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Metabolism of Chlorfenvinphos in Both Soil and Plant of Cauliflower and Brussels Sprouts Field Crops

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The insecticide chlorfenvinphos [2-chloro-1-(2,4-dichlorophenyl) ethenyl diethyl phosphate] (1) is efficiently used for the insect control by foliar spray of potato, cabbage, onion and carrot. It is applied onto soil for the control of the flies of the onion, carrot, cabbage and bean crops. When applied at planting onto soil around the stem of the plant, it is one of the best insecticide for the protection of cauliflower and brussels sprouts crops against root fly (Rouchaud et al., 1988a); it sterilizes the soil around the plant stem, killing the larvae of the root fly which, otherwise, would penetrate into the stem and the roots of the plant. In the soil, chlorfenvinphos is metabolized by the soil microbial fauna, progressively into the non insecticide 2,4-dichlorophenacyl chloride (2), 2,4-dichloroacetophenone (3), α-(chloromethyl)-2,4-dichlorobenzyl alcohol (4), 1-(2',4'-dichlorophenyl)-ethan-1-ol (5), 2,4-dichlorobenzoic acid (6), 2-hydroxy-4-chlorobenzoic acid (7), and 2,4-dihydroxybenzoic acid (8)(Beynon et al., 1966, 1967, 1968; Rouchaud et al., 1988b). Chlorfenvinphos is generally considered to be non systemic i.e., when it is applied onto soil, it should not migrate into the plant; when it is sprayed onto the plant foliage, it should not move from the treated leaves to the untreated parts of the plant. This point of view is rough, and mainly based on residue analyses made at plant maturity and harvest. Indeed, turnips, carrots, cabbages, white radish and potato planted or sown into chlorfenvinphos treated soil, contained generally at harvest very low residues ($\leqslant 0.02$ mg kg^{-1}) of chlorfenvinphos and compound 2 (Beynon et al., 1966, 1967, and 1968). However, during the first month that follows planting and soil treatment of spring and summer cauliflower and brussels sprouts crops, usually these are not attacked by the foliar insects like caterpillars. Is the chlorfenvinphos transported in low amounts from the soil to the foliage, where it there gives some protection against insects? In order to give some answer to this question, in the

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present work we studied the metabolism of chlorfenvinphos, applied onto soil around the stem of the plant at planting, in both soil and plant during the plant growth.

MATERIALS AND METHODS

Cauliflower crops (cv. Alpha-Balanza; planting at the 4-6 leaves stage; plant interdistance 50x50 cm) were made in Belgium in 1988 at St Katelijne-Waver (Research Station for Vegetables; sand 72.3%, silt 22.3%, clay 5.4%, organic matter 3.2%, pH 5.8, soil classified as loamy sand), Opdorp (sand 75.1%, silt 14.9%, clay 10.0%, organic matter 3.0%, pH 6.1, soil classified as sandy loam), and Gembloux (School for Horticulture; sand 10.5%, silt 74.5%, clay 15.0%, organic matter 2.1%, pH 5.9, soil classified as silt loam). Chlorfenvinphos was applied just after planting, except at Gembloux where the treatment (26-6-88) was made 5 days after planting (21-6-88). 50 mg of chlorfenvinphos/plant were applied onto the soil by pouring around the stem of the plant an emulsion of Birlane 25EC (Shell; 25 g% of chlorfenvinphos) in water. At the spring, one cauliflower crop was made at St Katelijne-Waver, and one at Opdorp; in the summer, two cauliflower crops were made at St Katelijne-Waver, one at Opdorp, and one at Gembloux.

One brussels sprouts crop (cv. Acropolis; planting at the 4-6 leaves stage; plant interdistance: 50 cm in the line, 65 cm between the lines) was done in Belgium in 1988 at Staden (sand 60.2%, silt 29.4%, clay 10.4%, organic matter 2.7%, pH 5.8, soil classified as sandy loam). Chlorfenvinphos was applied just after planting (17-5-88) for the treatment in line, and 7 days (24-5-88) after planting (17-5-88) for the treatment at the foot. For the foot treatment, 50 mg of chlorfenvinphos/plant were applied by pouring onto the soil around the stem of the plant the dry granulates of Birlane 10G (Shell; 10 g% of chlorfenvinphos). For the treatment in line, 100 mg of chlorfenvinphos/metre were applied onto the soil along the planting line, using granulates of Birlane 10G.

