

Impact of Toasting Oak Barrels on the Presence of Polycyclic Aromatic Hydrocarbons in Wine

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Toasting *Quercus* sp. oak wood is one of the key stages in manufacturing barrels intended for aging wines and spirits. During this operation, the increase in temperature causes variable modifications in the physical structure and, more importantly, the chemical composition of the wood. Polycyclic aromatic hydrocarbons (PAH) are high-risk molecules likely to be formed during toasting of the wood and later extracted by wine or spirits in direct contact with the barrel. In the context of an analysis of all potential sources of risk associated with the manufacture of barrels for winery use [prevention policy defined using a Hazard Analysis Critical Control Point (HACCP) approach], we carried out a preliminary study to provide a more accurate assessment of potential risks related to the presence of PAH in cooperage and winemaking. Wood toasted to different levels under different conditions, as well as wines aged in barrels made using different methods, was analyzed by gas chromatography and mass spectrometry (GC–MS) for the identification of the main PAH present, quantification of each of the molecules extracted, and estimation of any possible toxicological risks, via a comparison of values with those measured in other types of food. The results clearly showed that the heating processes associated with barrel production actually resulted in the formation of various molecules in the PAH family. However, only a minority of the target PAH presented high toxicity, particularly carcinogenic potential. Because of the specific toasting process used, benzo[a]pyrene, the best-known, and one of the most dangerous, contaminants, was not significantly present in toasted barrel wood. In view of the PAH concentrations in wood and the low solubility of these compounds, their extraction in wine is apparently relatively slow and limited. Finally, comparing the overall PAH concentrations, and particularly those of the most toxic compounds, with estimated absorption from food or the environment, we found it was obvious that the contribution of toasted barrels to the total amount was extremely low and should not, therefore, be considered a major health concern.

KEYWORDS: PAH; assay; wood; wine; cooperage; toxicity

INTRODUCTION

Toasting *Quercus* sp., oak, by far the most widely used wood in modern cooperage, is one of the key stages in manufacturing barrels intended for aging wines and spirits. During this operation, the increase in temperature causes variable modifications in the physical structure and, more importantly, the chemical composition of the wood. Barrels are generally toasted by burning wood or, more rarely, using gas burners, hot-air convection, or electric infrared heaters (1). Toasting is indispensable, not only for softening the ligneous fibers so that the staves can be bent but also, above all, for forming new volatile, odoriferous substances, mainly via the thermal degradation of parietal polymers in the oak (2). The aromatic profile of the wood is profoundly modified, depending on the specific combination of toasting time and heating method used by the cooper (time, toasting intensity, variable anoxia, humidification, etc.). As most of these molecules can be easily extracted during

the shorter barrel aging of wine and prolonged aging of brandies, the flavors and, above all, aromas are modified, with variable effects depending on the type of wood used and barrel toasting intensity.

Besides consequences based solely on the organoleptic preferences of barrel users, the wood toasting process is also capable of producing chemicals that cannot be detected by sensory means but could have an impact on health. Under certain conditions, heating organic materials may produce specific compounds [carbolines, acrylamide, dioxins, polycyclic aromatic hydrocarbons (PAH), etc.] with toxicological properties. It is, therefore, essential to analyze the risks they present, as well as the parameters that affect their accumulation and extraction under normal conditions of use. Oak barrels are situated at the interface between “intermediate technologies”, involved in the production process, and “materials in contact with foodstuffs”, required to be “inert” in relation to their contents. Oak barrels cannot meet the criteria for perfect inertness, as some of the substances extracted have a direct (flavor and odor compounds) or indirect (ellagitannins involved in oxidation processes) impact

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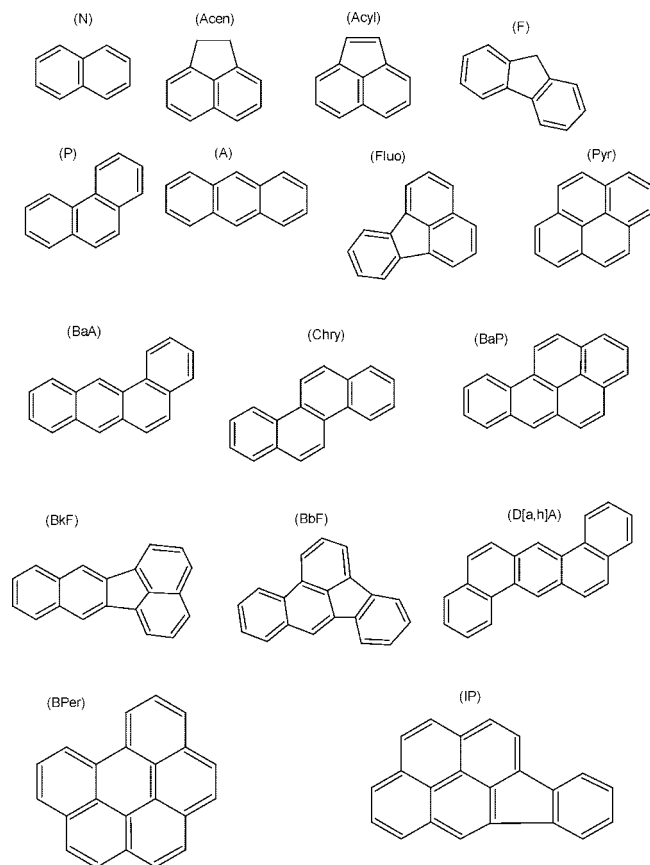


Figure 1. Molecular structure of the main PAH in oak wood: naphthalene (N), acenaphthene (Acen), acenaphthylene (Acyl), fluorene (F), phenanthrene (P), anthracene (A), fluoranthrene (Fluo), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (Chry), benzo(a)pyrene (BaP), benzo(k)fluoranthrene (BkF), benzo(b)fluoranthrene (BbF), dibenzo(a,h)anthracene [D(a,h)A], benzo(g,h,i)perylene (Bper), and indeno(1.2.3-*cd*)pyrene (IP).

on the production and aging of their contents. In any case, from a legal standpoint, the raw or processed wood must not release any substances in quantities that would be prejudicial to the healthful quality of the product.

