

Identification and Responsibility of 2,4,6-Tribromoanisole in Musty, Corked Odors in Wine

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In this work, gas phase chromatography analysis coupled with selective selected ion monitoring (SIM) identified 2,4,6-tribromoanisole (TBA) in wines found on tasting to have significant “musty or corked” character, although they did not contain noteworthy quantities of chloroanisoles or chlorophenols, the contaminants generally reported to cause this type of defect. The perception thresholds were studied, together with contamination conditions during winemaking, storage, and bottle-aging. A “musty” off-odor was perceptible on smelling wine containing as little as 4 ng L⁻¹ TBA, and spoilage may be detected by retro-olfaction at even lower concentrations. TBA, produced by O-methylation of its direct precursor, 2,4,6-tribromophenol, generally comes from sources in the winery environment. This paper is the first to identify the sources of a large number of cases of wines polluted during storage in premises where the atmosphere was contaminated with TBA used recently to treat wood, or originating from much older structural elements of the winery, or from used wooden containers. In certain cases, although the initial source had been eliminated, residual pollution adsorbed on walls could be sufficient to make a building unsuitable for storing wooden barrels and plastics, as well as corks, which have been found to be particularly susceptible to contamination by the TBA in the winery atmosphere.

KEYWORDS: 2,4,6-Tribromoanisole; 2,4,6-tribromophenol; musty odor; wine; wood preservation; atmosphere

INTRODUCTION

“Musty” odors are some of the most unpleasant organoleptic defects in wines and attract strong criticism from connoisseurs and consumers alike. Several molecules have been identified as giving wines this unpleasant smell. Among these, chloroanisoles, especially 2,4,6-trichloroanisole (TCA) and 2,3,4,6-tetrachloroanisole (TeCA), are the most frequently identifiable in wines criticized on tasting as “musty” or “corked”. The term “corked”, meaning that spoilage is due to the natural cork traditionally used as a bottle stopper, is frequently inappropriate. In fact, although corks, made from the bark of cork oak *Quercus suber*, may release TCA into wines if the quality of the raw material or the manufacturing process is unsatisfactory (1–3), other sources of chloroanisole pollution have also been identified. Pentachloroanisole (PCA), which has relatively little odor, and especially 2,3,4,6-tetrachloroanisole (TeCA) are produced by the biochemical breakdown of certain pesticides containing 2,3,4,6-tetrachlorophenol (TeCP) or pentachlorophenol (PCP), with TeCP as an impurity. These compounds may contaminate wine that has not been in contact with cork (4, 5). The breakdown mechanisms of these precursors and the conditions for distant contamination via the atmosphere have been de-

scribed in detail (5). Excessive use of hypochlorite-based disinfectants may also lead to the contamination of organic materials in contact with wine or the cellar atmosphere after the formation of 2,4,6-trichlorophenol (TCP) (6), and its breakdown by certain filamentous fungi (7, 8). TCA has a relatively low olfactory detection threshold in water [300 pg L⁻¹ (9) and 30 pg L⁻¹ (10)]. Depending on the type of wine, changes in odor and, above all, retro-olfaction were significant between 1.5 and 3 ng L⁻¹ (11). TeCA is less odoriferous [4 ng L⁻¹ in water (9)], but noticeable spoilage has been reported at concentrations above 10 ng L⁻¹ in still wines and 5 ng L⁻¹ in sparkling wines (12).

However, some wines have exactly the same type of tasting fault but do not contain sufficient quantities of chloroanisoles to account for this “musty” character. Soleas et al. (13) reported that only a small fraction (27%) of a large number of wine samples analyzed contained a significant quantity of TCA (>2 ng L⁻¹). However, these authors did not assay TeCA, so it is not possible to draw any conclusions concerning the involvement of other chloroanisoles.

Exhaustive identification and assay in our laboratory of the various chloroanisoles in a large number of wines found to have off-odors on tasting made it possible to identify situations leading to a high frequency of spoilage when neither TCA nor TeCA was detectable at sufficiently high levels to account for the defect.

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Table 1. Characteristics of the Halophenol and Haloanisole Assays by GC/MS-SIM

matrix	TCA	TCP	TeCA	TeCP	PCA	PCP	TBA	TBP	TeBP	PBP
wines (ng L ⁻¹)										
detection limit	0.95	1.25	0.85	2.10	0.95	3.60	0.90	1.50	<i>a</i>	<i>a</i>
quantification limit	2.95	4.80	3.30	6.00	5.00	8.75	2.00	3.50	<i>a</i>	<i>a</i>
uncertainty ±	3.90	5.20	3.20	5.00	4.40	4.90	3.50	5.50	<i>a</i>	<i>a</i>
solid materials ^b (ng g ⁻¹)										
detection limit	0.45	2.45	0.90	0.55	1.95	1.40	0.40	2.55	<i>a</i>	<i>a</i>
quantification limit	2.40	7.45	3.20	4.10	5.20	5.90	2.00	7.55	<i>a</i>	<i>a</i>
uncertainty ±	2.00	3.50	1.60	1.60	2.20	3.80	2.00	3.60	<i>a</i>	<i>a</i>

^a Not studied. ^b And control of atmospheres by static trapping.

