JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Fate of Iprovalicarb, Indoxacarb, and Boscalid Residues in Grapes and Wine by GC−ITMS Analysis

Alberto Angioni,*[,†] Fabrizio Dedola,[‡] Vincenzo Luigi Garau,[†] Mario Schirra,[§] and Pierluigi Caboni[†]

[†]Department of Pharmaceutical Chemistry and Technology, Università degli Studi di Cagliari, via Ospedale 72, 09124 Cagliari, Italy

[‡]Agricultural Research Agency of Sardinia (AGRIS), 07100, Bonassai SS 291 km 18.6, Sassari, Italy

[§]Institute of Sciences of Food Production, National Research Council, Traversa La Crucca 3, Regione Baldinca, 07040, Li Punti, Sassari, Italy

ABSTRACT: The behavior in field and the transfer from grapes to wine during winemaking of iprovalicarb, indoxacarb, and boscalid was studied. The residue levels found in grapes were far below the MRLs set for grapes in EU, accounting at harvest time 0.81, 0.43, and 4.23 mg/kg for iprovalicarb, indoxacarb, and boscalid, respectively. The residue levels in the samples treated with boscalid may have residual problems (due to an accumulation effect) if repeated field treatments will be performed. Winemaking experiments showed a complete transfer of all pesticide from grapes to the must, while in wine the residues were low or negligible due to the adsorbing effect of lees and pomace. The clarification experiments showed a good removal of pesticide residues from the wine media, for all pesticides. The GC−ITMS method showed good performance with adequate recoveries ranging from 75 to 115%, and good method limits of quantitation (LOQs) and of determination (LODs) far below MRLs.

KEYWORDS: GC−ITMS, residues, wine, grapes, winemaking, clarification

INTRODUCTION

The evaluation of food quality has as an essential prerequisite "food safety". The attention of consumers has brought the topic of pesticide residues to a dominant role in the evaluation of toxicological risk. The legal parameter on pesticide residues, which determines whether a food product may be placed in the market or not, is represented by the maximum residual limit (MRL), which is not a toxicological end point. Actually MRL is calculated from the combination of toxicological data, such as acceptable daily intake (ADI, mg/kg/day) and daily food intake (FDI, kg/day), and agronomic data, such as active dose and decline curves in field. For this reason the same active ingredient may have different MRLs in different countries, with different climate conditions. The European Union is investing to improve safety and healthfulness of agricultural products grown by member states, and at the same time is trying to increase consumers' trust toward agricultural products and food coming from the European market, trying to find a better economic strategy of crop trade.[1,2] Although Italy is, among EU countries, the most rigorous in performing food analysis, Italian people show the lowest trust about food safety in Europe.[3] Official monitoring control carried out by the Italian Ministry of Health on various crops during 2007 showed that 66.8% of wine samples sold all over the country did not show pesticide residues over the limit of detection (LOD) and 33.2% had residues that were detectable but under the MRL set by European law.[4]

Pesticides are normally used to control pests and diseases in modern viticulture, and during the wine making process there is the chance that pesticides can be found, even if under the MRL level, in wine products.

Many mono- and multiresidue methods have been proposed for the determination of pesticides in grapes in field experiments, during winemaking and in wine, with new and highly selective analytical instruments;[5−26] this has allowed determinations of pesticides at the parts per billion (ppb) level, far below the MRL level usually set at parts per million (ppm). The need of minimal pesticide residues in grapes and wines could be achieved by diminishing the use of chemical products in field and at the same time using pesticides effective on pests when applied in field on grapes, but able to disappear in the field or to be adsorbed by the lees or pomace during winemaking, thereby obtaining wines free of detectable pesticide residues, below the analytical limit of determination (<LOD), and thus joining consumers' safety with farmers' necessity.

Indoxacarb is a new oxadiazine insecticide with potent insecticidal activity also in insecticide-susceptible (SRS) insects to pyrethroids and organophosphorus insecticides. It is used in grapes against *Planococcus* spp. and *Lobesia botrana*. Iprovalicarb and boscalid are two new fungicides from the carbamate and anilide families, respectively. They are highly active against *Plasmopara viticola*, *Uncinula necator*, and *Botrytis cinerea*.[27,28]

In order to define strategies to improve wines' quality and healthfulness, the aim of this research was to investigate the fate of indoxacarb, iprovalicarb, and boscalid (Figure 1), used in Sardinia, after field treatment and during the winemaking process, to produce wines with no residues. Moreover, the analytical method was validated, and data were reported.

MATERIALS AND METHODS

Fruit Material and Chemical Analysis. *Field Trials.* Trials were carried out on a red grape vineyard (cv. Carignano) located at Elmas

Received: March 22, 2011
Accepted: May 18, 2011
Revised: May 17, 2011
Published: May 18, 2011

