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Ochratoxin A Contamination in Italian Wine Samples and Evaluation of the Exposure in the Italian Population

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The scope of this study was to evaluate the exposure of the Italian population to ochratoxin A (OTA) attributable to wine consumption. With this aim 1166 wine samples (773 red wines, 290 white, 75 rosé, and 28 dessert wines), collected in 19 different Italian regions and mostly produced between 1988 and 2004, were analyzed for OTA content. The obtained results are reported by year of harvest, geographical area of production, and type of wine. Red wine showed the highest maximum level of contamination (7.50 ng/mL), even though rosé wines were characterized by a higher mean value (0.01 ng/mL). A gradually increasing mean concentration was also observed from the north (0.05 ng/mL) to south of Italy (0.54 ng/mL). Exposure calculations, performed using two different consumption databases, indicate a daily intake for *consumer only* of 0.59 up to 1.24 ng/(kg of b.w.)/day and of 0.33 up to 0.90 ng/(kg of b.w.)/day for the total population. Even in the worst case, corresponding to the calculation of the intake for *consumers only* in southern Italy and Islands and considering the mean consumption data increased by 1 standard deviation, a quite low exposure (1.68 ng/(kg of b.w.)/day, accounting for 9.8% of TDI) was obtained. Considering the overall OTA dietary exposure, obtained exposure rates indicate that wine did not pose a risk to the Italian population health.

KEYWORDS: Ochratoxin A; wine; daily intake

INTRODUCTION

Ochratoxin A (OTA), (−)-*N*-[(5-chloro-8-hydroxy-3-methyl-1-oxo-7-isochromanyl)carbonyl]-3-phenylalanine, consists of a *p*-chlorophenolic moiety containing a dihydroiso-coumarin group that is amide-linked to L-phenylalanine. It is a natural contaminant of foods, drinks, and feeds worldwide (*1*−*3*). Ochratoxin A is a potent nephrotoxic, teratogenic, and immunotoxic agent and exerts its toxic effect particularly on the renal system, being a potent renal toxin in all of the tested animal species. It induces a typical karyomegaly and a progressive nephropathy. The extent of renal injury is dose-dependent, but is also associated with the duration of exposure, as OTA accumulates in renal tissue. It has also been identified in other tissues, and because of its long half-life in mammalian tissues, contamination may also carry over into pork edible food products and blood of animals fed by contaminated feed (*4*−*7*); in addition, OTA has also been detected in human blood and breast milk (*8*−*10*). Serum half-lives after oral administration of OTA were 510 h in one monkey, 72−120 h in pigs, 55−120 h in rats, 24−39 h in mice, and 4.1 h in chickens. In one human volunteer, half-life was 840 h (about 35 days) (*11*).

The International Agency for Research on Cancer (IARC) has classified OTA as a possible human carcinogenic (group 2B) (*5*). OTA genotoxicity has been debated for a long time with pros and cons about this issue (*12*−*18*). The European Food Safety Authority's (EFSA) opinion of April 2006 (*19*) evidenced the lack of the existence of OTA-DNA adducts despite the availability of advanced chemical analytical procedures. Therefore, a threshold-based approach in the EFSA risk assessment of OTA was used, with the opinion that it would be prudent to reduce exposure to OTA as much as possible, setting a new Tolerable Weekly Intake (TWI) of 120 ng/kg of b.w., corresponding to an increase of the daily tolerable amount of about 3-fold compared to the previous threshold set by the Scientific Committee for Food in 1998.

Depending on the climatic conditions of the geographical areas worldwide, OTA is produced by a wide number of fungal species basically belonging to the genera *Aspergillus* (*Aspergillus ochraceus* and *Aspergillus carbonarius* spp) and *Penicillium* (*Penicillium verrucosum*) (*20*). The main dietary sources of OTA are cereals, followed by wine, spices, coffee, grape juice, dairy products, cocoa, beer, dried fruits, and pulses (*1*) with cereals accounting for 50% of the overall human intake with diet.

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In regard to OTA presence in grape and wine, this was first detected in 1996 by Zimmerli et al. (*21*). Since the discovery of this type of contamination, much research and surveys have been conducted in European countries. A concentration gradient was observed from the north to the south of Europe, especially for red wines. This could be attributed to the hotter and more humid climatic conditions in southern countries that can favor better the growth of the *Aspergillus carbonarius* and the consequent production of OTA. In particular, some specific geographical zones in southeast Spain, southeast France, south of Italy and Greece were identified as high-risk areas and, in some cases, very high contamination peaks for red wines, far above the maximum levels of 2 ng/mL set by the EU regulation, were found (*22*−*27*).