In this work, we identify 2,4,6-tribromoanisole (TBA) for the first time in wines with a significant “musty” character, which contained insufficient quantities of chloroanisoles to produce this defect. The perception threshold and conditions for contamination during winemaking, storage, and bottle-aging are specified.

EXPERIMENTAL PROCEDURES

Materials and abbreviations: 2,4,6-trichloroanisole (TCA) [CAS Registry No. 87-40-1], 99%, Aldrich; 2,4,6-trichlorophenol (TCP) [88-06-2], >98%, Aldrich; 2,3,4,5-tetrachloroanisole [938-86-3], 98% (used as equivalent of 2,3,4,6-TeCA but with exactly the same retention time 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 (TBP) [118-79-6], 98%, Aldrich; 2,4,6-tribromophenol-*d*₂, 98%, CDN Isotopes Inc.; pentabromophenol (PBP) [608-71-9], Aldrich; deuterated iodomethane CD₃I [865-50-9], >99.5%, isotope enrichment, CDN Isotopes Inc.

Synthesis of Deuterated Chloroanisole Analogues. A blend of pure chlorophenol (TCP, TeCP, or PCP) (6 mmol), anhydrous potassium carbonate (5 mmol), and deuterated iodomethane was prepared in 10 mL of dimethyl sulfoxide (Pestipur, SDS). It was stirred for 3 h at room temperature. 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 (MilliQ), 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 10-plate reflux column and a nickel helix. The solid residue was recrystallized in ethanol. The deuterated chloroanisole on the methoxy group was purified by adsorption chromatography on a 50 × 0.5 cm flash chromatography column filled with activated silica (activity V). The synthesis product was rinsed with *n*-pentane (SDS, Pestipur > 99.5%) and eluted (5 mL/min) with a 96:4 blend of 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 keV, 150 °C) and scan mode (40–400 *u*m). The mass spectrum obtained from the analogues was as expected, showing that the purity of the 2,4,6-TCA-OCD₃, 2,3,4,6-TeCA-OCD₃, and PCA-OCD₃ synthesized was >99% of the total signal (scan mode).

Simultaneous Assay of Chlorophenols and Chloroanisoles in Wines and Dry Materials. The chlorophenols and chloroanisoles in the wines and dry materials were assayed simultaneously using a method derived from that previously described by Chatonnet et al. (14). The halophenol assay was carried out using 2,4,6-tribromophenol-*d*₂ (TBP-*d*₂) as an internal standard, and the haloanisoles were assayed using the deuterated chloroanisole analogues -OCD₃ and 2,4,6-TBA-*d*₅ for 2,4,6-tribromoanisole. The proposed analysis method was assessed using

the validation protocol for a standard method described in the OIV repository (15) and the French standards for intralaboratory validation: NF ISO 5725-1 and NF V 03-110 (16a,b), as described previously by Chatonnet et al. (14) (Table 1).

The wine (200 mL) was centrifuged (15 min at 5000g) and put into a 200 mL borosilicate glass graduated vial deactivated by silanizing with hexamethyldisilazane, and then exactly 1 mL of an internal standard solution in absolute ethanol containing 0.1 mg L⁻¹ TBP-*d*₂ as well as 0.05 mg L⁻¹ TCA-OCD₃, TeCA-OCD₃, PCA-OCD₃, and TBA-*d*₅ was added. The contents of the vial were homogenized and transferred to a 250 mL Erlenmeyer flask, and then ~1 mL of 1/3 sulfuric acid was added using a graduated pipet to acidify the medium, as well as a Teflon stir bar (6 g, 25 mm) previously rinsed with ethanol. Then 10 mL of a 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 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 broken by asymmetrical agitation, and the aqueous phase 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 Zymark concentrating tube; the flask was rinsed carefully twice with 1 mL of dichloromethane/pentane. The solution was concentrated using a Zymark Turbovap II, operating at 25 ± 2 °C in a steam of nitrogen (quality I, Air Products) at 1 bar. It was concentrated automatically to 0.5 mL in ~15 min. The sample was transferred to a 500 μL disposable injection flask and kept at -20 °C in a dark place prior to analysis.

The dry materials (corks, barrel wood, wood from roof timbers, cardboard, chipboard, etc.) were shredded in a granulating mill with a stainless steel bowl. The mill was decontaminated between samples by soaking it in a detergent solution (5% Decon 90) with added ethanol (50% vol) and dried overnight in an oven at 105 °C. The material was grated onto a sheet of aluminum foil.

