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## *Research Article*

# Is Airborne 2,4,6-Trichloroanisole (TCA) a Threat for Bottled Wine?

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*Background and Aims*. This work investigated the TCA permeability of twelve types of commercial bottle closures during 24 months of bottle storage in the $d_5$-TCA-contaminated atmosphere: medium pollution (max. ~50 ng/L of $d_5$-TCA in the air) and high pollution (max. ~500 ng/L of $d_5$-TCA in the air). *Methods and Results*. The $d_5$-TCA content of wine samples and bottle closures was monitored by GC-MS analysis, and the closures of one group (comprising natural corks, agglomerated stoppers, and BVS Tin Saran™ screw caps) were found to be excellent barriers against airborne $d_5$-TCA, i.e., no contaminant was detected in wine under any storage conditions. In contrast, a second group of closures (synthetic stoppers with low OTR, BVS Saranex™, and plastic body screw caps) allowed permeation of $d_5$-TCA, polluting the wine when air contamination was high, albeit no $d_5$-TCA was detected in wines following storage under medium air contamination conditions. A third group of closures (synthetic stoppers with medium and medium + OTR, MCA screw caps, and glass stoppers) allowed $d_5$-TCA to accumulate in wine under both medium and high contamination environments. *Conclusions*. Some commercial bottle closures were found to permeate airborne $d_5$-TCA, thereby contaminating bottled wine under certain storage conditions. *Significance of the Study*. This work provides the wine industry with insight into the potential for postbottling contamination of wine by airborne TCA.

## 1. Introduction

Cork stoppers are a well-established source of 2,4,6-trichloroanisole (TCA) in wine and other alcoholic drinks [1]. TCA causes a musty/mouldy defect, which is detrimental to wine aromas. In the wine world, this defect is known as "cork taint." Wine is able to extract noticeable amounts of TCA from contaminated corks already after one day of immersed soaking and 30 days of bottle storage [2]. Very low concentrations of TCA, i.e., from 1 to 1.5 ng/L, can evoke aroma defects in wine [3, 4]. The kinetics of TCA migration into wine is complex, and only a proportion of the contaminant might be extracted into the alcoholic solution [2]. Based on these observations, two concepts for determining TCA in cork stoppers were proposed:

(1) *Total TCA content* refers to the entire amount of TCA present in a cork, measured as ng of contaminant per g of cork material;

(2) *Releasable TCA content* corresponds to the TCA that is extracted into wine from the cork stopper, expressed as ng of TCA per 1 L of wine or model wine solution.

Previous studies demonstrated that *releasable TCA* usually corresponds to about 0.05–8% of the *total TCA content* of cork stoppers [2, 4–6]. This range in TCA values can be attributed to the variability in cork materials, as well as the methods used to determine *releasable TCA* and *total TCA* [7]. In order to overcome the latter uncertainties, the standardised methods of TCA analysis in cork material were introduced by ISO and OIV organisations [8–10].

Producers of cork stoppers traditionally focus on the wine industry, where cork is one of the main materials used to seal wine bottles: natural cork stoppers, agglomerated and technical cork stoppers, etc. Modern technologies for the production of cork stoppers, applied in recent decades, have considerably reduced the TCA defects. Most of the current technologies follow two main strategies:

(1) *Analysis and Selection of "Cork Taint"-Free Corks.* Initially, trained employees began to do this, relying on sensory methods [11]. Later, automated equipment was developed to provide fast and sensitive selection of "TCA-free" stoppers (usually with *releasable TCA* levels not exceeding 0.5 or 1 ng/L, which are below sensory thresholds). The first such industrial scale system was NDtech from Amorim Cork. Other automated systems of a similar principal were presented later: One by One from M.A. Silva [12, 13]; the system of the CEVAQOE laboratory; Perfect GO from Laffite; Vocus Cork Analyser from Tofwerk [14]; DS100+ from Cork Supply Portugal, S. A.; the systems of Bruker and Egitron;

(2) *Treatment of Cork Material to Remove TCA.* Hot steam [15, 16] and supercritical $CO_2$ treatments [17–19] demonstrated high efficiency in reducing the TCA content in cork material. These procedures are most effective for cork granules, which are later used to produce agglomerated stoppers.

Besides cork stoppers, contaminated air in the cellar can also be a source of TCA in wine. This becomes evident when an entire batch of bottled wine demonstrates elevated levels of TCA (and/or other haloanisoles), especially in bottles with closures other than corks. For example, wines bottled with plastic stoppers in a polluted atmosphere (40–45 ng/L of TCA, bentonite analysis [20]) were contaminated with TCA at a level of about 2.2–2.3 ng/L [21]. At the same time, no TCA was detected in tank samples of the wines.

Scientific investigations have shown that TCA and other haloanisoles can be found in walls (plaster, paint) and wooden materials inside the cellar: roof constructions, pallets, barrels, etc. [5, 22–26]. Due to biosynthetic pathways, halophenoles (precursors) in wood can be converted by certain fungi into various haloanisoles, including TCA. The latter remains the most prominent musty/mouldy contaminant in the haloanisole family. Once TCA forms in wooden materials inside the cellar, it can migrate into the air and contaminate winery equipment and oenological materials such as hoses, fining agents, filter sheets, etc. Subsequent contact of wine with these contaminated materials can lead to a musty/mouldy taint. Bottle closures stored in a contaminated environment can also absorb a certain amount of TCA [27] and later release it into wine.

