Soil Metabolism of the Herbicide Isoxaben in Winter Wheat Crops

Jean Rouchaud,*,† Fabrice Gustin,† Dany Callens,‡ Michel Van Himme,‡ and Robert Bulcke‡

Laboratory of Phytopharmacy, Université Catholique de Louvain, 1348 Louvain-la-Neuve, Belgium, and Research Center for Weed Science, Rijksuniversiteit Gent, 9000 Gent, Belgium

Winter wheat fields were treated with the herbicide isoxaben after sowing. Trials were made in 1990–1991 and 1991–1992. The main isoxaben soil metabolite was demethoxyisoxaben (*N*-[3-(1-ethyl-1-methylpropyl)isoxazol-5-yl]-2-hydroxy-6-methoxybenzamide), i.e., the monodemethoxylation product of isoxaben. 5-Isoxazolone (3-(1-ethyl-1-methylpropyl)isoxazolin-5-one) was the second main isoxaben metabolite. When 5-aminoisoxazole (5-amino-3-(1-ethyl-1-methylpropyl)isoxazole) was detected in soil, it always was at very low concentrations. It never accumulated in soil; 4 months before winter wheat harvest, it could not be detected in soil. Benzamides 2,6-dimethoxybenzamide and 2-hydroxy-6-methoxybenzamide and 2,6-dimethoxybenzoic acid also were detected in soil. Organic fertilizer treatments increased isoxaben soil persistence. At the crop's end, their effects, however, progressively disappeared, the soil residues of isoxaben and of its metabolites becoming very low and similar in the organic fertilizer treated and untreated plots. 5-Aminoisoxazole was not detected. This work thus indicated that isoxaben was soil-metabolized into nontoxic products, unable to generate toxic ones, during the wheat crops whose soil had been treated or not treated with organic fertilizers.

INTRODUCTION

Isoxaben (*N*-[3-(1-ethyl-1-methylpropyl)isoxazol-5-yl]-2,6-dimethoxybenzamide) is a selective preemergence herbicide widely used in Europe for season-long control of dicotyledonous weeds in winter cereals (Huggenberger et al., 1982). Isoxaben has a very low water solubility (1 mg/L at 25 °C), very low vapor pressure ($<3.9 \times 10^{-7}$ mmHg), leaches only slightly, and degrades primarily microbiologically (*Herbicide Handbook*, 1989). In winter and rape plant shoots grown in laboratory conditions, isoxaben was hydroxylated at the methyl or methylene carbon atoms of one of the ethyl groups of the 1-ethyl-1-methylpropyl 3-substituent of the isoxazole ring (Cabanne et al., 1987). Except for the TLC R_f with one elution solvent, no analytical data or synthesis procedures for the standards were published for these plant metabolites. Published information about the soil metabolism of isoxaben is very scarce. Rutherford (1990) published an HPLC analysis method for the soil residues of isoxaben and *N*-[3-(1-hydroxy-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide (compound **II**). However, no analytical data about compound **II** soil concentrations in crops or about the procedure for synthesis of its standard of analysis were given.

In a previous work, we measured the dissipation of isoxaben in the soil of a winter wheat crop made at Melle, Belgium, in 1990–1991 (Rouchaud et al., 1993). We observed that the isoxaben dissipation in soil was slower in the plots treated with one of the organic fertilizers, green manure, pig slurry, or cow manure. In the present paper, we report further work about isoxaben: the study of its soil metabolism pathways.

EXPERIMENTAL PROCEDURES

Winter Wheat Trials Made in the 1990–1991 and 1991–1992 Seasons. This research was performed in both of the winter trials made in 1990–1991 and 1991–1992 at Melle. Both trials were made successively on the same field plots. The organic fertilizers were applied and soil incorporated on the same field plots just before the beginning of each trial. A field at Melle, Belgium (clay 8%, silt 35%, sand 57%, pH 6.6, sandy loam type) was tilled on August 8, 1990, and divided into plots (10 m × 15 m). Onto each plot was applied one of the organic fertilizers, green manure, cow manure, or pig slurry; moreover, there were control plots not treated with organic fertilizers. Four plots were sown (17 kg of seed ha^{-1}) with yellow mustard (*Sinapis alba* cv. Emergo) on August 8, 1990. On October 23, 1990, the height of the yellow mustard was 45–50 cm. On October 23, 1990 cow manure (50 tons ha^{-1}) or pig slurry (50 tons ha^{-1}) was applied onto plots not sown with yellow mustard. The composition of the cow manure was (weight percentages relative to the manure fresh weight): dry matter 25.4%, organic matter 19.6%, N 0.55%, P_2O_5 0.38%, K_2O 0.35%, CaO 0.40%, MgO 0.15%; pH (H_2O) 7.5. The composition of the pig slurry was dry matter 9.1%, organic matter 6.8%, N 0.53%, P_2O_5 0.18%, K_2O 0.65%, CaO 0.21%, MgO 0.12%; pH (H_2O) 7.1. There were four replicate plots for each treatment and the control. On October 24, 1990, the field was rotary tilled to a 17-cm depth, incorporating in this way the organic fertilizers in soil, wheat (cv. Capitaine) was sown, and 125 g ha^{-1} of isoxaben was applied by spraying the emulsion of AZ 500 SC (500 g L^{-1}, isoxaben, DowElanco, Belgium) in water (750 L ha^{-1}) using proper protective gear. Harvest was made on August 13, 1991. The field was plowed on September 12, 1991. At intervals indicated in Table V during the trial, samples were taken separately (and analyzed once separately) from the 0–10-cm soil layer of each of the four replicate plots. In addition, at two times (May 15 and July 1, 1991) single samples were taken separately (and analyzed once separately) from the 10–20-cm soil layer of each of two of the four replicate plots. As well as for the 0–10-cm and 10–20-cm soil layers, 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. At harvest, an aliquot of 1 kg of cereal grain was taken at random, sampling being made in the four replicate plots, but bulking together the samples from each replicate plot. Grain samples were stored at −25 °C until analyzed. Four replicate analyses were made on the cereal grain from each organic fertilizer object.

The concentrations of soil organic matter were measured in each replicate plot of each of the organic fertilizer treatments using the chromic acid titration method. The soil concentrations of C (to which the correction factor 1.33 was not applied) multiplied by 1.72 gave the organic matter soil concentrations. On October 29, 1990, the organic matter concentrations in soil were (means of four replicates) 1.71, 1.81, 1.94, and 2.38% dry soil in the control, green manure, pig slurry, and cow manure-

† Université Catholique de Louvain.
‡ Research Center for Weed Science.