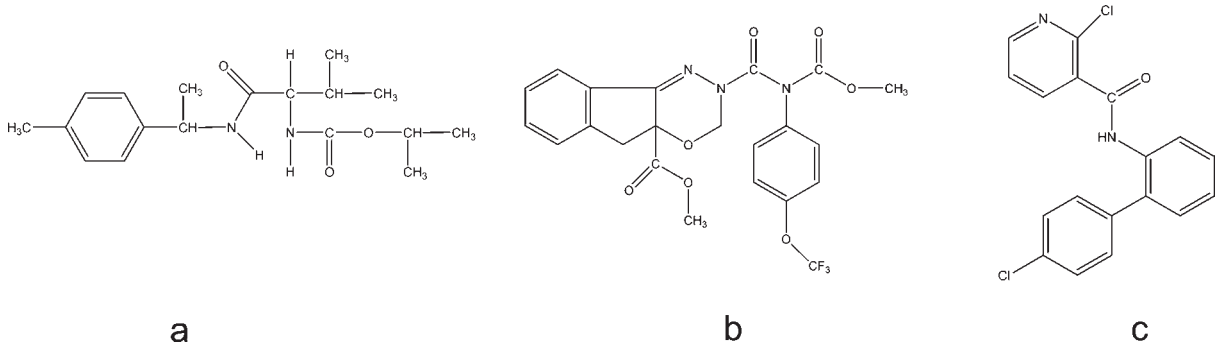

Figure 1. Chemical structures of iprovalicarb (a), indoxacard (b), and boscalid (c).

(Cagliari, Italy). The plant density was 1.90 × 0.90 m, and they were in perfect nutritional and physiological condition. A random block scheme with four replicates for each experiment was used. Each plot consisted of 40 plants; one parcel was left without treatment as a control. Pesticides were applied using doses recommended by the manufacturers and were sprayed with a Robin 5.0 EY20 manual sprayer (Subaru, Japan) using 10 hL/ha of water. Iprovalicarb (isopropyl 2-methyl-1-{[(*RS*)-1-*p*-tolylethyl]carbamoyl}-(*S*)-propylcarbamate), indoxacarb (methyl (*S*)-*N*-[7-chloro-2,3,4a,5-tetrahydro-4a-(methoxycarbonyl)indeno[1,2-*e*]-[1,3,4]oxadiazin-2-ylcarbonyl]-4-(trifluoromethoxy)carbanilate), and boscalid (2-chloro-*N*-(4′-chlorobiphenyl-2-yl)nicotinamide) were able to ensure a good management of principal parasites of vine (*Plasmopara viticola*, *Uncinula necator*, *Botrytis cinerea*, *Planococcus* spp., and *Lobesia botrana*) and are included in Annex I of Directive 91/414/EEC.[29] Steward (30% indoxacarb, DuPont), Melody compact (4.20% iprovalicarb, Bayer), and Signum (6.70% boscalid, BASF) were the commercial formulations applied, at the doses recommended in the vineyard by the respective manufacturers. To gain homogeneity grape samples (3 kg) from each block were collected randomly taking small parts in every bunch belonging to plots, before and about 1 h after the treatment (when the canopy was dry) and subsequently after 3, 7, 10, 14, 21 days for indoxacarb and iprovalicarb, and 7, 21, 28, and 35 days for boscalid (EU Directive 2002/63/CE).[30] Meteorological data were collected by an agrometeorological station AD-2 (Silimet, Modena) located near the vineyard. During the experiments the maximum and minimum average temperatures were 31.4 and 15.7 °C, respectively. Total rainfall was 18.8 mm in two events, 11 and 12 days after the first treatment.

Winemaking. In order to study the fate of pesticide residues from grape to wine, at harvest (21 days for iprovalicarb and indoxacarb, and 35 days for boscalid) samples were of about 6 kg. Each of the four grape samples collected at harvest was divided into three parts of 2 kg. One part was used for the determination of the pesticide residues; in the other two fractions, after pressing and removal of the stems, grapes were processed in the absence of skins (vinification without maceration), and with the skins (vinification with maceration) as described previously by Cabras et al.[13] Prior to vinification in the absence of the skins, residue analyses were carried out in the must and in the centrifuged must to avoid the lees. Fermentation had a regular course in all samples, and after 15 days, all fermented must were pressed and centrifuged to obtain clear wines, which were analyzed for pesticide residue determination.

Chemicals. The active ingredient (ai) standards (purity ≥99%) were kindly provided by the manufacturer. Ethyl acetate, hexane, acetonitrile, and methanol were for pesticide residue analysis (Carlo Erba, Milan, Italy). Water was distilled and filtered through a Milli-Q apparatus (Millipore, Bedford, MA). Standard stock solutions (∼1000 mg/L) were prepared in acetone. Working standard solutions were obtained by dilution with the ethyl acetate/hexane extracts from untreated (control) grapes, must, and wine. Several dilutions were prepared to check the linearity response of the detector and to obtain the detection limits for the three pesticides.

GC−ITMS Analysis. A gas chromatograph Varian model 3800 equipped with a Varian 7800 autosampler, a split/splitless, with a temperature program control, injector Varian 1079, operated in large volume injection mode and an ion trap mass detector ITMS 2000, was used. The analytical column was a Varian VF17 ms (30 m × 0.25 mm i.d. × 0.15 μm film thickness) (Varian, Milan, Italy). Helium was the carrier gas at 1 mL/min. The sample 1 μL was injected in splitless mode with purge valve on at 2 min. The injector temperature was set at 200 °C. The mass spectrometer was calibrated weekly, following the autotune test of the software (Saturn GC/MS Workstation 5.41). The mass spectrometer detector was operated in the positive chemical ionization (CI) mode between 90 and 400 amu; methanol was used as reagent gas. Trap, manifold and transfer line temperatures were at 170, 100, and 200 °C, respectively. The oven was programmed as follows: 90 °C (1 min), raised to 290 °C at 20 °C/min. Matrix-matched standards were prepared at the same concentrations as that of the calibration solutions by adding the appropriate amounts of standard solution to the control matrix extracts. Quantitative determinations were made in the SIS mode using *m*/*z* 528 for indoxacarb, *m*/*z* 320 and 119 for iprovalicarb, *m*/*z* 343 for boscalid, integrating peak area of the GC−ITMS chromatograms versus concentration (Figure 2).