Potentially, contamination of wine can also occur after bottling when airborne TCA migrates through the bottle closure into the wine. Several studies on this topic demonstrated that natural corks and agglomerated stoppers are impermeable to airborne TCA after at least 2-3 years of storage (Table 1). In those experiments, wine bottles were placed in an atmosphere contaminated with $d_5$-TCA, or its solution was applied to the top of stoppers. At the same time, certain amounts of TCA were found to migrate through synthetic stoppers and BVS screw caps and into the wine. The main criticism of these experiments is usually related to the use of storage conditions with extremely high and unrealistic air contamination and, therefore, the extent to which this reflects TCA migration through synthetic stoppers and BVS screw caps in environments that are naturally contaminated with TCA. Thus, in the current study, we evaluated lower levels of TCA in the air. However, to our knowledge, there are no comprehensive studies demonstrating what the TCA levels in the air are in real polluted environments.

As for real cases of wine contamination due to the migration of pollutants through bottle closures, they have also been observed. For example, sparkling wine sealed with crown caps was contaminated by airborne tetrachloroanisole (TeCA) after 14 months of storage, giving the wine a musty smell similar to TCA [32].

The aim of the current research was to summarise the existing knowledge concerning the migration of airborne TCA through bottle closures and to perform an extended storage experiment with a wide range of commercial closures. Thus, twelve types of commercial bottle closures were selected for this study in order to clarify a number of aspects, which may be of interest to the wine industry and wine consumers:

(1) How does the quality *(high vs. basic)* of natural corks, as well as agglomerated stoppers, affect the permeability of TCA?

(2) What are the differences in TCA migration of various synthetic stoppers? Is there any relationship between TCA permeability and the oxygen transmission rates (OTR) of synthetic stoppers?

(3) How effective are glass stoppers with sealing rings against airborne TCA?

(4) How does the type of liner and construction of screw caps influence TCA permeability?

## 2. Materials and Methods

*2.1. Chemicals, Wine, and Other Materials.* The following chemicals were used for the experiment and analyses: $d_5$-TCA (CDN Isotopes, Canada); ethanol (Martin und Werner Mundo OHG, Germany); deionised water purified with a Milli-Q water system (Millipore, Bedford, MA); $d_5$-2,4,6-tribromoanisole ($d_5$-TBA) (Neochema, GmbH, Germany); and NaCl p.a. (Sigma-Aldrich). Metallic containers (125 L) for bottle storage were supplied by Bayern Fass, Germany. Parafilm "M"® was purchased from Carl Roth Karlsruhe, Germany.

A white wine cuvée (2015 vintage) from the Rheingau wine region in Germany was used for the study. The wine had the following principal parameters: alcohol content 12.2% (v/v), titratable acidity 7.7 g/L, sugar content 1.9 g/L, and pH 3.1. Physicochemical analysis of the wine was undertaken at the Department of Enology, Hochschule Geisenheim University. Wine pH and titrable acidity were analysed by an automatic titrator "848 Titrino plus" coupled to an "869 compact Sample Changer" (Metrohm, Switzerland). Sugar content was determined according to the Dr.

Table 1: Permeation of the airborne TCA by bottle closures. Analysis of wine (yes/no indicates that $d_5$-TCA was/was not detected in the wine after bottle storage in the contaminated atmosphere).

| | | Bottle stoppers (closures) | | | |
|---|---|---|---|---|---|
| | | Natural corks | Technical/agglom. corks | Synthetic stoppers | BVS screw caps |
| Storage conditions: $d_5$-TCA concentration in air/storage time | 1080 ng[1]/36–44 months [28] | No | No | — | — |
| | 32 $\mu$g/L/3–24 months [29] | No | No | Yes | — |
| | 1.75 $\mu$g/L/1–30 months [30] | No | No | Yes | Yes |
| | ≈400 ng/L/3–15 months [31] | — | — | Yes | — |

[1] Amount of $d_5$-TCA in solution applied on the top of the stoppers.

Rebelein method [33]. A DMA 48 density meter (Anton Paar, Austria) coupled with a refractometer (Carl Zeiss, Germany) were used for the measurement of alcohol content.

Twelve commercial bottle closures, corresponding to the most common types of wine closures on the market, were used for bottling wine in the current experiment, comprising

(1) Two types of natural cork stoppers supplied by Amorim Cork: *high* quality (HQ), visual grade "flor" (45 × 24 mm); and *basic* quality (BQ), visual grade "2nd" (45 × 24 mm);

(2) Two types of *agglomerated cork stoppers* supplied by Amorim Cork: *high* quality (HQ) micro-agglomerated Neutrocork stoppers made with 0.5–2 mm granules (45 × 24 mm); and *basic* quality (BQ) agglomerated stoppers made with 2–3 mm granules (45 × 24 mm);

(3) Three types of *synthetic stoppers* (coextruded, 22.5–23 × 43–44 mm): with "low" (<1.5 mg), "medium" (1.5–2 mg), and "medium+" (>2 mg) oxygen transmission rates (OTR, measured as mg of $O_2$ per year per bottle, after one year);

(4) Two types of *BVS screw caps*: with Saranex™ and Tin Saran™ liners;

(5) Two types of other screw caps: *MCA screw cap* with a typical liner and *plastic body screw cap* with a glass lens and a sealing ring; and

(6) A *glass stopper* (18.2 mm length) with a sealing ring (ethylene-vinyl acetate (EVA) copolymer) and an external metallic capsule.

Transparent glass bottles (750 mL) with either a "Cork" finish (for *natural* and *agglomerated corks*, *synthetic stoppers*) or a "BVS" finish (for *BVS* and *plastic body screw caps*) were produced by "Verallia" and supplied by Saint Gobain-Oberland (Bad Wurzach, Germany). Dark green glass bottles (750 mL) with the finish required for *glass stoppers* and green glass bottles (750 mL) with "MCA" finish (for *MCA screw caps*) were supplied by Rheingauer Winzerbedarf GmbH (Hessen, Germany).