A material sample weighing 0.5–5 g depending on the contamination level (±0.01 g) was put into an 150 mL Erlenmeyer flask stopped with emery and 100 μL of internal standard (2,4,6-tribromophenol-*d*₂ at 1.180 mg L⁻¹ in absolute ethanol), 70 mL of dichloromethane, and 0.1 mL of glacial acetic acid (Rectapur, Prolabo) were added to facilitate extraction of the halophenols. It was stirred for 120 min at room temperature with a stir bar (25 mm, PTFE, 6 g, 250 rpm). The extract was purified by continuous extraction with ethanol in a Soxhlet for 12 h, rapidly filtered through cellulose paper to eliminate any cork dust, concentrated to 0.5 mL, and then transferred to a flask for storage as described above.

Atmospheric Quality Control by Static Trapping on an Inert Adsorbent. The technique used was described by Chatonnet et al. (5) and Chatonnet and Labadie (12) for trapping atmospheric chloroanisoles and chlorophenols in wineries. The following system was placed in the atmosphere to be monitored: 25 g of a blended adsorbent compound containing bentonite, silica gel, and Tenax (85:10:5), purified by continuous methanol extraction in a Soxhlet for 24 h and dried in an oven at 105 °C prior to use. The blend was spread in a thin layer on a 30 × 30 cm sheet of aluminum foil and left in the atmosphere for 120 h to trap any contaminants presents in the premises under investigation.

A cellulose paper scoop was then used to take a 5 g sample of adsorbent. The blend of internal standards (100 μL) was added to the scoop, which was installed in a 75 mL Soxhlet continuous extractor. The evaporator flask was filled with 70 mL of dichloromethane, and 0.1 mL of glacial acetic acid was added. The condenser was fed with water at 12 ± 5 °C. The flask was heated to produce a reflux of condensed solvent at a rate of ~ 3 mL/min for 120 min. The extractor was then removed from the heating system and cooled. The solvent was transferred from the extractor and flask directly into a 100 mL concentration tube, and the flask was rinsed out twice with ~ 2 mL of hexane. The solution was concentrated using a Zymark Turbovap II, operating at 25 ± 2 °C in a stream of nitrogen (quality I, Air Products) at 1 bar. It was concentrated automatically to 0.5 mL in ~ 15 min. The sample was transferred to a 500 μL disposable injection flask and kept at -20 °C in a dark place prior to analysis. The quantity of contaminants assayable in air is conventionally expressed in nanograms per gram of adsorbent.

Analysis by Gas Phase Chromatography and Selected Ion Monitoring (GC-MS/SIM). A 1 μL (extracted from dry materials or trapping residues) or 2 μL (wine) sample was injected into an HP5-MS (5% phenyl-methyl-siloxanes) capillary column 30 m \times 0.25 mm (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 time = 1.5 min, bleed = 50 mL/min, bleed time = 1.5 min) and an Agilent 5183 4711 insert using an automatic Combi PAL injector (10 μL syringe, CTC Analyticals Inc.). The balance gas (helium N55, Air Product) was used at constant flow rate (initial pressure at column head = 7.1 psi, flow rate = 1.0 mL/min, linear velocity = 36 cm s^{-1}). 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 (pentadecane, decylamine, 3-octanol, 2,4-dichlorophenol). Analysis of chlorophenols requires a perfectly inert system, assessed by the retention time, surface area, and width of the decylamine and 2,4-dichlorophenol peaks, as well as changes in the response factors of the chlorophenols in relation to the internal standard.

An HP5973 quadrupole mass detector operating in electron impact mode was used for detection (source temperature = 250 °C, quadrupole = 150 °C, constant ionization potential = 70 keV, electron multiplier = 1500 V) and fragmentometry mode 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, 329; TBP, 328, 330, 332; TeBP, 408, 410, 411; PBP, 486, 488, 490; 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); 232/336 (TeCP/TBP- d_2); 280/283 (PCA/PCA- OCD_3); 266/336 (PCP/TBP- d_2); 346/349 (TBA/TBA- d_5), and 328/336 (TBP/TBP- d_2). In the absence of a reference standard, TeBP and PBP were respectively estimated using the same response factor as TeCP and PCP. **Table 1** presents the detection and quantification thresholds and uncertainties associated with the various compounds studied using this method.

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^{-1} diluted in uncontaminated wine, and 0, 1, 5, 10, 20, 50, and 100 ng g^{-1} impregnated in cork, wood, or adsorbent material previously extracted by continuous extraction (24 h) in a Soxhlet by methanol (dried for 1 h in an oven at 24 °C and then stored in glass). The pure halophenols were stored 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 analysis system was always properly calibrated.

