Pesticide Residues in Lettuce. 2. Influence of Formulations

Paolo Cabras,* Mara Gennari, Marco Meloni, Franco Cabitza, and Mario Cubeddu

The influence of the type of formulation (granulated and liquid) on the residual behavior of Ethiofencarb and its metabolites in lettuce was studied. Lettuces treated with granulated formulations contained no residues of active ingredient (AI) but only those of sulfoxide and sulfone metabolites. Otherwise, lettuce treated with liquid formulations contained residues of AI, the above-mentioned metabolites, and phenol sulfoxide. AI and phenol sulfoxide residues, determined via HPLC, were not detectable (<0.02 ppm) 3 and 1 days after the last treatment, respectively. The accumulation effect due to repeated treatments is evaluated. At harvest, only residues of sulfoxide and sulfone metabolites were found in all the experiments and were lower in the lettuce treated with granulated formulations than in those treated with the liquid ones. The great differences among maximum residue limits fixed by the European countries and the need of establishing a sole limit in the whole of Europe are discussed.

The aphid is one of the most dangerous phytophagouses known, not only for the importance of immediate damages but even more for those connected to its action as viral vector.

In horticulture, controlling these pests became particularly important because such cultures have to be eaten while fresh and can produce high profits. Thus, farmers frequently use insecticides to guarantee a high-quality standard of their product. On the other hand, repeated chemical treatments induce a progressive impoverishment of entomophagouses and a subsequent need of further treatments (Barbagallo, 1985). Hence, it becomes necessary to use active ingredients (AI) able to fight aphids without destroying the useful entomofauna. In this way conditions for progressive reduction of chemical treatment could be determined.

Ethiofencarb is an insecticide that possesses the above-mentioned characteristics together with absorption by roots and translocation to the aerial part of the plant. Here it is quickly converted to the sulfoxide and sulfone derivatives. These products retain insecticidal activity (Aharonson et al., 1979) and undergo further transformation to the corresponding phenolic derivatives, by hydrolysis of the carbamic acid group (Figure 1). Such properties make this AI particularly suitable for controlling different lettuce aphids, such as epigean (*Nasonovia ribis-nigri*, *Acyrthosiphon lactucea*, *Mizus persicae*, etc.) or radical (*Pemphigus bursarius*).

Only few data on degradative behavior of Ethiofencarb in lettuce are reported in the literature. West and Meier (1983) did not find detectable residues (<0.03 ppm) 7 days after the last treatment. In FAO/WHO reports of 1978 and 1979, data on residues are reported as the sum of Ethiofencarb and its sulfone and sulfoxide derivatives. Furthermore, they are related only to liquid treatments, and nothing is reported on granular treatments.

This work reports the results of several experiments carried out to evaluate the degradative behavior of Ethiofencarb and its metabolites, depending on the different formulations used.

EXPERIMENTAL SECTION

Materials and Methods. The trial was carried out in alluvial ground, on lettuce cv. Odessa, type cos. This type of lettuce was chosen because the residual content of the edible part is much higher than in type crisp lettuce when pesticides are sprayed; this was found to be due to its particular shape (Cabras et al., 1988).

Seeding was done on April 12, 1988, and transplantation on May 15, 1988, on double rows, 75 × 35 cm apart. A random-block scheme was used, with four replications, and each block measured 10 m^2 (20 m × 0.50 m) and contained 60 plants.

An irrigation system by microjet sprinkling, with daily water administration of 5–7 L/m^2, depending on evaporation, was used. During the whole experiment, it never rained.

Granulated (Croneton 10 Granulare) and liquid (Croneton) formulations were used. Treatment with granulated formulation were broadcast on May 31, 1988, by two different techniques: by localization along the row in doses of 15 kg/ha (G1) and double-strengthened (G1d) and on the entire field in doses of 30 kg/ha (G2) and double-strengthened (G2d). Treatments with liquid formulations, applied with portable mechanical sprayers, were done in the dose recommended by the manufacturer (0.15 L/hL; 10 hL/ha) and double-strengthened; in one experiment (Lr) it was repeated weekly four times, starting on June 7, 1988, while in another experiment (Ls) it was done once on June 21, 1988. Sampling started 7 days after treatments and was carried on weekly, for the experiments in which granulated formulation was used, whereas it was carried out 0, 1, 3, 7, and 8 days after treatment for those in which liquid formulation was used.