#### 2.1.1. Bottling of Wine.

Bottling was completed in December 2015 using facilities at the Hochschule Geisenheim University (Germany); bottles were rinsed with 5% aqueous $SO_2$ prior to use. The bottles sealed with cylindrical closures (*natural cork*, *agglomerated*, and *synthetic stoppers*) were filled to 63 ± 5 mm from the top of the bottleneck. Stoppers were inserted using a corking machine GAI 4040 without any prior headspace treatment with inert gas or vacuum. Capsules were not applied above the stoppers. The bottles with a "BVS," "MCA," or *glass stopper* finish were filled to 30 ± 5 mm from the top of the bottleneck. They were then mechanically bottled using an Adelski (Kellerei-Maschinen, Mannheim, 964/2008), with the corresponding modules for *BVS screw caps*, *MCA screw caps*, or capsules for *glass stoppers*. *Plastic body screw caps* with glass lenses and *glass stoppers* were applied manually.

#### 2.1.2. Design of the Experiment.

The actual experimental design involved storage of the bottles with twelve types of closures under two conditions, which had the following objectives:

(1) *Medium conditions*: medium air contamination conditions (initial $d_5$-TCA concentration about 50 ng/L) and cool storage (average temperature 16.5 ± 2°C), to simulate storage in a TCA-contaminated environment, but at levels lower than those reported in previous studies (Table 1);

(2) *Intense conditions*: high air contamination conditions (initial $d_5$-TCA concentration about 500 ng/L) and warm storage (average temperature of 26.5 ± 3°C). It was applied to test the permeability of different bottle closures in relation to airborne TCA in principle.

Thirty-six bottles (three replicates with 12 types of closures) were placed horizontally in each of six metallic containers of about 125 L capacity (Figure 1). The bottles occupied about half of the container and were organised in such a way that each of the three replicates was located in the bottom, middle, and upper part of the pile. This was done in order to homogenise the exposure of bottles to the contaminated atmosphere. A 100 mL glass beaker containing 0.53 or 5.3 mL of $d_5$-TCA solution in ethanol was placed inside each container (above the bottles) to achieve artificial contamination of the atmosphere. The concentration of $d_5$-TCA solution was about 10 $\mu$g/mL, which provided the maximal expected levels of the initial air contamination inside containers around 50 ng/L and 500 ng/L, respectively. $d_5$-TCA was preferred over TCA as a contaminant to avoid any interference with TCA, which can be naturally present in cork material or cellar atmosphere.

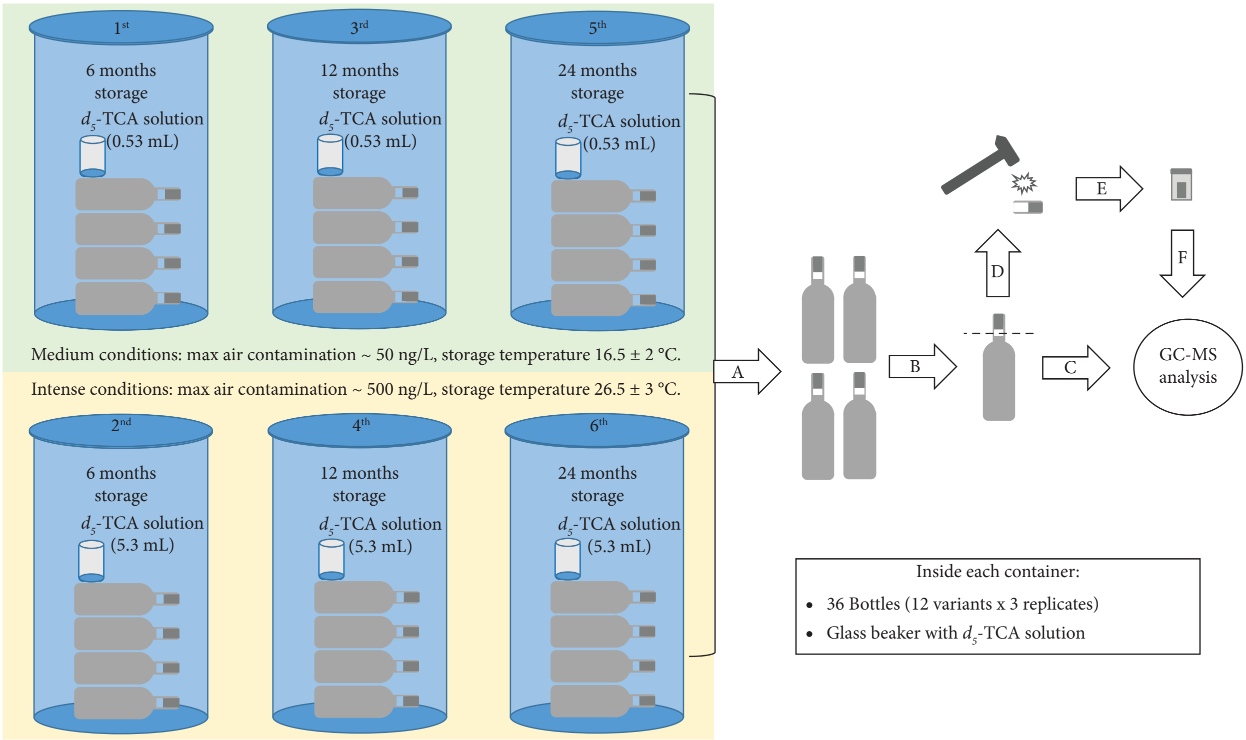

Figure 1: Design of the experiment: (A) removing bottles from the containers after the specified storage time; (B) opening the wine bottles (by cutting the bottlenecks of bottles with stoppers or manually for other closures); (C) sampling and GC-MS analysis of the wines; (D) breaking the bottlenecks and preparing closures for soaking; (E) soaking parts of bottle closures in 12% aqueous ethanol; (F) sampling and GC-MS analysis of closure extracts.