In the other parts of the world (South America, Israel, Australia and former Soviet Union countries), there was no particular concern with respect to OTA in wine, except for the countries of north Africa such as Morocco where peak contamination levels of OTA were observed (*28*).

It should be noted that in all the involved countries preventive actions have been taken in order to reduce the contamination phenomenon. This has been done through national guidelines or specific international codes of practice, such as the proposed Codex Draft Code of Practice for the prevention and reduction of ochratoxin A contamination in wine, introduced within the Codex Committee of Contaminants in Food (CX/CF 07/1/11) of January 2007 (*29*), based on the guidelines of the Organisation Internationale de la Vigne et du Vin (OIV).

Also in Italy a wide spectrum of different initiatives, through both the launch of national research projects and the adoption of self-control activities within Hazard Analysis Critical Control Points (HACCP) process, started in 1996 in order to control and reduce the growth of OTA-producing black *Aspergilli* and to guarantee consumer health.

It is now ascertained that OTA has the higher incidence in red and dessert wines followed by rosé and white wines. Red wine processing conditions favor the transfer of OTA from the skin of the grape to the must, because of the prolonged contact time required for the development of its red color. Crushed grapes are left mashed for several days, and maceration can induce an increase of OTA contamination up to 20% (*30*).

Furthermore, it is well-known that OTA occurs in the vineyard and its formation in grapes is mainly due to berry contamination by certain mold species, belonging essentially to the black *Aspergillus* species (in particular *A. carbonarius* species and to a lesser extent *A. niger*), but also to *Penicillium* species in cooler regions (*29*, *31*).

The growth of *Aspergillus* species occurs at air humidity levels of 72% to 90% and temperatures of 12 to 39 °C (28 °C being the optimum) (*31*). The presence and spread of such fungus in vineyards are influenced by environmental and climatic factors (high humidity, hot and dry environments), nocturnal dampening conditions of grapes, grape bunch shape, aeration level of grape bunch, susceptibility of vine varieties and health status of grapes. Berry injuries are the main entry points for ochratoxinogenic fungus together with skin thickness, use of fungicides, winemaking procedures and *Lobesia botrana* (grape worm) berry damage (*29*).

Depending on climatic conditions, OTA development on grape does not occur before the early veraison and reaches its maximum during the ripening period. In the winemaking process, more intensively in red wine production, the contamination levels of OTA in grapes continues to increase until fermentation.

The contamination begins to decrease during the racking process and malo-lactic fermentation due to the action of lactic acid bacteria that can induce a loss of OTA up to 50% (*27*). An additional decrease seems to occur after the bottling and during the aging period of the wine, with a possible reduction up to 17% after 12 months of storage (*32*).

With the exception of red wines, a final tool to inactivate the residue of OTA in wines is the use of fining agents such as oenological charcoal, that can reduce OTA up to 90% (*33*).

The aim of this study was to observe the OTA contamination in Italian produced wines over a wide period and to evaluate the potential contribution of wine to the dietary OTA exposure in Italian consumers.

MATERIALS AND METHODS

Samples. Wine samples were purchased in food stores in Rome during the period 2002−2004. All the information about the samples was taken from the bottle labels. Most of the samples were from the grape harvest 2000−2004 (35% before 2000, 55% from 2000 to 2004, and 10% without any vintage indication). Samples were representative of the most important Italian wine-producing regions. A majority of samples were those produced in south of Italy, because wines from those regions were previously found to be more susceptible to OTA contamination. A total of 1166 samples were analyzed (773 red wines, 290 white, 75 rosé, and 28 dessert wines).

Reagents and Standards. Acetonitrile, acetic acid, methanol and toluene were of HPLC grade from Carlo Erba Reagents (Milano, Italy). OTA standard was purchased from Sigma (Milan, Italy). The standard solutions were made in toluene/acetic acid, and nominal concentration was measured by a UV spectrophotometer (*34*).

OTA Determination. Wine samples were analyzed according to the recently published paper by Brera et al. (*35*). In this paper, performance characteristics of the method are described. In brief, 5 mL of wine was diluted with 60 mL of phosphate buffered saline (PBS) adjusting the pH to 8.2 for some types of red wines. The mixture was shaken vigorously and filtered through glass microfiber filters if solid residues were formed after dilution or if solutions were cloudy. The immunoaffinity column (IAC) (Ochraprep, RBiopharm Rhône, Scotland, U.K.) was preconditioned with 4 mL of PBS. A 20 mL aliquot of the diluted sample was passed through the IAC column (3 mL/min). The column was washed with 9 mL of PBS and 8 mL deionized water and then dried with air. OTA was then eluted with 1.5 mL of methanol. The eluate was diluted with 0.5 mL of deionized water, mixed vigorously and stored at 4 °C prior to HPLC analysis. One hundred fifty microliters of the aliquot was injected into an HPLC system consisting of a Gilson 402 Diluitor (Villiers-le-Bel, France), a Rheodyne 7010 injector (California) fitted with a 200 μL loop and a Jasco FP 1520 scanning fluorescence detector (Tokyo, Japan), set to excitation and emission wavelengths, 333 and 470 nm, respectively. The analytical column Kromasil KR100 C18, 150 × 4.6 mm i.d., 5 μm was maintained at 40 °C. The mobile-phase was water:acetonitrile:glacial acetic acid (99:99:2 v/v/v) at 1 mL/min flow rate. The estimated parameter values for repeatability and recovery factors, obtained in this study satisfy the CEN criteria (*36*).

