ELSEVIER

Food Chemistry 82 (2003) 367–371

Food Chemistry

www.elsevier.com/locate/foodchem

Decomposition of myclobutanil and triadimefon in grapes on the vines and during refrigerated storage

Panagiotis E. Athanasopoulos*, Christos J. Pappas, Nikolaos V. Kyriakidis

Agricultural University of Athens, Department of Food Science and Technology, Iera Odos 75, Votanikos, Athens 118 55, Greece

Received 5 August 2002; received in revised form 25 November 2002; accepted 25 November 2002

Abstract

Decompositions of the triazole fungicides, myclobutanil and triadimefon, in table grapes, of a Greek variety, on the vines and during refrigerated storage, were studied. Vines of the Greek variety, Opsimo Edessas, were separately sprayed onto the vines with trade preparations of myclobutanil and triadimefon. Samples were collected from the vines after 1, 5, 11, 25, 35 and 46 days. Also, a representative 15-kg sample of grapes, for each fungicide, was collected 2 h after the pesticide applications and stored in a refrigerator at 0 °C. Samples from the refrigerator were removed every 10–30 days for 7.5 months. Residues were determined by a simple gas-chromatographic method. The recovery of myclobutanil was found to be from 88 up to 106% and, of triadimefon, from 94 up to 105%. The limit of determination was 0.02 mg/kg for myclobutanil and 0.04 mg/kg for triadimefon. Half-lives for myclobutanil and triadimefon were 10.5 and 16.5 days respectively, for decomposition on the vines and 92.4 and 216 days, respectively, for decomposition during storage in a refrigerator.

Keywords: Myclobutanil; Triadimefon; Decomposition; Grapes

1. Introduction

Myclobutanil and triadimefon are systemic pesticides (fungicides) belonging to the triazole family of chemicals and are merchandised under different trade names. The chemical name of myclobutanil is 2-*p*-chlorophenyl-2-(1*H*-1,2,4,-triazole-1-ylmethyl)hexanenitrile, with code number RH-3866 (The Pesticide Manual, 1997). Myclobutanil is a moderately toxic compound in toxicity class II. Both of them inhibit ergosterol biosynthesis (EBI-s) (Hasali, 1990), and are used for prevention of fungal attack. The basis of their fungicidal action is the inhibition of 24-methylene hydrolanosterol demethylase, a cytochrome P-450 enzyme responsible, in part, for the biosynthesis of ergosterol. This sterol is essential for fungal growth because it is a critical component of fungal cell membrane structure.

For myclobutanil, $LD_{50} = 290$ mg/kg bw and, for triadimefon, $LD_{50} = 300$–600 mg/kg bw (in rats). It acts against fungi classified as Ascomycetes, Deuteromycetes (fungi imperfecti), and Basidiomycetes. The susceptible organisms include Venturia sp.(brown rot, blossom blight), Stigmina sp. (shot hole), Coccomyces sp. (leaf spot), and Tranzschelia sp. (rust). The susceptible organisms in grapes include Guignardia sp. (black rot), and Uncinula sp. (powdery mildew). They are used for the treatment of *Oidium* in grapes.

Myclobutanil is rapidly excreted by animals. The main metabolites which occur in animals are the keto derivatives α-(3-oxobutyl)-α-(4-chlorophenyl)-1*H*-1,2,4-triazole-1-propanenitrile (RH 9089) and the hydroxy derivative α-(hydroxybutyl)-α-(4-chlorophenyl)-1*H*-1,2,4-triazole-1-propanenitrile (RH 9090).

Myclobutanil is metabolized in apples and grapes in an identical manner. Initial metabolism involves oxidation on the butyl chain to RH-9090 and RH-9089 and a glucoside conjugation product. Grapes were treated once with myclobutanil at 0.02 kg active ingredient (ai)/ha, 34 days before harvest. Samples of grapes, foliage and soil were collected at regular intervals and radio analysed. The half-life of myclobutanil in grape foliage was 15 days. The recovery of radioactivity was 85% for apples and 80% for grapes (Nelson, 1984). Myclobutanil is extensively degraded in plants, animals and soil. The half-life of the total ^{14}C residue on foliage was 15–28 days (Stavinski, 1987; Stavinski, Brackett, & Deakyre, 1998).

Myclobutanil is sparingly soluble in water (142 mg/l). It degrades in aqueous solutions on exposure to light,

* Corresponding author.