In each field, there were 4 replicates; one analysis of soil and of plant was made in each replicate. Soil sampling: 1. treatment at the foot: soil was taken in the soil half sphere of 10--12 cm of radius around the stem of the plant; 2. treatment in line (only for the brussels sprouts at Staden): soil was taken with a borer in the planting line in the 0-10 cm superficial soil layer; nothing was detected at greater depth. Sampling of the leaves: aliquot of leaves were taken on all the plant; the largest part of the leaf-stems was thrown avay, the concentrations being measured mainly in the limb tissues of the leaves. At harvest, an aliquot of 1 kg of the "flower" of cauliflower and 1 kg of brussels sprouts self were taken.

The analytical procedure already described was followed with minor changes (Rouchaud et al., 1988b). Standard of chlorfenvin-

phos (1) was prepared from Birlane WP (Shell; 25 g% of chlorfenvinphos); compound 2 to 8 were obtained from Janssen Chimica, Belgium. Thin layer chromatography (t.l.c.) was performed using silicagel plates (Merck); the analyzed solution was applied as a band; the standards were applied on a part of the t.l.c. plate. Chlorfenvinphos and its metabolites 2 to 5 were analyzed as such by gas liquid chromatography (g.l.c.). Metabolites 6, 7 and 8 were methylated with diazomethane before g.l.c. analysis. Chlorfenvinphos was detected using a flame photometric detector. Compounds 2 to 8 were detected by electron capture. Injection and detection at 250°C, glass column 1.80x2mm i.d. filled with 5% SE30 on 80-100 mesh Gas Chrom Q, nitrogen carrier gas at 80 ml/min. Metabolite, oven temperature, retention time: 1, 200°C, 4.2 min; 2, 150°C, 2.3 min; 3, 125°C, 3.6 min; 4, 160°C, 3.5 min; 5, 160°C, 1.8 min; 6 (methylated), 140°C, 2.2 min; 7 (methylated) 140°C, 3.3 min; 8 (methylated), 140°C, 4.5 min. Several times, compounds 1, 2, 6, 7 and 8 were analyzed by mass spectrometry (m.s.). When separated on t.l.c., they were scraped, extracted with ethyl acetate, this was concentrated and analyzed by m.s. (without methylation of acids 6-8).

Soil (100 g) was extracted with acetone+water 8+2 v/v (200 ml) with stirring and heating to reflux during 30 min. The extraction was repeated. The extracts were gathered after filtration, water (100 ml) was added, the mixture was concentrated to 130 ml, saturated with NaCl, extracted two times with methylene chloride (2x200 ml). This was dried with Na_2SO_4, concentrated and applied on a first t.l.c. plate. Elution with ethyl acetate gave a band at Rf=1.0 containing the compounds 1-5. The band was separated, extracted with ethyl acetate, concentrated, and applied on a second t.l.c. plate. Elution with chloroform gave (metabolite, Rf): 2 and 3, 1.0; 4, 0.72; 5, 0.45; 1, 0.25. A band containing metabolites 2+3 was isolated; an other large one was isolated which contained metabolites 1+4+5. The silicagel was extracted, concentrated, and analyzed by g.l.c. and m.s.

The acetone+water extracted soil was extracted with NaOH 1 N in water (200 ml) with stirring and heating to reflux during 30 min. After centrifugation, the supernatant was made acid with HCl, the mixture was extracted with ethyl acetate; this was dried (Na_2SO_4) and concentrated, and applied on a t.l.c. plate. Elution with 2-propanol+33% ammonia+water 7+1.5+3 v/v/v separated the acid metabolites (metabolite, Rf): 6: 0.82; 7, 0.68; 8, 0.32. The bands were separated, extracted with ethyl acetate, methylated with diazomethane and analyzed by g.l.c. and, occasionally, by m.s.