Among the molecules currently identified as potentially produced during the combustion of wood in air or toasting by indirect heating, PAH are the most likely to be formed and subsequently extracted by wine or spirits in direct contact with the oak. PAH molecules have at least two fused aromatic rings (**Figure 1**). PAH may be divided into two groups: low-molecular weight PAH that are relatively soluble in water, with little tendency to fix, (3) and high-molecular weight PAH that are relatively insoluble but fix easily on particles in suspension. PAH formed by incomplete combustion of organic materials contain large quantities of alkyl derivatives. PAH formation mechanisms during combustion and toasting processes are highly complex and variable. Flame reactions under anoxic conditions are triggered by free radicals (3, 4). This system suggests that PAH are formed from C_2 species during the pyrolysis of hydrocarbons. Once the PAH have formed, they may undergo pyrolytic reactions to form larger molecules by condensation and cyclization (5). A very wide range of PAH may be formed, and their abundance and quantity depend on the material, particularly its aliphatic hydrocarbon content, and, above all, combustion temperature and conditions. At low temperatures (<200 °C), the distribution of the compounds is controlled by their thermodynamic stability, whereas compounds with a very high molecular weight may be generated at high temperatures (>800

°C) (3, 6). Indeed, at very high temperatures (2000 °C), certain activation energies may be reached and heavy, relatively unsubstituted PAH may be formed, and when the temperature decreases, a higher proportion of alkyl PAH accumulates. Several different PAH derivatives (amino, nitro, and hydroxy), identified during the combustion of gas and petroleum derivatives, are widespread in urban environments (7).

It is almost impossible to analyze all the PAH that have been identified (more than 500). Following a critical evaluation of the toxic effects of each parent and derivative PAH, an analysis of priority substances is generally restricted to the list established by the United States Environmental Protection Agency (EPA). This list includes 16 of the most toxic PAH, but the generic term "PAH" is frequently applied to 12–21 compounds (8).

There is considerable concern about PAH, as the toxicity of a relatively large number of molecules in this family, clearly demonstrated since the 1930s, has recently been confirmed (9–12). Their capacity to induce cancerous tumors has been demonstrated, as well as their teratogenic and mutagenic effects on animals and humans. Benzo(a)pyrene (BaP) is considered a priority contaminant by the World Health Organization (WHO), due to its high toxicity (8). As the main origin of this type of compound is anthropic, humans are exposed to their pollutant effects every day. Every aspect of the environment is involved, especially food (13, 14).

Consequently, in the context of an examination of all potential sources of risk involved in manufacturing barrels for use in winemaking [prevention policy required as part of an HACCP (Hazard Analysis Critical Control Point program)], we carried out a preliminary study to obtain a more accurate assessment of the possible risks related to PAH in cooperage and wine-making. Wood toasted at different intensities and wines aged in barrels toasted using various methods were analyzed to identify the main PAH present, quantity each of the molecules extracted, and estimate the possible toxicological risks in comparison with those measured in other types of foods.

MATERIALS AND METHODS

Extracting PAH from Oak Wood. We used a method based on the one developed by Chen and Chen (15). A 5 g wood sample was ground in an Ultraturax grinder for 2 min, and then 100 μ L of fluorene- d_{10} (internal standard) at 250 μ g/L in methanol was added and extracted in a Soxhlet extractor for 4 h using 100 mL of dichloromethane heated with reflux. The extract was preconcentrated rapidly to approximately 1 mL in a rotating vacuum evaporator at 35 ± 2 °C. The concentrate was transferred to a 10 mL Turbovap concentrating tube, being rinsed twice with 1 mL of acetone. The sample was then evaporated dry under a 100 mL/min nitrogen stream at 25 °C. The concentrate was dissolved in 1 mL of acetone and the mixture transferred directly into a 2 mL HP6890 sample tray-injector flask (Agilent).

Extracting PAH from Wine. PAH were assayed in wine according to the method of Garcia-Falcon et al. (16). A 200 mL wine sample supplemented with 100 μ L of fluorene- d_{10} at 250 μ g/L was placed in an SPE C18 SEP-PACK (Varian) cartridge containing 360 mg of adsorbent phase conditioned with 5 mL of acetonitrile and then 10 mL of ultrapure water (MilliQ). The column was rinsed with 10 mL of an acetonitrile/water mixture (70/30) and dried, and then the PAH were eluted using 5 mL of hexane. The extract obtained was concentrated to approximately 0.5 mL in the Turbovap and then rinsed twice with 250 μ L of hexane and transferred to a 2 mL sample tray flask.

