Evolution of Residual Levels of Six Pesticides during Elaboration of Red Wines. Effect of Wine-Making Procedures in Their Dissappearance

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The effect of wine-making procedures on the concentrations of six pesticides (chlorpyrifos, penconazole, fenarimol, vinclozolin, metalaxyl, and mancozeb) in red wines has been studied. During maceration stage (4 days), the percentage remaining of chlorpyrifos, penconazole, and metalaxyl was ∼90%, whereas that of fenarimol, vinclozolin, and mancozeb is somewhat smaller (74−67%). The residual levels found in pressed must were ≤20%, except for metalaxyl (69%). From the whole concentration of chlorpyrifos in must, 85% is eliminated from the racking step; clarification (bentonite plus gelatin) eliminates 43% of the product found in the decanted wine, and with filtration, all of the residue is eliminated. Penconazole and mancozeb are retained on the lees in proportions of 43 and 31%, respectively. The eliminated percentage of vinclozolin after racking is 29%, whereas clarification and filtration reduce its proportion in the wine to 33 and 28%, respectively. Finally, fenarimol and metalaxyl are eliminated in smaller proportion with the realization of these processes.

Keywords: *Pesticide residues; red wine; wine-making procedures*

INTRODUCTION

On the whole world surface dedicated to the crop of grapevine (8016×10^3 Ha) in 1994, 70% (5452×10^3 Ha) corresponds to Europe. Spain is the European Union (EU) country that dedicates the largest surface to its cultivation (1281×10^3 Ha), 16% of the whole world and 24% of the EU, followed by Italy and France, although these two countries obtain greater production due to the use of soils with better agricultural quality (Tinlot and Rousseau, 1996). A total of 649 792 Ha of vineyard are inscribed in 41 Apellations d'Origine Controlles (AOC) in Spain, and 7% (45 361 Ha) corresponds to the Jumilla wine-producing region in Murcia (southeastern Spain), an area of great viticultural importance characterized by a quite low annual mean precipitation (273 mm) and a relatively high annual mean temperature (16 °C). The peculiar climatic characteristic of this area favor the development of certain pests and diseases, the most important ones, for their economic repercussion are lepidopterons (*Lobesia botrana*), powdery mildew (*Uncinula necator*), downy mildew (*Plasmopora viticola*), and, on some occasions, gray mold (*Botrytis cinerea*).

As important as effective combat of weeds, careful working of the soil, correct fertilization, or a system of rational pruning is the sanitariness of the vine stock. For that reason, the vine growers need to protect their crops with pesticides, but it is necessary to keep in mind that when the established preharvest time for each product is not respected, residues on grapes can pass to the must and later to the wine, causing a rising risk for the health; in certain cases, the final quality of the wine could be altered. In this sense, the elaboration system (white, rosé, or red wine) and the correct realization of the wine-making process influence in a decisive way the dissipation and/or elimination of the current residues in grapes and must. Many researchers have studied these topics, and the results of these studies have been published in some reviews and papers (Cabras et al., 1987; Flori and Cabras, 1990; Farris et al., 1992; Coscollá, 1993; García and Xirau, 1994; Navarro et al., 1997). With this aim, this study was designed to realize two objectives: (i) to know the evolution of the residues of six pesticides, one insecticide (chlorpyrifos) and five fungicides (fenarimol, mancozeb, metalaxyl, penconazole, and vinclozolin) often used in Jumilla, during the elaboration of red wines obtained with Monastrell grapes and (ii) to evaluate the influence of wine-making procedures (maceration, pressing, racking, clarification, and filtration) in the elimination of the residues of those compounds.

EXPERIMENTAL PROCEDURES

Phytosanitary Treatments and Sampling. Prior to the phytosanitary treatments, we chose parcels of land in a 15-year-old plantation of vineyards (Monastrell variety) in full production. The experimental plot was situated in Jumilla, Murcia (southeastern Spain). The vine stocks had a plantation density of 2.5×2.5 m^2, and they were in perfect nutritional and physiological condition. The experiment was carried out in 18 unitary parcels of 25 m^2 each (10 vine stocks/parcel), and the treatment was carried out three times. Within each parcel a line was left without treatment. One parcel was left without treatment as a control. During September 1996 we

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Table 1. Phytosanitary Products and Treatment Dose

active ingredient	formulation	dose	preharvest time (days)	MRL[a] (mg/kg)
penconazole	Topas 10% w/v EC	30 mL/hL	14	0.2
fenarimol	Rubigan 12% w/v EC	25 mL/hL	28	0.2
metalaxyl	Ridomil Plus 5% WP	400 g/hL	21	0.5
vinclozolin	Ronilan 50% WP	125 g/hL	21	5
chlorpyrifos	Dursban 48% w/v EC	175 mL/hL	21	0.5
mancozeb	Ventine MZ 80% WP	350 g/hL	15	5 (as CS_2)

[a] Established for wine grapes by Spanish legislation.

carried out the phytosanitary treatments specified in Table 1. The dose used was that recommended in the vineyard by the respective agrochemical companies (Liñan, 1998). The application of the products was carried out in every case by using a Senior knapsack sprayer of 20 L with a conic opening of 2 mm and an exit pressure of 3 atm. Water was used as a dispersion medium, and all of the vine stocks were wetted to the dripping point. The quantity used per vine stock was ~3 L for parcel (300 mL/vine stock). The mean speed of the wind during the application was 0.56 m/s and the temperature 22.5 °C.