The plant (50 g) was cut into small pieces. These were homogenized with acetone+water 8+2 v/v using an omnimixer. The mixture was heated to reflux (30 min, stirring), filtered, and the solid was extracted again. The extracts, and the solid residue left by the extraction were analyzed further in the same way as with soil. The recoveries in soil and in plant of chlorfenvinphos and of its metabolites were 62-105%.

RESULTS AND DISCUSSION

In the soil of the crops, chlorfenvinphos was transformed into the non insecticide compounds 2 to 8 (Table 1; Rouchaud et al., 1988b). Compounds 2, 6, 7 and 8 were the major soil metabolites.

The soil half life time of chlorfenvinphos for the spring cauliflower crops was 1.7 times greater at St Katelijne-Waver (20 days) than at Opdorp (12 days). For the summer cauliflower crops, the soil half life time of chlorfenvinphos was 1.2 times greater at Gembloux (22 days), 1.3 times greater for the first crop at St Katelijne-Waver (24 days), and 1.6 times greater for the second crop at St Katelijne-Waver (28 days), than at Opdorp (18 days). The history of the fields, as to the number of years during which previously continuous cauliflower crops (including their soil insecticide treatments) were made onto it, were the following: 1. field of the second summer crop at St Katelijne--Waver: 1 year; 2. the same field of both the spring crop and of the first summer crop at St Katelijne-Waver, and the field at Gembloux: 2 years; 3. the same field of the spring and of the summer crops at Opdorp: 8 years. Soil half life time of chlorfenvinphos thus was the smallest as the history of the field was greater. A similar relationship was already observed during the assays made in the previous years (Rouchaud et al., 1988b). For the soil concentrations of the total of chlorfenvinphos+all its metabolites, the differences due to the soil history were levelled.

In the plant foliage, chlorpyrifos (1) and only compounds 2 and 6 were observed (Table 1). Concentrations of the other chlorfenvinphos metabolites in the foliage were equal or lower than 0.02 mg kg^{-1}, the limit of the analytical sensitivity. In the foliage of the spring cauliflower cultivated at St Katelijne-Waver, the concentration of chlorfenvinphos fastly increased with time, reaching 1.5 mg kg^{-1} 15 days after treatment; then, it progressively decreased, reaching 0.2 mg kg^{-1} at the end of the crop. The weight of chlorfenvinphos present in the foliage of one spring cauliflower grown at St Katelijne-Waver changed in the same way during the crop; however, the maximum (600 µg $plant^{-1}$) was reached only 1.4 month after the treatment, when the weight of the aerial part of the plant was about 1 kg. This total weight was the result of the absorption by plant of chlorfenvinphos and of its metabolites from the soil, and to the biodegradation of these compounds self by the plant; the weight of insecticide compounds absorbed by the plant decreased, when the soil concentrations of these compounds decreased. The concentrations of chlorfenvinphos in the plant foliage were the result of the same phenomenons; to these was added the growth of the plant foliage which diluted the concentrations of the xenobiotics. In the spring cauliflower crop, the chlorfenvinphos concentrations in the foliage were lower at Opdorp (maximum 1.0 mg kg^{-1}) than at St Katelijne-Waver (maximum 1.5 mg kg^{-1}). This could be explained by the fact that the soil concentrations of the insecticide

Table 1. Concentrations of chlorfenvinphos and of its metabolites in the soil and in the plant of cauliflower and brussels sprouts crops.