0308-8146/03/$ - see front matter
doi:10.1016/S0308-8146(02)00556-3

but it is stable to hydrolysis at pH 5–9. It does not degrade under anaerobic conditions. It has medium toxicity against animals. Its LD_{50} is 290 mg/kg for female rats and it is not mutagenic in the Ames test (FAO, 1992).

Triadimefon is a moderately toxic compound belonging to toxicity class II. The corresponding name of triadimefon is 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl) butanone (Kidd & James, 1991). Triadimefon is a mixture of two isomers of equal toxicity (Kramer, Buchel, & Draber, 1983).

Bayleton (25% triadimefon) has an acute oral LD_{50} of 300–600 mg/kg in rats and about 500 mg/kg in rabbits and dogs (Kidd & James, 1991; Weed Science Society, 1994). The teratogenic potential of triadimefon is relatively low (Stevens & Summer, 1991). Triadimefon shows low to moderate persistence in soils. In a sandy loam type of soil, half of the initial amount of the compound was lost within 18 days. In loamy soils the half-life was much sorter (about 6 days) which indicates that breakdown of the compound varies with soil type (US National Library of Medicine, 1995). Other reported soil half-lives are 14–60 days, with an average of 26 days (Wauchope, Buttler, Harnsby, Augustijn-Beckers, & Burt, 1992). Triadimefon and its residues are moderately mobile and may have potential to leach to groundwater (US National Library of Medicine, 1995). The compound is very stable in water and does not readily hydrolyse. In water with a pH 3.0, 6.0, or 9.0, almost 95% of the compound remains after 28 weeks (US National Library of Medicine, 1995). Triadimefon is used to control powdery mildews, rusts and other fungal pests on cereals, fruits, vegetables, turf, shrubs, and trees (Kidd & James, 1991). It is available in wettable powder, emulsifiable concentrate, granular and paste forms. In plants it degrades by reducing the carbonyl group to a hydroxyl group to form triadimenol, being of comparable toxicity to triadimefon. In grains it is used against the fungus *Erysiphae* sp., *Septoria* and *Rynchosporium*. In fruits and vegetables it is used against the fungus *Uncinula necator*.

Myclobutanil and triadimefon are certified pesticides in Greece and are widely used by farmers during July and August in vineyards for protection against *Oidium* attacks. As far as we know, the degradation of these fungicides on grapes has not been reported up to now. The degradation of myclobutanil and triadimefon was studied in grapes on the vines and in grapes stored in a refrigerated room.

2. Materials and methods

2.1. General

Applications of pesticides were performed on the vineyard of the Agricultural University of Athens. Six lines, of 24 vines each, of the Greek table grape variety Opsimo Edessas, were sprayed. Two lines were sprayed with Systhane EC (12.5% ai), two with Bayleton WP (25% ai) and two were left as control. Application doses were 80 ml emulsion/100 l water for the Systhane and 70 g powder/100 l water for Bayleton. The vines were sprayed to run off by a motorized sprayer. During the sampling period, air temperature was from 27 to 34 °C and the relative humidity was 60–70%. All days were sunny and dry.

A lot of 15 kg of grapes, for each pesticide, was collected, 2 h after their application (EC, 95). The sample was divided into thirteen sub-samples. One of these was analyzed immediately for pesticide residues. The rest of them were packed in plastic bags, and a special sodium metabisulfite impregnated paper was placed on the top of the grapes; bags were closed and stored in a refrigerated room at 0 ± 1 °C (Nelson & Ahmedulah, 1973). Samples were removed and analyzed every 10–15 days and up to 7.5 months.

Samples of 1-kg each were also collected for analysis from the vines every 5–7 days, for up to 1.5 months.

2.2. Analytical procedures

All samples were analyzed by a general method, suitable for gas chromatographic analysis, with a nitrogen-phosphorus detector (NPD) (Ministry of Wellfare, 1988), modified as concern the timing of the programme. According to the method the berries of the samples were removed and pulped in a laboratory mixer. Fifty grams of the pulp of each sample were mixed with 100 ml of ethyl acetate and 50 g of sodium sulfate. The mixture was blended for 2 min and the extract was filtered through Whatman No. 1 filter paper, containing 2 g of sodium sulfate, into a conical flask. During filtration, all parts were kept under crushed ice to avoid undue evaporation of ethyl acetate. The clear filtrate was injected into the chromatograph.