PAH Assay by Gas Phase Chromatography Coupled with Mass Spectrometry. Injection, separation, and detection parameters were as described by Chen and Chen (15). A 1 μ L sample of extract was injected into the splitless injector of an HP6890 chromatograph (Agilent) heated to 290 °C and equipped with a nonpolar HP5-MS column (Agilent) with very low bleeding (30 m \times 0.25 mm, 0.25 μ m) programmed from 70 to 250 °C at a rate of 10 °C/min and then to 290 at a rate of 5

Table 1. Analysis of PAH that Can Be Extracted from Different Types of Oak Wood with and without Toasting in a 12% Volume Dilute Alcohol Solution (maceration for 15 days at 25 °C using a 20 g/L sample of sawdust taken from a layer approximately 0–5 mm deep)

extractable PAH ^a	stave wood entering dryer (ng/g)	dried stave wood in dryer (ng/g)	nontoasted stave (ng/g)	heavily toasted stave (ng/g)	medium-toasted head (ng/g)
naphthalene	145.1	53.3	38.7	73.3	36.5
acenaphthylene	8.2	3.7	10.2	5.3	3.0
acenaphthene	13.5	3.7	2.6	2.4	1.1
fluorene	28.5	7.8	14.8	5.3	4.7
phenanthrene (Ph)	171.9	96.7	148.1	39.8	42.5
anthracene (An)	10.2	7.9	25.3	26.5	29.6
fluoranthene (F)	28.2	24.7	38.3	2.9	4.7
pyrene (Pyr)	25.4	23.6	40.1	3.0	2.4
benzo(<i>c</i>)phenanthrene	1.0	2.6	1.0	4.9	nd ^b
benzoanthracene (E)	1.5	1.5	0.6	nd ^b	0.9
chrysene (L)	7.5	3.9	nd ^b	nd ^b	nd ^b
benzo(<i>b</i>)fluoranthene (E)	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b
benzo(<i>j+k</i>)fluoranthene (E)	41.8	nd ^b	nd ^b	nd ^b	nd ^b
benzo[<i>a</i>]pyrene (E)	0.9	1.4	0.2	nd ^b	nd ^b
dibenzo(<i>a,h</i>)anthracene (L)	1.8	nd ^b	nd ^b	nd ^b	nd ^b
benzo(<i>g,h,i</i>)perylene	nd ^b	0.7	nd ^b	nd ^b	0.2
indeno(1,2,3- <i>cd</i>)pyrene (E)	nd ^b	1.0	nd ^b	nd ^b	nd ^b
dibenzo(<i>a,l</i>)pyrene (E)	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b
dibenzo(<i>a,e</i>)pyrene (E)	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b
dibenzo(<i>a,l</i>)pyrene (E)	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b
dibenzo(<i>a,h</i>)pyrene (E)	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b
total PAH [E + L]	53.5	8.5	0.8	nd ^b	1.1
total PAH (ng/g)	485.5	232.5	320.0	163.5	125.5
Ph/An ratio	16.9	12.2	5.8	1.5	1.4
F/Pyr ratio	1.1	1.0	1.0	1.0	1.9

^a E, definite carcinogenic potential; L, limited carcinogenic potential; no indication, no confirmed potential according to the classification of Bjorseth and Beaker (3). ^b Not assayable.

°C/min, with a final isotherm of 10 min. The balance gas (Helium U) flow was kept constant at 1.25 mL/min. PAH detection was by electron impact mass spectrometry in fragmentometry mode, using the specific groups of ions indicated by Chen and Chen (15) with an HP5973 mass spectrometer (Agilent).

Under these conditions, according to Chen and Chen (15), the assay limit was 0.5 ng/g for the main PAH, 1 ng/g for benzo[*a*]pyrene, and 4 ng/g for indenopyrene isomers. In wine, according to Garcia-Falcon et al. (16), the assay limit was 1 ng/L for the majority of PAH, 2 ng/L for benzo[*a*]pyrene, 6 ng/L for benzo[*a*]pyrene, and 60 ng/L for indenopyrene isomers.

To specifically decrease the level of quantification of benzo[*a*]pyrene in some particular samples of oak wood, after the concentrate had been dissolved in 1 mL of acetone, the final concentration is directly determined under a gentle nitrogen flux (80 mL/min) in a conic vial adapted to the HP6890 sample tray up 100 μ L; in that case, the quantification level of this particular compound is decreased to 0.1 ng/g.

Calibration curves for each analyte are realized with internal standard in the range of 5–200 ng/L for the different molecules considered and produced a linear response with a correlation coefficient of >0.9 in all cases.

RESULTS AND DISCUSSION

Impact of Toasting on the Presence of PAH in Barrel Wood. Analysis of stave wood stored in a woodlot in the open air for 9–24 months and then in a final conditioning unit to homogenize humidity levels produced results that initially seemed relatively surprising (Table 1). Indeed, general PAH levels were expected to be low, with almost no carcinogenic PAH, but the results revealed significant quantities of semivolatile PAH, such as naphthalene, phenanthrene, and chrysene, as well as moderate quantities of benzo[*a*]pyrene, dibenzo(*a,h*)anthracene, and benzo(*j+k*)fluoranthene. When staves were cut from the same stave wood after a period in the drying unit,

where the wood was maintained at a higher temperature in a ventilated atmosphere, the concentrations of these same molecules decreased. These results indicated that contamination of the wood was superficial. Furthermore, examination of the phenanthrene/anthracene (>10–16) and fluoranthene/pyrene (<1–2) ratios confirmed the petrogenic origin of the contamination (Tables 1 and 2). As the woodlot is not *a priori* located in an environment polluted by this type of compound (near a road with heavy traffic), this accumulation of PAH on the surface of microporous stave wood was certainly due to the exhaust gases of the diesel-engined forklift trucks used to move the wood into the conditioning units, where the doors were kept closed during loading.

