Pesticides in the Distilled Spirits of Wine and Its Byproducts

Paolo Cabras,*,† Alberto Angioni,† Vicenzo L. Garau,† Elizabeth V. Minelli,‡ Marinella Melis,† and Filippo M. Pirisi†

Dipartimento di Tossicologia, Università di Cagliari, viale Diaz 182, 09126 Cagliari, Italy, and Departamento de Química Orgânica, Instituto de Química—UNESP, Araraquara, SP, Brazil

The fate of eight fungicides (benalaxyl, fenarimol, iprodione, metalaxyl, myclobutanil, procymidone, triadimefon, and vinclozolin) and five insecticides (dimethoate, fenthion, methidathion, parathion methyl, and quinalphos) in wine and its byproducts (cake and lees) during the production of distilled spirits was studied. Among the pesticides studied, only fenthion, quinalphos, and vinclozolin residues were present in the distilled spirits. During wine distillation, respectively 13% and 5% of the initial residues of fenthion and vinclozolin were transferred to the distilled spirit. Low percentages (2% for fenthion and 0.1% for vinclozolin) of these active ingredients (AI) also passed from the lees to the final-distilled spirit, when samples were fortified at 10.1 and 26.1 ppm for fenthion and vinclozolin, respectively. Quinalphos passed only from the lees to the final-distilled spirit in percentages lower than 1% when samples were fortified at the highest concentration (4.6 ppm).

Keywords: *Pesticides; residues; distilled spirits*

INTRODUCTION

The fate of pesticide residues on grapes during wine-making has been widely studied. The results of these studies have been reported in some reviews (Cabras et al., 1987; Flori and Cabras, 1990; Zironi et al., 1991; Farris et al., 1992). In these papers is reported that most of the pesticides remain adsorbed in the cake and lees, which are byproducts of wine-making, at relevant levels, and are transferred to the wine in low percentages. Only a few active ingredients (benalaxyl, dimethoate, and fenthion) are completely transferred from the grapes to the wine (Cabras et al., 1991, 1994, 1995). Therefore, the cake and lees contain most of the pesticide residues present on grapes. These byproducts are used in industry to produce alcohol and distilled beverage spirits. Since about 17 kg of cake with an average of 4.5% alcohol content is obtained from 100 kg of grapes (Garoglio, 1973), 2220 kg of cake is necessary to obtain 100 L of alcohol. If the pesticide residues present in grapes were to pass completely from the grapes to the cake, and from the latter to the alcohol, their residue levels would be 130 times higher than in grapes. Also, 140 kg of grapes produces approximately 1 hL of wine, which gives 5.5 kg of liquid lees at 4.5% alcohol content (Garoglio, 1973). If all pesticide residues were to be transferred from the grapes to the lees and from the lees to the alcohol, their residue levels would be 574 times higher than in grapes. Even wine is submitted to distillation to produce alcohol and brandy, but in this case if all residues were to be transferred to alcohol, as wine contains ca. 10% (v/v) of alcohol, they would be ca. 10 times concentrated. These theoretical concentration factors indicate that distilled spirits, which are used to produce alcoholic beverages such as brandy, cognac, and grappa, would have a toxic potential if the technological process did not contribute to decrease pesticide residues considerably. Despite the potential toxic risks presented by the distilled spirits of wine and its byproducts, no studies concerning the fate of pesticide residues during the distillation process have been reported in the current literature. This paper aims to contribute to the knowledge of this issue.

* Corresponding author (fax +39 70 300740).
† Università di Cagliari.
‡ Instituto de Química—UNESP.

MATERIALS AND METHODS

Chemicals. The pesticides and anilazine were analytical standards purchased from Ehrenstorfer (Augsburg, Germany). Triphenylphosphate (99%) was of analytical grade (Janssen, Geel, Belgium). Acetone, acetonitrile and benzene were HPLC grade solvents (Carlo Erba, Milano, Italy), while petroleum ether and *n*-hexane (Carlo Erba) were special reagents for pesticide determination. Cyclohexane and ethyl alcohol were analytical grade solvents (Carlo Erba). Anhydrous sodium sulfate and sodium chloride were of analytical grade (Carlo Erba). Stock standard solutions were prepared in absolute ethyl alcohol. Working standard solutions were obtained by dilution with acetone/petroleum ether (50/50, v/v) containing triphenylphosphate as internal standard (IS) at 0.3 ppm for GC-NPD determinations, or *n*-hexane containing anilazine as the internal standard at 0.5 ppm for GC-ECD determinations and with an acetonitrile/water mixture (50/50, v/v) for HPLC determinations.

