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Identification of a New Source of Contamination of *Quercus sp.* Oak Wood by 2,4,6-Trichloroanisole and Its Impact on the Contamination of Barrel-Aged Wines

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Thanks to practical experience in various wineries in recent years, it is now clear that, similarly to the well-known phenomenon in corks, there are several sources of unpredictable contamination of oak wood by 2,4,6-trichloroanisole (TCA). TCA affects staves in the same barrel very sporadically, with extremely limited contaminated areas on the surface that may reach several millimeters in depth. The precise origin of the TCP and TCA in oak wood is not known at this stage. Available data indicate that the phase where stavewood is naturally dried and seasoned is the source of these undesirable organochlorine contaminants. The strictly chemical formation of 2,4,6-trichlorophenol (TCP), derived from organochlorine biocides, was demonstrated to be impossible under traditional cooperage conditions, and its accumulation remained highly improbable. Similarly to previous discoveries in corks, all the analyses of oak wood suggested that the TCP was of biochemical origin. The capacity to biomethylate chlorophenols is well-known and relatively widespread among the usual microflora in stavewood, but the precise origin of the intermediary leading to TCP formation is still unknown. One probable hypothesis is that this reaction involves chloroperoxidase (CPO). Several ideas have been proposed, but the microorganisms responsible for the formation of the TCA precursor in oak wood have not yet been identified. The extent of this problem is still severely underestimated by coopers and barrel-users, due to the extremely unpredictable, localized contamination of the staves.

KEYWORDS: Oak wood; cooperage; new oak barrels; 2,4,6-trichloroanisole; microbiological origin

INTRODUCTION

Oak wood, mainly from European, e.g., *Quercus robur Linn.* and *Q. petrae Liebl.*, and American species, e.g., *Q. alba* and *macrocarpa L.*, has been used for storing, transporting, and making wines and spirits for several centuries (*1*). Advances in knowledge of oak wood and cooperage techniques, particularly the importance of seasoning conditions (*2*, *3*) and toasting (*4*, *5*) during barrel manufacture, as well as better control of fermentation and aging conditions for wines and spirits (*6*−*8*), have contributed to the widespread use of oak wood for making high-quality, high added-value products all over the world. Oak wood is certainly not an inert material. In addition to micropores, which facilitate gas exchanges and evaporation of the majority components in wines (water) or spirits (ethanol) (*9*), the oak releases various substances into products stored in contact with it for several months, or even years. Among the many extractable compounds in wood, a large number of volatile and odoriferous compounds (*10*), as well as various polyphenols, particularly certain ellagitannins (*11*), play an active role in enhancing the physicochemical stability of these products, as well as modifying their organoleptic characteristics in ways that are generally considered favorable to quality. The barrels used may vary in capacity and wood thickness, but the most widely used formats worldwide are the 225 L Bordeaux (*barriques*) and 228 L Burgundy barrels (*pièces*), built from staves varying in thickness from 22 to 27 mm (*1*). Around 2% of wines worldwide and 6% of French wines are currently aged in new barrels, despite competition from alternatives to traditional barrel-aging (maceration with wood chips). French cooperages are still the main global source of European oak barrels intended for aging wines, with annual sales on the order of 330 million euros, representing approximately 580,000 barrels manufactured, including 80% for export markets.

However, while aging wines in barrel may contribute considerably to enhancing their quality and complexity, this technique also introduces certain risks. First, the characteristics of this material make it complicated to clean and disinfect. If the aging conditions are not perfectly controlled, barrel-aged wines may be more easily exposed to several types of microbiological contamination likely to have a negative impact on their composition (*12*). However, these problems are not caused by the wood itself or the cooperage methods, but only the conditions in which the barrels are used. Second, it is true that some poorly selected wood, that has been insufficiently seasoned or toasted, may sometimes give unpleasant, "planky" or "sappy" odors (*13*). This is due to a defect in the composition of the raw material, or an accident during production, so the cooper is entirely liable in this case.

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There are also other sources of contamination of the wood and barrels, directly or indirectly responsible for the spoilage of the products stored in them. The composition of wine makes it more sensitive than spirits to this type of problem. Thus, in some cases, contamination of oak wood by 2,3,4,6-tetrachloroanisole (TeCA) and pentachloroanisole (PCA) has been traced back to preventive treatment applied to unbarked logs, or indirect contamination of staves split in industrial premises where pentachlorophenol (PCP) had been used to treat other types of wood (*14*). Chlorophenols are commonly biomethylated into the corresponding chloroanisoles *via* the intermediary of an *O*-phenylmethylase (*15*). Many microorganisms, particularly molds that develop easily under the conditions in damp, poorly ventilated aging cellars, are capable of this type of reaction. Assay methods have been demonstrated for identifying this type of pollutant in wood before it is assembled into barrels (*16*). Barrels may also be contaminated at a distance *via* the atmosphere, if they are stored in premises where these pollutants have concentrated (*17*), or transported in containers that have absorbed these pesticides, due to either preventive treatment of the floors or the accumulation of contaminants from various goods shipped in them over a period of time (*18*). Methylation of 2,4,6-tribromophenol (TBP), the direct precursor of 2,4,6-tribromoanisole (TBA), was recently identified as the origin of this compound in South American wineries, as well as in a large number of international shipping containers (*19*, *20*). This molecule has similar physicochemical properties to those of 2,4,6-trichloroanisole (TCA) and gives off particularly unpleasant "moldy" odors (*21*).

TCA is one of the most important trace contaminants among the chloroanisoles, identified as sources for "moldy" off-odors. In the wine and spirits industry, the presence of TCA is usually associated with cork or cork-related contamination problems ("cork taint") (*22*). A great deal of research has investigated the origins of TCA in cork and cork stoppers, and many publications have reported on various possible sources of 2,4,6-trichlorophenol (TCP), the direct precursor of TCA, in cork sheets and stoppers. The conditions required for TCP to be converted into TCA by microflora in cork are now relatively well-known (*23*). The conditions where these organohalogen contaminants are formed in the forestry environment or during cork manufacture have been relatively clearly elucidated (*24*). Finally, the conditions that enable TCA to migrate into wine have been identified and led to the development of a TCA assay technique that is usable as a routine quality control check (*25*, *26*).

Cases of TCA contamination in wines and spirits that have had no contact with contaminated corks are much less common. This original work, based on analyses of practical cases examined recently in the world wine industry, highlights the existence of olfactory defects with a "moldy" character acquired from oak wood in new barrels used to age various wines, although the surrounding environment was perfectly healthy. Between 2004 and 2009, we have been able to study 11 different cases of wine contamination by TCA in relation with the use of new oak barrels (several hundred barrels concerned) all over the world. To simplify the presentation of these data, we have decided to use one very typical example found in the industrial contamination of a particular wine. The presence of TCA was detected for the first time in new oak wood at a very early stage in the cooperage process. The various sources potentially responsible for the sporadic presence of TCA in staves at different stages in the barrel manufacturing process are studied and discussed.

MATERIALS AND METHODS

Materials and Abbreviations. 2,4,6-Trichloroanisole (TCA) [87-40-1] 99% Aldrich, 2,4,6-trichlorophenol (TCP) [88-06-2] >98% Aldrich, 2,3,4,5-tetrachloroanisole [938-86-3] 98% (in equivalent 2,3,4,6-TeCA but with exactly the same retention time in under the analysis conditions used), 2,3,4,6-tetrachlorophenol (TeCP) [58-90-2] 95% Lancaster, 2,3,4,5,6-pentachloroanisole (PCA) [1825-21-4] 98% Aldrich, pentachlorophenol (PCP) [87-86-5] 98% Aldrich, 2,4,6-tribromoanisole (TBA) [607-99-8] >99% Aldrich, 2,4,6-tribromoanisole-d_5 99% CDN Isotopes Inc., 2,4,6-tribromophenol [118-79-6] 98% Aldrich, 2,4,6-tribromophenol-d_2 98% CDN Isotopes Inc., pentabromophenol (PBP) [608-71-9] 96% Aldrich, deuterated iodomethane (CD_3I) [865-50-9] >99.5% isotope enrichment CDN Isotopes Inc., isoborneol [124-76-5] 95% Aldrich.

