

Evaluation of Teflubenzuron Residue Levels in Grapes Exposed to Field Treatments and in the Must and Wine Produced from Them

Nicholas G. Tsiropoulos*

Department of Theoretical and Applied Sciences, University of Thessaly, Pedion Areos, Volos 38334, Greece

Pipina G. Aplada-Sarlis and George E. Miliadis

Benaki Phytopathological Institute, 7 Ekalis Street, Kifissia 14561, Greece

Dissipation of benzoylurea insecticide teflubenzuron in grapes exposed to field treatments was evaluated. Vines of a white grape vineyard located in central Greece were sprayed twice, at a 28-day interval, with a commercial formulation of the insecticide at 12 g of active ingredient/100 L. Residues were determined by HPLC diode array detection, after ethyl acetate extraction and cleanup on silica phase cartridges, with a detection limit of 0.005 mg/kg. Under field conditions teflubenzuron residues in grapes were found to be very stable with no significant reduction for the whole experimental period of 49 days. However, residues were slightly lower than the maximum residue limits set by individual countries. The fate of teflubenzuron during the vinification process was also studied. Residues were found to be transferred completely into the must but, due to their high affinity for the suspended matter, were removed by ~98%; thus, very low concentrations were detected in the produced wine. Among various clarifying agents studied, charcoal was the only one found to be effective for removing teflubenzuron residues from wine.

Keywords: Teflubenzuron; insecticides; residues; grapes; wine; vinification

INTRODUCTION

The use of insecticides in viticulture is a major issue for grape protection against pest diseases, and their use is therefore important in grape productivity and wine quality. Thus, assessment for their presence and the concentration of residues of phytosanitary products on grapes and in their processed products, such as wine, is of particular interest. However, studies on insecticide residues in grapes and their fate during wine-making are quite limited (Cabanis and Cooper, 1991; Cabras et al., 1983, 1995; Sala et al., 1996) and more are required.

Teflubenzuron [1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6-difluorobenzoyl)urea] is a nonsystemic insect growth regulator with stomach action, belonging to the benzoylurea family of insecticides, which is used for the control of the grape moth (*Lobesia botrana*), the most widespread and harmful insect with regard to grapes. It is also recommended for the control of Lepidoptera, Coleoptera, Diptera, Aleyrodidae, Phyllidae, and Hemiptera larvae in other crops such as pome, stone, citrus fruits, and vegetables (British Crop Protection Council and Royal Society of Chemistry, 1994). The preharvest interval (PHI), that is, the number of days from final application to harvest, for teflubenzuron in grapes is 40 days in Greece. No maximum residue limits (MRLs) have been set by the European Union or by the FAO for this compound in grapes.

Very limited data are reported concerning the dissipation of teflubenzuron in agricultural products, for example, in pears (Aplada-Sarlis et al., 1999), and there

is lack of published data for the fate of this insecticide in grapes and in the produced must and wine.

The aim of this work is to evaluate teflubenzuron residue levels in grapes exposed to field treatments and to study the fate of teflubenzuron during the vinification process. The influence of different clarifying substances on the removal of residues from wine will also be studied.

EXPERIMENTAL PROCEDURES

Field Experiment. The experimental trial was carried out in a white grape vineyard (cv. Roditis), located at Aghialos, in the vicinity of Volos, in central Greece. The vines were spaced 1.2 m from each other and 1.8 m between rows, and during the experimental period they received routine horticultural practices. The experimental area consisted of two plots of 32 plants each; one was treated with teflubenzuron, and the other was used as the control. In the first plot, the grapevines were sprayed twice, on August 1 and 29, 1997, with a 15% teflubenzuron formulation (Nomolt 15SC) at the recommended dose (12 g of active ingredient/100 L of water) using a pressurized hand-gun applicator at high volume to run off. To protect the grapes against pest attack, the grapevines of the control plot were often sprayed with a *Bacillus thuringiensis* formulation. The average minimum/maximum daily air temperatures were 17.4 °C/28.4 °C for August, 12.9/23.5 °C for September, and 10.5/21.8 °C for October. The average relative humidity ranged from 63.5% in August to 70% in October, and the rainfall was 10.6 mm in August, 13.0 mm in September, and 18.6 mm in October.

