Bull. Environ. Contam. Toxicol. (1994) 52:568–573

Environmental Contamination and Toxicology

Pig Slurry and Cow Manure Effect on Atrazine and Metolachlor Soil Biodegradation in Maize

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Received: 19 May 1993/Accepted: 12 August 1993

Atrazine (6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine--2,4-diamine) is still one of the main herbicides used very efficiently in maize crops against both grass and broadleafed weeds. It is used alone, or in mixture with other herbicides, e.g. metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1--methylethyl)acetamide), in order to effectively avoid resistance development in weeds. We previously observed that treatments with organic fertilizers increase the insecticides' soil persistence and efficiency in sugar beet crops (Rouchaud et al. 1993). In the present work, we studied the influence of cow manure and pig slurry on the atrazine and metolachlor soil persistences in maize crops. Both these organic fertilizers are frequently applied before maize sowing. On account of the atrazine soil persistence, some care must be given to the choice of the rotational crop which follows maize when high doses of atrazine have been applied. One of the aims of the present work was to determine if the organic fertilizers would increase the long-term soil persistence of atrazine.

MATERIALS AND METHODS

A field (70 x 60 m) at Wavre (12% clay, 78% silt, 10% sand, silt type, 1.9% organic matter, pH(H_2O) 6.2), Belgium, was divided into plots (10 x 15 m). On 10-3-1992 (day-month-year), onto each plot one of the organic fertilizers, cow manure or pig slurry, was applied at the rate of 40 tons ha^{-1}. On 20-3-1992 the field was tilled to a 20-25 cm depth. Control plots that were not treated with organic amendments were included. There were 4 replicate plots for each organic fertilizer treatments and control. On 8-5-1992, the entire field was rotary-tilled to a depth of 10 cm, and maize (cv. Aviso) was sown. On 14-5-1992, one of the herbicides, atrazine or metolachlor, was applied onto each plot. Atrazine was applied at 1.25 kg ha^{-1} by spraying the emulsion of Atratex FL (50 wt.% atrazine, Protex) in water (750 L ha^{-1}). Metolachlor was applied at 2.9 kg ha^{-1} using Dual 720 EC (72 wt.% metolachlor, Ciba-Geigy). For each atrazine or metolachlor treat-

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ments, there were 4 replicate plots. At intervals during the trial (Tables 1 and 2), samples were taken separately (and analyzed once separately) in the 0-12 cm soil layer of each of the four replicate plots, of each of the two organic fertilizer treatments and control. In addition, on 9-7, 9-9 and 19-11-1992 single samples were taken separately (and analyzed once separately) from the 12-25 cm soil layer in each of the four replicate plots. 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. At harvest, aliquots of maize foliage (about 2 kg) and grain (about 1 kg) were taken at random in each organic fertilizer treatment, sampling being made in the four replicate plots, but bulking together the samples from each replicate. The foliage was cut into small pieces which were mixed, and an aliquot of each of them was separately stored at -25°C until analyzed. Four replicate analyses were made on each of the foliage and grains from each of the organic fertilizer treatments.

Thin-layer chromatography (TLC) was done by using silica gel 60, F254, 20 x 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. Atrazine and metolachlor extracted from soil and maize foliage and grain were analyzed using a Varian 2700 gas-liquid chromatography (GLC) apparatus. Detection was by flame ionization and electron-capture. Inlet and detector were at 240°C. Glass column used was 1.8 m x 2 mm i.d., and contained 5% SE30 on 80-100 mesh Gas-Chrom Q; nitrogen was the carrier gas at 40 mL min^{-1}. With column oven at 170°C, retention times of atrazine and metolachlor were 3.6 and 8.9 min, respectively. Frequently, atrazine and metolachlor extracted from soil were analyzed by mass spectrometry (MS). Infrared (IR) spectra were recorded with the Midac FTIR apparatus (KBr disks: cm^{-1}). ^1H NMR spectra were recorded in CDCl3 with the Varian XL200 apparatus, using tetramethylsilane as internal standard: δ , ppm/tetramethylsilane. MS were recorded with the VG Micromass 7070F spectrometer at 70 eV used in the electron impact mode: m, relative abundance, %.