Each sample consisted of two to four tufts, depending on plant development; it was triturated, homogenized, and analyzed just after sampling.

Chemicals. The analytical standard of Ethiofencarb was purchased from Ehrenstorfer (Augsburg, FRG), and its metabolites were synthesized as described by Cabras et al. (1989). Acetonitrile and methylene chloride were HPLC-grade solvents (Carlo Erba, Milan, Italy), and water was distilled twice and filtered through a Milli Q apparatus (Millipore, Milan, Italy) before use.

Apparatus and Chromatography. Analyses were carried out on a HPLC Varian 5020, equipped with a Varian UV 100 variable-wavelength UV/vis detector, Rheodyne injector (loop 50 μL), and Hewlett-Packard 3390 A reporting integrator. An Erbasil 10 C_8/H (250 × 4.6 mm (i.d.), 10 μm, Carlo Erba) column, a flow of 1.0 mL/min, and an eluting mixture of water/acetonitrile (65:35, v/v) were used for the simultaneous determination of AI and its five metabolites. The same mixture, in a 70:30 ratio, was then used for best determination of low concentrations of sulfoxide and sulfone metabolites and in a 60:40 ratio when the only AI had to be detected.

The best wavelength for such simultaneous determinations was found to be 195 nm. Using these analytical conditions, we could detect concentrations down to 0.02 ppm. Standard curves were constructed (external standard method) by plotting peak areas vs concentrations. Good linearity was achieved for each compound in the range 0–2.5 ppm. Using the extraction procedure, by methylene chloride, and the HPLC method previously described

Istituto di Chimica Farmaceutica Tossicologica ed Applicata, Via Ospedale 72, 09100 Cagliari, Italy (P.C., M.M.), Istituto di Chimica Agraria, Via P. Giuria 15, 10126 Torino, Italy (M.G.), and Centro Regionale Agrario Sperimentale, 09100 Cagliari, Italy (F.C., M.C.).

0021-8561/89/1437-1405$01.50/0

Table I. Residues (ppm ± SD) of Ethiofencarb and Some of Its Metabolites on Lettuce after Liquid Repeated Treatments (Lr) in Single (S) and Double (D) Doses

days after treatment	tuft wt, g	sulfoxide	sulfoxide phenol	sulfone	Ethiofencarb
		Lr S			
0 pre	256 ± 30	1.49 ± 0.33	nd[a]	0.83 ± 0.04	nd
0 post	343 ± 58	2.14 ± 0.55	2.27 ± 0.42	0.92 ± 0.43	4.40 ± 0.82
1	509 ± 86	6.46 ± 1.37	nd	1.36 ± 0.20	0.83 ± 0.18
3	400 ± 47	4.34 ± 0.42	nd	0.86 ± 0.16	nd
7	500 ± 44	1.71 ± 0.50	nd	0.71 ± 0.33	nd
		Lr D			
0 pre	263 ± 33	3.33 ± 0.45	nd	0.96 ± 0.65	nd
0 post	283 ± 45	5.73 ± 1.80	7.34 ± 2.43	1.96 ± 0.37	15.96 ± 3.93
1	371 ± 46	18.38 ± 1.35	nd	3.96 ± 0.71	4.36 ± 1.05
3	355 ± 8	11.36 ± 1.38	nd	1.70 ± 0.60	nd
7	483 ± 60	3.55 ± 0.73	nd	1.14 ± 0.30	nd

[a] nd = not detectable.

Table II. Residues (ppm ± SD) of Ethiofncarb and Some of Its Metabolites on Lettuce, after One Liquid Treatment (Ls) in Single (S) and Double (D) Doses

days after treatment	tuft wt, g	sulfoxide	sulfoxide phenol	sulfone	Ethiofencarb
		Ls S			
0	96 ± 8	9.21 ± 2.82	8.77 ± 2.98	1.66 ± 0.94	6.02 ± 1.90
1	86 ± 23	12.79 ± 1.77	nd[a]	1.73 ± 0.44	1.62 ± 0.46
3	136 ± 12	4.60 ± 0.99	nd[a]	0.71 ± 0.14	nd
8	371 ± 83	0.71 ± 0.37	nd[a]	0.22 ± 0.05	nd
		Ls D			
0	90 ± 7	22.02 ± 3.48	14.48 ± 1.61	2.83 ± 0.22	8.53 ± 3.23
1	86 ± 24	22.54 ± 1.68	nd	2.56 ± 0.53	4.79 ± 0.42
3	136 ± 12	7.87 ± 2.87	nd	0.59 ± 0.09	nd
8	395 ± 43	2.30 ± 0.25	nd	0.48 ± 0.04	nd

[a] nd = not detectable.