Sampling. The grape samples intended for residues analysis were taken by randomly collecting parts of bunches from separated vines according to FAO/WHO (1986) recommendations. The overall sample weight was 1–1.5 kg. Grape samples were forwarded to the laboratory, blended, subdivided into 50-g aliquots as analytical replicates, and stored in individual bags

* Corresponding author [telephone (+30)42174242; fax (+30)42174270 1; e mail ntsirop@uth.gr].

at -18°C until extraction. For studying the dissipation of teflubenzuron on grapes, samples were collected from both experimental plots just before the last application (-0 days sample), at 2 h post-spray from dry plants (0 days sample), and at 2, 4, 7, 10, 14, 17, 21, 24, 28, 31, 35, 42, and 49 days after the last treatment. For studying the fate of teflubenzuron residues from vine to wine, samples (~ 30 kg of grapes collected from all vines of the plot) were taken at 14, 28, 35, and 42 days after the last treatment and processed to produce must and wine. Wine was also produced from grapes collected from the control plot, to be used as control wine.

Vinification Processing. The vinification procedure was performed at laboratory scale with the usual white wine production method. After the grape samples had been pressed, 0.1 g/L sodium metabisulfite and 0.2 g/L dry yeast were added in the produced must and the must was allowed to ferment at room temperature. Alcoholic fermentation had a regular course in all samples, and after 20 days, the obtained wine was filtered and analyzed for teflubenzuron residues. For evaluating the affinity between teflubenzuron residues and the suspended matter of the must, a 2×100 mL aliquot of cloudy must was taken and centrifuged at 4000 rpm for 10 min, and the obtained clear must was kept frozen until analysis. Clarification tests were also carried out on 1-L samples of the fermented wine to evaluate the effect of clarifying substances on removing teflubenzuron residues. For clarifying wine, the following substances were used at the indicated doses: bentonite at 0.4 g/L, charcoal at 0.2 g/L, polyvinylpyrrolidone (PVPP) at 0.4 g/L, cellulose at 0.3 g/L, potassium caseinate at 0.4 g/L, and colloidal silicon dioxide plus gelatin at 0.5 and 0.05 g/L, respectively. Must, clear must, and unclarified and clarified wine, as well as control wine, were conserved at -18°C until all samples were analyzed together.

Chemicals. Analytical standard of teflubenzuron (certified $>97.4\%$) was supplied by Cyanamid. Stock (1000 ppm) and working standard solutions were prepared in methanol. Methanol and water were of HPLC grade, and the other solvents were of pesticide residue grade; all solvents were obtained from Lab-Scan. Anhydrous sodium sulfate and bisulfate were of proanalysis grade (Merck). C_{18} and silica SPE cartridges (500 mg/3 mL) were purchased from Isolute. Bentonite, charcoal, PVPP, cellulose, potassium caseinate, gelatin, and colloidal silicon dioxide were commercial grade products.

Analytical Procedures. All grape, must, and wine samples were analyzed for teflubenzuron residues by high-performance liquid chromatography, with a UV diode array detector. Grape and must samples after solvent extraction were cleaned up in silica cartridges, and wine samples were solid phase extracted in C_{18} cartridges, according to a method for benzoylurea determination in grapes and wine (Miliadis et al., 1999). Extraction and cleanup are briefly described below.

Grape and Must Extraction Procedure. Fifty grams of homogenized sample was blended with 100 mL of ethyl acetate and 50 g of anhydrous sodium sulfate, and 50 mL of the filtered extract was evaporated to dryness. The residue was redissolved in 5 mL of hexane, and 1 mL of the solution was cleaned up in a silica cartridge, preconditioned with 10 mL of hexane. The residues were eluted with 2 mL of 2-propanol/dichloromethane (1:9), and the solution was dried under a gentle nitrogen stream, redissolved in 1 mL of MeOH, and filtered through a $0.2\ \mu\text{m}$ filter in a sealed vial ready for HPLC analysis.

