Analysis of Buprofezin Residues in Vegetables. Application to the Degradation Study on Eggplant Grown in a Greenhouse†

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Three different multiresidue methods (the Mills, Luke, and Leary methods) have been assessed for their extraction of buprofezin residues from peppers, beans, and eggplants. Recovery studies were carried out on fortified samples, using capillary gas chromatography with nitrogen–phosphorus detection for buprofezin analysis in the vegetable extracts. Recoveries of greater than 81% with standard deviations of less than 11% were obtained in all instances, but the Leary procedure was more efficient than the Mills and Luke methods. On the other hand, residue levels and the degradation rate of buprofezin in eggplants grown in a commercial greenhouse have also been studied. Buprofezin residues were extracted by the Leary method, analyzed by GC-NPD, and confirmed by GC-FPD. The degradation rate constant and the half-life period obtained for buprofezin in eggplants were, respectively, 0.15 days^{-1} and 4.6 days.

INTRODUCTION

Buprofezin [2-[(1,1-dimethylethyl)imino]tetrahydro-3-(1-methylethyl)-5-phenyl-4*H*-1,3,5-thiadiazin-4-one) is the common name (ISO) of the insecticide recently introduced by Nihon Nohyaku Co., Ltd. (Tokyo, Japan) as code no. NNI-750 and trademark Applaud (Worthing and Hance, 1991). This compound is a thiadiazine insect regulator, molting inhibitor, that has a persistent larvicidal action against some Coleoptera and Hemiptera and effectively controls harmful insect pests including the brown rice planthopper *Nilaparvata lugens* and the greenhouse whitefly *Trialeurodes vaporariorum* (Funuyama et al., 1986; Michihino, 1989). It is already widely used all the world over, and in Spain it is mainly applied on citrus and greenhouse plantations of different vegetables; in fact, the Spanish legislature has established buprofezin maximum residue levels (MRL) only for tomato (1.00 mg/kg), citrus fruits and cucurbitaceae (0.20 mg/kg), and other vegetables (0.01 mg/kg) (Ministerio de Relaciones con las Cortes, 1989).

At the present time, the literature on buprofezin is very sparing, but some papers describing analytial methods have been published. Uchida et al. (1982) studied buprofezin soil adsorption and mobility in rice plants by gas chromatography with electron capture detection, and Funuyama et al. (1986) used thin-layer cochromatography and radioassay of [^{14}C]buprofezin to study its degradation in soils. Nevertheless, publications about buprofezin residue analysis are not found, except for an internal publication of Imperial Chemical Industries—Plant Protection Division, in which Atreya et al. (1984) describe a specific analytical method to determine buprofezin residues in crops by gas chromatography with a selective NP detector. It is interesting to note here that buprofezin was first included in *The Pesticide Manual*, published by the British Crop Protection Council, in the seventh edition (Worthing, 1983), but neither this edition nor the most recent one (Worthing and Hance, 1991) gives any information under the analysis section.

The objective of the present research was to assess the efficiency of multiresidue extraction methodologies for analysis of buprofezin residues in vegetables. Specifically, three methods have been assessed: the Mills method (Mills et al., 1963), the Luke method (Luke et al., 1975, 1981), and the Leary method (Leary, 1971).

At the present time, the multiresidue methods most commonly used for analysis of pesticides in nonfatty food are, doubtless, the Mills and Luke methods. These are considered official by AOAC for a number of pesticides (AOAC, 1984, 1990), and they are widely used in governmental monitoring programs to determine pesticide levels in food products and to trace possible residue tolerance level excesses (Reed et al., 1987; Carson, 1992; Andersson, 1986). The Leary extraction method was first developed for residue analysis of methamidophos, a very polar pesticide, but it has been recently tested as a multiresidue procedure (Andersson and Palsheden, 1991; Valverde et al., 1991); it is already used in the Swedish National Food Administration monitoring program of pesticide residues (Andersson and Bergh, 1991; Andersson, 1992).

