the sulcotrione soil concentrations, the sum of the GLC peaks was made for the 3 isomers of methylated sulcotrione. In several cases, sulcotrione extracted from soil, and analyzed by GLC successively before and after methylation, was further analyzed by GC-MS before and after methylation.

Infra-red (IR) spectra: KBr discs, cm-l. Nuclear magnetic resonance (lH-NMR) spectra: δ, ppm/tetramethylsilane in CDC13. Mess (MS) and gas-liquid mass (GC-MS) spectra: 70 eV, electron impact; m/e, relative abundance, %.

Sulcotrione analytical standard was made as following: Mikado (60 g; 30 g% sulcotrione, Zeneca) was concentrated to dryness in a vaccuum rotary evaporator (60°C). The solid was stirred with acetone (250 ml), the mixture was filtered, the filtrate was concentrated to dryness in a vacuum rotary evaporator (30°C), the solid was recrystallized two times in chloroform giving sulcotrione (12.3 g, 68%) whose purity was greater than 99.5% as shown by TLC. Spectra of sulcotrione (mainly the second isomer): IR: 3462, 3007, 2992, 2917, 1657(CO), 1593, 1551, 1397, 1308, 1279, 1200, 1154, 1096, 961, 777, 752.
lH-NMR: 2.08 (q, 2H, $COCH_2\underline{CH}_2CH_2CO$); 2.46 (t, 2H, $COCH_2CH_2\underline{CH}_2CO$); 2.82 (t, 2H, $CO\underline{CH}_2CH_2CH_2CO$) 3.11(s, 3H, $SO_2CH_3$); 3.64 (s,1H, CH(CO)3); 7.38-x42 (m, lH, Harom); 7.86-7.97 (m, 2H, Harom).
MS: 329 (M+l, 0.4); 331 (329+2, 0.1); 313 (M-$CH_3$, 0.4); 315 (313+2, 0.1): 293 (M-Cl, 100); 264 (293-CO-H, 32); 214 (293-$SO_2CH_3$, 43); 199 (214-$CH_3$, 8); 155 ($\overline{CHCCCHCHC}(SO_2CH_3)$+H, 16); 138 ( $\overline{COCH_2CH_2CH_2COC}HCO$-H,8)

Methylated sulcotrione analytical standard was made as the following. To sulcotrione (0.115 g, 0.35 mmole) in a mixture of acetone (5 ml) and ethyl acetate (20 ml) was added a solution of diazomethane in ether (~25 ml) until persistence of the yellow colour. The mixture was stirred for 24 hr at room temperature, concentrated in vacuum to dryness and applied to a silica gel TLC plate. Elution with ether gave a band at Rf=0.23 containing the second isomer of the methylated sulcotrione. The band at Rf=0.23 was separated, the silica gel extracted with ethyl acetate, and the extract concentrated to dryness giving mainly the second isomer of the methylated sulcotrione (0.102 g, 85%). Spectra of the second isomer of methylated sulcotrione: IR: 3485, 3013, 2938, 1655(CO), 1624(C=C of ketone enol form), 1458, 1400, 1377, 1314, 1154, 1096, 965, 801, 754.
lH-NMR: 2.13 (s, lH, $\overline{C(OCH_3)CHCH_2CH_2COC}\underline{H}$; 2.39(m, 2H, $\overline{C(OCH_3)CHCH_2\underline{CH}_2COCH}$); 2.63 (m, 2H, $\overline{C(OCH_3)CH\underline{CH}_2CH_2COCH}$); 3.08 (s, 3H, $SO_2$) $CH_3$); 3.29 (s, 3H, $OCH_3$); 4.64 (s, 1H, $\overline{C(OCH_3)\underline{CH}CH_2CH_2COCH}$); 7.72 -8.05 (m, 3H, Harom).
MS: 325 (M-OH, 9); 327 (325+2, 3); 289 (325-HCl, 100); 281 (325-$COCH_3$-H, 4); 258 (289-$OCH_3$, 6); 239 (258-$H_3O$+, 7); 226 (289-$SO_2$+H, 12); 210 (289-$SO_2CH_3$, 56); 152 ($\overline{CHCC1C(CO)CHCHC}CH_3$-H, 18); 126 ($\overline{C(OCH_3)CHCH_2CHCOHC}H$+H, 23); 79 ($SO_2CH_3$, 24); 63($SOCH_3$, 38).

