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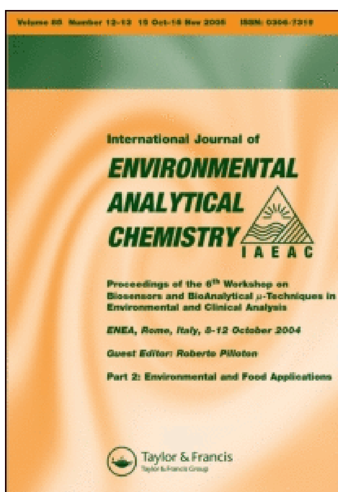

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Analysis of the Sulfonylurea Herbicide Metsulfuron-Methyl and Its Metabolites in the Soil of Cereal Crops. Comparative Analytical Chemistry of the Sulfonylureas

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ANALYSIS OF THE SULFONYLUREA HERBICIDE METSULFURON-METHYL AND ITS METABOLITES IN THE SOIL OF CEREAL CROPS. COMPARATIVE ANALYTICAL CHEMISTRY OF THE SULFONYLUREAS

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(Revised 11 April 2000; In final form 13 July 2000)

For the analysis of metsulfuron-methyl in the crop soils with a sensitivity limit of 0.3 $\mu g\ kg^{-1}$ dry soil, in the soil extract metsulfuron-methyl was separated from its soil metabolites and the soil impurities by repeated thin-layer chromatographies (TLC). In the cleaned soil extract, diazomethane transformed metsulfuron-methyl **1** into *N,N'* -dimethyl metsulfuron-methyl **2** (methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)methylamino]carbonyl]methylamino]sulfonyl]benzoate). In the gas-liquid chromatograph with detection by electron capture (GC-EC) and in the combined gas chromatograph-mass spectrometer (GC-MS), **2** was transformed into 1-dioxy-2-*N*-methyl-3-keto-1,2-benzisothiazole **3** which was measured by GC-EC with confirmation by GC-MS. The metsulfuron-methyl soil metabolites 2-sulfonamido-methylbenzoate **6**, 1-dioxy-3-keto-1,2-benzisothiazole (saccharin) **7** and 2-sulfonamidobenzoic acid **8** were analyzed in the soil of winter wheat crops by a procedure similar to the one for metsulfuron-methyl. After their separation and purification in the soil extracts by TLC, **7** and **8** were methylated, and analyzed as **3** in the GC-EC and GC-MS apparatus where the generated **6** was quantitatively transformed into **3**; **6** was analyzed as such with the GC and GC-MS apparatus wherein it was transformed into **3**. The sensitivity limit for each metabolite was 0.3 μg of equivalents of metsulfuron-methyl kg^{-1}dry soil. The syntheses of the analysis standards of the metsulfuron-methyl derivatives **2** and **3**, and of the metsulfuron-methyl metabolites **6**, **7** and **8** are described. The transformation pathways of metsulfuron-methyl and of its derivatives are different from those of the pyridine-pyrimidine sulfonylurea herbicides flupyrsulfuron-methyl and rimsulfuron. The soil analysis of a sulfonylurea -by means of one of its transformation product- needs a previous study of the chemical reactivity of the sulfonylurea. This leads to the analysis procedures for the main soil metabolites of the sulfonylurea.

Keywords: Metsulfuron-methyl; soil metabolites; gas chromatography; combined gas chromatography -mass spectrometry

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INTRODUCTION

The sulfonylurea metsulfuron-methyl **1** (methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino] sulfonyl]benzoate) is an herbicide used with efficiency in winter wheat at the dose of 4 to 7.5 g ha^{-1} against annual and perennial broad-leaved weeds (Figure 1)[1]. The rate of metsulfuron-methyl soil degradation is much dependent on the soil pH, moisture content and microbiological activity[2]. The measurement of the metsulfuron-methyl soil residues thus are needed, the forecast of its soil persistence being difficult. Metsulfuron-methyl has a low rate of field application; it has a high molecular weight, is highly polar and thermally unstable, and gives a small molecular ion at the mass spectrometry. Therefore its analysis in field soil is a problem. Sensitive plants have been used to measure it in soil by bioassays[3,4]. Pyrolysis at 800°C of the sulfonylureas produces a range of fragments characteristic of the original molecule which are analyzed by GC-MS: the heterocyclic amine or isocyanate, and the sulfonamide[5]. Diazomethane transforms metsulfuron-methyl **1** into its *N,N'*-dimethyl derivative **2** which, in special conditions, may be measured as such (the intact methylated herbicide) by GC and GC-MS (Figure 1)[6,7]. The detection limit is 2 µg kg^{-1} soil, thus too high as it corresponds to 33% of the applied dose[8]. HPLC (high pressure liquid chromatography), eventually combined with MS, measures the intact herbicide with a limit of sensitivity of about 1 µg kg^{-1}, i.e. 17% of the applied dose[9,10]. Lower sensitivity limits are desired in order to determine the possible presence of low but biologically active persistent herbicide residues in soil. The analytical sensitivity required to detect trace levels of the sulfonylurea herbicides continues to pose problems in routine detection of herbicide residues in soils[11]. These could be phytotoxic to the following sensitive crops, sugar beet and chicory for instance. On the other hand, there is no publication about the metabolism of metsulfuron-methyl in crop soils.

In the present work, a procedure has been developed for the analysis of metsulfuron-methyl in the soil of winter wheat crops. The soil extracts were cleaned by repeated TLC, with simultaneous separation of metsulfuron-methyl from its soil metabolites. Metsulfuron-methyl was transformed into a derivative which could be analyzed by GC-EC with a high sensitivity, and with confirmation by GC-MS. A similar analysis method has been developed for the soil metabolites of metsulfuron-methyl generated from its methyl 2-sulfonamidobenzoate moiety. The syntheses of the analysis standards of the derivatives of metsulfuron-methyl and of its soil metabolites, and their interconversion reactions are described. The reactivities of metsulfuron-methyl and of its metabolites are compared to those of the pyridine-pyrimidine sulfonylureas flupyrsulfuron-methyl and rimsulfuron.

