C A U S E S and origins of wine contamination by HALOANISOLEs (chloroanisoles and bromoanisoles)

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INTRODUCTION

Wine contamination with fungal aromas is currently recognized as one of the wine’s worst threat for the cellars all around the world. Traditionally, and erroneously, this phenomenon has been considered exclusively associated with the cork. From this point of view, the cork stopper would act as the source of wine contamination by chloroanisoles (specially 2,4,6-trichloroanisol, or TCA). This organic compound is able to confer a very unpleasant fungal aroma to the wine, even at concentrations as low as 2-4 ng/l.

As a consequence, this phenomenon has been named “cork taint” or wine contamination by the cork stopper. However, bearing in mind the different and multiple possible contaminating sources, the most appropriate term that should be used is wine contamination by chloroanisoles. The massive and indiscriminate use of this term (cork taint) has contributed to the spreading of the idea, between the technical personnel of cellars, and also among the general public, that contamination of the wine by chloroanisoles is attributed, always and exclusively, to the cork stopper. However, according to scientific investigations this particular notion has proven to be false.

The above mentioned pollutants can also appear in other foods, or even in the water for public consumption. In fact, it is a well documented problem and more widespread than what might be thought, which does not concern exclusively to the wine sector.

The studies conducted by different research groups indicate that, the wine pollution by haloanisoles is more complex than was presumed to be. In fact, this problem is caused by several chemical compounds and factors, which can not be attributed exclusively to the cork stopper. This way, it is becoming more evident that a high proportion of contaminated wines derive from contamination events suffered in the proper cellar.
In this report we will describe in an exhaustive way the current state of the topic, reviewing the last conducted studies and the future prospects regarding this problem.

The fungal aroma as an indicator of food contamination

The fungal aroma of the wine is a consequence of the presence in the environment (air, water, wood, etc.) of microorganisms (specially filamentous fungi), which when getting into contact with halophenols halophenols (a kind of highly toxic pesticides), develop a defensive mechanism that leads to the production of haloanisoles. These pesticides are chlorophenols, fluorophenols, iodophenols and bromophenols (see figure 1).

The haloanisoles are very significant pollutants, being able to wreck the natural aromas and flavors of any wine. Their more important characteristics are:

A They produce unpleasant odors frequently defined as musty or moldy. In the case of 2,4,6-tribromoanisole (TBA), if the contamination level is higher than 20 ng/L, besides an intense musty odor, the wine also can exhibit a phenolic or iodized character (Chatonnet et al., 2004).

B Haloanisoles have a very low perception thresholds in alcoholic solution, in the range of ng/L (1 ng or nanogram corresponds to $10^{-9}$ grams). This fact is highly significant, since a very small amount of certain haloanisoles is perceived with clarity in taste and smell. The perception thresholds of the main haloanisoles identified as contaminants in both wine and water are listed in table 1.

C Generally, these compounds are very volatile, easily being transmitted through the air, and show a great capability to adhere and contaminate wood, cork and many other materials (plastic polymers, silicons, cardboard and paper, gums, etc.).
These characteristics determine that when these compounds appear as pollutants in foods, even at very low levels, they produce a disgusting fungal sensations (smells and flavors) which makes their consumption inadvisable.

The pollution by halogenoanisoles can affect a large variety of food products

The first data regarding the contamination of wines by halogenoanisoles, and more specifically by TCA, were reported by Tanner et al., 1981; and Buser et al., 1982. In a later work Amon and co-workers (1989) identified several chemical compounds responsible for the contamination of wines, denoting the role of 2,4,6-TCA as the main contaminant agent.

However, the detection of chloroanisoles (2,4,6-TCA and 2,3,4,6-tetrachloroanisole or 2,3,4,6-TeCA) as contaminants of other foods was previous to their detection in wine:

- In 1966 Engel and colleagues detected a batch of chickens and eggs contaminated with 2,3,4,6-TeCA.
- Later, it was reported that 2,3,4,6-TeCA was the responsible compound for the unpleasant odor of a batch of chickens from a broiler house (Curtis et al., 1972 and 1974).
- Pentachloroanisole has been described to produce a musty odor in pulp chips stored in a mill (Cserjesi and Johnson, 1972).
- Also, 2,4,6-TCA and 2,3,4,6-TeCA have been reported to be the responsible for the bad odor of a lot of dried fruits (Whitfield et al., 1985; Tindale et al., 1989) and a batch of Brazilian coffee (Spadone et al., 1990).
- More recently, 2,4,6-TCA has been pointed out as responsible for a musty off-flavor in drinking water in Sweden (Nystrom et al., 1992), and also as the cause of a musty/muddy odor detected in the traditional Japanese alcoholic beverage, sake (Miki et al., 2005).
HALOANISOLES AS WINE CONTAMINANTS: CAUSES, PRODUCING MICROORGANISMS AND SOURCES OF THE CONTAMINATION

Halophenols and haloanisoles: chemical structure

The haloanisoles (chloroanisoles and bromoanisoles) more frequently identified as wine contaminants are the 2,4,6-trichloroanisol (2,4,6-TCA), the 2,3,4,6-tetrachloroanisol (2,3,4,6-TeCA), the pentachloroanisol (PCA) and finally the 2,4,6-tribromoanisol (2,4,6-TBA). Their chemical structures are shown in figure 1.

Structurally, they can be considered as derivatives of the anisole (or methoxybenzene), which contains in their molecule at least one atom of an halogen. The anisole is a phenol-derived compound present in a great variety of volatile substances that are usually very scented. There are four halogens: fluorine (F), chlorine (Cl), bromine (Br) and iodine (I). When at least one atom of an halogen is present in the anisole’s molecule, the resultant compounds are called haloanisoles, which can then be fluoroanisoles, chloroanisoles, bromoanisoles or iodoanisoles, depending on the halogen present in the molecule.

The chemical structures of the main haloanisoles involved in wine contamination are indicated in figure 1.

From a biological point of view haloanisoles originate from a biochemical defensive reaction known as biotransformation, that occurs in certain microorganisms (mainly filamentous fungi). This reaction takes place when the filamentous fungi get into contact with halophenols (mainly chlorophenols – see and bromophenols) which are massively used as highly toxic pesticides and fungicides. As a result of this biotransformation reaction the highly toxic halophenols

Causes and origins of wine contamination by haloanisoles (chloroanisoles and bromoanisoles)
are converted into non-toxic haloanisoles (see figure 4). Therefore, the formation of haloanisoles is a survival mechanism for many microorganisms when exposed to environments polluted by halophenols.