For analysis of sulcotrione in soil, soil (100 g) was stirred in methanol/water (8/2 vol./vol., 200 ml) at room temperature (35 min). The mixture was filtered, and the extraction repeated. The filtrates were combined, water (100 ml) was added, and the methanol removed in a vacuum rotary evaporator (35°C). The volume of the water extract was adjusted to 180 ml by eventual addition of water and was made HC1 0.24 N by addition of hydrochloric acid (3.6 ml). NaCl (15 g) was added, the acid aqueous solution was extracted two times with ethyl acetate (2x200 ml), the ethyl acetate solution was dried (stirring during 1 hr at 20°C with $Na_2SO_4$), concentrated to 40 ml in a vacuum evaporator (30°C), and then concentrated further to 0.5 ml under a slow stream of nitrogen (20°C). The concentrate was applied to a TLC plate. A first elution with ethyl acetate moved most of the yellow and green pigments in the solvent front, and sulcotrione at Rf=0-0.25. The plate was taken off the bath, quickly air dried, and placed in a second TLC bath made up of ethyl acetate/acetic acid, 10/l, vol./vol. When the solvent front reached Rf=0.50 (relative to the first elution solvent front) the plate was taken off the TLC bath. The mixture of isomers of sulcotrione was in the band at Rf=0.15-0.45. This band was scraped off, the silica gel extracted with acetone (40 ml) and the extract concentrated to 1 ml under a slow stream of nitrogen (20°C). The unmethylated extract was first analyzed for sulcotrione by GLC and, in several cases by GC-MS. It then was methylated by successive addition of ethyl acetate (4 ml) and a solution of diazomethane in ether (about 3 ml) until a persistent yellow colour. After 1 hr at room temperature, the methylated extract was concentrated to 1 ml under a slow stream of nitrogen (20°C), and analyzed by GLC and, in several cases, by GC-MS. At the 0.1 mg and 0.05 mg sulcotrione kg-l level in soil, the recovery was 82-96%. The analytical limit of sensitivity was 0.01 mg kg-l dry soil. Harvested maize foliage and grain were analyzed in the same way as soil. Before extraction, the foliage however was cut into small pieces, and the grain was ground into flour. Sulcotrione recoveries and limit of sensitivity respectively were 81-95% and 0.01 mg kg-l fresh weight,

## RESULTS AND DISCUSSION

In both maize crop trials, no sulcotrione was detected in the 10-20 cm soil layer. There was thus no detectable leaching of sulcotrione in soil. No residues of sulcotrione were detected in the maize foliage and grain at harvest, the limit of sensitivity for both soil and plant materials being 0.01 mg kg-l dry soil and fresh weight, respectively.

The triketone structure of sulcotrione leads to the formation of several isomers. The secondary and tertiary protons ∝ to the keto functions indeed are able to enolize. If there is some rigidity along the keto bridge between the cyclohexane and phenyl rings, and if there are simultaneously mono- and dienols, the number of isomers increases. For unmethylated sulcotrione, these were observed on the TLC plate as a broad band. GLC however separated 3 main unmethylated isomers. For methylated sulcotrione, major and minor isomers were separated by TLC elution with ether. GLC also separated 3 main methylated isomers.