In this paper, we have also assessed the efficiency of short Florisil columns to be applied to the cleanup step in buprefezin residue analysis. Likewise, this work has been completed with a degradation study of buprofezin residues on eggplants grown in a commercial greenhouse.

EXPERIMENTAL PROCEDURES

Reagents and Apparatus. (*a*) Standard buprofezin (purity = 99.0%) was supplied by ICI—Plant Protection Division (Bracknell, Berkshire, England). The other pesticide standards were obtained from Riedel de Haën (Seelze, Germany).

(*b*) Buprofezin standard solutions were prepared using petroleum ether (40–60 °C) as solvent.

(*c*) All of the solvents used were Sharlau, pesticide residue grade.

(*d*) Anhydrous sodium sulfate, Merck AR (99.5%), 12–60 mesh, was heated at 300 °C overnight.

(*e*) Florisil, 60–100 mesh, Baker analyzed reagent, was activated at 650 °C and reheated at 130 °C for 5 h before use.

(*f*) An Omni mixer, 400-mL maximum capacity, and a rotary vacuum evaporator Heidolph W-2000 were used.

(*g*) The gas chromatograph was a Hewlett-Packard 5890A with nitrogen–phosphorus detection (NPD), equipped with a HP-1 wide-bore fused silica capillary column (10-m length, 0.53-mm i.d., and 2.65-μm film thickness). A Perkin-Elmer 8600 gas chromatograph with flame photometric detection (FPD) in mode

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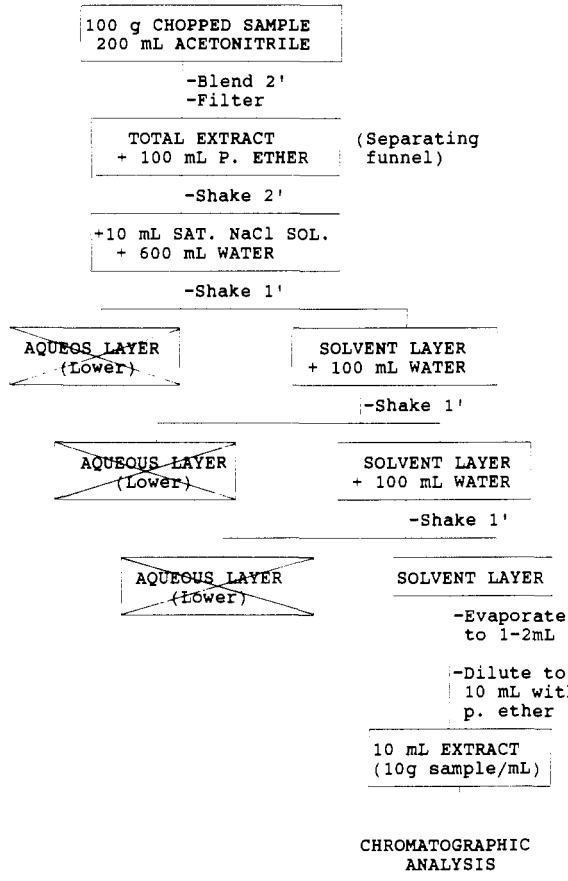

Figure 1. Scheme of the Mills extraction method.

Table I. NPD Gas Chromatograph Conditions

oven program	100 °C (1 min) → 30 °C/min → 160 °C (1 min) → 10 °C/min → 220 °C (1 min)
injector temp, °C	220
detector temp, °C	250
carrier gas flow (N_2), mL/min	15
split flow, mL/min	30
air flow, mL/min	70
H_2 flow, mL/min	3
auxiliary gas flow (N_2), mL/min	15
injection vol, μL	2

Table II. FPD Gas Chromatograph Conditions

oven program	150 °C (1 min) → 30 °C/min → 190 °C (0 min) → 5 °C/min → 230 °C (10 min)
injector temp, °C	240
detector temp, °C	300
carrier gas flow (N_2), mL/min	10
air flow, mL/min	100
H_2 flow, mL/min	60
injection vol, μL	2

S, equipped with a HP-1 wide-bore fused silica capillary column (30-m length, 0.53-mm i.d., and 2.65-μm film thickness) was also used.