Wine Extraction Procedure. Ten milliliters of wine was diluted with 30 mL of water, and teflubenzuron was extracted on a C_{18} SPE cartridge preconditioned with 3 mL of acetone, 4 mL of 20% ethanol in water, and finally 4 mL of 2.5% ethanol in water. Diluted wine samples (40 mL) were passed through the cartridge under manual positive pressure with the aid of a glass syringe. After a rinse with 4 mL of 2.5% ethanol in water and removal of solvent residues by passing air, teflubenzuron residues were eluted from the cartridge with methanol until 2 mL of eluate had collected in a volumetric flask. The extract was filtered through a $0.2\text{-}\mu\text{m}$ filter in a sealed vial ready for HPLC analysis.

HPLC Determination. Chromatographic analyses were performed with a Hewlett-Packard, Model HP 1090 Series II

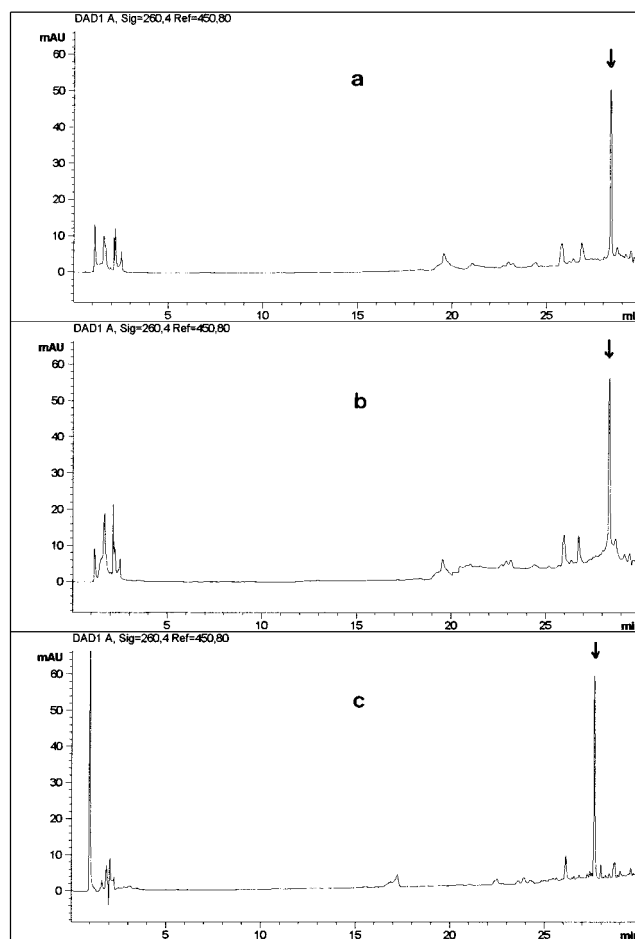


Figure 1. Typical HPLC chromatograms from analysis of teflubenzuron residues: (a) fortified grapes at 1 mg/kg; (b) field grapes 14 days after treatment; (c) must from grapes collected 14 days post-treatment.

liquid chromatograph, equipped with a UV diode array detector and an ODS Hypersil C_{18} column, 250×2.1 mm, $5\text{-}\mu\text{m}$ particle size. The mobile phase was methanol/water, delivered at a flow rate of 0.3 mL/min with a gradient composition, consisting of 10% v/v methanol for 5 min, a linear increase over 15 min to 64% methanol, then an increase to 100% methanol over 20 min, and finally a decrease at 10% v/v methanol over 2 min, giving a total run time of 42 min. The injection volume was $20\ \mu\text{L}$, the column temperature was 42°C , and the detection wavelength was set at 260 nm. Under these conditions the retention time for teflubenzuron was ~ 28 min.

RESULTS AND DISCUSSION

Method Efficiency. The described methods of analysis of teflubenzuron residues in grape and must and in wine samples are relatively simple, and the cleanup of the extracts is sufficient, as can be seen from the chromatograms in Figure 1. The detector response was linear in the investigated range of concentrations, with a correlation coefficient $r = 0.9995$ ($N = 5$). Residue determination in samples was made by comparing the area of teflubenzuron peak in the sample's chromatogram with the respective area of standard solutions and quantification with the external standard method.