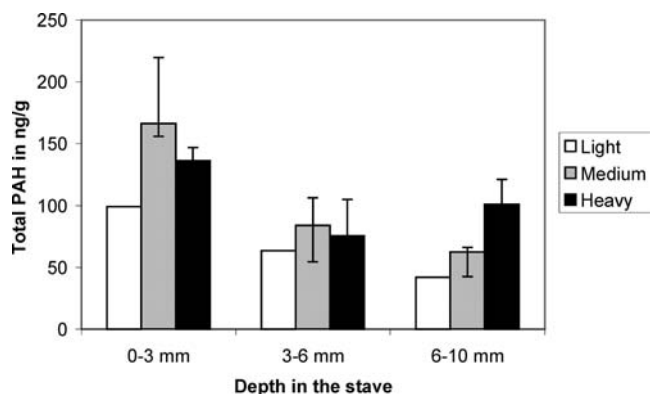
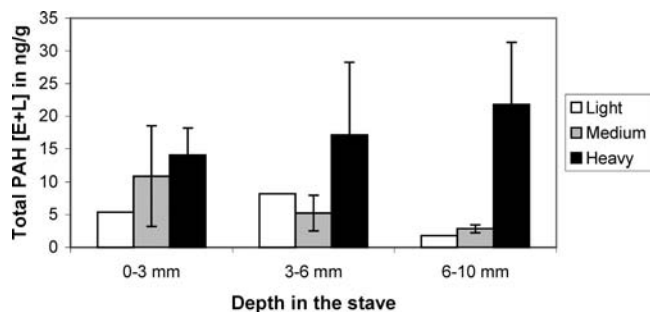
Toasting modified the wood's composition in terms of PAH likely to migrate into wine (Table 1), but the toasting necessary to bend the staves and develop the toasted wood aroma winemakers appreciate in barrels used to age wines certainly did not cause the considerable increase in quantity that might have been expected. Toasting operations in the cooperage certainly caused an increase in the total PAH content (can be extracted by hot dichloromethane) of the oak wood (Table 2). Depending on toasting intensity, i.e., toasting time and the temperature reached by the wood, the PAH content of the staves tended to increase, but the modifications were mainly qualitative. The most volatile molecules tended to disappear, and heavier compounds formed as toasting became more intense.

Indeed, PAH formation was highly dependent on temperature, with greater accumulations in the surface layers directly exposed to the heat of the fire (Figure 2). If one starts from the medium-toast stage, large quantities of PAH derivatives were formed to a depth of 6–10 mm. The synthesis of PAH considered to be carcinogenic [according to the Bjorseth and Beaker classification (3), where L means there is evidence of limited carcinogenic potential and E means there is evident carcinogenic potential]

Table 2. Changes in the Total PAH Content (nanograms per gram) of Oak Wood (solvent extraction) after Traditional Toasting in the Cooperage

PAH ^a	0–3 mm					3–6 mm					6–10 mm				
	light	medium	σ	heavy	σ	light	medium	σ	heavy	σ	light	medium	σ	heavy	σ
naphthalene	42.6	103.1	40.5	72.3	8.4	1.3	50.7	19.1	23.5	12.3	2.9	25.6	4.5	40.9	8.0
acenaphthylene	0.9	4.3	2.3	1.6	0.2	–	0.9	0.2	0.7	0.0	1.2	1.0	0.2	0.9	0.0
acenaphthene	3.6	7.8	3.7	5.9	0.7	3.0	3.5	1.1	3.1	0.4	4.1	2.3	0.8	3.8	0.5
fluorene	3	5.3	2.2	4.3	0.3	2.8	2.3	0.3	2.2	0.4	2.7	1.6	0.2	2.6	0.6
phenanthrene (Ph)	24.7	24.8	6.3	25.1	2.3	34.7	16.6	5.2	20.6	5.4	22.5	16.3	2.4	22.4	7.7
anthracene (An)	1.4	2.9	0.5	2.4	0.4	1.6	1.9	0.6	2.7	1.0	0.1	2.0	0.6	2.8	0.8
fluoranthene (F)	10.5	4.3	1.3	5.8	1.2	6.4	1.3	0.4	3.0	1.5	4.0	1.6	0.4	3.1	1.3
pyrene (Pyr)	7	3.6	1.4	4.8	1.4	5.2	1.4	0.3	2.4	0.9	2.7	1.5	0.3	2.4	1.1
benzo(c)phenanthrene	0.2	0.3	0.0	0.2	0.1	0.3	0.0	0.0	0.2	0.1	nd ^b	0.2	0.0	0.5	0.3
benzoanthracene (E)	0.6	0.4	0.3	0.8	0.1	0.5	0.1	0.0	2.6	3.4	0.2	0.2	0.1	0.3	0.1
chrysene (L)	1.1	0.7	0.2	0.5	0.1	1.2	0.3	0.1	0.8	0.6	0.5	0.2	0.0	0.8	0.4
benzo(b)fluoranthene (E)	0.5	21.4	0.0	nd ^b	–	0.1	nd ^b	–	5.1	0.0	nd ^b	0.2	0.0	nd ^b	–
benzo(j+k)fluoranthene (E)	3	3.9	0.1	13.0	4.5	6.4	4.6	2.5	10.7	3.2	1.1	2.0	0.6	20.9	9.7
benzo[a]pyrene (E)	0.2	nd ^b	–	nd ^b	–	nd ^b	nd ^b	–	3.4	0.0	nd ^b	nd ^b	–	nd ^b	–
dibenzo(a,h)anthracene (L)	nd ^b	nd ^b	–	nd ^b	–	nd ^b	nd ^b	–	nd ^b	–	nd ^b	nd ^b	–	nd ^b	–
benzo(g,h,i)perylene	nd ^b	nd ^b	–	nd ^b	–	nd ^b	nd ^b	–	nd ^b	–	nd ^b	nd ^b	–	nd ^b	–
indeno(1,2,3-cd)pyrene (E)	nd ^b	0.9	0.0	nd ^b	–	nd ^b	0.6	0.1	0.2	0.1	nd ^b	0.5	0.2	nd ^b	–
dibenzo(a,i)pyrene (E)	nd ^b	nd ^b	–	nd ^b	–	nd ^b	nd ^b	–	nd ^b	–	nd ^b	nd ^b	–	nd ^b	–
dibenzo(a,e)pyrene (E)	nd ^b	nd ^b	–	nd ^b	–	nd ^b	nd ^b	–	nd ^b	–	nd ^b	nd ^b	–	nd ^b	–
dibenzo(a,l)pyrene (E)	nd ^b	nd ^b	–	nd ^b	–	nd ^b	nd ^b	–	nd ^b	–	nd ^b	nd ^b	–	nd ^b	–
dibenzo(a,h)pyrene (E)	nd ^b	nd ^b	–	nd ^b	–	nd ^b	nd ^b	–	nd ^b	–	nd ^b	nd ^b	–	nd ^b	–
total PAH [E + L]	5.4	10.9	7.7	14.0	4.1	8.2	5.2	2.7	17.1	11.1	1.8	2.8	0.6	21.8	9.6
total PAH (ng/g)	99.3	166.3	53.3	136.4	10.5	63.5	83.9	22.6	75.6	29.4	42.0	62.5	3.5	101.0	20.1
Ph/An ratio	17.6	8.5	0.7	10.6	1.3	21.7	8.9	1.3	8.0	0.9	225.0	8.7	1.9	7.9	0.5
F/Pyr ratio	1.5	1.2	0.1	1.3	0.2	1.2	0.9	0.1	1.2	0.1	1.5	1.1	0.0	1.3	0.1