From each parcel, we harvested fruits at every height, depth, and orientation, taking into account that the size of the fruits was always as homogeneous as possible. The weight of the field samples was ~20−25 kg. We took the sample for wine making 24 h after the application. Prior to the treatments, we gathered samples of the whole plantation to check the possible existence of residues coming from a previous application.

Must and Wine Production. When the fruit was delivered to the cellar, 15 kg of grapes was pressed with a drum press and stems were removed at this time to avoid giving the must woody flavors. The crushed harvest was allowed to ferment with the skins (vinification with maceration) in 30 L capacity flasks. The weights of sample (kilograms ± SD, $n = 3$) obtained after the realization of the wine-making processes for the six compounds were as follows: crushing, 14.51 ± 0.04; pressing, 2.89 ± 0.09 for pomace and 11.62 ± 0.08 for must; and racking, 0.95 ± 0.08 for lees and 10.65 ± 0.12 for decanted wine. Fermentation had a regular course (14−16 days) in all flasks, and 3 days after racking, the wines were clarified (bentonite plus gelatin) and filtered (nylon). Figure 1 shows the followed procedure for standard wine making.

Pesticides and Reagents. Pesticide analytical standards were purchased from BASF AG (vinclozolin), DowElanco (chlorpyrifos and fenarimol), and Novartis BCM (metalaxyl and penconazole). These chemicals were at least 99.5% pure. The internal standard (I.S.) β-endosulfan 99.6% ([1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-ylenebis(methylene)] sulfite) was kindly supplied by (Supelco). Acetone, dichloromethane, isooctane, and toluene were for pesticide residues (SDS, France); anhydrous sodium sulfate was analytical grade (Panreac). Stock standard solution containing all pesticides (~50 ng/μL each) were prepared in isooctane plus toluene (1 + 1, by volume). Several dilutions were prepared to check the linearity of response of detectors and to obtain the detection limits in each case by dilution in the same solvent with β-endosulfan (1.46 mg/L) as I.S.

Extraction Procedure. Once in the laboratory, after eliminating the stems, we proceeded to obtain the representative subsample by aleatory selection of enough number of units and, later on, to the crushing of the same one by utilization of a crusher (Osteirrizer). The sample so obtained (500 g) was subdivided in two approximately equal parts and frozen in polyethylene boats to −30 °C until its analytical determination. For the extraction of penconazole, fenarimol, metalaxyl, vinclozolin and chlorpyrifos residues in grapes, must, and wine, a micro on-line extraction method (Oliva et al., 1998) has been used. The vegetable material is extracted with an acetone/dichloromethane mixture, filtering, and concentrating the obtained extract next.

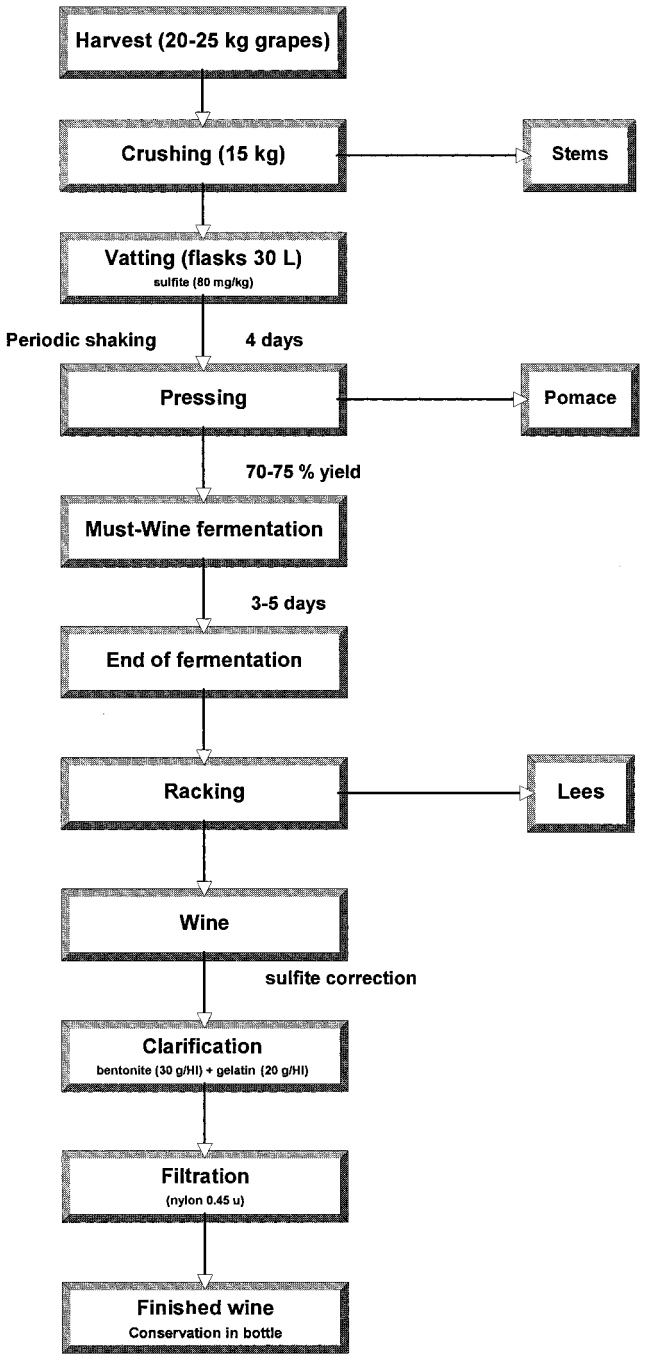

Figure 1. Scheme for standard wine making.