The efficiency of the method was evaluated by spiking control grape and wine samples with teflubenzuron at various levels covering the concentrations determined in the field samples. All recovery values (Table 1) are

Table 1. Mean^a Recovery Percent and Relative Standard Deviation for Teflubenzuron in Grapes and Wine at Various Fortification Levels

concn (ppm)	grapes	wine
0.01	<i>b</i>	104.6 ± 1.95
0.025	87.0 ± 1.54	101.6 ± 4.34
0.05	85.6 ± 1.34	100.6 ± 2.36
0.25	98.0 ± 2.95	97.4 ± 0.53
0.75	96.2 ± 5.43	<i>b</i>
1.0	101.1 ± 2.43	<i>b</i>
1.5	100.0 ± 1.82	<i>b</i>

^a Three samples for each fortification level. ^b Not evaluated.

Table 2. Teflubenzuron Residue Levels in Grapes (Milligrams per Kilogram) and in the Produced Musts and Wines (Milligrams per Liter) at Various Time Intervals after the Last Application^a

days	grapes	must	centrifuged must	wine
-0 ^b	0.36	<i>c</i>	<i>c</i>	<i>c</i>
0	0.89	<i>c</i>	<i>c</i>	<i>c</i>
2	0.95	<i>c</i>	<i>c</i>	<i>c</i>
4	0.98	<i>c</i>	<i>c</i>	<i>c</i>
7	1.00	<i>c</i>	<i>c</i>	<i>c</i>
10	0.92	<i>c</i>	<i>c</i>	<i>c</i>
14	1.01	1.06	0.02	0.015
17	0.79	<i>c</i>	<i>c</i>	<i>c</i>
21	0.91	<i>c</i>	<i>c</i>	<i>c</i>
24	0.85	<i>c</i>	<i>c</i>	<i>c</i>
28	0.83	0.94	0.019	0.013
31	0.91	<i>c</i>	<i>c</i>	<i>c</i>
35	0.80	0.90	0.020	0.017
42	0.87	1.01	0.019	0.016
49	0.86	<i>c</i>	<i>c</i>	<i>c</i>

^a Values are the means of duplicate analyses. ^b -0 days: residues remaining from previous application. ^c No vinification.

within accepted levels for residue determination ranges (Greve, 1984). The method's detection limit (Su, 1998) was evaluated to be 0.005 mg/kg in grapes and 0.005 mg/L in wine.

Dissipation of Residues in Grapes. Teflubenzuron residues in grape samples at various time intervals following the last application are presented in Table 2. As seen from these data teflubenzuron deposit remaining on grapes from the first application (-0 days sample) was 0.36 mg/kg, and initial deposit, immediately after the last application, was 0.89 mg/kg. Thereafter, teflubenzuron residues remained remarkably stable for the whole experimental period of 49 days, indicating high stability of the molecule. The variation of residue values in grapes was from 0.79 to 1.01 mg/kg and seems to be independent of time for the experimental period of 49 days. For this period time, it should be noted that the grapes had attained their final growth state and, therefore, any diluting effect was negligible. No residues of teflubenzuron were detected in all analyzed control grape samples. The recorded teflubenzuron residue levels in grapes can be compared only with individual countries' MRLs due to the absence of EU or FAO/WHO MRLs. Italy, a country with climatic conditions similar to those of Greece, has set 1 mg/kg as the MRL. Teflubenzuron residues found in grapes were in general lower but quite close to this MRL value.

Residues in Wine. Teflubenzuron residues transferred from grapes into the must and through the vinification process into the wine are also presented in Table 2. As seen from these data, residues in must were found to be slightly higher than in grapes, indicating possibly that teflubenzuron does not remain on the solid commodity of the fruit but is completely transferred in

Table 3. Mean^a Residues (Milligrams per Liter) of Teflubenzuron in Wine Produced from Grapes Taken 14, 28, and 42 Days after the Last Application, after Clarification with Different Clarifying Substances, and the Percent Mean Overall Reduction in Residues Resulting from These Procedures

treatment	wine from grapes at			reduction %
	14 days	28 days	42 days	
no clarification	0.015	0.013	0.016	
PVPP	0.011	0.011	0.012	22.7
charcoal	nd ^b	nd	nd	~100
cellulose	0.012	0.011	0.013	18.2
potassium caseinate	0.013	0.012	0.013	13.6
bentonite	0.014	0.012	0.014	9.0
silicon dioxide ^c	0.014	0.013	0.014	6.8