Synthesis of Deuterated Chloroanisole Analogues. A blend of chlorophenols (TCP, TeCP, and PCP: 6 mmol), anhydrous potassium carbonate (5 mmol), and deuterated iodomethane was prepared in 10 mL of dimethyl sulfoxide (Pestipur, SDS). The solution was stirred at room temperature for three hours. The mixture was then poured into 50 mL of dilute hydrochloric acid N. The solution was extracted three times with 50 mL of diethyl ether (Pestipur, SDS). The ether phases were static-settled, combined, and then rinsed with 250 mL of purified distilled water (Milli-Q), sodium hydroxide N, and then water again. The mixture was dried on anhydrous sodium sulfate (Rectapur, Prolabo), and the ether was slowly evaporated at 38 °C in a Kuderna−Danish concentrator equipped with a ten-plate reflux column and a nickel helix. The solid residue was recrystallized in ethanol. The deuterated chloroanisoles-OCD_3 on the methoxy group were purified by adsorption chromatography on a 50 × 0.5 cm flash-chromatography column filled with activated silica (activity V). The synthesized product was rinsed with *n*-pentane (SDS Pestipur >99.5%) and then specifically eluted (5 mL/min) with the following mixture: 96:4 pentane:diethyl ether (SDS, Pestipur >99%).

The solvent was evaporated in a cold vacuum rotary evaporator, and the purity of the solid diluted in *n*-pentane was measured by gas-phase chromatography (DB5-MS J&W column, 30 m × 0.25 mm, phase thickness 0.25 μm) coupled with a mass spectrometer (HP 5973-II) operating in electron impact (70 eV, 150 °C) and scan mode (40−400 uma). The mass spectrum obtained from the analogues was as expected, showing that the purity of the synthesized 2,4,6-TCA-OCD_3 and 2,3,4,6-TeCA-OCD_3 was over 99% of the total signal recorded.

Assays for Chlorophenols and Chloroanisoles in Wood and Wine. The chlorophenols and chloroanisoles present in the wood and wine were assayed simultaneously, using a method derived from the one described by Chatonnet et al. (2005). The halophenol assay used 2,4,6-tribromophenol-d_2 (TBP-d_2) as an internal standard, and the haloanisoles were assayed using deuterated analogues: OCD_3 for chloroanisoles and 2,4,6-TBA-d_5 for 2,4,6-tribromoanisole. The performance of the assay method was assessed and corresponded to those previously described (*26*).

Wine (100 mL) was centrifuged (15 min at 5000*g*), and precisely 1 mL of internal standard was added in the form of a solution in absolute ethanol, consisting of TBP-d_2 (0.1 mg L^{-1}), TCA-OCD_3, TeCA-OCD_3, PCA-OCD_3, and TBA-d_5 (all at 0.05 mg L^{-1}), in a 200 mL graduated borosilicate glass vial, deactivated by silanizing with hexamethyldisilazane. The contents of the vial were homogenized and transferred to a 250 mL deactivated-glass Erlenmeyer flask, then approximately 1 mL of 1/3 sulfuric acid was added, using a graduated pipet, to acidify the medium, and the mixture was stirred using a PFTE stir bar (6 g, 25 mm), previously rinsed with ethanol. Then 10 mL of 1:1 dichloromethane/*n*-pentane blend (Pestipur, SDS) was added and stirred for 5 min (250 rpm). The contents of the Erlenmeyer flask were then static-settled in a silanized separating funnel, rinsed with dichloromethane. The organic phase was collected carefully in a sealed 50 mL flask. The solution was extracted twice more using 5 mL of solvent. The organic phases were combined, the emulsion was broken by asymmetrical agitation, and the aqueous phase was eliminated by means of a disposable Pasteur pipet. The organic phase was dried on anhydrous sodium sulfate (Aldrich) and then transferred into a 100 mL silanized Zymark concentrating tube, the flask being carefully rinsed twice with 1 mL of dichloromethane/pentane. The solution was concentrated using a Zymark Turbovap II, operating at 25 °C ± 2 in a nitrogen stream (quality I, Air Products) at 1 bar. It was concentrated automatically to 0.5 mL in approximately 15 min. The sample was transferred to a 500 μL disposable injector/sample-changer flask and kept at −20 °C in the dark prior to analysis.

The wood samples (seasoned in the open air) were shredded in a granulating mill with a stainless steel bowl, to produce a powder with a particle size below 1 mm. The system was decontaminated after each

sample by soaking in a detergent solution (5% Decon 90) enriched with ethanol (5° %/vol.) and dried overnight in an oven at 105 °C. The material was collected on a sheet of aluminum foil and stored until it was extracted.

A wood sample weighing 0.5−1 g (±0 g) was put into a 100 mL Erlenmeyer flask stoppered with emery, and 100 μL of internal standard (2,6-dibromophenol-3,4,3-d_2 at 1.180 mg L^{-1} absolute ethanol), 50 mL of dichloromethane, and 100 μL of glacial acetic acid (Rectapur, Prolabo) were added to facilitate extraction of the halophenols. The mixture was stirred for 120 min at room temperature with a stir bar (25 mm, PFTE, 6 g, 250 rpm). The extract was rapidly filtered through cellulose paper, purified by continuous extraction with absolute ethanol in a Soxhlet for 12 h, then concentrated to 0.5 mL in a nitrogen stream, and transferred to a flask for storage as described above.

Analysis by Gas Phase Chromatography and Mass Fragmentometry (GC/MS/SIM)). A sample consisting of 1 μL (wood extract) or 2 μL (wine extracts) was injected, using a Combi PAL automatic injector-syringe 10 μL, CTC Analyticals Inc., into a 30 m × 0.25 mm HP-MS capillary column (5% phenyl-methyl-siloxanes; phase thickness: 0.25 μm) installed on an HP 6890 chromatograph, equipped with an injector operating in splitless mode (250 °C; initial pressure, 7.1 psi; pulsed splitless, 25 psi; pulse duration, 1.5 min; bleed, 50 mL/min; bleed time, 1.5 min) and an Agilent insert (ref 5183 4711). The carrier gas (helium N55, Air Product) was used at constant flow rate (initial column-head pressure, 7.1 psi; flow rate, 1.0 mL/min; linear velocity, 36 cm/s). The temperature was programmed from 40 °C (initial isotherm: 3 min) to 110 °C at a rate of 25 °C/min, then up to 230 °C at a rate of 5 °C/min, and up to 310 °C at a rate of 25 °C/min (final isotherm: 5 min). Analysis system inertness was checked by weekly injection of a column test mix (pentadecane, decylamine, octanol-3, and dichloro-2,4-phenol). Analysis of chlorophenols requires a perfectly inert system, assessed by retention time, surface area and width of the decylamine and 2,4-dichlorophenol peaks, as well as changes in the response factors of the halophenols in relation to the internal standard.