Extraction Procedures. After harvesting, grape samples were chopped and homogenized with a semi-industrial blender (Malavasi, Bologna, Italy). A 5 g aliquot of grape homogeneous sample, or lees and pomace, and a 5 mL aliquot of must or wine samples were weighed or measured in a 30 mL screwcapped tube; after the addition of 10 mL of a mixture of ethyl acetate/hexane (1:1, v/v) and 2 g of NaCl, the tubes were agitated for 15 min in a rotary shaker (Falc Instuments, Bergamo, Italy). The phases were allowed to separate, and the organic phase was injected without any cleanup step in the GC−ITMS system for the analysis.

Wine Clarification. Clarification tests were carried out on 1 L samples of residue-free assessed red and white wine spiked with the studied pesticides. The clarifying agents and the doses were those used in standard enological practice. The clarifying agents used were bentonite (30 g/hL), casein (20 g/hL and 50 g/hL) and gelatin (20 g/hL). After clarification, the cleared wine and the control samples (without clarification) were analyzed for pesticide residues. Each clarification test was performed with four replications.

Method Validation. The experimental method was validated by determining the relative standard deviation (RSD) of repeatability and intermediate precision, recovery, and linearity. Repeatability (*r*) involved the repeated analysis of 6 samples for grape, must, and wines each day, while intermediate precision (IP) was calculated by the analysis of 6 samples/day for each typology on 6 different days. Each sample belongs to an independent experiment. Untreated samples of grapes, must, and wine were fortified with 0.04, 0.5, and 1.0 mg/kg and mg/L of the aforementioned pesticides and processed as reported above. The results of the recovery analysis were compared with matrix control standard dilutions. The recovery assays were replicated 6 times. The

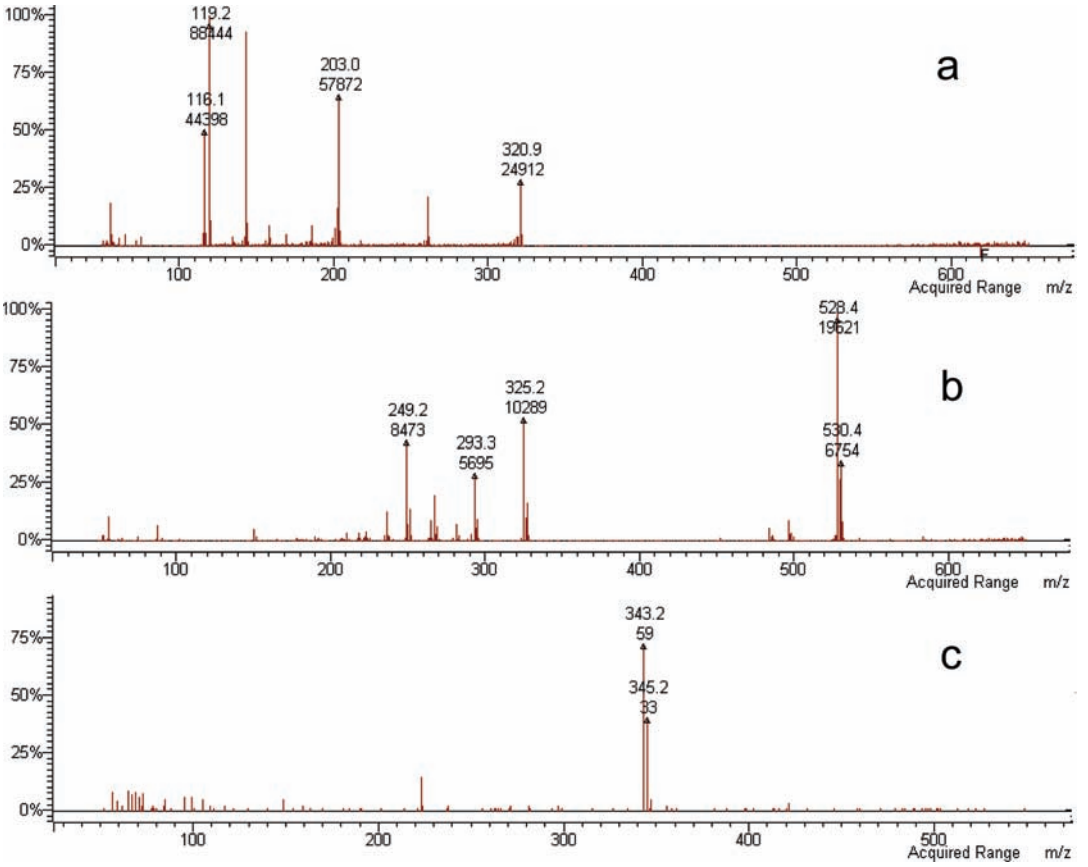

Figure 2. GC−ITMS SIM chromatogram of iprovalicarb (a), indoxacard (b), and boscalid (c) at 0.5, 0.39, and 0.45 mg/kg, in grape matrix.