2.3. Gas chromatographic determination

A Hewlett-Packard (model 5890, series II) gas chromatograph was used, equipped with a splitless injector, an NPD, and a 30 m×0.5 mm i.d.×0.88 μm film thickness, glass capillary column (Hewlett - Packard), coated with cross-linked 5% phenyl methyl silicone. The injection port temperature was 250 °C and the detector temperature 290 °C. The column temperature was programmed as follows: The initial temperature of 120 °C was increased at a rate of 20 °C /min to 210 °C with a residence time of 2 min. From 210 to 270 °C, a rate of 10 °C/min was used, with a residence time of 2 min. From 270 to 285 °C, a rate of 13 °C/min was used, with a residence time of 5 min at the final temperature. Helium carrier gas at a flow rate of 7 ml/min was used. Samples

of 2 μl of the extract (in triplicate) were injected, and quantitation of the insecticide was performed by automatic integration of the peak areas (EC, 1999). Certified standards of triadimefon and myclobutanil were used for external calibration. Quantitations of the insecticides in the examined samples were done by comparing the detector responses for the samples to that measured before and after each injection with a calibration standard within the linear range.

2.4. Degradation kinetics

To determine degradation kinetics, plots of concentration against time were constructed for each data set, and the maximum square of correlation coefficients found were used to determine the equations of best fit curves. For all cases studied, exponential relations were found to apply, corresponding to first-order rate equations. Confirmations of the first-order rate kinetics were further made, graphically, from the linearity of the plots of lnC against time.

The rate constant k, was calculated from the first order rate equation:

$$C_t = C_0 e^{-kt} \quad (1)$$

where C_t represents the concentration of pesticide at any time t, C_0 represents the initial concentration and k is the rate constant in days. The half-life ($t_{1/2}$) was determined from the k value for each experiment; $t_{1/2} = \ln 2/k$.

Table 1
Recovery of triadimefon and myclobutanil in the grapes on the vines

Level(mg/kg)	Myclobutanil		Triadimefon			Samples
	Recov.%	RSD	Level (mg/kg)	Recov.%	RSD	
0.01	106	6.2	0.1	102	5.3	3
0.05	103	3.5	0.2	96	2.5	3
0.1	99	2.4	0.5	98	3.2	3
0.5	97	3.7	0.8	95	5.2	3
1	88	4.2	1	101	3.8	3
1.5	89	3.3	1.5	105	4.8	3
2	94	2.8	2	94	4.5	3
2.5	96	4.6	2.5	97	3.1	3

RSD: Relative standard deviation.

3. Results and discussion

3.1. Determination and recovery

The method of analysis was simple and fast. The response of the detector for myclobutanil and triadimefon was linear in the studied ranges of 0.02–0.5 and 0.1–2.5 mg/kg, respectively. Regression equations found for myclobutanil and triadimefon, were respectively, $y = 197.1x + 1.0467$, correlation coefficient $R^2 = 0.999$, and $y = 159.86 \times 0.2157$, with $R^2 = 0.9998$.

The efficiency of the method was evaluated by spiking control samples with myclobutanil and triadimefon at various concentration levels. The results of the recovery studies are presented in Table 1. As seen from this table, average recoveries were from 88 to 106% (RSD: 2.4–6.2%) for myclobutanil and from 94 to 105% (RSD: 2.5–5.3%) for triadimefon. Similar recoveries have been reported by Flori and Brunelli (1995). These values are within the accepted range for residue determination (EC, 1999). The method limit of determination, evaluated as the product of the standard deviation at the lowest validation level with the Student t-test (US EPA, 1984) and 99% confidence level, was 0.02 mg/kg for myclobutanil and 0.04 mg/kg for triadimefon.

3.2. Degradation of Myclobutanil and Triadimefon

Results of degradation of myclobutanil and triadimefon are presented in Table 2 and Figs. 1–4. The pesticides studied were found to follow pseudo-first order kinetics.Half life for the myclobutanil for the vines was 10.5 days while it was extended to 92.4 days for the stored grapes. Decomposition half-life of the fungicide Triadimefon on the vines was 16.5 days, but it was extended to 216 days for refrigerated storage.

The recommended (by the manufacturers) pre-harvest interval is 15 days for myclobutanil and 28 days for triadimefon. As can be seen from Table 2, results are in agreement with manufacturers recommendations only for grapes on the vines. For the grapes stored in the refrigerator, the times to attain MRLs are increased 5-fold for myclobutanil and 9.25-fold for triadimefon.