The limit of detection of the method was 0.01 ng/mL, and the limit of quantification was 0.03 ng/mL.

Typical chromatograms of OTA reference standard and naturally contaminated red wine sample are shown in **Figure 1**.

Safety Precautions. This method requires the use of solutions of ochratoxin A. All the procedures were carried out according to the safety precautions commonly used in mycotoxin analysis and described by AOAC (*34*). Decontamination procedures of all the glassware and work plans as described by IARC (*37*) were used during this study.

Statistical Analysis. Considering the qualitative nature of variables (5 out of 8) of the data set and with the aim to determine the relationship among variables, multidimensional analysis such as multiple correspondence analysis (MCA) and cluster analysis (CA) were considered

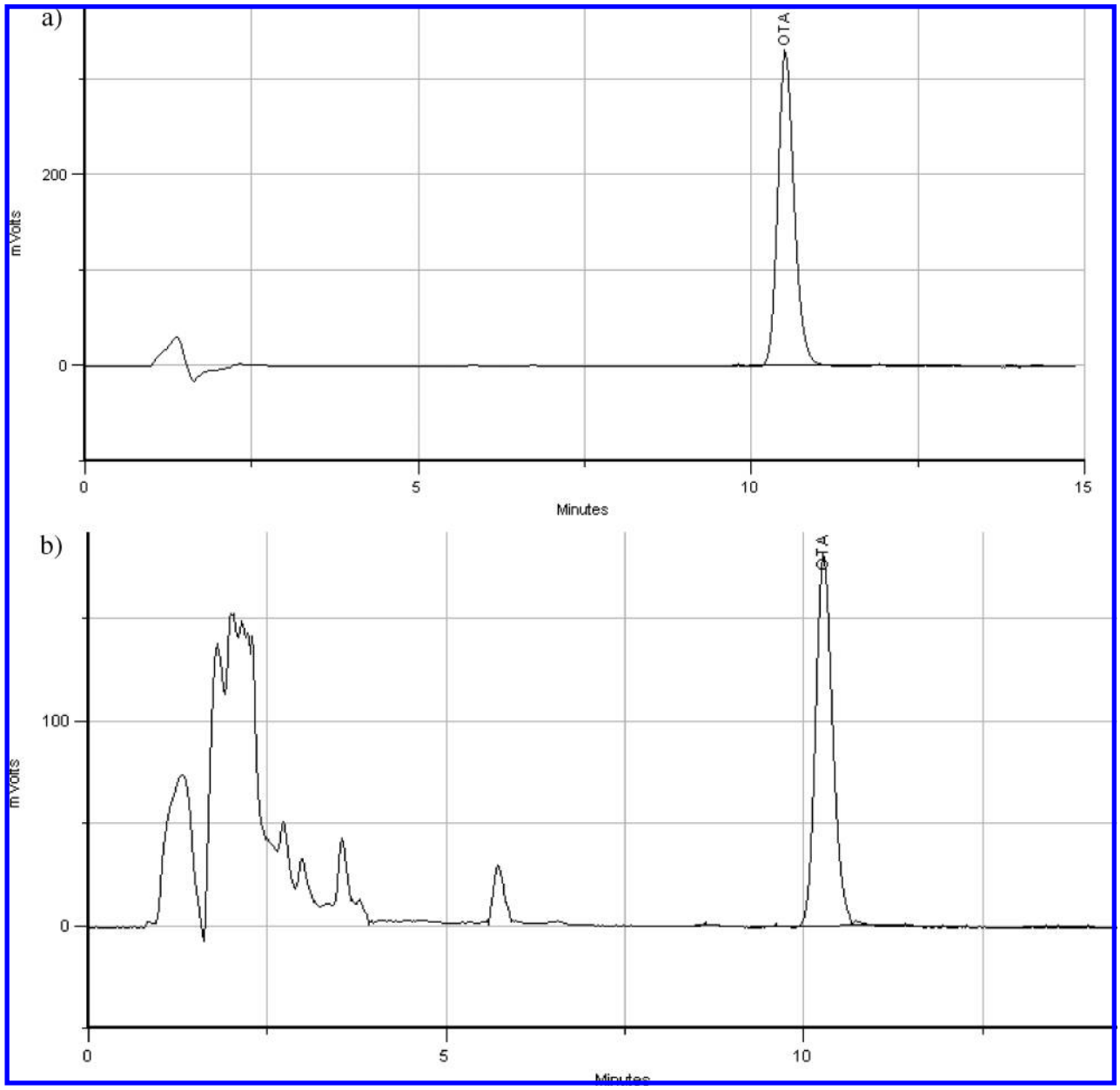

Figure 1. Chromatogram of (**a**) ochratoxin A standard solution 6.70 ng/mL (injected volume 150 μL) and (**b**) naturally contaminated red wine sample.

suitable for the purpose. To perform the MCA and the cluster analysis, Système Portable pour l'Analyze des Donnèes (SPAD) package version 5.0 was used.

The MCA is an exploratory technique to investigate the relationships among the multiple categorical variables (*38*−*41*). The aim was to identify the relationship among variables (i.e., wine type, OTA concentration, etc.) through factors (latent variables) that are not directly deducible from the tested samples. These factors permit one to summarize the information (total variability or inertia) and to obtain dependence relationships among the variables of interest. Furthermore, this analysis