Distillation Process. *Wine.* 200 mL of wine, at 12% alcohol (v/v), were distilled by using the Jaulmes apparatus (Lequeax, Paris). A fraction of 50 mL at 43.9% alcohol (v/v) was collected. Four of these fractions were redistilled together, and a second 50 mL fraction at 83% alcohol was collected.

Lees. 200 g of liquid lees at 11% of solid content and 10.1% of alcohol was distilled as described for wine. The first and second fractions collected had 29% and 76% alcohol, respectively.

Cake. 200 g of cake at 11.3% of alcohol was distilled using the Camboni apparatus, and a 50 mL fraction at 32.5% of alcohol was collected. This fraction was redistilled by the same procedure described for wine and lees, and a second fraction at 82% alcohol (v/v) was collected.

Apparatus. *Distillation.* Alcohol was stripped from wine and lees by a Jaulmes apparatus (described in Ribereau-Gayon et al., 1976), while a Camboni apparatus (described in Garoglio, 1973) was used for cake. These spirits were redistilled with a Jaulmes apparatus.

Chromatography. A gas chromatograph HRGC Mega 5160 (Carlo Erba, Milano, Italy) was employed, fitted with an N-40 nitrogen—phosphorus detector, an ECD 400 detector, an AS 550 autosampler (Carlo Erba), and a split—splitless injector, connected to an HP 3396-A reporting integrator (Hewlett-Packard, Avondale, PA).

S0021-8561(96)00457-8 CCC: $14.00

An HP model 1050 liquid chromatograph (Hewlett-Packard) was used. It consisted of a pump, an autosampler with a 100 μL loop, and a variable-wavelength UV/vis detector. The chromatograph was connected to an HP 3394 reporting integrator (Hewlett-Packard).

RESIDUE ANALYSIS

GC-NPD Determination: Dimethoate, Fenthion, Methidathion, Parathion Methyl, and Quinalphos. *Extraction Procedure.* A 5 mL aliquot of the distilled spirit was transferred to a screw-capped 30 mL test tube; 2 g of sodium chloride and 10 mL of an acetone/petroleum ether mixture (1:1, v/v) containing triphenylphosphate as the internal standard (IS) were added, and the flask was agitated in a rotating stirrer for 15 min. The phases were allowed to separate, and the organic layer was poured into another flask containing 2 g of anhydrous sodium sulfate and then injected for GC analysis.

GC Analysis. A Durabond fused silica column (30 m × 0.25 mm i.d.) (J&W Scientific, Folsom, CA) was employed, with a DB 1701 liquid phase (14% cyanopropylphenylmethylpolysiloxane, film thickness 0.25 μm). The injector and detector were operated at 250 and 290 °C, respectively. The sample (2 μL) was injected in the splitless mode (60 s), and the oven temperature programmed as follows: 90 °C for 1 min, raised to 180 °C (20 °C/min) and held for 1 min, and then raised to 280 °C (5 °C/min) and held for 4 min. Helium was the carrier and make-up gas at 120 and 130 kPa, respectively. Calibration graphs were constructed with the internal standard (IS) method by measuring peak heights *vs* concentrations. Good linearities were achieved in the range 0.01−3.0 ppm, with correlation coefficients between 0.9991 and 0.9995.

GC-ECD Determination: Procymidone and Vinclozolin. *Procedure.* A 100 μL aliquot of the distilled spirit was transferred to a 300 μL vial, evaporated to dryness under a nitrogen stream, taken up with 100 μL of *n*-hexane containing anilazine as is at 0.5 ppm, and injected for GC analysis.