Days after treatment	Foliage weight/plant, g[a]	Chlorfenvinphos and metabolites (as equivalents of chlorfenvinphos) concentrations in soil (mg kg-1 dry soil) and in plant foliage (mg kg-1 fresh weight)[b]							µg of 1 in the foliage of one plant
		1	2	3	6	7	8	Total	
1. Spring cauliflower crops. 1.1. Crop at St Katelijne-Waver.									
a. Soil concentrations:									
0		25.8	n.d.	n.d.	n.d.	n.d.	n.d.	25.8	
8		21.1	0.7	0.2	0.4	n.d.	n.d.	22.6	
15		16.2	2.1	0.2	2.1	0.8	n.d.	21.5	
22		11.2	2.2	0.3	3.7	2.3	0.5	20.4	
30		9.8	3.1	0.2	2.5	1.5	0.1	17.5	
43		6.3	2.6	0.1	2.4	2.2	0.5	14.2	
58c		3.5	2.4	0.2	2.7	2.3	0.9	12.1	
Soil half life time, days:		20						57	
Planting+treatment: 5-5d; total rainfall during crop: 102 mm.									
b. Plant foliage concentrations:									
0	30								
8	40	0.9	n.d.		n.d.				36
15	100	1.5	0.1		0.2				150
22	250	1.2	0.2		0.1				300
30	500	0.8	0.07		0.04				400
43	1200	0.5	n.d.		n.d.				600
58c	2400	0.2	n.d.		n.d.				480
1.2. Crop at Opdorp. a. Soil concentrations:									
1		23.1	n.d.	n.d.	n.d.	n.d.	n.d.	23.1	
8		16.6	1.6	0.2	2.1	1.6	n.d.	22.2	
16		9.3	3.1	0.4	3.9	3.2	0.1	20.3	
29		5.8	4.7	0.3	3.5	2.9	0.8	18.4	
44		1.2	4.3	0.4	4.3	3.5	0.9	14.9	
58c		0.7	2.5	0.2	4.2	2.4	1.1	11.3	
Soil half life time, days:		12						53	
Planting+treatment: 19-5d; total rainfall during crop: 131 mm.									
b. Plant foliage concentrations:									
1	25								
8	30	0.7	n.d.		n.d.				21
16	50	1.0	0.2		0.1				50
29	200	1.0	0.1		0.2				200
44	700	0.4	0.04		n.d.				280
58c	1600	0.2	n.d.		n.d.				320
2. Summer cauliflower crops. 2.1. First summer crop at St Katelijne-Waver. a. Soil concentrations:									
0		23.6	n.d.	n.d.	n.d.	n.d.	n.d.	23.6	
23		14.5	2.3	0.1	2.1	1.6	0.2	20.9	
30		11.7	2.1	0.1	1.8	1.4	0.1	17.3	
37		8.1	3.4	0.2	2.6	1.9	0.8	17.2	
54		4.9	3.1	0.1	2.5	1.9	0.7	13.4	
69c		4.2	2.5	0.1	1.7	1.2	0.4	10.2	

Days	Foliage, g[a]	Concentrations, mg kg^{-1} [b]							µg of
		1	2	3	6	7	8	Total	1
t1/2		24						58	
Planting+treatment: 28-7[d]; total rainfall during crop: 131 mm.									
b. Plant foliage concentrations:									
0	20								
23	219	1.3	0.2		n.d.				285
30	450	0.9	0.2		0.1				405
37	900	0.6	0.1		0.1				540
54	2500	0.2	n.d.		0.03				500
69c	2600	0.1	n.d.		n.d.				260
2.2. Second crop at SKW. a. Soil concentrations:									
0		26.3	n.d.	n.d.	n.d.	n.d.	n.d.	26.3	
9		21.2	1.3	n.d.	n.d.	n.d.	n.d.	22.5	
16		18.5	1.5	0.2	1.1	0.7	n.d.	22.1	
24		13.3	3.7	0.1	1.3	0.6	0.1	19.4	
41		10.2	3.8	0.2	1.7	1.1	n.d.	17.3	
56		7.1	3.2	0.1	1.6	1.0	0.2	13.4	
73c		3.8	2.7	0.2	2.8	0.5	0.7	10.9	
t1/2		28						65	
Planting+treatment: 10-8[d]; total rainfall during crop: 186 mm.									
b. Plant foliage concentrations:									
0	20								
9	30	1.1	n.d.		n.d.				33
16	40	1.7	0.1		n.d.				68
24	136	1.6	0.2		n.d.				218
41	800	0.7	0.05		0.1				560
56	1800	0.4	n.d.		0.2				720
73c	2300	0.3	n.d.		n.d.				690
2.3. Crop at Opdorp. a. Soil concentrations:									
0		24.9	n.d.	n.d.	n.d.	n.d.	n.d.	24.9	
9		17.5	2.2	0.1	2.1	1.3	n.d.	23.3	
16		14.0	2.8	0.2	3.3	1.1	0.3	21.8	
24		9.5	4.5	0.4	3.5	2.0	0.5	20.7	
41		6.2	4.8	0.2	4.2	1.9	0.6	18.1	
56		2.3	4.4	0.3	4.7	2.0	0.4	14.4	
73c		1.9	2.8	0.1	3.2	2.2	1.0	11.3	
t1/2		18						69	
Planting+treatment: 10-8d; total rainfall during crop: 186 mm.									
b. Plant foliage concentrations:									
0	10								
9	12	0.7	n.d.		n.d.				8
16	22	0.8	0.1		n.d.				18
24	31	1.1	0.1		n.d.				34
41	450	0.7	0.2		0.1				315
56	800	0.4	n.d.		0.06				320
73c	1300	0.3	n.d.		n.d.				390
2.4. Crop at Gembloux. a. Soil concentrations:									
0		23.9	n.d.	n.d.	n.d.	n.d.	n.d.	23.9	
20		13.5	2.7	0.2	2.1	1.2	n.d.	19.8	
31		10.3	3.1	0.3	2.6	1.4	0.2	18.1	
47		5.6	3.6	0.2	2.5	1.2	0.2	13.5	
60c		3.9	3.2	0.2	2.8	1.2	n.d.	11.5	
t1/2		22						55	