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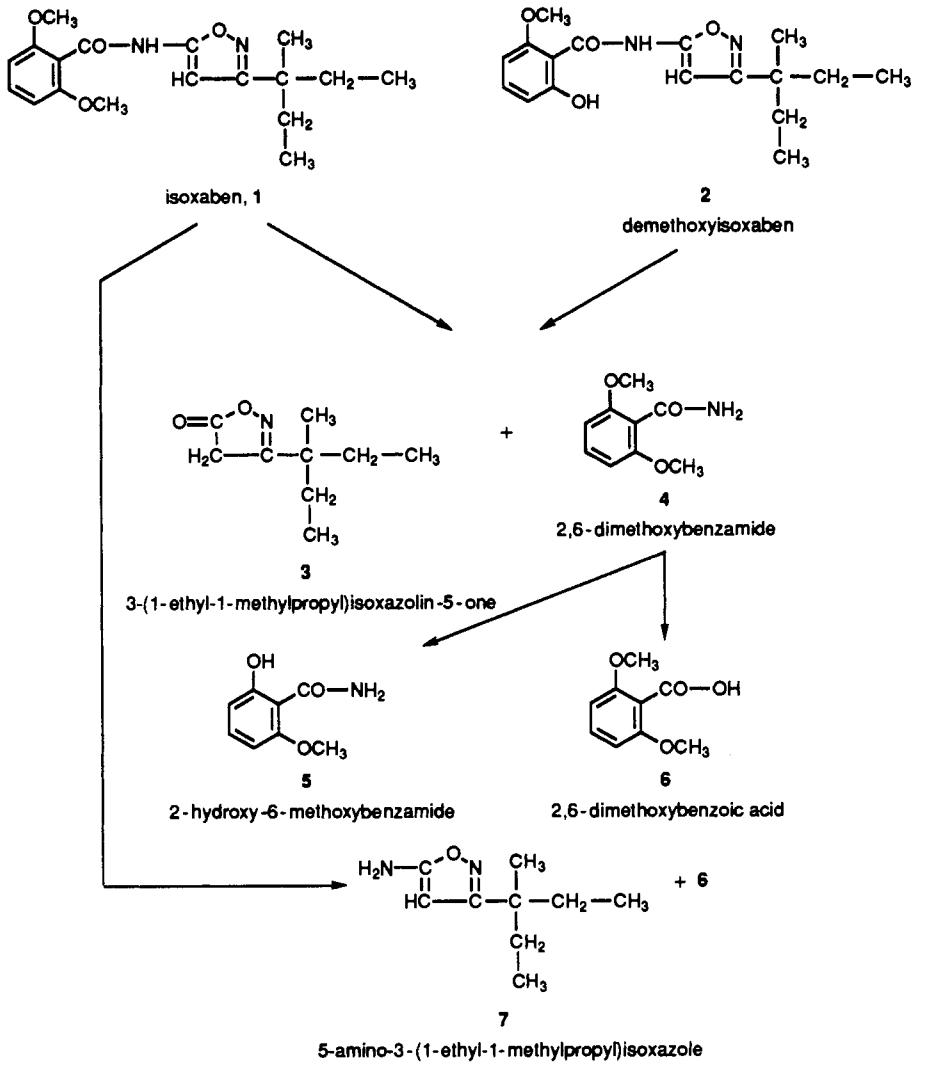

Figure 1. Proposed metabolism pathways for isoxaben in soil of winter wheat crops.

treated plots, respectively. On February 2, 1991, comparable values were 1.67, 1.76, 1.92, and 2.33%.

The winter wheat trial was repeated on the same field in 1991–1992 exactly in the same way as in 1990–1991. One of each of the pig slurry and cow manure organic fertilizers was applied some days before sowing onto the same field plots as in 1990–1991. The height of yellow mustard before soil incorporation was 70–80 cm. Winter wheat sowing and isoxaben application were made on October 18, 1991. Intervals for soil samplings in the 0–10-cm soil layer are indicated in Table I. At two times (April 27 and July 27, 1992) samples were taken from the 10–20-cm soil layer.

Thin-Layer (TLC) and Gas–Liquid (GLC) Chromatographies. Infrared (IR), Nuclear Magnetic Resonance (NMR), and Mass (MS) Spectrometries. TLC was done using silica gel 60F254 (20 × 20 cm, 0.2 mm thick) plates from Merck. The sample solution was applied as a band. Standards were applied on another part of the TLC plate, next to the band of the sample solution.

Isoxaben (1) and its metabolites (Figure 1) were analyzed by GLC using a Varian 2700 gas chromatograph with a flame ionization detector or a Hewlett-Packard 5730 gas chromatograph with a NPD thermionic detector in a safety-approved laboratory. GLC conditions: injector and detector at 250 °C, glass column 1.8 m × 2 mm internal diameter with 5% SE30 on 80–100 mesh Gas Chrom Q. The carrier gas (40 mL min^{-1}) was nitrogen for the flame ionization detector and helium for the thermionic detector. Column oven temperatures and retention times: isoxaben (1), 200 °C, 2.2 min; 180 °C, 5.8 min; metabolite 2 was detected as isoxaben after methylation; metabolite 3, 180 °C, 1.9 min; metabolite 4, 150 °C, 3.1 min; metabolite 5, 140 °C, 4.4 min; metabolite 6 (as its methyl ester), 120 °C, 4.1 min; metabolite 7, 150 °C, 4.3 min; compound 11, 150 °C, 3.5 and 5.4 min (both oxime isomers).

IR spectra were recorded with the Midac FTIR apparatus: KBr disks, cm^{-1}, ^1H and ^{13}C NMR spectra were recorded with the Varian XL200 apparatus: δ, ppm relative to tetramethylsilane in $CDCl_3$ (except metabolite 5 which was in DMSO-d_6). MS spectra (*m/e*, relative abundance, %) were recorded by direct introduction of the sample into the VG Micromass 7070F spectrometer at 70 eV. GC–MS spectra were obtained by sample injection into a Tracor 540 gas chromatograph (15-m SE54 capillary column; column oven temperature increasing from 50 to 300 °C at 20 °C min^{-1}) coupled to a ITD (ion trap detector) Finnigan Mat. MS spectra were recorded in the electron impact (EI) or chemical ionization (CI) modes. The samples from soil extracts, analyzed by GLC, frequently were further analyzed by MS. The final extracts were cleaned up by two or three successive TLC separations and then introduced directly into the mass spectrometer or injected into the GC–MS apparatus.

Standards for Analysis. Chemicals were purchased from Janssen Chimica and used as such. Column chromatography was made with Merck silica gel (230–400 mesh).

Isoxaben (1). A mixture (100 mL) of a commercial SC 500 formulation in 400 mL of chloroform was stirred for 20 min at room temperature. The chloroform layer was separated, washed with 100 mL of saturated aqueous NaCl solution, dried over Na_2SO_4, and concentrated to dryness in a vacuum rotary evaporator. Isoxaben was recrystallized from a mixture of ethyl acetate/hexane, giving 42 g of isoxaben of which the purity was greater than 99%. Spectra of isoxaben: IR 3245 (NH), 3001, 1692 (CO), 1605, 1539, 1473, 1285, 1115, 804; ^1H NMR 0.67, 0.77, 0.90 (t, 6H, $C(CH_3)(CH_2CH_3)_2$); 1.22 (s, 3H, $C(CH_3)(CH_2CH_3)_2$), 1.43, 1.55, 1.70, 1.83 (q, 4H, $C(CH_3)(CH_2CH_3)_2$); 3.77 (s, 6H, OCH_3), 6.33–

7.43 (m, 4H, aromatic H), 8.47 (b, 1H, NH); MS 332 (M^+, 37), 303 ($M - CH_2CH_3$, 31), 222 ($M - CNC(CH_3)(CH_2CH_3)_2 + H$, 87), 166 ($C_6H_3(OCH_3)_2CHO$, 100), 151 (166 - CH_3, 43).