For the preparation of the atrazine standard for analysis, Gesaprim 500 Flowable (100 g; 50 wt.% atrazine, Ciba-Geigy) was stirred (15 min) with chloroform (300 mL). Water (150 mL) saturated with NaCl, benzene (100 mL), hexane (50 mL) and dodecylsulfate (1 g) were added. The mixture was further stirred for 15 min. The organic layer was separated, dried (Na_2SO_4), and concentrated to dryness in a vacuum rotary evaporator. Recrystallization in chloroform+benzene gave atrazine (42 g, 84%) whose purity was greater than 99% as shown by TLC and GLC. Spectra of atrazine: IR: 3273(NH), 3119(NH), 2974, 1622, 1554, 1404, 1346, 1304, 1265, 1242, 1167, 1130, 1057, 992, 878, 839, 806. ^1H NMR: 1.18-1.28 (m, 9H, $CH(\underline{CH_3})_2$, $CH_2\underline{CH_3}$); 3.47(m, 2H, $\underline{CH_2}CH_3$); 4.20(m, 1H, $\underline{CH}(CH_3)_2$); 5.35(br, 1H, $\underline{NH}$); 6.10(br, 1H, $\underline{NH}$). MS: 215(M^+, 85); 217(215+2, 27); 200(M-CH_3, 100); 202(200+2, 32); 187(M-CH_2CH_3+H, 9); 189(187+2, 3); 173(M-$CH(CH_3)_2$+H, 73); 175(173+2, 23); 158

(M-NHCH(CH_3)$_2$+H, 33); 160(158+2, 11); 145(M-CH_3CH_2NHCN, 18); 147 (145+2, 6).

For the preparation of the metolachlor standard for analysis, Dual (3 g; 96 wt.% metolachlor, Ciba-Geigy) was purified by column chromatography on silica gel (30 g). Elution with dichloromethane gave metolachlor (2.7 g, 90%) whose purity was greater than 99.5% as shown by TLC and GLC. Spectra of metolachlor: IR: 2978, 1674(CO), 1462, 1364, 1242, 1113, 789, 702. ^{1}H NMR: 1.11--1.13 (d, 3H, CH($\underline{CH_3}$)); 1.15-1.27(t, 3H, $CH_2\underline{CH_3}$); 2.22, 2.24 (d, 3H, $C_6H_3\underline{CH_3}$); 2.52-2.60(m, 2H, $C_6H_3\underline{CH_2}CH_3$); 3.24, 3.27(d,3H,$OCH_3$); 3.59(s, 2H, CH_2Cl); 3.44-3.72(m, 2H, $\underline{CH_2}OCH_3$); 4.20(m, 1H, $\underline{CH}CH_3$); 7.13-7.26(m, 3H, aromatic H). MS: 283(M^+, 32); 285(283+2, 11); 238(M-CH_2OCH_3, 100); 240(238+2, 32); 211(M-$CHCH_3CH_2OCH_3$+H, 83); 213(211+2, 27); 176(211-Cl, 29); 174(211-HCl-H, 32); 163 (238-$COCH_2Cl$+2H, 69); 162(238-$COCH_2Cl$+H, 66); 160(238-$COCH_2Cl$-H, 78).

For the atrazine soil analysis, soil (100 g) was refluxed with stirring for 2 h in methanol/water (8/2, vol/vol, 200 mL). The mixture was filtered, and the extraction was repeated with heating to reflux during 10 min. The filtrates were combined; water (100 mL) was added, and the methanol removed in a vacuum rotary evaporator (30°C). NaCl (15 g) was added to the aqueous solution, which was then extracted twice with dichloromethane (200+150 mL). The dichloromethane solution was dried (Na_2SO_4), concentrated to 40 mL in a vacuum rotary 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, together with the standard of atrazine. After a first elution with dichloromethane, the solvent was evaporated from the plate. The plate then was eluted a second time with dichloromethane, giving a band containing atrazine at Rf=0.32. The band was scraped off. The silica gel was extracted with ethyl acetate. The extract was then concentrated and analyzed for atrazine by GLC and, in several cases, by MS.

