Behavior of Methamidophos Residues in Peppers, Cucumbers, and Cherry Tomatoes Grown in a Greenhouse: Evaluation by Decline Curves

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Residue levels of methamidophos were determined in peppers, cucumbers, and cherry tomatoes grown in commercial greenhouses, up to 6 weeks after being sprayed with Monitor (methamidophos 60%). Mathematically defined decline curves were established by determining optimal relationships between methamidophos residues and time, using different models. Model functions that best fit experimental data were 1st-order function for cucumber, 1.5th-order function for pepper, and 1st-order root function for tomato. However, in all cases, the 1st-order function was legitimized statistically. Half-life times determined from the optimal functions were 8.68 days (cucumber), 13.28 days (pepper), and 2.77 days (tomato), whereas half-life times determined from the 1st-order reaction function were 8.68 days (cucumber), 17.04 days (pepper), and 7.47 days (tomato). In this work, some experiments to determine residue levels of methamidophos in these vegetables after multiple applications were also carried out. The unexpected high residue levels found in all cases after five successive applications seem to indicate that methamidophos presents certain long-term accumulative effects in the three studied vegetables.

Keywords: *Methamidophos; residues decline curves; cucumber; pepper; tomato*

INTRODUCTION

Methamidophos (*O*,*S*-dimethyl phosphoramidothioate) is an organophosphorus compound with broad spectrum activity as an insecticide−acaricide. It was introduced by Chevron Chemical Co. and by Bayer AG (Tomlin, 1994), and its properties were first described by Hamman (1970). It is a highly active, systemic, residual insecticide−acaricide with contact and stomach action; its mode of action in insects and mammals is by decreasing the activity of acethylcholinesterase (Hussain, 1987).

Methamidophos is widely used all over the world for vegetables, corn, and other crops (Thomson, 1992), and its residues are one of the most commonly found by regulatory agencies in residue monitoring and total diet studies (Andersson et al., 1997; Food and Drug Administration, 1993). In Almería, a Spanish province where ∼2 million metric tons of vegetables was produced annually during the past three years, methamidophos has been also the most commonly found pesticide in residue monitoring carried out by the Spanish Ministry of Agriculture, Fisheries and Food (Herrera and Brotons, 1998; MAPA, 1997; Gamón, 1998).

The objectives of this work were to study the behavior of methamidophos residues in pepper, cucumber, and cherry tomatoes grown in commercial greenhouses under the particular climatic conditions of Almería and to assess the mathematical models proposed by Timme et al. (1986) to determine the statistical parameters that describe these processes.

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EXPERIMENTAL PROCEDURES

Reagents and Apparatus. Methamidophos standard was supplied by Riedel-de Haën (Seelze, Germany). Solvents (pesticide residue grade) and anhydrous sodium sulfate 12−60 mesh (99.5%) were obtained from Merck (Darmstadt, Germany).

The gas chromatograph was a Perkin-Elmer Model 8700 equipped with an HP1 wide-bore fused capillary column (30 m × 0.53 mm i.d. × 2.65 μm film thickness) attached to a nitrogen−phosphorus detector (NPD). The chromatographic conditions used for the analysis of methamidophos residues were as follows: detector temperature, 250 °C; injector temperature, 250 °C; oven temperature program, 2 min at 100 °C, 10 °C/min to 150 °C, hold for 1 min, 20 °C/min to 210 °C, hold for 6 min; carrier gas (nitrogen) flow rate, 20 mL/min; air flow rate, 150−160 mL/min; hydrogen flow rate, 1−3 mL/min; injection volume, 2 μL.

Decline Studies. Decline experiments were conducted in three commercial greenhouses belonging to Campos de Nijar S.A., located in Nijar, 40 km northeast of Almería. The greenhouse sizes and plantation densities were 0.52 ha, 17500 plants/ha (pepper); 0.55 ha, 16000 plants/ha (cucumber); and 0.38 ha, 20000 plants/ha (tomato). Pepper (variety Mazurca), cucumber (variety Brunex), and tomato (variety Cheresita) plants, receiving routine horticultural treatment, were sprayed with Monitor (60% methamidophos, w/v) at a dose of 1 mL/L and application rates of 507 g of active ingredient (ai) in 845 L/ha (pepper), 462 g of ai in 770 L/ha (cucumber), and 369 g of ai in 615 L/ha (cherry tomato).