An HP5973 quadripole mass detector was used for detection, operating in electron impact mode (source temperature = 150 °C, constant ionization potential = 70 eV, electron multiplier = 1500 V) and fragmentometry mode, focusing on selected ions characteristic of each molecule (TCA, 197, 210, 212; TCP, 196, 198; TeCA, 244, 246; TeCP, 230, 232; PCA, 278, 280; PCP, 264, 266; TBA, 344.346; TBP, 328, 330, 332; dwell time, 100 ms). The following ratios were used for quantification: m/z 212/213 (TCA/TCA-OCD_3); 196/336 (TCP/TBP-d_2); 246/249 (TeCA/TeCA-OCD_3) ; 232/338 (TeCP/DBP-d_2); 280/283 (PCA/PCA-OCD_3); 266/255 (PCP/TBP-d_2); 329/331 (TBA/TBA-d_2) and 328/338 (TBP/DBP-d_2). The detection and quantification thresholds and uncertainties associated with the various compounds analyzed using this method have been previously published (*26*).

The system was calibrated using a range of known concentrations, prepared from pure standard products at concentrations of 0, 2, 5, 10, 20, and 50 ng/L diluted in uncontaminated wine, and 0, 5, 10, 20, 50, and 100 ng/g impregnated in uncontaminated wood by adding know amounts of pure compounds in an *n*-pentane solution, then analyzed under the same conditions as the contaminated samples. The pure reference chemicals were stored as follows: halophenols in Teflon flasks and haloanisoles in borosilicate glass. The range of standard substances was injected every 15 samples, every 24 h, and at least once per week, to ensure that the system was always properly calibrated.

Determining Atmospheric Concentrations of Volatile Organic Compounds, Particularly Haloanisoles and Halophenols. The volatile organic compounds (VOCs) present in the atmosphere were analyzed using the method developed by Boutou (2008) (*27*), based on the solid phase microextraction (SPME) in gas phase technique, proposed by Chai M. and Pawliszyn J. (1995) (*28*). This method uses adsorption of the VOCs on a polydimethylsiloxane- and divinylbenzene-based solid phase (PDMS/DVB, Supelco, Bellefonte, PA) deposited on a silica fiber that is directly exposed in the atmosphere for a predetermined length of time. The fiber is then directly thermally desorbed in the splitless injector of a gas-phase chromatograph coupled with a mass spectrometer. This technique was developed to obtain a quantitative measurement of the haloanisoles and halophenols per unit volume of air (see below) but is also able to determine the concentrations of several other VOCs (results not shown). The VOCs were quantified by normalizing each analysis fiber used and calibrating a gas ampule containing known concentrations of each targeted analyte in the 0.7 to 2000 ng/m^3 range in gas phase. The analysis protocol is summarized below.

Atmospheric Trapping on SPME Fiber. A PDMS/DVB fiber (pretreated according to the manufacturer's recommendations under the reference PDMS/DVB 65 μm (Supelco 57311)) was exposed for 15 min (±10 s) to the atmosphere to be analyzed (kiln oven with the door closed). The fiber was held about 1 m above the floor by a metallic support with a clip. After adsorption, the sleeve of the SPME needle was plugged by piercing a septum (LB2 Thermogreen, Supelco) while awaiting the analysis and stored at +4 °C (< 48 h).

Analysis of the SPME Fiber. At the laboratory, the fiber was mounted on the MPSE injector for analysis. Before injection, the fiber was used to adsorb internal reference samples as follows. Ten milliliters of the solution containing both deuterated anisoles (TCAd, TeCAd, PCAd, and TBAd) at 200 μg/L and [$^2H_{10}$]fluorene at 10 mg/L were added to a 20 mL SPME vial containing 3 g of NaCl and 10 mL of ultrapure water made by a Milli-Q water apparatus (Millipore). The vial was then conditioned for three minutes at 30 °C (agitated at 250 rpm for 10 s intervals, with 2 s pauses between intervals). A new vial was prepared in this manner for each fiber analysis, permitting each fiber to be calibrated individually. The fiber was exposed to the solution for 10 s at 30 °C before being placed in the gas chromatograph (GC) injector (splitless mode) for 5 min (275 °C; purge flow, 25.0 mL/min; purge time, 5 min; specific SPME linear, 0.78 mm internal diameter). The injector is coupled to the GC by a capillary column of type Fast-GC DB5-ms (nonpolar column, 95% dimethylpolysiloxane/5% diphenyl, from Agilent JW Scientific) 30 m long, 0.25 mm in diameter, and having a film thickness of 0.25 μm. The gas vector (helium) flows at a constant rate (initial head pressure, 37.63 psi; flow. 0.4 mL/min; linear speed, 40 cm/s). The temperature is programmed to rise from 50 °C (initial isotherm: 0.30 min) to 110 at 25 °C/min, plateau for 2 min, rise to 165 at 5 °C/min, and finally rise to 300 at 60 °C/min. The mass detector is operated in electron impact (EI) mode (source temperature, 230 °C; quadrupole temperature, 150 °C; constant ionization energy, 70 eV; electron multiplier, 1500 V), and provides fragmentometry (SIM mode) on ions selected to be characteristic of each molecule (TCA, 210, 212; TCP, 196, 198; TeCA, 244, 246; TeCP, 230, 232; PCA, 278, 280; PCP, 264, 266; TBA, 344, 346; TBP, 328, 330, 332; NAPH, 128, 127, 129; internal references: TCA-d_3, 213, 215; TeCA-d_3, 249, 247; PCA-d_3, 283, 285; TBA-d_5, 349, 351; [$^2H_{10}$]fluorene, 176, 174 amu); isoborneol (IB) (95,135); methylisoborneol (MIB) (95,136). For quantification, the following m/z ratios are used: 210/213 (TCA); 196/213 (TCP); 246/213 (TeCA); 232/249 (TeCP); 280/283 (PCA); 266/283 (PCP); 346/349 (TBA); 332/349 (TBP); and 128/176 (IB).

Calibration of the SPME Fiber. Our method for generating gas standards in static mode issues from a proposal of Chai, M. and Pawliszyn, J. (1995) (*28*). We used a 1 L gas sampling bulb (ref: 22144-U, Supelco). To prepare the bulb between analyses, we executed the following steps:

- The bulb is placed in an air oven for 15 min at 100 °C to volatize any substances still present.
- On removal from the oven, industrial-quality nitrogen is injected into the bulb for 10 min. One tap is connected to the nitrogen source, while the other is left open to evacuate any remaining analytes.
- The bulb is brought back to the ambient temperature.
- With the taps closed, target compounds at known concentrations are injected through the septum with the aid of a GC syringe (SGE; Courtaboeuf, France). The volume of solution injected ranges from 1 to 40 μL, depending on the compound, with a precision of ±1%.
- The analytes are volatilized by placing the balloon back in the oven at 120 °C for 5 min.
- The bulb is returned to analysis temperature (20 °C) by placing it in a climatic chamber for 20 min.
- The fiber is exposed to the atmosphere in the bulb by piercing the septum. Throughout the analysis, the balloon is left inside the climatic chamber.
- After 15 min, the fiber is taken out of the bulb and placed on the sampler for injection into the GC/MS system.

