Bull. Environ. Contam. Toxicol. (1991) 46:705–712
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Environmental Contamination and Toxicology

Influence of Slurry and Ammonium Nitrate Fertilizations on Soil and Plant Metabolism of Chlorpyrifos in Field Cauliflower

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Chlorpyrifos [0,0-diethyl-0-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate] (1) (Dursban) is applied onto soil at the stem of the cauliflower plant at plantation, in the field, for plant protection against the root fly. During crop, chlorpyrifos was soil metabolized -mainly by the soil microbial activity- into the oxon insecticide analog [0,0-diethyl-0-(3,5,6-trichloro-2--pyridinyl)phosphate] (2), and into 3,5,6-trichloro-2-pyridinol (3) whose insecticide activity is about 20% of that of chlorpyrifos (Rouchaud et al., 1989a). Chlorpyrifos (1) was transported from soil into the plant foliage, where it could give to the young plant protection against the foliage insects during a certain period of time. When the field history as to cauliflower monoculture and insecticide treatments was long, the rate of chlorpyrifos soil metabolism could be enhanced. The hypothesis was made of the adaptation of the soil microbial fauna to the insecticides. On the other hand, the increase of the rate of insecticide soil metabolism could lower the efficiency of the plant protection against soil insects.

Looking for agricultural and horticultural factors which could change the rate of insecticide soil metabolism, in the present work we studied the influence of the soil fertilization with slurry, onto the rate of chlorpyrifos soil metabolism in cauliflower field crop. Slurry fertilization indeed is very frequently applied in North Europe, during the spring, a short time before cauliflower plantation in the field. At first sight, one should think that slurry would enhance the soil microbial activity, and thus increase the rate of insecticide soil metabolism.

MATERIALS AND METHODS

A cauliflower crop (cv. Ravella) was planted in 1990 on a field (100 m x 20 m) located at Elverdinge (Yper, Belgium; sand 54.9%, silt 34.5%, clay 10.6%, pH 6.28, organic matter 1.43%, sandy loam type soil). The plat and uniform field was divided into 5 plots (20 m x 20 m, each) onto which one of the following fertilizer was applied on 19-4-1990: 1. 100 tons of

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pig slurry/ha (pig slurry composition -% relative to fresh material- was: dry matter 9.4, organic matter 6.9, N 0.5, P 0.08, K 0.54, Ca 0.15, Mg 0.07; pH 7.1); 2. 50 tons of the same pig slurry/ha; 3. 50 kg of inorganic nitrogen/ha (185 kg of ammonium nitrate/ha, containing 27% of nitrogen); 4. 300 kg of nitrogen /ha (1111 kg of ammonium nitrate/ha); 5. control plot, onto which no fertilizer was applied. Each plot was divided into 4 replicate sub-plots (20 m x 5 m, each). On 21-4-1990, the 0-13 cm soil layer was harrowed, and thereafter the cauliflower (4-6 leaves stage) were planted (plant interdistance of 67.5 cm x 60 cm). On 25-4-1990, the plants of all the plots were treated against the root fly, by pouring onto soil around the plant foot the emulsion in water (100 ml/plant) of Dursban EC (48 g% of chlorpyrifos) at the rate of 0.1 g chlorpyrifos/plant. At several dates during crop, soil and plant samples were taken. At each sampling date, one soil sample was taken in each sub-plot, and it was analyzed separately two times. Thus there were 8 separate soil analyses at each sampling date, for each fertilizer plot; the same was done for the plant foliage. Soil was taken for sampling in the soil half sphere of 10 cm radius around the stem of the plant, thus at at a soil depth of 0-10 cm; nothing was detected at lower depth. For the plant foliage, the leaves limbs were analyzed, as the main parts of the leaves stems were thrown away. At harvest, 1 kg of the 'flower' of cauliflower was taken in each sub-plot.

The analytical procedure already described was followed with minor changes (Rouchaud et al., 1989a). T.l.c. was carried out using silicagel Merck 60F254 plates, 20 x 20 cm, 0.2 mm thick. The analyzed solution was applied as a band. The standards were applied on another part of the t.l.c. plate.

Chlorpyrifos and compound 2 were analyzed as such by g.l.c. using the Tracor 550 apparatus. Compound 3 was methylated before the g.l.c. analysis. Injection at 230°C, detection at 180°C. Glass column 1.8 m x 2 mm i.d., 3% OV17 on Gas Chrom Q 80-100 mesh, nitrogen carrier gas at 40 ml/min. Compound, column temperature, retention time, detection (FPD=flame photometry in the phosphorus mode; EC=electron capture); chlorpyrifos, 190°C, 3.9 min, FPD; compound 2, 200°C, 3.4 min, FPD; methylated compound 3, 130°C, 4.3 min, EC.

