

Pesticide Residue Levels in Peppers Grown in a Greenhouse after Multiple Applications of Pyridaben and Tralomethrin

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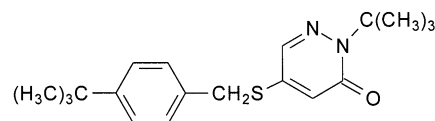
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Residue levels of pyridaben and tralomethrin were determined in peppers grown in an experimental greenhouse, during a 4 week period in which up to four successive treatments with both pesticides were applied. In all cases, plants were sprayed with a mixture of pyridaben and tralomethrin at application rates of active ingredients of 140 and 36 g/ha, respectively. Sampling was carried out at 1, 3, 7, 8, 10, or 14 days after each multiple application, simulating the typical harvesting practices in greenhouse plantations. Residue levels of pyridaben and tralomethrin were determined by using ethyl acetate extraction and GC-ECD. During the study, residue levels in the plantation ranged between 0.22 and 0.04 mg/kg for pyridaben and between 0.09 and 0.02 mg/kg for tralomethrin, with median values of 0.14 and 0.06 mg/kg, respectively. These values represent 28 and 600%, respectively, of the corresponding maximum residue limits currently established in Spain. The application of an intensive washing process to the pepper samples did not lead to a significant reduction of the residue level of either pesticide. Likewise, no significant differences were found between the residue levels in the “edible” and “inedible” parts of the peppers.

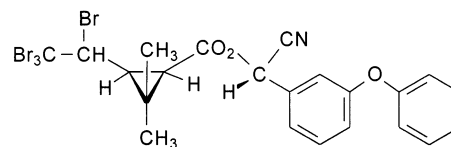
KEYWORDS: Pyridaben; tralomethrin; residues; peppers

INTRODUCTION

Pyridaben, 2-*tert*-butyl-5-(4-*tert*-butylbenzylthio)-4-chloropyridazin-3(2*H*)-one, is the common name of the relatively new acaricide/insecticide developed by Nissan Chemical Ind., Ltd. (1), which is effective for the control of Acari, Aleyrodidae, Aphididae, Cicadellidae, and Thysanoptera on field crops, fruit trees, ornamentals, and vegetables (2). On the other hand, tralomethrin, (*S*)- α -cyano-phenoxybenzyl(1*R*,3*S*)-2,2-dimethyl-3-[(*RS*)-1,2,2,2-tetrabromoethyl]cyclopropanecarboxylate, is a new nonsystemic pyrethroid insecticide discovered and introduced by Roussel Uclaf, which is effective for the control of a range of agronomic pests, particularly Lepidoptera, in cereals, fruits, vegetables, and other crops at application rates as low as 7.5–20 g of active ingredient (ai)/ha (2). The structures of pyridaben and tralomethrin are given in **Figure 1**. At present time, the literature on pyridaben and tralomethrin residues in foods is very sparse, but some papers describing analytical methods have been published (3–5). The only paper found in the open literature studying the behavior of some of these two pesticides in fruits and vegetables is that published by Cabras et al. (4), in which some data for pyridaben in clementine citrus are given.



Pyridaben



Tralomethrin

Figure 1. Molecular structures of pyridaben and tralomethrin.

In Spain, pyridaben is commercially available through Basf under the trade name Sanmite, whereas tralomethrin is commercially available from DuPont under the trade name Tracker (6), and both pesticides are already widely used on different vegetable crops as effective substitutes for some traditional pesticides whose use in Europe is being greatly restricted. Maximum residue limits (MRLs) for pyridaben and tralomethrin have been established in some European countries, such as Spain (7) and Italy (8), but MRLs are not yet set at the European

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Union level. MRLs established in Spain for pyridaben and tralomethrin in peppers are 0.50 mg/kg (7 day preharvest interval) and 0.01 mg/kg (3 day preharvest interval), respectively.