Note that significant differences in the PDMS/DVB fibers can arise depending on their fabrication series and prior use (number of analyses

Table 1. Characteristics of the Environmental Air Control by Solid-Phase Microextraction/GC/MS-SIM after Normalization of the PDMS-DVB (65 μm) Fibers[a]

molecule	ion for quantification	retention time (min)	dynamic range (ng/m^3)	slope	r^2	LOD (ng/m^3)	LOQ (ng/m^3)	RSD % (n = 3)
TCA	212	8.94	1.5−1981.6	1.9062	0.999	1.5	5	4.9
TCP	196	9.86	12.8−22507.2	1.5869	0.955	12.8	42.7	10.1
TeCA	246	12.86	2.2−2112.8	3.5108	0.981	2.2	7.3	6.9
TeCP	232	13.63	46.4−24088.0	0.2937	0.951	46.4	154.8	8.1
PCA	280	16,88	2.6−2012.8	1.5537	0.990	2.6	8.8	9.3
PCP	266	17.95	3444.5−21241.2	0.0112	0.990	3444.5	11481.7	11.3
TBA	346	14.84	4.4−2575.2	4.1208	0.997	4.4	14.6	8.8
TBP	332	15.38	39.4−24801.2	0.3184	0.955	39.4	131.3	8.3
isoborneol	95	7.22	16.2−8000	0.0262	0.998	16.2	52.5	6.5

[a] Regression mode: $y = ax$.

performed, damaged coatings, and so on). As a consequence the quantity of contaminant adsorbed will be different for each fiber, although linear with concentration. It is therefore essential to calibrate each fiber so that its results can be properly interpreted. The calibration procedure described above is tedious (many steps and measurements) and must be renewed often to learn the amount of wear on the fibers. Thus, the use of external reference compounds to normalize the response is more appropriate. Xiong et al. (2003) (*29*) proposed calibrating the fiber to correct for possible desorption of molecules during the time required for extraction and analysis. By adsorbing a known quantity of reference molecules before exposing the fiber to the sample, such losses can be taken into account. In our application, the fibers may be stored for a certain time before usage (< 8 days). To ease automation of the sampler, we decided to perform the calibration just before CG analysis by exposing the fiber to deuterated haloanisoles reference compounds. Because of competitive adsorption being possible for a PDMS/DVB fiber, the time required for this adsorption was reduced (thanks to efficient management of the sampler) to 10 s. Whether or not fibers were exposed to the references beforehand, no difference was observed in the quantities adsorbed and detected by the system (results not presented).

Normalization Procedure of the Fibers. In a 20 mL SPME vial we placed 10 mL of pure Milli-Q water, 3 g of NaCl, and 10 μL of the solution containing deuterated reference haloanisoles. As described in the GC/MS analysis sequence, the vial is conditioned for three minutes as 30 °C. The fiber to analyze is placed in the vial containing deuterated compounds for 10 s and then allowed to desorb in the injector for 5 min.

We calculate the normalization as the ratio between measured and reference surface adsorptions:

$$S_{\text{norm}} = \frac{S_{\text{measured}}}{S_{\text{reference}}}$$

Applying this ratio to the target compounds reduces the variability among fibers to 17%. Without calibration by the references, the variability was 52%. For an extraction time of 15 min, the characteristics of the method are gathered in **Table 1**; the limits of detection (LOD) and limits of quantification (LOQ) are defined with respect to the analytic background noise; the signal-to-noise ratio must be 3 for detection and 10 for quantification.

RESULTS AND DISCUSSION

Identification of Sporadic TCA Contamination in Barrel-Aged Wines. During tastings at very different wineries all over the world since 2003, sporadic olfactory defects with a "moldy" character have been detected in wines aged in barrels made of European oak wood, manufactured by French cooperage firms. This defect may be detected after a short time in barrel (one to three weeks), becomes increasingly intense over the first three months, and then remains relatively stable.

Table 2 shows analysis reports for several samples of a single red wine, made from the Tempranillo grape variety in the Rioja region of Spain in 2007, taken from the same 25,000 L batch distributed among several barrels from three different cooperages, that had been left untouched since it was put into barrel. All the barrels were "Bordeaux"-style, with a theoretical capacity of 225 L and a mean stave thickness of 27 mm (tapered inside),

Table 2. Content of Haloanisoles (ng/L) of Different Barrels Coming from Various Cooperages Used To Age the Same Red Wine in the Same Cellar

	cooperage A				cooperage B				cooperage C			
barrel no.	TCA	TBA	TeCA	PCA	TCA	TBA	TeCA	PCA	TCA	TBA	TeCA	PCA
1	nd	nd	nd	nd	nd	nd	nd	5.9	nd	nd	nd	nd
2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	2.5	nd	tr	6.5	nd	nd	5.1	5.5	nd	nd	4.1	tr
4	nd	nd	tr	nd	nd	nd	nd	nd	nd	nd	nd	nd
5	4.1	nd	nd	2	nd	nd	6.2	nd	nd	nd	nd	5.5
6	nd	nd	tr	5.2	nd	nd	nd	nd	nd	nd	8.2	tr
7	5.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
8	nd	nd	nd	1	nd	nd	nd	nd	nd	nd	nd	8.5
9	nd	nd	tr	5.5	nd	nd	4.2	6.0	nd	nd	nd	nd
10	12.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

toasted inside (medium intensity) but not on the ends, and partially planed on the inside.

After three months' aging, wines from certain French oak barrels made by cooperage A were considered spoiled on tasting. In view of the type of defect detected ("moldy" or "musty" odor), samples were assayed for halophenols and haloanisoles, known to be responsible for this type of spoilage. Analysis revealed that the suspect barrels from cooperage A were mainly contaminated with TCA, while concentrations of the other haloanisoles were well below their perception thresholds (*17*, *19*). At the same time, the same wine, stored in the same building, in French oak barrels from cooperage B, or American oak from cooperage C, did not exhibit any tasting defects and contained no noticeable concentrations of haloanisoles, particularly TCA.

The TCA content of wine from the barrels where off-odors were detected on tasting reached or considerably exceeded its perception threshold in this type of wine, i.e., 1−2 ng/L. For a professional wine taster, the spoilage threshold of TCA corresponds approximately to its perception threshold. Olfactory detection of TCA is considerably impacted by the subject and the composition of the matrix, with reported perception thresholds ranging from 0.03 ng/L in water (*21*) to over 22 ng/L in wine (*30*) in orthonasal perception. The composition and aromatic complexity of a wine may also have a significant impact on the TCA perception threshold for the same taster. However, the trained tasters in our experiment detected defects via the orthonasal pathway and *a fortiori* via the retronasal pathway at concentrations between 1 and 2 ng/L, which is in agreement with Simpson (*31*), who reported that "cork taint" was detected retronasally at 1.4 ng/L. Although they were not considered badly spoiled, the wines from barrels containing 1.5 and 2.5 ng/L TCA were markedly different from those aged in healthy barrels. The orthonasal aromatic profile was completely spoiled, it had very little "fruit", and the aroma perceived by retro-olfaction was described as "less clean" and "musty". Wines containing over 5 ng/L were immediately considered "moldy" or "corked" by tasters.

In this specific case, in view of the sporadic character of the contamination (4 barrels out of 40), neither the wine nor the winery environment could be considered responsible for this TCA contamination. The barrels had not been stored anywhere outside the aging cellar and were filled without any prior treatment. Consequently, prior contamination of the barrels by the winery atmosphere (*17*) or by chlorine-based cleaning products, leading to the risk of chemical formation of TCP by direct chlorination followed by methylation(*32*), were easily excluded as explanations for the presence of TCA in the wine.

All the barrels were stoppered on the first day, using new, food-grade, silicone bungs supplied by each cooper. In view of the capacity of silicone elastomers to fix this type of pollutant and release it into wine (*19*), one hypothesis that could not immediately be excluded was that the barrel bungs were responsible for the contamination. However, analysis of the bungs from control and contaminated barrels failed to reveal sufficient quantities of haloanisoles or halophenols to account for the spoilage. The same protocol was used as in the wood assays (silicone sample 2 cm deep, weighing 5 g, from the side of the bung in contact with the wine), but no TCP was detected in any of the bungs (< 2.45 ng/g). TCA was assayed at 3.0 ng/g ± 2 (i.e., maximum 150 ng per bung) in the bung from barrel no. 10, where the concentration in the wine was 12.1 ng/L ± 3.9 (i.e., 2722.5 ng in the entire barrel), but remained undetectable in a bung from a control barrel in the same batch (< 0.45 ng/g). The mass balance, therefore, clearly indicated that the silicone bung had been contaminated by TCA from the wine in the barrel. Consequently, the silicone bungs were ruled out as a pollution source.