^a E, definite carcinogenic potential; L, limited carcinogenic potential; no indication, no confirmed potential. ^b Value lower than the assay limit.

**Figure 2.** Changes in formation of total PAH with an increase in the toasting intensity on the inside of the barrel staves.**Figure 3.** Changes in the formation of total PAH with carcinogenic potential with an increase in the toasting intensity on the inside of the barrel staves.

and, therefore, particularly undesirable followed the same rules (**Figure 3**). They accumulated mainly at the “heavy-toast” stage, especially in the deeper layers, where volatilization phenomena and combining reactions that could have decreased concentrations had a weaker effect.

Table 3. Benzo[a]pyrene Content of Oak Chips Toasted in Hot Air in the Same System (convection oven, maximum air temperature of 230 °C, sides locally at 300 °C)

toasting intensity	oak origin	benzo[a]pyrene (ng/g)
light	France	0.5
	United States	0.5
medium	France	0.1
	France	<0.1
	United States	0.6
medium+	United States	0.3
	France	0.4
	France	0.5
	United States	0.9
	United States	0.6

Benzo[a]pyrene is one of the most toxic, most studied molecules. This compound is particularly well-known in products preserved by smoking and is subject to legislative control. EEC directive n°88/388, dated June 22, 1988, on flavorings for use in foodstuffs (18) established a maximum concentration of 0.03 mg/kg (30 ng/g), and AFFSA set a guideline value for PAH (19) of 2 μ g/kg fresh weight (i.e., 2000 ng/L of wine or 2 ng/g of wood).

The analyses carried out did not detect large quantities of benzo[a]pyrene in toasted barrel wood (detection threshold of 1 ng/g in wood using a 5 g sample). The maximum concentration assayed was 3 ng/g in heavily toasted wood, 3–6 mm below the surface (**Table 2**), while in other cases, concentrations were in the vicinity of the detection threshold.

Analysis of the same contaminant in oak chips toasted in a thermal convection oven also revealed very low concentrations of benzo[a]pyrene (**Table 3**). In view of the concentrations used (between 1 and 5 g/L), the assayed concentrations were always below the EEC threshold. Furthermore, this molecule is not very soluble in water, and therefore, very little of it migrates into wine.

Table 4. Impact of the Barrel Toasting Technique on the Formation of PAH Derivatives (nanograms per gram)

PAH ^a	traditional toasting over a wood fire	hot-air convection toasting
naphthalene	63.8	61.4
acenaphthylene	1.7	3.1
acenaphthene	1.0	1.0
fluorene	4.9	6.8
phenanthrene	37.7	73.4
anthracene	5.9	6.3
fluoranthene	12.3	35.9
pyrene	12.6	35.9
benzo(c)phenanthrene	0.4	1.0
benzoanthracene (E)	2.0	2.7
chrysene (L)	1.4	0.3
benzo(b)fluoranthene (E)	<0.5	<0.5
benzo(j+k)fluoranthene (E)	1.0	nd ^b
benzo(a)pyrene (E)	<1	<1
dibenzo(a,h)anthracene (L)	1.1	<1
benzo(g,h,i)perylene	0.1	<0.1
indeno(1,2,3-cd)pyrene (E)	<5	<5
dibenzo(a,l)pyrene (E)	<5	<5
dibenzo(a,e)pyrene (E)	<5	<5
dibenzo(a,i)pyrene (E)	<5	<5
dibenzo(a,h)pyrene (E)	<5	<5
total PAH [E + L]	5.5	3.0
total PAH (ng/g)	145.9	227.8

^a Toxic potential of the molecules/carcinogenic activity: E, definite risk; L, limited risk; no indication, insufficient or no evidence of risk. ^b Could not be assayed below 0.1 ng/g.

The moderate PAH content, particularly of potential carcinogens, may be due to the specific conditions during toasting in the cooperage. Our previous research (20) on this subject, confirmed by Sarni et al. (21), clearly showed that the barrel wood rarely reached temperatures over 230–250 °C during the various toasting operations.