The objective of this work was to evaluate the degradation behavior and residue levels of pyridaben and tralomethrin in peppers grown in a plastic greenhouse using the typical horticultural practices of this type of plantation (i.e., multiple pesticide applications and short preharvest intervals). An additional objective in this work was to assess the influence of some household processing usually applied to the peppers before being consumed in raw (such as washing or elimination of some "inedible" parts) on the residue levels of pyridaben and tralomethrin in peppers produced with applications of both pesticides.

EXPERIMENTAL PROCEDURES

Chemicals and Apparatus. Acetone, ethyl acetate, cyclohexane, and anhydrous sodium sulfate (pesticide residue grade) were obtained from Panreac (Barcelona, Spain). Certified standards of pyridaben (99.6% purity) and tralomethrin (90.0% purity) were supplied by Riedel-de Haën (Seelze, Germany) and Dr. Ehrenstorfer (Augsburg, Germany), respectively. Individual stock standard solutions of pyridaben and tralomethrin were prepared in acetone. Standard solutions for gas chromatographic (GC) analysis were prepared by suitable dilution of the stock standard solutions with blank pepper extracts.

The gas chromatograph was a Varian model 3800 (Walnut Creek, CA) equipped with a model 1079 injection port, a model 8200 Cx autosampler, an electron-capture detector (ECD), and a DB-5MS fused-silica capillary GC column (J&W, Folsom, CA) of 30 m length, 0.25 mm internal diameter, and 0.25 μ m film thickness. The chromatographic conditions were as follows: detector temperature, 300 °C; injector temperature, 250 °C; oven temperature program, 1 min at 90 °C, 30 °C/min to 180 °C, 4 °C/min to 260 °C, and hold for 15 min; carrier gas (helium) flow rate, 1.2 mL/min; makeup gas (nitrogen) flow rate, 30 mL/min; injection volume, 1 μ L; and splitless time, 0.75 min. The retention times of pyridaben and tralomethrin in this column under these GC conditions were 28.9 and 37.8 min, respectively. A Varian Star 4.5 chromatography workstation was used for chromatographic data processing.

Greenhouse Plantation, Treatments, and Sampling. The study was conducted in a commercial greenhouse belonging to Hortamar S.C.A., located in Roquetas de Mar (Almeria, Spain). The greenhouse size was 0.50 ha, and the pepper planting density (variety Barbadillo) was ~20000 plants/ha. Residue levels of pyridaben and tralomethrin were determined in peppers of commercial size (110–130 g), during a period of time in which four consecutive treatments with both pesticides were applied to the plantation (treatments I, II, III, and IV), with intervals of 7 or 8 days between each application. In all cases, pepper plants, receiving routine horticultural treatment, were sprayed with an application mixture containing 0.7 g/L Sanmite (pyridaben 20%) and 1 mL/L Tracker (tralomethrin 3.6%) at application rates of 140 g of pyridaben/ha and 36 g of tralomethrin/ha. Treatments I, II, and III were applied to the whole plantation, whereas treatment IV was applied to only half of the greenhouse. Samples were collected at 8 days after treatment I (sample I+8); at 1, 3, and 7 days after treatment II (samples II+1, II+3, and II+7); at 1, 3, 7, 8, 10, and 14 days after treatment III (samples III+1, III+3, III+7, III+8, III+10, and III+14); and at 1, 3, 7, and 14 days after treatment IV (samples IV+1, IV+3, IV+7, and IV+14). In all cases, the greenhouse samples consisted of 40 pieces of peppers taken at random from the whole plantation, or from the corresponding half of the greenhouse (for those samples collected after the fourth treatment). The days of treatment and sampling are summarized in **Table 1**. Note that samples I+8, II+7, and III+7 were collected just before treatments II, III, and IV, respectively, were applied.

The daily maximum/minimum/medium temperatures inside the greenhouse throughout the study ranged between 25/12/17 and 37/19/

Table 1. Days of Treatments and Sampling

day	treatment	samples collected
0	I	
8	II	I+8
9		II+1
11		II+3
15	III	II+7
16		III+1
18		III+3
22	IV	III+7
23		III+8 ^a /IV+1 ^a
25		III+10 ^a /IV+3 ^a
29		III+14 ^a /IV+7 ^a
36		IV+14 ^a

^a Samples collected from the corresponding half of the greenhouse.