**Figure 1.** Chemical structure of the anisole and the main halophenols (chlorophenols and bromophenols) and haloanisoles involved in the contamination of wine.
The halophenols are phenol-derivative chemical compounds that carry in their structure one or more atoms of a halogen (see figure 2). Halophenols are currently widely used as pesticides due to their high toxicity for almost any living organism.

The halophenols more widely used in industries are chlorophenols. They are named mono-, di-, tri-, tetra- or pentachlorophenols when carrying in their structure 1, 2, 3, 4 or 5 chlorine atoms respectively (see figure 2). In a similar way we call them fluorophenols, iодophenols or bromophenols when the halogen present is fluorine, iodine or bromine respectively.

![Figure 2](image-url)

**Figure 2.** Structure of phenol and several derivative chlorophenols carrying from one to five chlorine atoms in their molecule.

In a similar way bromophenols, fluorophenols and iодophenols would be structurally identical except for the halogen present in their molecules (bromine, fluorine or iodine respectively).
Table 1. Perception thresholds of the main chloroanisoles and bromoanisoles involved in the contamination of wine according to the data of several authors.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>DETECTION THRESHOLD</th>
<th>STRUCTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4,6-TCA</td>
<td>In water: 30 – 300 pg/L* ((a, b)) In alcoholic solution: (\text{wine}): 1.5 – 3 ng/L* ((c))</td>
<td>![2,4,6-TCA structure]</td>
</tr>
<tr>
<td>2,3,4,6-TeCA</td>
<td>In water: 4 ng/l ((a)) In alcoholic solution: (\text{wine}): - 10 – 15 ng/L (white and red wines) ((d)) - 5 ng/L (sparkling wines) ((d))</td>
<td>![2,3,4,6-TeCA structure]</td>
</tr>
<tr>
<td>PCA</td>
<td>Weakly scented compound Detection threshold &gt; 50 µg/l* ((d))</td>
<td>![PCA structure]</td>
</tr>
<tr>
<td>2,4,6-TBA</td>
<td>In water: 8 – 30 pg/l ((e,f,g,i)) In alcoholic solution: (\text{wine}): 3.4 ng/l ((d))</td>
<td>![2,4,6-TBA structure]</td>
</tr>
</tbody>
</table>

* µg (microgram) is equal to \(10^{-6}\) grams; ng (nanogram) is equal to \(10^{-9}\) grams; pg (picogram) is equal to \(10^{-12}\) grams
\((a)\): Curtis et al., 1972; \((b)\): Griffiths, 1974; \((c)\): Duerr, 1985
\((d)\): Chatonnet, P., 2004
\((e)\): Saxby et al., 1982; \((f)\): Whitfield et al., 1997; \((g)\): Malleret and Bruchet, 2002

Causes and origins of wine contamination by haloanisoles (chloroanisoles and bromoanisoles)
Origin of the Halophenols and haloanisoles detected as contaminants in cork and wines.

Chlorophenols and bromophenols are the halophenols more widely employed in industrial processes. These compounds, as indicated before, can be transformed into haloanisoles by the biological action of some microorganisms, mainly filamentous fungi (as a *Trichoderma longibrachiatum* strain isolated in our laboratory from cork samples) which are able to carry out the biomethylation reaction described in figure 4 (Coque *et al.*, 2003).

**Figure 3. Environmental origin of chlorophenols and chloroanisoles contaminating cork, wood and other materials**

Causes and origins of wine contamination by haloanisoles (chloroanisoles and bromoanisoles)
Chlorophenols are not natural compounds. In fact, they can not be found in nature but for their anthropogenic origin. This fact determines that we can hardly find microorganisms able to degrade these chemical compounds. As a consequence, they are very recalcitrant and can persist in the ecosystems for a very long time (even decades).

Some authors have proposed that chlorophenols could be formed during the chlorination treatment of drinking water due to the reaction of hypochlorite with phenols dissolved in water (Nystrom et al., 1992). In a similar fashion, in the cork they could originate during the hypochlorite-bleaching of the stoppers (Sponholz y Muno, 1994). It is important to mention that this bleaching practice has fallen out of date in the cork companies, being replaced by a bleaching wash in a hydrogen peroxide bath (10%) containing ammonia (5%).

However, the main reason for the presence of chlorophenols in cellars and also in cork slabs is the massive use of the PCP and 2,4,6-TCP as pesticides all around the world, although its use in the European Union has been forbidden several years ago. Additionally, the PCP batches are frequently contaminated with traces (2-8%) of 2,6-DCP and 2,3,4,6-TeCP. Recently it has been shown that 2,4,6-TCP can be produced from the aquatic degradation of the pesticide triclosan [2-(2,4-dichlorophenoxy)-5-chlorophenol] in the presence of low concentrations of free chlorine (Canosa et al., 2005).

There are several important reasons why chlorophenols are so widely used as pesticides:

- They are very easy to synthesize by the chemical industries.
- They are very cheap.
- They are liposoluble (soluble in fats) and for that reason they can easily cross the biological membranes entering into the cells.
- They are very toxic. This toxicity is due to their capability to react and destroy proteins and the genetic material (DNA) of the cells.
- They are very effective and have a very wide spectrum action. In fact they are toxic not only to microorganisms, but also to insects and plants.
Chlorophenols have been widely used during decades, mainly as fungicides to preserve wood from fungal attack, and also to prevent fungal growth on package materials as cardboard, leather and textile goods, and crops and grains of cereals stored in silos. As a consequence they have become one of the more important group of pollutants detected in many different environments - see figure 3 - (Chaudhry and Chapalamadugu, 1991). In fact, several studies have detected chlorophenols as contaminants in aquatic ecosystems of Sweden (Nystrom et al., 1992); also TCA, TeCA, PCA and their related chlorophenols have been found in cork slabs collected from Portuguese oak tree forests before stopper manufacture (Rigaud et al., 1984); contamination by PCP and PCA has been detected in Canadian forests (Cserjesi and Johnson, 1972); and also is well known that chlorophenols are present in high amounts in chlorine bleaching Kraft pulp mill effluents (Huynh et al., 1985).

The use of chlorophenols is currently forbidden in Europe because of their suspected carcinogenic characteristics. However, they continue to be highly employed in Asia, Africa and South America.

Bearing in mind all these data we can state that the putative contamination of wine can originate at two different levels.