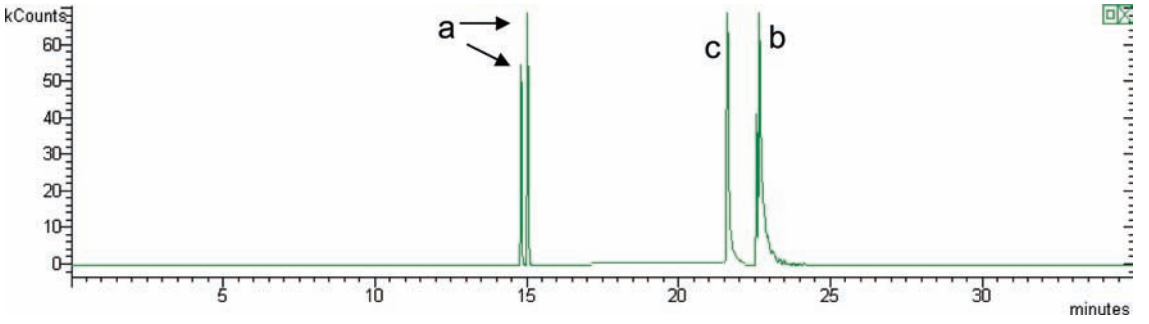

Figure 3. CI-MS spectra of iprovalicarb (a), indoxacard (b), and boscalid (c).

matrix effect was evaluated by comparing the analytical responses of the pesticides dissolved in acetone/hexane with those prepared with control matrix extracts.

Statistical Assays. This method was validated under EURACHEM Guide (1998) and CITAC/EURACHEM Guide (2002) recommendations.[31,32] Analysis of variance (ANOVA) was carried out with the software STATISTICA, using Tukey's test at $p < 0.05$.

RESULTS AND DISCUSSION

Analytical Method. The experimental design allowed studying the decline curves of pesticides after field treatment, and to determine the amount of pesticide at harvest and evaluate the effect of the technological process of winemaking on the residues left in the grapes. The chromatographic method allowed a good separation of the three pesticides (iprovalicarb isomers 14.83 and 15.01 min, boscalid 21.49 min, and indoxacarb 22.70 min) (Figure 3). Five point standard calibration curves ranging from 0.04 to 10.00 mg/L were prepared, the correlation coefficient (R^2) obtained, ranging from 0.9979 (boscalid in grape matrix) to 0.9999 (iprovalicarb in wine matrix) showing a good linearity, and the CV % max was detected for indoxacarb (10%) (Table 1). No interfering peaks were detected in the chromatographic range of interest, and no cleanup was necessary. The method limits of quantitation (LOQs) and of determination (LODs) were calculated as 10-fold and 3-fold the signal-to-noise ratio (Table 2). All pesticide showed LOD and LOQ far below the MRLs set for grapes and wines by the European Community (Table 1). Accuracy data were provided by recovery experiments from 6 replicates each for the three pesticides at 0.04, 0.5, and 1.0 mg/kg

Table 1. Analytical Method Limits of Quantification (LOQ) and Determination (LOD) of the Studied Pesticides in Grapes (mg/kg) and Wine (mg/L). Correlation Coefficient and CV %

		iprovalicarb	indoxacarb	boscalid
LOD	grapes	0.019	0.018	0.013
	wine	0.019	0.018	0.013
LOQ	grapes	0.039	0.036	0.041
	wine	0.039	0.036	0.041
$R^2 \pm$ CV %	grapes	0.9996 ± 7	0.9993 ± 10	0.9979 ± 8
	wine	0.9999 ± 8	0.9987 ± 6	0.9994 ± 5

Table 2. Validation Parameters for the Three Pesticides in Grapes (mg/kg), Must, and Wine (mg/L) at Three Different Levels of Fortification

		iprovalicarb	indoxacarb	boscalid
		Repeatability (n = 6) CV %		
0.04	grapes	9.4	8.2	8.1
	must	8.5	4.5	4.1
	wine	4.6	5.4	7.8
0.5	grapes	3.2	9.8	6.4
	must	1.3	4.1	2.7
	wine	6.0	2.9	5.8
1.00	grapes	4.5	3.2	1.1
	must	2.5	6.0	7.7
	wine	4.1	7.7	5.3
		Intermediate Precision (n = 6) CV %		
0.04	grapes	4.5	9.8	9.3
	must	4.1	5.0	4.8
	wine	4.2	4.1	5.5
0.5	grapes	3.8	2.1	5.0
	must	4.6	5.0	3.2
	wine	9.2	3.8	9.4
1.00	grapes	6.6	3.8	2.8
	must	5.4	9.2	2.0
	wine	5.0	8.7	8.7
		Recoveries % (n = 6) ± CV %		
0.04	grapes	102 ± 7	75 ± 5	82 ± 7
	must	77 ± 6	78 ± 7	89 ± 7
	wine	79 ± 5	83 ± 6	111 ± 3
0.5	grapes	76 ± 8	107 ± 4	102 ± 9
	must	101 ± 2	115 ± 8	105 ± 3
	wine	80 ± 3	108 ± 1	108 ± 2
1.00	grapes	75 ± 6	108 ± 2	115 ± 5
	must	78 ± 9	107 ± 3	86 ± 2
	wine	97 ± 2	92 ± 5	87 ± 4

for grapes and even mg/L amounts for must and wine. Good recoveries were achieved for all pesticides studied, according to EC SANCO/10684/2009 values.[33] Recoveries ranged for iprovalicarb from 75 to 102% in grapes, from 77 to 101% in must, and from 79 to 97% in wines. Boscalid residues ranged from 82 to 115% in grapes, from 86 to 105% in must, and from 87 to 111% in wines, while indoxacarb residues ranged from 75 to 107% in grapes, from 78 to 115% in must, and from 83 to 108% in wines.