Investigation into the Presence of TCA inside the Suspect Barrels. Barrel no. 10, the worst affected, was emptied, drained, and air-dried for 48 h, then entirely dismantled in situ. Each stave in the shell and both ends was numbered for accurate identification. The staves were then individually wrapped in food-grade aluminum foil to avoid any risk of contamination during transfer and storage prior to analysis.

Analysis of the Staves from a Suspect Barrel. In the laboratory, a wood cutter was used to remove wood samples approximately 3 cm^2 in size and a maximum of 1.5 mm thick every 10 cm along the full length of the side of each stave in contact with the wine, staggered from side to side. The wood samples from each stave were dried in a desiccator at 25 °C for 12 h, shredded, and analyzed, as described above.

Table 3 shows that, out of 30 staves in the shell and 7 or 8 staves in each of the ends of the incriminated barrels, only stave 4 in end no. 1 of the barrel contained a significant quantity of TCA (6.0 ng/g ± 2), as well as an assayable quantity of TCP (8.5 ng/g ± 3.5). Stave 5 in the same end contained 7.5 ng/g ± 3.5 TCA but only trace amounts of TCP (< 2.4 ng/g).

Some of the other staves in the incriminated barrel contained moderate quantities of PCP, TeCP, and smaller amounts of PCA, but the few traces of TCA were unquantifiable (< 2.4 ng/g) or even undetectable (< 0.45 ng/g).

Detailed Analysis of the Stave Identified as Contaminated. Stave 4 from end no. 1, which was 10 cm wide and approximately 40 cm long, was planed carefully every 10 cm along its length, removing a maximum thickness of 2 mm. The four samples obtained, each representing a surface of approximately 100 cm^2, were analyzed for halophenols and haloanisoles, as previously.

Figure 1 shows that only a limited part of the surface of the stave considered to be in contact with the wine was actually contaminated with TCA and TCP, while the rest of the stave did not contain any significant concentrations of contaminants (< LD or LQ).

Table 3. Analysis of Halophenols and Haloanisoles (ng/g) in the Different Staves of the Same Barrel with High TCA Content in the Corresponding Wine

stave no.	TCA	TCP	TeCA	TeCP	PCA	PCP	TBA	TBP
				Head No. 1				
1	tr	nd	nd	nd	tr	6.1	nd	tr
2	tr	tr	nd	nd	tr	tr	nd	nd
3	tr		nd	nd	tr	tr	nd	nd
4	6	8.5	nd	nd	5.2	tr	nd	tr
5	tr	7.5	nd	nd	tr	tr	nd	nd
6	nd	tr	nd	nd	tr	tr	nd	nd
7	tr	tr	nd	nd	tr	tr	nd	nd
8	nd	tr	nd	nd	tr	tr	nd	nd
				Head No. 2				
1	tr	tr	nd	nd	tr	5.9	nd	nd
2	nd	tr	nd	nd	tr	tr	nd	tr
3	nd	tr	nd	nd	tr	tr	nd	nd
4	nd	tr	nd	nd	6.1	tr	nd	nd
5	nd	tr	nd	nd	tr	tr	nd	nd
6	nd	tr	nd	nd	tr	tr	nd	nd
7	nd	tr	nd	nd	tr	tr	nd	nd
				Staves of the Barrel				
1	tr	nd	nd	nd	tr	tr	nd	nd
2	nd	tr	nd	nd	tr	tr	nd	nd
3	tr	tr	nd	nd	tr	tr	nd	nd
4	nd	tr	nd	nd	tr	tr	nd	nd
5	tr	tr	nd	nd	tr	tr	nd	nd
6	nd	nd	nd	nd	tr	nd	nd	nd
7	nd	nd	nd	4.2	6.1	tr	nd	nd
8	nd	nd	nd	nd	nd	nd	nd	nd
9	nd	nd	nd	nd	nd	nd	nd	nd
10	nd	nd	nd	nd	nd	nd	nd	nd
11	nd	nd	nd	nd	nd	nd	nd	nd
12	nd	nd	nd	tr	nd	nd	nd	nd
13	nd	nd	nd	nd	nd	nd	tr	nd
14	nd	nd	tr	nd	nd	nd	nd	nd
15	nd	nd	nd	nd	nd	nd	nd	nd
16	nd	nd	nd	nd	nd	nd	nd	nd
17	nd	nd	nd	nd	nd	nd	nd	nd
18	nd	nd	nd	nd	nd	nd	tr	tr
19	nd	nd	tr	tr	5.2	nd	nd	nd
20	nd	nd	nd	nd	nd	nd	nd	nd
21	nd	nd	nd	nd	nd	nd	nd	nd
22	nd	nd	nd	nd	nd	nd	nd	nd
23	nd	nd	nd	nd	nd	nd	nd	nd
24	nd	tr	nd	nd	nd	nd	tr	nd
25	nd	nd	nd	nd	nd	nd	nd	nd
26	nd	nd	nd	6.0	nd	tr	nd	nd
27	nd	nd	nd	tr	nd	nd	nd	nd
28	nd	nd	nd	tr	nd	nd	nd	nd
29	nd	nd	nd	tr	nd	nd	nd	nd
30	nd	nd	nd	tr	nd	nd	nd	nd

Further samples were taken from the contaminated area, every 2 cm between 20 and 30 cm along the stave, in 2 mm layers up to a total of 10 mm in depth, to determine the depth to which TCA and TCP had penetrated the wood. This produced five samples, each analyzed at four depths.

Figure 2 shows that only a small part of the selected area, representing a maximum of 20 cm^2, was severely contaminated with TCA, which was detectable up to 8 mm in depth and reached a maximum concentration of 25 ng/g at depths between 4 and 6 mm.

All these results revealed that only a very small fraction of the surface of the staves in contact with the wine (just under 2 m^2 on the inner surface) was significantly contaminated with TCA.

It was quite clear that this very limited area (5% of the surface) was responsible for contaminating the wine with TCA. In this specific case, an end stave approximately 100 cm^2 in area with a patch of approximately 20 cm^2 severely contaminated to a depth of 8 mm, i.e., a maximum of 8 cm^3 wood weighing under 6 g, that had been in contact with 225 L of wine for 3 months, still contained a total of 99.5 ± 8 ng of TCA. The outer layer (0−4 mm approximately), humidified by the wine, had certainly released larger quantities of contaminants. The wine in the barrel had accumulated a total of approximately 2720 ng of TCA, indicating that, theoretically, the initial total contamination of the incriminated wood was 470 ng/g.

The Sporadic Nature of Contamination in New Barrels. Analysis of other cases involving spoiled wine (mainly red) in similar situations around the world (France, South Africa, Italy, Australia, USA, and Austria; results not shown), using the most sensitive assay technique for haloanisoles (HSSPME/GC−MS, range: 0.1−20.0 ng/L 2,4,6-trichloroanisole, LD = 0.18 ng/L, LQ = 1.45 ng/L, uncertainty = 0.33%), according to the protocol developed by Chatonnet et al. (*26*), revealed TCA contamination, varying from 1.5 to 15.5 ng/L (results not shown), that was strictly attributable to the barrels used. White wines fermented and aged in new barrels are hardly ever affected by this problem, provided they are maintained on the lees with stirring, essentially due to the remarkable capacity of yeasts to fix haloanisoles on their cell walls (*33*). Red wines that are put into barrel very early with a large biomass load are also protected from contamination in the same way, but they are rarely aged on as large quantities of lees for such a long time as white wines.