Table 2
Decomposition equations and half-lives for myclobutanil and triadimefon in the grapes

Pesticide	Equation	R^2	k	Half-life ($t_{1/2}$) (days)	Time to attain MRL (days)
Myclobutanil					
(a) In Grapes on the vines	$C = 1.649\exp(-0663t)$	0.984	0.0663	10.5	7.5
(b) Grapes refrigerated	$C = 1.749\exp(-0075t)$	0.989	0.0075	92.4	74.5
Triadimefon					
(a) In Grapes on the vines	$C = 1.030\exp(-0421t)$	0.902	0.0421	16.5	17.2
(b) Grapes refrigerated	$C = 1.146\exp(-0032t)$	0.989	0.0032	216	259

MRL for myclobutanil = 1 mg/kg and for triadimefon = 0.5 mg/kg [FAO, (2001) Pesticide Residues in Food (MRLs/EMRLs)].

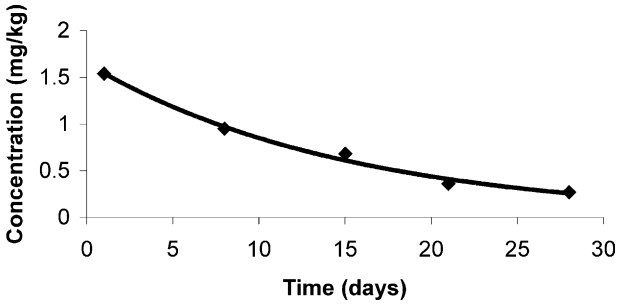

Fig. 1. Decomposition of myclobutanil in grapes on the vines.

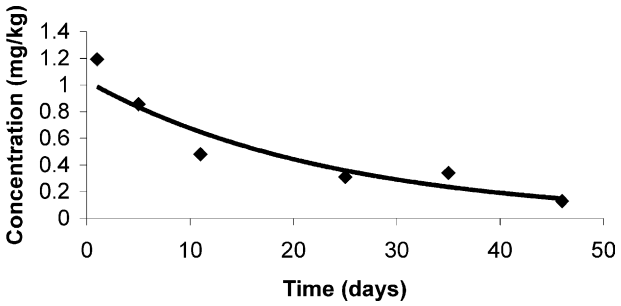

Fig. 2. Decomposition of triadimefon in grapes on the vines.

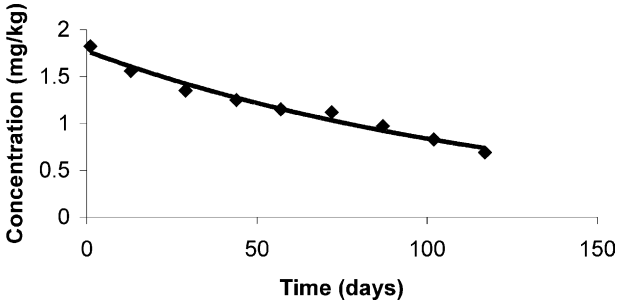

Fig. 3. Decomposition of myclobutanil in grapes stored in refrigerator.

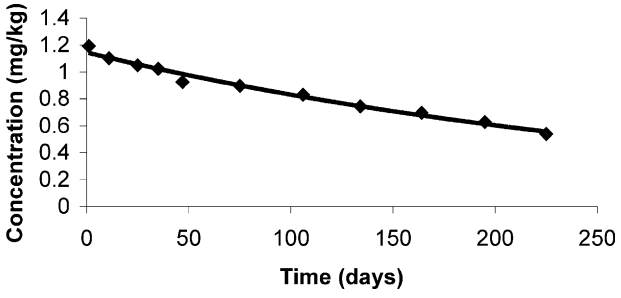

Fig. 4. Decomposition of triadimefon in grapes stored in refrigerator.

Similar results have been reported for methidathion (Kyriakidis, Athanasopoulos, Thanos, Pappas, & Yialitaki, 2000) and pyrazophos (Athanasopoulos, Kyriakidis, & Pappas, 2000).

The decomposition rate of triadimefon in the grapes (Table 2) on the vines is 57% lower than the corresponding rate for myclobutanil. During storage in the refrigerator, this difference is lowered further to 134%.

Other workers have found similar results. Bayleton 5 WP (triadimefon), at 0.1% concentration, was applied to plants four times; the last treatment was made 14 days before harvest. The residues of triadimefon in the fruits decreased within 12 days after last treatment from 0.57 to 0.02 mg/kg and from 0.61 to 0.02 mg/kg, depending on the year of experiment (Kepczynska, 1989).

Myclobutanil was studied in grapes (Brackett, 1986a) and wine (Brackett, 1986b). When wine was prepared from treated grapes, residues were found to be lower in the fresh juice and the wine than in the whole fresh grapes.