Once the beaker with $d_5$-TCA solution was placed, each container was tightly sealed with a metallic lid and stored between 6 and 24 months. Earlier experiments suggested six months was sufficient time for $d_5$-TCA to migrate through certain bottle closures and reach the wine, whereas three months was not long enough to result in $d_5$-TCA being detected in wine stored under "medium+" OTR *synthetic stoppers* in an environment similar to the aforementioned *intense conditions* [31]. A 24 months time point was selected for the collection of the final samples since the majority of wine is known to be consumed within this time period. Containers with lower levels of air contamination were placed in a cellar with an average temperature (throughout the duration of storage) of 16.5 ± 2°C, while highly contaminated containers were kept in a room heated to 26.5 ± 3°C.

After the corresponding storage periods, bottles were removed from containers, and bottlenecks were immediately covered with aluminum foil and wrapped with Parafilm "M"® (to prevent $d_5$-TCA losses from the surface of closures). Bottles were then transported to the laboratory for analysis. The bottles stored under *screw caps* and *glass stoppers* were opened manually. In the case of *natural cork*, *agglomerated*, and *synthetic stoppers*, the glass bottlenecks were partially cut on the level of headspace and then broken with a hammer in order to remove the untouched stoppers. It was important to preserve the integrity of the stoppers for the determination of their $d_5$-TCA content. The use of a corkscrew was avoided in order to prevent contamination of the inner parts of the stoppers due to the insertion of the screw. Once each bottle was opened, wine was sampled into 20 mL flasks for GC-MS analysis. Bottle closures were either wrapped in aluminum foil prior to analysis or immediately subjected to sample preparation.

*2.1.3. Analysis of $d_5$-TCA in Wines.* Gas chromatography-mass spectroscopy (GC-MS) analysis of wine samples was performed according to the previously described methodology [30]. Wine samples (10 mL) were placed in 20 mL solid phase microextraction (SPME) vials containing 3.0 g of NaCl. $d_5$-TBA (100 $\mu$L of a 2 $\mu$g/L solution) was added as the internal standard, and the vial was sealed with a screw cap. $d_5$-TCA was analysed using a QP-2010 Plus gas chromatograph mass spectrometer (GC-MS) (Shimadzu, Kyoto, Japan) equipped with a Multipurpose Autosampler (MPS2) (Gerstel, Mülheim an der Ruhr, Germany) in SPME operating mode. Sample vials were transported from the tray to an agitator held at 55°C. Samples were incubated for 3 min at 55°C and then extracted for 11 min at 55°C with agitation (250 rpm). Desorption of the polydimethylsiloxane (PDMS) 100-$\mu$m fibre (Supelco, Bellefonte, PA, USA) into the injector was carried out at 270°C for 4 min in splitless mode. Compounds were separated on an RTX-5MS capillary column (30 m × 0.25 mm i.d.) (Restek, Bellefonte, PA, USA) with a 0.25-$\mu$m film thickness. The carrier gas was helium, which was programmed to flow at a constant linear speed of 47 cm/sec during each run (flow 1.61 mL/min). The oven program started at an initial temperature of 90°C, then increased at a rate of 10°C/min to 205°C, and finally at 30°C/min to 280°C.

The MS was operated with electron impact ionisation in selected ion monitoring (SIM) mode. The quantification ions selected were 215 for $d_5$-TCA and 349 for the internal standard $d_5$-TBA. The GC-MS interface temperature was 280°C, and the ion source temperature was 200°C. The limits of detection and quantification for $d_5$-TCA were 0.4 and 1 ng/L, respectively. Each wine was analysed in duplicate.

*2.1.4. Analysis of $d_5$-TCA in Bottle Closures.* Closures were analysed to investigate $d_5$-TCA migration as the *releasable $d_5$-TCA content*. In the case of *MCA* and *BVS screw caps*, the liners were separated from the metallic caps prior to analysis. Likewise, the sealing rings were removed from the *glass stoppers* and *plastic body screw caps* for analysis. Metallic and glass parts of closures were not analysed; however, plastic caps from the *plastic body screw caps* were analysed, since plastic can absorb TCA, as shown previously [34]. Each of the cylindrical closures (*natural cork, agglomerated*, and *synthetic stoppers*) was halved through the middle (Figure 2) to enable separate analysis of the outer part (which was in contact with the contaminated air) and the inner part (which was in contact with the wine). Closure parts were placed into 60 mL glass flasks, which were then filled with 12% aqueous ethanol (Figure 1). The solution volume varied for different closures, but the measurement of *releasable TCA* has been shown to be insensitive to variations in the solvent volume [2]. Flasks were covered with aluminum foil, caps applied, and stored at room temperature for 24 hours. The extracts were subsequently sampled into 20 mL vials for GC-MS analysis according to the same procedure described above for wine.

*2.1.5. Data Processing.* Calculation of means (±standard deviations) and preparation of figures were performed using Microsoft Office (Version 15.0.5153.1000, Microsoft Corporation, Redmond, Washington, DC, USA). Where $d_5$-TCA values were below the LOD, they were considered to be “0 ng/L” for the calculation of means. Statistical analysis was performed using JASP software (Version 0.16, University of Amsterdam, Netherlands). Analysis of variance (ANOVA) was carried out with the Tukey HSD test for post hoc comparison to discriminate among the means of $d_5$-TCA content in wine (Supplementary Table 1) and *releasable $d_5$-TCA* content of bottle closure extracts (Supplementary Table 2). The values for ANOVA analysis were grouped according to bottle closure types.