GC Analysis. A Durabond fused silica column (30 m × 0.25 mm i.d.) (J&W Scientific, Folsom, CA) was employed, with a DB 5 liquid phase (5% phenylmethylpolysiloxane, film thickness 0.25 μm). The sample (1 μL) was injected in the split mode (1:20), and the oven temperature programmed as follows: 150 °C for 1 min and then raised to 250 °C (10 °C/min) and held for 2 min. Helium was the carrier gas at 100 kPa, and nitrogen was the make-up gas at 150 kPa. Calibration graphs were constructed with the internal standard method by measuring peak heights *vs* concentrations. Good linearities were achieved in the range 0.01−3.0 ppm, with correlation coefficients of 0.9992 and 0.9998.

HPLC Determination: Benalaxyl, Fenarimol, Iprodione, Metalaxyl, Myclobutanil, and Triadimefon. *Procedure.* A 1 mL aliquot of distilled spirit was transferred to an 8 mL vial, 2 mL of a cyclohexane/benzene mixture (1:1, v/v) was added, and the flask was agitated in a rotating stirrer for 10 min. The phases were allowed to separate, and 1 mL of the upper layer was transferred to a 1.8 mL vial. The solvent was evaporated to dryness under a nitrogen stream, taken up with 0.5 mL of an acetonitrile/water mixture (50/50, v/v), and injected for HPLC analysis.

HPLC Analysis. A Spherisorb S_5-C_8 column (250 × 4.6 mm i.d., 5 μm) (Phase Separations, Waddinxveen, Netherlands) was employed. An acetonitrile/water mixture (50:50, v/v) was used at the flow rate of 1 mL/min, and the wavelength was 200 nm. Quantitative determinations were performed by the external standard method by measuring peak heights *vs* concentrations. Good linearities were achieved in the range 0.01−1.5 ppm, with correlation coefficients between 0.9999 and 1.

Under the operating conditions of different methods, the limits of detection ranged between 0.001 and 0.003 mg/kg for the studied compounds.

Recovery Assays. Untreated distilled samples were fortified with pesticide and processed according to the above procedure. Recovery assays, carried out at 3.5, 1.5, 0.3, and 0.05 ppm, showed values ranging between 86% and 115% with a maximum CV of 11%.

RESULTS AND DISCUSSION

In this work thirteen pesticides among the active ingredients commonly used for controlling the parasites of grapes were studied. The study was carried out fortifying the wine, cake, and lees samples with the pesticides at different concentrations. Fortification levels were selected according to the maximum residue limits (MRL) established for grapes in Italy. The behavior of the residues was evaluated at the selected concentrations and at concentrations ten times lower. On the lees and cake samples, experiments were also performed at average levels 21 and 5 times higher than MRL, respectively. In industry to produce brandy or grappa, wine and its byproducts are usually submitted to a first distillation in a stripping column, which produces a spirit at ca. 30% of alcohol, followed by a second distillation in a rectifying column, to give a spirit at ca. 80% (De Rosa and Castagner, 1994). In our experiments we tried to reproduce the same scheme, by producing two distilled spirits at alcoholic contents similar to those in the industrial process and determining the pesticide residues in both. The results obtained in these experiments are reported in Tables 1 and 2.

The residues of fenarimol, methidathion, and myclobutanil present in the wine, lees, and cake samples at the studied concentrations did not pass to the distilled spirits.

Benalaxyl residues were found in first-distilled spirits obtained from the lees and cake fortified at the highest concentrations (30.7 and 7.7 ppm, respectively). In both distilled spirits the residue amount was the same (0.06 ppm), which is 0.2% and 0.8% of the residues present in the lees and cake, respectively. No residue was found in the final-distilled spirits of wine, lees, and cake at the studied concentrations.

The behavior of iprodione was similar to that of benalaxyl. In the first-distilled spirits that were obtained from lees and cake fortified at 19.2 and 11.8 ppm, respectively, 0.08 and 0.03 ppm of this fungicide were found. These amounts represented 0.4% and 0.3% of the initial residue. No residue was found in any of the final-distilled spirits.