The mass spectra (m.s.) were recorded using the VG Micromass 7070F spectrometer at 70 eV. For one analysis out of eight (i.e. for at least one of both the analyses made into one of the 4 replicate sub-plots at each sampling date), chlorpyrifos and its metabolites, extracted from soil and plant, were analyzed by m.s.

The mixture of soil (100 g) and acetone/water 8:2 (v/v) (200 ml) was heated under reflux (stirring; 15 min), filtered, and the solid was extracted again in the same way. The filtrates (which contained chlorpyrifos and compound 2) were combined water (150 ml) was added, the acetone was evaporated in a va-

cuum rotary evaporator (30°C), NaCl and dodecylsulfate (0.3 g) were added to the aqueous phase, this was extracted two times with methylene chloride (2 x 200 ml); the methylene chloride solution (solution 1) was dried with anhydrous sodium sulfate, concentrated to 40 ml in a vacuum rotavapor, and to 0.5 ml by means of a slow current of nitrogen (20°C), and was applied as a band onto the t.l.c. plate. Elution with hexane/ethyl acetate 100:13 (v/v) gave the bands of compound 2 (Rf=0-0.1) and of chlorpyrifos (Rf=0.76). The bands were scraped off separately, extracted with ethyl acetate (40 ml) in a small glass column, the ethyl acetate was reduced to 0.1 ml, and analyzed by g.l.c. and, for one analysis out of eight, by m.s.

In order to extract compound 3, the soil -already extracted by acetone/water- was extracted by a solution (150 ml) of 3 g% KOH in water (heating to reflux; 15 min; stirring). The cooled mixture was centrifugated, the liquid was brought to pH 2 by means of hydrochloric acid, and extracted two times with ethyl acetate (2 x 200 ml); the ethyl acetate solution (solution 2) was dried, concentrated, and applied onto a t.l.c. plate. Elution with hexane/ethyl acetate/acetic acid 100:70:5 (v/v/v) gave the band of compound 3 (Rf=0.81), which was separated, and extracted with ethyl acetate; this was concentrated to 5 ml, an ethereal solution of diazomethane was added, the volume was reduced to 0.1 ml, and the final solution was analyzed by g.l.c. and, for one analysis out of eight, by m.s. Plants were analyzed in the same way as soil; before extraction however, the plant was cut into small pieces.

At the 0.1 mg/kg dry soil level, the recoveries of chlorpyrifos and compounds 2 and 3 in soil were respectively 83-95, 82-96 and 75-88%. At 0.1 mg/kg fresh weight of plant, the same recoveries in plant were respectively 85-99, 83-95 and 72-84%. Recovery assays, made separately with each of the chlorpyrifos and compounds 2 and 3, indicated that one compound did not generate another during the analytical procedure.

RESULTS AND DISCUSSION

Regression analysis showed that there was a linear relationship between the logarithms of the chlorpyrifos soil concentrations against the number of days following chlorpyrifos soil treatment (Figure 1). There were two distinct periods during crop: the first 40 days dry period, followed by the second 30 days rainy period. Indeed, during the first 40 days crop period, only 22% of the total cumulative rainfall during the whole crop, had fallen. The rates of chlorpyrifos soil metabolism were greater during the rainy period, than during the dry one.

The chlorpyrifos soil concentrations were always greater in the slurry treated plots, than in the non-fertilized ones (control) (Table 1). The effect was greater when the slurry application rate on the field was greater (100 tons versus 50 tons of slurry