(a) Extraction in Grapes. Five grams of grapes was homogenized at 3000 rpm during 10 min in a high-speed electric mixer (Omni-Mixer, Sorvall) with 30 mL of the mixture solvent acetone/dichloromethane (1:1, v/v), 2 g of Celite, and 1 g of NaCl. The mixture was filtered through a funnel of porous plate no. 4, and the filtrate is passed through 1 PS Phase Separator paper (Whatman 2100150), washing flask and filter with 10 mL of the mixture solvent. All of the fractions were picked up in a concentration flask and concentrated to dryness by rotary vacuum evaporation. The dry extract was dissolved in 5 mL of the mixture isooctanee/toluene (1:1, v:v) that contains β-endosulfan (1.46 mg/l) as I.S.

(b) Extraction in Must and Wine. Five milliliters of must or wine was placed in a glass tube of 30 mL with hermetic closing, with 20 mL of the mixture solvent being acetone/dichloromethane (1:1, v/v) and 2 g of anhydrous NaCl. The tube is agitated smoothly for 20 min, in a shaker (Unite-Mixer Lab Line 1306, Biomedical Products, Inc.), and the liquid is passed

Table 2. Residual Concentrations ($n = 3$, Milligrams per Kilogram) Found in the Different Control Stages

	$\bar{x} \pm$ SD					
stage	penconazole	chorpyrifos	vinclozolin	mancozeb	fenarimol	metalaxyl
crushed grape	0.575 ± 0.061	2.005 ± 0.262	1.133 ± 0.042	3.047 ± 0.516	0.828 ± 0.187	0.650 ± 0.181
maceration	0.505 ± 0.063	1.858 ± 0.143	0.805 ± 0.045	2.247 ± 0.068	0.553 ± 0.119	0.620 ± 0.053
pomace	**1.083 ± 0.214**	**3.335 ± 0.110**	**1.567 ± 0.157**	**1.753 ± 0.132**	**0.908 ± 0.053**	**0.763 ± 0.212**
must, 4 days	0.175 ± 0.024	0.338 ± 0.026	0.380 ± 0.099	0.857 ± 0.023	0.308 ± 0.034	0.563 ± 0.060
must, 6 days	0.140 ± 0.021	0.225 ± 0.079	0.355 ± 0.072	0.617 ± 0.122	0.167 ± 0.037	0.550 ± 0.062
must, 8 days	0.125 ± 0.008	0.143 ± 0.012	0.342 ± 0.065	0.360 ± 0.079	0.157 ± 0.027	0.527 ± 0.045
must, 11 days	0.117 ± 0.015	0.070 ± 0.024	0.332 ± 0.080	0.233 ± 0.117	0.150 ± 0.040	0.503 ± 0.050
must, 14 days	0.108 ± 0.023	0.033 ± 0.014	0.287 ± 0.020	0.123 ± 0.006	0.140 ± 0.060	0.483 ± 0.057
lees	**0.908 ± 0.073**	**3.540 ± 0.337**	**1.298 ± 0.107**	**3.930 ± 0.252**	**0.808 ± 0.082**	**1.617 ± 0.204**
decanted wine	0.105 ± 0.037	0.030 ± 0.009	0.257 ± 0.010	<DL[a]	0.148 ± 0.026	0.457 ± 0.059
wine, 21 days	0.093 ± 0.010	0.027 ± 0.005	0.227 ± 0.020	<DL	0.143 ± 0.035	0.450 ± 0.044
not clarified	0.093 ± 0.010	0.022 ± 0.004	0.218 ± 0.010	<DL	0.133 ± 0.026	0.430 ± 0.052
clarified wine	0.078 ± 0.013	0.012 ± 0.006	0.145 ± 0.012	<DL	0.132 ± 0.028	0.410 ± 0.044
filtered wine	0.058 ± 0.010	<DL	0.105 ± 0.010	<DL	0.118 ± 0.025	0.403 ± 0.038
filtered, 180 days	0.043 ± 0.006	<DL	0.073 ± 0.006	<DL	0.080 ± 0.010	0.347 ± 0.032
not filtered, 180 days	0.040 ± 0.010	<DL	0.057 ± 0.006	<DL	0.080 ± 0.010	0.330 ± 0.030

[a] DL, detection limit.

through 1 PS Phase Separator paper (Whatman 2100150), washing tube and filter with 10 mL of the mixture solvent. All organic fractions are evaporated through rotary vacuum evaporation, and the residue is dissolved in 5 mL of isooctane/toluene (1:1, v/v) that contains β-endosulfan (1.46 mg/L) as I.S.