In the lH-NMR spectrum of unmethylated sulcotrione in CDC13, 2 broad hydroxyl bands indeed were sometimes observed between 2 and 3 ppm. Their intensities changed according to the sulcotrione concentration in CDC13, or according to the manipulations made before: TLC on silica gel, or contact with water acidified or not. On the other hand, separate experiments indicated that more

Table 1. Concentrations of sulcotrione in the 0-10 cm surface soil layer of the maize field at Melle post-emergence treated with 450 g sulcotrione ha$^{-1}$. Each field plot had been treated either with cow manure or pig slurry applied either in November or in March before sowing. Other plots were treated with green manure. There were also control plots not treated with organic fertilizers.

| Days after sulcotrione treatment[a] | Organic fertilizer treatment | | | | | |
|---|---|---|---|---|---|---|
| | Control | Green mamanure | Cow manure in November | Pig slurry in November | Cow manure in March | Pig slurry in March |
| | Concentrations of sulcotrione (mg kg$^{-1}$ dry soil) in the 0–10 cm soil layer[b] | | | | | |
| 0 | 0.46 | 0.47 | 0.45 | 0.44 | 0.43 | 0.44 |
| 9 | 0.30 | 0.36 | 0.39 | 0.37 | 0.40 | 0.38 |
| 20 | 0.19 | 0.25 | 0.26 | 0.26 | 0.29 | 0.27 |
| 28 | 0.14 | 0.18 | 0.21 | 0.22 | 0.24 | 0.23 |
| 35 | 0.11 | 0.15 | 0.17 | 0.19 | 0.20 | 0.20 |
| 46 | 0.06 | 0.11 | 0.13 | 0.15 | 0.15 | 0.15 |
| 55 | 0.04 | 0.06 | 0.06 | 0.08 | 0.09 | 0.07 |
| 72 | 0.02 | 0.03 | 0.01 | 0.04 | 0.02 | 0.03 |
| Corr. coeff.[c] | | | | | | |
| | −0.9956 | −0.9947 | −0.9928 | −0.9922 | −0.9881 | −0.9928 |
| y Intercept[c] | | | | | | |
| | 3.82 | 3.85 | 3.84 | 3.78 | 3.83 | 3.80 |
| Slope, days$^{-1}$ [c] | | | | | | |
| | −0.04271 | −0.03193 | −0.02817 | −0.02394 | −0.02385 | −0.02368 |
| Soil half-lives, days[c] | | | | | | |
| | 16.2±0.8 | 21.7±1.1 | 24.6±1.2 | 29.0±1.5 | 29.1±1.5 | 29.3±1.5 |

a. Maize was sown on 11-5-1994. Sulcotrione was applied on 17-6-1994 at the maize 5 to 6 leaves stage. The dates of soil sampling (day-month of year 1994), the number of days after sulcotrione application and the cumulative rainfall (mm) were, respectively: 17-6, 0, 0; 26-6, 9, 2; 7-7, 20, 19; 15-7, 28, 59; 22-7, 35, 59; 2-8, 46, 100; 11-8, 55, 118; 28-8, 72, 142.

b. Means of 4 replicates. S.d. generally were ± 0.02 for means greater than 0.29 mg kg$^{-1}$ dry soil, and ± 0.01 for means lower than 0.29.

c. For the first 46 days period, linear regression $\ln y = kt + b$ of the naperian logarithms of the sulcotrione soil concentrations ($y = 100 \times$ mg kg$^{-1}$ dry soil) against time t (days) following sulcotrione treatment: correlation coefficient, y intercept, and slope. The sulcotrione soil half-lives with their 95% confidence intervals were obtained using the SAS logicial CMS SAS 5.18 (1984, 1986, SAS Institute Inc., Cary, NC 27512).