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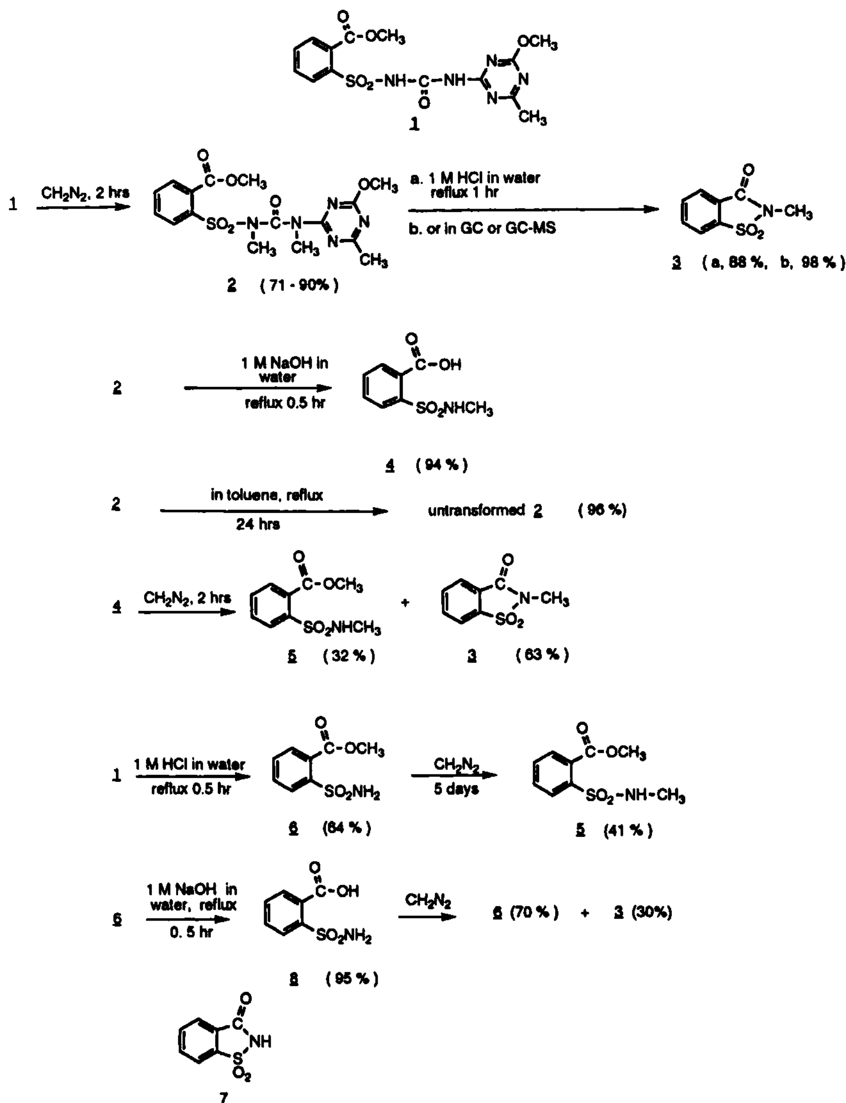

FIGURE 1 Metsulfuron-methyl 1, its analyzed transformation compound 3, the metsulfuron-methyl soil metabolites and their transformation products

EXPERIMENTAL

Instrumental analysis

Column chromatography was carried out using silica gel 60 F254, 70–230 mesh, from Merck. Thin layer chromatography was carried out using 20 × 20 cm, 0.2 mm layer silica gel 60 F254+366 plates (Merck). Standards and samples were applied on different lanes of the TLC plate.

Diazomethane methylation of metsulfuron-methyl generated *N,N'*-dimethyl metsulfuron-methyl **2** (Figure 1). In the GC and GC-MS apparatus, **2** was transformed into **3** which was detected.

Gas chromatography was carried out using a Varian 2700 instrument with ^{63}Ni ECD, injector and detector at 280°C, and a glass column 1.80 m × 2 mm i.d. containing 5% SE30 on Chromosorb W-HP 80–100 mesh, 160°C isothermal. Nitrogen was used as carrier gas at 50 ml min^{-1}. The retention time of compound **3** was 2.9 min. Residues in some extracts were confirmed by GC-MS (VG AutoSpec; Fisons GC 8065). One-microliter injections were made into the GC injector operated in splitless mode at 280°C. A 15 m capillary column 0.45 mm i.d. containing SE 54 at 1.0 μm film thickness (Alltech); column oven temperature program was 50°C (3 min) increasing to 250°C at 20°C min^{-1}, and maintained at this temperature for 8 min. Helium carrier gas head pressure 15 psi. The transfer oven was operated at 280°C. Electron impact ionization parameters were 30 eV, source temperature 200°C, full scan 50–500 amu, and scan rate 1 scan/s. Chemical ionization with reagent gas isobutane at 0.95 Torr. Selected ion monitoring (SIM) experiments used the ions 197 (M^+), 169 (M-CO), and 133 ($M\text{-}SO_2$). When the analysis standards were directly injected into the mass spectrometer, electron impact was at 70 eV (m/e; relative abundance, %). Infra red IR spectra were recorded by means of the Midac FTIR apparatus with KBr discs, cm^{-1}. The ^1H-NMR nuclear magnetic resonance spectra were recorded by means of the Varian 300 MHz spectrometer (δ, ppm relative to tetramethylsilane, in $CDCl_3$ unless mentioned otherwise). Elemental analyses were performed on a Heraeus CHN-O-Rapid analyzer. For compounds **2, 3, 4, 5** and **8**, satisfactory elemental analysis were obtained: C ± 0.21, H ± 0.18, N ± 0.24. Among the metsulfuron-methyl transformation products studied, only **6** and 1-dioxy-3-keto-1,2-benzisothiazole **7** were commercially available (Aldrich). The commercial analytical grade reagents were used as such. However, all the solvents were distilled before use.

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Standards for analysis

Metsulfuron-methyl (1)(methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino]carbonyl]amino] sulfonyl]benzoate)

The formulation Ally (water dispersible granules containing 20 g% metsulfuron-methyl, Du Pont, Belgium; 10 g) in ethyl acetate (300 ml) was heated to reflux with stirring (5 min). The mixture was filtered, and the extraction of the filtered solid was repeated two times. The filtrates were gathered, the ethyl acetate was evaporated under vacuum, and the solid was recrystallized in 1:1 diethyl ether/hexane (v/v) giving metsulfuron-methyl **1** (1.91 g, 96%) of a purity greater than 99.5%.