Decomposition half-life of the insecticide myclobutanil on the vines was 10.5 days, but was extended to 92.4 days for storage at 0 °C. It can be seen that half-lives at 0 °C were 9-fold higher than that on the vines.

References

Athanasopoulos, E. P., Kyriakidis, B. N., & Pappas, C. (2000). Disappearance of the pesticide pyrazophos from Greek Soultanina grapes on the vines and during storage. *Italian Journal of Food Science*, *3*(12), 353–357.

Brackett, C. K. (1986a). *Analytical reports for residues due to RH-3866 in grapes*. Rohm and Haas Report No.31A-86-42.

Brackett, C. K. (1986b). *RH-3866 residue decline study in grapes and wine*. Rohm and Haas Report No.31A-86-50.

European Commission (1999). *Recommendation 99/333/EC of 3 March 1999 concerning a coordinated Community monitoring program for 1999 to ensure compliance with maximum levels of pesticide residues in and on certain products of plant origin, including fruit and vegetables* (OJ No L 128/25 of 21-05-1999).

FAO Pesticide residue in food. (1992). *Plant production and protection Paper,116, 1993. Pesticide residue in food—1992 evaluations, Part II-Toxicology*. Rome, Italy: FAO/WHO.

Flori, P., & Brunelli, A. (1995). Residues of EBI (Ergosterol Biosynthesis inhibitors) fungicides on grape, in must and wine [Triadimenol, Myclobutanil, Hexaconazole, Penconazole, Cyproconazole. Mededelingen-Faculteit-Landbouwkundige-en-Toegepaste-Biologische-Wetenschappen-Universiteit-Gent (Belgium). v. 60(2b) pp. 503–509.

Hassali, A. K. (1990). *The biochemistry and uses of pesticides* (2nd ed.), pp. 336–339.

Kepczynska, E. K. (1989). Rate of dissipation of triadimefon and its metabolite [triadimenol] in strawberry fruits. *Fruit-Science-Reports (Poland)*, *16*(1), 17–21.

Kidd, H., James, D. R. (Eds.). (1991). *The agrochemicals handbook* (3rd ed.). Cambridge, UK: Royal Society of Chemistry Information Services.

Kramer, W., Buchel, K. H., & Draber, W. (1983). *Pesticide Chemistry: Human Welfare and the Enviroment*. Oxford: Pergamon.

Kyriakidis, B. N., Athanasopoulos, E. P., Thanos, A., Pappas, C., & Yialitaki, M. (2000). Decay of Methidathion on Greek Soultanina Grapes during storage and on the Vines. *Journal of Agricultural & Food Chemistry*, *48*, 3095–3097.

Ministry of Welfare, Health and Culture Affairs. (1988). Analytical Methods for Residues of Pecticides in Foodstuffs. In P. A. Greve (pp. 3). The Netherlands: Rijswijk.

Nelson, S. S. (1984). *Residue decline study of RH-3866 in grapes*. Rohm and Haas Report No.310-84-29.

Nelson, K. G., & Ahmedulah, M. (1973). Effect of temperature change on the release rate of sulfur dioxide from two-stage sodium bisulfate generator. *Am. J. Food Viticult*, *24*, 75–80.

The Pesticide Manual (1997) British Crop Protection Council, Eleventh Edition, Editor C.D.S. Tomlin Lavenham, Press, Lavenham, Suffolk, UK.

Stavinski, S. S. (1987). *RH-3866 residue data for grapes*. Rohm and Haas Report No No 31A-87-67. December 1.

Stavinski, S. S., Brackett, C. K., & Deakyne, R.O. (1998). *RH-3866 Total residue analytical method a for apple and grape*. Rohm and Haas Company Technical Report No. 34S-88-10.

Stevens, J. T., & Sumner, D. D. (1991). Herbicides. In W. J. Hayes Jr., & E. R. Laws Jr. (Eds.), *Handbook of pesticide toxicology*. New York, NY: Academic Press.

US EPA Fed. Regist. (1984), 49 (209), 198.

US National Library of Medicine. *Hazardous substances databank*. Bethesda, MD, 1995. pp. 8–17.

Wauchope, R. D., Buttler, T. M., Hornsby, A. G., Augustijn-Beckers, P. W. M., & Burt, J. P. (1992). SCS/ARS/CES Pesticide properties database for environmental decision making. *Rev. Environ. Contam. Toxicol.*, *123*, 1–157.

Weed Science Society of America. (1994). *Herbicide handbook* (7th ed.).