N-[3-(1-Ethyl-1-methylpropyl)isoxazol-5-yl]-2-hydroxy-6-methoxybenzamide (Demethoxyisoxaben, 2). Isoxaben (4 g, 12 mmol) in a solution of HBr in acetic acid (75 mL; containing 30 g % HBr) was heated to reflux for 1.75 h. After 1 h, 25 mL of the HBr in acetic acid solution was added again. The cooled mixture was vacuum evaporated to dryness. Column chromatography on silica gel (30 g) and elution with methylene chloride/hexane 1:2 by volume gave compounds **2** (1.34 g, 4.2 mmol, 35%) and **12** (5-(acetylamino)-3-(1-ethyl-1-methylpropyl)isoxazole: 0.88 g, 4.2 mmol, 35%). Spectra of compound **2**: IR 3289 and 3179 (OH, NH), 2965, 1649 (CO), 1547, 1464, 1238, 1090, 1013, 839, 802, 754; ^{1}H-NMR 0.78, 0.81, 0.84 (t, 6H, $C(CH_3)(CH_2CH_3)_2$); 1.28 (s, 3H, $C(CH_3)(CH_2CH_3)_2$, 1.51–1.82 (m, 4H, $C(CH_3)(CH_2CH_3)_2$), 4.02 (s, 3H, OCH_3), 6.32 (s, 1H, isoxazole aromatic H), 6.42–6.72 (m, 2H, phenyl aromatic H), 7.25–7.40 (m, 1H, phenyl aromatic H), 10.7 (br, 1H, OH); MS 318 (M^+, 100), 290 ($M - CH_2CH_3 + H$, 32), 231 ($290 - 2(CH_2CH_3) - H$, 8), 229 ($231 - 2H$, 11), 207 ($290 - C(CH_3)(CH_2CH_3)_2 + 2H$, 15), 179 (CN-(CCHCNO)$C(CH_3)(CH_2CH_3)_2 + H$, 18), 167 (HN-(CCHCNO)$C(CH_3)(CH_2CH_3)_2$, 18), 166 ($C_6H_3(OH)(OCH_3)$(CO-NH), 13).

Spectra of compound **12**: IR 3283 (NH), 2969, 1696 (CO), 1613, 1551, 1458, 1375, 1267, 995, 783; ^{1}H-NMR 0.67, 0.78, 0.90 (t, 6H, $C(CH_3)(CH_2CH_3)_2$), 1.23 (s, 3H, $C(CH_3)(CH_2CH_3)_2$), 1.47, 1.57, 1.70, 1.73 (q, 4H, $C(CH_3)(CH_2CH_3)_2$); 2.23 (s, 3H, CH_3CO), 6.17 (s, 1H, isoxazole aromatic H), 9.40 (br, 1H, NH); MS 210 (M^+, 19), 182 ($M - CH_2CH_3 + H$, 100), 168 ($M - CH_3CO + H$, 17), 152 ($M - CH_3CONH$, 2), 140 ($182 - CH_3CO + H$, 52), 139 ($182 - CH_3CO$, 58), 123 ($182 - CH_3CONH_2$, 27).

3-(1-Ethyl-1-methylpropyl)isoxazolin-5-one (the 5-Isoxazolone 3) and 2-Hydroxy-6-methoxybenzamide (5). Isoxaben (4 g, 12 mmol) in a mixture of acetic acid (75 mL) and hydrochloric acid (35 mL; 37 g % HCl in water) was heated to reflux for 4 h. The cooled mixture was vacuum evaporated to dryness. Column chromatography on silica gel (30 g) and elution with toluene/hexane 1:1 by volume gave compounds **3** (1.26 g, 7.5 mmol, 62%), **4** (2,6-dimethoxybenzamide; 0.31 g, 1.7 mmol, 14%), and **5** (0.86 g, 5.1 mmol, 43%). Further elution with ethyl acetate gave compound **6** (2,6-dimethoxybenzoic acid; 0.18 g, 1.0 mmol, 8%).

Spectra and CHN analysis of compound **3**: IR 2971, 1802 (CO), 1464, 1385, 1163, 880, 822, 698; ^{1}H-NMR 0.80, 0.84, 0.89 (t, 6H, $C(CH_3)(CH_2CH_3)_2$); 1.17 (s, 3H, $C(CH_3)(CH_2CH_3)_2$), 1.48, 1.52, 1.58, 1.62 (q, 4H, $C(CH_3)(CH_2CH_3)_2$); 3.36 (s, 2H, $COCH_2$); ^{13}C-NMR 10.9 (s, $C(CH_3)(CH_2CH_3)_2$), 22.6 (s, $C(CH_3)(CH_2CH_3)_2$), 33.8 (s, $C(CH_3)(CH_2CH_3)_2$), 36.2 (s, $C(CH_3)(CH_2CH_3)_2$), 44.5 (s, CH_2CO), 175.2 (s, C=N), 178.6 (s, C=O); MS-EI 169 (M^+, 4), 154 ($M - CH_3$, 12), 141 ($M - CO$, 78), 140 ($M - CH_2CH_3$, 100), 126 ($M - CH_2CO - H$, 37), 112 ($C(CH_3)(CH_2CH_3)_2CN + H$, 28); MS-CI ($NH_3$) 187 ($M + NH_4^+$, 78), 170 ($M^+ + 1$, 27), 129 (128 + 1, 28); 128 ($170 - CH_2CO$, 100). Satisfactory microanalyses obtained for $C_9H_{15}NO_2$: C, ±0.28; H, ±0.25; N, ±0.26.

Spectra of compound **5**: ^{1}H-NMR 3.83 (s, 3H, OCH_3), 6.37, 6.50, 7.10, 7.25 (m, 3H, phenyl H); 8.05 (br, 2H, NH_2); MS-EI 167 (M^+, 58), 150 ($M - OH$, 100), 122 ($150 - CO$, 33), 107 ($150 - CONH_2 + H$, 53); MS-CI (CH_4) 168 (MH^+, 100), 150 ($M - OH$, 23).

2,6-Dimethoxybenzamide (**4**) and 2,6-dimethoxybenzoic acid (**6**) were purchased from Janssen Chimica, Belgium.

Methyl 2-Ethylbutyrate (8). A mixture of 2-ethylbutyric acid (116 g, 1 mol) and $SOCl_2$ (202 g, 1.7 mol) was progressively heated with stirring and maintained at reflux during 2 h. The excess of $SOCl_2$ was evaporated under vacuum. The acyl chloride was ice-cooled, and dry methanol (96 g, 3 mol) was added dropwise with stirring. The mixture was heated to reflux (2 h). The excess of methanol was evaporated under vacuum. Distillation gave **8** (125 g, 0.96 mol, 96%). Spectra of compound **8**: ^{1}H-NMR 0.85, 0.89, 0.92 (t, 6H, $CH(CH_2CH_3)_2$); 1.42–1.74 (m, 4H, $CH(CH_2CH_3)_2$), 2.15–2.98 (m, 1H, $CH(CH_2CH_3)_2$), 3.68 (s, 3H, OCH_3); MS 130 (M^+, 17), 129 ($M^+ - 1$, 86), 115 ($M - CH_3$, 24); 99 ($M - OCH_3$, 38), 97 ($129 - CH_3OH$, 62); 73 ($M - COCHO$, 58), 71 ($M - CO_2CH_3$, 72), 69 ($71 - 2H$, 93), 59 (CO_2CH_3, 97).