Mancozeb residues in grapes, must, and wine (100 g) were determined by decomposition with HCl and measuring the carbon disulfide liberated (Keppel, 1971).

Recovery of Pesticides. To determine percent recovery of chlorpyrifos, fenarimol, metalaxyl, penconazole, and vinclozolin, untreated grapes, must, and wine were spiked with concentrations of pesticides in the range 0.1−1 mg/kg. The results of recovery analysis were compared with those in standard dilutions used in the fortification. The recovery assays were replicated five times. After the evaporation of the spiking solvent, the samples were processed in the way indicated previously.

Pesticide Analysis. Chlorpyrifos, fenarimol, penconazole, and vinclozolin were determined by GC with an electron capture detector (ECD). In all cases, a Perkin-Elmer Nelson 1020 integrator was used in combination with the gas chromatograph (Autosystem Perkin-Elmer). A fused silica capillary column (SPB-5, Supelco Inc.) packed with 5% diphenyl, 94% dimethyl, 1% vinyl polyxiloxane, 30 m by 0.32 mm i.d., and film thickness 0.25 μm was used. The operating temperatures were as follows: injection port, 250 °C; detector, 320 °C; column oven, initial 90 °C, hold 1 min, programming rate 30 °C/min (from 90 to 210 °C), hold 0 min, 10 °C/min (from 210 to 240 °C), hold 0 min, 5 °C/min (from 240 to 270 °C), and hold 7 min at 270 °C. The carrier gas was N_2 at 15 psi; makeup detector was 30 mL/min. Injection mode was splitless (45 mL/min) with valve opening of 30 s.

Metalaxyl was determined by GC with a mass selective detector (MSD). A Hewlett-Packard 6890 gas chromatograph was employed. It was fitted with an MSD HP 5971 (Hewlett-Packard) and a split−splitless injector, connected to an HP Vectra 500 integrator (Hewlett-Packard). An HP-5MS fused silica column (30 m × 0.25 mm i.d.) was employed, with 5% diphenyl, 95% dimethyl siloxane liquid-phase (film thickness = 0.25 μm) (Hewlett-Packard). The injector and interface were operated at 250 and 280 °C, respectively. The operation conditions were as follows: acquisition mode, SIM; voltage, 1247 V; ionization foil temperature, 230 °C; quadrupole temperature, 150 °C; solvent delay, 8 min; and selected ion m/z 132, 160, 206, 234, 249, and 279. The carrier gas was He at 1.2 mL/min. The sample (2 μL) was injected in the splitless mode (60 s), and the oven temperature was programmed as follows: 90 °C for 1 min, raised to 210 °C (10 °C/min), to 240 °C (5 °C/min), and to 270 °C (30 °C/min), and held for 3 min.

The identity of chlorpyrifos, fenarimol, penconazole, and vinclozolin residues in grapes, must, and wine extracts was confirmed by GC-MSD in the same conditions mentioned previously. Scan mass range was 50−290 and selected ion monitoring (SIM) as follows: β-endosulfan, m/z 121, 159, 195, 237, 267, 339; chlorpyrifos, m/z 97, 197, 258, 286, 314; fenarimol, m/z 107, 139, 219, 251, 330; penconazole, m/z 115, 159, 213, 248; and vinclozolin, m/z 53, 124, 178, 212, 285.

Quantitative determination of mancozeb residues has been realized by colorimetric measure of the yellow complex [cupric salt of *N*,*N*-bis(hydroxyethyl)dithiocarbamic acid] at 435 nm with utilization of a Hitachi 2000 spectrophotometer. Previously, we realized a linear regression with carbon disulfide, between 9.6 and 192 μg ($r = 0.998$).

RESULTS AND DISCUSSION

Analytical Determination. The obtained correlation coefficients (0.9986 for penconazole and 0.9977 for chlorpyrifos) showed a great correlation between concentration and area for the studied compounds. The obtained detection limits were 0.025, 0.0047, 0.0049, 0.0076, and 0.0033 ng for metalaxyl, chlorpyrifos, fenarimol, penconazole, and vinclozolin, respectively, and 1 μg for mancozeb.

Mean recovery ($n = 5$) in grapes was >85% in all cases with the exception of fenarimol, for which the values were located at ∼80%. The coefficients of variability ranged between 3.9 and 8% in the most unfavorable case. In must and wine, the recoveries from fortified samples were in the range of 87.4−100.6 with coefficient of variability of 3.7−7.4%. The obtained values allowed us to affirm that the used extraction method is appropriate for the determination of residual levels of the studied compounds.

The limits of determination calculated for grapes were 0.002, 0.001, 0.011, 0.003, and 0.001 mg/kg for chlorpyrifos, fenarimol, metalaxyl, penconazole, and vinclozoline, respectively. In must and wine, the limits for the same compounds were 0.002, 0.002, 0.012, 0.004, and 0.001. Those limits are, in all cases, very inferior to the maximum residue limit (MRL) established by the different legislations for those compounds.