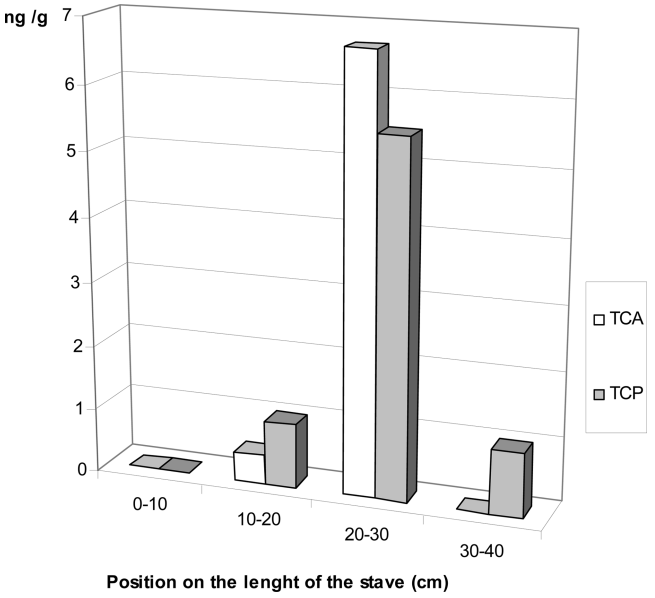

Figure 1. Fine localization of the contamination of oak wood by TCA and TCP on the stave identified as contaminated.

TCA contamination in barrels is extremely sporadic. Only a few barrels are likely to be affected in a given production batch, made from a single batch of stavewood. Furthermore, contamination does not affect entire batches of stavewood, but only a few staves, and possibly only a very limited fraction of their surface, as described above.

Over a four-year period, from 2002 to 2006, a single French cooperage, whose French oak barrels for the California (USA) market were always the same type, manufactured in the same way, received complaints concerning 0.15% of their production, following excessive TCA contamination (1.5−14.4 ng/L) in the various wines aged in them. In the space of three years, this situation led to a 50% loss of sales in this market, due to doubts concerning the quality of their barrels (**Figure 3**). Major changes in the production system and quality-control methods put an end to the complaints in 2005, but this only resulted in a slow recovery in sales. Consequently, even a small percentage of defective products may have major mid- to long-term repercussions on an industrial and business level.

Testing Oak Wood for Contamination during Open-Air Drying and Seasoning in the Woodlot. Stavewood, the raw material for staves, is obtained by splitting or sawing bolts of wood cut from unbarked logs (*1*). The trees, felled in the forest in early autumn and only prepared as stavewood in the following summer, are usually sprayed with water during storage to avoid splitting and damage by xylophages. Stavewood is stored in well-ventilated piles, raised off the ground, with a batten between each layer of staves. These piles are stored in the open air and may initially be sprayed with water for a time, to facilitate the rapid elimination of certain compounds considered organoleptically undesirable and accelerate favorable changes during the period of exposure to the elements. Indeed, before oak wood is usable for making barrels, it must be dry and undergo several modifications in its

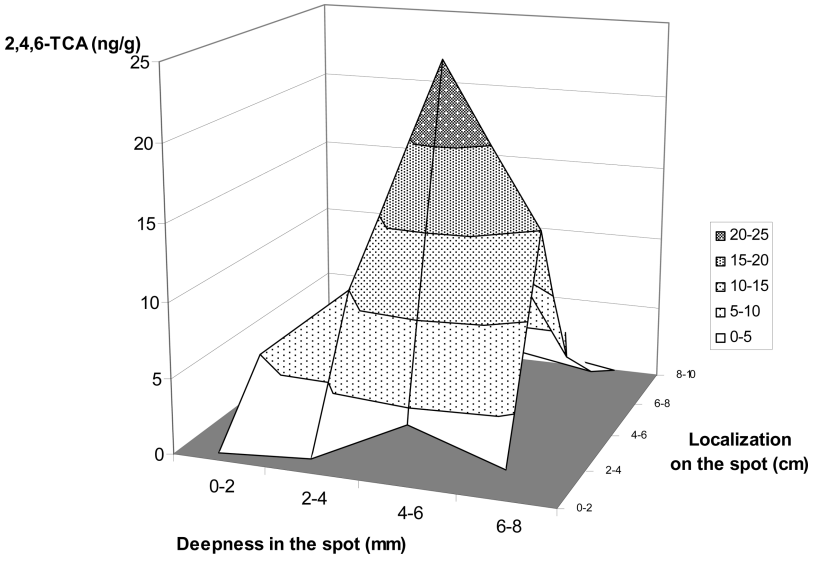

Figure 2. Deepness and fine localization of the contaminated area by TCA in the polluted part of the stave.

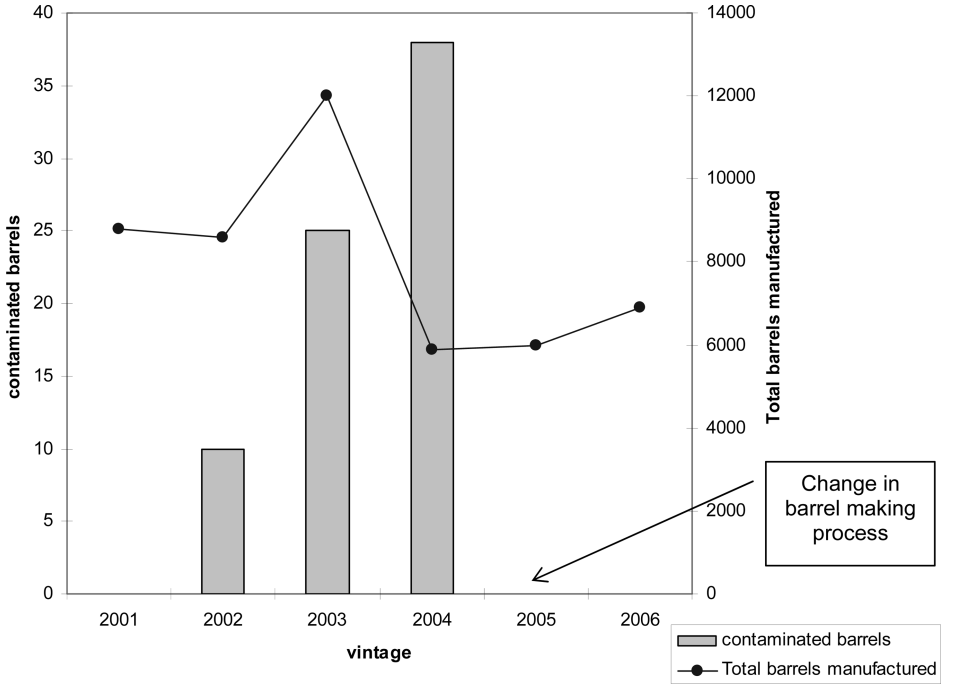

Figure 3. Frequency of barrel contamination by TCA observed in a French cooperage vs time: influence on the sales.