Methyl 2-Ethyl-2-methylbutyrate (9). To a stirred solution of lithium diisopropylamide (21.42 g, 0.2 mol; 2 M solution in THF/*n*-heptane) in anhydrous tetrahydrofuran (400 mL) at −78 °C under analytical grade nitrogen gas (maintained throughout the reaction) was added dropwise methyl 2-ethylbutyrate (**8**) (26 g, 0.2 mol). The mixture was stirred at −78 °C for 0.5 h. Methyl iodide (46.3 g, 0.33 mol) was added dropwise to the stirred mixture at −78 °C. Stirring of the mixture at −78 °C was maintained for 0.5 h. The mixture was progressively allowed to warm to room temperature over 2 h and stirred overnight. Water (100 mL) and ether (300 mL) were added. The ether layer was separated and the aqueous solution was extracted again with ether (300 mL). The ether extracts were gathered and dried (Na_2SO_4), and the ether was evaporated under vacuum. Distillation gave **9** (26.8 g, 0.186 mol, 93%). Spectra of compound **9**: ^{1}H-NMR 0.78, 0.82, 0.86 (t, 6H, $C(CH_2CH_3)_2$); 1.10 (s, 3H, $C(CH_3)(CH_2CH_3)_2$), 1.35–1.76 (m, 4H, $C(CH_2CH_3)_2$), 3.67 (s, 3H, OCH_3); MS 145 ($M^+ + 1$, 70), 144 (M^+, 6), 129 ($M - CH_3$, 8), 116 ($M - CO$, 68), 101 ($M - CO_2$, 37), 85 ($M - CO_2CH_3$, 43), 59 (CO_2CH_3, 65).

4-Ethyl-4-methyl-3-oxohexanenitrile (10). To a stirred solution of lithium diisopropylamide (21.42 g, 0.2 mol) in anhydrous tetrahydrofuran (400 mL) at −78 °C under nitrogen gas (maintained throughout the reaction), acetonitrile (8.2 g, 0.2 mol) was added dropwise. The mixture was stirred at −78 °C for 0.5 h. Methyl 2-ethyl-2-methylbutyrate (**9**; 28.8 g, 0.2 mol) was added dropwise to the stirred solution at −78 °C. The mixture was further stirred at −78 °C for 0.5 h. The mixture was slowly warmed to room temperature over 2 h and stirred for an additional 13 h. Water (100 mL) and ether (300 mL) were added, and the aqueous phase (in contact with the ether solution) was brought to pH 1.0 with hydrochloric acid. The ether layer was separated and the aqueous phase again extracted two times with ether (2 × 300 mL). The ether extracts were gathered and dried (Na_2SO_4). The ether was evaporated under vacuum, giving crude compound **10** (26.8 g, 0.18 mol, 88%). Aliquots of 4 g were chromatographed on a silica gel column (20 g) using ether/hexane 1:1 by volume as eluent. Spectra of compound **10**: IR 2973, 2883, 2261 (C≡N), 1719 (CO), 1458, 1387, 1302, 1047, 936, 791. ^{1}H-NMR 0.79, 0.82, 0.87 (t, 6H, $C(CH_2CH_3)_2$); 1.12 (s, 3H, $C(CH_3)(CH_2CH_3)_2$), 1.43–1.76 (m, 4H, $C(CH_2CH_3)_2$), 3.63 (s, 2H, $COCH_2CN$); MS-EI 154 ($M^+ + 1$, 8), 153 (M^+, 3), 138 ($M - CH_3$, 7), 125 ($M - CO$, 94), 113 ($M - CH_2CN$, 33), 86 ($(CH_3CH_2)_2CHCH_3$, 98), 71 ($86 - CH_3$, 100), 69 ($HCOCH_2CN$, 96), 68 ($COCH_2CN$, 97); MS-CI ($CH(CH_3)_3$) 154 ($M^+ + 1$, 100).

4-Ethyl-4-methyl-3-oxohexanenitrile Oxime (11). To a solution of 4-ethyl-4-methyl-3-oxohexanenitrile (**10**; 1 g, 6.5 mmol) in ethanol (40 mL) were added hydroxylamine hydrochloride (1.2 g, 17.1 mmol) and a solution of sodium hydroxide (0.68 g, 17 mmol) in water (8 mL) successively. The mixture was heated to reflux with stirring over 20 min. Methylene chloride (200 mL) and water (100 mL) were added. The water phase (in contact with the methylene chloride solution) was adjusted to pH 1 with hydrochloric acid and, after partition, the methylene chloride solution was discarded. The water phase was brought to pH 8.2 by successive addition of sodium bicarbonate and sodium carbonate and extracted two times with ethyl acetate (2 × 200 mL). The ethyl acetate solution was dried (Na_2SO_4), and ethyl acetate was evaporated in vacuum, giving compound **11** (0.350 g, 2.1 mmol, nonoptimized yield of 32%). When the reaction mixture was heated for 1.5 h instead of 20 min, no oxime was isolated, but 5-amino-3-(1-ethyl-1-methylpropyl)isoxazole (**7**) contaminated with secondary products was obtained. Spectra of compound **11**: IR 3488 and 3381 (=NOH and NHOH), 2971, 1699 (C=NOH), 1663, 1458, 1385, 1076, 1047, 1005, 965, 909; ^{1}H-NMR 0.76, 0.79, 0.82 (t, 6H, $C(CH_2CH_3)_2$); 1.08 (s, 3H, $C(CH_3)(CH_2CH_3)_2$); 1.40–1.78 (m, 4H, $C(CH_3)(CH_2CH_3)_2$); 3.26 (s, 1H, CH_2CN), 5.17 (s, 1H, C(NHOH)=CHCN, enolized oxime, 7.73 (br, 1H, =NOH); MS-EI 168 (M^+, 22), 153 ($M - CH_3$, $M - NH$, 6), 139 ($M - CH_2CH_3$, 70), 126 ($M - CNO$, $M - CH_3CN - H$, 13), 111 ($M - CH_2OHCN$, 18), 97 ($CC(CH_3)(CH_2CH_3)_2$, 39), 85 ($C(CH_3)(CH_2CH_3)_2$, 34), 83 ($C(NOH)CH_2CN$, 27); MS-CI ($CH(CH_3)_3$) 169 ($M^+ + 1$, 100).