For the metolachlor soil analysis, the procedure was the same as with atrazine, but with the following changes. Soil was extracted two times with acetone/water (8/2, vol/vol, 2x200 mL) with heating to reflux (2x15 min). The mixture was filtered. Water was added to the filtrate, and the acetone was evaporated. NaCl was added to the aqueous solution, and it was extracted with dichloromethane. The dichloromethane solution was dried, concentrated and applied onto a TLC plate. Elution with dichloromethane gave a band containing metolachlor at Rf=0.38. This was extracted and analyzed by GLC and, in several cases, by MS.

Maize leaves and grains were analyzed in the same way as soil. Leaves were first cut into small pieces and extracted with solvent in a laboratory blender. Grains were first ground into flour. The flour then was extracted as the leaves.

At the 0.1 mg kg^{-1} level in soil, the recoveries were 83-95% for atrazine and 82-93% for metolachlor. The analytical limit of quan-

Table 1. Concentrations of atrazine in the 0-12 cm surficial soil layer of a maize field preemergence treated with 1.25 kg atrazine ha^{-1}. Each field plot had been treated 2 months before atrazine treatment with either pig slurry (40 tons ha^{-1}) or cow manure (40 tons ha^{-1}). There were also control plots not treated with organic fertilizers.

Date[a]	Days after atrazine treatment	Cumulative rainfall, mm	Organic fertilizer: Control (no organic fertilizer)	Pig slurry	Cow manure
			Concentrations of atrazine (mg kg^{-1} dry soil) in the 0-12 cm soil layer[b]		
14-5	0	0	1.05±0.05	0.95±0.05	0.98±0.05
29-5	15	10	0.82±0.04	0.89±0.04	0.86±0.04
2-6	19	28	0.73±0.04	0.85±0.04	0.86±0.04
23-6	40	110	0.61±0.03	0.76±0.04	0.69±0.03
9-7	56	138	0.51±0.02	0.64±0.03	0.61±0.03
24-7	71	189	0.41±0.02	0.60±0.03	0.52±0.02
7-8	85	209	0.37±0.01	0.49±0.02	0.48±0.02
9-9	118	380	0.26±0.01	0.41±0.02	0.40±0.02
20-10	159	417	0.15±0.01	0.18±0.01	0.21±0.01
19-11	189	538	0.11±0.01	0.13±0.01	0.10±0.01
Corr. coeff.[c]			-0.9915	-0.9737	-0.9789
y Intercept[c]			4.57	4.58	4.56
Slope, $days^{-1}$ [c]			-0.01159	-0.007234	-0.007614
Soil half-lives, days[c]			60±3	96±4	91±4

a. Sampling date, day-month, year 1992. The organic fertilizers were applied on 10-3-1992. Maize was sown on 8-5-1992. Atrazine was applied on 14-5-1992.

b. Means of 4 replicates±s.d.

c. For the first 118 days crop period, linear regression $\ln y = kt + b$ of the naperian logarithms of the atrazine soil concentrations ($y = 100 \times$ mg kg^{-1} dry soil) in the 0-12 cm surface soil layer, against time t (days) following atrazine treatment: correlation coefficient, y intercept, and slope. The atrazine soil half-lives with their 95% confidence intervals were obtained using the SAS logical CMS SAS 5.18 (1984, 1986, SAS Institute Inc., Cary, NC 27512).

titation for atrazine and metolachlor in soil was 0.01 mg kg^{-1} dry soil. At the 0.1 mg kg^{-1} fresh weight level, the recoveries and limits of detection of atrazine and metolachlor in the maize foliage and grains were similar to the corresponding values in soil, the limit of quantitation being 0.01 mg kg^{-1} of atrazine

Table 2. Concentrations of metolachlor in the 0-12 cm surficial soil layer of a maize field preemergence treated with 2.9 kg metolachlor ha^{-1}. Each field plot had been treated 2 months before the metolachlor treatment with either pig slurry (40 tons ha^{-1}) or cow manure (40 tons ha^{-1}). There were also control plots not treated with organic fertilizers.