IR: 3350 (NH), 3071, 2955, 1734 (CO), 1715 (CO), 1590, 1562, 1452, 1364, 1296, 1173, 1119, 1061, 883, 819, 788. ^{1}H-NMR: 2.61 (s, 3H, triazin-CH_3); 3.92 (s, 3H, triazin-OCH_3); 4.08 (s, 3H, CO_2CH_3); 7.62–7.78 (m, 3H, benzene-4,5,6-H); 8.39 (m, 1H, benzene-3-H); 12.45 (br, 1H, SO_2NH). MS: 382 (M^++1, 7); 366 (M-CH_3, 4); 350 (M-OCH_3, 23); 317 (M-SO_2, 36); 275 (M-SO_2NHCO+H, 8); 210 (M-CH_3OH-$C_3N_3(OCH_3)(CH_3)(NH)$, 100); 199 ($C_6H_4(CO_2CH_3)(SO_2)$, 35).

N,N'-Dimethyl metsulfuron-methyl(methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)methylamino]carbonyl]methylamino]sulfonyl]benzoate)(2)

To the solution of metsulfuron-methyl **1** (0.5 g, 1.31 mmole) in ethyl acetate (100 ml) was added an ethereal solution of diazomethane (70 ml) until persistence of the yellow color. After 2 hr at 20°C, the solution was concentrated to dryness in vacuo, and the solid was purified by preparative TLC. Elution with 2:1 diethyl ether/ tetrahydrofuran (v/v) separated both isomers of *N,N'* -dimethyl-metsulfuron-methyl **2** in the TLC bands at R_f = 0.18 and 0.41. These bands were scraped off separately, the silica gel was extracted with acetone, the acetone was evaporated in vacuo, giving equal amounts of each isomer (ratio of 1:1), and a total yield of 382 mg (0.93 mmole, 71%) of isolated **2**. When 1 mg or lower amounts of metsulfuron-methyl were methylated in the same way, the isomer corresponding to the TLC band at R_f = 0.41 was formed with a yield greater than 90%, the second isomer at R_f = 0.18 being no more detected probably because at a too low concentration. Both *N,N'*-dimethyl-metsulfuron-methyl isomers had similar IR and MS spectra, but slightly different ^{1}H-NMR spectra. IR: 2956, 1737 (CO), 1682 (CO), 1631, 1596, 1541, 1470, 1358, 1265, 1163, 1117, 1077, 947, 832, 735, 601. ^{1}H-NMR: a. isomer at R_f = 0.18: 2.42 (s, 3H, triazin-CH_3); 3.17 (s, 3H, N'-CH_3); 3.48 (s, 3H, SO_2NCH_3); 3.83 (s, 3H, triazin-OCH_3); 3.92 (s, 3H, CO_2CH_3); 7.48–7.63 (m, 3H, benzene-4,5,6-H); 7.94 (m, 1H, ben-

zene-3-H). b. Isomer at $R_f = 0.41$: 2.31 (s, 3H, triazin-CH_3); 3.02 (s, 3H, N'-CH_3); 3.51 (s, 3H, SO_2NCH_3); 3.93 (s, 3H, triazin-OCH_3); 4.08 (s, 3H, CO_2CH_3); 7.58–7.62 (m, 3H, benzene-4,5,6-H); 7.99 (m, 1H, benzene-3-H). MS: 409 (M^+, 6); 378 (M-OCH_3, 8); 350 (M-CO_2CH_3, 4); 286 ($C_3N_3(OCH_3)(CH_3)$, 3); 199 ($C_6H_4(CO_2CH_3)(SO_2)$, 22); 181 ($C_3N_3(OCH_3)(CH_3)(NCH_3)CO$, 100); 135 ($C_6H_4(CO_2CH_3)$, 7).

N-Methyl-2-sulfonamido-benzoic acid (4)

N,N'-Dimethyl-metsulfuron-methyl **2** (0.5 g, 1.22 mmole) in 1 M NaOH in water (50 ml) was heated to reflux (30 min) with stirring. The cooled mixture was brought to pH 8.5 with 3 M HCl, washed with ethyl acetate, and the ethyl acetate washings were discarded. The pH of the water phase was brought to 2.2, and extracted with ethyl acetate. The ethyl acetate extract was dried (Na_2SO_4), and evaporated to dryness in vacuo. Column chromatography (10:1 ethyl acetate/acetic acid, v/v) of the solid residue gave **4** (247 mg, 1.15 mmole, 94%). IR: 3307 (OH, NH), 2923, 1708 (CO), 1590, 1471, 1401, 1325, 1165, 1121, 1057, 801, 759, 732, 646. ^{1}H-NMR (CD_3OD): 2.57 (NHCH_3); 7.56–8.03 (m, 4H, benzene-H). MS (EI): 197 (42, M-H_2O); 183 (8, M-CH_3OH); 171 (12, M-CO_2); 149 (7, M-SO_2-2H); 132 (88, 197-SO_2-H); 122 (36, M-SO_2NHCH_3+H). MS (CI, NH_3): 233 (53, M+NH_4^+); 216 (92, M+1); 198 (16, M-OH); 189 (48, M+1-CO+H); 172 (33, M+1-CO_2H+H); 136 (100, M-SONHCH_3-H).

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N-Methyl-2-sulfonamido-methylbenzoate 5 and 1-dioxy-2-N-methyl-3-keto-1,2-benzisothiazole (3)

To the solution of *N*-methyl-2-sulfonamido-benzoic acid **4** (500 mg, 2.33 mmole) in ethyl acetate (50 ml) was added an ethereal solution of diazomethane (about 60 ml) until persistence of the yellow color. After 2 hr at 20°C, the solution was evaporated to dryness in vacuo. Column chromatography (1:1 chloroform/hexane, v/v) of the solid residue successively gave **3** (290 mg, 1.47 mmole, 63%) and **5** (170 mg, 0.74 mmole, 32%).