5-Amino-3-(1-ethyl-1-methylpropyl)isoxazole (5-Aminoisoxazole 7). 4-Ethyl-4-methyl-3-oxohexanenitrile oxime (**11**; 1 g; 6.0 mmol) in 1.67 N HCl in water (100 mL) was heated to reflux during 1.5 h. The cooled water solution was brought to

pH 8.2 successively with $NaHCO_3$ and Na_2CO_3 and extracted with methylene chloride. The methylene chloride solution was dried (Na_2SO_4) and concentrated under vacuum to an oil. Column chromatography on silica gel with ether/hexane 1:1 by volume as eluent gave compound 7 (0.91 g, 5.5 mmol, 91%). Spectra of compound 7: IR 3435 (NH_2), 2967, 2932, 1618, 1468, 1383, 1260, 1100, 1015, 797; ^{1}H-NMR 0.73, 0.77, 0.81 (t, 6H, $C(CH_3)(CH_2CH_3)_2$); 1.18 (s, 3H, $C(CH_3)(CH_2CH_3)_2$), 1.42–1.77 (m, 4H, $C(CH_3)(CH_2CH_3)_2$), 4.08 (s, br, 2H, NH_2), 5.52 (s, 1H, aromatic isoxazole H); MS-EI 168 (M^+, 35), 139 ($M-CH_2CH_3$, 100), 111(139 - CH_2CH_3 + H, 23), 97($CC(CH_3)(CH_2CH_3)_2$, 48), 83 (NH_2-CCHCNO, 39; MS-CI (CH_4) 169 (M^+ + H, 100). Satisfactory microanalyses obtained for $C_9H_{16}N_2O$: C, ±0.28; H, ±0.25; N, ±0.26.

Soil and Grain Analyses. Soil (100 g) was extracted by heating to reflux and stirring for 20 min in 200 mL of acetone/water 8:2 by volume. The mixture was filtered and the extraction repeated. The filtrates were combined, 100 mL of water was added, and the acetone was removed in a vacuum rotary evaporator at 30 °C. NaCl was added to the aqueous solution which was then partitioned against methylene chloride (200 mL). The aqueous phase was brought to pH 3.5 with hydrochloric acid and extracted again with methylene chloride (200 mL). The methylene chloride solutions were gathered, dried over Na_2SO_4, concentrated to 40 mL in a vacuum rotary evaporator at 30 °C, and then concentrated to 0.5 mL under a stream of nitrogen at room temperature. The concentrated was applied to 20 × 20 cm TLC plate together with the standards of isoxaben (1) and of its metabolites **2**, **3**, **4**, and **5** (Figure 1). Development in methylene chloride/hexane 1:1 by volume gave band 1 containing metabolite **4** (R_f = 0), band 2 containing isoxaben (1) (R_f = 0.23), and band 3 (R_f = 0.35–0.41) containing metabolites **2** (R_f = 0.38), **3** (R_f = 0.35), and **5** (R_f = 0.41). The TLC bands were scraped off separately, the silica gel extracted with ethyl acetate, and the extract concentrated and applied onto a second TLC plate, repeating the same procedure; however, the extract from band 1 was chromatographed for the second time using ethyl acetate for the solvent, giving metabolite **4** at R_f = 0.43. The final extracts were analyzed by GLC and, in several cases, by MS and GC-MS; however, for GLC analysis of metabolite **2**, an aliquot of the extract of TLC band 3 was treated for 45 min with an ethereal solution of diazomethane; this transformed metabolite **2** into isoxaben (1), which then was analyzed by GLC.

The soil, already extracted with acetone/water 8:2 by volume was extracted (20 °C, 20 min, stirring) with 3 g % by weight KOH in water (200 mL) and filtered. The filtrate was brought to pH 3.2 with hydrochloric acid and extracted two times with methylene chloride (2 × 200 mL). The methylene chloride solution was dried (Na_2SO_4), concentrated, and applied onto a TLC plate. Elution with ether gave band 1 containing metabolites **2** (R_f = 0.72), **3** (R_f = 0.81), and **7** (R_f = 0.74), and band 2 containing metabolite **6** (R_f = 0.30). TLC bands were extracted separately with ethyl acetate, and the extracts were concentrated. MS analysis of the metabolites **2**, **3**, **6**, and **7** were done. In the extract of TLC band 1, metabolites **3** and **7** were analyzed by GLC. This extract also was treated with a solution of diazomethane in ether (45 min), transforming metabolite **2** into isoxaben (1) which was analyzed by GLC. The extract from band 2 was treated by diazomethane in ether, transforming metabolite **6** into its methyl ester which was analyzed by GLC.

Metabolites **2** and **3** extracted from soil were found generally in both the acetone/water extract and in the 3 g % KOH in water extract. Soil concentrations indicated in Tables I–VIII are the sum of their values observed in both extracts.

At the 50 ppb level in dry soil, recoveries of isoxaben (1) and of its metabolites **2**, **3**, **4**, **5**, **6** and **7** were respectively 83–97, 80–89, 84–92, 79–88, 78–91, 80–92, and 79–89%. The limit of detection for each of these compounds was 5 ppb (5 μg kg^{-1} dry soil).

The research of the 5-aminoisoxazole **7** in the 1990–1991 and 1991–1992 trials soils samples was made by the method described above. Moreover, the 1991–1992 trial soil samples all were analyzed a second time for the 5-aminoisoxazole **7** by the second and separate analytical procedure which follows. Soil (100 g) was extracted by heating to reflux (20 min) with stirring in the mixture acetone/water 8:2 by volume (200 mL) made 0.12 N HCl by addition of concentrated HCl. The extraction was repeated, the mixture was filtered, water (100 mL) was added to the filtrate, the acetone was evaporated in vacuo, the pH of the water solution was brought to 7.0 by successive addition of $NaHCO_3$ and Na_2CO_3, NaCl was added, the water solution was extracted with ethyl acetate (2 × 200 mL), and the dried (Na_2SO_4) ethyl acetate solution was concentrated and applied onto a TLC plate. Elution with ether/hexane 1:1 by volume gave a band (R_f = 0.56) containing the 5-aminoisoxazole **7**. The band was isolated and extracted, and the TLC was repeated. In the purified extract, the 5-aminoisoxazole **7** was analyzed by GLC and, in several cases, by MS and GC-MS. Recovery experiments results and soil residues of the 5-aminoisoxazole **7** were similar to the ones obtained with the first analytical procedure.

Wheat grains were analyzed in the same way as soil, except that grains were first ground into flour. The flour then was extracted at room temperature in a laboratory blender. Recoveries and detection limits were similar to soil samples.

Results were analyzed statistically by the linear regression ln $y = kt + b$ of isoxaben soil concentrations (y = ppb = μg kg^{-1} dry soil) against time t (days) for the 5–6 month-period following isoxaben treatment (the first 179-day period for the 1990-1991 trial; the first 152-day period for the 1991-1992 trial). Statistical results (r = corr coeff, b and k) are given here for the 1991-1992 winter wheat trial (Tables I–IV). For the 1990–1991 trial, they were previously published by Rouchaud et al. (1992). The 95% confidence intervals for the soil half-lives were evaluated using the SAS logical CMS SAS 5.18 (1984, 1986, SAS Institute Inc., Cary, NC); Rouchaud et al., 1991) (Tables I–VIII).