## 3. Results and Discussion

The concentrations of migrated $d_5$-TCA in wine, together with the *releasable $d_5$-TCA* from the parts of bottle closures are illustrated in Figures 2 and 3. The only missing results are the concentrations of $d_5$-TCA extracted from the bottle closures after six months of storage. At that time point, the analysis of *total $d_5$-TCA* content in the bottle closures was carried out, which initially involved maceration of the closure parts in cyclohexane. However, this approach was not effective for all closure materials, so comparisons between samples were not possible. Therefore, at subsequent sampling time points (being 12 and 24 months), maceration of closures was performed in 12% aqueous ethanol to determine the *releasable $d_5$-TCA* content.

Before discussing the results of the experiment, it is noteworthy to define the possible pathways of TCA migration through the closure/bottle system. There are three main mechanisms of gas and taint agent migration (Figure 4) described in the literature [30, 35, 36]:

1. Interface diffusion effect: This involves the migration of taint agents along the small gap between the glass bottleneck and the closure (closure/bottleneck interface);
2. Solution-diffusion effect: This mechanism is based on the absorption properties and involves three steps. First, there is dissolution of a compound in the closure material (outer part). Then pollutant diffusion occurs through the stopper under the influence of a concentration gradient. Finally, the compound evaporates from the inner part of the closure, which is in contact with wine or air in the headspace. The solution-diffusion effect is considered to reflect “true permeability” and is directly dependent on the thickness of the closure material;
3. Pore effect: This mechanism provides the transfer of gaseous compounds through the pores, pinholes, and cracks within a closure. If bottle closures are intact and have no pores in their structure, then this TCA migration mechanism is not relevant.

*3.1. Natural Cork and Agglomerated Cork Stoppers of High and Basic Quality.* In accordance with previous studies (Table 1), the *natural cork* and *agglomerated cork* stoppers were excellent barriers to airborne TCA. Thus, no $d_5$-TCA was detected in the bottled wines sealed with these closures, even after two years of storage under *intense conditions*. In addition, this study demonstrated that *natural cork* and *agglomerated cork* stoppers of *high* and *basic* quality performed equally well (Figure 2).

Regarding the aforementioned mechanisms of TCA travel, all three were either absent or only occurred at a very low level. Cork typically comprises a significant number of air-filled cells, which are completely isolated and act as sealed units. This ensures the impermeability of cork to atmospheric gases and volatiles such as TCA. Therefore, no *pore effect* was expected for sound natural cork stoppers. The only natural disruptions to the homogeneous structure of cork are fissures and lenticels. The latter are columns of thin-walled parenchyma involved in gas exchange [37]. When producing closures, cork planks are cut at right angles to lenticels and fissures to maintain impermeability. In agglomerated stoppers, there are no lenticels and fissures.

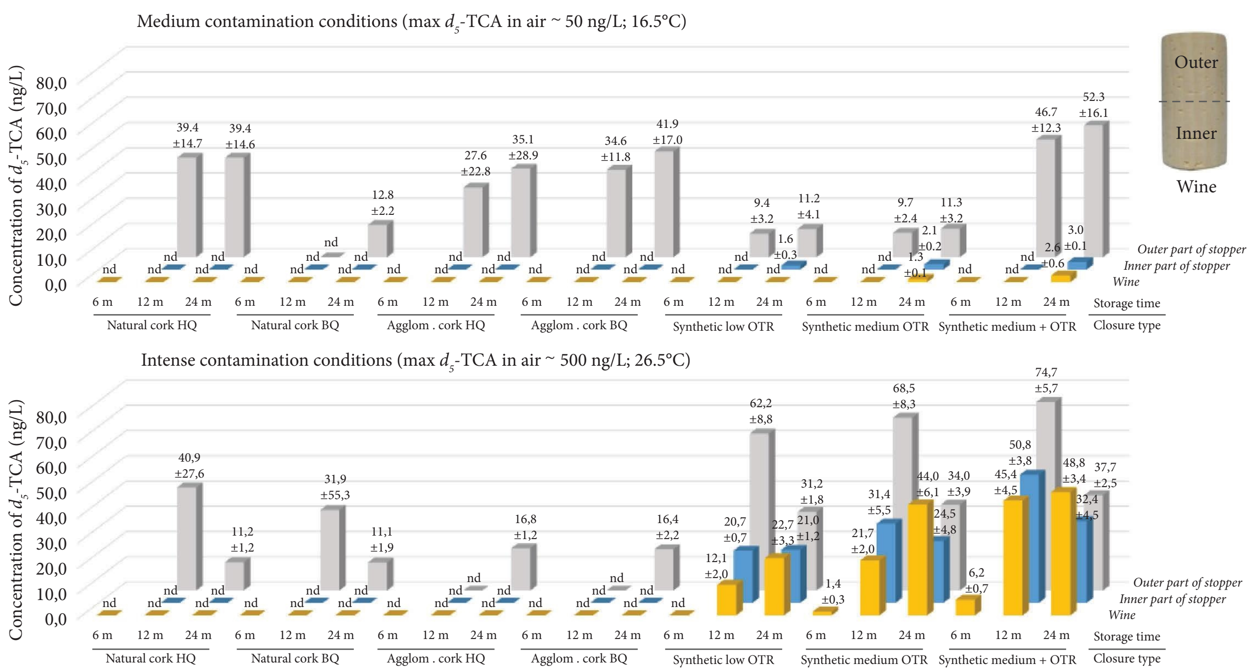

Figure 2: Concentration (ng/L) of $d_5$-TCA in wine samples and closure extracts after 6, 12, and 24 months of storage (Natural Cork, Agglomerated Cork and Synthetic Stoppers). The values are means of three replicates ± standard deviations (<1 means < LOQ; nd means < LOD).