Dissipation of Residues. From the crushing and destemming of wine grapes until 6 months later, we took different samples (including pomace and lees) to study the evolution of residual levels during the different elaboration steps. The residual concentrations found during this period are shown in Table 2. In all cases, the residual levels found in the crushed grape are superior to MRL established by the Spanish legislation for wine grapes, except for vinclozolin and mancozeb, both of which had MRLs of 5 ppm.

Table 3. Amount (Milligrams) of Pesticide (n = 3) in the Whole Weight of Sample (Kilograms) for Each Control Stage and Percentage Remaining

	penconazole		chlorpyrifos		vinclozolin		mancozeb		fenarimol		metalaxyl	
stage	mg	%	mg	%	mg	%	mg	%	mg	%	mg	%
crushed grape	8.35	**100**	29.1	**100**	16.4	**100**	44.2	**100**	12.1	**100**	9.46	**100**
maceration	7.33	**87.8**	26.9	**92.6**	11.6	**71.0**	32.6	**73.7**	8.05	**66.8**	9.01	**95.2**
pomace	**3.14**	**37.6**	**9.77**	**33.6**	**4.45**	**27.2**	**5.25**	**11.9**	**2.54**	**21.0**	**2.22**	**23.5**
must, 4 days	2.03	**24.3**	3.91	**13.5**	4.42	**26.9**	9.87	**22.3**	3.62	**30.0**	6.54	**69.1**
must, 6 days	1.62	**19.2**	2.60	**8.9**	4.13	**25.2**	7.10	**16.1**	1.96	**16.3**	6.39	**67.5**
must, 8 days	1.45	**17.2**	1.65	**5.7**	3.97	**24.2**	4.14	**9.4**	1.85	**15.4**	6.12	**64.7**
must, 11 days	1.36	**16.3**	0.81	**2.8**	3.86	**23.6**	2.68	**6.1**	1.76	**14.6**	5.85	**61.8**
must, 14 days	1.25	**15.0**	0.38	**1.3**	3.33	**20.3**	1.41	**3.2**	1.65	**13.7**	5.61	**59.3**
lees	**0.88**	**10.5**	**3.31**	**11.4**	**1.30**	**7.93**	**3.60**	**8.2**	**0.72**	**6.0**	**1.60**	**16.9**
decanted wine	1.12	**13.3**	0.32	**1.10**	2.75	**16.8**	ND[a]	**ND**	1.60	**13.3**	4.86	**51.4**
wine, 21 days	0.99	**11.9**	0.28	**1.00**	2.41	**14.7**	ND	**ND**	1.55	**12.9**	4.79	**50.6**
not clarified	0.99	**11.9**	0.23	**0.80**	2.31	**14.1**	ND	**ND**	1.44	**11.9**	4.57	**48.3**
clarified wine	0.83	**9.90**	0.13	**0.50**	1.54	**9.40**	ND	**ND**	1.43	**11.9**	4.36	**46.1**
filtered wine	0.62	**7.29**	ND	**ND**	1.11	**6.80**	ND	**ND**	1.39	**11.5**	4.29	**45.4**
filtered, 180 days	0.46	**5.28**	ND	**ND**	0.77	**4.70**	ND	**ND**	0.87	**7.20**	3.69	**39.0**
not filtered, 180 days	0.42	**5.10**	ND	**ND**	0.60	**3.70**	ND	**ND**	0.87	**7.20**	3.51	**37.1**

[a] ND, not detected.

Keeping in mind the yields obtained after the wine-making procedures of crushing, pressing, and racking, the total residual amount (milligrams) present in each of the wine-making flasks was calculated, as shown in Table 3, with the purpose of being able to know the evolution and losses of residues during the whole process. The total amount present in the crushed grape was considered as 100% in each case and the starting point to study the disappearance of the pesticides.

As we can see in Table 3, the remaining amounts at the end of the sampling on wine, bottled and filtered, 6 months later, do not overcome 7.5% in any case with the exception of metalaxyl, for which the value risess to 39%.

The residual level of penconazole in must, after pressing, is already below its MRL (0.2 ppm). After racking and filtration, the values are 0.105 and 0.058 ppm, respectively. Six months later, this level is 0.043 ppm, which supposes 23% of the concentration found in the must immediately after pressing. Studies carried out with this fungicide indicate that, in finished red wines, percentage remaining is nearly 15% of the initial dose (0.3 mg/kg) in must (Flori et al., 1992; Scarponi and Martinetti, 1992; Lanari et al., 1993). However, for white wines elaborated in cold or ambient temperature and with or without clarifying substances, the losses are smaller, between 60 and 70% (Lanari et al., 1993), and for rosé wines, the decrease is 48%, leaving 1 ppm as initial concentration (Navarro et al., 1997). Another fungicide of the same family, propiconazole, decreases during the wine-making procedure by 90% for red wines, a value very similar value to those of the pesticides studied by us (FAO/WHO, 1988).

In the same way, the residual level of chlorpyrifos in must, once separated from the pomace, is below the MRL (0.5 ppm). Once the wine is decanted, after racking, the value falls to 0.03 ppm (9% of the residue found in must after pressing). Residues in clarified wine (0.012 ppm) disappear with the filtration process. These values are similar to those found in the bibliography. In vineyards treated 2 days before the gathering, it was observed that chlorpyrifos and other organophosphorus insecticides (parathion-methyl and fenitrothion) were not detected at the end of fermentation (Sala et al., 1996). In the case of chlorpyrifos-methyl, parathion-methyl, and quinalphos, reductions were 80%, whereas only 50% of metidathion was lost during the wine making. The elaboration with or without maceration does not have any influence (Cabras et al., 1995). Other organophosphorus compounds such as omethoate and dimethoate show quite smaller reductions than chlorpyrifos, ∼30% (Steller and Pasarela, 1972).