Compound **5**: IR: 3298 (NH), 2957, 1723 (CO), 1438, 1398, 1272, 1169, 1118, 1057, 962, 762, 739, 687. ^{1}H-NMR: 2.69 (d, 3H, NHCH_3); 3.99 (s, 3H, CO_2CH_3); 5.87 (br, 1H, NH); 7.64 (m, 2H, benzene-4,5-H); 7.83 (m, 1H, benzene-6-H); 8.11 (m, 1H, benzene-3-H). MS (EI): 199 (91, M-$NHCH_3$); 169 (15, M-CO_2CH_3-H); 164 (13, M-SO_2-H); 150 (38, M-SO_2-CH_3); 135 (100, M-SO_2NHCH_3); 120 (28, $C_6H_4CO_2$). HRMS: 199 ($C_6H_4CO_2CH_3SO_2$): obsvd 199.006392; calcd 199.006506, 0.6 ppm. 150 ($C_6H_4CO_2CH_3NH$): obsvd 150.055471; calcd 150.055504, 0.2 ppm. 135 ($C_6H_4COOCH_3$): obsvd

135.044554; calcd 135.044605, 0.4 ppm. 120 ($C_6H_4CO_2$): obsvd 120.021127; calcd 120.021129, 0.0 ppm.

Compound **3**: IR: 3096, 2925, 1740 (CO), 1596, 1468, 1328, 1276, 1188, 1175, 978, 889, 751, 687. ^{1}H-NMR: 3.28 (s, 3H, NCH_3); 7.82–8.0 (m, 3H, 1,2-benzisothiazole-4,5,6-H); 8.03–8.13 (m, 1H, 1,2-benzisothiazole-7-H). MS: 197 (M^+, 100); 169 (M-CO, 18); 133 (M-SO_2, 88); 120 ($COSO_2NCH_2$, 14); 117 ($C_6H_4(CN)(CH_3)$, 22); 105 (C_6H_4CHO, 74); 104 (C_6H_4CO, 72); 76 (C_6H_4, 92).

1-Dioxy-2-N-methyl-3-keto-1,2-benzisothiazole 3 from N,N'-dimethyl-metsulfuron-methyl (2)

N,N'-Dimethyl-metsulfuron-methyl **2** (0.5 g, 1.22 mmole) in 1 M HCl in water (50 ml) was heated to reflux (1 hr, stirring). Cooling at 0°C gave a precipitate of **3** (62 mg) which was filtered. The filtrate was brought to pH 2.2 with Na_2CO_3, and extracted with ethyl acetate. The ethyl acetate extract was dried (Na_2SO_4), and concentrated to dryness in vacuo. Column chromatography of the solid (1:1 chloroform/hexane, v/v) gave **3** (149 mg). The total amount obtained of **3** was 211 mg (1.07 mmole, 88%).

2-Sulfonamido-methylbenzoate (6)

The mixture of metsulfuron-methyl **1** (1.0 g, 2.62 mmole) in 1 M HCl in water (100 ml) was heated to reflux (0.5 hr, stirring). After cooling, the pH was brought to 2.2 with Na_2CO_3 and extracted with ethyl acetate. The ethyl acetate extract was dried (Na_2SO_4) and concentrated to dryness under vacuum. Column chromatography (3:1 ethyl acetate/hexane, v/v) of the solid gave **6** (361 mg, 1.68 mmole, 64%). IR: 3336 (NH_2), 3235 (NH_2), 3095, 2963, 1715 (CO), 1557, 1433, 1336, 1273, 1190, 1160, 1139, 1122, 1060, 961, 898, 764, 739, 703. ^{1}H-NMR: 3.99 (s, 3H, OCH_3); 5.78 (br, 2H, SO_2NH_2); 7.63 (m, 2H, benzene-4,5-H); 7.88 (m, 1H, benzene-6-H); 8.14 (m, 1H, benzene-3-H). MS (EI): 215 (13, M^+); 199 (100, M-NH_2); 184 (83, M-OCH_3); 151 (28, M-SO_2); 135 (25, M-SO_2NH_2); 120 (47, 199-SO_2NH_2+H). MS (CI, NH_3): 233 (84, M+NH_4^+); 216 (100, M+1); 199 (67, M-NH_2).

N-Methyl-2-sulfonamido-methylbenzoate 5 from 2-sulfonamido-methylbenzoate 6

To the solution of 2-sulfonamido-methylbenzoate **6** (500 mg, 2.33 mmole) in ethyl acetate (60 ml) was added an ethereal solution of diazomethane in ether (about 60 ml) until persistence of the yellow color. The progress of the methylation of the sulfonamide was monitored byTLC. The R_f of **6, 5** and **3** with the following elution solvents respectively were: with diethyl ether, 0.69, 0.64 and 0.81; with dichloromethane, 0.39, 0.64, 0.74; with chloroform, 0.23, 0.60, 0.69.

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At each of the delays of 1, 2, 3 and 5 days of contact with diazomethane, the ether was evaporated under vacuum by reducing the volume to its half, and a new ethereal solution of diazomethane was added for further methylation. After 5 days of methylation, the mixture was evaporated to dryness under vacuum. The solid was purified by column chromatography (1:1 chloroform/hexane, v/v) giving successively **3** (18 mg, 0.09 mmole, 4%), **5** (219 mg, 0.96 mmole, 41%), and untransformed **6** (256 mg, 1.19 mmole, 51%).

2-Sulfonamido-benzoic acid 8

The mixture of compound **6** (1 g, 4.65 mmole) in 1M NaOH in water (50 ml) was heated at reflux for 0.5 hr with stirring. The cooled solution was brought to pH 2.2 with 2 M HCl, and extracted with ethyl acetate. After drying (Na_2SO_4), the ethyl acetate extract was evaporated under vacuum to dryness and the solid was purified by column chromatography (diethyl ether) giving **8** (0.89 g, 4.42 mmole, 95%). IR: 3362 and 3258 (OH and NH_2), 3098, 2967, 1712, 1548, 1397, 1336, 1251, 1162, 1120, 1068, 908, 800, 757, 720, 654. ^{1}H-NMR (DMSO-d6): 7.18 (br, 2H, NH_2); 7.69 (m, 2H, H-3,4); 7.96 (m, 1H, H-5); 8.13 (m, 1H, H-2). MS: 201 (18, M^+); 185 (47, M-NH_2); 183 (52, M-H_2O); 157 (62, M-CO_2); 141 (52, 185-CO_2); 120 (38, M-SO_2NH_2-H); 119 (59, 120-H).