Above that level, the risk of combustion is so high that coopers take action to reduce the temperature sharply, by humidifying the wood and opening the shell if it is closed. According to the bibliography on this subject (3), any significant formation of benzopyrene requires temperatures of at least 400 °C, never reached during traditional toasting over a wood fire or convection system (<235 °C), while direct radiant heating with infrared sources, used to toast barrel heads, may produce temperatures in the vicinity of 300 °C. However, under these conditions, the wood is not really heated through; there is only surface charring. The wood is not maintained at high temperatures long enough for deep heat penetration, resulting in limited PAH production.

Consequently, the only possible source of synthesis of significant amounts of PAH would be condensation of smoke from the brazier (core temperature between 800 and 1200 °C, radiation temperature of approximately 600 °C). This should not normally generate large amounts of smoke under the conditions of oxygen supply during barrel toasting. Thus, indirect toasting with air heated to a maximum of 350 °C (convection heating) certainly resulted in a decrease in the concentration of potentially carcinogenic PAH (Table 4), but the total quantity of PAH remained close to the values obtained following traditional toasting over an open fire, which produced similar final temperatures on the stave surface (210–220 °C). Irrespective of the method, the amount of potentially toxic PAH generated by traditional toasting was very small in all cases.

Impact of Barrel Aging on the Wines' PAH Content. We analyzed concentrations in the same type of wine aged for 12 months in 225 L Bordeaux barrels, representing a contact surface

of just more than 2 m², made by two methods (two different cooperages, SA and MS) with two toasting intensities [medium (M) and medium plus (M+)] using two types of oak wood (French sessile oak and American white oak). Similarly, we compared the PAH content of the same wines aged in similar barrels (French oak) previously used to age one or two wines for one year each, made by one of the two cooperages compared (cooperage 1). The results are listed in Tables 5 and 6.

Aging wine in new barrels resulted in a PAH content higher by a factor of 2–8 in comparison with PAH content after aging in more inert containers (stainless steel or concrete tanks). It was observed that, for identical types of wood and similar toasting levels, the toasting technique used by the cooperage had a significant impact on both the quantity and type of PAH that migrated into the wine. The cooperage 1 used more intense toasting than 2, resulting in more naphthalene, phenanthrene, and chrysene, and nearly 70% more PAH was released into the wine. Total quantities of PAH were still small (<200 ng/L), and the carcinogenic PAH content was very low (<10 ng/L) with <6 ng/L benzo[a]pyrene (detection threshold) and no detectable dibenzopyrene derivatives (<60 ng/L).

The PAH content of the wine aged in American oak barrels from the cooperage 1 was higher, due to the longer toasting time for this type of wood, aimed at reducing the amount of methyl octalactones released into the wine, although toasting temperatures were relatively similar. Toasting the head did not apparently increase the amount of PAH released to any significant extent. Increasing the toasting intensity led to a clear increase in the levels of naphthalene, acenaphthylene, fluorene, and fluoranthene. Levels of benzoanthracene (E) and chrysene (L) also increased, while levels of benzo[a]pyrene and the dibenzopyrenes were still undetectable.

Release of PAH was still highly significant in barrels previously used to age one wine. The concentrations decreased markedly (more than 50%) only after the second year of use (third wines aged). This constant PAH release from the wood into the wine may be related to the low solubility of these compounds and the fact that they are located in both the surface and deep layers of the staves. On the basis of assays in the 0–3 mm layer of the barrels and in wine that had been aged for 1 year, the migration rate of total PAH was estimated to vary from 3 to 7%.

Critical Character of Release of PAH from Barrels into Wines. To evaluate the hazard represented by release of PAH from toasted barrel wood into wines, these figures were compared with those generally observed in the environment. Humans are likely to encounter PAH not only in foodstuffs but also in the atmosphere. Exposure to these pollutants has been monitored in various countries for many years, and the European Commission recently produced a summary of the main research published on this subject (13).

Independently of any differences related to the evaluation and research methods, the amount of PAH present in foodstuffs is relatively comparable throughout Europe. There are, however, sources of regional or seasonal variations that may cause significant fluctuations in these average figures. For example, consumption of smoked pork products in Germany and certain parts of Italy, or smoked fish in the Scandinavian countries, or a tendency to consume larger quantities of barbecued meats in southern Europe in summer, may have a considerable impact on the statistics.

Monitoring in six European countries made it possible to estimate the mean daily intake of benzo[a]pyrene from foodstuffs to be 0.05–0.42 µg/day per adult. In the United States,

Table 5. Impact of Aging in New Oak Barrels (European and American) of Various Origins on the PAH Content (nanograms per liter) of Wines (mean of five barrels per category)