- Contamination of the cork slabs produced either directly in the oak tree forests, or in the installations of the cork company during the manufacturing process.

- Contamination of the wine, or the stopper, during the storage in the cellar. In this case contamination usually originates from wooden structures (walls, floors, ceilings), barrels or stands for bottles storage.

The most routinely used compounds after chlorophenols are the bromophenols (specially the 2,4,6-tribromophenol or 2,4,6-TBP). TBP can be naturally found in marine environments since it is synthesized by brown algae as a way to remove excessive bromine (Flodin and Whitfield, 1999). It has also been described that TBP can be chemically formed in wastewater treated with chlorine in the presence of bromide ions and traces of organic phenols (Patnaik et al., 2002).

The bromophenols are also produced in huge amounts by chemical industries. Their main applications are as antifungal agents and flame retardants on wood and many plastics and paintings (Chatonnet et al, 2004). In Europe these compounds are substituting the forbidden chlorophenols.
The detection of TBP as a wine contaminant in several French wineries (Chatonnet, 2004) is in direct relation to its presence in the cellars, where it might have originated from paintings, varnishes, plastics and treated woods.

**Molecular mechanism for the formation of chloroanisoles**

Studies carried out by our research group led us to obtain several important conclusions about the molecular mechanism involved in the formation of chloroanisoles (Álvarez-Rodríguez et al., 2002a; 2004 y Coque et al., 2003).

**a)** Although several hypothesis have been proposed to explain the origin of chloroanisoles, both in cork and cellars, currently the only scientifically proved origin of the 2,4,6-TCA is the biomethylation of 2,4,6-TCP (see biochemical reaction in figure 4).

**b)** This biomethylation reaction is mainly performed by filamentous fungi, that can grow on the surface of both cork planks and wooden elements in the cellar (Álvarez-Rodríguez et al., 2003). It has also been reported that some actinomycetes isolated from drinking water could transform TCP into TCA/ (Nystrom et al., 1992).

**c)** This reaction is carried out by a protein (enzyme) named chlorophenol O-methyltransferase (CPOMT), that can methylate a wide range of halophenols (including chlorophenols, bromophenols and iodosphenols) (Coque et al., 2003) to produce their corresponding haloanisoles.

**d)** Therefore one single enzyme, the CPOMT, is responsible for the formation of all the anisoles that can be found in wine: 2,4,6-TCA, 2,3,4,6-TeCA, PCA and 2,4,6-TBA.

*Figure 4*. Mechanism for the formation by a *Trichoderma longibrachiatum* fungal strain of 2,4,6-TCA by O-methylation of 2,4,6-TCP in a reaction catalyzed by the enzyme Chlorophenol O-methyltransferase (CPOMT).
¿Why filamentous fungi produce chloroanisoles?

Today it is well documented that filamentous fungi only produce chloroanisoles when they get into contact with chlorophenols.

Due to their high toxicity (that is the reason why they are used as pesticides) when filamentous fungi detect the presence of chlorophenols in the environment they actively try their inactivation (process called detoxification or removal of the toxicity), since otherwise they might die or suffer important damages affecting their physiology. In order to perform this detoxification the filamentous fungi have developed two different resistance strategies, as shown in figure 5.

I) When the fungal cell comes in contact with 2,4,6-TCP, it produces a type of oxidative enzymes (as for example laccases), that are actively secreted from the cell to attack and degrade the chlorophenols outside the cell. Thus, most of the 2,4,6-TCP is degraded without hurting the fungus.

II) Nevertheless, since chlorophenols are liposolubel, there is always a small proportion that can cross the cell wall and the cytoplasmic membrane reaching the content of the cell (cytoplasm and nucleus), where they can irreversibly damage important proteins, or even the genetic material (DNA). To avoid this threat can occur, the fungal defense system immediatly produces the enzyme CPOMT (Chlorophenol O-methyltransferase) which has been discovered by our research group. Such enzyme is responsible for the transformation of the highly toxic 2,4,6-TCP into a completely harmless compound, the 2,4,6-TCA. This compound is then secreted from the cell and it is rapidly absorbed by cork, wood, or any other material on which the filamentous fungi are growing.

Such defensive strategy is very common among filamentous fungi, so that most of the fungi present in both cork or cellars can synthesize anisoles. Besides, in our laboratory we have shown that this enzyme can act on several chlorophenols and bromophenols. Therefore, one single enzyme, the CPOMT is responsible for the formation of all the wine-contaminant chloroanisoles and bromoanisoles.
Figure 5. Mechanisms for the biotransformation of 2,4,6-TCP by filamentous fungi and origin of 2,4,6-TCA (and others anisoles) in cork and wood.

Causes and origins of wine contamination by haloanisoles (chloroanisoles and bromoanisoles)
Tracking down the origin of the contamination

The scientific data showed indicate that the true origin of the wine contamination by chloroanisoles is a problem of environmental contamination.

Nevertheless, departing from the fact that a limited number of cases can owe to the employment of defective stoppers, we can not stop affirming that the approach of blaming almost exclusively to the cork stopper is too simplistic and needs a more rigorous analysis, since there are many different sources of contamination. In fact, it is possible to detect in some cellars, contaminated wines that never have been in contact with cork (Chatonnet et al., 2004).

Contaminations attributable to the cork

Several studies indicate that cork stoppers would be responsible for a significant number of cases of wine contamination, although there are not rigorous studies to confirm with veracity the percentage of cases that would be really attributable to the stopper. In fact, there is certain confusion when one speaks about pollutions attributable to the cork, being able to distinguish two clearly different situations:

I) The cork stopper as a contaminating element. In this case, when the stopper arrives to the cellar, it already has an unacceptable levels of haloanisoles. The origin of this contamination can be different:

- In certain occasions the pollution would originate at the forest, when the bark of a tree absorbs halophenols that are transported through the atmosphere or by the rain water, or that reach in an accidental way to the cork oak (see figure 3). Then the filamentous fungi that dwell on the bark would detoxificate these pesticides by degradation, or by transformation into haloanisoles, which remain adhered to the cork. This kind of contamination can be drafted along all the manufacturing process, and so the stopper is finally tainted.

- In other cases, the cork planks arrive in a clean condition to the cork factories. However, the stopper is contaminated. In this situation we can
confirm that there are one or more sources of haloanisoles in the factories, being these compounds absorbed by the cork during the manufacturing steps.

II) The cork stopper as an element which is able to transmit a contamination existing in the cellar.