Days	Folia-ge, g[a]	Concentrations, mg kg-1 [b]							µg of
		1	2	3	6	7	8	Total	1
Planting+treatment: 26-6[d]; total rainfall during crop: 152 mm.									
b. Plant foliage concentrations:									
0	30								
20	70	n.m.	n.m.		n.m.				n.m.
31	300	1.1	0.2		0.1				330
47	1100	0.6	0.03		0.06				660
60[c]	1950	0.3	n.d.		n.d.				585
3. Brussels sprouts crops at Staden. 3.1. Treatment at the foot.									
a. Soil concentrations:									
0		24.9	n.d.	n.d.	n.d.	n.d.	n.d.	24.9	
18		18.6	1.8	0.1	1.2	0.6	n.d.	22.4	
32		12.3	2.7	0.2	1.9	1.0	0.2	18.5	
49		10.3	2.4	0.1	1.7	0.8	0.2	15.7	
60		5.9	3.1	0.1	2.2	1.1	0.7	13.3	
84		3.7	1.9	0.2	2.5	1.2	0.4	10.1	
99		2.1	1.8	0.2	2.7	1.1	0.4	8.5	
116[c]		1.3	1.6	0.1	2.6	1.4	0.5	7.6	
t1/2		32						72	
Planting+treatment: 24-5[d]; total rainfall during crop: 313 mm.									
b. Plant foliage concentrations:									
0	30								
18	50	0.9	0.2		0.1				45
32	500	1.5	0.3		0.2				750
49	1100	0.8	0.1		0.1				880
60	1300	0.7	0.02		n.d.				910
84	2000	0.4	n.d.		n.d.				800
99	2400	0.3	n.d.		n.d.				720
116[c]	2950	0.1	n.d.		n.d.				295
3.2. Treatment in the line. a. Soil concentrations:									
0		15.3	n.d.	n.d.	n.d.	n.d.	n.d.	15.3	
7		13.8	1.8	n.d.	n.d.	n.d.	n.d.	15.6	
25		11.2	1.2	0.1	0.7	0.3	n.d.	13.5	
39		6.9	1.9	0.1	2.6	0.9	0.3	12.8	
56		5.8	1.4	0.1	1.8	0.7	0.1	10.1	
67		3.9	1.1	0.1	1.9	1.1	0.6	8.7	
91		1.9	0.8	0.1	2.1	0.8	0.7	6.5	
106		2.2	0.9	0.1	1.6	1.1	0.3	6.2	
123[c]		0.8	0.6	0.1	1.5	1.3	0.2	4.5	
t1/2		35						76	
Planting+treatment: 17-5[d]; total rainfall during crop: 316 mm.									
b. Plant foliage concentrations:									
0	25								
7	30	0.5	n.d.		n.d.				15
25	50	0.7	0.1		n.d.				35
39	500	1.3	0.2		0.1				650
56	1100	0.7	0.08		0.2				770
67	1300	0.6	n.d.		0.04				780
91	2000	0.4	n.d.		n.d.				800
106	2400	0.3	n.d.		n.d.				720
123[c]	2950	0.1	n.d.		n.d.				295