PAH ^a	control wine (vat)	cooperage 1/French oak				cooperage 2/French oak				cooperage 1/American oak			
		M ^b	σ^c	M+ ^b	σ^c	M+ ^b	σ^c	M+/TH ^b	σ^c	M ^b	σ^c	M+/TH ^b	σ^c
naphthalene	10.0	81.0	64.0	65.0	14.0	52.0	10.0	11.2	35.0	192.0	56.0	175.0	83.0
acenaphthylene	<1	10.0	5.0	7.0	2.0	6.0	4.0	6.0	2.0	15.0	4.0	16.0	11.0
acenaphthene	3.0	4.0	3.0	2.0	1.0	2.0	1.0	4.0	2.0	6.0	2.0	8.0	6.0
pyrene	<2	2.0	1.0	1.0	0.0	1.0	0.0	1.0	0.0	2.0	0.0	2.0	1.0
phenanthrene	18.0	51.0	27.0	40.0	14.0	21.0	7.0	39.0	16.0	53.0	13.0	57.0	29.0
fluorene	8.0	14.0	8.0	11.0	3.0	7.0	4.0	11.0	4.0	26.0	6.0	28.0	20.0
anthracene	1.0	1.0	0.0	0.0	0.0	1.0	0.0	10.0	0.0	1.0	0.0	2.0	1.0
fluoranthene	<1	12.0	6.0	9.0	3.0	7.0	4.0	8.0	4.0	17.0	5.0	15.0	9.0
benzoanthracene (E)	<1	2.0	1.0	2.0	1.0	1.0	0.0	1.0	0.0	5.0	1.0	3.0	3.0
chrysene (L)	2.0	3.0	1.0	2.0	1.0	1.0	0.0	2.0	0.0	9.0	2.0	6.0	4.0
benzofluoranthene ^d (E)	<2	2.0	1.0	<2	0.0	<2	–	<2	–	<2	0.0	<2	–
benzo[a]pyrene (E)	<6	<6	–	<6	–	<6	–	<6	–	<6	–	<6	–
dibenzo(a,h)anthracene (L)	<60	<60	–	<60	–	<60	–	<60	–	<60	–	<60	–
benzo(g,h,i)perylene	<67	<67	–	<67	–	<67	–	<67	–	<67	–	<67	–
indeno(1,2,3-cd)pyrene (E)	<30	<30	–	<30	–	<30	–	<30	–	<30	–	<30	–
dibenzo(a,i)pyrene (E)	<60	<60	–	<60	–	<60	–	<60	–	<60	–	<60	–
dibenzo(a,e)pyrene (E)	<60	<60	–	<60	–	<60	–	<60	–	<60	–	<60	–
dibenzo(a,l)pyrene (E)	<60	<60	–	<60	–	<60	–	<60	–	<60	–	<60	–
dibenzo(a,h)pyrene (E)	<60	<60	–	<60	–	<60	–	<60	–	<60	–	<60	–
Σ PAH	42.0	182.0	116.0	139.0	39.0	99.0	30.0	93.2	63.0	326.0	89.0	312.0	167.0
Σ PAH [E+L]	2.0	5.0	2.0	4.0	2.0	2.0	0.0	3.0	0.0	14.0	3.0	9.0	7.0

^a E, definite carcinogenic potential; L, limited carcinogenic potential; no indication, no confirmed potential. ^b M, medium toast; M+, medium plus toast; TH, toasted heads. ^c Standard deviation. ^d Benzo(b)fluoranthene and benzo(k)fluoranthene.

Table 6. Impact of Aging in Previously Used Barrels (cooperage 1/French oak^a) on the Wines' PAH Content (nanograms per liter)

PAH ^b	Control wine(vat)	two wines, medium toast	one wine, medium toast	σ	one wine, medium plus toast	σ
naphthalene	10.0	28.0	85.0	60.0	87.0	10.0
acenaphthylene	<1 ^c	2.0	12.0	2.5	5.0	1.0
acenaphthene	3.0	3.0	17.0	10.0	10.0	6.0
pyrene	<2 ^c	2.0	6.0	1.0	3.0	0.0
phenanthrene	18.0	29.0	41.0	25.0	35.0	2.0
fluorene	8.0	10.0	13.0	3.5	16.0	2.0
anthracene	1.0	1.0	2.0	0.0	1.0	0.0
fluoranthene	<1 ^c	9.0	13.0	7.0	13.0	0.0
benzoanthracene (E)	<1 ^c	1.0	6.0	4.0	2.0	1.0
chrysene (L)	2.0	3.0	1.3	6.0	5.0	0.0
benzofluoranthene (E)	<2 ^c	<2 ^c	<2 ^c	0.0	<2 ^c	0.0
benzo[a]pyrene (E)	<6 ^c	<6 ^c	<6 ^c	–	<6 ^c	–
dibenzo(a,h)anthracene (L)	<60 ^c	<60 ^c	<60 ^c	–	<60 ^c	–
benzo(g,h,i)perylene	<67 ^c	<67 ^c	<67 ^c	–	<67 ^c	–
indeno(1,2,3-cd)pyrene (E)	<30 ^c	<30 ^c	<30 ^c	–	<30 ^c	–
dibenzo(a,i)pyrene (E)	<60 ^c	<60 ^c	<60 ^c	–	<60 ^c	–
dibenzo(a,e)pyrene (E)	<60 ^c	<60 ^c	<60 ^c	–	<60 ^c	–
dibenzo(a,l)pyrene (E)	<60 ^c	<60 ^c	<60 ^c	–	<60 ^c	–
dibenzo(a,h)pyrene (E)	<60 ^c	<60 ^c	<60 ^c	–	<60 ^c	–
Σ PAH	42.0	88.0	176.3	119.0	177.0	22.0
Σ PAH [E+L]	2.0	4.0	7.3	10.0	7.0	1.0

^a Blend of 25 barrels for each category. ^b E, definite carcinogenic potential; L, limited carcinogenic potential; no indication, no confirmed potential. ^c Below the assay detection threshold.

estimated absorption varies in the same proportions, between 0.05 and 0.14 $\mu\text{g/day}$ per adult, with seasonal peaks varying from 0.57 to 1.15 $\mu\text{g/day}$.

