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Concentrations of the Herbicides Propyzamide, Chlorpropham, and of Their Metabolites in Soil and Lettuce Under Field Conditions

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Propyzamide [3,5-dichloro-N-(1,1-dimethyl-2-propynyl)benzamide] is a useful and effective herbicide which is widely used for weed control in the cultures of lettuce, endive, witloof, scorzonera, and alfalfa. As with most pesticides, propyzamide possesses properties which make it necessary to establish rules for its safe use.

Propyzamide has some carcinogenicity (Reuber 1980); that activity would correspond to the inactivation of hepatic cytochrome P-450 in animals (Montellano and Kunze 1980). Following dietary administration of propyzamide to a lactating cow, about 5% of the administered dose was detected in the milk (St John and Lisk 1975). To reduce these risks, the lettuce tolerance for the combined residues of propyzamide and its metabolites has been reduced to 1 ppm in most of the countries (USEPA 1983).

Winter crops, planted in the year of herbicide application to the preceding crop, were especially sensitive to herbicide residues; winter wheat and barley, grasses, spring cereals and other crops were injured by residues of propyzamide (Eagle 1981).

The half-life of propyzamide in different soils has been measured in laboratory experiments; thus in the absence of any crop culture; it varied from 10 to 40 days at 25°C, and from 60 to 120 days at 15°C.

It is known that cultivation changes the persistence and phytotoxicity of propyzamide residues (Hance et al. 1978).

Little has been published about the concentrations of propyzamide and of its initial ketone metabolite [3,5-dichloro-N-(1,1-dimethylacetonyl)-benzamide] in the soil of lettuce cul-

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ture. In the present study, we measured these concentrations in lettuce cultures grown in two culture regions having completely different soil types. Simultaneously, we measured the concentrations of the herbicide chlorpropham [1-methylethyl (3-chlorophenyl) carbamate] and of its metabolite 3-chloro--aniline. Chlorpropham is widely used in the commercial culture of lettuce, witloof, scorzonera, spinach, carrot, bean, strawberry, and alfalfa.

MATERIALS AND METHODS

Cultures made in St. Kathelijne-Waver. The lettuce culture was made at the Research Station for Vegetables, St. Kathelijne--Waver, Belgium. The lettuce (Reskia variety) was sown in a greenhouse, and they were transplanted in the field when they had about six leaves. The space between the lettuce plants was 30x30 cm. The plots were of the same soil type and had a common agricultural history. Soil analysis indicated pH 5.6, 72.3% sand, 22.3% silt, 5.4% clay, loamy sand classification, and 3.0% organic matter. All experiments were arranged in a randomized block design. There were four plots (four replications) for each of the treatments. The size of each plot was 1.3x10.0 m.

Only one herbicide was applied to each plot. For each herbicide treatment, an overall surface spray with an aqueous emulsion of the herbicide was made on the finely granulated soil, just before planting of the lettuce plants; the soil then was raked so that the herbicide was incorporated to a depth of about 6 cm. The treatment was made with one of the following two herbicides. Kerb 50 (formulation containing 50 g% of propyzamide) was used at the dose of 20 g of Kerb 50/are (are= 100 square meters); CIPC (formulation containing 400 g of chlorpropham/L) was used at the dose of 40 mL of CIPC/are. Three lettuce cultures were made successively, the same treatment being repeated on the same plot. For the first one, the herbicide treatment and the planting of the lettuce in the field were made on April 1, 1985. The lettuce was harvested on May 31, 1985, when they had a mean unitary weight of about 400 g. The cultures were repeated two times on the same plots. A third lettuce culture and treatment was repeated on the same plots. The harvested lettuce from each plot were healthy.

Culture made in Louvain-la-Neuve. The lettuce culture was made in an experimental field at Louvain-la-Neuve, Belgium. Soil analysis indicated pH 6.1, 4.9% sand, 70.1% silt, 25.0% clay, silt loam classification and 2.2% organic matter. The culture was made exactly in the same way as in St. Kathelijne-Waver. However, only one culture (crop) was made, and only the herbicide Kerb 50 was assayed.

Soil sampling was made by using a bore auger. Samples were taken

at depth of 0-6, 7-15 and 16-30 cm.