Occasionally the stopper can arrive clean at the cellar where it would be contaminated due to the existence of polluted material there. In this situation the stopper can absorb haloanisoles, and later transfer them to the wine. Thus, the cork stopper acts as a transmitter vehicle for the pollution, but its origin lays in the cellar. It is possible that a high percentage of contaminations attributed to the cork belong to this category, being then the cellar responsible for the pollution.

On the other hand, the analysis that we can do is different depending on the type of the stopper:

- **Agglomerate cork stopper.** The analysis of different samples of granulated cork from which the composite or agglomerate stoppers are produced indicated that this material has basal levels, although generally low, of 2,4,6-TCA contamination (Álvarez-Rodríguez et al., 2002a) that can be transmitted to the wine.

- **Natural cork stopper.** In the case of natural cork stoppers it has been recently estimated a medium level of contamination of 5.6% (Soleas et al., 2002). According to Sefton and Simpson (2005), nearly all natural bark closures contain some TCA but in most cases this TCA does not contaminate bottled wine.

However, the problem that raise the lots of natural stoppers is that corks of the same batch, and even of the same tree, can present different levels of contamination depending on several factors:

- first, the putative contamination of the tree from which they are produced.
- second, from their origin inside the same tree. Often the cork planks exhibit a gradient of chloroanisoles pollution that diminishes from the foot of the plant towards the branches.
- third, depending on punctual problems of contamination at the cork factory.
Behavior of the cork stopper with respect to chloroanisoles

The behaviour of cork stoppers with respect to chloroanisoles is ruled by a few basic principles:

- The cork stopper, as the wood and plastic polymers, has a high capability to absorb chloroanisoles that are propagated through the atmosphere without existing a direct physical contact with the source of pollution. This absorption is very fast and can take place in brief periods of time (24 hours) (Barker et al., 2001). This means that in the case of an existing contamination in the cellar, the cork might absorb the chloroanisoles and later transfer them to the wine, and therefore in this case the cork stopper is not the primary source of the problem.

- This absorption is limited to the 2 external mm of the stopper, and 2,4,6-TCA does not penetrate towards the center of the cork (Barker et al., 2001).

- The cork only can transfer to the wine the 2,4,6-TCA that is located at the surface that gets into contact with the wine (Pollnitz et al., 1996; Capone et al., 1999 and 2002). When the contamination is located in the center of the cork, it is not going to be significantly transmitted to the wine. In fact, some studies indicate that the average percentage of 2,4,6-TCA that a cork closure can yield to the wine after a period of 12 months of bottling ranges from 12 to 50% (Capone et al., 1999; Soleas et al., 2002; Hervé et al., 2004.)

- The cork closures can rapidly absorb a high percentage of the chloroanisoles present in a tainted wine. About half of 2,4,6-TCA was absorbed by cork closures over a 30-month period, and significantly 2,3,4,6-TeCA and PCA were even more efficiently absorbed (about 90%) from the wine (Capone et al., 1999). In some experiments 2,4,6-TCA was rapidly extracted from the wine by a group of four cork stoppers soaked in 200 mL of wine: about 80% was absorbed after 24 hours and nearly 90% after 48 hours. These studies suggest that when we detect a tainted cork...
stopper which has been in contact with the wine in the bottle, it could happen that the primary source of the contamination was the wine itself and not the closure. The only way to know which is the origin of the contamination is to carry out an extensive analysis of stoppers and wine samples. If there is no direct relation between the quantities of 2,4,6-TCA detected in the stopper and in the wine, the pollution would be attributable to the cork. If, on the contrary, the stoppers and the wine are contaminated in a uniform way the primary source of pollution would be the wine.

- The 2,4,6-TCA content of a stopper can either be uniform, and then the pollution would have happened along a very long period of time in the tree, or on the contrary it can concentrate in the external part of the stopper (Howland et al., 1997). In this later case the contamination would have happened after the extraction of the stopper from the plank, either at the cork factory or at the cellar (presumably during their storage).

- The addition of 2,4,6-TCA to the external part of corks being used as closures for wine bottles did not produce contamination of the wine after 3 years of bottling. This data clearly indicated that the cork stopper does not let the migration of 2,4,6-TCA inside the bottle, and therefore it represents an effective barrier able to avoid the tainting of a wine by exogenous chloroanisoles (Capone et al., 2002).

Taking together all this information, we can conclude that when we detect a tainted wine bottled with a cork stopper we can not affirm, without any doubt, that the primary source of pollution is the cork. In fact, additional analyses should be necessary to discard the hypothesis that the problem of pollution derives from the wine.

**Contamination of wines not attributable to the cork: contamination before the bottling**

As it has been previously indicated, every day there are more evidences indicating that an important percentage of wine contaminations by haloanisoles, are attributable to other elements different from the corks. Among them we can mention:

Causes and origins of wine contamination by haloanisoles (chloroanisoles and bromoanisoles)
There have been detected tainted wines with 2,4,6-TCA, PCA and 2,3,4,6-TeCA whose contamination originated from polluted wooden structures (roofs) or elements (barrels) at the cellar (Chatonnet et al., 1994). The analysis conducted indicate that woods also contained high amounts of chlorophenols. It is presumed that wood was treated with the fungicides PCP or 2,4,6-TCP. These compounds in the wet environment of the cellar would have been transformed to chloroanisoles by filamentous fungi. Since chloroanisoles are highly volatile they can easily travel through the air to contaminate many elements in the cellar.

In the case of 2,4,6-TBA contamination, three different situations have been described (Chatonnet et al., 2004):

- **Cellar A.** The contamination was firstly detected in wines stored in oak barrels (12 ng/l). A detailed analysis showed the contamination of elements such as the stainless steel vats (2 ng/l), the atmosphere of the cellar (46-162 ng/g adsorbent) and the oak barrels (143-224 ng/g wood). The detected levels of the precursor 2,4,6-TBP were even higher: the contamination was maximun in 24-month-old oak wood barrels, which suggests that this could be the origin of the contamination. The 2,4,6-TBP would be biologically transformed into 2,4,6-TBA by microbes. This later compound, once produced, would be easily transported through the air to contaminate the wine.

- **Cellar B.** In this case the primary source of the contamination were oak wood barrels that had been removed from the winery several years before. However, residual amounts of 2,4,6-TBP and 2,4,6-TBA (15-122 ng/g adsorbent) were adsorbed into the microporous winery structure covered with clay bricks, and they were released through the air to contaminate the new barrels and the wine (13-78 ng/l).