Sensory Analysis. Initially, a range of concentrations of TBA was prepared at 0, 2, 4, 8, 16, and 32 ng L^{-1} in a standard red wine (red wine, 11.85% vol, pH 3.68) to provide reference values for olfactory intensity (0 = undetected, up to 5 in increments of 1) in the red wine thought to be contaminated that were investigated in this study and training of the tasters. The wines were tasted by two series of three

Table 2. Concentrations of Halophenols and Haloanisoles in Various Samples of Red Wines Suspected of Musty, Corky Off-Flavors^a

wine sample (ng L^{-1})	TCA	TCP	TeCA	TeCP	PCA	PCP	TBA	TBP	TeBP	PBP
1	nd	11.5	nd	nd	tr	16.8	12.9	68.5	nd	nd
2	nd	nd	nd	nd	nd	113.5	34.4	17.8	nd	nd
3	nd	nd	nd	22.4	tr	19.5	2.1	tr	nd	nd
4	nd	nd	nd	13.2	nd	33.7	3.9	8.3	nd	nd
5	nd	tr	nd	71.0	tr	8.3	12.1	5.4	nd	nd
6	nd	nd	tr	7.5	tr	675.4	2.2	252.8	nd	nd
7	nd	tr	nd	6.9	tr	147.6	12.0	67.0	nd	nd
8	nd	nd	nd	nd	tr	48.0	0.0	68.3	nd	nd
9	nd	nd	nd	nd	tr	nd	2.5	11.1	nd	nd
10	nd	6.5	nd	nd	tr	nd	tr	7.2	nd	nd
11	nd	7.6	nd	nd	nd	40.2	12.3	37.1	nd	nd
12	nd	8.5	nd	nd	tr	28.5	26.7	31.3	nd	nd
13	nd	7.1	nd	nd	nd	nd	13.1	13.4	nd	nd
14	nd	5.4	nd	nd	tr	nd	9.1	14.6	nd	nd
15	tr	tr	nd	nd	nd	tr	26.1	32.4	nd	nd
16	2.9	tr	nd	nd	tr	tr	17.9	36.1	nd	nd
17	nd	tr	nd	nd	nd	nd	3.8	7.5	nd	nd
18	nd	12.3	nd	nd	tr	8.3	nd	3.6	nd	nd
19	2.6	17.1	nd	nd	tr	29.8	3.5	37.4	nd	nd
20	tr	21.5	nd	23.9	tr	45.2	6.3	18.8	nd	nd
21	tr	72.0	nd	nd	nd	10.7	nd	tr	nd	nd
22	nd	14.3	nd	nd	nd	12.9	13.7	43.5	nd	nd
23	nd	tr	nd	nd	nd	nd	16.8	104.1	nd	nd
24	nd	nd	nd	27.0	nd	87.7	12.8	78.2	nd	nd
25	nd	tr	nd	nd	nd	nd	37.9	108.1	nd	nd
26	nd	11.7	nd	nd	nd	23.7	28.3	100.8	nd	nd
27	nd	64.6	nd	nd	11.0	nd	4.0	9.6	nd	nd
28	nd	3.2	nd	nd	122.9	38.0	4.0	30.1	nd	nd
29	nd	tr	nd	59.2	tr	81.2	6.5	392.6	nd	nd
30	nd	nd	nd	nd	nd	nd	6.3	44.9	nd	nd

^a nd, <DL; tr, >DL but <QL.

trained tasters using a paired test to determine whether samples are perceptibly different in comparison with the reference range of TBA in wine.

In a second stage, a range of TBA concentrations (0, 2, 4, 8, and 16 ng L^{-1}) was prepared in a dilute alcohol solution at 12% vol ethanol with 5 g/L tartaric acid neutralized to pH 3.65 with sodium hydroxide N, used as a model solution designed to simulate wine, as well as in the same standard wine used above. The olfactory perception threshold was estimated by a blind duo-trio test in which the range of concentrations was presented in increasing order to six trained, but not expert, tasters, according to the procedure described by Boidron et al. (17). The threshold determined in model dilute alcohol solution, known as the perception threshold, and the covering threshold determined in wine corresponded to the detection threshold obtained by 50% of the tasters.

RESULTS AND DISCUSSION

Identifying and Isolating 2,4,6-Tribromoanisole in Wines Found To Have a "Musty" Character on Tasting. Table 2

presents the assay results for several chloroanisoles and chlorophenols, reported to be standard contaminants in wines with a musty character, together with TBP and TBA. With the exception of a few samples that contained small amounts of TCA, the wines selected in this study contained little (<3 ng L^{-1}) or no chloroanisoles (<1 ng L^{-1} detection threshold). It was observed that the relative intensity of the musty defect on an arbitrary scale varying from 0 to 5 was significantly correlated ($p < 0.05$) with the TBA content of the wine, rather than with the other chloroanisoles studied (**Figure 1**). In cases of high-level TBA contamination (>20 ng L^{-1}), besides an intense musty odor, the wines also had a "phenolic" or "iodized" character of varying intensity. This defect was mainly perceived on the rear palate when the wine was tasted and was particularly persistent on the aftertaste. The odor is similar to that of TBP,