Soil (100 g) and acetone (200 mL) in an 500 mL Erlenmeyer flask were magnetic stirred during 30 min. The extract was filtered. The solid residue was extracted again during 30 min with acetone+water 1+1 (200 mL). The mixture was centrifugated at 6000 rpm. The supernatant and extract were combined and concentrated to about 80 mL with a vacuum rotary evaporator at 35°C. The aqueous solution was extracted three times with 3x200 mL of dichloromethane. The volume of combined dichloromethane extracts was reduced to ca. 2 mL. The concentrate was applied as a line on a t.l.c. plate (DC-Plastikfolien Kieselgel 60 F_{254} 20x20 cm, 0.2 mm thick, Merck). For the samples from soils treated with propyzamide, elution with benzene+acetone 95+5,v/v gave a band (Rf=0.65) containing propyzamide, and a band containing the ketone metabolite (Rf=0.43). For the samples from soils treated with chlorpropham, the same t.l.c. gave a band containing chlorpropham at Rf=0.32. The bands were scraped separately and extracted with acetone. The extracts were concentrated by using a stream of nitrogen and t.l.c. again in the same way as the first time. The final extract was concentrated to dryness with a stream of nitrogen, and the residue was dissolved in 0.1 mL of chloroform, of which 0.1 µL was injected in a gas chromatograph which was equipped with a flame ionization detector and with an electron capture detector, both being used alternatively. Glass column of 2 m x2 mm i.d. packed with 5% SE 30 on 80-100 mesh Gas Chrom Q. The inlet was 250°C. The column oven temperature was programmed from 130 to 170°C at 8°C/min. Retention times were 5.4 min for propyzamide, 8.2 min for propyzamide ketone metabolite and 3.3 min for chlorpropham. Quantitation was made by using standard solutions of each of the three compounds. Recoveries of propyzamide, ketone metabolite and chlorpropham in soil at the 0.1 ppm level were 81-92, 88-103 and 79-96% respectively. The limit concentration detected in soil was 0.01 ppm for the three compounds.

3-Chloro-aniline present as such in the soil was bound to it so that acetone and acetone+water did not extract any 3-chloro--aniline. After extraction of the soil with both these solvents which took off chlorpropnam, the extracted soil was heated with 50 g% NaOH in water, 3-chloro-aniline being extracted in this way and measured after purification and derivatization.

The acetone extracted soil was heated to reflux during 3 hr with 50 mL of a 50 g% solution of NaOH in water and 1.5 mL of 1-butanol as antifoam. The cooled mixture was centrifugated. The supernatant was extracted three times with 3x50 mL of benzene. The benzene extract was extracted two times with successively 100 and 50 mL of 1.2 N HCl. The aqueous extracts were gathered and 30 mL of 50 g% NaOH in water were added. The aqueous solution was extracted two times successively with 150 mL and 50 mL

of benzene. 3-Chloro-aniline in this extract was derivatized by acetylation. The benzene extract plus 3.5 mL of acetic anhydride was heated to reflux during 30 min, with a calcium chloride protecting tube. The cooled mixture was washed with 30 ml of 10 g% aqueous Na_2CO_3. The benzene solution was concentrated successively to 40 mL at 30°C in a vacuum rotary evaporator, and to 0.5 mL by a slow stream of nitrogen. It was applied as a band onto a silica gel t.l.c. plate, standard of 3-chloro-acetanilide being applied on a part of the t.l.c. plate. Benzene+ethyl acetate 65+50, v/v (Rf of 3-chloro-acetanilide =0.67) was the eluting solvent. The band corresponding to 3-chloro-acetanilide was separated, extracted with ethyl acetate, the extract was concentrated and analyzed by g.l.c. in the same way as chlorpropham except that the column oven temperature was 160°C and the retention time for 3-chloro--acetanilide was 3.7 min. In several cases, the final extract was analyzed further by mass spectrometry. Moreover, in several cases an aliquot of the final benzene extract was derivatized using 4-bromo-benzoylchloride, which transformed 3-chloro-aniline into 4-bromo-N(3-chlorophenyl) benzamide. In this way, 3-chloro-aniline present as such in the soil was measured simultaneously as two different derivatives using both g.l.c. and mass spectrometry. Recovery at the 0.1 ppm level in soil and in lettuce for 3-chloro-aniline were 75-89 and 79-92% respectively. The limit of sensitivity was 0.01 ppm. Lettuce samples were analyzed in the same way as the soil ones.

RESULTS AND DISCUSSION

Almost all of the residues in propyzamide-treated soils were present in the 0-6 cm soil layer (Tables 1 and 2). Thus, propyzamide and its ketone metabolite were poorly transferred to depths lower than 6 cm in the time period of this study. This is consistent with the low solubility of propyzamide in water (15 mg/L). However, transfer was slightly higher in St. Kathelijne-Waver than in Louvain-la-Neuve.

The soil concentrations of propyzamide and of the ketone metabolite were somewhat higher in the second and third cultures (crop) than in the first one. This suggest that there is some accumulation of the residues in the soil after successive cultures and treatments.

At lettuce harvest, the concentrations of propyzamide and of its ketone metabolite were rather high in the soil; they were higher in the soil of St. Kathelijne-Waver than in the one of Louvain-la-Neuve. The half-life of propyzamide in the soil of St. Kathelijne-Waver was of 38-40 days, and in the one of Louvain-la-Neuve of 9 days. The herbicidal efficacy of propyzamide, qualitatively evaluated by the amount of weeds during culture, was rather similar in both locations.