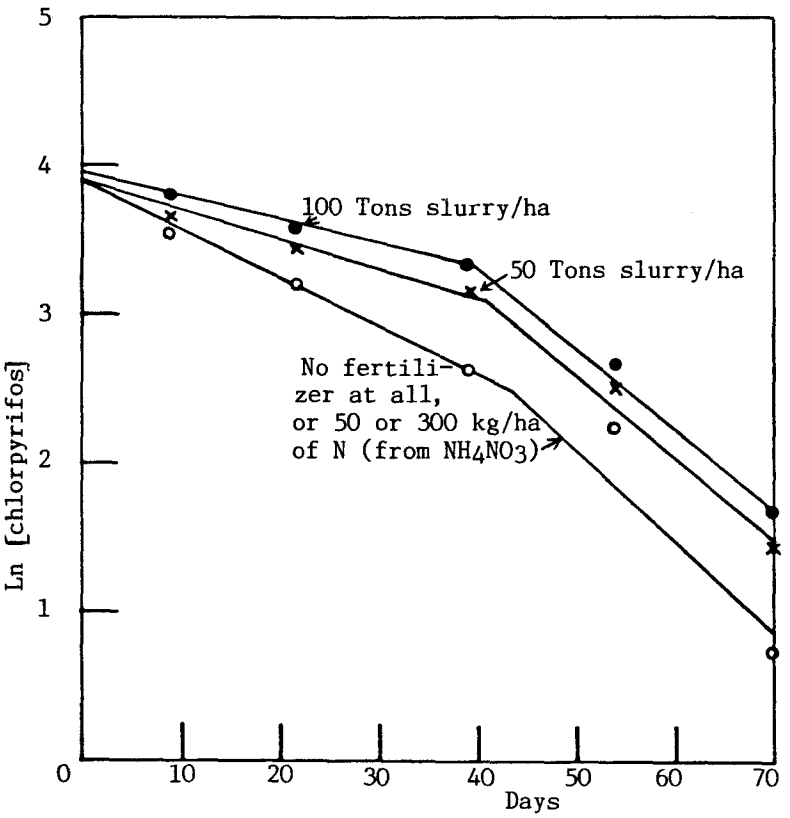

Figure 1. Correlation lines between the naperian logarithms of the chlorpyrifos soil concentrations (mg kg^{-1} dry soil) against time (days) following chlorpyrifos soil treatment. Linear regression calculations were made using separately each of the replicate soil concentrations measured at each sampling date in each of the four replicate sub-plots; soil concentration in each replicate sub-plot was the mean of two analyses. Points indicated in the figure correspond to the mean values of the chlorpyrifos soil concentrations from the 4 replicates at each sampling date. For each of both the dry and rainy crop periods, respectively fertilizer treatment (crop plot), correlation coefficients, y intercept, slope (i.e. the first order rate constant k, day^{-1}), chlorpyrifos soil half-life (days): 1. 100 tons of slurry/ha: dry crop period: -0.9848, 3.93, $-15.3\ 10^{-3}$, 45.3; rainy period: -0.9932, 5.53, $-54.9\ 10^{-3}$, 12.6. 2. 50 Tons/ha: dry period: -0.9707, 3.89, $-19.4\ 10^{-3}$, 35.7; rainy period: -0.9887, 5.43, $-56.1\ 10^{-3}$, 12.4. 3. 50 Kg of nitrogen/ha: dry period: -0.9918, 3.86, $-30.7\ 10^{-3}$, 22.6; rainy period: -0.9552, 5.25, $-61.4\ 10^{-3}$, 11.3. 4. 300 Kg of nitrogen/ha: dry period: -0.9831, 3.88, $-31.7\ 10^{-3}$, 21.9; rainy period: -0.9196, 5.14, $-59.8\ 10^{-3}$, 11.6. 5. No fertilizer at all: dry period: -0.9941, 3.88, $-32.2\ 10^{-3}$, 21.6; rainy period: -0.9341, 5.17, $-60.5\ 10^{-3}$, 11.5.

Table 1. Soil and plant concentrations of chlorpyrifos (1) and its metabolites 2 and 3 in the cauliflower crop, field grown on soil fertilized either with pig slurry, or with ammonium nitrate; each of these fertilizers was applied alone and separately at different rates.