provided a graphical representation of factors that allows a clearer interpretation through the visualization of position modalities (for example, Piedmont, DOC, 1.0−2.0 ng/mL and so on) of variables on a two-dimensional graphical display. Factors were represented as axis of a Cartesian plan on which all the modalities were projected. The proximity of modalities indicated similarity while distance indicated difference.

Based on factors identified by MCA, CA was applied to better define the partitions of units detected through the interpretation of the first factorial plan (first and second factors) (**Figure 2**).

The inertia (variance) of factors detected by MCA is artificially diminished, and therefore the percentage of inertia depicted by every axis is severely underestimated. A Benzécri formula is often used to correct this underestimation (*38, 39*).

Daily Intake. Daily intake as reported in **Tables 2** and **3** was calculated multiplying the mean of the consumption rate, derived from Turrini et al. (*42*), by the mean of OTA contamination level and dividing by the average body weight (70 kg). It should be noted that, in order to evaluate the exposure of high consumers, an additional calculation of the intake was done multiplying the sum of the mean consumption rate and 1 standard deviation (sd) by the mean OTA concentration level.

RESULTS AND DISCUSSION

Descriptive Analysis. Data population of the present study was composed of 1166 units and 8 variables as follows: region of production, geographical area (north, center, south and islands), kind of wine (red, white rosé and dessert), quality of wine (TW, IGT, DOC and DOCG, see below for explanation),