Table I. Winter Wheat Crop Trial Made in 1991–1992: Concentrations of Isoxaben (1) and of Its Metabolites 2–7 in the 0–10-cm Superficial Soil Layer of the Field Plots Not Treated with Organic Fertilizers (Control) (Isoxaben (1) Soil Half-Life: 75 ± 3 Days)[a]

days after 1 treatment[b]	concentrations of isoxaben and of its metabolites 2–7 (ppb, μg kg^{-1} dry soil, as equivs of isoxaben) in the 0–10-cm superficial soil layer[c]						
	1	2	3	4	5	6	7
3	136 ± 6	nd	nd	nd	nd	nd	nd
34	117 ± 5	16	nd	nd	nd	nd	nd
48	94 ± 4	18	7	10	5	nd	11
83	71 ± 3	15	25	25	5	nd	15
118	52 ± 2	13	38	20	10	5	20
152	35 ± 1	12	45	30	10	10	15
191	28 ± 1	13	35	25	8	5	5
248	17 ± 1	11	25	15	5	5	nd
282	10 ± 1	10	15	10	5	nd	nd

[a] Soil half-lives were calculated from the regression lines ln $y = kt + b$ correlating the naperian logarithms of isoxaben soil concentrations (y = ppb isoxaben in dry soil; 1 ppb = 1 μg kg^{-1}) against time (t = days following isoxaben treatment). Corr coeff r = 0.9925; b = 4.999; k = −0.009224 days^{-1}. The 95% confidence intervals for the soil half-lives were evaluated using the SAS logical CMS SAS 5.18 (1984, 1986, SAS Institute Inc., Cary, NC). [b] At soil sampling, number of days after isoxaben treatment (day-month-year), and cumulative rainfall since isoxaben application, respectively: 3 days, 21-10-1991, 24 mm; 34 days, 21-11-1991, 182 mm; 48 days, 5-12-1991, 183 mm; 83 days, 9-1-1992, 230 mm; 118 days, 13-2-1992, 260 mm; 152 days, 19-3-1992, 298 mm; 191 days, 27-4-1992, 395 mm; 248 days, 23-6-1992; 564 mm; 282 days, 27-7-1992, 637 mm. [c] Means of four replicates ± SD. 1: isoxaben; 2: demethoxyisoxaben; 3: the 5-isoxazolone; 4: 2,6-dimethoxybenzamide; 5: 2-hydroxy-6-methoxybenzamide; 6: 2,6-dimethoxybenzoic acid; 7: the 5-aminoisoxazole. For metabolites 2–7, SDs were between ±1 and ±2 ppb. nd = not detected.

RESULTS AND DISCUSSION

Isoxaben (1) and its metabolites 2–7 were observed in the 0-10-cm superficial soil layer (Figure 1, Tables I–VIII). None of the isoxaben (1) nor its metabolites 2–7 were detected in the 10-20-cm superficial soil layer during the cropping period (data not shown). There was thus no leaching of each of these compounds beneath the 0-10-cm

Table II. Winter Wheat Crop Trial Made in 1991–1992: Concentration of Isoxaben (1) and of Its Metabolites 2–7 in the 0–10-cm Superficial Soil Layer of the Field Plots Treated with Green Manure (Isoxaben (1) Soil Half-Life: 106 ± 5 Days)[a]

days after 1 treatment[b]	concentrations of isoxaben and of its metabolites 2–7 (ppb, μg kg^{-1} dry soil, as equivs of isoxaben) in the 0–10-cm superficial soil layer[c]						
	1	2	3	4	5	6	7
3	142 ± 7	nd	nd	nd	nd	nd	nd
34	115 ± 5	nd	nd	nd	nd	nd	nd
48	107 ± 5	14	5	6	5	nd	8
83	85 ± 4	18	11	10	8	5	12
118	69 ± 3	17	19	12	5	5	17
152	53 ± 2	13	31	15	12	5	17
191	34 ± 1	10	16	18	10	nd	5
248	15 ± 1	8	14	12	5	nd	nd
282	12 ± 1	5	10	8	nd	nd	nd

[a–c] As in Table I. For *a*, however, corr coeff r = 0.9932; b = 4.978; k = −0.006541 days^{-1}.

Table III. Winter Wheat Crop Trial Made in 1991–1992: Concentration of Isoxaben (1) and of Its Metabolites 2–7 in the 0–10-cm Superficial Soil Layer of the Field Plots Treated with Pig Slurry (Isoxaben (1) Soil Half-Life: 203 ± 10 Days)[a]

days after 1 treatment[b]	concentrations of isoxaben and of its metabolites 2–7 (ppb, μg kg^{-1} dry soil, as equivs of isoxaben) in the 0–10-cm superficial soil layer[c]						
	1	2	3	4	5	6	7
3	131 ± 6	nd	nd	nd	nd	nd	nd
34	123 ± 6	10	nd	nd	nd	nd	nd
48	118 ± 5	12	nd	nd	nd	nd	nd
83	100 ± 5	10	12	10	5	nd	5
118	92 ± 4	11	10	8	7	5	10
152	80 ± 4	10	16	10	8	7	13
191	37 ± 1	11	23	12	11	10	10
248	18 ± 1	9	12	8	5	5	nd
282	13 ± 1	7	10	7	5	nd	nd

[a–c] As in Table I. For *a*, however, corr coeff r = −0.9751; b = 4.908; k = −0.003411 days^{-1}.

Table IV. Winter Wheat Crop Trial Made in 1991–1992: Concentration of Isoxaben (1) and of Its Metabolites 2–7 in the 0–10-cm Superficial Soil Layer of the Field Plots Treated with Cow Manure (Isoxaben (1) Soil Half-Life: 137 ± 6 Days)[a]

days after 1 treatment[b]	concentrations of isoxaben and of its metabolites 2–7 (ppb, μg kg^{-1} dry soil, as equivs of isoxaben) in the 0–10-cm superficial soil layer[c]						
	1	2	3	4	5	6	7
3	134 ± 6	nd	nd	nd	nd	nd	nd
34	120 ± 6	13	nd	nd	nd	nd	nd
48	113 ± 5	15	nd	nd	nd	nd	nd
83	100 ± 5	12	9	8	5	nd	5
118	79 ± 3	10	21	15	10	5	12
152	63 ± 3	13	21	13	12	10	16
191	37 ± 1	10	15	8	7	8	10
248	23 ± 1	8	12	5	5	5	nd
282	12 ± 1	5	10	5	5	nd	nd

[a] As in Table I. For *a*, however, corr coeff r = −0.9790; b = 4.957; k = −0.005070 days^{-1}.

superficial soil layer. No residues of isoxaben (1) nor of its metabolites 2–7 were detected in the cereal grains, the limit of sensitivity for each of these compounds being 5 ppb (5 μg kg^{-1} fresh weight).