The contribution of different types of foods and beverages to the overall absorption of PAH (17 molecules) has also been analyzed. According to a Dutch study (22), the major contributors are oils and fats (47%), then cereal products (36%), followed by sweet products (14%). Research in the United Kingdom and Sweden (23, 24) also considered cereal products as major contributors (34%), followed by vegetables (18%) and fats (16%). Smoked fish and grilled meat were the main sources of PAH (11 molecules) in Sweden. However, as they represent only a small percentage of the diet, their contribution to overall

absorption was still small. This confirmed the findings of Dennis et al. (25) concerning the limited contribution of barbecued products to overall ingestion of PAH. This cooking method may theoretically contribute significantly to absorption, if barbecued foods represent a significant proportion of the daily diet, especially if cooking is long and intense. In agreement with the Swedish study, work by Kazerouni et al. (26) in the United States showed that the highest levels of benzo[a]pyrene that can be assayed (approximately 4 ng/g of grilled meat) were found in intensely grilled steaks and chicken grilled with its skin. Under these conditions, unlike the European diet, barbecued meat may represent up to 21% of the average daily benzo[a]pyrene intake.

Table 7. Estimated Daily Intake (nanograms per day) from Different Sources for a Nonsmoking Adult (according to ref 13)

	foodstuffs		drinking water		air
	low	high	low	high	mean
anthracene	30	640			20
phenanthrene	330	4510			400
fluoranthene	600	1660	2	20	100
pyrene	600	1090	0.2	20	100
benzo(a)anthracene	20	410	0.2	10	20
chrysene	200	1530	20	20	20
benzo(b)fluoranthene	5	360	0.1	2	20
benzo(j)fluoranthene	30	30	0.02	0.2	
benzo(k)fluoranthene	40	140	0.02	2	20
benzo(b+j+k) fluoranthene	70	1100			60
benzo(a)pyrene	50	290	0.2	2	20
benzo(e)pyrene	200	200			20
benzo(g,h,i)perylene	120	360	0.2	2	20
indeno(1,2,3-cd)pyrene	20	460	0.2	2	20
dibenzo(a,h)anthracene	10	80			2
subtotal	2255	11760	23.14	80.2	782
mean total absorption	3060	12622			

Water contributes only approximately 0.2 $\mu\text{g}/\text{day}$ per person (1% of the total); food represents 90% of daily intake, and the remainder is inhaled from the atmosphere (13). Inhalation from the atmosphere represents 12–140 ng/day per person. However, the exact figures depend a great deal on atmospheric contamination and tobacco smoking. Passive smoking is comparatively more dangerous than active smoking as the benzo[a]pyrene concentration in indirectly inhaled smoke is 10 times higher. A regular smoker may absorb 210 ng/day, which is the same level as overall intake from food, and a passive smoker approximately 40 ng/day.

Thus, considering the PAH concentrations assayed in this work, with an average wine consumption of 50 L/year per person and a total PAH concentration (20 molecules targeted) varying from 80 to 400 ng/L of wine aged in a barrel or placed in direct contact with toasted wood (alternative flavoring products), overall daily intake may vary between 11 and 55 ng/person. These figures include naphthalene, which represents 40–60% of the total PAH in wood but is generally excluded from these evaluations, as it is relatively nontoxic, and assuming that this type of wine represents 100% of the wines consumed (not a realistic hypothesis). This total thus represents 0.5–2.5% of the minimum estimated average daily dietary intake (2.25 $\mu\text{g}/\text{day}$ per adult) (Table 7).

No benzo[a]pyrene concentrations over 6 ng/L (detection threshold) were detected. If the mean concentration is estimated to be 5 ng/L (the maximum risk hypothesis), under the same consumption conditions, the average daily intake would never exceed 0.7 ng/person, i.e., more than 20 times smaller than the amount that would be absorbed by breathing air containing 1 ng/m³ at a rate of 20 m³/day (mean contamination level registered and overall quantity representing 20 ng/day) or, at most, approximately 0.03% of the dietary intake.

These results show clearly that the heating processes involved in barrel manufacture are likely to cause the formation of several molecules in the PAH family. Among the various molecules targeted, a minority of the compounds are highly toxic, particularly those with carcinogenic potential. Benzo[a]pyrene, the best-known, and one of the most dangerous contaminants, is not present in significant quantities in toasted barrel wood.

It was already known that the PAH content and profile were related to toasting intensity (temperature). Thus, although the

quantity of PAH increases with toasting intensity, the proportion of carcinogenic PAH does not increase in the same way, as toasting intensity in the barrels is mainly dependent on time rather than maximum temperature, which does not increase as fast. The barrel toasting method may also affect PAH synthesis, so there may, logically, be differences in PAH content from one cooperage to another at theoretically identical toasting levels. Finally, types of wood used to age wines that require more intense toasting, such as American oak, tend to have a higher final PAH content. We did not study specific production methods, e.g., those used in making barrels for spirits (whisky, bourbon, or rum), which involve combustion and charring of the inside of the staves. Under these conditions, the final temperatures are higher (>300 °C) and the resulting formation of PAH is certainly different.

Due to the PAH content of the wood and the low solubility of these compounds, they apparently migrate very slowly into wine in very small quantities. This explains why the level of barrels' potential extractable PAH only decreases very slowly during use and, consequently, PAH concentrations in wine remain relatively low.

Finally, in a comparison of overall absorption of PAH, especially the most toxic molecules, with estimated intake from diet or the environment, it is clear that the contribution of toasted barrel wood to the total is extremely low and does not, therefore, represent a major public health hazard.

The quantities of PAH, and particularly benzo[a]pyrene, absorbed when other forms of toasted wood are used as an alternative to barrel aging, e.g., powder, chips, or staves immersed directly in wine, do not represent any greater matter for concern. Provided that the thermal degradation occurs under conditions comparable to those of traditional toasting and, in any case, at temperatures not much in excess of 200 °C, and the quantities of wood used are consistent with standard barrel aging, there is no reason that wine should be exposed to any greater increase in PAH content than it would during barrel aging.

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