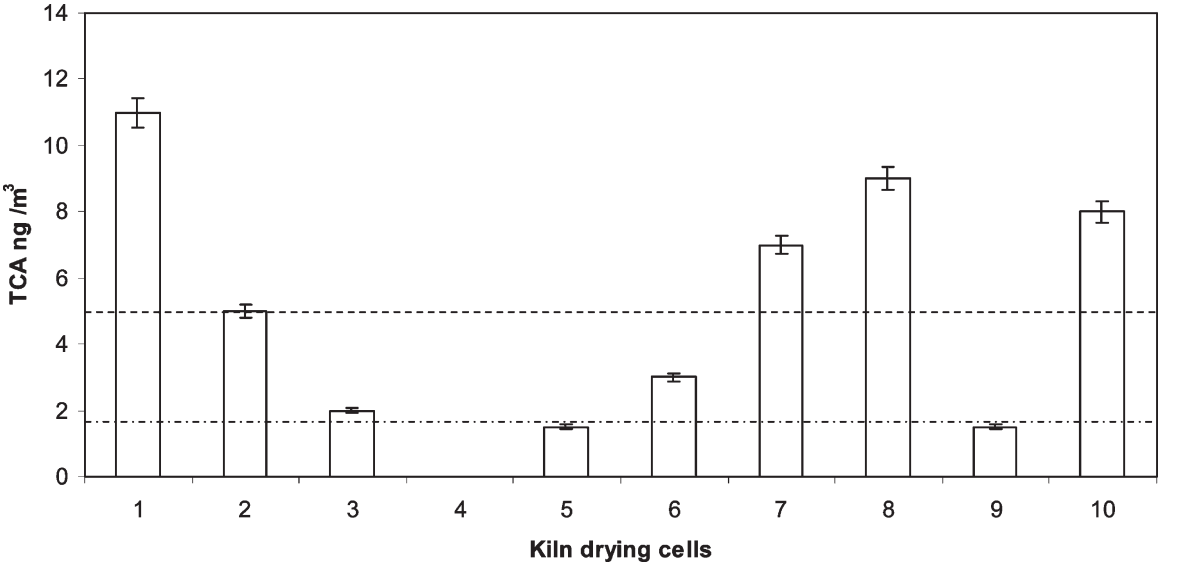

Figure 4. TCA concentration in the kiln oven atmospheres used for oak wood stabilization before barrel making after 24 months of natural seasoning. - - - Limit of Detection LOD = 1.75 ng/m^3 ± 0.75. --- Limit of Quantification LOQ = 5 ng/m^3 ± 2.5.

physicochemical composition (*3*, *4*), over a period that usually varies from 18 to 36 months but, in some cases, up to 60 months. During this time, the humidity levels of the wood decrease from 35% to approximately 14% and its surface is exposed to the development of several microorganisms (*34*). When the oak stavewood is split into staves, it is essential that the humidity level should be in the vicinity of the equilibrium moisture content of the fibers, i.e., 14−16%, to avoid the risk that they will become misshapen due to shrinkage or swelling (*51*). However, with mean summer temperatures of 25 °C and 70% relative humidity in the air, humidity in the oak wood frequently drops to 12−13%, which is slightly too low. In winter, with a mean temperature of 5 °C and 85% relative humidity, it is difficult to obtain stavewood with under 19−20% humidity using strictly natural drying, so the oak is too humid. To offset these seasonal variations, as well as, in some cases, to accelerate the natural process, a majority of coopers make temporary use of industrial drying kilns, but the quality of the extractable compounds is not strictly identical (*35*, *36*). In just a few weeks, these closed units, with controlled air temperature and humidity, adjust and homogenize the physical characteristics of the wood before it is processed to make staves (*37*).

In view of the sporadic, highly localized character of TCA contamination in stavewood, it is difficult to detect it directly by taking samples from the untreated wood during the seasoning process. For this reason, we decided to detect the pollutants targeted in this work indirectly, by analyzing the composition of the air in the unit just before the end of the drying cycle (19th day in a 21-day cycle). The kilns studied in this work belonged to the same cooperage. They were all built out of insulation panels, sandwiched between galvanized steel, and the inner surface, which came into contact with the wood, was hot lacquered with inert, chlorophenol-free epoxy resin (results not shown).

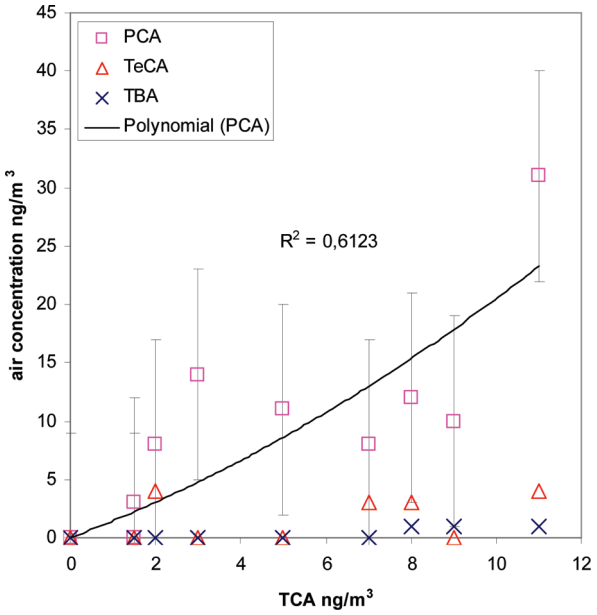

Figure 5. Correlations between TCA and other haloanisoles measured in kiln atmospheres filled with oak wood merrains (bar errors correspond to 95% confidence intervals of the measurement).

Figure 4 shows the quantity of TCA measurable in the atmosphere of several drying kilns used at the end of the winter in a cooperage that had experienced irregular but recurrent TCA contamination of their barrels. Note that, while no contaminants were detected in the empty kiln (< LOD = 1.75 ng/m^3 ± 0.75), in 4 out of 9 units filled to a capacity of approximately 1000 m^3, operating at temperatures varying from 29 to 36 °C ± 1, at a relative humidity on the order of 62% ± 5, assays revealed significant quantities of TCA (> LOQ = 5 ng/m^3 ± 2.5) while traces of this compound were detected in the others (> LOD). These results clearly indicate the presence of small quantities of TCA, detectable at an early stage in stavewood preparation, i.e., at the end of the wood drying cycle.

This analysis of the atmosphere in the kilns also detected other volatile organic compounds and quantified those which were present in sufficiently high concentrations. TBP, TeCP, PCP, TCP, geosmin, and 2-methylisoborneol were not detected (< LOD) in any of the locations tested, and this research did not, unfortunately, investigate dichloroanisoles (particularly 2,4- and 2,6-dichloroanisole) or their precursors. However, measurable quantities of PCA, TeCA, and isoborneol, as well as traces of TBA, were found in some assays. The quantities of TCA were not, apparently, correlated with any of these molecules, as the possible positive correlation with PCA was not statistically significant (**Figure 5**).

Possible Origins of TCA in Oak Wood. This section discusses a possible origin of TCA in oak wood following the O-methylation of its direct precursor, TCP, according to a standard process of microbiological detoxification (*15*, *38*) or even a purely chemical nucleophilic substitution reaction (*39*).

Chemical Formation of Chlorophenols. The reaction of chlorine with a phenol molecule produces a mixture of 2- and 4-chlorophenol, 2,4-, and 2,6-dichlorophenol and 2,4,6-trichlorophenol (TCP), which accumulates (*32*). However, unlike corks, where it was common practice at one time to use chlorine or hypochlorite, which represented a major source of TCA contamination (*31*), these treatments are not used on oak wood. Finally, the water used to spray the unbarked logs and stavewood (well water) does not usually contain sufficiently high concentrations of chlorine to induce any significant synthesis of TCP.

Chemical oxidation of halides may form diatomic molecules from anions (*40*). While iodides oxidize relatively easily in an acid medium (E_0 = 1.36 V), bromides require a stronger oxidant than hydrogen peroxide (E_0 = 1.06 V), and chlorides (E_0 = 0.58 V) can only be oxidized by an even stronger chemical oxidant, or even by electrolysis. Consequently, even if oxidation of ellagitannins in an aqueous medium may produce hydrogen peroxide (*41*) and chlorides are always abundant, direct oxidation to form Cl_2 is apparently completely impossible under the conditions prevalent during wood seasoning.