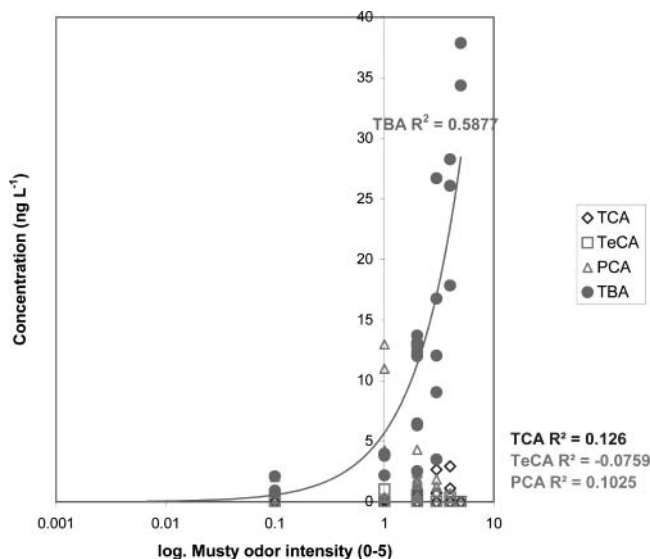


Figure 1. Relationship between the concentration of haloanisoles in various suspected red wines and the intensity of the musty odor measured by tasting.

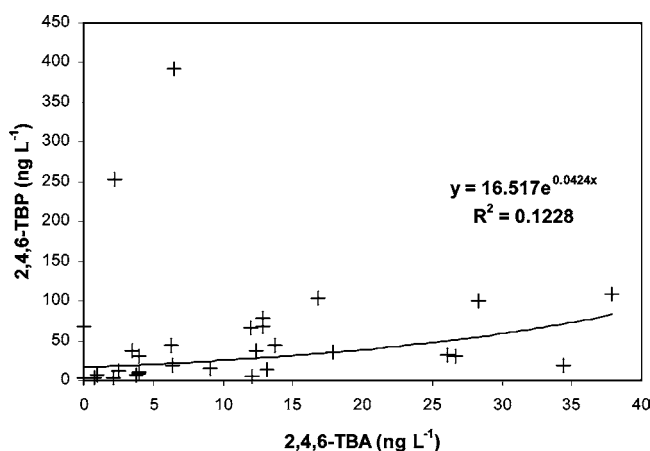


Figure 2. Relationship between the 2,4,6-tribromoanisole (TBA) and 2,4,6-tribromophenol (TBP) contents in various contaminated red wines.

but the intensity of the defect is not linked in any obvious way with the concentrations assayed in wines. Similarly, the TBA content of a wine is not significantly correlated with the assayable TBP level. We suppose that the conversion from TBP does not take place in wine but results from outside pollution (**Figure 2**). Unlike pentabromophenol, tetrabromobiphenol A (TBBPA) and polybrominated diphenyl ethers (PBDEs), widely used molecules known as brominated flame retardants (BFR), which may be endocrine disruptors (18), with regard to their toxicological properties TBA and TBP (19) do not seem to have any severe toxicological effects at the concentrations measured in the wines studied.

TBA has been identified as a trace contaminant in marine fauna and certain marine sediments (20, 21), as well as in the atmosphere (22). TBA is a derivative of its direct precursor, TBP, via O-methylation by bacterial microorganisms (23). TBP is naturally present in many marine organisms (24–28) and, in particular, certain brown algae, which may synthesize TBP de novo from bromides in seawater by means of specific enzymes (29).

TBA is known to cause powerful musty and earthy flavors in fruit packed in cases contaminated with TBP that has been broken down into TBA by *Paecilomyces variotii* (30), a filamentous fungus also known to be capable of converting TCP

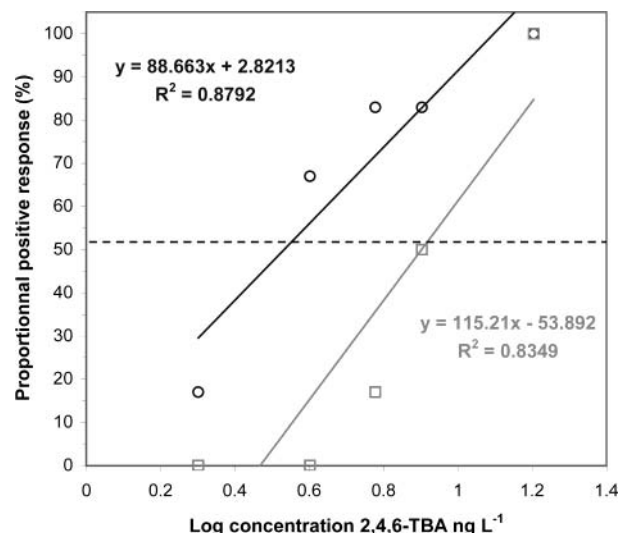


Figure 3. Estimation of the perception threshold of 2,4,6-tribromoanisole (TBA) in a model hydroalcoholic solution simulating wine (○) and in a standard red wine (□).

into TCA by O-methylation (31). Wylie (32) also reported the presence of TBP and TBA in pears, although these products had not been used in the orchard. Indirect pollution of pharmaceuticals by polyethylene stoppers contaminated through contact with wooden pallets impregnated with TBP has also been reported by Hoffman and Sponholz (33). Finally, trace amounts of TBA are known to cause this type of organoleptic defect in drinking water (34–36).