Table 3. Residues of Iprovalicarb, Indoxacarb, and Boscalid in Grapes after Field Treatment (n = 4),

	residue (mg/kg ± SD)		
days after treatment	iprovalicarb (20,[a] 2.0[b])	indoxacarb (10,[a] 2.0[b])	boscalid (35,[a] 5.0[b])
−0	nd	nd	nd
0	2.21 ± 0.32	1.51 ± 0.20	3.50 ± 1.91
3	1.95 ± 0.44	1.10 ± 0.27	
7	1.25 ± 0.30	0.90 ± 0.16	5.04 ± 1.26
10	1.56 ± 0.07	1.33 ± 0.37	
14	1.30 ± 0.25	0.80 ± 0.09	
21	0.81 ± 0.14	0.43 ± 0.17	4.00 ± 1.04
28			4.93 ± 1.36
35			4.23 ± 2.02

[a] PHI (days). [b] MRL (mg/kg) grapes.

The coefficient of variability ranged from 1 to 9% in the most unfavorable case (Table 2). The obtained values confirmed that the proposed extraction method is suitable for the determination of the residues of the studied pesticides in grape and wine matrices. Repeatability (r) and intermediate precision (IP) were valued for n = 6 for grape, must, and wine samples. Good results were obtained for almost all tests (CV ≤ 20) according to EC SANCO/10684/2009.[33] The maximum variation coefficients (CV) were for iprovalicarb 9.4% in repeatability and 9.2% in intermediate precision, for boscalid 8.1% in repeatability and 9.4% in intermediate precision, and for indoxacarb 9.8% both in repeatability and in intermediate precision (Table 2).

In order to evaluate the influence of the pomace on the removal of pesticide residues, two different winemaking technique were used, fermentation together with the grape skins (red wine), and fermentation of the grape juice with the removal of the grape skins (white wine). After pressing, the must, immediately separated from the grape skins, was centrifuged to evaluate the ability of the lees in adsorbing pesticide residues.

Residues in Grapes Must and Wine. Treatments were made on grapes at the veraison stage, and no decrease of pesticide residues due to fruit growth was observed. After treatment, iprovalicarb showed in grapes a residue of 2.21 ± 0.32 mg/kg, higher than the MRL set for grapes (2.0 mg/kg), which decreased to 0.81 ± 0.14 mg/kg at PHI (20 days); indoxacarb had a residual of 1.51 ± 0.20 mg/kg, lower than the MRL set for grapes (2 mg/kg), and at PHI (10 days) the residues remained almost unchanged, 1.33 ± 0.93 mg/kg, decreasing to 0.43 ± 0.17 at harvest after 21 days (Table 3). The MRL fixed for boscalid in grapes is 5 mg/kg. Boscalid levels after treatment were 3.50 ± 1.91 mg/kg, under the MRL value. At PHI after 35 days, boscalid residues were 4.23 ± 2.02 mg/kg, statistically even to those at time 0 (Table 3). The decline curves for iprovalicarb and indoxacard (Figure 4) showed a slow decrease in the first week with a steady residue after ten days and a slow decrease until 21 days. Boscalid showed a residue stable during the 35 day treatment; this data is in accordance with field studies reported by Cus et al. which found a high persistence of this fungicide in grapes and during the winemaking process.[34,35] The high standard deviations are compatible with field treatment experiments.[36] All experiments showed a complete transfer of pesticide residues from the grapes to the resulting must solution (Table 4). The centrifugation of the must led to an average lees amount of 4% with residue