The averages of the daily maximum/minimum temperatures outside and inside the greenhouses throughout the study were 20/10 and 25/10 °C, respectively, whereas the maximum/minimum absolute temperatures outside and inside the greenhouses were 23/3 and 29/5 °C, respectively. Average relative humidities outside and inside the greenhouses were 70 and 80%, respectively, and average solar irradiations outside and inside the greenhouses were 3 and 2 kWh/m^2·day, respectively.

For each vegetable, three samples were collected at random

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Table 1. Application Rates of Monitor for Each Treatment Day and Plantation (Multiple Treatments)

treatment	application rate, g/ha (active ingredient)		
	pepper	cucumber	tomato
I (day 0)	507	462	369
II (day 14)	600	554	485
III (day 28)	646	646	600
IV (day 44)	646	646	600
V (day 71)	646	646	600

from sampling plots of 200 m^2 at 1, 3, 7, 14, 22, 32, and 38 days after application. The samples consisted of 15−20 pieces of peppers (160−180 g per piece), 10 pieces of cucumbers (400−500 g per piece), and 60−70 pieces of cherry tomatoes (7−9 g per piece). Inmediately after picking, the samples were put into polyethylene bags and transported to the laboratory, where they were chopped, thoroughly mixed, and divided into three subsamples each. The subsamples were kept deep-frozen until analysis. In all cases, <90 min passed between harvest and storage in the freezer, analyses being always carried out between 24 and 48 h after subsamples were stored in the freezer. Stability of methamidophos in the matrices during the homogenization and storage procedures was previously tested on spiked samples.

Extraction of methamidophos residues from peppers, cucumbers, and cherry tomatoes was carried out according to the Leary procedure (Leary, 1971). A brief description of the procedure is as follows. Fifty grams of chopped sample was weighed into a high-speed blender jar; 100 mL of ethyl acetate and 80 g of anhydrous sodium sulfate were added and then blended for 5 min. The mixture was decanted and filtered through a 12 cm Büchner funnel with 20 g of anhydrous sodium sulfate. Two successive 100 mL portions of ethyl acetate were added to the solid residue, blended for 2 min, and filtered through anhydrous sodium sulfate. The combined extracts were evaporated to dryness using a vacuum rotatory evaporator (40 °C water bath) and diluted to exactly 10 mL with light petroleum ether in a 10 mL volumetric flask. In all instances it was assumed that this extract represented 5 g of sample/mL.

Methamidophos residues in the light petroleum ether extracts were determined by GC-NPD using the operating conditions described above.

Multiple Treatments. To study the behavior of methamidophos in pepper, cucumber, and tomato after multiple applications, experimental plots of 100 m^2, inside the greenhouses above-mentioned, were selected. Plants were sprayed five times with Monitor (60% methamidophos, w/v) at a concentration of 1 mL/L, at the intervals and application rates indicated in Table 1.

For each vegetable, three samples were collected at random from experimental plots at 8 days after the second treatment (II), 9 days after treatment IV, and 9 and 18 days after the last treatment (V). Each sample consisted of ∼500 g of tomato, ∼3 kg of pepper, and ∼4.5 kg of cucumber. After picking, the samples followed the same treatment as described for the decline experiments with single treatment.

RESULTS AND DISCUSSION

Single Treatment. The residue data obtained in the decline studies of methamidophos on pepper, cucumber, and cherry tomato are summarized in Table 2.

Methamidophos residue levels found in all cucumbers and pepper samples analyzed during this study were <1 mg/kg, this value being the maximum residue limit (MRL) established by the Spanish legislation for these vegetables (Ministerio de la Presidencia, 1996). For tomato, methamidophos residue levels were higher than MRL (0.50 mg/kg) at the begining of the study, and only 7 days after treatment were values below the MRL found.

Table 2. Levels of Methamidophos Residues in Pepper, Cucumber, and Cherry Tomato Samples from the Single-Treatment Study

time (t), days	residue level (R),[a] mg/kg		
	pepper	cucumber	tomato
1	0.48 ± 0.08	0.44 ± 0.05	0.80 ± 0.09
3	0.43 ± 0.08	0.35 ± 0.03	0.61 ± 0.06
7	0.33 ± 0.03	0.40 ± 0.05	0.16 ± 0.02
14	0.21 ± 0.03	0.21 ± 0.03	0.04 ± 0.01
22	0.19 ± 0.02	0.07 ± 0.03	0.03 ± 0.01
32	0.15 ± 0.02	0.06 ± 0.01	0.02 ± 0.01
38	0.09 ± 0.02	0.02 ± 0.01	0.03 ± 0.01

[a] Mean of triplicate analyses from three replicates.