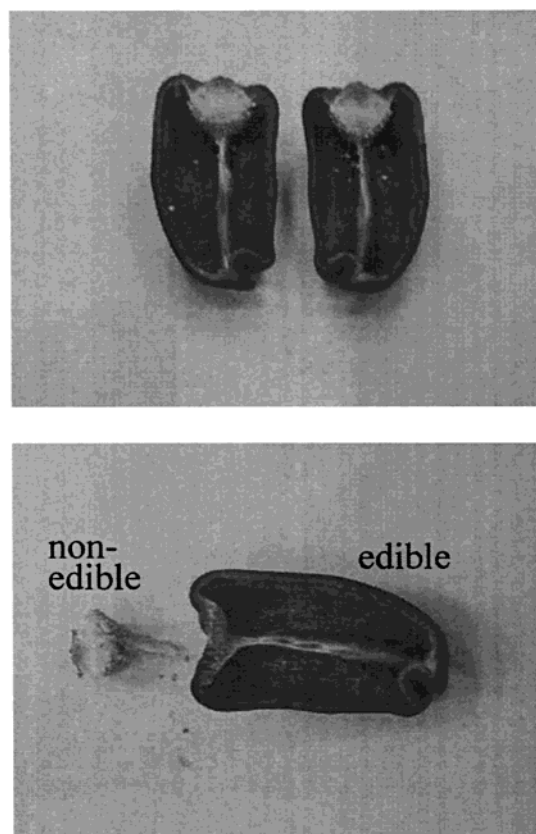


Figure 2. Edible and inedible parts of the peppers.

25 °C, whereas the daily maximum/minimum/medium relative humidity inside the greenhouse ranged between 85/42/64 and 98/87/93%.

Sample Preparation, Processing, and Analysis. Immediately after picking, the greenhouse samples were put into polyethylene bags and transported to the laboratory, where they were divided into four identical subsamples containing 10 pieces of pepper each. Two of these four subsamples ("unprocessed" samples A and B) were directly chopped and thoroughly mixed. On the other hand, before being chopped and mixed, the 10 pieces of pepper from the third subsample were intensively washed with tap water and further dried with absorbent paper ("washed" sample). Finally, each piece of pepper from the fourth subsample was divided into an "edible" part and an "inedible" part (see **Figure 2**), and then the edible parts sample and the inedible parts sample were chopped and thoroughly mixed separately. As shown in the scheme of **Figure 3**, after the sample preparation and processing procedure above-described had been applied, five different samples were obtained from each greenhouse sample. Immediately after chopping, all of these samples were kept deep-frozen until analysis. In all cases,

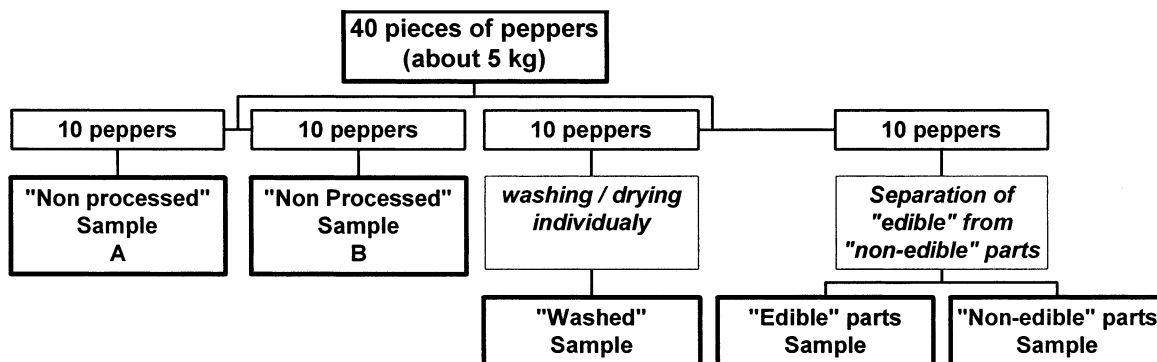


Figure 3. Sample preparation and processing scheme.