Anthropogenic Origin of Chlorophenols. Chlorophenols have been widely manufactured and used worldwide to protect wood from xylophagous insects and fungi. TCP has sometimes been used as a pesticide (Dowicide 2S, Omal, Phenachlor, etc.), but PCP is more widely used, due to its greater persistence. Industrial PCP generally contains between 3 and 10% TeCP and an even smaller proportion of TCP (*13*). Oak wood does not generally require special protection, as it is naturally resistant to xylophages. Any treatments used are only applied to the outside of the unbarked logs, while only the inside (heartwood) is used in cooperage. The USA Environmental Protective Agency (US EPA) has listed chlorophenols as priority trace contaminants since 1984 (*42*). The European Economic Community (EEC) prohibited the use of PCP in 1991 (*43*). Finally, no chlorophenol-based products, and certainly none containing TCP, were known to have been used in the wood environment. Prochloraz (CASE No. 67747-09-5), a fungicide known to generate TCP as a degradation byproduct (*44*), has no useful applications in cooperage. PCP and TeCP, which may have been used on sites where the wood was sawn, split, or stored, may be partially degraded into TCP under anaerobic conditions, but TCA have never been clearly identified as degradation byproduct (*45*, *46*). In view of all these elements, it seemed extremely unlikely that the particularly random, localized TCA contamination investigated in this work was connected to deliberate treatment or accidental contamination with organochlorine-based treatment products that had accumulated in the environment around the cooperage.

Formation of TCA from TCP by Electrophilic Substitution. An alkoxide such as TCP may undergo an S_N2 electrophilic substitution in the presence of a halogenoalkane, such as bromomethane ($BrCH_3$ 74-83-9), at alkaline pH via a Williamson reaction (*36*) (this reaction is also possible at lower pH, but the yield is reduced to only 3% according to Boutou (*27*)). The theory that TBA was generated from the TBP accumulated in the wooden floor of certain shipping containers due to mandatory fumigation with bromomethane (*47*) has been confirmed in practice.

The probability that both TCP and a halogenoalkane will be present during the production and seasoning of stavewood under normal conditions is zero. However, it is by no means impossible (and even relatively common) for wood potentially containing a certain quantity of TCP (or TBP) to be subjected to chemical fumigation during the loading (or before the unloading) of shipping containers, especially in the case of untreated (nontoasted) wood shipped to certain destinations (particularly Australia and USA), resulting in the accumulation of unpleasant-smelling haloanisoles. Bromomethane, now considered a hazardous chemical, is prohibited by the Montreal protocol and is gradually being replaced by phosphyne (PH_3, 7803-51-2), which does not have the same disadvantages.

***De Novo* Biosynthesis of Organochlorine Compounds.** A relatively large number of organisms with chloroperoxidase-type enzyme activities are known for their *de novo* production of organohalogen derivatives, especially chlorophenols (CPO) (*48*). Haloperoxidases (HPO) and perhydrolases more or less directly

produce a peracid capable of oxidizing a halide into hypohalous acid, leading to a relatively nonspecific, nonregioselective halogenation, or, possibly, a dependent $FADH_2$ halogenase, as found in certain bacteria (*49*). As mentioned above, chlorides are not directly oxidizable by standard peracids or peroxides (peracetic acid, hydrogen peroxide). The reaction is necessarily globally catalyzed by the intra- or extracellular enzyme system of the microorganisms.

The microorganisms frequently identified in wood (*50*) include a large number of molds (mainly deuteromycetes, with a few zygomycetes, ascomycetes, and some *fungi imperfecti*, or yeasts). A majority of these are capable of converting TCP into TCA (via *O*-phenylmethyl methylase), but none have been described as exhibiting the CPO activity required for *de novo* synthesis of TCP. Maujean et al. (*51*) described a very unusual mechanism for TCA formation by *Penicilium roqueforti*, the mold most frequently identified in stavewood seasoning in the open air. In this specific case, TCP synthesis occurred due to chemical chlorination in the presence of hypochlorite introduced into the medium. *Penicilium sp.* simply biosynthesize phenol, *via* the standard shikimic acid pathway, and then *O*-methylate the chemically formed TCP into TCA. These conditions are very different from those likely to be found in a cooperage, where the use of chlorinated products is not only unnecessary but also prohibited. Prat et al. (*52*) have identified *Cryptococcus sp.*, *Rhodotorula sp.*, *Penicilium glabrum*, *Penicilium variabile* and *Pseudomonoas jessenii* isolates to be able to produce TCA in the presence of cork material but there is any indication in this work about the pathway of TCA synthesis. Because of the conditions of cultivation of these microorganisms, in a synthetic medium supplemented with cork granules, we can suppose that TCA appears by biomethylation of TCP residues accumulated in the organic material.

In a previous work (*50*), we also reported the presence of the less common *Alternaria alternata* (5 to 11% of isolates, depending on the season and humidity of the wood) among the identifiable deuteromycetes in naturally seasoned stavewood. Following prolonged incubation under conditions including ideal humidity, this fungus is capable of degrading the S_2 (lignified) cell walls of hardwood (*53*). Some particular strains of *Alternaria alternata* possess a significant CPO activity (*54*) likely to be capable of oxidizing chlorides. This type of microorganism could be therefore potentially capable of forming TCP under the conditions prevalent during stavewood seasoning, which could then be converted into TCA.

Basidiomycetes are by no means the most common fungi in stavewood. However, in our previous work (*50*), we reported the presence of an unusual basidiomycete with arthroconidia, related to a *Phanerochaete*-type xylophagous fungus (white rot) in very old stavewood. This particular fungus (which has not been perfectly identified, see ref *50*) is capable of degrading lignin and has a strong polyphenol oxidase activity sometimes correlated with HPO activity. *Phanerochaete chrysosporium*, a basidiomycete capable of degrading hardwood lignin, exhibits an intense lignin peroxidase activity, corresponding to an HPO capable of oxidizing iodides and bromides, but not chlorides (*55*). It is, therefore, improbable that the presence of TCP and TCA in oak wood is due to the development of this specific type of basidiomycete.

We also reported that the bacteria identifiable on oak stavewood seasoned in the open air included *Streptomyces sp.* (actinomycetes) (*50*). This type of microorganism is also reputed to possess relatively nonspecific BPO (*56*) and more specific CPO activity, as in the case of *Streptomyces lividans* (*57*), *Serrata marcescens* (*58*), and *Pseudomonas pyrrocinia* (*59*). This type of microorganism may, therefore, also represent a source of TCP contamination in wood.

In conclusion, while the results presented indicate that, as is well-known in the case of cork, there must be one or more sources of sporadic TCA contamination of oak wood, we have not yet identified the precise origin or formation mechanism of its precursor. Available data indicates that the formation and accumulation of these undesirable organochlorine contaminants in stavewood takes place during the natural drying and seasoning phase. We demonstrated that strictly chemical formation was also impossible under standard cooperage conditions. However, bromomethane fumigation in shipping containers may, under highly specific conditions, lead to the presence of the corresponding anisoles. Among the various hypotheses capable of explaining the presence of these substances, wood treatment products and contamination with pesticide residues are highly improbable, as the stavewood had not been exposed to these types of treatment. Similarly to previous discoveries in corks, all the analyses of oak wood suggested that the formation of TCP and its conversion into TCA was of biochemical origin. While biomethylation is well-known and relatively widespread among the usual microflora in stavewood, the precise origin of the intermediary leading to TCP formation is still unknown. In our opinion, the intervention of a (fungal or bacterial) haloperoxidase with a chloride activity is the most probable hypothesis. Several ideas have been proposed, but the microorganisms responsible for the formation of the TCA precursor in oak wood have not yet been identified and the conditions under which they develop in oak wood require further clarification. In view of the extremely sporadic nature of the contamination, it is relatively unlikely that standard quality control techniques based on the analysis of random samples of oak fragments would be capable of detecting it. Consequently, the problem is seriously underestimated by coopers. In view of the contaminant potential of TCA in wine, it is absolutely indispensable to develop a new approach for detecting its presence in wood and barrels well before they are used in wineries.

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