^a Each value is the mean of duplicate analyses. ^b nd, ≤ limit of detection (0.005 mg/L). ^c Colloidal silicon dioxide plus gelatin.

the juice product. However, after centrifugation of the must, teflubenzuron residues disappeared almost completely, indicating that teflubenzuron is thoroughly adsorbed by the suspended solids in the must. The same great affinity of teflubenzuron for the suspended matter is shown from the great reduction of residues during wine-making. In fact, on passing from grapes to wine, teflubenzuron concentration was reduced by ~98% and residues found in wine, as shown in Table 2, were from 0.013 to 0.017 mg/L, values slightly above the method's detection limit. Wine that was obtained after fermentation with the maceration technique (with the skins) showed the same residues (0.015–0.020 mg/L), indicating that residues are not dependent on the vinification technique.

The effect of clarification treatment on teflubenzuron residues in wine is presented in Table 3. As seen from the data of this table no residue was detectable when charcoal was used for clarification, whereas the other clarifying agents showed scarce effectiveness. These results are in agreement with the results of Cabras et al. (1995), who studied the effectiveness of these agents on removing residues of other insecticides from wine.

Conclusions. Teflubenzuron residues found in grapes for seven weeks following application were very stable, and this is attributed to the stability of the insecticide molecule against biological and environmental factors and to the absence of the dilution effect, because the grapes attain their final growth state before the last treatment. Grape processing into wine caused almost complete residue reduction as shown from the very low concentrations found in wine, and this is attributed to the significant adsorption of teflubenzuron on the suspended matter during the vinification procedure.

ACKNOWLEDGMENT

We thank Mr. D. Zoes for assistance in carrying out of field experiments and Mr. P. Tomaras for aid and guidance during the oenological procedure.

LITERATURE CITED

- Aplada-Sarlis, P.; Miliadis, G. E.; Tsiropoulos, N. G. Dissipation of teflubenzuron and triflumuron residues in field-sprayed and cold stored pears. *J. Agric. Food Chem.* **1999**, *47*, 2926–2929.
- British Crop Protection Council and The Royal Society of Chemistry. *The Pesticide Manual*, 10th ed.; BCPC and RSC: London, U.K., 1994.

- Cabanis, J. C.; Cooper, J. F. Residus de produits phytosanitaires dans les vins. Approches toxicologiques and analytiques. *Rev. Fr. Oenol.* **1991**, *130*, 43–50.
- Cabras, P.; Meloni, M.; Pirisi, F. M. The effect of clarifying substances on the content of some insecticides and fungicides in white wine. *Am. J. Enol. Vitic.* **1983**, *34* (2), 370–373.
- Cabras, P.; Garau, L. V.; Pirisi F. M.; Cubeddu M.; Cabitza F.; Spanedda, L. Fate of some Insecticides from vine to wine. *J. Agric. Food Chem.* **1995**, *43*, 2613–2615.
- FAO/WHO. Recommended method of sampling for the determination of pesticide residues. *Codex Alimentarius Commission of the Joint FAO/WHO Food Standards Programme*, 2nd ed.; Food and Agricultural Organization/World Health Organization: Rome, Italy, 1986; Vol. XIII, Part VI.
- Greve, P. A. Good Laboratory Practice in Pesticide Residue Analysis. In *Pesticide Residue Analysis*; Ambrus, A., Greenhalgh, R., Eds.; Proceedings of a Joint WHO/FAO Course; WHO/FAO: Rome, Italy, 1984; p 281.
- Miliadis, G. E.; Tsiropoulos, N. G.; Aplada-Sarlis, P. High performance liquid chromatographic determination of benzoylurea insecticides residues in grapes and wine using liquid and solid-phase extraction. *J. Chromatogr.* **1999**, *835*, 113–120.
- Sala, C.; Fort, F.; Busto, O.; Zamora, F.; Arola, L.; Guasch, J. Fate of some common pesticides during vinification process. *J. Agric. Food Chem.* **1996**, *44*, 3668–3671.
- Su, G. C. C. A comparison of statistical and empirical detection limit. *J. Assoc. Off. Anal. Chem.* **1998**, *81*, 105–110.

Received for review January 6, 1999. Accepted August 18, 1999.

JF990010N