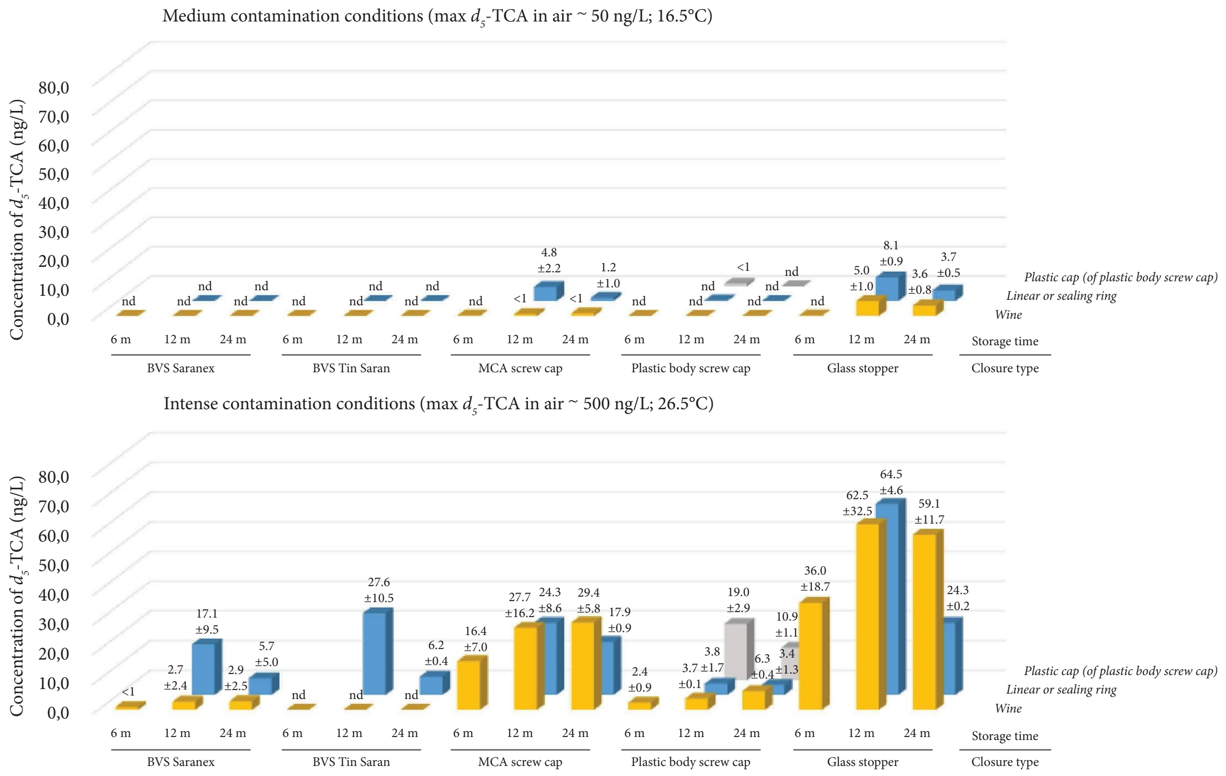

Figure 3: Concentration (ng/L) of $d_5$-TCA in wine samples and closure extracts after 6, 12, and 24 months of storage (Screw Caps and Closures with Sealing Rings). The values are means of three replicates ± standard deviations (<1 means < LOQ; nd means < LOD).

For cork closures, the *interface diffusion effect* is also limited due to cork structure: cork material adheres tightly to the glass with microscopic suction cups, which are formed on the surfaces from cut cells [35]. Lopes et al. [29] demonstrated that TCA can move the 10–15 mm in length of natural cork, and this effect was assumed to reflect the *interface diffusion*