Metalaxyl residues only passed to the first-distilled spirit when this was obtained from cake fortified at the highest concentration. From a initial residue of 10.97 ppm, 0.03 ppm (0.3%) was obtained. No residue was found in any of the final-distilled spirits.

Procymidone residues were found in the first-distilled spirits of wine and lees but not in those of cake. The residue percentages found in the distilled spirits were

Table 1. Residues (mg/kg ± sd) of Fungicides in the Distilled Spirits of Wine and Its Byproducts

fungicide	sample	concn	1st distn.	2nd dist.	fungicide	sample	concn	1st dist.	2nd dist.
benalaxyl	wine	1.16	n.d.[a]	n.d.	myclobutanil	wine	0.48	n.d.	n.d.
		0.15	n.d.	n.d.			0.06	n.d.	n.d.
	lees	30.7	0.06 ± 0.01	n.d.		lees	12.6	n.d.	n.d.
		1.16	n.d.	n.d.			0.48	n.d.	n.d.
		0.15	n.d.	n.d.			0.06	n.d.	n.d.
	cake	7.7	0.06 ± 0.06	n.d.		cake	2.5	n.d.	n.d.
		1.16	n.d.	n.d.			0.48	n.d.	n.d.
		0.15	n.d.	n.d.			0.06	n.d.	n.d.
fenarimol	wine	0.34	n.d.	n.d.	procymidone	wine	1.25	0.17 ± 0.01	n.d.
		0.04	n.d.	n.d.			0.17	0.02 ± 0.0	n.d.
	lees	6.1	n.d.	n.d.		lees	24.3	1.39 ± 0.21	n.d.
		0.34	n.d.	n.d.			1.25	0.08 ± 0.03	n.d.
		0.04	n.d.	n.d.			0.17	n.d.	n.d.
	cake	1.6	n.d.	n.d.		cake	4.86	n.d.	n.d.
		0.34	n.d.	n.d.			1.25	n.d.	n.d.
		0.04	n.d.	n.d.			0.17	n.d.	n.d.
iprodione	wine	2.44	n.d.	n.d.	triadimefon	wine	1.58	n.d.	n.d.
		0.31	n.d.	n.d.			0.2	n.d.	n.d.
	lees	19.2	0.08 ± 0.01	n.d.		lees	45.3	1.13 ± 0.14	n.d.
		2.44	n.d.	n.d.			1.58	n.d.	n.d.
		0.31	n.d.	n.d.			0.2	n.d.	n.d.
	cake	11.8	0.03 ± 0.01	n.d.		cake	9.06	n.d.	n.d.
		2.44	n.d.	n.d.			1.58	n.d.	n.d.
		0.31	n.d.	n.d.			0.2	n.d.	n.d.
metalaxyl	wine	2.2	n.d.	n.d.	vinclozolin	wine	1.6	3.16 ± 0.85	0.08 ± 0.03
		0.27	n.d.	n.d.			0.2	0.83 ± 0.27	n.d.
	lees	41.2	n.d.	n.d.		lees	26.1	2.09 ± 0.10	0.03 ± 0.01
		2.2	n.d.	n.d.			1.6	0.42 ± 0.18	n.d.
		0.27	n.d.	n.d.			0.2	n.d.	n.d.
	cake	10.97	0.03 ± 0.01	n.d.		cake	6.5	0.63 ± 0.21	n.d.
		2.2	n.d.	n.d.			1.6	0.43 ± 0.05	n.d.
		0.27	n.d.	n.d.			0.2	0.12 ± 0.14	n.d.

[a] n.d. = not detectable.