Transformation 2-sulfonamido-benzoic acid 8 into the mixture of compounds 6 and 3

To the solution of 2-sulfonamido-benzoic acid **8** (0.1 g, 0.5 mmole) in ethyl acetate (7 ml) was added a solution (about 5 ml) of diazomethane in ether until persistence of the yellow color. After 2 hr at r.t., the solution was evaporated to dryness by a current of nitrogen (20°C). The solid was purified by preparative TLC (chloroform) giving **6** (75 mg, 70%) and **3** (30 mg, 30%).

Analysis of metsulfuron-methyl in soil

The mixture of soil (100 g) in 0.1 M $NaHCO_3$ in water (200 ml) was stirred at room temperature during 20 min. The mixture was centrifuged (3000 rpm, 15 min), the supernatant was separated, and the extraction of the soil was repeated. The supernatants were gathered and washed with dichloromethane (200 ml). The aqueous solution was brought to pH 2.2 with 1M HCl, and extracted two times with ethyl acetate (2 × 200 ml). The ethyl acetate extract was dried (Na_2SO_4) and concentrated in a 1 litre flask to 40 ml in a vacuum rotary evaporator at 30°C. Thereafter the extract in a 50 ml flask was concentrated to 15 ml in a vacuum rotary evaporator at 20°C. The extract was further concentrated to 0.5 ml under a

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slow stream of nitrogen at 20°C. The concentrations of the extracts cited further were made according to the same procedure. The extract was applied to a TLC plate with the standard of metsulfuron-methyl. Development with 1:1 acetone/hexane (v/v) gave a band containing metsulfuron-methyl at $R_f = 0.44$ (Table I). The band was scraped off and the silica gel was extracted with acetone (40 ml). The extract was concentrated to 15 ml in a vacuum rotary evaporator at 20°C, and then to 1 ml under a slow stream of nitrogen (20°C). Ethyl acetate (4 ml) was added, and then a solution (about 7 ml) of diazomethane in ether until persistence of the yellow color. After 2 hr at 20°C, the solution was concentrated to 0.5 ml under a slow stream of nitrogen (20°C). The methylations cited further were made according to the same procedure. The concentrated solution was applied to a second TLC plate, together with the standard of *N,N'*-dimethyl-metsulfuron-methyl **2**. Development with 2:1 diethyl ether/tetrahydrofuran (v/v) gave a band at $R_f = 0.41$ containing the isomer of *N,N'*-dimethyl metsulfuron-methyl **2** which is formed quantitatively by methylation of metsulfuron-methyl at the residue level. The band was separated, the silica gel was extracted with acetone, the extract was concentrated, and analyzed by GC and, in several cases, by GC-MS. In the GC and GC-MS apparatus, **2** was transformed quantitatively into **3**. When the final extract was unsufficiently clean (interferences at the GC or GC-MS analyses), the latest TLC was repeated. For recovery experiments, soil samples of different soil types were spiked with the known concentrations of metsulfuron-methyl of 2, 1 and 0.3 μg (the limit of sensitivity) metsulfuron-methyl kg^{-1} dry soil (Table II). Incorporation in soil was made by means of an aqueous solution of metsulfuron-methyl made by repeated dilutions of an initial solution containing the commercial formulation of metsulfuron-methyl. After 24 hr at room temperature, the soil was extracted.

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Analysis of metabolites 6, 7 and 8 in soil

The metabolites **7** and **8** were isolated in the 0.1 M $NaHCO_3$ soil extract made for the analysis of metsulfuron-methyl. They were as a mixture in the band at $R_f = 0$–0.10 of the first TLC made after the soil extraction, with 1:1 acetone/hexane (v/v) as development solvent. This band was separated, extracted with 9:1 acetone/methanol (v/v), and the concentrated extract was applied to a second TLC plate. Development with 99:1 tetrahydrofuran/acetic acid (v/v) gave the two isomers of the analysis standard of **8** -synthesized at the 200 mg level- in the bands at $R_f = 0.36$ and 0.71. At the residue level in the soil extract of the recovery experiments, only the isomer of **8** at $R_f = 0.36$ was observed. The metabolite **7** was in the band at $R_f = 0.48$. The bands containing **7** and **8** were scraped off separately, extracted with 9:1 acetone/methanol (v/v), and the concentrated extracts were methylated, trans-

forming **7** into **3**, and **8** into the mixture of **6** and **3**. The two concentrated extracts were applied to separate TLC plates. Development with 2:1 diethyl ether/tetrahydrofuran (v/v) gave **3** -alone or in mixture with **6**- in the band at R_f = 0.83–0.87. This band was separated, extracted with acetone, and the two concentrated extracts were analyzed by GC and GC-MS for **3** which corresponded to the separated **7** and **8**, **6** being transformed into **3** in the GC and GC-MS apparatus.

TABLE I Thin-layer chromatography of metsulfuron-methyl, of its soil metabolites, and of their methylation derivatives, with the development solvents used before and after diazomethane methylation

	TLC development solvent	
	1:1 Acetone/hexane, v/v	*2:1 Diethyl ether/tetrahydrofuran, v/v*
	R_f	
1. Development solvent used before diazomethane methylation:		
Metsulfuron-methyl **1**	0.44	
1-Dioxy-3-keto-1,2-benzisothiazole (saccharin) **7**	0.08	
N-Methyl-2-sulfonamido-benzoic acid **4**	0.29	
2-Sulfonamido-methyl benzoate **6**	0.84	
2-Sulfonamido-benzoic acid **8**	0.10	
1 -Dioxy-2-*N*-methyl-3-keto-1,2-benzisothiazole **3**	0.71	
2. Development solvent used after diazomethane methylation:		
N,N'-Dimethyl metsulfuron-methyl **2**		0.41
1-Dioxy-2-N-methyl-3-keto-1,2-benzisothiazole **3**		0.83
N-Methyl-2-sulfonamido-methylbenzoate **5**		0.78
2-Sulfonamido-methyl benzoate **6**		0.87

TABLE II Recovery percent of metsulfuron-methyl from soils at indicated fortification levels

Soil type (composition and sampling site in Belgium)	*Metsulfuron-methyl fortification level, μg kg^{-1} dry soil*		
	2	*1*	*0.3*
	Percent metsulfuron-methyl recovery[a]		
Sandy soil (clay 10%, loam 11%, sand 79%, organic matter 1.43%, pH(H_2O) 6.8; Zingem)	84–97	86–101	77–89
Sandy loam soil (clay 7%, silt 38%, sand 55%, organic matter 1.94%, pH 6.9; Melle)	83–98	84–98	79–94
Loam soil (clay 14%, loam 51%, sand 35%, organic matter 1.45%, pH 6.4; Zarlardinge)	80–96	86–99	81–92
Clay soil (clay 34%, loam 47%, sand 19%, organic matter 1.96%, pH 7.2; Koksijde)	87–102	81–95	76–93

a. Three recovery assays for each soil type at each fortification level.