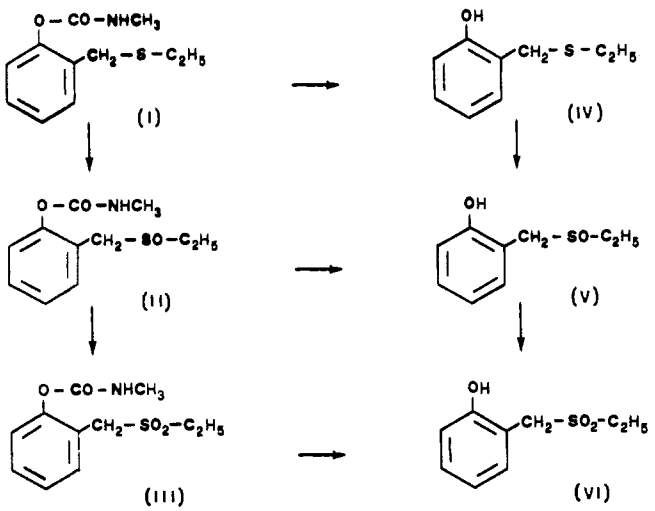

Figure 1. Ethiofencarb (I) and five of its metabolites: sulfoxide (II), sulfone (III), phenol (IV), phenol sulfoxide (V), phenol sulfone (VI).

(Cabras et al., 1989), we obtained quantitative recoveries of every product, in the concentration range used in the experiments.

RESULTS AND DISCUSSION

In all the tables we reported the residual data and the tuft average weight, in order to evaluate even the dilution effect due to the plant growth.

Treatment with Liquid Formulations. The sample, taken up after spraying, as soon as the plant was dried (about 1 h), contained residues of Ethiofencarb and three metabolites: sulfoxide, phenol sulfoxide, and sulfone (Tables I and II). Two other metabolites, phenol and phenol sulfone, detectable with this method, were not found. Since the presence of metabolites in high concentrations, just after treatment, could be due to their presence in the commercial formulation, analyses were carried out and showed that only Ethiofencarb and traces of its sulfoxide were contained.

Phenol sulfoxide, very concentrated in the first analysis (2.27–14.48 ppm), was completely degraded within 24 h; its parent compound was converted to the sulfoxide with a very fast degradation kinetic, as demonstrated by its metabolite content. In fact, in 1 day, sulfoxide increased its concentration by an amount proportional to that of the AI degradation. Ethiofencarb degradation was completed within 3 days, and no residue of AI above our limits of determination (0.02 ppm) was found, in any of the experiments.

The degradation of the main metabolite, sulfoxide, was slower, and, therefore, it must be evaluated considering the dilution effect due to the plant growth and its formation from AI.

The noteworthy difference in the residues found immediately after the last treatment, in experiments with single or repeated spraying, is due to the different weight of each tuft: The ratio among the average weight of the tufts is a factor of about 3, similar to that found for the corresponding residues.

Less important is the residual amount of sulfone, which degraded slower than its parent compound sulfoxide.

The incomplete degradation of metabolites at the treatment cadence (1 week) explains the accumulation effect registered when spraying were repeated. Thus, at harvest, the residual amount of these products was much higher in those lettuces that underwent repeated treatments than in those sprayed only once.