- **Cellar C.** In this winery a high contamination level by 2,4,6-TBP and 2,4,6-TBA (13-108 ng/l) was detected in wines aged in oak barrels. The highest level of pollution was detected in the wood framework of the cellar (82363 ng/g) and also in the painting that covered part of the walls (4933 ng/g). Analysis of the materials in contact with the atmosphere showed that wooden roof timbers had been massively...
impregnated with 2,4,6-TBP, which was slowly converted to 2,4,6-TBA due to the action of microorganisms in the atmosphere. These compounds later contaminated all the structures and elements in the cellar, specially the oak barrels and the silicone bungs used to seal the barrels. The primary source of the contamination seemed to be the use of paintings containing 2,4,6-TBP as fungicide or flame retardant.

SUMMARY

The existence of tainted wines with fungal off-odors is not solely attributable to the cork stopper:

- In fact, the average of the 2,4,6-TCA content of the cork has diminished about 75% in the last few years as a consequence of the many controls that the cork factories are adapting to avoid the contamination with haloanisoles (see www.celiege.com).

- 2,4,6-TCA can be also found in many elements in the wineries including water, the atmosphere, wooden structural elements, paintings, silicon and plastics.

- The cork stopper can only contaminate the wine when the haloanisoles are located at the surface that gets in contact with the wine. The contamination present in the central or inner part of the stopper cannot be transmitted to the wine.

- A tainted wine can transfer the contamination to the stopper. In fact, the cork has a great capability to absorb most of the haloanisoles present in a wine, diminishing its pollution level.

- A TCA-free cork stopper provides an absolute guarantee to preserve a bottled wine stored in a contaminated cellar since 2,4,6-TCA cannot migrate through the closure.
DIMENSION OF THE PROBLEM OF WINE CONTAMINATION BY CHLOROANISOLES

Wine contamination by chloroanisoles: current status of the problem

Despite being a big problem for the wineries all around the world, there are many discrepancies about its real incidence, and the quantification of the economic losses produced by TCA-mediated taint. This is mainly due to the lack of transparency of both the wineries and the cork companies facing this situation.

Economical losses of the wineries produced by chloroanisoles

The quantification of the economic losses of wineries all around the world by the wine contamination by chloroanisoles is almost impossible to calculate. Some published data that should be born in mind are the following:

- According to Butzke and coworkers (1999) it is estimated that cork-related wine spoilage exceeds over 10 billion $ in value. This includes losses resulting from physical defects of corks causing leakage and unwanted oxidations. It has been calculated that, only for the California state, a retail value of $180 to 630 millions is being spoiled every year by cork taint. Most of the people currently think that this quantification is totally wrong and overestimated.

- Other expertis, like Dr. Pascal Chatonnet, think that the accumulated losses for the wineries, since this problem was detected for the first time, could be about 1.000 millions $ (statement to El Mundo Vino, published January the 19th, 2004 in http://elmundovino.elmundo.es).
Frequency of wine contamination by chloroanisoles

There are many discrepancies about the real incidence of this problem. Depending on whether the study comes from a cork related sector, the winery world, or the sector of the synthetic stoppers. According to scientific data we can mention the following studies:

- Lee and Simpson (1993) estimated a level of taint ranging between 0.5-6%.

Later it has been suggested that the incidence of the problem would be about 2-7% of the bottles (Butzke et al., 1999).

- Recently, Soleas and colleagues (2002) have analyzed more than 2,400 different wines from several countries. The study consisted on tasting the wines by a panel of experts which could detect 2,4,6-TCA at a concentration of 2 ng/L or higher. The conclusions of this study were the next:

  I).- The 6.1% of the wines tasted were considered to be affected by cork taint.

  II):- A second analysis of the tainted wines by Gas Chromatography-Mass Spectrometry (GC-MS) showed that only 51% (74) of the wines had 2,4,6-TCA levels higher than 2 ng/L. Therefore, the 49% of the wines initially defined as cork tainted suffered contamination by other unidentified compounds different from 2,4,6-TCA, and probably this taint could not be attributed to the cork stopper.

NEW THREATS FOR THE WINES: TAINT BY 2,4,6-TRIBROMOANISOLE AND PYRAZINES

Although chloroanisoles have been traditionally blamed to be the main chemical compounds responsible for tainted wines with fungal off-odors, there are growing evidences showing that other compounds are also involved in this problem:

Causes and origins of wine contamination by haloanisoles (chloroanisoles and bromoanisoles)
As previously mentioned, Soleas and co-workers (2002) showed that from 145 wines defined as moldy tainted by a panel of tasting experts, only 51% (74 wines) exhibited 2,4,6-TCA levels higher than 2 ng/L. This result clearly indicates that for the 49% of the wines (71) the contamination should be attributable to other unknown compounds different from 2,4,6-TCA. Unfortunately in this study the 2,3,4,6-TeCA, PCA and 2,4,6-TBA levels were not assayed and therefore the ultimate cause of the taint remain to be identified.

More recently Chatonnet and colleagues (2004) reported an analysis of 30 tainted wines with fungal off-odors and flavors that had never been in contact with cork. Surprisingly only 2 wines (6.7%) showed 2,4,6-TCA amounts higher than the sensory threshold. However, 26 of these wines (86.7%) exhibited a 2,4,6-TBA contamination higher than 2 ng/L, being this compound identified as responsible for the bad odor and flavor detected.

This study is very important for at least three reasons:

I) It is the first analysis that shows that the fungal off-odor and flavor is not produced solely by chloroanisoles, but also by other structurally almost identical chemical compounds named bromoanisoles. In fact, the fungal aroma level of every wine was directly related to its 2,4,6-TBA content.

II) In this study it is demonstrated that the contamination is not attributable to the stopper but to the cellar.

III) This study suggests that all the analyses previously carried out in order to determine the nature of the fungal aromas in wines should be considered as incomplete and considered with caution.

Today it is clear that musty aromas in wines are not exclusively produced by haloanisoles. The compound 2-methoxy-3,5-dimethylpyrazine has been identified as a potent musty compound from wine corks (Simpson et al., 2004). Besides, its aroma threshold in a white wine is about 2.1 ng/L. Its contribution to the frequency of bottles tainted with fungal aromas is yet
to be established, although it seems that, at least for the Australian wines, this compound could be second to 2,4,6-TCA as the cause of cork taint.