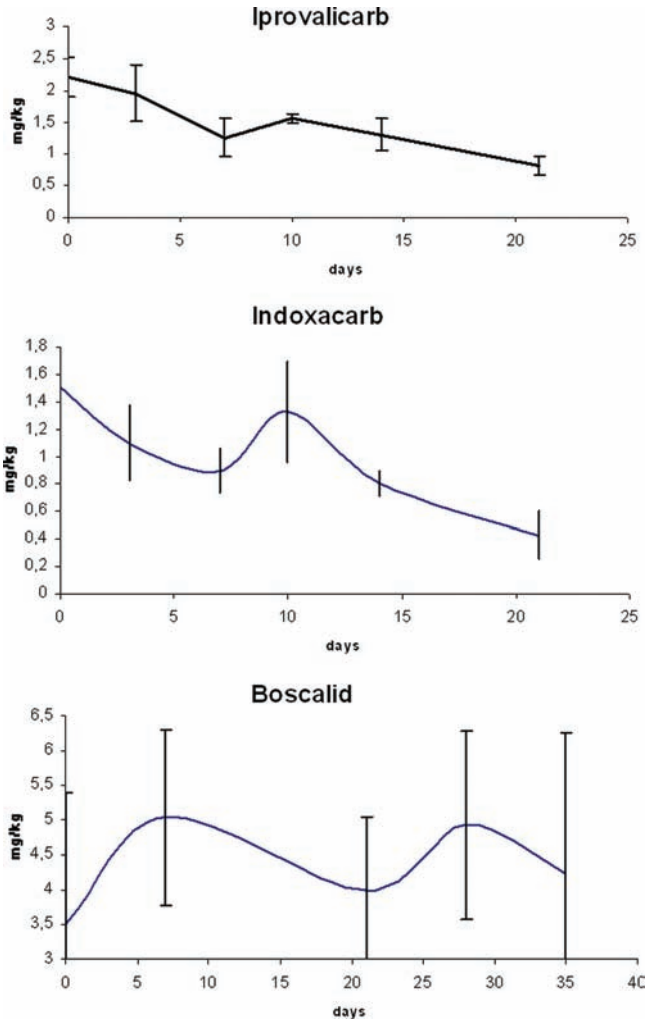

Figure 4. Decline curves in grapes after field treatment of iprovalicarb, indoxacarb, and boscalid. Error bars represent standard deviations of four replicate samples.

decrease of almost 60% (0.29 ± 0.09 mg/kg) for iprovalicarb, 74% for indoxacarb, and 65% for boscalid, thus indicating the ability of the lees in adsorbing pesticide residues from the must solution (Table 4). After vinification in the presence of the skins (red wines), iprovalicarb residues accounted for 0.36 ± 0.12 mg/L, the pomace fraction accounted for 16%, and pesticide residues were 2.90 ± 0.03 mg/kg. Lower removal was obtained in the white wines by the lees owing to higher wine residues (0.57 ± 0.18 mg/kg) (Table 4). Considering the yields obtained after winemaking procedures of crushing, pressing, and racking, the total theoretical residual amount present in each of the winemaking flasks was calculated. The sum of the amount of residues in the lees and white wine, and pomace and red wine, led to a theoretical value of 0.80 mg/L for white wine and 0.83 mg/L for red wines, which is comparable to the values found in the must, 0.79 ± 0.27 mg/L, and in the homogenized grapes, 0.81 ± 0.14 mg/kg, respectively (Table 4). This data agree with the data reported by Gonzalez-Rodrıguez et al.[37] which reported for iprovalicarb a loss due to absorption of almost 50% of the residue in the must media. Indoxacarb residues in white wines accounted for 0.05 ± 0.01, and were not detectable in red wines. Residue analysis of the lees and of the pomace showed residue levels of

Table 4. Residues of Iprovalicarb, Indoxacarb, and Boscalid in Must and Wine (mg/L) and in the Lees and Pomace Fraction (mg/kg), after Winemaking (n = 4)

	iprovalicarb (1.0[a])	indoxacarb	boscalid
must	0.79 ± 0.27	0.54 ± 0.14	4.26 ± 1.28
centrifuged must	0.29 ± 0.09	0.14 ± 0.10	1.50 ± 0.94
white wine	0.57 ± 0.18	0.05 ± 0.01	2.18 ± 0.49
lees	5.75 ± 0.06	11.73 ± 0.08	56.12 ± 1.50
theor value	0.80 ± 0.04	0,52 ± 0.06	4.42 ± 0.65
red wine	0.36 ± 0.12	nd	1.00 ± 0.32
pomace	2.90 ± 0.03	3.10 ± 0.04	20.05 ± 0.95
theor value	0.83 ± 0.03	0.50 ± 0.09	4.21 ± 0.80

[a] MRL (mg/L) in wines.

11.73 ± 0.08 mg/kg and 3.10 ± 0.04, respectively. The residue corrected for the amount of lees and pomace led to a calculated residue of 0.52 mg/L and 0.50 mg/L for white and red wine, respectively (Table 4). The analysis of white and red wine showed for boscalid a decrease of 50 and 70%, respectively, with a final residue in the lees and the pomace of 56.12 ± 1.50 and 20.05 ± 0.95 mg/L (Table 4). The calculated total residue for boscalid was 4.42 ± 0.65 mg/L and 4.21 ± 0.80 mg/L for white and red wines, respectively. In all experiments the theoretical values found were equal to the values found in the starting material, intending must without skins for white and crushed and destemmed grapes for red wines, thus indicating that the decrease of pesticide was due to the adsorbing effect of the lees and the pomace and no decrease was attributable to degradation processes.

Clarification Experiments. The clarification tests were carried out on the aforementioned pesticides at concentration similar to those found in the grapes at harvest. The concentrations used were 0.80, 0.45, and 4.00 mg/L for iprovalicarb, indoxacarb, and boscalid, respectively. Iprovalicarb and indoxacarb were completely adsorbed by casein 20 and 50, and gelatin. Moreover, iprovalicarb was completely adsorbed also by bentonite. Bentonite decreased indoxacarb residues of 50%, while boscalid was decreased of almost 50% using bentonite, casein 50 and gelatin, while it remained unchanged using casein 20. Several experiments have been made on different pesticides to evaluate the capacity of wine clarification in reducing the residues of pesticides.[22,23,25] The results of these studies confirmed the tendency of bentonite and gelatin to be more effective in eliminating pesticide residues, even if the amount removed is pesticide dependent.[38]