Days after 1 treatment a	Concentrations of chlorpyrifos and of its metabolites (as equivalents of chlorpyrifos) in the soil (mg/kg dry soil) and in the foliage (mg/kg fresh weight)b						µg of 1+2 in foliage/plant
	Soil			Plant			
	1	2	3	1	2	3	
1. 100 Tons of slurry/ha:							
0	51.3±2.6	nd	nd				
9	44.2±2.1	1.3±0.1	1.7±0.1	3.1±0.2	0.2±0.1	0.1±0.1	165
22	35.3±1.8	2.1±0.1	6.4±0.3	3.6±0.2	0.3±0.1	0.3±0.1	700
39	28.3±1.5	3.7±0.2	7.2±0.4	1.9±0.1	0.3±0.1	0.2±0.1	1430
54	14.6±0.7	3.9±0.2	7.4±0.4	0.3±0.1	nd	nd	480
70	5.2±0.3	1.9±0.1	3.4±0.2	0.1±0.1	nd	nd	240
Soil half-life time: 1: 45 days; 1+2+3: 54 days							
2. 50 Tons of slurry/ha:							
0	52.8±2.6	nd	nd				
9	37.7±1.8	3.2±0.2	5.0±0.3	2.9±0.1	0.2±0.1	0.1±0.1	155
22	31.4±1.6	3.5±0.2	6.2±0.3	3.1±0.2	0.3±0.1	0.2±0.1	610
39	23.8±1.3	3.4±0.2	5.5±0.3	1.8±0.1	0.2±0.1	0.1±0.1	1300
54	12.8±0.6	3.1±0.2	5.3±0.3	0.2±0.1	nd	nd	320
70	4.2±0.2	1.6±0.1	3.7±0.2	0.1±0.1	nd	nd	240
Soil half-life time: 1: 36 days; 1+2+3: 49 days							
3. 50 Kg of nitrogen/ha, using ammonium nitrate:							
0	50.3±2.6	nd	nd				
9	33.7±1.7	3.1±0.2	6.4±0.3	2.6±0.1	0.1±0.1	0.1±0.1	135
22	24.3±1.2	3.6±0.2	8.1±0.4	2.7±0.1	0.2±0.1	0.2±0.1	520
39	14.7±0.8	3.3±0.2	7.8±0.4	1.5±0.1	0.1±0.1	0.1±0.1	1040
54	9.8±0.4	1.3±0.1	3.1±0.2	0.1±0.1	nd	nd	160
70	2.2±0.1	0.3±0.1	2.5±0.1	0.1±0.1	nd	nd	240
Soil half-life time: 1: 23 days; 1+2+3: 38 days							
4. 300 Kg of nitrogen/ha, using ammonium nitrate:							
0	47.4±2.4	nd	nd				
9	34.8±1.6	1.9±0.1	5.0±0.3	2.3±0.1	0.2±0.1	0.2±0.1	125
22	27.6±1.5	3.1±0.2	7.1±0.4	2.5±0.1	0.2±0.1	0.1±0.1	490
39	13.2±0.7	4.5±0.2	9.1±0.5	1.7±0.1	0.1±0.1	0.1±0.1	1170
54	10.8±0.5	0.7±0.1	3.2±0.2	0.2±0.1	nd	nd	320
70	2.1±0.2	0.4±0.1	2.1±0.1	0.1±0.1	nd	nd	240
Soil half-life time: 1: 22 days; 1+2+3: 44 days							
5. Control: no fertilizer at all:							
0	48.7±2.4	nd	nd				
9	35.3±1.8	1.2±0.1	4.8±0.2	2.5±0.1	0.2±0.1	0.1±0.1	135
22	25.6±1.4	3.7±0.2	7.3±0.4	2.6±0.1	0.3±0.1	0.2±0.1	520
39	13.6±0.6	3.2±0.2	8.5±0.4	1.6±0.1	0.1±0.1	0.1±0.1	1110
54	10.2±0.5	0.9±0.1	3.8±0.2	0.2±0.1	nd	nd	320
70	2.1±0.1	0.2±0.1	2.8±0.1	0.1±0.1	nd	nd	240
Soil half-life time: 1: 22 days; 1+2+3: 41 days							

Footnotes of Table 1:
a. The dates (day-month-year) which corresponded to the number of days after chlorpyrifos soil treatment were (number of days after chlorpyrifos soil treatment, date, cumulative rainfall -mm- at that date, weight of the foliage of one plant, g, i.e. of the aerial part of the plant -stem+leaves, but without the 'flower' of the cauliflower): 0 day, 25-4-1990, 0 mm, 25 g; 9 days, 4-5-1990, 2 mm, 50 g; 22 days, 17-5-1990, 22 mm, 180 g; 39 days, 3-6-1990, 51 mm, 650 g; 54 days, 18-6-1990, 62 mm, 1600 g; 70 days, 4-7-1990 (cauliflower harvest), 100 mm, 2400 g.
b. Means of 4 replicates (see in Materials and Methods) $\pm$ s.d. nd=none detected. In soil, concentrations in the half sphere of 10 cm radius around the stem of the plant; nothing was detected at lower soil depth. In the plant foliage, concentrations mainly in the leaves limb, as the main part of the leaves stems were thrown away. 1=Chlorpyrifos; 2=0,0-diethyl-0-(3,5,6-trichloro-2--pyridinyl)-phosphate; 3=3,5,6-trichloro-2-pyridinol.