Chromatographic Analysis. The chromatographic conditions used for analysis of buprofezin are given in Tables I and II. The NPD equipment was used for all quantitative determinations, whereas the FPD system was only used for confirmation purposes in the degradation study.

Extraction and Recovery Study. The three extraction methods used to carry out the recovery study of buprofezin residues from different vegetables were those described by Mills et al. (1963), Luke et al. (1975, 1981), and Leary (1971), with few modifications (Valverde and Gonzalez, 1989). Schemes of the extraction methods used are given in Figures 1, 2, and 3, respectively. When the Leary method was applied, the following short-column Florisil cleanup preceded the chromatographic

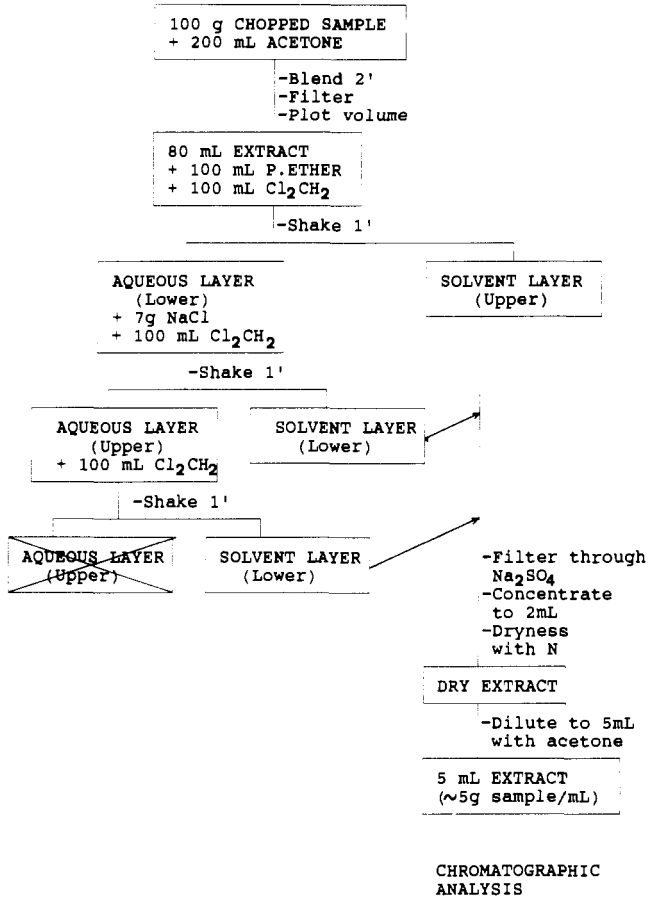

Figure 2. Scheme of the Luke extraction method.