Table 1. Soil and lettuce concentrations of propyzamide and of its ketone metabolite in lettuce cultures made in St. Kathelijne-Waver; both compounds were detected only in the 0-6 cm soil surface layer.

Sampling date, day-month, 1985	Propyzamide[d]	Ketone[d]
1. First culture: 1.1. Soil		
20-3[a]	<0.01	<0.01
1-4[b]	0.82[a]	0.09[a]
15-4	0.64[b]	0.16[b]
15-5	0.40[c]	0.27[c]
31-5[c]	0.23[d]	0.34[d]
1.2. Lettuce	0.21	0.19
2. Second culture: 2.1. Soil		
25-6[a]	0.04[a]	0.06[a]
28-6[b]	0.95[b]	0.13[b]
12-7	0.73[c]	0.20[c]
2-8[c]	0.51[d]	0.26[d]
2.2. Lettuce	0.27	0.14
3. Third culture: 3.1. Soil		
12-8[a]	0.06[a]	0.08[a]
13-8[b]	0.89[b]	0.18[b]
5-9	0.75[c]	0.27[c]
24-9[c]	0.65[d]	0.39[d]
3.2. Lettuce	0.32	0.27

[a]Before treatment+plantation.

[b]Just after treatment+plantation.

[c]Lettuce harvest.

[d]ppm of product relative to the dry weight of soil or the fresh weight of lettuce. Data are the means of 4 replications. Means within the column -relative to the same culture- followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

In the harvested lettuce, the concentrations of propyzamide and of its ketone metabolite were somewhat higher in Louvain-la-Neuve than in St. Kathelijne-Waver, opposite of the concentrations in the soils of both locations.

Table 2. Soil and lettuce concentrations of propyzamide and of its ketone metabolite in the lettuce culture made in Louvain-la-Neuve; both compounds were detected only in the 0-6 cm soil layer.

Sampling date, day-month, 1985	Propyzamide[d]	Ketone[d]
1. Soil		
19-6[a]	<0.01	<0.01
21-6[b]	0.77[a]	0.08[a]
1-7	0.35[b]	0.09[a]
11-7	0.16[c]	0.11[b]
1-8[c]	0.09[d]	0.14[c]
14-8	0.05[e]	0.13[b,c]
2. Lettuce	0.35	0.24

[a-d]As in Table 1.

The differences observed between St. Kathelijne-Waver and Louvain-la-Neuve, as to their soil and lettuce concentrations in propyzamide and in its ketone metabolite, probably is related to the different soil types. It is known that soil type influences the fate of pesticides in soil (Copin et al. 1984).

The rate of disappearance of chlorpropham in soil was much higher (half-life = 14-17 days) than that of propyzamide (Table 3). Transfer in soil of chlorpropham was higher than that of propyzamide. This corresponds to the solubility in water of chlorpropham (89 mg/L) which is higher than that of propyzamide (15 mg/L). As with propyzamide, there was some accumulation of chlorpropham in soil after repeated treatments. That accumulation was not observed for 3-chloro-aniline, the metabolite of chlorpropham.

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Table 3. Soil and lettuce concentrations of chlorpropham and of its metabolite 3-chloro-aniline in lettuce cultures made in St. Kathelijne-Waver; both compounds were not detected at depth lower than 15 cm.

Sampling date, day--month 1985	Soil depth			
	0-6 cm		7-15 cm	
	Chlorpropham[d]	3-Chloro-aniline[d]	Chlorpropham[d]	3-Chloro-aniline[d]
1. First culture: 1.1. Soil				
20-3[a]	<0.01	<0.01	<0.01	<0.01
1-4[b]	1.75[a]	0.21[a]	<0.01	<0.01
15-4	0.72[b]	0.92[b]	0.07[a]	0.02[a]
15-5	0.17[c]	1.03[b]	0.12[b]	<0.01
31-5[c]	0.12[d]	0.95[b]	0.09[a]	0.02[a]
1.2. Lettuce				
	0.13	0.14		
2. Second culture: 2.1. Soil				
25-6[a]	0.02[a]	0.12[a]	0.02[a]	0.12[a]
28-6[b]	1.81[b]	0.21[b]	0.01[a]	0.10[a]
12-7	0.66[c]	0.83[c]	0.08[b]	0.05[b]
2-8[c]	0.21[d]	1.09[d]	0.04[c]	0.04[b]
2.2. Lettuce				
	0.22	0.19		
3. Third culture: 3.1. Soil				
12-8[a]	<0.01	0.13[a]	<0.01	0.13[a]
13-8[b]	1.98[a]	0.22[b]	<0.01	0.12[a]
5-9	0.42[b]	1.04[c]	0.04[a]	0.13[a]
24-9[c]	0.26[c]	0.99[c]	0.03[a]	0.09[b]
3.2. Lettuce				
	0.21	0.23		

[a-d]As in Table 1.

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