Datea	Days after metolachlor treatment	Cumulative rainfall, mm	Organic fertilizer:		
			Control (no organic fertilizer)	Pig slurry	Cow manure
			Concentrations of metolachlor (mg kg^{-1} dry soil) in the 0-12 cm soil layer[b]		
14-5	0	0	2.31±0.11	2.09±0.10	2.20±0.11
29-5	15	10	1.72±0.09	2.01±0.10	1.98±0.10
2-6	19	28	1.71±0.09	1.96±0.10	1.82±0.09
10-6	27	64	1.52±0.08	1.95±0.10	1.81±0.09
23-6	40	110	1.13±0.05	1.68±0.08	1.69±0.08
9-7	56	138	0.95±0.05	1.46±0.07	1.37±0.06
24-7	71	189	0.76±0.03	1.32±0.06	1.16±0.06
7-8	85	209	0.60±0.03	1.09±0.05	0.98±0.05
9-9	118	380	0.36±0.02	0.82±0.04	0.78±0.04
20-10	159	417	0.18±0.01	0.31±0.02	0.29±0.02
19-11	189	538	0.11±0.01	0.10±0.01	0.12±0.01
Corr. coeff.[c]			-0.9968	-0.9823	-0.9876
y Intercept[c]			5.42	5.43	5.42
Slope, $days^{-1}$			-0.01564	-0.008355	-0.009155
Soil half-lives, days[c]			44±2	83±4	76±3

a,b,c: As in Table 1.

and metolachlor kg^{-1} fresh weight.

RESULTS AND DISCUSSION

In all the organic fertilizer treated and untreated control plots, no residue of atrazine was detected in the 12-25 cm soil layer. On the other hand, the soil concentrations of metolachlor in the 12-25 cm soil layer always were less than 8% of their corresponding values in the 0-12 cm surface soil layer. No residues of atrazine or metolachlor were detected in the maize foliage and grain at harvest; the limit of quantitation for each of these compounds was 0.01 mg kg^{-1} fresh weight of foliage or grain.

During the first four months period of the maize crop, the rates of disappearance of the atrazine or metolachlor soil concentrations were proportional to the atrazine or metolachlor soil con-

centrations (apparent first-order kinetics)(Tables 1 and 2). After the first four months period, these rates greatly increased and became greater than the ones forecasted by the first-order kinetics. During the first four months period which followed the herbicide treatments, the organic fertilizer treatments increased the atrazine and metolachlor soil persistences. In the control plots not treated with organic fertilizers, and in the plots treated with pig slurry or cow manure, the atrazine soil half--lives were 60, 96 and 91 days, respectively. The corresponding values for metolachlor were 44, 83 and 76 days. The organic fertilizer treatments probably increased the herbicides adsorption onto the soil organic matter. The soil concentration of this last indeed was increased by the organic fertilizer treatments. That herbicides adsorption should protect them against the soil microbial and enzyme activities which metabolize the herbicides. The effects of the organic fertilizer treatments were greater with metolachlor than with atrazine; perhaps because atrazine was already much adsorbed onto the soil organic matter in the organic fertilizer untreated control plots.

After the first four months crop period, the atrazine and metolachlor soil residues became very low, and similar in the organic fertilizers treated and untreated plots. Whereas the organic fertilizer treatments increase the atrazine soil persistence during the first crop period, they thus have no more influence on the long-term soil persistence of the very low remaining atrazine soil residues. The organic fertilizer treatments thus should not increase the care for the sensitivity of the following crops to atrazine. The absence of atrazine, and the only very low residues of metolachlor in the 12-25 cm soil layer indicated that these compounds were not leached in soil, or only very slightly, in both the organic fertilizers treated and untreated plots.

Acknowledgments. Mass spectra were recorded by C. Moulard (Université Libre de Bruxelles, Brussels, Belgium). The research was supported by the Institute for Applied Research in Industry and Agronomy, IRSIA-IWONL, Brussels, Belgium.

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