Table 3. Modified Coefficient of Determination (r^2) and Test Quantity for Correlation (D) Values Determined from the Six Functions

function	r^2 (D)		
	cucumber	pepper	tomato
1st	0.899 (0.194)	0.958 (0.225)	0.599 (0.020)
1.5th	−0.461	0.982 (0.461)	0.461 (−0.070)
2nd	−18.878	0.928 (0.209)	0.373 (−0.143)
1st RF	0.613 (0.030)	0.975 (0.233)	0.877 (0.182)
1.5th RF	−12.243	0.878 (0.182)	0.751 (0.112)
2nd RF	−2012.7	−0.215	−1.708

Residue data were subjected to statistical analysis to evaluate the decline of methamidophos residues as a function of time and to determine the parameters that describe these processes. Statistical analysis was carried out according to the formal approaches proposed by Timme et al. (1986) to study the behavior of pesticide residues on crops prior to harvest. These approaches were successfully applied by the authors in a previous work on endosulfan (Aguilera-del Real et al., 1997). Residue values and/or time were transformed using the six formal models described by these authors to achieve a linear relationship, as shown in Figure 1.

To obtain a measure of the quality of the fit, the modified coefficient of determination r^2 proposed by Frehse and Walter (1995) was calculated. The value of r^2 was tested with the aid of the test quantity D described by Timme and Frehse (1980). If the calculated test quantity is greater than nil, the correlation is confirmed. If the modified coefficient of determination becomes negative, the fit is automatically regarded as not assured. In Table 3 calculated values of r^2 and D are shown for each vegetable. As can be seen in this table, correlation is not assured ($r^2 < 0$) for 1.5th-order, 2nd-order and 1.5th-order root function (RF) function for cucumber and for 2nd-order RF function for all the vegetables. In all other cases, the test quantity values were greater than nil, except for 1.5th-order and 2nd-order functions for tomato.

Functions that best fit experimental data (maximum value of r^2) were 1st-order function for cucumber, 1st-order RF for tomato, and 1.5th-order function for pepper, but in this last case all functions gave practically tha same values of r^2 and D as obtained by the best fit function. In Figure 2, the decline curves are plotted in the original system (back-transformed functions) for those cases in which correlations were confirmed.

Methamidophos half-life times ($t/2$) in the three plantations determined from the first-order function and the optimal functions, by using the general formulas for decline times (t/X), derived by Timme and co-workers (Timme et al., 1986; Frehse and Walter, 1995), are given