/ha). The actual chlorpyrifos soil half-life times were 45, 36, and 22 days, respectively in the plots fertilized with 100 or 50 tons of slurry/ha, and in the non-fertilized plots.

The slurry fertilization also increased the soil concentrations of the sum chlorpyrifos+metabolites 2+3, relative to the non-fertilized plots; the effect however was smaller than with the chlorpyrifos soil concentrations. The absorption of chlorpyrifos and of its metabolites 2 and 3 -onto the organic matter of the soil treated with slurry- thus especially stabilized the organophosphorus function toward hydrolysis.

It has been related that when the pesticides incorporated in soil were incubated in laboratory conditions, the rate of pesticide soil metabolism could be greater when the soil had been amended with organic fertilizers (manure, sewage sludge...). The organic fertilizer should increase the soil microbial activity and, simultaneously, the rate of pesticide soil metabolism. Indeed, Doyle et al. (1978) generally observed higher rates of $^{14}CO_2$ formation during the laboratory soil incubation of fourteen ^{14}C herbicides and insecticides, when soil was amended with sludge or manure (relative to unamended soil). Lichtenstein et al. (1982) also observed lower soil persistence for the fonofos and parathion insecticides -soil incubated in laboratory conditions- when the soil had been amended with cow manure or sewage sludge, than when the soil was unamended.

On the other hand, it has been reported that the pesticide soil persistence in laboratory and field conditions frequently was greater when the soil organic matter concentration was greater. The absorption of the pesticide onto the soil organic matter should stabilize the pesticide relative to its soil degradation. Indeed, soil persistence in the field of chlorinated hydrocarbon

insecticides was greater in muck soil (organic matter 40%), than in silt loam (organic matter 3.8%)(Lichtenstein and Schultz, 1959). In field conditions, the soil persistence of the herbicide 3,6-dichloropicolinic acid was directly related to the soil organic matter content (between 0.7 and 4.8%)(Pik et al., 1977).

In the present work, results suggest that the slurry soil fertilization increased the chlorpyrifos absorption onto the soil organic matter, and correspondingly increased the stability of chlorpyrifos relative to its soil metabolism; this effect should have been greater than the one corresponding to the possible increase -by the slurry- of the soil microbial activity (to which should have corresponded the increase of the rate of chlorpyrifos soil metabolism). Other possible effect with increased organic matter, is that organisms base this organic matter as an energy source, and so do not have to use the pesticide.

Results obtained in this work showed that the slurry soil fertilization maintained higher insecticide compounds soil concentrations during crop, relative to the unfertilized soil; that effect occurred most intensively during the first 1.5 months period of the crop, when the young plants were the most sensitive to the root fly. To that slurry fertilization effect should correspond a better protection efficiency of the plant against the root fly (Rouchaud et al., 1989b).

At cauliflower harvest, the 'local' soil concentrations (in the soil half-sphere of 10 cm radius around the plant stem) of chlorpyrifos and of its metabolites 2 and 3 were low in both the slurry fertilized plots and in the unfertilized ones. During the period of 3 weeks between cauliflower harvest and the start of the next crop, the soil residue concentrations still will further decrease, at the fast rate of chlorpyrifos soil metabolism observed during the second period of the crop. Moreover, before the next crop, ploughing in the 0-30 cm soil layer will dilute the remaining 'local' residues in the soil mass, so that the soil will actually be cleaned from residues at the beginning of the next crop.

Chlorpyrifos and its metabolites 2 and 3 concentrations in the plant foliage were greater for the plants grown on slurry fertilized soil, than for the ones grown on non-fertilized soil (Table 1). The increase of the chlorpyrifos absorption onto the soil organic matter -due to the slurry soil fertilization-, thus did not decrease the insecticide transport from soil into plant, and thus the disponibility of the insecticide toward the plant.

No residue of chlorpyrifos or of its metabolites 2 and 3 were observed in the flower of cauliflower at harvest, the limit of the analytical sensitivity being 0.02 mg/kg fresh weight.

On the other hand, ammonium nitrate soil fertilization had no significant influence on the rate of chlorpyrifos soil metabolism, and onto the chlorpyrifos transport from soil into the plant foliage (Table 1).

Acknowledgments. Supported by the Institut pour l' Encouragement de la Recherche Scientifique dans l' Industrie et l' Agriculture, IRSIA-IWONL, Belgium (Grant n°4868A).

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Received September 28, 1990; accepted October 15, 1990.