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For the analysis of metabolite **6**, the soil extraction with 0.1 M $NaHCO_3$ in water gave unsufficient recovery yields. A separate soil extraction with 8:2 acetone/water (v/v) was satisfactory (Table III). Soil (100 g) was stirred with 8:2 acetone/water (v/v) (200 ml, 20°C, 1 hr). The mixture was filtered, and the extraction was repeated during 30 min. The filtrates were gathered, water (100 ml) was added, and the acetone removed in a vacuum rotary evaporator (30°C). NaCl (15 g) was added, and the aqueous solution was extracted two times with ethyl acetate (2 × 200 ml). The aqueous solution was brought to pH 2.2 with 1 N HCl and extracted again with ethyl acetate. The ethyl acetate extracts were gathered, dried (Na_2SO_4), concentrated and applied to a first TLC plate. Development with 1:1 acetone/hexane (v/v) gave a band containing **6** at $R_f = 0.84$. The band was separated, extracted with acetone, and the concentrated extract was applied to a second TLC plate. Development with 3:1 diethyl ether/tetrahydrofuran (v/v) gave a band containing **6** at $R_f = 0.66$. The band was separated, extracted with acetone, and the concentrated extract was analyzed for **6** by GC and GC-MS wherein **6** was transformed into **3**.

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TABLE III Recovery percent of the metabolites **6, 7** and **8** from the sandy loam soil at Melle at indicated fortification levels

Metabolite[a]	*Metabolite fortification level, μg of equivalents of metsulfuron-methyl kg^{-1} dry soil*		
	2	*1*	*0.3*
	Percent metabolite recovery[b]		
6	81–96	83–98	79–91
7	82–91	78–92	75–93
8	85–97	81–91	77–89

a. **6**: 2-Sulfonamido-methylbenzoate. **7**: 1-Dioxy-3-keto-1,2-benzisothiazole (saccharin). **8**: 2- Sulfonamido-benzoic acid.

b. For each metabolite, three recovery assays at each fortification level.

For recovery experiments, sandy loam soil from Melle not treated with metsulfuron-methyl nor any other herbicide was spiked separately with known concentrations of metabolites **6, 7** and **8** at the concentrations of 2, 1 and 0.3 μg (the limit of sensitivity) of equivalents of metsulfuron-methyl kg^{-1} dry soil (Table III). Incorporation in soil was made by means of an aqueous solution of each metabolite in water. This solution was made by repeated dilutions in water of an initial solution of the metabolite in acetone. After 24 hr at room temperature, the soil was extracted.

RESULTS AND DISCUSSION

Metsulfuron-methyl **1** was extracted from soil by means of 0.1 M $NaHCO_3$ in water. The pH of this extraction solution was about 8, wherein metsulfuron-methyl ($pK_a = 3.3$) was ionized. Washing of the solution with dichloromethane eliminated soil organic contaminating substances. Thereafter, the pH of the aqueous solution was brought to 2.2, and the acid metsulfuron-methyl was extracted with ethyl acetate. The extract obtained in this way was cleaner than the one given by an initial soil extraction made with an organic solvent, like acetone plus water. Metsulfuron-methyl **1** was detected at the residue level after its quantitative transformation by diazomethane into its *N,N'*-dimethyl derivative **2**. In the GC-EC and GC-MS apparatus, **2** was quantitatively transformed into **3**, which was measured.

Repeated TLC of the soil extract were made in order to clean it further, and to separate simultaneously metsulfuron-methyl from its soil metabolites which could also generate **3** after methylation. At each TLC, the separation of the products was made accurately by applying the standards (each applied as a spot) and samples (concentrated soil extract applied as a band) on different lanes of the TLC plate. After development of the plate, the separated sample bands and spots were visualized under a UV lamp. The silicagel of the sample band was scraped off separately and extracted with acetone. In this way, the accurate separation of the searched compound could be made in the soil extract. The standard compounds for analysis were synthesized at the level of at least 100 mg, in order to compensate for their consumption at each TLC. The synthesis trials indicated simultaneously the chemical reactivities of the compounds.

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The soil metabolites of metsulfuron-methyl **1** which after methylation could generate -directly or in the GC and GC-MS apparatus- the measured compound **3**, are **6, 7** and **8**. In the TLC of the unmethylated soil extract (the first TLC with 1:1 acetone/hexane, v/v), they were separated from metsulfuron-methyl **1** ($R_f = 0.44$), **6, 7** and **8** having the R_f 0.84, 0.08 and 0.10, respectively (Table I). In the TLC of the methylated soil extract (the second TLC with 2:1 diethyl ether/tetrahydrofuran, v/v), *N,N'*-dimethyl metsulfuron-methyl **2** ($R_f = 0.41$) (which was generated selectively by methylation of metsulfuron-methyl at the residue level, without simultaneous formation of **3**) was separated from the methylation products of the soil metabolites, which would have escaped in the first TLC of the unmethylated extract. These methylated metabolites are **3** ($R_f = 0.83$), **5** ($R_f = 0.78$) and **6** ($R_f = 0.87$).

In order to determine the efficiency of the analytical method, soil samples were spiked with known concentrations of metsulfuron-methyl (2, 1 and 0.3 μg metsulfuron-methyl kg^{-1} dry soil) (Table II). Incorporation in soil was made using

an aqueous solution of metsulfuron-methyl. This solution was made by repeated dilutions of an initial solution containing the commercial formulation of metsulfuron-methyl. After the incorporation, the soil remained 24 hr at room temperature, and was then extracted. Sandy, sandy-loam, loam and clay soils were assayed separately. The soil type and composition had no effect on the metsulfuron-methyl recovery. Good recovery percentages were obtained and the sensitivity limit was 0.3 μg metsulfuron-methyl kg^{-1} dry soil. This analysis method for metsulfuron-methyl in the soils from fields of winter wheat crop trials has been applied successfully more than 200 times with high recovery levels (Table II). When 6 g of metsulfuron-methyl ha^{-1} was applied post-emergence on winter wheat at the beginning of March, the decrease of its soil concentration during crop has been measured; metsulfuron-methyl was no more detected in soil at the mid of November.