TBA has been reported to have extremely low perception thresholds in water. Saxby et al. (37) measured a threshold of 8 $\mu\text{g L}^{-1}$; Whitfield et al. (30), 20 $\mu\text{g L}^{-1}$; and Malleret and Bruchet (35), 30 $\mu\text{g L}^{-1}$. These thresholds make TBA one of the most powerful contaminants identified, with a similar pollution capacity to that of TCA which also has, according to Griffiths (10), a perception threshold in water in the vicinity of 30 $\mu\text{g L}^{-1}$. The thresholds estimated according to 50% of the tasters (trained but not expert) in an acidic dilute alcohol solution (pH 3.5–4.0) containing 10–15% ethanol volume, that is, a model medium (Pt) or a standard wine (Rt), were considerably higher. The perception threshold $\text{Pt}_{\alpha:50\%}$ was estimated at 3.4 ng L^{-1} and the covering threshold $\text{Rt}_{\alpha:50\%}$ at 7.9 ng L^{-1} (**Figure 3**). These results were compatible with tasting notes for the suspect wines studied (**Table 2**), although TBA could affect the quality and character of wines even below its detection or identification thresholds.

TBP may be formed chemically in wastewater treated with chlorine in the presence of bromide ions and traces of organic phenols. Chlorine may oxidize bromide ions, releasing bromine, which may then combine rapidly with a number of organic pollutants, producing not only chlorophenols but also bromophenols and bromochlorophenols (38). In the presence of sodium hypochlorite and UV radiation, the bromides present in salting pans may form 2-bromo-2-methylphenol, which is capable of giving off the powerful “phenolic” or “chemical” odor sometimes identified in some Gouda cheeses (39). No attempt was made to identify this compound in this research, but it could explain the iodized and phenol character found in certain wines in addition to musty off-odors.

Source of TBA Contamination in Wines. Analysis of several samples of red wines from different wineries with known winemaking and storage conditions indicated several ways in which wines could be contaminated.

Table 3. Control of Various Cellar Atmospheres by Static Trapping for the Presence of Halophenols and Anisoles^a

atmosphere (ng g ⁻¹ adsorbant)	TCA	TCP	TeCA	TeCP	PCA	PCP	TBA	TBP	TeBP	PBP
1	nd	nd	nd	2	15	179	26	nd	nd	nd
2	nd	nd	nd	nd	nd	28	72	1	nd	nd
3	nd	nd	nd	nd	nd	26	52	48	nd	nd
4	nd	nd	54	5	210	30	0	1	nd	nd
5	nd	nd	64	2	393	10	0	2	nd	nd

^a nd, <DL; tr, >DL but <QL.

As in the case of chloroanisole derivatives of PCP and TeCP, which are capable of polluting wines directly or at a distance via the atmosphere (5, 12), TBA, apparently produced by the microbiological breakdown of TBP, has been detected in the atmosphere in several wineries. Atmospheres contaminated by PCP and TeCP derivatives did not contain any TBP derivatives and, conversely, TeBP and PBP were never identified in the cases studied here (Table 3).

The physicochemical characteristics of these contaminants (Table 4) explain that, although the halophenols and haloanisoles considered have high boiling points, they may be relatively easily found in the atmosphere in buildings containing sources of emission of these molecules. All of the contaminants under consideration are similarly hydrophobic (log *P*). The low saturation vapor pressure of TeCA explains why it is easy to detect this molecule in places where there is TeCP in the atmosphere [wood treated with industrial PCP contains 10–15% TeCP as an impurity (5)]. TCP may sublime at room temperature, but it is highly soluble in water and its low Henry constant restricts its presence in the gas phase. TBA has a log *P* similar to that of TCA, but it is less soluble in water and has a lower saturation vapor pressure but a higher Henry constant. These characteristics explain the ease of detecting TBA in the atmosphere and its perception threshold equivalent to that of TCA in water but higher in matrices with a higher ethanol content.

In winery A (Table 5), wines kept either in unpolluted stainless steel vats or in barrels where pollutants had accumulated presented high levels of contamination. In this case, relatively nonvolatile TBP from a primary source of contamination was broken down microbiologically into TBA, a more volatile, unpleasant-smelling compound, easily transported through the air. Wine handled in this atmosphere may dissolve contaminants present in the gas phase or be contaminated directly via contact with easily contaminated porous materials (barrel wood) as initially demonstrated for chloroanisoles (5).

In winery B (Table 6), the original source of contamination (old wood vats) had been eliminated several years earlier and the barrels remaining in the winery were uncontaminated on arrival. It was, however, still possible to detect significant quantities of TBA in the atmosphere. Residual pollutants, adsorbed into the microporous winery structure covered with clay bricks, could be gradually released via the considerable surface in contact with the air.