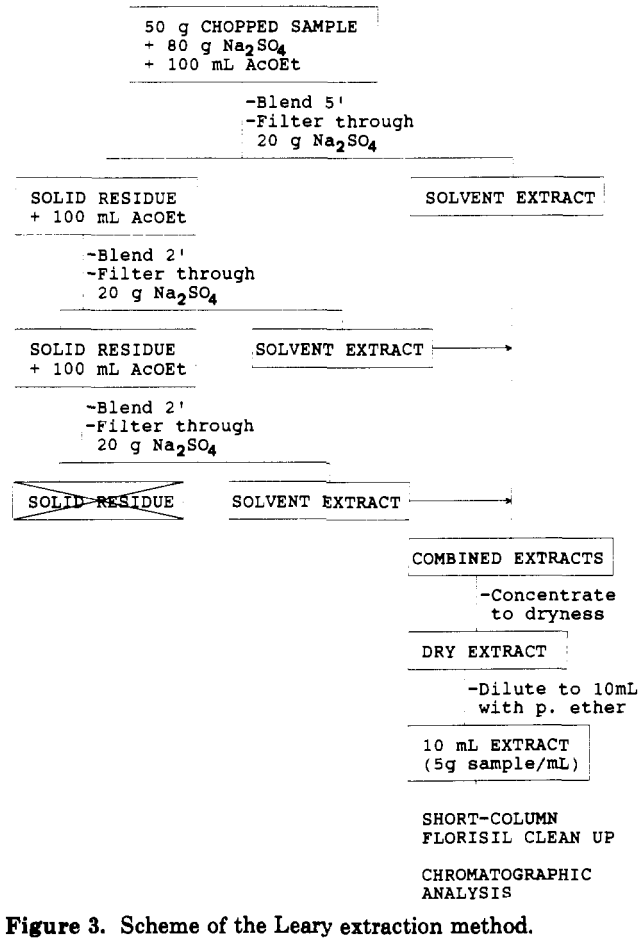

Figure 3. Scheme of the Leary extraction method.

Table III. Retention Time (RT), Relative Retention Time (RRT), and Relative Response Factor (RRF) Values of Buprofezin and Other Pesticides in the NPD Chromatographic Conditions

pesticide	RT, min	RRT[a]	RRF[a]
methamidophos	1.92	0.26	0.57
acephate	2.87	0.40	0.15
monocrotophos	4.32	0.60	0.32
dimethoate	4.68	0.64	1.37
chlorpyrifos-methyl	6.31	0.87	3.56
fenitrothion	6.77	0.93	0.96
malathion	7.07	0.97	0.77
chlorpyrifos	7.26	1.00	1.00
buprofezin	9.22	1.27	1.40
carbophenothion	10.19	1.40	0.88

[a] Reference: chlorpyrifos.

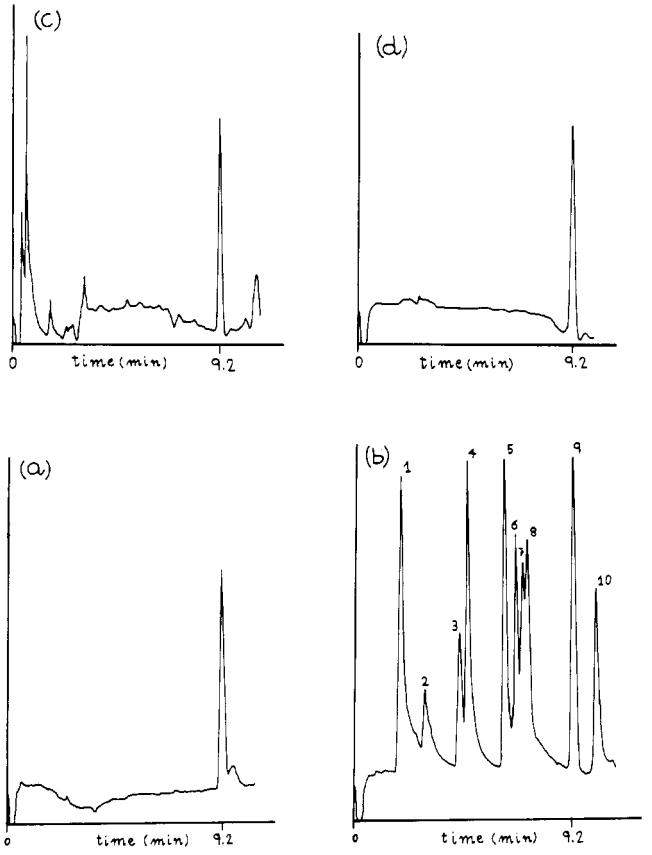

Figure 4. NPD chromatograms obtained for (a) a buprofezin standard solution of 0.50 mg/L; (b) a pesticide standard solution containing 2.0 mg/L methamidophos (peak 1), 2.0 mg/L acephate (peak 2), 1.7 mg/L monocrotophos (peak 3), 1.0 mg/L dimethoate (peak 4), 0.4 mg/L chlorpyrifos-methyl (peak 5), 1.0 mg/L fenitrothion (peak 6), 1.0 mg/L malathion (peak 7), 1.0 mg/L chlorpyrifos (peak 8), 1.0 mg/L buprofezin (peak 9), and 1.0 mg/L carbophenothion (peak 10); (c) extract of a fortified eggplant sample (0.1 mg/kg buprofezin), obtained by using the Leary method and before cleanup step; and (d) the same extract as in (c) but after cleanup stage.