Table 2. Concentrations of sulcotrione in the 0-10 cm surface soil layer of the maize field at Cortil-Noirmont post-emergence treated with 450 g sulcotrione ha-1. Each field plot had been treated either with cow manure or pig slurry applied in March before sowing. There were also control plots not treated with organic fertilizers.

| Days after sulcotrione treatment[a] | Organic fertilizer treatment | | |
|---|---|---|---|
| | Control | Cow manure | Pig slurry |
| | Concentrations of sulcotrione (mg $kg^{-1}$ dry soil) in the 0-10 cm soil layer[b] | | |
| 0 | 0.47 | 0.43 | 0.44 |
| 10 | 0.29 | 0.39 | 0.36 |
| 17 | 0.21 | 0.31 | 0.29 |
| 24 | 0.16 | 0.25 | 0.23 |
| 31 | 0.11 | 0.21 | 0.21 |
| 41 | 0.07 | 0.16 | 0.15 |
| 52 | 0.04 | 0.12 | 0.11 |
| 62 | 0.03 | 0.05 | 0.07 |
| 70 | 0.02 | 0.01 | 0.03 |
| Corr. coeff.[c] | -0.9983 | -0.9851 | -0.9900 |
| y Intercept[c] | 3.85 | 3.84 | 3.81 |
| Slope, $days^{-1}$[c] | -0.04599 | -0.02533 | -0.02624 |
| Soil half-lives, days[c] | 15.1±0.8 | 27.4±1.4 | 26.4±1.3 |

a to c: As in Table 1, except the following:

a. Maize was sown on 29-4-1994. Sulcotrione was applied on 7-6-1994. The dates of soil sampling (day-month of year 1994), the number of days after sulcotrione application, and the cumulative rainfall (mm) were, respectively: 7-6, 0, 0; 17-6, 10, 7; 24-6, 17, 7; 1-7, 24, 7; 8-7, 31, 19; 18-7, 41, 32; 29-7, 52, 37; 8-8, 62, 79; 16-8, 70, 95.

c. Linear regression for the first 41 days period.

than 95% of sulcotrione was recovered untransformed after heating to reflux during 3 hr in 1.2 N HCl in water; during soil analysis contact with 0.24 N HCl in water during a short time at room temperature thus did not destroy sulcotrione. Moreover, isomerization of the secondary cyclohexane protons $\alpha$ to the ketonic functions was observed in methylated sulcotrione by the appearance of one olefinic proton at 4.64 ppm. Methylation of sulcotrione with diazomethane increased the GLC detection sensitivity by a factor of about 4. Sulcotrione methylation also gave 3 main isomers, whose proportions changed according to the sulcotrione concentrations and previous treatments. For soil analysis, care thus was given for these isomerizations. Unmethylated and methylated sulcotrione were measured successively in the soil extracts by GLC and, in several cases, by GC-MS.

In the control plots not treated with organic fertilizers of both crops, the rate of sulcotrione dissipation in soil followed first order kinetics (Tables 1 and 2). In the plots treated with organic fertilizers, the first order kinetics was observed during the first 1.5 month period which followed sulcotrione application; after that period, the rate of sulcotrione dissipation in soil became greater than the one forecasted by the first order kinetics. In the crop made at Melle, during the first 1.5 month period which followed sulcotrione application, its soil half-life in the control plots was 16 days; in the plots treated with green manure it was 22 days; in the plots treated with cow manure applied either in November or in March, it was 25 or 29 days, respectively; in both the plots treated with pig slurry applied either in November or in March, it was 29 days (Table 1). In the crop made at Cortil-Noirmont, the sulcotrione soil half-life in the control plots was 15 days; in the plots treated with cow manure or pig slurry applied in March, it was 27 days (Table 2). These sulcotrione soil half-lives are greater than the ones first announced. They correspond to the biological effects (strong braking) observed on the sensitive sugar beet (and some other sensitive crops) sown 1.5 and still 4 months after sulcotrione application in March (Callens and Bulcke, 1995). The effect of the organic fertilizers to slow down the sulcotrione soil dissipation corresponds to increased adsorption of sulcotrione onto soils containing greater organic matter concentrations (Wilson and Foy, 1992). In the solid adsorbed phase, sulcotrione should be in some way protected against the soil microbial activity which metabolizes the herbicide (Honnay, 1993).

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