- Also 2-isopropyl-methoxypyrazine has been identified as a wine contaminant producing a typical earthy, and peanut aroma and flavor (Pickering et al., 2004). This taint, named as ladybug-taint, is not related to haloanisoles and cork-derived flavors. Instead this taint is associated with the incorporation of *Harmonia axyridis* beetles (the Multicolored Asian Lady Beetle) from the vineyard, with grapes at harvest and subsequent processing. The identified pyrazine is a normal component of the beetle haemolymph and has an olfactory threshold for humans in the low ng/L range.

The cork does not have a typical aroma. Therefore, the term cork taint should be solely employed in those cases where it is well demonstrated that the primary source of contamination is the stopper. Instead, the more appropriate way to name the presence of fungal/musty off-odor in wines should be *fungal taint* or *musty taint*.

The fungal taint can have many different origins. According to the latest studies probably more than half of the tainted wines with fungal/musty aromas are not TCA-associated contaminations and have other different origins.

Besides, different investigations have showed that it is possible to detect tainted wines that have never been in contact with cork.
METHODS TO REMOVE WINE CONTAMINATION BY HALOANISOLES FROM THE CORK STOPPERS

Although the cork companies are making in the last few years big research and technical efforts in order to remove the contamination by haloanisoles, currently there is no totally effective treatment to completely eliminate these compounds from the cork stopper.

Nevertheless, we can mention several different strategies to remove these contaminants:

- The cork company AMORIM have developed the ROSA technology based on the use of a distillation process with water steam. The effectiveness of the process has been independently verified by several studies (Hall et al., 2004; Sefton and Simpson, 2005). According to these studies ROSA technology removed 75-80% of the contamination.

- The OENEO group has developed a method based on the use of a supercritical-state carbon dioxide extraction. This process called “Diamant” (diamond) has shown good results in some independent studies.

- The company NovoNordisk developed in 1999 a commercial preparation named SUBERASE, based on a laccase enzyme that should be used in alcoholic solution to wash the stoppers (Conrad et al, 1999). Unfortunately this product has not offered good results.

- A patented process (European Patent WO 01/41989 A2) has been developed (2001) based on the use of an activated charcoal suspension obtained from the bark of coconut palm to wash the cork stoppers.

- The DOLFIN project, financed by the European Union, has developed a system to remove TCA from the closures by using microwave technology. However, according to some independent studies no significant reduction in the TCA content has been detected in closures treated by this method.

- The IONMED company has developed a technology based on the use of radiation (b emissions) to sterilize the stopper, removing the microorganisms producing TCA. However, this method does not eliminate the chloroanisoles already present in the cork before treatment.
Also the National Institute of Engineering, Technology and Innovation from Portugal (INETI) has developed a technology based on the use of gamma radiation to partially or totally destroy the TCA, depending on the dose used.

Finally, it should be mentioned that many research projects are currently under progress. We can mention the INNOCUOUS Project coordinated by the Rovira I Virgili University (Tarragona, Spain) that it is trying to develop different strategies to obtain taint free stoppers by using several technologies, like for example the cleaning of corks by using ozone. Also, at the Institute of Biotechnology of León or INBIOTEC (Spain) some research is being conducted in order to develop biotechnological strategies based on the isolation of microorganisms able to degrade TCA.

WINE CONTAMINATION BY CHLOROANISOLEs: CHOOSING A NATURAL PRODUCT (CORK STOPPER) OR A SYNTHETIC CLOSURE FOR BOTTLING

In recent times, the use of synthetic stoppers for bottling wines is becoming a regular occurrence as a consequence of the wine contamination by chloroanisoles. These synthetic closures can be made of several materials like glass or aluminium (ROTE closures), but mainly polyethylene (PET).

The main advantage of these closures is that they are apparently absolutely TCA-free. However, in the growing controversy cork stopper – synthetic stopper several questions can be raised in order to clarify this subject.

a).- Are synthetic stoppers an effective closure to avoid the transfer of haloanisoles to prevent wine contamination?

Today it is well known that the cork stopper does not allow the 2,4,6-TCA migration into the bottle from the cellar atmosphere (Capone et al., 2002). However, similar studies have not been reported for synthetic stoppers.

This is an important point because if a contamination exists in the cellar we should be completely sure that this taint is not going to pass through the synthetic closure to reach the wine.

b).- Can synthetic closures absorb haloanisoles?

This is an important question because if they absorb chloroanisoles or bromoanisoles these later could be transmitted to the wine. Currently it is
known that many materials like paraffin, wood, polypropylene-derived plastics, silicons, or metallic closures internally covered with plastic can actively absorb chloroanisoles. Remarkably Capone and co-workers (1999) have shown that polyethylene (the material used to make most of the synthetic closures) has a great capability to efficiently absorb 2,4,6-TCA. These authors also suggest that the addition of small pieces of polyethylene film could be an efficient and cheap way of reducing chloroanisole taint from wines. However, it is important to mention the loss of floral/fruity characters, as has been detected for Riesling wines which were polyethylene-treated (Capone et al., 1999).

\[\text{c).- Do synthetic closures allow a similar wine aging to that of cork stoppers?}\]

Although several studies have been conducted to compare different wine closures, currently, with the available data, we cannot be totally sure about the capability of the synthetic closures to allow a right aging of bottled wines.

A first study was carried out by Mas and co-workers (2002a and 2002b) by using several wines (white wine, red wine and sparkling wines –cava and champagne-) and different types of closures: natural cork stopper; colmated-cork stopper; agglomerated-cork stopper; plastic stopper (ethyl vinyl polyaacetate), polyurethane powdered-cork stopper (PPC) and metal screw-top with a expanded-polyethylene connection. Several chemical and sensory parameters were analyzed for every wine and kind of closure at different times (for a maximum of 24 months for white and sparkling wines, and 36 months for red wines). The conclusion of this study was that the sealing systems that allowed the most suitable evolution of wine were the natural-cork stopper, the colmated-cork stopper and the PPC stopper. The plastic and screw-top closures allowed oxidation of the wine more quickly than the other sealing systems.

A similar study has been conducted at the Australian Wine Research Institute (AWRI). In this case a Semillon wine was bottled by using 14 different closures: a ROTE screwcap closure, 2 natural-cork stoppers, 2 technical-cork stoppers (Altec and the Twintop one plus one closure from Amorim), 3 moulded synthetic closures (Nukork, Nomacorc and Ecorc) and 6 extruded synthetic stoppers (Aegis, Auscork, Betacorque, Integra, Supremecorq and Tage).