In winery C (Table 7), wines aged in barrels were strongly contaminated with TBA and TBP. Pollutant concentrations in the wine and barrel wood increased regularly with the age of the barrels, that is, the length of time they had been in the contaminated atmosphere of the winery. However, TBA pollution in the wine must also come from the silicone bungs used to seal the barrels as they were in direct contact with the wine. As is the case with chloroanisoles, plastic materials in general and silicone elastomer resins in particular readily adsorb TBA and, to a lesser extent, TBP, which is just a little bit less hydrophobic but much less volatile (lower saturation vapor pressure and much higher Henry constant). Polyethylene- or polyester-based winemaking equipment, vulcanized rubber gaskets, and the silicone bungs used in barrels readily fix pollutants from the air and release them into wine over time. Analysis of the materials in contact with the atmosphere in this winery showed that the wooden roof timbers had been massively impregnated with TBP, which had gradually broken down into TBA due to the action of microflora in the atmosphere. Some

Table 4. Physicochemical Characteristics of Halophenols and Anisoles from PhysProp Database Syrris Inc. (45)

molecule [CAS Registry No.]	boiling point, °C (760 mmHg)	melting point, °C (760 mmHg)	vapor pressure, 10 ⁻³ mmHg	log <i>P</i> octanol/water	water solubility, mg L ⁻¹	Henry's law constant, 10 ⁻⁶ atm (mol·m ³) ⁻¹
TCA [87-40-1]	241	61	0.228	4.11	10.0	130.000
TCP [88-06-2]	246	69	2.500	3.69	800.0	2.600
TBA [607-99-8]	298	88	0.644	4.74	1.0	20.200
TBP [118-79-6]	286	95	0.303	4.18	70.0	0.035
TeCA [938-86-3]		84	3.190	4.65	1.4	96.100
TeCP [58-90-2]	232	70	0.666	4.09	23.0	8.840
PCA [1825-21-4]		108	0.592	5.29	0.4	1930.000
PCP [87-86-5]	309	174	0.110	5.12	14.0	0.025

Table 5. Concentration of Halophenols and Anisoles in the Atmosphere and Various Materials Sampled in Cellar A^a

material	TCA	TCP	TeCA	TeCP	PCA	PCP	TBA	TBP	TeBP	PBP
cellar atmosphere (ng g ⁻¹ adsorbant)										
tank cellar	nd	nd	nd	nd	nd	nd	46	13	nd	nd
barrel cellar	nd	nd	nd	nd	nd	nd	162	4	nd	nd
wines stored in the cellar (ng L ⁻¹)										
stored in stainless steel tanks	nd	nd	tr	8	tr	675	2	253	nd	nd
stored in 12-month-old barrels	nd	3	nd	7	tr	148	12	67	nd	nd
potential sources of contamination ^b (ng g ⁻¹)										
12-month-old oak wood barrels	nd	nd	nd	nd	nd	tr	143	902	nd	nd
24-month-old oak wood barrels	nd	tr	nd	nd	nd	6	224	1158	nd	nd
new wood frameworks of the tank cellar	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
old wood frameworks of the tank cellar	nd	nd	nd	nd	nd	nd	33	835	nd	nd

^a nd, <DL; tr, >DL but <QL. ^b External part of the material on 0–3 mm thickness.

Table 6. Concentration of Halophenols and Anisoles in the Atmosphere and Various Materials Sampled in Cellar B^a

material	TCA	TCP	TeCA	TeCP	PCA	PCP	TBA	TBP	TeBP	PBP
cellar atmospheres (ng g ⁻¹ adsorbant)										
underground cellar 1	nd	nd	nd	nd	nd	nd	15	tr	nd	nd
underground cellar 2	nd	nd	nd	nd	nd	tr	122	15	nd	nd
wines stored in the cellar (ng L ⁻¹)										
stored in new barrel cellars 1 + 2	nd	5	nd	8	nd	tr	13	78	nd	nd
residual sources of contamination ^b (ng g ⁻¹)										
ground (earth) cellars 1 + 2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
concrete wall cellars 1 + 2	nd	nd	nd	nd	nd	tr	tr	tr	nd	nd
brick wall cellar 1	nd	nd	nd	nd	nd	nd	2	tr	nd	nd
brick wall cellar 2	nd	nd	nd	nd	nd	nd	15	tr	nd	nd

^a nd, <DL; tr, >DL but <QL. ^b External part of the material on 0–3 mm thickness.

Table 7. Concentration of Halophenols and Anisoles in the Atmosphere and Various Materials Sampled in Cellar C^a