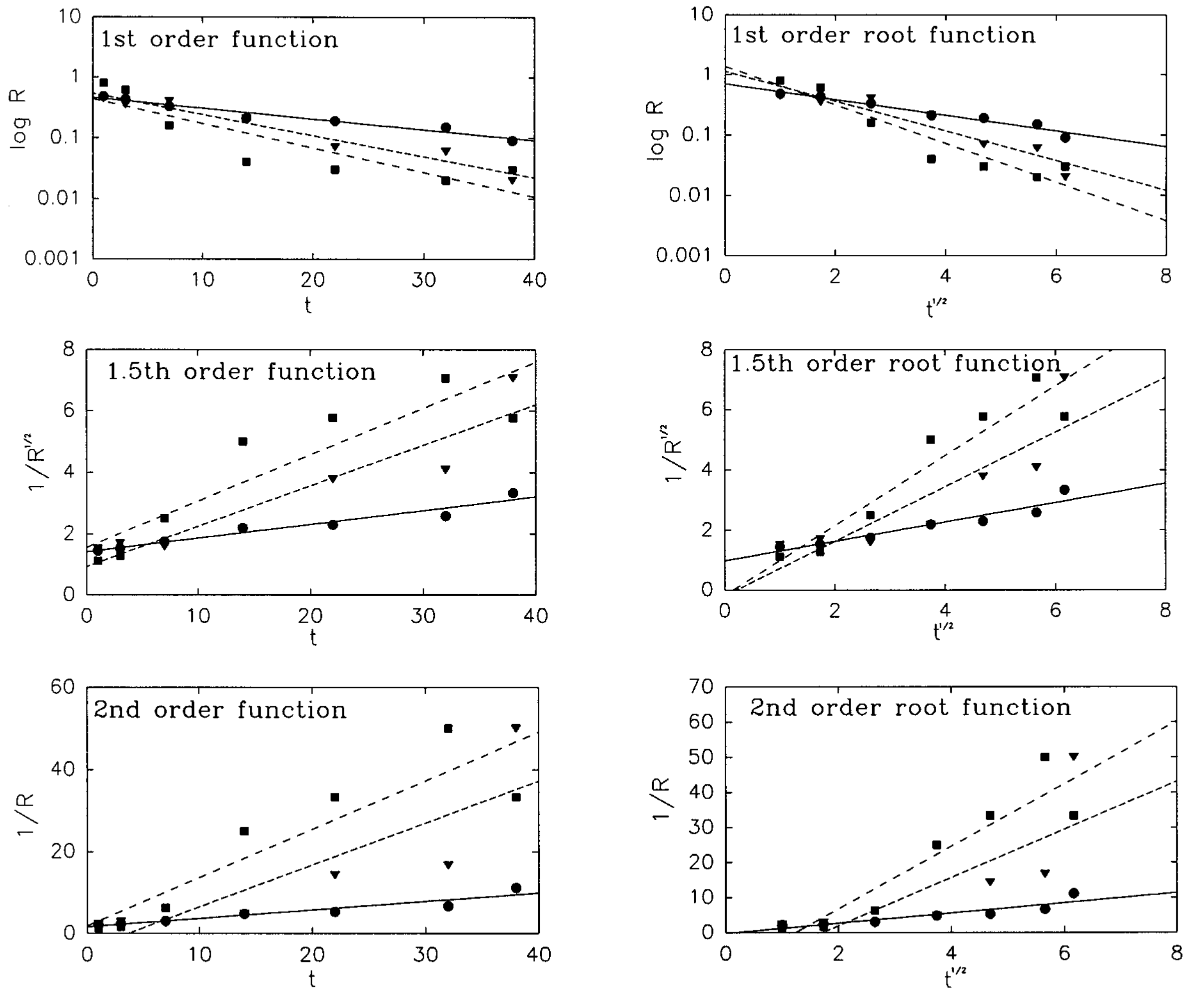

Figure 1. Straight lines obtained for methamidophos residue data in cucumber (▼), pepper (●), and tomato (■) using the coordinate transformation proposed by Frehse and Walter (1995).

Table 4. Half-Life Times (*t*/2) Determined from the First-Order and Optimal Functions

	t/2 ± CI,[a] days	
vegetable	first-order function	optimal function
cucumber	8.68 ± 2.31	8.68 ± 2.31 (1st-order)
pepper	17.04 ± 4.27	13.28 ± 4.50 (1.5th-order)
tomato	7.47 ± 4.76	2.77 ± 2.24 (1st-order RF)

[a] CI, confidence interval at a significance level of 0.05.

Table 5. Residue Values at Preharvest Time (R_7) Determined from the First-Order and Optimal Functions

	R_7 ± CI,[a] mg/kg	
vegetable	first-order function	optimal function
cucumber	0.30 (+0.13/−0.09)	0.30 (+0.13/−0.09) (1st-order)
pepper	0.34 (+0.06/−0.05)	0.34 (+0.09/−0.07) (1.5th-order)
tomato	0.23 (+0.37/−0.15)	0.19 (+0.17/−0.09) (1st-order RF)

[a] CI, confidence interval at a significance level of 0.05.

in Table 4. *t*/2 values given in most studies carried out on the decline of pesticide residues in crops are usually obtained by assuming a pseudo-first-order reaction to describe the behavior of residues (Timme and Frehse, 1980; Valverde-García et al., 1993a,b). In our experiments, the first-order model, although optimal for cucumber only, was confirmed in all cases, and *t*/2 values obtained for methamidophos residues from this model were 8.68 days (cucumber), 17.04 days (pepper), and 7.47 days (tomato). However, *t*/2 values obtained from the optimal function were 8.68 days (cucumber), 13.28 days (pepper), and 2.77 days (tomato). It is interesting to notice that for those plantations in which the first-order and the optimal function were not the same, *t*/2 values obtained from the optimal function were lower than those obtained from the first-order function. This means that in these cases, shortly after application, the residues decline at a faster rate than suggested by an assummed pseudo-first reaction.