Table 2. Pyridaben and Tralomethrin Recoveries

matrix	pyridaben		tralomethrin	
	recovery (%)	RSD (%)	recovery (%)	RSD (%)
whole peppers	88	13	118	21
edible parts	90	10	100	13
inedible parts	92	8	87	7

<90 min passed between harvest and storage in the freezer, analyses being always carried out between 24 and 48 h after chopped samples had been stored in the freezer.

Extraction of pyridaben and tralomethrin residues in pepper samples was carried out according to a modification of the ethyl acetate/GC multiresidue extraction method developed by the Swedish National Food Administration for fruits and vegetables (5, 9). A brief description of the extraction procedure is as follows: Weigh 37.5 g of thoroughly homogenized sample and blend with 100 mL of ethyl acetate and 20 g of anhydrous sodium sulfate for 5 min. Filter the solvent phase through a glass fiber filter with a 10 g sodium sulfate layer, and dry the filtrate by shaking with 5 g of sodium sulfate. Transfer 25 mL of the ethyl acetate layer to a 100 mL round-bottom flask and concentrate to ~2 mL on rotary vacuum evaporator at 37 °C. Transfer the concentrate quantitatively to a graduated test tube, and adjust the volume to 5 mL with ethyl acetate and then to 10 mL with cyclohexane. Filter the extract through a 0.45 μm microfilter by suction with a 10 mL syringe. The extracts so obtained, which contained 0.94 g sample/mL, were analyzed by GC-ECD using the operating conditions described above.

During the study, a number of quality control recovery tests were conducted on pepper samples previously analyzed and demonstrated not to contain any residues of pyridaben or tralomethrin. In total, 10 recovery tests were performed on whole-pepper samples at spiking levels ranging from 0.05 to 0.46 mg/kg for pyridaben and from 0.01 mg/kg to 0.11 mg/kg for tralomethrin. In addition, three recovery tests on both edible part pepper samples and inedible part pepper samples were performed at spiking levels of 0.46 mg/kg for pyridaben and 0.11 mg/kg for tralomethrin. In all cases, recoveries were calculated using analytical standards prepared in extracts of the corresponding blank samples.

RESULTS AND DISCUSSION

Mean recovery values and the corresponding relative standard deviations (RSD) obtained for pyridaben and tralomethrin in the recovery tests performed during the study are indicated in **Table 2**. These values can be considered acceptable according to the validation and quality control criteria recently established for pesticide residue analysis (10, 11). Pyridaben and tralomethrin residue levels determined in all of the pepper samples analyzed during the study are indicated in **Tables 3** and **4**, together with the maximum, minimum, median, and mean values obtained for each type of sample. **Figure 4** shows a typical

Table 3. Pyridaben Residue Levels, in Milligrams per Kilogram, Determined in the Pepper Samples Analyzed during the Study

sample	unprocessed		washed	edible parts	inedible parts
	A	B			
I+8	0.10 ^a	0.10 ^a			
II+1	0.22	0.22	0.10	0.11	0.10
II+3	0.23	0.22	0.20	0.21	0.18
II+7	0.13	0.13	0.12	0.10	0.12
III+1	0.16	0.12	0.17	0.09	0.17
III+3	0.17	0.14	0.16	0.13	0.11
III+7	0.10	0.04	0.07	0.06	0.06
III+8	0.10	0.11	0.12	0.11	0.11
III+10	0.08	0.06	0.09	0.08	0.08
III+14	0.05	0.03	0.03	0.04	0.06
IV+1	0.18	0.25	0.16	0.11	0.16
IV+3	0.21	0.17	0.13	0.14	0.08
IV+7	0.18	0.20	0.13	0.12	0.08
IV+14	0.16	0.12	0.07	0.12	0.10
max level	0.23	0.25	0.20	0.21	0.18
min level	0.05	0.03	0.03	0.04	0.06
mean	0.15	0.14	0.12	0.11	0.11
median	0.16	0.13	0.12	0.11	0.10

^a Not taken into account for statistics.