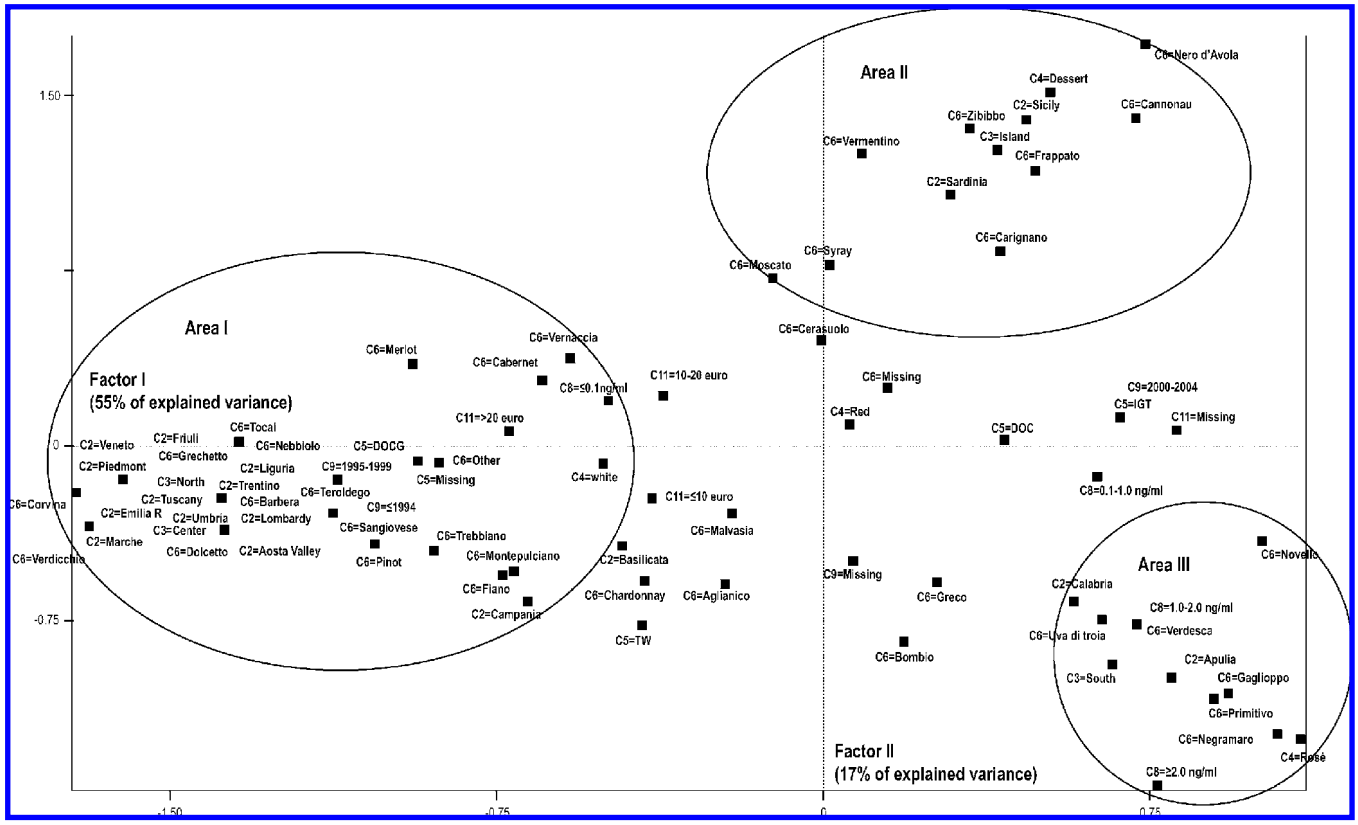

Figure 2. First factorial plan (first and second factors) and clusters.

Table 1. Ochratoxin A Concentrations per Wine Type, Geographic Area, Quality of Wine (Denomination), Year, and Price

		N	mean (ng/mL)	median (ng/mL)	95th percentile (ng/mL)	*N* > 0.01 (ng/mL)	% > 0.01 (ng/mL)	max (ng/mL)
all		1166	0.28	0.07	1.18	750	64.32	7.50
type	red	773	0.34	0.10	1.30	535	69.21	7.50
	dessert	28	0.26	0.05	1.72	18	64.28	1.90
	white	290	0.08	0.005[a]	0.37	128	44.13	1.95
	rosé	75	0.50	0.25	2.31	69	92.00	4.07
area	north	178	0.01	0.005[a]	0.07	13	7.30	0.42
	center	138	0.07	0.005[a]	0.41	42	30.43	1.19
	south	460	0.54	0.24	2.03	408	88.69	7.50
	island	390	0.19	0.08	0.69	287	73.59	5.94
quality (denomination)	TW	39	0.74	0.02	4.52	20	51.28	5.00
	IGT	410	0.34	0.14	1.16	336	81.95	7.50
	DOC	299	0.29	0.11	1.25	219	73.24	4.13
	DOCG	6	0.005[a]	0.005[a]	0.005[a]	0.00	0.00	0.005
	no information	412	0.19	0.005[a]	0.98	175	42.75	5.94
year	≤1994	94	0.13	0.005[a]	0.78	31	32.98	2.04
	1995−1999	324	0.19	0.005[a]	0.84	124	38.27	5.94
	2000−2004	633	0.31	0.12	1.16	518	81.83	5.06
	no information	115	0.53	0.09	4.00	77	66.95	7.5
price	≤10 euros	408	0.22	0.03	1.12	227	55.64	5.26
	10−20 euros	271	0.15	0.02	0.51	144	35.29	4.33
	>20 euros	89	0.17	0.006	0.66	39	43.82	5.94
	no information	398	0.47	0.19	1.72	340	85.43	7.5

[a] 0.005 ng/mL was assigned to all samples with a contamination below LOD.

grape varieties, OTA concentration in ng/mL, year of harvesting, production, and price.

The data was pooled according to three time periods (before 1994, 1995–1999, 2000–2004) rather than individual year of harvest. This was done to give a general overview of the trends and to obtain a suitable size group for statistical analysis. Although it would have been preferable to discuss data from individual years, because OTA content may depend on the particular weather conditions of the year of harvest, there were not enough data for some years for this approach to be meaningful.

Tables 1 and **2** show the results obtained for 6 out of 8 variables of the 1166 units.