The residual level of vinclozolin is found below the MRL (5 ppm) in the control carried out on the crushed vintage. Once the fermentation of must is finished (after racking and before clarification), it remains 68% of the initial concentration approximately in the must, being finally 6 months later 19% of the original amount. Numerous studies exist on the vinclozolin behavior and other dicarboximidic fungicides (iprodione, procymidone, and chlozolinate) during wine making. These studies show that the losses oscillate around 90−99% for vinclozolin and around 80% for the others (Zironi et al., 1981; Flori et al., 1982b, 1994; Lemperle et al., 1982; Cabras et al., 1986; FAO/WHO, 1986; Flori and Cabras 1990; Bernard et al., 1995). Some works carried out in Spain, in the viticultural areas of Penedés and Rioja (Zaballa et al., 1992; García and Xirau, 1994), show that the vinclozolin reduction in red wines is located around 80% of the initial concentration in the must. This value is larger than the one observed by us, and it is calculated at 72% after the clarification and filtration of the wine.

Mancozeb is one of the studied products that disappears more quickly, although its initial concentration in grapes (3.05 ppm) is the highest. In decanted wine, there are no residues, although it is necessary to keep in mind that its limit of determination (0.01 ppm) is higher than the limits of the other studied compounds. For this product, less studied than others, the values found in the bibliography coincide with those obtained by us; in finished wine, the elimination is practically 100%. Something similar happens with other dithiocarbamates such as zineb and propineb (Lemperle et al., 1975; Ripley et al., 1978; FAO/WHO, 1985; Zaballa et al., 1992; McLean, 1996).

With regard to fenarimol, during the fermentation, 52% of the initial residue in must is eliminated. Finally, 6 months later, 26% of the initial amount remains in the filtered wine. Bibliographical data indicate that the elimination of fenarimol residues in red wines is ∼40% from the pressed must until the finished wine. These values are lower than those obtained by us (52%). The same thing happens if we compare the initial values in the grape with those values at the end of fermentation,

whereas in the bibliography a remnant of 17% appears, in our assay it is 7%. These values indicate how easily this product is adsorbed on pomace and lees (Scarponi and Martinetti, 1992; Lanari et al., 1993). For rosé wines, the fenarimol loss, from the must to finished wine, is 33% (Navarro et al., 1997), whereas in white wines, the dissipation is 50% of the initial value in the must and 70% in the vintage grapes (Lanari et al., 1993).

In relation with the six studied products, metalaxyl is the one that suffers smaller losses. Eleven days after crushing, the residual level is still very high, being located very close to its MRL (0.5 ppm). The losses during the fermentation are small (19%), and even 6 months later the remnant concentration is apparently 62% of the initial. Data from the scientific literature show that this product and others from the same family (benalaxyl and furalaxyl) present some differences with those pesticides obtained in our experiment. The reduction in finished wine is located in our case around 45% with regard to the initial dose in grapes (0.65 ppm), whereas in other experiments are observed reductions that oscillate from 65% for concentrations of 0.52 ppm up to 86% for 2.10 ppm (FAO/WHO, 1983, 1986; Cabras et al., 1986; Kakalikova et al., 1996). However, in other authors' studies, the values found are very similar to ours. Among them we can mention those of Flori and Cabras (1990) and Lanari et al. (1993), with a reduction of 40% in relation to the amount found in grapes. For white wines, obtained in ambient temperature, the reduction is 30% and in elaboration in cold the reduction is 10% (Lanari et al., 1993).

In relation to the losses, mainly for degradation, that took place during the conservation in bottle, from the obtaining of the filtered wine (30 days) until 180 days, it could be observed that the compound that suffers the largest decrease is fenarimol, followed by vinclozolin, penconazole, and metalaxyl with percentages of 37, 31, 26, and 14%, respectively. Mancozeb and chlorpyrifos no longer appear after filtration. In this sense, Kawar et al. (1979) indicate that dimethoate residues, in wine stored at 24 °C, are hydrolyzed with a half-life of 30 days, remaining stable during 1 year in a freezer. Gnaegi and Lipka (1974) showed that residues of BMC in wine remain unaffected during 1 year. The influence of other factors, besides the temperature, on the degradation has also been studied. In this way, Cabras et al. (1984) studied the influence of pH of the wine in the disappearance of chlozolinate, vinclozolin, procymidone, and iprodione residues; the kinetic data show that at pH 3, the degradation order is chlozolinate > vinclozolin > procymidone, with $t_{1/2} = 0.35$, 48.5, and 71.3 days, respectively. When the pH is increased from 3 to 4, the degradation rate, mainly for chlozolinate, vinclozolin, and procymidone, also increases.