Table 4. Tralomethrin Residue Levels, in Milligrams per Kilogram, Determined in the Pepper Samples Analyzed during the Study

sample	unprocessed		washed	edible parts	inedible parts
	A	B			
I+8	0.03 ^a	0.03 ^a			
II+1	0.08	0.06	0.04	0.03	0.04
II+3	0.10	0.06	0.08	0.08	0.06
II+7	0.05	0.05	0.04	0.03	0.05
III+1	0.04	0.04	0.07	0.02	0.06
III+3	0.07	0.05	0.07	0.04	0.04
III+7	0.05	0.03	0.03	0.03	0.01
III+8	0.05	0.05	0.08	0.05	0.04
III+10	0.04	0.03	0.06	0.04	0.03
III+14	0.02	0.01	0.02	0.01	0.02
IV+1	0.07	0.10	0.09	0.04	0.05
IV+3	0.09	0.07	0.07	0.06	0.02
IV+7	0.07	0.09	0.08	0.05	0.02
IV+14	0.07	0.05	0.04	0.05	0.03
max level	0.10	0.10	0.09	0.08	0.06
min level	0.02	0.01	0.02	0.01	0.01
mean	0.06	0.05	0.06	0.04	0.04
median	0.07	0.05	0.07	0.04	0.04

^a Not taken into account for statistics.

chromatogram of the analysis of a positive finding (unprocessed sample III+1, B) and a control (a blank of pepper).

Unprocessed Peppers. The results obtained for the unprocessed samples A and unprocessed samples B were not signifi-

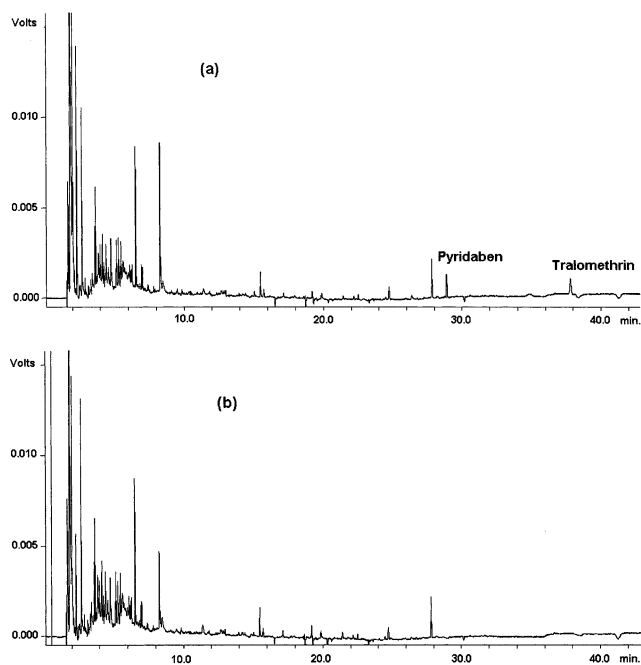


Figure 4. (a) Chromatogram of the analysis of the unprocessed sample III+1 (B); (b) chromatogram of the analysis of a blank of pepper.

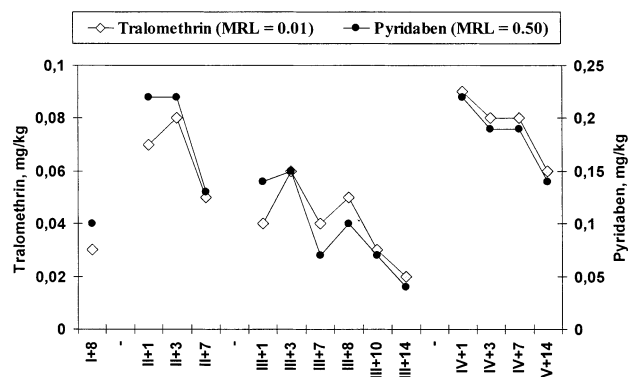


Figure 5. Residue levels of pyridaben (right scale) and tralomethrin (left scale) in unprocessed peppers (average of unprocessed samples A and B).