Wines were analyzed at 20 and 36 months after bottling (Godden et al., 2001; Francis et al., 2003) and the main conclusions of the study are presented below:
Evaluation of the physical properties of the stoppers

- **Extraction force required.** It was similar for all the closures tested and tended to increase during the first 12 months after bottling, especially when the temperature decreases.

- **Ease of reinsertion into the neck of the bottle.** The technical-cork stoppers and natural-cork stoppers were more easily reinserted into the bottle, whereas the synthetic closures, specially Ecorc and Tage were very difficult to reinsert. This study also showed that the synthetic closures exhibited a greater difficulty to be removed from the corkscrew as compared to the cork stoppers.

- **Incidence of leakage.** Only the synthetic closures Betacorque and Normacorc showed signals of an imperfect closing with leakages.

Retention of sulfur dioxide and browning of wine. Wine sealed with ROTE closures retained SO₂ to a greater extent than all other closures. The technical-cork closures and the natural cork-stoppers retained more SO₂ than the most of the synthetic closures (data for 24 months after bottling). Very low levels of total SO₂ were obtained for some of the synthetic closures like Tage and Ecorc. These are important results because browning and deterioration of the sensorial attributes in the wine accelerate with concentrations of free SO₂ falling below 10 ng/L.

Sensorial Analyses.

- **Contamination by chloroanisoles.** TCA taint was only detected in bottles closed with cork stoppers.

- Synthetic polymer-closures did not confer to the wine any plastic-type taint.

- **Oxidation of aromas.** The greater problems of aroma’s oxidation were detected for one of the natural-cork stoppers and for the synthetic Betacorque. Most of the synthetic closures exhibited an intermediate oxidation level, and the lowest were detected for the rest of the cork stoppers (technical or natural) and the ROTE closure. It seems that there is a direct relationship between the concentration of free SO₂ and the oxidation level: the higher free SO₂ concentrations corresponds to the lower oxidation levels.

- It is important to mention that although the ROTE closure exhibited a good performance in all the tests, it produced a rubber-like aroma/flavour in the wine.
It seems that it could be the consequence of a lack of oxygen as result of the reduced headspace, probably because of chemically reduced sulfur compounds.

More recently Skouromounis and colleagues (2005) have reported a similar study carried out at AWRI consisting of the analysis of the impact of closure type and storage conditions on the composition, color and flavor properties of a Riesling and wooded Chardonay wines during five years storage. In this case, wines were closed under five different types of closures: a ROTE screw-cap closure, two different natural cork stoppers, a synthetic polyethylene (PET) closure, and finally some wine samples were bottled by using glass ampoules.

The more important conclusions of this study are the following:

- Wines sealed with the synthetic closure were relatively oxidized in aroma, brown in colour, and low in sulfur dioxide when they were compared to wines bottled under the other closures.

- A struck flint/rubber (reduced) aroma was detected in the wines sealed under the screw cap or in glass ampoules.

- Remarkably, the wines preserved under natural cork stoppers only showed negligible reduced characters.

- Also the concentrations of SO₂ and ascorbic acid were determined in these wines after three years of bottling. Wines sealed under the synthetic closure had consistently lower levels of free and total SO₂ and ascorbic acid. No significant differences were detected between cork stoppers and the screw-cap closure.

Hence, bearing in mind all these data it seems that synthetic closures, although do not contaminate the wine with fungal/musty taints, have some important disadvantages with respect to cork stoppers:

- mechanically they are more imperfect.

- the loss of free SO2 is generally more rapid (except for ROTE closure), thus causing the shortening of wine’s life into the bottle, and allowing the development of oxidized aromas/flavours.

*d)* - What is the consumer’s opinion about the use of synthetic or cork closures on bottled wine?

A very important aspect that must be taken into account by the cellar is the consumer’s opinion.
Several public opinion polls have been conducted:

- The magazine *Wine Spectator* (http://www.winespectator.com) reported in March 2001 the opinion of wine consumers: 55% would prefer cork stoppers whereas the 38% preferred the synthetic closures. When they were asked whether they would feel annoyed if the cork oak forests would be protected, even although the cork stopper was not used for bottling wine, the 34% stated that they would not be annoyed whereas the 31% indicated the opposite.

- The consulting agency *Wine Intelligence* (http://www.wineintelligence.com) published in 2003 a study among British consumers:
  
  - 99% stated that the traditional “pop” sound produced by opening a wine bottle closed with a cork stopper is a very important ritual for wine consumers.
  
  - Almost 60% are reluctant to acquire wine bottled with screw caps.
  
  - Most of them were neutral with respect to the synthetic closures. These are not refused but they are not liked.
  
  - Young generations of wine consumers (below 45 years of age) showed a higher acceptance of the cork stopper.

- Finally, TNS Sofres published in 2004 a public opinion poll about the image of the cork stopper among French wine consumers:
  
  - 90% indicated that the last wine bottle opened was closed with a cork stopper.
  
  - 29% stated that the cork stopper is indispensable as a closure for a bottle of wine, whereas the 48% choose it to be their preferred closing method.
  
  - When they were asked about their preferences about the type of closure, 79% identified the cork stopper as the preferred material. On the contrary only 1% preferred the screw cap, and other 1% the plastic closures.

To conclude, noteworthy that some important ecologist organizations, such as WWF, have been promoting in the last years a world wide advertising campaign in order to arouse the consumer’s demand of wines closed with cork stoppers, in order to favor the survival of oak cork forests, and hence of several animal species in danger of extinction in this type of Mediterranean forest.
CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

On the basis of the studies conducted in the last years, we can summarize a series of well documented conclusions, that are important to bear in mind when we face a concrete situation of wine pollution by haloanisoles:

I

The problem of wine contamination by haloanisoles is complex since it has multiples causes and origins. In fact, it can be produced by one or more different chemical compounds able to generate fungal/musty aromas. Among these substances we can mention 2,4,6-TCA; 2,3,4,6-TeCA; PCA; 2,4,6-TBA and 2-methoxy-3,5-dimethylpyrazine

II

The last reported data indicate that only about 50% of the tainted wines with fungal aromas can be attributed to contamination by chloroanisoles. Probably the rest of the cases are due to the presence of other compounds such as 2,4,6-TBA, 2-methoxy-3,5-dimethylpyrazine, or a mixture of several of these contaminants, or maybe to another presently unknown compounds.