Conclusions. The data reported showed that at harvest time the three pesticides studied had residue levels under the MRL, also when used on already grown grapes. Particular attention may be paid for boscalid grape levels if repeated field treatments are carried out. All pesticides studied are completely transferred from grapes to the must, but present a good affinity for the lees and the pomace, thus reducing the final residue in wine. In particular indoxacarb showed residues near the LOD for white wines and was not detectable in red wines. Field agricultural practice and different winemaking processes influence in a critical way the decrease and, in some cases, the disappearance of pesticide residues. This fact depends on the pesticide initial grape concentration at harvesting, and dramatically on the winemaking technology used. The clarification experiments showed a good removal of pesticide residues from the wine media for all

pesticides. The aim of this paper was to investigate the disappearance of three pesticides to make wines with no residues. Among the studied compounds, only indoxacarb satisfies this condition, while iprovalicarb can meet this condition if used at lower levels. On the other hand boscalid showed high levels of residues both in grapes and in wine. The use of technological adjuvants such as clarifying agents could lead to wines with pesticide residues under the LOD. Further work and testing of other pesticides will be needed to establish the correct behavior in the field and during winemaking in purely chemical terms, and to establish new field strategy to overcome detectable levels of pesticides in wines.

AUTHOR INFORMATION

Corresponding Author

*Department of Toxicology, Via Ospedale 72, 09124 Cagliari, Italy. Tel: +390706758615. Fax +390706758612. E-mail: aangioni@unica.it.

REFERENCES

(1) Commission of the European Communities (2000), *White paper on food safety*, Bruxelles 12.1.2000 COM (1999) 719 final.

(2) *Regulation (EC) No 178/2002, Official Journal of the European Communities L 31*, 01/02/2002 pp. 1−24.

(3) Rapid Alert System for food and Feed (RASFF). Annual report 2007. http://ec.europa.eu/food/food/rapidalert/report2007_en.pdf.

(4) Ministero della Salute (2007), *Controllo ufficiale sui residui di prodotti fitosanitari negli alimenti di origine vegetale.* http://www.salute.gov.it/imgs/C_17_pubblicazioni_950_ allegato.pdf.

(5) Lian, Y. J.; Pang, G. F.; Shu, H. R.; Fan, C. L.; Liu, Y. M.; Feng, J.; Wu, Y. P.; Chang, Q. Y. Simultaneous determination of 346 multiresidue pesticides in grapes by PSA-MSPD and GC-MS-SIM. *J. Agric. Food Chem.* **2010**, *58* (17), 9428–9453.

(6) Dasgupta, S.; Banerjee, K.; Patil, S. H.; Ghaste, M. S.; Dhumal, K. N.; Adsule, P. G. Optimization of two-dimensional gas chromatography time-of-flight mass spectrometry for separation and estimation of the residues of 160 pesticides and 25 persistent organic pollutants in grape and wine. *J. Chromatogr., A* **2010**, *1217* (24), 3881–3889.

(7) Grimalt, S.; Sancho, J. V.; Pozo, O. J.; Hernandez, F. Quantification, confirmation and screening capability of UHPLC coupled to triple quadrupole and hybrid quadrupole time-of-flight mass spectrometry in pesticide residue analysis. *J. Mass Spectrom.* **2010**, *45* (4), 421–436.

(8) Savant, R. H.; Banerjee, K.; Utture, S. C.; Patil, S. H.; Dasgupta, S.; Ghaste, M. S.; Adsule, P. G. Multiresidue Analysis of 50 pesticides in grape, pomegranate, and mango by gas chromatography-Ion Trap Mass spectrometry. *J. Agric. Food Chem.* **2010**, *58* (3), 1447–1454.

(9) Wong, J. W.; Webster, M. G.; Halverson, C. A.; Hengel, M. J.; Ngim, K. K.; Ebeler, S. E. Multiresidue pesticide analysis in wines by Solid-Phase extraction and capillary Gas Chromatography−Mass spectrometric detection with selective ion monitoring. *J. Agric. Food Chem.* **2003**, *51* (5), 1148–1161.

(10) Angioni, A.; Sarais, G.; Dedola, F.; Caboni, P. Pyrimethanil residues on table grapes Italia after field treatment. *J. Environ. Sci. Health, Part B* **2006**, *41* (6), 833–841.

(11) Abreu, S. D.; Caboni, P.; Pirisi, F. M.; Cabras, P.; Alves, A.; Garau, V. L. Residues of the fungicide famoxadone in grapes and its fate during wine production. *Food Addit. Contam.* **2006**, *23* (3), 289–294.

(12) Cabras, P.; Angioni, A.; Garau, V. L.; Pirisi, F. M.; Cabitza, F.; Pala, M.; Farris, G. A. Fenhexamid residues in grapes and wine. *Food Addit. Contam.* **2001**, *18* (7), 625–629.

(13) Cabras, P.; Angioni, A. Pesticide residues in grapes, wine, and their processing products. *J. Agric. Food Chem.* **2000**, *48* (4), 967–973.

(14) Cabras, P.; Angioni, A.; Garau, V. L.; Pirisi, F. M.; Cabitza, F.; Pala, M.; Farris, G. A. Fate of quinoxyfen residues in grapes, wine, and their processing product. *J. Agric. Food Chem.* **2000**, *48* (12), 6128–6131.

(15) Cabras, P.; Angioni, A.; Garau, V. L.; Pirisi, F. M.; Farris, G. A.; Madau, G.; Emonti, G. Pesticides in fermentative processes of wine. *J. Agric. Food Chem.* **1999**, *47* (9), 3854–3857.