cantly different according to the paired t statistical test ($t = 1.20$ for pyridaben and $t = 1.64$ for tralomethrin, with 12 degrees of freedom and $P = 0.05$). As indicated in **Figure 5**, residue levels in the pepper plantation (mean of unprocessed samples A and B) ranged between 0.04 and 0.22 mg/kg for pyridaben and between 0.02 and 0.09 mg/kg for tralomethrin, the decline behaviors exhibited by both pesticides being very similar. Because pyridaben and tralomethrin were applied simultaneously in all cases and because the residue levels of both pesticides were determined on the same samples, this similarity in the decline behavior could indicate that (a) the way in which treatments were applied and the position in the plantation of the peppers collected on each sampling day are important factors at the time of defining the decline behavior of these pesticides; and (b) the differences between the physical–chemical properties of pyridaben and tralomethrin (2) are not great enough to define clearly differentiated decline behaviors.

In **Figure 5**, it can be seen that pyridaben residues in the pepper plantation were always below 50% of the Spanish MRL (0.50 mg/kg), including 1 day after the application of treatments II, III, and IV. Therefore, this MRL can be considered to be compatible with the horticultural practices usually applied in

the greenhouse pepper plantations, which include continuous harvesting during three or four months and the application of multiple treatments with the same active ingredient during the harvesting period.

Figure 5 also shows that tralomethrin residue levels in the pepper plantation were always above the Spanish MRL, which is currently fixed at the limit of determination (0.01 mg/kg). These results indicate that an MRL >0.10 mg/kg could be compatible with the use of this pesticide on peppers grown in a greenhouse. At this point, it is important to note that the GC multiresidue methodologies routinely applied in the analysis of pyrethroid pesticides in foods cannot distinguish between tralomethrin and deltamethrin, because tralomethrin is transformed into deltamethrin (in a reproducible way) in the injector port of the GC systems (5). This means that the chromatographic signal obtained for all of the pepper samples of this study could have also been quantified as deltamethrin using the corresponding deltamethrin standards. According to the tralomethrin/deltamethrin relative response factor obtained in our GC-ECD system (~ 0.6), the residue levels in the unprocessed peppers would have ranged between 0.01 mg/kg (III+14) and 0.05 mg/kg (IV+1) if they had been quantified as deltamethrin, and these levels are, in all cases, well below the MRL established for deltamethrin in peppers (0.20 mg/kg). To avoid this confusion, Valverde et al. (5) have already suggested the introduction of a change in the residue definition of both tralomethrin and deltamethrin.

Processed Peppers. Pyridaben and tralomethrin residue levels in the washed pepper samples and in the unprocessed peppers (average of samples A and B) were not significantly different ($t = 2.04$ for pyridaben and $t = 0.29$ for tralomethrin, with $df = 12$ and $P = 0.05$). Therefore, the application of an intensive washing to the peppers does not seem to reduce to any extent the residue levels of these two pesticides. The mean “processing factors” (12) calculated from the data presented in **Tables 3** and **4** for pyridaben and tralomethrin in peppers undergoing an intensive washing were 0.9 and 1.0, respectively. These results could be justified by the high K_{ow} (octanol/water partition coefficient) values presented by these two pesticides, which are $\sim 10^5$ – 10^6 (2). Because of their high liposolubility, pyridaben and tralomethrin can be quickly absorbed and strongly retained by the waxes of the pepper skin, their elimination by washing, including 1 day after the treatment, being impossible. “Washing factors” of ~ 1 can be found in the literature for other pesticide/crop combinations (13–15).

Finally, results in **Tables 3** and **4** for edible part samples and inedible part samples indicate that, for both pesticides, there are not significant differences between the residue levels in the edible and inedible parts of the peppers ($t = 0.07$ for pyridaben and $t = 0.74$ for tralomethrin, with $df = 12$ and $P = 0.05$). Therefore, we can conclude that the household processing usually applied to the peppers before being consumed raw does not reduce, significantly, the residue levels of pyridaben and tralomethrin.

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