analysis: Place 5 mL of sample extract on a glass column (2-cm i.d.) containing 1.1 g of Florisil (1-cm length) and 4.5 g of anhydrous sodium sulfate (1-cm length), and previously saturated with petroleum ether. Elute with 50 mL of petroleum ether solution containing 10% (v/v) of diethyl ether (5 mL/min flow), concentrate the eluate to 1–2 mL using a vacuum rotary evaporator (40–60 °C water bath), and dilute the extract to exactly 5 mL with petroleum ether in a volumetric flask.

The recovery study was carried out by fortifying fresh pepper, bean, and eggplant samples grown in greenhouse by Campos de Nijar SA (Almería, Spain), which were not treated with buprofezin. The fortification process of plant tissues was carried as follows (Valverde et al., 1991, 1993): Buprofezin standard solution

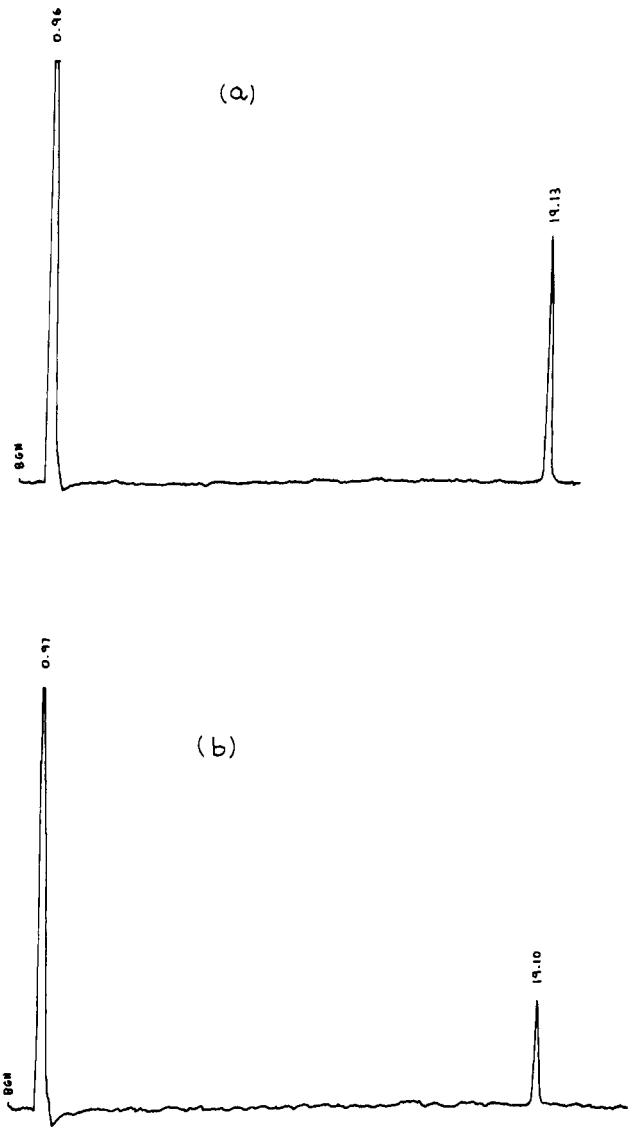

Figure 5. FPD chromatograms obtained for (a) a buprofezin standard solution of 0.5 mg/L and (b) an eggplant subsample from the degradation study, 4 days after treatment.

(10 mL) was added to 100 g of chopped sample in a high-speed blender jar (5 mL and 50 g, respectively, when the Leary method was assessed). After evaporation of the petroleum ether by an air stream, the sample was thoroughly mixed and homogenized. After 30 min, the sample was again homogenized and immediately analyzed. The Leary extraction method was assessed at three different fortification levels (0.02, 0.10, and 1.00 mg/kg), whereas the Mills and Luke methods were only assessed at a fortification level of 0.10 mg/kg. Three replicates of each recovery assay and three check samples of each vegetable were analyzed.