As far as wine type is concerned, despite the higher mean OTA concentration of rosé wines, maximum values of concentration were detected in red wine. The 95.0% of red wines

Table 2. Ochratoxin A Concentrations per Region of Italy

		N	mean (ng/mL)	median (ng/mL)	95th percentile (ng/mL)	$n > 0.01$ (ng/mL)	% > 0.01 (ng/mL)	max (ng/mL)
all		1166	0.28	0.07	1.18	750	64.32	7.50
north	Emilia Romagna	30	0.005[a]	0.005[a]	0.005[a]	0	0.00	0.005
	Friuli	27	0.005[a]	0.005[a]	0.005[a]	0	0.00	0.005
	Liguria	5	0.02	0.005[a]	0.08	1	20.00	0.08
	Lombardy	11	0.01	0.005[a]	0.08	1	9.09	0.08
	Piedmont	34	0.005[a]	0.005[a]	0.005[a]	0	0.00	0.005
	Trentino	21	0.04	0.005[a]	0.21	6	28.57	0.36
	Aosta Valley	13	0.005[a]	0.005[a]	0.005[a]	0	0.00	0.005
	Veneto	37	0.03	0.005[a]	0.36	3	8.11	0.42
center	Abruzzo	11	0.02	0.005[a]	0.12	3	27.27	0.12
	Latium	21	0.05	0.005[a]	0.17	6	28.57	0.41
	Marche	34	0.04	0.005[a]	0.16	10	29.41	0.65
	Tuscany	54	0.09	0.005[a]	0.54	20	37.04	0.83
	Umbria	18	0.10	0.005[a]	1.19	3	16.67	1.19
south	Apulia	381	0.58	0.28	2.09	348	91.33	7.50
	Campania	29	0.12	0.02	0.49	15	51.72	1.16
	Calabria	41	0.52	0.21	1.28	39	95.12	5.26
	Basilicata	9	0.10	0.09	0.33	6	66.67	0.33
islands	Sicily	233	0.21	0.07	0.78	163	69.95	5.94
	Sardinia	157	0.16	0.08	0.53	124	78.98	2.03

[a] 0.005 ng/mL was assigned to all samples with a contamination below LOD.

Table 3. Ochratoxin A Dietary Intake in Wine: Comparison between Data from SCOOP Task 3.2.7 and the Present Study

			consumption of wine (mL/day)		OTA contamination in wine samples (ng/mL)		OTA intake[a] (ng/(kg of b.w.)/day)		
survey	matrix	population	av	95th percentile	av	range	av	95th percentile	intake for worse consumption[d]
SCOOP Task 3.2.7	red wine	total	46.93	292	1.29	<LOD−15.60	0.86	5.38	
		consumers only	159.76	450.4			2.94	8.30	
present study 1999−2006	red wine	total	46.93[a]	292	0.34	<LOD−7.50	0.27	1.65	
		consumers only	159.76[b]	450.4			0.91	2.55	
	wine	total	83.7 ± 142.5[c]		0.28	<LOD−7.50	0.33		0.90
		consumers only	148 ± 162.5[c]				0.59		1.24

[a] Body weight (70 kg). [b] Consumption data as from ref *1*. [c] Consumption data as from updated study ref *42*. [d] Intake data derived multiplying the sum of average consumption data and 1 sd by average OTA concentration level.

showed values of concentration between the limit of quantification (LOQ) and 1.30 ng/mL and of the remaining 5% only 2.84% of wines ($N = 22$) showed OTA concentration higher or equal to 2.0 ng/mL. However among the rosé wines, despite the lower number of samples collected, a higher percentage (5.33%) of wines with a concentration up to or equal to 2.0 ng/mL was observed; in addition, the 95th percentile was also higher accounting for a value of 2.31 ng/mL.

As far as geographical locations are concerned, a gradual increase of mean concentration from north (0.01 ng/mL) to south of Italy (0.54 ng/mL) was observed.

Wines produced in the regions of southern Italy showed a clear trend of higher concentrations than northern and central regions, with the 95th percentile value exceeding the statutory limit currently in force. Among the regions of southern Italy, Apulia showed the highest mean concentration (0.58 ng/mL) in combination also with also the highest maximum value (7.50 ng/mL). The profile of the concentration values showed a similar trend also in Calabria. It is assumed that the difference in latitude from 46.8° to 36.8° parallel and the consequent significant climatic differences between the north and south of Italy can influence mold contamination and OTA production.

As for wine quality categories (in the tables indicated as denomination variable), Italy has four major categories: (i) table wine (TW, *Vino da Tavola*) representing the lowest quality of wine, made by the producer as he sees fit; (ii) IGT (*Indicazione Geografica Tipica*) characterizing a wine produced in a specific geographical area; (iii) DOC (*Denominazione di Origine Controllata*) indicating wines produced in specific well-defined regions and according to specific rules and protocols designed to preserve the traditional wine-making practices; (iv) DOCG (*Denominazione di Origine Controllata e Garantita*) which is similar to the DOC type but with even more stringent conditions, since DOCG wines must pass an evaluation by a wine tasting committee before they can be bottled.