The metabolism pathways of metsulfuron-methyl in wheat and barley have been described[12]. The soil metabolism of sulfometuron-methyl has been published[13]; sulfometuron-methyl contains the same 2-sulfonamido-methylbenzoate group as metsulfuron-methyl. Sulfometuron-methyl decomposition in soil generates the metabolites **6, 7** and **8**. No publication exists about the soil metabolism pathways of metsulfuron-methyl. An internal but non obtainable report of Du Pont however has been briefly summarized[14]; it indicates that **6, 7** and **8** indeed are soil metabolites of metsulfuron-methyl. In the present work, the analysis method for metsulfuron-methyl in crop soils has been extended to the analysis of the metabolites **6, 7** and **8** in winter wheat field crop soils.

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The metabolites **7** and **8** were efficiently extracted from soil with 0.1 M $NaHCO_3$ in water, simultaneously with metsulfuron-methyl (Table III). This extraction solvent however gave unsufficient recovery yields with metabolite **6**. For metabolite **6**, a second separate extraction of the fresh soil was made with 8:2 acetone/water (v/v) at 20 °C (Table III). The metabolites **6, 7** and **8** were separated in each soil extract by repeated TLC. Metabolite **6** was injected as such in the GC and GC-MS apparatus, wherein it was transformed into **3**. Methylation transformed **7** into **3** which was analyzed by GC and GC-MS. At the residue level, methylation transformed **8** quantitatively into **6**, the compound **3** being not formed simultaneously. In the GC and GC-MS apparatus, **6** was transformed into **3**. Recovery experiments were made with sandy loam soil from Melle not treated with metsulfuron-methyl nor any other herbicide. The soil was spiked separately with known concentrations of metabolites **6, 7** and **8** at the concentrations of 2, 1 and 0.3 μg (the limit of sensitivity) of equivalents of metsulfuron-methyl kg^{-1} dry soil (Table III).

Several soil samples from crop trials analyzed for metsulfuron-methyl, were further analyzed for the metabolites **6, 7** and **8** (unpublished results). When 6 g of

metsulfuron-methyl ha^{-1} was applied post-emergence on winter wheat at the beginning of March, **6** and **7** were detected in soil only in May and June; the concentrations of each of them (as equivalents of metsulfuron-methyl) corresponded to 10 to 20% of the applied dose of metsulfuron-methyl. The metabolites **6** and **7** were no more detected in soil after the winter wheat harvest at the end of July. During the crop and after the harvest, the metabolite **8** either was not detected in soil, or its soil concentration in the field was at the limit of the analytical sensitivity.

The transformation pathways of metsulfuron-methyl **1**, of its soil metabolites and of their methylation derivatives were studied, and these compounds were simultaneously synthesized. Diazomethane methylation of **1** in ethyl acetate during 2 hr yielded the *N,N'*-dimethyl derivative **2** (Figure 1). This was formed with a yield greater than 90% when 1 mg, or lower amounts of metsulfuron-methyl was methylated. It indicated the acidity of the two urea nitrogen protons due to the sulfonyl function and the triazine cycle. When 500 mg metsulfuron-methyl was methylated, TLC separated two stereoisomers of **2**. When methylation was made with 1 mg or lower amounts of metsulfuron-methyl, only one stereoisomer was obtained, the second one being probably formed in very low amount. In the GC-EC and GC-MS apparatus, **2** was quantitatively transformed into **3** which was measured in the soil extracts for metsulfuron-methyl **1**.

The diazomethane methylation of metsulfuron-methyl occurred in a different way as compared to the pyridine-pyrimidine sulfonylurea flupyrsulfuron-methyl **9** (methyl 2-[[[[4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-6-(trifluoromethyl)-3-pyridinecarboxylate) (Figure 2)[15]. Methylation of 500 mg of **9** yielded compounds **11** and **12** besides the *N,N'*-dimethyl derivative **10**. When methylation was run on 1 mg or lower amounts of **9**, **11** was generated quantitatively in the soil extract and was measured as such by GC-EC and GC-MS for flupyrsulfuron-methyl. Metsulfuron-methyl **1** and its *N,N'*-dimethyl derivative **2** did not generate during methylation, or by further transformations, the compounds corresponding to **11** and **12**. On the other hand, methylation of the pyridine-pyrimidine sulfonylurea rimsulfuron **13** (*N*-[[4,6-dimethoxypyrimidine-2-yl)amino]carbonyl]-3-(ethylsulfonyl)-2-(pyridinesulfonamide) generated the *N*-mono-methyl derivative **14** (Figure 3)[16,17]. In the GC-EC and GC-MS apparatus, **14** decomposed into **15**, which was measured in the soil extracts for rimsulfuron[18].

In aqueous alkaline solution, the *N,N'*-dimethyl derivative **2** of metsulfuron-methyl was transformed into the *N*-methyl-2-sulfonamidobenzoic acid **4**, which was rapidly transformed by diazomethane into the mixture of **3** and *N*-methyl-2-sulfonamido-methylbenzoate **5** (Figure 1). In aqueous acid solution, **2** was transformed into **3**, like in the GC-EC and GC-MS apparatus. In toluene

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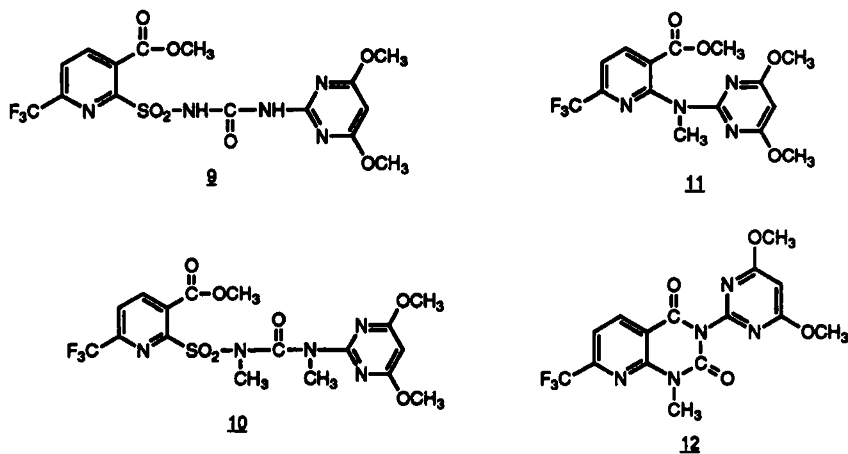