Table 2. Residues (mg/kg ± sd) of Insecticides in the Distilled Spirits of Wine and Its Byproducts

insecticide	sample	concn	1st dist.		insecticide	sample	concn	1st dist.	2nd dist.
dimethoate	wine	1.03	n.d.[a]	n.d.	parathion methyl	wine	0.25	0.05 ± 0.0	n.d.
		0.13	n.d.	n.d.			0.03	n.d.	n.d.
	lees	24.9	0.02 ± 0.01	n.d.		lees	5.0	0.51 ± 0.02	n.d.
		1.03	n.d.	n.d.			0.25	0.04 ± 0.0	n.d.
		0.13	n.d.	n.d.			0.03	n.d.	n.d.
	cake	4.90	n.d.	n.d.		cake	1.00	0.03 ± 0.02	n.d.
		1.03	n.d.	n.d.			0.25	n.d.	n.d.
		0.13	n.d.	n.d.			0.03	n.d.	n.d.
fenthion	wine	0.62	0.71 ± 0.06	0.08 ± 0.02	quinalphos	wine	0.25	0.11 ± 0.0	n.d.
		0.08	0.13 ± 0.02	n.d.			0.03	0.02 ± 0.0	n.d.
	lees	10.1	2.82 ± 0.5	0.19 ± 0.03		lees	4.6	0.48 ± 0.06	0.04 ± 0.01
		0.62	0.20 ± 0.02	n.d.			0.25	0.04 ± 0.0	n.d.
		0.08	0.05 ± 0.0	n.d.			0.03	n.d.	n.d.
	cake	2.50	0.07 ± 0.05	n.d.		cake	1.00	n.d.	n.d.
		0.62	0.03 ± 0.02	n.d.			0.25	n.d.	n.d.
		0.08	<0.01	n.d.			0.03	n.d.	n.d.
methidathion	wine	0.20	n.d.	n.d.					
		0.03	n.d.	n.d.					
	lees	4.90	n.d.	n.d.					
		0.20	n.d.	n.d.					
		0.03	n.d.	n.d.					
	cake	1.00	n.d.	n.d.					
		0.03	n.d.	n.d.					

[a] n.d. = not detectable.

12% for wine and 6% for lees. No residue was found in any of the final-distilled spirits.

For triadimefon, residues were found only in the first-distilled spirit obtained from lees fortified with 45.3 ppm. The amount present in the first-distilled spirit represented 2.4% of the initial residue. No residue was found in any of the final-distilled spirits.

Dimethoate showed a similar behavior to that of triadimefon. This AI passed only from lees fortified with 24.9 ppm to the first-distilled spirit at a percentage lower than 0.1%.

In the first-distilled spirit, vinclozolin, presented the highest amount of residue among the pesticides studied. In wine-distilled spirits, this AI was found respectively at concentrations of two and four times the initial residue present in the samples fortified at the highest and lowest levels. Considering that in the first-distilled spirit the alcoholic content is about four times higher than that of wine, this means that vinclozolin residues passed completely from wine to the distilled spirit at the lowest concentration, while at the highest concentration only 50% passed.

Lees behaved in the same way as cake, but their behavior was very different from that of wine. At the highest concentrations, ca. 9% of the initial residue was

transferred to the first-distilled spirit and this percentage increased on decreasing the concentration.

Residues of vinclozolin were found in the final-distilled spirit only when the initial residue in the first-distilled spirit was higher than 2 ppm. They were ca. 2% of the residue found in the first-distilled spirit. The residue levels found in the final-distilled spirits represented 5% and 0.1% of the initial residues in wine and lees, respectively.

Fenthion showed an analogous behavior to that observed for vinclozolin. The residue in the first wine-distilled spirit were higher than those in wine. The concentration factors of the distilled spirits were 1.1 and 1.6, respectively, for the highest and lowest levels of wine. Subsequent distillation in the rectifying column determined a remarkable residue decrease in the final-distilled spirit (0.08 ppm). This represented 13% of the initial residue of fenthion.

The results obtained for lees distillation showed that as the initial concentration of fenthion decreased, the percentage of residues transferred to the first-distilled spirit increased (28%, 32%, 63% for concentrations of 10.1, 0.62, and 0.08 ppm, respectively). After the second distillation, the residue found in the final-distilled spirit was 0.19 ppm. This amount represented 7% and 2% of the initial residues in the first-distilled spirit and lees respectively.

A low percentage of residue was transferred from the cake to the first-distilled spirit (3% and 5% for the highest and intermediate concentrations, respectively). No residue was found in any of the final-distilled spirits from cake.