material	TCA	TCP	TeCA	TeCP	PCA	PCP	TBA	TBP	TeBP	PBP
cellar atmosphere (g g ⁻¹ adsorbant)	nd	tr	nd	tr	nd	nd	190	38	nd	nd
wines stored in the cellar (ng L ⁻¹)										
stored in new barrels	nd	nd	nd	nd	nd	nd	13	78	nd	nd
stored in 12-month-old barrels	nd	4	nd	nd	nd	nd	38	108	nd	nd
stored in 24-month-old barrels	nd	12	nd	nd	nd	nd	28	101	nd	nd
potential sources of contamination ^b (ng g ⁻¹)										
new oak wood barrels (9 months in the cellar)	nd	nd	nd	nd	nd	tr	22	240	nd	nd
12-month-old oak wood barrels	nd	nd	nd	nd	nd	tr	143	902	nd	nd
24-month-old oak wood barrels	nd	tr	nd	nd	nd	6	224	1158	nd	nd
36-month-old oak wood barrels	nd	tr	nd	nd	nd	tr	1973	2943	nd	nd
barrel racks	nd	nd	nd	nd	nd	tr	2185	13127	nd	nd
silicon bungs	nd	nd	nd	nd	nd	nd	1337	57	nd	nd
wood framework of the cellar										
superficial part (0–3 mm)	nd	nd	nd	nd	nd	nd	234	82363	nd	nd
inner part (3–15 mm)	nd	nd	nd	nd	nd	nd	201	963	nd	nd
varnish	nd	nd	nd	nd	nd	nd	tr	39	nd	nd
white paint	nd	nd	nd	nd	nd	nd	1002	4933	nd	nd

^a nd, <DL; tr, >DL but <QL. ^b External part of the material on 0–3 mm thickness.

Table 8. Detection of Halophenols and Anisoles in Used and Unused Stoppers^a

material	TCA	TCP	TeCA	TeCP	PCA	PCP	TBA	TBP	TeBP	PBP
atmosphere of storage (ng g ⁻¹ adsorbant)	nd	nd	tr	tr	tr	5	14	2	nd	nd
new natural cork stopper (ng per cork)	16	13	3	nd	15	24	9	33	nd	nd
new PET synthetic cork (ng per cork)	nd	nd	51	106	107	872	39	294	nd	nd
wine in bottle with contamination										
wine (ng L ⁻¹)	nd	12	nd	nd	2	17	13	68	nd	nd
used cork ^a (ng per cork)	27	8	nd	nd	5	48	275	117	nd	nd

^a nd, <DL; tr, >DL but <QL. ^b One-third from the external part of the stopper removed to avoid the risk of a contamination from the atmosphere.

of the paints assayed in the same winery also contain TBP as flame retardant or/and fungicide.

Analysis of wines in corked bottles contaminated with TBA and possibly TCA is more difficult to explain (**Table 8**). At this stage, we do not know whether the wine spoilage was due to pollution from corks stored in a contaminated atmosphere before use (on the cork manufacturer's or user's premises) or, in a scenario similar to that described for TCA, attributable to contamination by TBA formed in situ in the cork. Quite obviously, the discovery of large quantities of pollutants in extruded polyethylene (PET) corks stored in a contaminated atmosphere showed that, as was the case of the silicone bungs mentioned above, airborne pollution of stoppers over large distances via the atmosphere and through packaging materials was entirely possible. This result should be compared with observations made by Whitfield et al. (30) for TBA and TCA with PET packaging films and by Hoffman and Sponholz (33) with PET screw caps stored on wooden pallets treated with TBP. However, the extent to which pollutants adsorbed on PET may later migrate into wine has not yet been determined.

TBP Sources in Winery Atmospheres. The cases of wines contaminated with TBA and TBP described in this work were obviously due to wooden or wood-based materials impregnated or surface-treated with TBP, similar to the situations described for PCP in direct (40) or indirect contact with wine (4, 5). Neither PBP nor TeBP, both potential precursors of TBP, was found in the wines and materials studied, or they were present in only nonquantifiable trace amounts. TBP and other bromophenolic derivatives are widely used on wood as antifungal agents (41, 46) and flame retardants, as well as on many plastics (33, 42, 43).

Conclusions. TBA, produced by the microbiological breakdown of TBP, may cause serious organoleptic defects. Like TCA and TeCA, low concentrations of TBA may give wines pungent musty odors. In view of the perception thresholds and physicochemical characteristics of this molecule, it may be considered similar to TCA in its capacity to spoil wine. The identification of trace amounts of TBA in certain wines may thus explain cases of reported spoilage when TCA or TeCA was not present in significant quantities.

TBA and TBP seem mainly to derive from environmental pollution in wineries. This paper is the first to identify the sources of pollution in a large number of cases when wines became polluted during storage in premises where the atmosphere was contaminated with TBA coming from TBP used recently to treat wood or originating from much older structural elements of the winery or from used wooden containers. In certain cases, although the initial source had been eliminated, residual pollution adsorbed on walls could be sufficient to make a building unsuitable for storing wines or sensitive materials intended for direct contact with wine. Both plastics and corks are highly susceptible to TBA contamination. Atmospheric quality in storage areas is thus vital to ensure the chemical and organoleptic inertia of materials that come into contact with wine.

Unlike PCP, the use of which is restricted and even prohibited in many parts of the world, especially Europe (44), there are apparently no restrictions on the use of TBP. Furthermore, the fact that TBP derivatives are widely used as flame retardants in a wide range of materials raises the risk of widespread environmental pollution. The increasing use of recycled wood- and polymer plastic-based materials, which may previously have been impregnated with TBP or its derivatives, may represent a source of latent pollution likely to contaminate not only sensitive food products but also the water table as a result of irresponsible storage. The extensive use of TBP and its derivatives thus deserves further investigation.

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