Degradation Study of Buprofezin Residues on Eggplants. The degradation experiment was conducted on eggplants (var. Madonna) in a commercial greenhouse belonging to Campos de Nijar SA, located in Níjar, 40 km northest of Almería. The greenhouse size and plantation density were, respectively, 135 × 48 m and 22 000 plants/ha.

Eggplants, receiving routine horticultural practices, were sprayed with Applaud 25 wp at a dose of 0.6 g/L (formulate product) and consumption of 900 L/ha, when the fruits had commercial size (about 300 g/piece). Three eggplants samples, 2 kg each, were collected at random from a sampling plot of 200 m^2 at 0, 2, 4, 7, and 14 days after application. As soon as the samples had been picked, they were stored into polyethylene bags and immediately driven to the laboratory, where they were chopped, blended, thoroughly mixed, and divided into three subsamples, which were kept deep-frozen until analysis (in all cases, no more than 90 min passed between harvest and storage

Table IV. Buprofezin Recoveries Obtained by Using the Mills and Luke Extraction Methods (Fortification Level = 0.10 mg/kg)

vegetable	recovery,[a] (SD) Mills method	Luke method
pepper	87.7 (7.0)	86.4 (9.9)
bean	85.8 (8.1)	82.4 (3.9)
eggplant	81.6 (2.2)	86.0 (5.8)

[a] Average of three replicates.

Table V. Buprofezin Recoveries Obtained by Using the Leary Extraction Method

fortification level, mg/kg	recovery,[a] % (SD) pepper	bean	eggplant
0.02	99.1 (9.1)	93.5 (10.5)	100.4 (11.4)
0.10	89.4 (10.0)	96.7 (8.0)	97.4 (6.1)
1.00	92.7 (6.3)	91.7 (3.4)	94.9 (5.5)

[a] Average of three replicates.

in the freezer). Buprofezin residues were extracted with the Leary method, and determined by GC-NPD, using the operating conditions indicated in Table I. The analysis was always carried out between 36 and 72 h after subsamples were stored in the freezer, and both matrix stability and buprofezin residue stability during the homogenization and storage procedures were previously tested on spiked samples. For confirmation purposes, one subsample of each sample was anlayzed by GC-FPD, using the operating conditions indicated in Table II.

RESULTS AND DISCUSSION

Analysis. Figure 4 shows the NPD chromatograms of a buprofezin standard solution of 0.50 mg/L (Figure 4a) and a pesticide standard solution containing methamidophos (2.0 mg/L), acephate (2.0 mg/L), monocrotophos (1.7 mg/L), dimethoate (1.0 mg/L), chlorpyrifos-methyl (0.4 mg/L), fenitrothion (1.0 mg/L), malathion (1.0 mg/L), chlorpyrifos (1.0 mg/L), buprofezin (1.0 mg/L), and carbophenothion (1.0 mg/L) (Figure 4b). Retention time values obtained for buprofezin and the other pesticides in the NPD chromatographic conditions are given in Table III, in which the relative retention times and relative response factors, using chlorpyrifos as reference, are also given. NPD response for buprofezin was linear between 0.2 and 10.0 ng, with a sensitivity of about 1.7×10^5 peak area units/ng.

On the other hand, Figure 5a shows the FPD chromatogram of a buprofezin standard solution of 0.5 mg/L. The buprofezin retention time obtained under the FPD conditions was 19.13 ± 0.05 min.

Recovery Study. Average recoveries obtained for buprofezin from pepper, bean, and eggplant with the Mills and Luke extraction methods are given in Table IV, whereas those obtained with the Leary extraction method are given in Table V. In all cases, average recoveries were greater than 81% with standard deviations of less than 11%, values that are generally considered satisfactory for residue quantification (Ambrus and Thier, 1986). However, the Leary method was the most efficient, with average recoveries ranging from 89% to 100% depending on type of vegetable and fortification level. These values are comparable with those obtained by Atreya et al. (1984) for buprofezin from tomato and cucumber samples, using the specific extraction method proposed by Imperial Chemical Industries for determination of buprofezin in crops.