Quinalphos showed the same behavior as fenthion. On decreasing the initial concentration of wine, the percentage of residues transferred to the first-distilled spirit increased (44% and 67% for concentrations of 0.25 and 0.02 ppm, respectively). An analogous behavior was observed with lees, but the percentage values were lower (10% and 16%). Residues were found only in the final-distilled spirit obtained from lees at 4.6 ppm. The amount present in this distilled spirit (0.04 ppm) was 8% of the residue in the first-distilled spirit, i.e., less than 1% of the initial residue.

Residues of parathion methyl were found in the first-distilled spirits obtained from wine, lees, and cake. The residue amounts represented 20%, 10%, and 3% of the initial concentrations in wine, lees, and cake, respectively. No residue was found in any of the final-distilled spirits.

CONCLUSIONS

Samples of wine, lees, and cake containing residues of the 13 studied pesticides yielded distilled spirits that were practically free of pesticide residues. During the distillation process, only quinalphos, fenthion, and vinclozolin were transferred to the final-distilled spirit. 13% of fenthion and 5% of vinclozolin passed from wine to the distilled spirit. These AI were also transferred to the final-distilled spirit obtained from lees but only when the initial concentration was very high and at low percentages (2% and 0.1% for fenthion and vinclozolin at 10.1 and 26.1 ppm, respectively). Quinalphos residues pass from lees to the distilled spirit at lower than 1% and only when at the highest concentration (4.6 ppm).

The remarkable decrease in residues during the rectifying process could depend on fact that very low amounts of residue are transported by alcoholic vapors while higher amounts are transported by water vapors. Consequently we should expect that distilled spirits of an alcoholic content of 95−96% would give lower residue concentrations. The highest residue concentrations used in our experiments are not expected to occur in industrial practice. In fact, it is supposed that the product submitted to a distillation process in industry is made up of several batches containing different AI. This would dilute the residue concentrations. It is assumed, therefore, that the distilled spirits produced in industrial systems should be free from residues of the studied pesticides.

LITERATURE CITED

Cabras, P.; Meloni, M.; Pirisi, F. M. Pesticide fate from vine to wine. *Rev. Environ. Contam. Toxicol.* **1987**, *99*, 83−117.

Cabras, P.; Porcu, M.; Spanedda, L.; Cabitza, F. The fate of the fungicide benalaxyl from vine to wine. *Ital. J. Food Sci.* **1991**, *3*, 181−186.

Cabras, P.; Garau, V. L.; Melis, M.; Pirisi, F. M.; Cubeddu, M.; Cabitza, F. Pesticide residues of dimethoate and chlorpyrifos in grapes and wine. *Proc. Giornate Fitopatologiche* **1994**, *1*, 27−32.

Cabras, P.; Garau, V. L.; 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.

De Rosa, T .; Castagner, R. *Tecnologia delle grappe e dei distillati d'uva*; Edagricole: Bologna, Italy, 1994; pp 176−179.

Farris, G. A.; Cabras, P.; Spanedda, L. Pesticide residues in food processing. *Ital. J. Food Sci.* **1992**, *3*, 149−169.

Flori, P.; Cabras, P. Pesticide residues in wine. *Vignevini* **1990**, *17* (7−8), 31−37.

Garoglio P. G. *Enciclopedia Vitivinicola Mondiale*; Ed. Sci. U.I.V.: 1973, Vol. 8, pp 61−66, 75.

Ribereau-Gayon, J.; Peynaud, E.; Ribereau-Gayon, P.; Sudraud, P. In *Sciences et techniques du vin. Vol. 1. Analyse et controle des vins*; Dunod: Paris, 1976, pp 51−58.

Zironi, R.; Farris, G. A.; Cabras, P.; Fatichenti, F. Pesticide residues from vine to wine. *Atti. Accad. Ital. Vite Vino, Siena* **1991**, *43*, 351−369.

Received for review June 24, 1996. Revised manuscript received March 3, 1997. Accepted March 5, 1997.[⊗] E.V.M. acknowledges the CNPq−Brasil for a fellowship.

JF960457L

[⊗] Abstract published in *Advance ACS Abstracts,* May 1, 1997.