With the purpose of knowing the dissipation rate of residues in the must wine, the experimental data have been fitted to the model $\ln R_t = \ln R_0 - Kt$ (Timme and Frehse, 1980). The study begins the fourth day (after pressing), the moment when the density begins to considerably decrease and it concludes on day 21 (after racking), once fermentation is concluded. This period (17 days) coincides with the maximum activity of the yeasts and, therefore, the losses of residues can be greater for several reasons (sedimentation, degradation, etc.). In Table 4, the derived values of the carried out fit are shown. In all cases it is verified that a good linear correlation exists between $\ln R_t$ and the time ($r \geq 0.95$) except for fenarimol, for which the corresponding coefficient is smaller ($r = 0.78$).

Table 4. Linear Fit of the Data for the Dissipation of Pesticides during Fermentation of the Must (17 Days)

parameter	penconazole	chlorpyrifos	vinclozolin	mancozeb	fenarimol	metalaxyl
K	−0.0304	−0.1286	−0.0229	−0.1355	−0.0311	−0.0111
r	−0.9766	−0.9527	−0.9621	−0.9885	−0.7799	−0.9843
R_0	0.1676	0.3192	0.3950	0.9300	0.2293	0.5667
$t_{1/2}$ (days)	22.8	5.4	30.3	5.1	22.3	62.4

In relation with the calculated values of the constant rate (K), according to a pseudo-first-order kinetic, the following dissipation rate is observed: mancozeb > chlorpyrifos > fenarimol > penconazole > vinclozolin > metalaxyl. It is corroborated by the half-life times ($t_{1/2}$) obtained.

Dealing with the differences between the real concentration obtained and the theoretical concentration calculated (R_0) at 4 days, the deviations are quite small: 0.074, 0.018, −0.015, − 0.073, 0.078, and − 0.003 for penconazole, chlorpyrifos, vinclozolin, mancozeb, fenarimol, and metalaxyl, respectively.

Influence of Wine-Making Procedures. Percentages of residues remaining for the six products after the wine-making processes are shown in Figures 2 and 3. During maceration step (4 days), the concentrations of metalaxyl, chlorpyrifos, and penconazole remaining are located around 90%, whereas mancozeb, vinclozolin, and fenarimol decrease more, between 74 and 67%. Because most pesticides are more soluble in ethyl alcohol than in water, one would expect less residue reduction in wine making with maceration than without, when prefermentation separation is used. However, it is true that maceration of the dregs can lead to a greater amount of suspended matter, which could adsorb the residual pesticides and compensate the effect of the ethyl alcohol (Farris et al., 1992). In bibliographical references some contradictions exist on the influence of this wine-making step in the elimination of residues. In this way, Flori et al. (1982a) and Cabras et al. (1986) indicate that there is a loss in maceration for vinclozolin, metalaxyl, penconazole, and fenarimol, whereas other authors (Miller et al., 1985; Cabras et al., 1995) conclude that for methiocarb and some organophosphorus insecticides (chlorpyrifos, methidation, quinalphos, and parathion-methyl) do not exist significant differences when the maceration is or is not carried.

Once the must is pressed, the pesticide that remains in greatest proportion in pomace is penconazole (37.6%), with a smaller amount of mancozeb (12%). It is necessary to keep in mind that with this process an important amount of the initial residue is eliminated; on the other hand, it will be necessary to evaluate if the found levels allow the later use of the pomace as a subproduct for obtaining feeds, colorings, etc. According to diverse authors (Flori and Cabras, 1990; Scarponi and Martinetti, 1992; Lanari et al., 1993), some products such as penconazole, fenarimol, vinclozolin, and metalaxyl have a high power of adsorption in the residue. That is the reason, during pressing, the loss will be greater than for other assayed compounds. These data are fully coincident with those data obtained in our assay.

The levels found in must, once pressed, are located around 20% and even less except for metalaxyl (69%), which has a higher solubility in water (7.1 g/L) and is