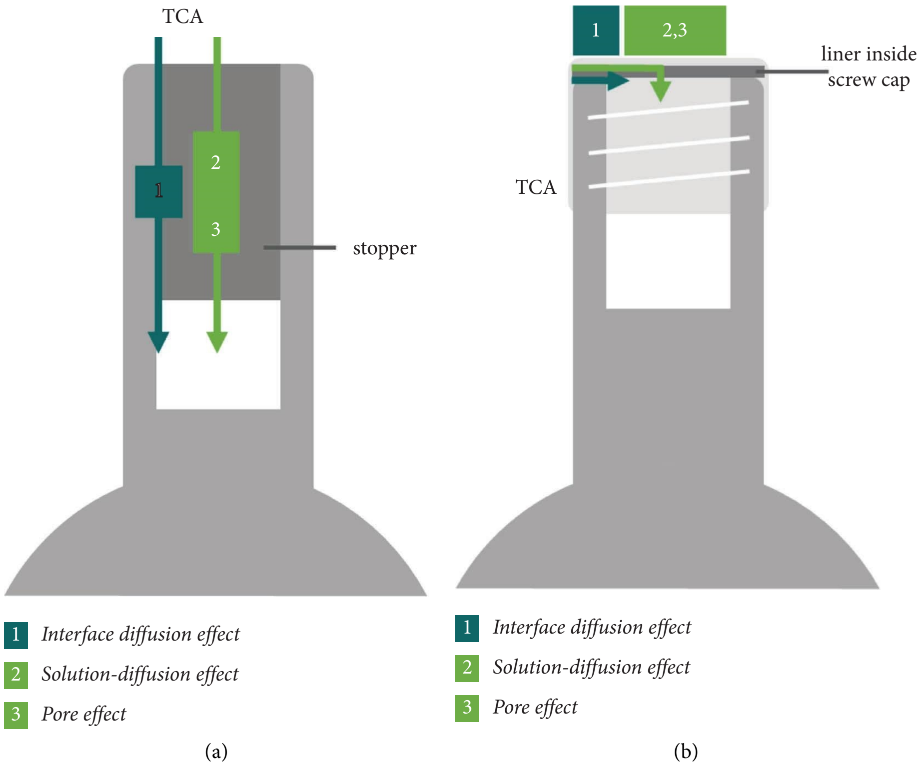

Figure 4: Visualisation of the possible TCA migration mechanisms through (a) bottle stoppers and (b) screw caps.

mechanism [29]. In turn, the *solution-diffusion effect* for cork closures seems to be limited to the first step. The cell walls of cork comprise alternating layers of wax and suberin, which can absorb TCA molecules from the atmosphere. However, the subsequent migration of TCA along the stopper is problematic due to the lack of intercellular space in the cork material and excessive pressure in the gas-fill lumen of the cork cells due to compression of the cork stopper in the bottleneck. In support of this, Barker et al. [27] demonstrated that the majority of $d_5$-TCA found in the cork stoppers exposed to $d_5$-TCA-contaminated air was localised in the outer 2 mm of cork material (although a significant amount of $d_5$-TCA also migrated inside the cork, possibly via lenticels) [27].

In the current work, *releasable* $d_5$*-TCA* was found in the outer parts of both *natural* and *agglomerated cork* stoppers, but it was not detected in their inner parts. These results demonstrate that for both cork material and agglomerated stoppers (cork material + binder), limited TCA diffusion occurs along the closures. The *releasable* $d_5$*-TCA* content in the outer part of the stoppers deviated significantly between the replicates, probably due to the peculiarities in the cork material. In addition, these values were high under both *medium* and *intense* storage conditions, which confirms the high affinity of cork material to TCA.

### 3.2. Synthetic Stoppers with Different Oxygen Transmission Rates (OTRs).

The polymeric materials from which *synthetic stoppers* are made, and the stoppers' internal structure, play essential roles in the migration of TCA through these closures. The polymeric materials are hydrophobic and easily dissolve TCA molecules, and unlike natural cork and agglomerated stoppers, diffusion of TCA along synthetic stoppers is not limited, such that TCA can reach the inner part of the closure and then the wine. The *solution-diffusion mechanism* likely plays a major role in the TCA travel process for *synthetic stoppers*. The *interface diffusion effect* is expected to be less prominent, based on previous studies on oxygen ingress [35].

All three of the synthetic stoppers were found to permeate $d_5$-TCA, which was present in the wine samples under the *intense storage conditions*. The stoppers with *low OTR* provided a better barrier towards $d_5$-TCA, probably due to the denser structure of the polymer material and therefore slower diffusion of the contaminant. The $d_5$-TCA content of wines sealed with the *low OTR synthetic stoppers* was the lowest, and $d_5$-TCA was even absent following 24 months of storage under *medium conditions*. In the case of the *synthetic stoppers* with higher OTR, $d_5$-TCA was found in wines at levels around sensory threshold concentrations only after two years of the *medium conditions* storage. In the current experiment, capsules were not introduced above the bottlenecks closed with stoppers, but ordinary capsules would serve as additional protection against airborne TCA contamination. Indeed, in a previous study, we found sound capsules (without holes) were able to reduce contamination of wine through the migration of airborne $d_5$-TCA by tenfold or more [31].

$d_5$-TCA gradually diffused through the synthetic stoppers from the outer to the inner parts. The mathematical model used to describe the migration of wine contaminants through closures is based on Fick's law and has been discussed in previous studies [30, 38]. It is also worth considering the desorption process, which under the *intense storage* conditions for 24 months, was evidenced by *synthetic stoppers* starting to lose $d_5$-TCA. Since $d_5$-TCA was only introduced at the start of the experiment, its concentration in the air within the barrel presumably decreased over the subsequent two years. As a consequence, the *releasable $d_5$-TCA* content of these stoppers was found to be lower at the 24-month sampling point than at 12 months, and lower also than in the corresponding wines (specifically for wines sealed with *medium OTR* and *medium + OTR synthetic stoppers*).

*3.3. Screw Caps with Various Liners.* The main difference amongst the screw caps with sealing liners was the composition of the different liners themselves. In contrast to *MCA* closures, *BVS* closures have a saran layer which comes into direct contact with wine. *BVS Tin Saran*™ closures had an additional layer of metal in their structure. One of the aims of this work was to investigate the efficacy of these additional layers. From the results, it is clear that the metallic layer provided a high level of impermeability to TCA even under *intense storage conditions*, such that no $d_5$-TCA was detected in wine. This finding demonstrates the *interface diffusion mechanism* for TCA migration through BVS screw caps is negligible (if the linear is properly and tightly sealed over the bottleneck). At the same time, soaking tests demonstrated that a certain amount of $d_5$-TCA did accumulate in the upper layers of the liners (Figure 3).

In turn, Saranex™ liners were able to reduce the $d_5$-TCA ingress and wine contamination on average by about ten times, compared to the *MCA* liners (*intense storage condition*). Also, this resulted in nondetectable levels of $d_5$-TCA in wine with *BVS Saranex*™ under the *medium storage conditions*. Contamination of wine samples in bottles with *MCA* closures was also limited under the same storage conditions, being below or about TCA sensory threshold levels in some replicates (<1–1.6 ng/L).