In this study the IGT wines showed the highest value of OTA (7.50 ng/mL) but only 2.68% ($N = 11$) exceeded the statutory limit, while table wine showed the most alarming situation with 12.82% ($N = 5$) of samples exceeded 2 ng/mL with a 95th percentile up to 4.52 ng/mL. Vice versa, the DOCG wine samples, coherently with their higher quality, resulted with concentration levels below the limit of detection (LOD) in all samples.

An increase of OTA concentration levels throughout the harvest years was observed especially in the years after 2000. In the group of samples where information on the year of harvest was missing ("no information" in **Table 1**, see footnote *b*), very

Table 4. Ochratoxin A Dietary Intake in Wine: Comparison among Italian Geographic Areas

survey	area	population	consumption of wine (mL/day),[a] av ± 1 sd[b]	OTA contamination in wine samples (ng/mL) av	OTA contamination in wine samples (ng/mL) range	OTA intake (ng/(kg of b.w.)/day) c	OTA intake (ng/(kg of b.w.)/day) d
present study 1999−2006	north	total	83.7 ± 142.5	0.02	<LOD−0.42	0.02	0.06
		consumers only	148 ± 162.5			0.04	0.09
	center	total	83.7 ± 142.5	0.06	<LOD−1.19	0.07	0.19
		consumers only	148 ± 162.5			0.13	0.27
	south and islands	total	83.7 ± 142.5	0.38	<LOD−7.50	0.45	1.23
		consumers only	148 ± 162.5			0.80	1.68

[a] Consumption data as from ref *42*. [b] sd: standard deviation as from ref *42*. [c] Body weight 70 kg. [d] Intake data as derived multiplying average consumption data + 1 sd by average OTA concentration level.

high concentration levels were found with 95th percentile level of 4.0 ng/mL.

More specifically, the correlation between the harvest years with OTA concentration levels showed that wine production in the years 1997, 2002 and 2004 reported higher results with higher standard deviation values. An explanation of this trend could be attributed to the climate conditions that occurred in the harvest season of those years characterized by very hot and humid conditions.

Samples produced from 1971 to 1992 showed low levels of OTA (ranging from 0.01 to 0.88 ng/mL) proving that on average no health problem arose for wine produced in those years.

The price of wines and grape variety did not show direct correlation with the sanitary quality with differences in concentration of OTA showing no univocal correspondence with the commercial value, indeed the means of OTA concentration in the different classes of prices are quite similar. Despite the presence of OTA high levels in table wines, the lack of correlation between price and OTA concentration was evidenced by the Pearson's coefficient of correlation (−0.05011, $p = 0.1653$).

By comparing the results of the present study with past surveys performed, a non-homogeneous trend of the mean OTA levels was observed, these being higher than those reported by Pietri (*24*), Cecco and Bocchi (*43*) and Lo Curto et al. (*44*), but lower than the most recent surveys (*28*). This could be ascribed to many factors including the different origin of cultivars, the geographical location of vineyards, and the year of harvesting and production, and the different implementation by the involved stakeholders of the good agricultural practices used in the viticulture and winemaking processes in the past years.

Statistical analysis. *Multiple Correspondence Analysis.* Multiple correspondence analysis, MCA, was applied to identify factors that are used by cluster analysis, CA, to better define the partitions of units detected through the interpretation of the first factorial plan (first and second factors) or potentially particular groups (**Figure 2**).

The inertia (variance) of factors detected by MCA was artificially diminished, and therefore the percentage of inertia depicted by every axis was severely underestimated. A Benzécri formula has been often used to correct this underestimation.

According to Benzècri (*38, 39*) revaluation, the first 2 factors represent 72.1% of the total variability, the first factor alone representing 55.0% of all information provided by the data set.

Statistical analysis therefore was conducted only on the first factorial plan (**Figure 2**), permitting the individualization of 3 areas of correlated modalities. The first area (Area I) contains less contaminated wines (≤0.1 ng/mL), wines produced in north and central Italy or before the year 2000, and the white wines. The second area (Area II) contains dessert wines and wines produced in Sicily and Sardinia. The third area (Area III) contains wines produced in southern Italy, rosé wines and wines with the highest contamination (1.0−2.0 ng/mL and >2.0 ng/mL).

The variable "price" did not have a relevant role in the definition of areas.

Cluster Analysis. With MCA, CA was applied with the hierarchical method considering the first two factors. The dependence relationships detected by MCA are a starting point to define homogeneous groups (cluster) of wines.

The CA substantially confirmed MCA outcomes and better defined the characteristics of the groups identified by the first factorial plan.