The limit of quantification of the analytical method proposed to carry out the degradation study (Leary extraction method, short column Florisil cleanup, and NPD chromatographic analysis) was estimated as the lowest concentration actually tested and validated, that is to say, 0.02 mg/kg. The NPD chromatograms corresponding to the extract obtained by applying the Leary method to a fortified eggplant sample (0.10 mg/kg), are given in parts c (before cleanup step) and d (after cleanup step) of Figure 4.

Table VI. Residues of Buprofezin on Eggplant at Various Times after Application

t, days	buprofezin levels in subsamples, mg/kg sample 1	sample 2	sample 3	R, mg/kg (SD)
0	0.13/0.09/0.10	0.07/0.07/0.08	0.09/0.06/0.06	0.08 (0.02)
2	0.08/0.08/0.09	0.07/0.04/0.05	0.05/0.05/0.03	0.06 (0.02)
4	0.05/0.05/0.05	0.04/0.05/0.03	0.04/0.04/0.03	0.04 (0.01)
7	0.02/—/0.02	0.02/0.02/0.02	0.04/0.04/0.03	0.02 (0.01)
14	—/—/0.02	0.02/0.02/0.03	—/—/—	0.01 (0.01)

[a] R values are the means of triplicate analyses from three replicates.

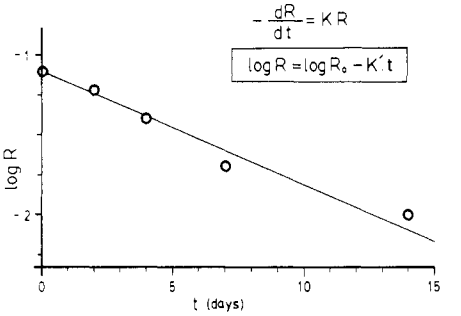

Figure 6. Degradation of buprofezin on eggplants.

Table VII. Statistical Quantities and Degradation Parameters Corresponding to the Degradation Study of Buprofezin on Eggplants

parameter	mean (±ci)[a]
degradation rate const, K, days^{-1}	0.15 (±0.05)
initial residue, R_0, mg/kg	0.08 (+0.04/−0.02)
coefficient of determ, r^2	0.9684
test quantity for correl, D	0.1059
half-life period, $T/2$, days	4.6 (±1.5)
tenth-life period, $T/10$, days	15.1 (±5.0)

[a] Confidence intervals at a level of significance of 95% ($p = 0.05$).

Degradation Study. Residue data found all along the degradation study of buprofezin on eggplants are given in Table VI, and Figure 5b shows the FPD chromatogram corresponding to a subsample from the degradation study, 4 days after treatment, in which a level of 0.04 mg/kg of buprofezin was determined by GC-NPD.

Statistical interpretation of buprofezin levels in the plantation was done by assuming that the degradation behavior of buprofezin residues can be described as a pseudo-first-order reaction and quantified by a linear semilogarithmic regression analysis (Timme and Frehse, 1980). As can be seen in Figure 6, mean residue levels in the plantation (R) at t days after treatment were fitted to the equation

$$\log R = \log R_0 - 0.434Kt$$

and the statistical quantities and degradation parameters given in Table VII were obtained.

The coefficient of determination (r^2) and the test quantity for correlation (D) values confirm that the degradation behavior of buprofezin residues on eggplant, under the experimental conditions studied, fits a pseudo-first-order reaction. The half-life ($T/2$) and tenth-life ($T/10$) periods were 4.6 and 15.1 days, respectively, and the residue level in the plantation at $t = 0$ (R_0) was 0.08 mg/

kg. According to these values and the maximun residue level established by the Spanish legislature for buprofezin on eggplant (0.01 mg/kg), we may conclude that a preharvest time about 14 days may be suitable for eggplants sprayed with buprofezin under the experimental conditions used in this study.

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