(16) Cabras, P.; Angioni, A.; Garau, V. L.; Pirisi, F., M.; Espinoza, J.; Mendoza, A.; Cabitza, F.; Pala, M.; Brandolini, V. Fate of azoxystrobin, fluazinam, kresoxim-methyl, mepanipyrim, and tetraconazole from vine to wine. *J. Agric. Food Chem.* **1998**, *46*, 3249–3251.

(17) Garau, V. L.; Abreu, S. D.; Caboni, P.; Angioni, A.; Alves, A.; Cabras, P. Residue-free Wines: Fate of Some quinone outside inhibitor (QoI) fungicides in the winemaking process. *J. Agric. Food Chem.* **2009**, *57* (6), 2329–2333.

(18) Poulsen, M. E.; Hansen, H. K.; Sloth, J. J.; Christensen, H. B.; Andersen, J. H. Survey of pesticide residues in table grapes: Determination of processing factors, intake and risk assessment. *Food Addit. Contam.* **2007**, *24* (8), 886–895.

(19) Fernandez, M,J.; Oliva, J.; Barba, A.; Càmara, M. A. Fungicide dissipation curves in winemaking processes with and without maceration step. *J. Agric. Food Chem.* **2005**, *53* (3), 804–811.

(20) Flori, P.; Frabboni, B.; Cesari, A. Pesticide decay models in the wine-making process and wine storage. *Ital. J. Food Sci.* **2000**, *12* (3), 279–289.

(21) Navarro, S.; Oliva, J.; Barba, A.; Garcia, M. A.; Zamorano, M. Evolution of chlorpyrifos, fenarimol, metalaxyl, penconazole, and vinclozolin in red wines elaborated by carbonic maceration of Monastrell grapes. *J. Agric. Food Chem.* **2000**, *48* (8), 3537–3541.

(22) Oliva, J.; Paya, P.; Camara, M. A.; Barba, A. Removal of famoxadone, Fluquinconazole and trifloxystrobin residues in red wines: Effects of clarification and filtration processes. *J. Environ. Sci. Health, Part B* **2007**, *42* (7), 775–781.

(23) Fernandez, M. J.; Oliva, J.; Barba, A.; Càmara, M. A. Effects of clarification and filtration processes on the removal of fungicide residues in red wines (Var. Monastrell). *J. Agric. Food Chem.* **2005**, *53* (15), 6156–6161.

(24) Cesnik, H. B.; Gregorcic, A.; Cus, F. Pesticide residues in grapes from vineyards included in integrated pest management in Slovenia. *Food Addit. Contam.* **2008**, *25* (4), 438–443.

(25) Navarro, S.; Barba, A; Oliva, J.; Navarro, G.; Pardo, F. Evolution of residual levels of six pesticides during elaboration of red wines. Effect of wine-making procedures in their disappearance. *J. Agric. Food Chem.* **1999**, *47* (1), 264–270.

(26) Navarro, S.; Barba, A.; Navarro, G.; Vela, N.; Oliva, J. Multiresidue method for the rapid determination − in grape, must and wine − of fungicides frequently used on vineyards. *J. Chromatogr., A* **2000**, *882*, 221–229.

(27) Tiemann, R.; Pflüger, W. Ecological profile of the fungicide iprovalicarb. *Pflanzenschutz-Nachr. Bayer* **1999**, *52* (1), 61–70.

(28) Zhang, C. Q.; Yuan, S. K.; Sun, H. Y.; Qi, Z. Q.; Zhou, M. G.; Zhu, G. N. Sensitivity of Botrytis cinerea from vegetable greenhouses to boscalid. *Plant Pathol.* **2007**, *56* (4), 646–653.

(29) Directive 91/414/EEC, concerning the placing of plant protection products on the market, 15 July 1991 (OJ L 230, ISSN).

(30) EU Directive 2002/63/CE, fixant des méthodes communautaires de prélèvement d'échantillons pour le contrôle officiel des résidus de pesticides sur et dans les produits d'origine végétale et animale et abrogeant la directive 79/700/CEE. 11 July 2002.

(31) EURACHEM Guide, *Harmonized, guidelines for the use of recovery information in analytical measurement*, 1998.

(32) CITAC /EURACHEM Guide, Guide to quality in analytical chemistry. 2002 Edition.

(33) SANCO /10684/2009 (01/01/2010), European Commission. *Method validation and quality control procedures for pesticide residues analysis in food and feed* (2009).

(34) Cus, F.; Cesnik, H. B.; Bolta, S. V.; Gregorcic, A. Pesticide residues in grapes and during vinification process. *Food Control* **2010**, *21* (11), 1512–1518.

(35) Cus, F.; Cesnik, H. B.; Bolta, S. V.; Gregorcic, A. Pesticide residues and microbiological quality of bottled wines. *Food Control* **2010**, *21* (2), 150–154.

(36) GUIDELINES ON ESTIMATION OF UNCERTAINTY OF RESULTS. CAC/GL 59-2006.

(37) Gonzalez-Rodrıguez, R. M.; Cancho-Grande, B.; Simal-Gandara, J. Efficacy of new commercial formulations to control downy mildew and dissipation of their active fungicides in wine after good agricultural practices. *J. Sci. Food Agric.* **2009**, *89*, 2625–2635.

(38) Cabras, P.; Angioni, A. Pesticide Residues in Grapes, Wine, and Their Processing Products. Review. *J. Agric. Food Chem.* **2000**, *48* (4), 967–973.