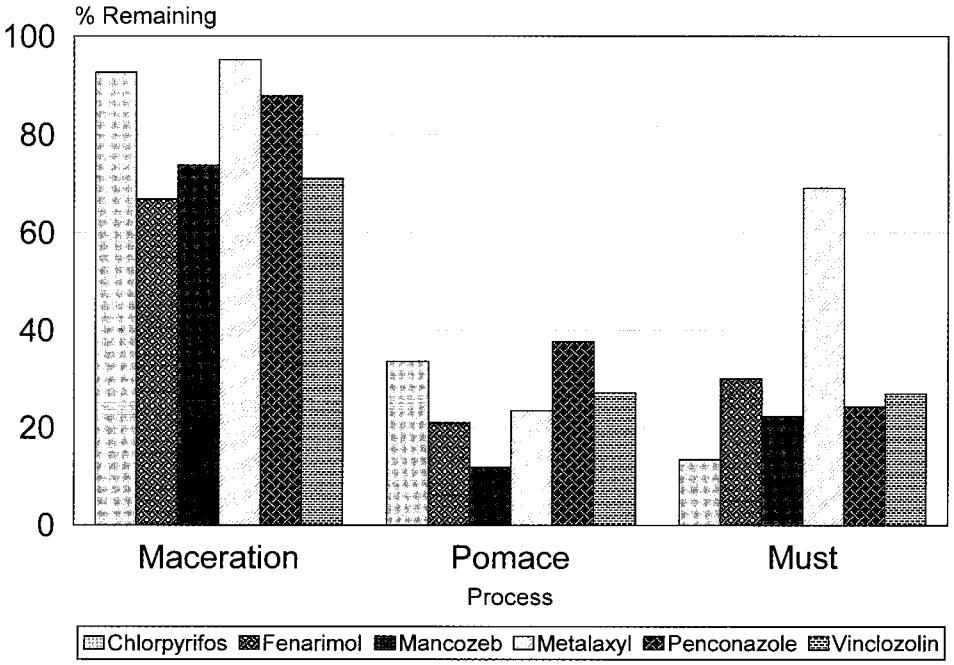

Figure 2. Percentage of pesticide residues remaining after maceration and pressing of the grapes.

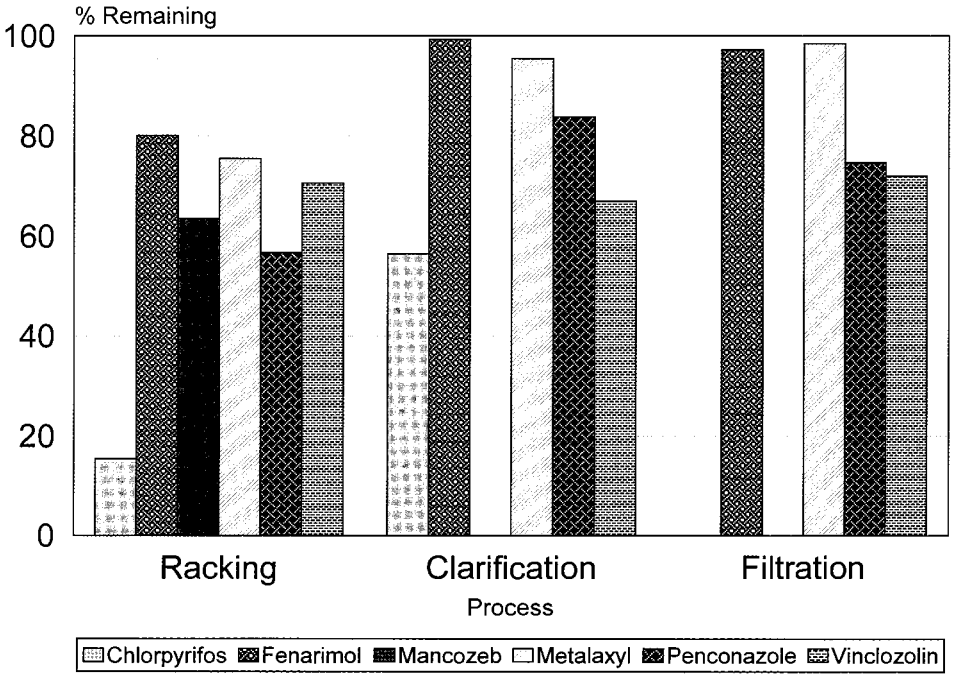

Figure 3. Percentage of pesticide residues remaining after racking and stabilization of the wine.

also probably dissolved in the must in great proportion after the maceration process.

In relation with the influence of racking and stabilization processes, the behavior of chlorpyrifos: is prominent 85% of the amount present in must after pressing is eliminated through racking, when it is retained in the lees. With clarification, 43% of the remaining product is eliminated in the decanted wine, and finally when filtering, the whole residue is eliminated, so that when the wine is bottled, it is free of residues.

Penconazole and mancozeb are retained on the lees in proportions of 43 and 31%, respectively, but whereas the former continues to be eliminated during clarifying and filtering, the latter is eliminated totally with racking. This compound is practically insoluble in water and, therefore, during the process of fermentation it is deposited with the lees.

For vinclozolin, the percentage eliminated with racking is 29%, whereas the clarification and filtration decrease the proportion in the wine in 33 and 28%, respectively; after these processes have been carried out, 6.8% of the initial amount is found in the crushed grapes.

Finally, fenarimol and metalaxyl are the compounds that are eliminated in smaller proportion with the realization of these processes: 19.5 and 24.5% after racking; 0.7 and 4.6% after clarification; and 2.8 and 1.6% with filtration, respectively.

Whereas in red wines, racking, clarification, and filtration processes are fundamental for the loss of residues, they are not so much in the case of rosé wines

(Navarro et al., 1997). On the other hand, the use of diverse clarifiers (bentonite, potassium caseinate, colloidal silicon dioxide with gelatin) does not influence the loss of metalaxyl, but it is significant (>50%) in the cases of vinclozolin and chlorpyrifos (Farris, 1992; Cabras et al., 1983, 1986).

Conclusions. We can positively say that the correct realization of wine-making processes (maceration, pressing, racking, clarification, and filtration) influences in a decisive way the decrease and, in some cases, the elimination of pesticide residues. This depends to a great extent on the initial concentration of the pesticide residues in the harvested grapes, on the physical−chemical characteristics of each product, and on the wine-making procedure (white, rosé, or red elaboration). Therefore, it is very unlikely that after an appropriate elaboration, there can be levels of residuals harmful to the health that could overcome the legislated maximum limits.

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