In general, the *releasable $d_5$-TCA* values for liners were also lower than for *synthetic stoppers*. The presence of a threaded metal protective cup may provide an additional protection due to the limited contact of the contaminated air with the liner. The *solution-diffusion* mechanism of TCA migration through the screw cap liners is expected to be the main route of wine contamination (provided the closure sealing operation was performed properly during the bottling) (Figure 4).

*3.4. Closures with Sealing Rings.* The last two types of bottle closures, the *plastic body screw cap* and the *glass stopper*, differed in construction, but both use plastic rings to seal the wine. Besides the sealing rings, only glass material comes into contact with the wine for both of these closures: the glass body of the *glass stopper* and the glass lens for the *plastic body screw cap*. Consequently, TCA can only penetrate inside the bottle through the sealing rings. This was shown to be possible under *intense storage conditions,* with $d_5$-TCA found in the wines sealed with both types of closures after just six months. Wine samples sealed with *glass stoppers* had the highest $d_5$-TCA concentration amongst all twelve closure types. This is likely because the sealing rings of *glass stoppers* are relatively thin, so $d_5$-TCA reaches wine faster than in the case of *synthetic stoppers*, where the contaminant must pass through about 4 cm of plastic material before reaching the wine. In addition, the metallic capsules above the *glass stoppers* are not as tightly fastened as screw caps. The latter explains the rather low $d_5$-TCA content in wines sealed with *plastic body screw caps*, although the sealing rings of these closures are even thinner than those used with *glass stoppers*. Furthermore, the plastic cap itself absorbed and retained a certain amount of $d_5$-TCA. As with several other variants, analyses after 24 months of storage showed losses of $d_5$-TCA due to desorption. This was especially obvious for *glass stoppers*, when the *releasable $d_5$-TCA* content in the sealing rings was less than half compared to levels detected after 12 months (under *intense storage conditions*).

Considering the *medium storage conditions*, no detectable levels of $d_5$-TCA were found in wines bottled under *plastic body screw caps*. The plastic cap appears to serve as a good barrier with the *releasable $d_5$-TCA* detected in the sealing ring only once after 12 months of storage. In turn, the wines with *glass stoppers* possessed the $d_5$-TCA concentrations above the sensory threshold levels at 12 months, and these values were the highest amongst the different bottle closures.

## 4. Conclusions

This study sought to extend existing knowledge about the migration of TCA through bottle closures and the potential risk of wine contamination. The conclusions of this work are formulated according to the questions and problems posed in the *Introduction section*:

(1) *High* and *basic* quality types of *natural cork* stoppers, *microagglomerated* and *agglomerated* stoppers demonstrated equally good barrier properties against airborne $d_5$-TCA. No contaminant was detected in the wine under the studied storage conditions;

(2) In principle, $d_5$-TCA was able to migrate through all three *synthetic* stoppers. However, wine contamination did not occur during the first year of storage under *medium-contamination conditions.* After 24 months, some $d_5$-TCA was found in wines with *medium* and *medium* + OTR, but not with *low* OTR. Under *intense-contamination conditions*, each of these closures gave contaminated wine, but less contamination was observed for *synthetic stoppers* with higher OTR. Desorption of $d_5$-TCA from stoppers was also observed, presumably due to a decrease in ambient contamination conditions. Additionally, the use of sound capsules (without holes) above synthetic stoppers mitigates wine contamination considerably [31];

(3) The permeability of $d_5$-TCA by *screw caps* was highly dependent on liner composition, with tin found to be impermeable to the contaminant (i.e., no $d_5$-TCA was detected in corresponding wine samples). The

use of a saran layer resulted in a significant decrease in wine contamination under the highly polluted atmosphere and in nondetectable levels of $d_5$-TCA in wine following storage under *medium contamination conditions*. The *MCA* screw cap with a simple liner allowed some $d_5$-TCA to be permeated under *medium storage conditions*;

(4) The sealing ring of the *glass stopper* gave the highest level of wine contamination among the various bottle closures, with the $d_5$-TCA level observed in wine after one year of storage in the *medium-contaminated* atmosphere being above the TCA sensory threshold concentration. In contrast, the construction of the *plastic body screw cap* afforded retention of $d_5$-TCA in the plastic cap and thus, much lower migration of $d_5$-TCA through the sealing ring was observed (compared with the *glass stopper* sealing ring);

(5) There is currently no comprehensive data available in the literature concerning naturally occurring concentrations of TCA in the atmosphere; therefore, further research is required to address this knowledge gap. In the current study, we simulated air contamination (for *medium conditions*: initial $d_5$-TCA concentration was about 50 ng/L of air), at a level lower than those applied in previous studies (Table 1). This demonstrated that wines sealed with certain closures might be at risk of contamination due to the migration and accumulation of TCA above its sensory threshold concentration.

## Data Availability

The detailed data from the GC-MS analysis can be available from the authors upon request.

## Disclosure

The authors declare cooperation between Hochschule Geisenheim University and Amorim Cork (Portugal) in the framework of various joint scientific projects, including technical and funding support by the latter. In the current work, Amorim Cork had no role in the interpretation of data or in the decision to publish the results.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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## Supplementary Materials

Supplementary Table 1: results of ANOVA (or Kruskal–Wallis test) for $d_5$-TCA values in wines grouped according to the types of bottle closures. Supplementary Table 2: results of ANOVA (or Kruskal–Wallis test) for *releasable* $d_5$-TCA content in bottle closure extracts grouped according to their types. (*Supplementary Materials*)

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