The first cluster was composed of 363 wines, and this represented 31.13% of all samples, the second of 386 wines (33.10%) and the third of 417 wines (35.76%) (**Figure 2**).

The analysis of the output as obtained from the cluster analysis revealed that (i) the first cluster was mainly composed of wines with low contamination, wines from north and central productions before the year 2000, table wines and white wines; (ii) the second cluster was characterized by IGT and DOC, productions after 2000, red and dessert wines; (iii) the third cluster was represented by wines with an OTA concentration higher than 1.0 ng/mL, IGT, DOC and table wines, wine productions later than 2000 and rosé wines.

As shown in **Figure 2**, the third cluster contained more contaminated wines and wines produced in the south of Italy, in particular from Apulia, showing an interrelationship between these modalities.

As from the scenario depicted by the statistical analysis, the following considerations can be drawn: in our study, only 2.2% of the overall samples exceeded the European regulatory limit (2.0 ng/mL) (*27*), while 6.5% showed an OTA contamination between 1.0 and 2.0 ng/mL.

Occurrence data in our study demonstrated that the contamination of OTA in Italian wines is generally quite low and generally follows the following sequence: red wine, south of Italy (Apulia and Calabria) and then table wine.

Daily Intake. From the obtained occurrence data, the evaluation of the exposure risk by the Italian consumers has been made taking into account official consumption data for *consumers only*, and total population.

At the European level, a unique pan-European study has been performed for the evaluation of the exposure of the European population, namely the SCOOP Task 3.2.7 (Scientific Cooperation on Question Related to Food) on "Assessment of dietary intake of OTA by the population of EU Member States" (*1*). In the SCOOP database, the estimation of daily intake was possible only for red wine as its consumption data was only available.

It should be noted that at the time of the study a lower toxicological threshold existed, namely a TDI of 5 ng/(kg of b.w.)/day.

In our study, the exposure assessment calculation was performed crossing the produced occurrence data with different data sets of wine consumption. Results are shown in **Table 3**.

Exposure assessment was calculated as follows:

(i) The calculation was performed only on red wine using the consumption data reported in the SCOOP Task, with the aim to compare directly the corresponding results. In comparison to the intake data from the SCOOP study, a decrease of exposure could be noted both for total population (from 0.86 to 0.27 ng/(kg of b.w.)/day) and consumer only (from 2.94 to 0.91 ng/(kg of b.w.)/day).

(ii) The exposure calculation was performed crossing the occurrence data produced within this study without any differentiation of the type of wine with more updated consumption data provided with standard deviations (*42*). The average estimated daily intake was 0.33 ng/kg of b.w. for total population and 0.59 ng/kg of b.w. for *consumers only*. By considering the mean consumption data + 1 sd an intake of 0.90 ng/kg of b.w. and 1.24 ng/kg of b.w. for total population and *consumers only* was obtained. It should be noted that wine consumption for *consumers only* is characteristic of the male and elderly population, and it is not differentiated by the geographical area.

In addition, since geographical region of production was observed to have a strong influence on OTA levels, the exposure calculation was performed separately for the 3 different regions (north, central, south and islands) (**Table 4**). This calculation was also performed using the updated INRAN consumption data (*42*). The results for the exposure assessment were the following: north Italy 0.02 ng/(kg of b.w.)/day for total population and 0.04 ng/(kg of b.w.)/day for *consumers only*; central Italy 0.07 ng/(kg of b.w.)/day for total population and 0.13 ng/(kg of b.w.)/day for *consumers only*; south of Italy and islands 0.45 ng/(kg of b.w.)/day for total population and 0.80 ng/(kg of b.w.)/day for *consumers only*. Furthermore, with the aim of considering the most realistic worse exposure situation, the intake for *consumers only,* obtained multiplying the sum of the average consumption data and 1 sd (standard deviation) (see **Table 4**) by the mean OTA concentration level, still gave a quite lower intake than the new TDI even for the south of Italy and island consumers. This accounted for 9.8% of contribution to the daily tolerable toxicological threshold (1.68 ng/(kg of b.w.)/day vs 17.1 ng/(kg of b.w.)/day). Even in the worst case, therefore, the reported exposure values clearly indicate that wine contribution does not represent a serious risk factor for the Italian population.

It should be remembered that in the SCOOP Task the intake from the red wine for *consumers only* accounted for 58.8% with respect to the TDI of 5 ng/(kg of b.w.)/day taken into consideration at that time and that even considering the new TDI of 17.1 ng/(kg of b.w.)/day, a contribution equal to 17% higher than the one reported with this study is obtained.

This finding shows a noteworthy decrease of the contribution of wine in the overall intake from the diet for OTA intake by Italian consumers, as a result of the abovementioned actions for preventing the risk of mold growth on the grape bunch and OTA presence in wine.

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