FIGURE 2 Flupyrsulfuron-methyl 9 and its methylation derivatives[14]

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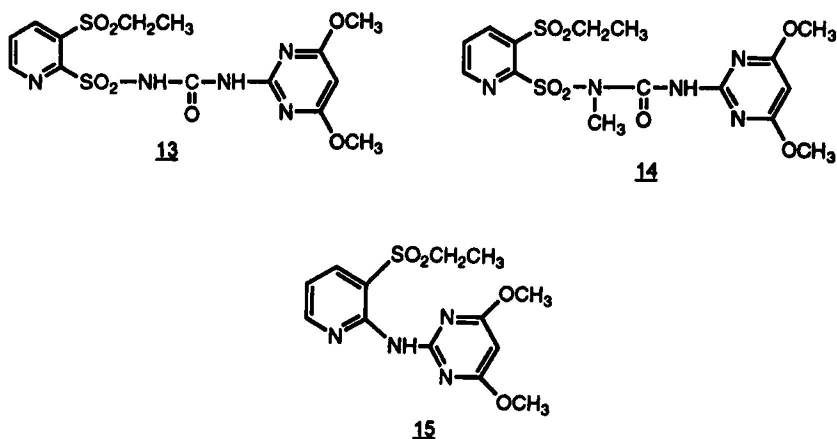

FIGURE 3 Rimsulfuron 13 and its analysis transformation products[15,16,17]

heated to reflux during 24 hr, **2** was not transformed into **3**, suggesting that this reaction was not a thermal one. On the other hand, metsulfuron-methyl **1** in aqueous dilute hydrochloric acid was transformed into 2-sulfonamido-methylbenzoate **6**, which was slowly *N*-methylated by diazomethane into **5**. Alkaline hydrolysis of **6** generated 2-sulfonamidobenzoic acid **8**, which was transformed by diazomethane into **6** (70%) and **3** (30%).

Acid hydrolysis of flupyrsulfuron-methyl **9** also gave the corresponding sulfonamide (2-sulfonamido-3-carbomethoxy-6-trifluoromethylpyridine). This was fastly *N*-methylated by diazomethane, at the opposite of the sulfonamide **6** from metsulfuron-methyl (unpublished results). However, the hydrolysis of rimsulfuron **13** in dilute acid or alkaline aqueous solutions always generated **15**, the sulfonamide (2-sulfonamido-3-ethylsulfonylpyridine) being never isolated (Figure 3)[16,17]. These results indicate that the transformation pathways of the sulfonylureas are not general, but are influenced by the aromatic rings and their substituents. The soil analysis of a sulfonylurea -by means of one of its transformation product- thus needs a previous study of the chemical reactivity of the sulfonylurea, different transformation pathways being possible. A high sensitivity however may be obtained with widespread analytical apparatus, allowing the analysis of a large number of samples. Moreover, the study of the chemical transformation pathways of the sulfonylurea leads to the establishment of the analysis procedures for their soil metabolites.

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References

[1] R.I. Doig, G.A. Carraro and N.D. Mc. Kinley, 10 th Int. Cong. Plant Prot., Brighton, Proc. Conf., 1, 324–331 (1983).

[2] N. Pons and E. Barriuso, *Pestic. Sci.*, **53**, 311–323 (1998).

[3] J.C. Streibig, A. Walker, A.M. Blair. G. Anderson-Taylor, D. J. Eagle, H. Friedländer, E. Hacker, W. Iwanzik, P. Kudsk, C. Labhart, B. M. Luscombe, G. Madafiglio, P. C. Nel, W. Pestemer, A. Rahman, G. Retzlaff, J. Rola, L. Stefanovic, H. J. M. Straathof and E. P. Thies, *Weed Res.*, **35**, 215–224 (1995).

[4] A.F. Wiese, M.L. Wood and E.W. Chenault, *Weed Technol.*, **2**, 251–256 (1988).

[5] G.C. Galetti, G. Chiavari, F.A. Mellon and K. Parlsey, *J. Anal. Appl. Pyrolysis*, **21**, 239–247 (1991).

[6] P. Klaffenbach, P.T. Holland and D.R. Lauren, *J. Agr. Food Chem.*, **41**, 388–395 (1993).

[7] P. Klaffenbach and P.T. Holland, *J. Agr. Food Chem.*, **41**, 396–401 (1993).

[8] T.K. James, P. Klaffenbach, P.T. Holland and A. Rahman, *Weed Res.*, **35**, 113–120 (1995).

[9] L.J. Marek and W.C. Koskinen, *J. Agr. Food Chem.*, **44**, 3878–3881 (1996).

[10] C.R. Powley and P.A. de Bernard, *J. Agr. Food Chem.*, **46**, 514–519 (1998).

[11] A.K. Sarmah, R.S. Kokana and A.M. Alston, *Aust. J. Agr. Res.*, **49**, 775–790 (1998).

[12] J.J. Anderson, T.M. Priester and L.M. Shalaby, *J. Agr. Food Chem*, **37**, 1429–1434 (1989).

[13] R.K. Trubey, R.A. Bethem and B. Peterson, *J. Agr. Food Chem.*, **46**, 2360–2367 (1998).

[14] B. Rhodes, Du Pont Internal Report AMR 408–85, in *Metabolic pathways of agrochemicals*, Part one. (T. Roberts Edit., Royal Soc. Chem., UK, 1998.).

[15] J. Rouchaud, O. Neus, K. Cools and R Bulcke. *J. Agr. Food Chem.*, **47**, 3872–3878 (1999).

[16] J. Rouchaud, O. Neus and C. Moulard, *Bull. Soc. Chim. Belges*, **106**, 59–65 (1997).

[17] J. Rouchaud, O. Neus and C. Moulard, *Bull. Soc. Chim. Belges*, **106**, 151–157 (1997).

[18] J. Rouchaud, O. Neus, D. Callens and R. Bulcke. *J. Agr. Food Chem.*, **45**, 3283–3291 (1997).