On the other hand, methamidophos residues in pepper, cucumbers, and tomatoes at preharvest time (Liñán and Vicente, 1996) (R_7), calculated from both the first-order and the optimal functions were very similar in all cases, as can be seen in Table 5. Values obtained from the optimal function in cucumber, pepper, and tomato were lower than the corresponding MRLs established by Spain, the EU, and Codex. R_7 values (optimal functions) were 0.30 mg/kg (cucumber), 0.34 mg/kg (pepper), and 0.19 mg/kg (tomato).

Multiple Treatments. Residue levels of methamidophos in plants of pepper, cucumber, and tomato sprayed five times with Monitor for each day of sampling (II + 8, IV + 9, V + 9, and V + 18) are shown in Table 6. We note that after four successive treatments at ~15 day intervals, no accumulative effects of methamidophos were observed in tomato, pepper, and cucumber plants, and residue levels similar to those obtained for single treatment were found. However, 18 days (for tomato) or 9 days (for pepper and cucumber) after the fifth treatment, with 4 week interval between treat-

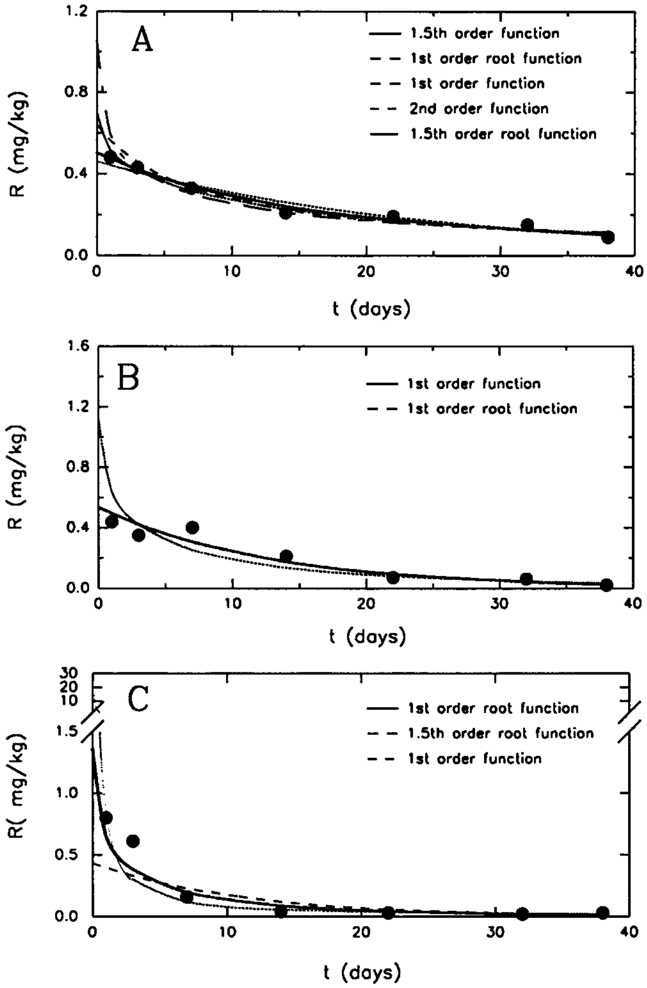

Figure 2. Decline curves of methamidophos in pepper (A), cucumber (B), and tomato (C) (back-transformed function) obtained by applying the models for which correlation was confirmed. The solid line is used for the best fit function.

Table 6. Levels of Methamidophos Residues in Pepper, Cucumber, and Cherry Tomato Samples from the Multiple-Treatment Study

	residue level,[a] mg/kg		
sample	cucumber	pepper	tomato
II + 8	0.25 ± 0.02	0.25 ± 0.01	0.12 ± 0.02
IV + 9	0.24 ± 0.02	0.27 ± 0.02	0.17 ± 0.02
V + 9	0.89 ± 0.07	1.36 ± 0.15	0.16 ± 0.01
V + 18	2.95 ± 0.32	1.04 ± 0.09	1.65 ± 0.18

[a] Mean of triplicate analyses from three replicates.

ments IV and V, unexpected high levels of methamidophos residues in all plantations were found. These data seem to indicate the existence of a certain long-term accumulative effect for this pesticide. These results are in agreement with the high residue levels of methamidophos sometimes found by regulatory agencies in residue monitoring (Andersson et al., 1997).