When isoxaben (1) was heated to reflux (1–48 h) in water containing KOH (1–8 g %) under synthesis conditions, it was recovered untransformed. In the present work, the

Table V. Winter Wheat Crop Trial Made in 1990–1991: Concentration of Isoxaben (1) and of Its Metabolites 2–7 in the 0–10-cm Superficial Soil Layer of the Winter Wheat Field Plots Not Treated with Organic Fertilizers (Control) (Isoxaben (1) Soil Half-Life: 86 ± 4 Days)[a]

days after 1 treatment[b]	concentrations of isoxaben and of its metabolites 2–7 (ppb, μg kg^{-1} dry soil, as equivs of isoxaben) in the 0–10-cm superficial soil layer[c]						
	1	2	3	4	5	6	7
5	136 ± 6	nd	nd	nd	nd	nd	nd
41	100 ± 5	20	nd	nd	nd	nd	nd
51	88 ± 4	34	nd	nd	nd	nd	nd
83	70 ± 3	18	14	9	5	nd	5
124	54 ± 3	13	12	8	5	nd	5
149	41 ± 2	15	16	11	5	nd	5
179	33 ± 2	15	15	10	5	5	nd
203	29 ± 2	13	13	10	6	5	nd
229	24 ± 1	12	11	7	6	5	nd
250	20 ± 1	10	8	6	5	5	nd
281	15 ± 1	6	6	5	nd	nd	nd

[a–c] As in Table I, except for the following: *a*, isoxaben (1) soil half-life with its 95% confidence intervals. Results of the statistical analysis by regression lines were published by Rouchaud et al. (1993); *b*, at soil sampling, number of days after isoxaben treatment, date (day-month-year) and cumulative rainfall (mm) since isoxaben application, respectively: 5 days, 29-10-1990, 55 mm; 41 days, 4-12-1990, 138 mm; 51 days, 14-12-1990, 175 mm; 83 days, 15-1-1991; 278 mm; 124 days, 25-2-1991, 304 mm; 149 days, 22-3-1991, 329 mm; 179 days, 21-4-1991, 351 mm; 203 days, 15-5-1991, 426 mm; 229 days, 10-6-1991, 446 mm; 250 days, 1-7-1991, 572 mm; 281 days, 1-8-1991, 657 mm.

Table VI. Winter Wheat Trial Made in 1990–1991: Concentration of Isoxaben (1) and of Its Metabolites 2–7 in the 0–10-cm Superficial Soil Layer of the Winter Wheat Field Plots Treated with Green Manure (Isoxaben (1) Soil Half-Life: 121 ± 6 Days)[a]

days after 1 treatment[b]	concentrations of isoxaben and of its metabolites 2–7 (ppb, μg kg^{-1} dry soil, as equivs of isoxaben) in the 0–10-cm superficial soil layer[c]						
	1	2	3	4	5	6	7
5	132 ± 6	nd	nd	nd	nd	nd	nd
41	108 ± 5	15	nd	nd	nd	nd	nd
51	109 ± 5	17	nd	nd	nd	nd	nd
83	87 ± 4	21	nd	nd	nd	nd	nd
124	65 ± 3	15	15	10	5	nd	5
149	55 ± 3	14	14	9	5	nd	5
179	52 ± 3	12	12	8	5	5	nd
203	42 ± 2	10	8	8	5	5	nd
229	37 ± 2	9	7	7	5	5	nd
250	29 ± 2	8	6	5	5	5	nd
281	22 ± 1	8	5	5	nd	nd	nd

[a–c] As in Table V.

products of the isoxaben acid hydrolysis and demethoxylation reactions run in synthesis conditions were tested as potential isoxaben soil metabolites. These reactions are known to generate products which generally also are formed as herbicide soil metabolites (Rouchaud et al., 1991). In the 1990-1991 trial, three months (83 days) after isoxaben application in the untreated control plots, the sum of the residues of isoxaben and its metabolites 2–7 corresponds to 89% of the applied dose. Four months (124 days) after, it represents 71% of the applied dose. This suggests that metabolites 2–7 are the main ones generated in the soil of wheat crops. No ^{14}C-labeled isoxaben was used in this work; other isoxaben soil metabolites which were not detected thus could be formed. However, their soil concentrations should be small.

Demethoxyisoxaben (2) (*N*-[3-(1-ethyl-1-methylpropyl)-isoxazol-5-yl]-2-hydroxy-6-methoxybenzamide) was the

Table VII. Winter Wheat Trial Made in 1990–1991: Concentration of Isoxaben (1) and of Its Metabolites 2–7 in the 0–10-cm Superficial Soil Layer of the Winter Wheat Field Plots Treated with Pig Slurry (Isoxaben (1) Soil Half-Life: 199 ± 9 Days)[a]

days after 1 treatment[b]	concentrations of isoxaben and of its metabolites 2–7 (ppb, μg kg^{-1} dry soil, as equivs of isoxaben) in the 0–10-cm superficial soil layer[c]						
	1	2	3	4	5	6	7
5	127 ± 6	nd	nd	nd	nd	nd	nd
41	117 ± 6	10	nd	nd	nd	nd	nd
51	112 ± 6	17	nd	nd	nd	nd	nd
83	106 ± 5	10	nd	nd	nd	nd	nd
124	87 ± 4	8	9	6	5	nd	nd
149	78 ± 4	7	7	5	5	nd	5
179	71 ± 3	9	9	6	5	nd	5
203	58 ± 3	9	8	6	5	5	nd
229	45 ± 2	10	9	7	5	5	nd
250	39 ± 2	8	10	8	5	5	nd
281	22 ± 1	7	8	8	nd	nd	nd

[a–c] As in Table I.

Table VIII. Winter Wheat Crop Trial Made in 1990–1991: Concentration of Isoxaben (1) and of Its Metabolites 2–7 in the 0–10-cm Superficial Soil Layer of the Winter Wheat Field Plots Treated with Cow Manure (Isoxaben (1) Soil Half-Life: 143 ± 7 Days)[a]

days after 1 treatment[b]	concentrations of isoxaben and of its metabolites 2–7 (ppb, μg kg^{-1} dry soil, as equivs of isoxaben) in the 0–10-cm superficial soil layer[c]						
	1	2	3	4	5	6	7
5	124 ± 6	nd	nd	nd	nd	nd	nd
41	111 ± 6	8	nd	nd	nd	nd	nd
51	108 ± 5	13	nd	nd	nd	nd	nd
83	98 ± 5	14	nd	nd	nd	nd	nd
124	74 ± 4	14	15	10	5	nd	5
149	61 ± 3	15	16	11	5	nd	5
179	57 ± 3	12	12	8	5	5	nd
203	53 ± 3	8	8	7	5	5	nd
229	42 ± 2	8	7	6	5	5	nd
250	34 ± 2	7	6	6	5	5	nd
281	21 ± 1	7	5	7	nd	nd	nd

[a–c] As in Table I.

main isoxaben soil metabolite observed in winter wheat crops (Tables I–VIII). It corresponds to the monodemethoxylation of isoxaben (1). In the 1990–1991 trial, 2 months (51 days) after isoxaben treatment, metabolite 2 corresponded to 28% of the total identified soil residue in the organic fertilizers untreated plots (Table V). Similar results were obtained in the 1991–1992 trial.