Footnotes of Table 1:
a. Weight of the aerial part of the plant (stem+leaves), but without the "flower" of the cauliflower, and without the brussels sprouts themselves. b. 1=chlorfenvinphos; 2=2,4-dichlorophenacyl chloride; 3=2,4-dichloroacetophenone; 4= α-(chloromethyl)-2,4--dichlorobenzyl alcohol; 5=1-(2',4'-dichlorophenyl)-ethan-1-ol; 6=2,4-dichlorobenzoic acid; 7=2-hydroxy-4-chlorobenzoic acid; 8=2,4-dihydroxybenzoic acid. 1. Soil concentrations. 1.1. For the foot treatment: concentrations in the soil half sphere of 10-12 cm radius around the stem of the plant. 1.2. For the treatment in line (only for the brussels sprouts at Staden): concentrations in the planting line in the 0-10 cm soil layer. Nothing was detected at lower soil depth. Soil concentrations of compounds 4 and 5 were between 0 (not detected) and 0.2 mg kg^{-1}. 2. Plant foliage concentrations: concentrations in the leaves limb, as the main part of the leaves stems were thrown away; metabolites 3, 4, 5, 7 and 8 were not detected in the plant foliage. Means of 4 replicates. n.d.=not detected. n.m.=not measured. c. Cauliflower and brussels sprouts harvests. d. Day-month, year 1988.

substances were lower at Opdorp that at St Katelijne-Waver. The same occurred for the maximum total weight of chlorfenvinphos in the foliage per plant: 320 and 600 μg $plant^{-1}$ respectively at Opdorp and at St Katelijne-Waver. The soil biodegradation of the insecticide, which was faster at Opdorp than at St Katelijne-Waver, and which was mainly due to the greater history of the field at Opdorp, thus generated insecticide concentrations both in the soil and in the plant foliage which were lower at Opdorp than at St Katelijne-Waver.

In the foliage of the brussels sprouts plants (maximum 1.5 mg of chlorfenvinphos kg^{-1}) the decrease of the chlorfenvinphos concentration, during the weight increase of the plant, was less fast than for the summer cauliflower crops at St Katelijne-Waver. The concentrations of chlorfenvinphos in the foliage of the brussels sprouts treated in line were somewhat lower than the ones of the plants treated at the foot.

In the 'flower' of cauliflower, and in the brussels sprouts themselves, the concentrations of chlorfenvinphos and of the metabolites 2 and 6 were lower than 0.02 mg kg^{-1}, the limit of the analytical sensitivity. The lack of residues in the 'flower' of cauliflower and in the brussels sprouts themselves -at the opposite of what was observed in the plant foliage- should be related to the physiological differences as to the plant tissues, and as to the kinetics of their formation. Indeed, the 'flower' of cauliflower and the brussels sprouts themselves appear at the plant maturity, i.e. when the xenobiotic residues are at their lowest concentrations in both the plant foliage and the soil. Such differences as to the residue concentrations in the diffe-

rent plant parts are well known, for instance in the straw and in the grain of cereals, and in the root and in the leaves of the sugar beet (Rouchaud and Meyer, 1982); concentrations in the grain and in the root are much lower -and frequently not detected- than in the straw and in the foliage.

The results obtained here thus indicated that chlorfenvinphos was absorbed from the soil by the plant, and transported into the foliage. During the period of about 1 month which followed planting and soil insecticide treatment, the chlorfenvinphos concentrations in the foliage were around 1.0 mg kg^{-1}. At the end of that period of time, the weight of the aerial part of the cauliflower and brussels sprouts plants were around 200 and 500 g, respectively. During that period of time, the insecticide concentration in the foliage should protect the plant against the foliage insects.

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