Treatment with Granulated Formulations. As shown in Table III, residues of sulfoxide and sulfone metabolites, but not of Ethiofencarb, were found in lettuces treated with granulated formulations. This is probably due to the degradation of the AI in soil that produces sulfoxide and sulfone derivatives that can be absorbed by plants and to an absorption of Ethiofencarb in such low concentra-

Table III. Residues (ppm ± SD) of Ethiofencarb and Some of Its Metabolites on Lettuce, after Ground Treatment in Granular Formulation, Localized (G1) and on the Entire Field (G2) (S represents the Dose Recommended by Manufacturers and D Its Double Strength)

days after treatment	tuft wt, g	sulfoxide	sulfoxide phenol	sulfone	Ethiofencarb
		G1 S			
7	14 ± 2	3.20 ± 1.01	nd[a]	0.89 ± 0.14	nd
14	35 ± 7	3.42 ± 1.75	nd[a]	1.53 ± 0.57	nd
20	100 ± 18	1.37 ± 0.25	nd[a]	1.09 ± 0.25	nd
27	166 ± 31	0.56 ± 0.29	nd[a]	0.91 ± 0.29	nd
34	509 ± 80	0.08 ± 0.03	nd[a]	0.15 ± 0.10	nd
		G1 D			
7	14 ± 2	5.60 ± 2.65	nd	0.97 ± 0.17	nd
14	30 ± 6	3.43 ± 1.12	nd	1.43 ± 0.41	nd
20	99 ± 11	2.66 ± 0.95	nd	1.51 ± 0.42	nd
27	144 ± 17	1.41 ± 0.70	nd	1.54 ± 0.34	nd
34	499 ± 56	0.61 ± 0.28	nd	0.54 ± 0.24	nd
		G2 S			
7	17 ± 1	3.50 ± 1.18	nd	0.84 ± 0.17	nd
14	39 ± 7	2.12 ± 1.32	nd	1.11 ± 0.41	nd
20	94 ± 10	0.67 ± 0.21	nd	0.52 ± 0.18	nd
27	169 ± 28	0.13 ± 0.08	nd	0.47 ± 0.09	nd
34	494 ± 99	0.04 ± 0.02	nd	0.22 ± 0.07	
		G2 D			
7	13 ± 1	6.41 ± 2.23	nd	1.12 ± 0.29	nd
14	36 ± 2	2.98 ± 0.46	nd	1.47 ± 0.13	nd
20	98 ± 12	1.74 ± 0.57	nd	1.27 ± 0.57	nd
27	172 ± 22	0.52 ± 0.12	nd	0.65 ± 0.29	nd

[a] nd = not detectable.

tions to be immediately metabolized by plant itself.

No significant differences in residual content were found among the experiments carried out with the different distribution techniques (localized/entire field).

The residual content at harvest was much lower in comparison to that found when liquid formulations were used. Also the sulfoxide to sulfone ratio was found to be different: With the liquid treatment the sulfoxide metabolite was much more concentrated whereas, with the granular one, the ratio is more or less equal to 1 or in favor of sulfone metabolite.

CONCLUSIONS

The maximum residual contents of Ethiofencarb allowed among European nations are so different that understanding the technical reasons for which they were determined is very difficult, even considering data reported in the literature and in this paper. In fact, the limits are 0.5 ppm in Italy, 1.0 ppm in Switzerland and Luxemburg, 2.0 ppm in France, 5.0 ppm in Sweden, and 10.0 ppm in West Germany (European Directory of Agrochemical Products, 1984).

If the residue is considered as the only AI residue, lettuces were within the limit of all mentioned nations 3 days after liquid treatment, because they were already completely degraded.

This kind of evaluation become quite different when the residue is considered as the sum of AI, sulfoxide, and sulfone residues, as indicated by FAO/WHO report (1978) for Ethiofencarb residue. In this case, only lettuces treated with granulated formulation (localized and entire field), using the doses recommended by manufacturers, contained residues lower than the above-mentioned legal limit for Italy. All other differently treated lettuces used for this work were found over this limit.

These considerations indicate that it is necessary to establish an unequivocal and clear method to determine compounds constituting the residue, in all countries, as indicated by FAO/WHO. Furthermore, it would be highly useful to have uniform legal limits in the European Countries, in that it should facilitate free commerce of goods. By establishing a sole limit in the whole of Europe, every overlimiting problem should be eliminated.

ACKNOWLEDGMENT

This work is supported by grants from Ministero dell'Agricoltura e Foreste, P.F. Lotta biologica ed integrata per la difesa delle piante agrarie e forestali, Gruppo residui.

Registry No. I, 29973-13-5; II, 53380-22-6; III, 53380-23-7; V, 53380-26-0.

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Received for review September 19, 1988. Accepted January 24, 1989.