The 5-isoxazolone 3 (3-(1-ethyl-1-methylpropyl)isoxazolin-5-one) was the main metabolite generated in soil by cleavage of the isoxaben amide bond (Tables I–VIII). In the 1990–1991 trial, 4 months (124 days) after isoxaben (1) treatment, its soil concentration was similar to that of demethoxyisoxaben (2). The 5-isoxazolone should be generated by cleavage in isoxaben of the bond between the isoxazole 5-C atom and the amide N atom. Nucleophilic attack of the isoxazole 5-C atom by OH^- should generate the 5-isoxazolone 3.

This mechanism of amide hydrolysis is unusual. Indeed, the usual one should lead to the 5-aminoisoxazole 7 (5-amino-3-(1-ethyl-1-methylpropyl)isoxazole), and 2,6-dimethoxybenzoic acid (6). The hydrolysis mechanism observed here with isoxaben (1) should be due to the chemical structure of the isoxazole ring; this should generate a positive charge at the isoxazole 5-C atom.

This mechanism is corroborated by the fact that the main benzoic compounds generated by the isoxaben amide bond cleavage were the N-unsubstituted amides 2,6-dimethoxybenzamide (4) and 2-hydroxy-6-methoxybenzamide (5) (Figure 1, Tables I–VIII). 2-Hydroxy-6-methoxybenzamide (5) is the product of monodemethoxylation of 2,6-dimethoxybenzamide (4). 2,6-Dimethoxybenzoic acid (6) was observed in soil only at the end of the crop. This suggested that it was generated more by the hydrolysis of 2,6-dimethoxybenzamide (4), than by direct hydrolysis of isoxaben (1) into 2,6-dimethoxybenzoic acid (6) and the 5-aminoisoxazole 7.

The 5-aminoisoxazole 7 never accumulated in soil. When it was detected, it only was in very low concentrations (Tables I–VIII). Four months before winter wheat harvest, it could not be detected in soil. On account of its original chemical structure, most of the isoxaben (1) was metabolized in soil into the nontoxic 5-isoxazolone 3. Further soil metabolism of the 5-isoxazolone 3 should open the isoxazole ring and lead to unstable nontoxic products (oximes). These should be quickly further metabolized into nontoxic, more stable, and common products. On account of its original 5-aminoisoxazole chemical structure, isoxaben thus is a good example of herbicide soil metabolism into products nontoxic for the environment.

In both the 1990–1991 and 1991–1992 trials, during the first 6-month crop period, the organic fertilizer treatments increased the isoxaben soil persistence (Tables I–VIII). The maximum soil concentration of metabolites 2–7 generally occurred later, in the plots treated with the organic fertilizers compared to the control. After that first 6 months, the effects of the organic fertilizers progressively disappeared, isoxaben and its metabolites 2–6 (no metabolite 7 was detected) soil concentrations progressively becoming very low and similar as in the control plots. This corresponded to the increase of the rate of isoxaben soil metabolism at the end of spring and in summer. This, however, did not generate, in the organic fertilizer-treated plots, greater soil concentrations of metabolites 2–7 relative to those in the organic fertilizer-untreated plots. This probably was the result of the kinetics of the simultaneous consecutive and competitive reactions of formation and decomposition of the isoxaben metabolites.

In the control plots and in the plots treated with green manure, the rates of isoxaben (1) soil biodegradation and metabolism were slightly higher in the 1991–1992 trial than in the 1990–1991 one (Tables I–VIII). In the pig slurry- and cow manure-treated plots, these rates, however, were similar in both trials. The ratios of the isoxaben (1) soil half-lives in the organic fertilizer-treated plots to the one in the control plots were 1.41, 2.31, and 1.66, respectively, in the green manure-, pig slurry-, and cow manure-treated plots of the 1990–1991 trial (Tables V–VIII). In the 1991–1992 trial, these ratios were 1.41, 2.71, and 1.83, respectively. The second organic fertilizer treatment thus had no supplementary effect relative to the first organic fertilizer treatment or only slightly increased the effect of the organic fertilizer treatments to slow down the isoxaben soil metabolism. Results thus suggest that the repeated organic fertilizer treatments have no cumulative effect to slow down the isoxaben soil metabolism. This should be related to the continuous mineralization of the soil organic matter into CO_2 and to the progressive transformation of the chemical structure of the organic matter remaining in soil. This first is the increase in the soil organic matter of the density of oxygenated (carboxylic, phenol, quinone, etc.) chemical functions able to chelate the herbicide (Inbar et al., 1989) and then the decrease of the density of these oxygenated

chemical functions by the soil organic matter coalification procedure (Stevenson, 1982).

The organic fertilizers a priori could have had several effects on isoxaben soil metabolism. They could increase the soil microbial and enzymatic activities and so increase the rate of isoxaben soil metabolism. Results obtained in the 1990–1991 and 1991–1992 trials obviously suggest that this at least was not a major effect. The supplement of recent organic matter given to soil by the recent organic fertilizer treatments should increase the herbicide adsorption onto the soil organic matter. This should in some way protect isoxaben toward the soil microbial activity which metabolizes it. This should correspond to the reduction of isoxaben bioavailability toward the soil microorganisms. The greater isoxaben soil persistence and its smaller rates of soil metabolism observed in the plots treated with organic fertilizers suggest that the herbicide adsorption onto the soil organic matter indeed has a major influence. On account of this effect on the kinetics of isoxaben soil metabolism, the organic fertilizers increase the isoxaben soil concentrations during the main first-crop period. This should have a positive effect on the herbicide efficiency, if the increase of the adsorption of the herbicide onto the soil organic matter should not too much reduce the isoxaben bioavailability toward weeds. It is known indeed that the herbicide efficiency is reduced in soils containing increasing concentrations of old organic matter humus (Harrison et al., 1976; Weber et al., 1987). This has been observed when comparing soils containing 2, 4, 6, and till 40% organic matter. In the trials described here, the increases of the soil organic matter concentrations in the 0–20-cm soil layer, due to the organic fertilizers treatments, were at maximum 0.6%. Using the published correlations between herbicide efficiencies and old soil organic matter concentrations, one should expect that the reduction of herbicide efficiency due to such a small change should be low. On the other hand, the chemical structure of the young soil organic matter due to the recent organic fertilizers treatments is different from the one of the old humus (Stevenson, 1982; Inbar et al., 1989). The final effect of the organic fertilizers, due to the increase of the herbicide adsorption onto the soil organic matter, on the herbicide efficiency will depend on the reduction of the rate of herbicide soil metabolism (positive effect corresponding to a herbicide slow release in soil) and the reduction of the herbicide bioavailability toward weeds (negative effect). The first biological measurements of weeds in the field made by M. Van Himme in the 1991–1992 trial indicated somewhat greater isoxaben efficiencies in the organic fertilizer-treated plots. Trials are repeated to obtain ascertained measurements.

ACKNOWLEDGMENT

Mass spectra were recorded by C. Moulard (Université Libre de Bruxelles, Brussels, Belgium). This work was sponsored by the Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, IRSIA-IWONL, Belgium.

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Received for review January 25, 1993. Revised manuscript received June 11, 1993. Accepted August 30, 1993.*

* Abstract published in *Advance ACS Abstracts*, October 15, 1993.