III

Haloanisoles, can be produced by several kind of microorganisms, but especially by filamentous fungi. They possess a protein (enzyme) named chlorophenol-O-methyltransferase which is able to transform an halophenol-type pesticide (mainly chlorophenols and bromophenols) into the corresponding haloanisole.
IV

This biological reaction discovered in the fungus Trichoderma longibrachiatum is very common, and explains the formation and contamination of haloanisoles on many materials: cork, water, wood, etc.

V

A very high percentage (nearly 80%) of all the filamentous fungi analyzed can produce haloanisoles and can be routinely isolated from cork and cellars.

VI

It can be stated that the true origin of wine contamination by haloanisoles is not the presence of fungi growing on cork or wood, but the high environmental contamination by chlorophenols and bromophenols, which are transformed by these microorganisms into the corresponding haloanisoles. In fact, filamentous fungi never synthesize 2,4,6-TCA unless they are exposed to its precursor, the pesticide 2,4,6-TCP. For this reason it is very important to carry out preventive measures in order to avoid the contamination by these pesticides.

VII

The presence of haloanisoles in wine is not always an evidence for the contamination by the cork stopper since in many occasions the closures that arrive perfectly clean to the cellars can absorb chlorophenols, chloroanisoles, bromophenols or bromoanisoles that are already present as contaminants in the cellar’s environment or installations.

VIII

In chromatographic analyses of a tainted wine and its corresponding cork, if the amount of haloanisoles detected in the stopper is higher than in the wine, we cannot automatically blame the cork, since it has been demonstrated that a cork stopper can absorb most of the 2,4,6-TCA present in a contaminated wine in a short period of time (24-48 hours). Similarly, about 80-90% of 2,3,4,6-TeCA and PCA present in a tainted bottled wine can be absorbed by the cork.
The only way to know if the primary source of contamination is the cork stopper or the wine, is to carry out a set of chromatographic analyses to measure the contamination levels of stoppers and wine, and also the pattern of the taint: if most (or all) the bottles are tainted, and high levels of chlorophenols (like 2,4,6-TCP or PCP) are detected, with a very high probability, the origin of the contamination would be the wine. If, on the contrary, the pollution level is at random (some tainted bottles and other not tainted), and high levels of chlorophenols are not detected, then there is a high probability for the primary source of pollution would be the cork.

A tainted cork stopper by chloroanisoles will only contaminate the wine if these compounds are located at the part of the closure that is in contact with the wine. It is well documented that 2,4,6-TCA can not cross through the cork stopper. Therefore, the cork can protect a wine even in the case of a contaminated cellar.

The use of polyethylene-synthetic closures (PET) is not a guarantee for chloroanisoles-free wines in case of polluted cellars since it has been reported that PET absorbs chloroanisoles in large quantities, which might be transferred later to the wine.

A comparative analysis of several types of closures performed by the Australian Wine Research Institute indicates that cork stoppers are physically more perfect than the synthetic closures analyzed. Besides, with the exception of ROTE closures, they retain a higher amount of SO₂ (for at least 36 months). This avoids some oxidative events, increasing wine’s life into the bottle.

Bearing in mind all this information and the lack of more comparative studies, specially for red wines, we can affirm that today the cork stopper continues to be the best option to close bottles of any type of wine.
RECOMMENDATIONS

In order to eradicate this problem a joint action is needed combining the efforts of the cork industries and the wineries. In this respect, a series of recommendations tending to minimize the effect of this problem can be suggested.

Recommendations for the cellars

It would be advisable that the wineries would also adopt a preventive actions in order to minimize the pollution of their facilities by these compounds:

A
The cellars should carry out periodical analyses (half-yearly or at the least annually) to determine that their facilities are free of chlorophenols, chloroanisoles, 2,4,6-TBP and 2,4,6-TBA.

B
The analyses should consist of atmospheric traps and also analyses of the materials susceptible of being contaminated, such as wood structures (roofs, floors, walls) and barrels.

C
The analyses performed should detect the presence of the following compounds: 2,4,6-TCP; 2,4,6-TCA; 2,3,4,6-TeCP; 2,3,4,6-TeCA; PCP; PCA, 2,4,6-TBP and 2,4,6-TBA. The detection of 2-methoxy-3,5-dimethylpyrazine should also be important.

D
A certification of taint-free materials should be demanded to the manufacturers of barrels, and other elements that get into contact with the wine, such as the silicon bungs. This is important because several cases of cellars are known where these silicon bungs and the barrels exhibited high levels of pollution.

E
Whenever is planned the introduction of new materials in the cellar (including glazes and paintings), the corresponding analysis should be performed or alternatively cleanliness of the materials should be demanded.

Causes and origins of wine contamination by haloanisoles (chloroanisoles and bromoanisoles)
A certification of chlorophenols, chloroanisoles, bromophenols and bromoanisoles free-materials should also be demanded to the suppliers of the boxes of cardboard and wood used for packaging the bottles of wine.

A putative pollution of these elements cannot affect the wine, since the stopper and the capsule will avoid the entry of the contaminants into the bottle, but it would give a bad image to the consumer that the boxes have a fungal/musty off-odor. Besides the pollution of these materials would contribute to the contamination of the facilities of the cellar.

**Recommendations for the cork companies**

Among them we can mention:

A. To abide by the recommendations that are established by the SYSTECODE system. This is the first accreditation system for cork companies developed by the European Federation of the Cork (CELIÈGE). It aims at cork enterprises reaching high standards of quality, which would minimize the possibilities of chloroanisoles pollution.

B. To avoid the introduction of contaminated materials at the industrial facilities, fundamentally PCP-wood treated, or paintings and glazes that may contain 2,4,6-TBP.

C. To store a small, but representative enough sample (at least 20 units), of every lot of stoppers manufactured. This would allow, in the case of a later claim, to carry out later analyses in order to confirms the possible guilt of the cork.

D. To perform periodically (every 6 months or 1 year) an atmospheric analysis of the facilities to know the possible presence of a contamination source for the same compounds already indicated for the cellars.


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Causes and origins of wine contamination by haloanisoles (chloroanisoles and bromoanisoles)


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Causes and origins of wine contamination by haloanisoles (chloroanisoles and bromoanisoles)


Causes and origins of wine contamination by haloanisoles (chloroanisoles and bromoanisoles)


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Causes and origins of wine contamination by haloanisoles (chloroanisoles and bromoanisoles)