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LITERATURE CITED

Aguilera-del Real, A.; Valverde-García, A.; Fernández-Alba, A. R.; Camacho-Ferre, F. Behaviour of Endosulfan Residues in Peppers, Cucumbers and Cherry Tomatoes Grown in Greenhouse: Evaluation by Decline Curves. *Pestic. Sci.* **1997**, *51*, 194−200.

Andersson, A.; Palsheden, H.; Bergh, T.; Jansson, A. Pesticide Residues in Food of Plant Origin 1996. *Rapport* **1997**, *25*.

Food and Drug Administration. FDA Pesticide Program: Residue Monitoring 1992. *J. AOAC Int.* **1993**, *76*, 1193−1205.

Frehse, H.; Walter, H. F. The Behaviour of Pesticide Residues in Fruits and Vegetables: Evaluation by Decline Curves. In *Residuos de Plaguicidas '94/Pesticide Residues '94*; Valverde, A., Rodriguez, A., Eds.; IEA/UAL/COEXPHAL: Almería, Spain, 1995.

Gamón, M. Plan Nacional de Vigilancia de Residuos de Plaguicidas: Diseño y Metodología. In *Residuos de Plaguicidas '96/Pesticide Residues '96*; Valverde, A., Ed.; IEA/UAL: Almería, Spain, 1998.

Hamman, I. Tamaron, A New Insecticide and Acaricide. *Pflanzenschutz-Nachr. Bayer* **1970**, *23*, 133−143.

Herrera, J. C.; Brotons, M. Results of the Residue Monitoring Programme of the Andalusian Agricultural Department in Almeria for Fruits and Vegetables. Presented at the 2nd European Pesticide Residue Workshop, Almeria, Spain; May 1998; paper B-36.

Hussain, M. A. Anticholinesterase Properties of Methamidophos and Acephate in Insects and Mammals. *Bull. Environ. Contam. Toxicol.* **1987**, *38*, 131−138.

Leary, J. B. Gas Chromatographic Determination of Monitor (O,S-Dimethyl Phosphoramidothioate) Residues in Crops. *J. Assoc. Off. Anal. Chem.* **1971**, *54*, 1396−1398.

Liñán y Vicente, C. *Vademecum de Productos Fitosanitarios y Nutricionales*; Carlos Liñán y Vicente: Madrid, Spain, 1996.

MAPA. *Informes Reuniones Anuales de los Grupos de Trabajo Fitosanitarios 1997*; Ministerio de Agricultura, Pesca y Alimentación: Madrid, Spain, 1997; pp 197−214.

Ministerio de la Presidencia. Orden de 5 de Diciembre de 1996 sobre Límites Máximos de Residuos de Productos Fitosanitarios. *Bol. Of. Estado* **1996**, *29*, 36918.

Thomson, W. T. *Agricultural Chemicals Book I: Insecticides*; Thomson Publications: Fresno, CA, 1992.

Timme, G.; Frehse, H. Statistical Interpretation of the Degradational Behaviour of Pesticide Residues, I. *Pflanzenschutz-Nachr. Bayer* **1980**, *33*, 47−60.

Timme, G.; Frehse, H.; Laska, V. Statistical Interpretation and Graphic Representation of the Degradational Behaviour of Pesticide Residues II. *Pflanzenschutz-Nachr. Bayer* **1986**, *39*, 187−203.

Tomlin, C., Ed. *The Pesticide Manual*, 10th ed.; British Crop Protection Council: Surrey, U.K.; The Royal Society of Chemistry: Cambridge, U.K., 1994.

Valverde-Garcia, A.; Gonzalez-Pradas, E.; Aguilera-del Real, A.; Ureña-Amate M. D. Determination and Degradation Study of Chlorothalonil Residues in Cucumbers, Peppers and Cherry Tomatoes. *Anal. Chim. Acta* **1993a**, *276*, 15−23.

Valverde-García, A.; González-Pradas, E.; Aguilera-del Real, A. Analysis of Buprofezin Residues in Vegetables. Application to the Degradation Study on Eggplant Grown in a Greenhouse. *J. Agric. Food Chem.* **1993b**, *41*, 2319−2323.

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