Assessment of the Exposure to Benzo(a)Pyrene (BaP) Contained in Sardines (Clupeidae) Consumed by the Ivorian Adult in the Area of Abidjan

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Abstract: ***In Côte d'Ivoire, fish products provide a substantial part of needs for animal proteins. Yet, owing to the different processing and preservation procedures used, neoformed molecules appear in the flesh of fish sold for consumption. Therefore, it is necessary to check the quality of the fish obtained from traditional processing procedures. This check involves regular determination of the levels of Polycyclic Aromatic Hydrocarbons (PAH) which cause some diseases such as cancer. This study aims to assess the contamination level of BaP present in smoked fish from the different production sites of the area of Abidjan so as to estimate, in the Ivorian adult, the intake of this neoformed contaminant during processing. For that purpose, 248 samples of fresh and smoked fish were collected. The calculation of the exposure from the determination of BaP in the samples of fish collected was made possible thanks to a High Performance Liquid Chromatograph (Adept), equipped with a UV-visible dectector (CE 4200 CECIL), a pump (CECIL CE 4104), an automatic injector (SPARK Optimas) and a column of Prevail C18 type (150 mm x 4.6 mm x 5 µm). The results revealed the presence of BaP in highly variable concentrations, between 0.65 and 510.3 µg/kg in the samples of fish tested. The estimated intake for the average Ivorian is 10.69 ng/kg BW/d for fresh fish and 47.4 ng/kg BW/d for smoked fish. That intake is at least twice the Toxicity Reference Value (5 ng/kg BW/d). In view of the characteristics of this danger, it is urgent to sensitise, on a larger scale, not only the actors and the consumers but also the competent authorities in charge of the safety control of foodstuffs.***

Keywords: Sardine, PAH, BaP, HPLC, traditional smoking**.**

1. Introduction

In Côte d'Ivoire, the preservation of fish through hot smoking raises health issues as the levels of organic contaminants such as PAHs measured in finished products remain high (**1**). According to **(2)**, more than 2/3 of the Ivorian production estimated at 50,000 tones is subjected to smoking while drying is very little practised. Smoking is frequently used for numerous species of fat fish such as horse mackerel *(Decapterus spp*), mackerel (*Scomber japonicus*) and sardinella (*Sardinelle aurita*). Hot smoking is widely used in the south of the country (**3**); this ancestral technique of preservation is also used owing to its flavouring potential (**4**). The combination of intense heating and smoking over a foodstuff allows to obtain a dried, cooked and smoked product. The final product obtained is dehydrated and impregnated with volatile substances responsible for the colour, the taste and the particular smell. Smoking is a technique of fish preservation leading to the partial loss of the water content in fish which results in the increase in the levels of nutrients, particularly those of proteins and salt **(5).** Thus, populations are provided with highly appreciated products used as ingredients or dishes (**3**). But these products have potential risks related to the presence of compounds such as Polycyclic Aromatic Hydrocarbons (HAPs) caused by the smoke released during fish smoking. The neoformed compounds such as BaP present in the flesh of the processed product are toxic. Some research has confirmed that these compounds have toxicity, particularly genotoxicity leading to teratogenicity, mutagenicity and/or carcinogenicity **(6)**.

Consumption of these fish is potentially risky. Therefore, this study is carried out to determine the level of contamination of BaP in sardines smoked in the different smoking areas of the District of Abidjan, from which the exposure level of Ivorian adults has been assessed.

2. Materials and Methods

2.1 Sampling

The samples of fresh and smoked fish were collected on 5 selected smoking sites of Abidjan. In total, 248 samples were used for the study which was conducted from December 2009 to May 2012. Table 1 shows the distribution of the samples.

Table 1: Distribution of samples

	Fresh fish	*Smoked fish*	*Total*
Macaci	26	26	52
Abobo Doumé	30	30	60
Ile Boulay	30	30	60
Koweït	7	7	14
Port-Bouët	30	30	60
Total	124	124	248

2.2 Reagents and Equipment

The determination of HAPs was carried out by a High Performance Liquid Chromatograph (Adept), equipped with a UV-Visible detector (CE 4200 CECIL), a pump (CECIL CE 4104), an automatic injector (SPARK Optimas) and a column of Prevail C18 type (150 mm x 4.6 mm x 5 µm). The operational conditions of the HPLC were the following: temperature of C18 column: 40°C; flow rate: 1.5 ml/minute; wavelength: 215 nm.

The reagents used for the PAHs dosage are made up of individual standard solutions of PAHs certified by EPA : Benzo(a)anthracene (0.02mg/mL), benzo(b)fluoranthene (0.2 mg/mL), benzo(k)fluoranthene (0.2 mg/mL), benzo(a)pyrene (0.01 mg/mL), benzo(g,h,i)perylene (0.02mg/mL) and indeno (1,2,3-cd) pyrene (0.04mg/mL); acetonitrile grade HPLC (Scharlau Chemie S.A); acetone grade HPLC (Scharlau Chemie S.A); methanol grade HPLC (Scharlau Chemie S.A.); n-hexane grade HPLC (Carlo Erba); toluene grade HPLC (Labosi); dichloromethane grade HPLC (Carlo Erba); deionised water grade HPLC (Panreac Quimica SA).

2.3 Fat and Moisture Content

The extraction of fat was made in accordance with NF V 04 – 035 methods (2008), with a Soxhlet extractor (Sibata 34/45) made up of four sample positions and with the following solvents: hydrochloric acid 37% (Carlo Erba Réactifs SA) and n-hexane 96% extra pure (Scharlau chemie SA). As for the moisture content, it was assessed gravimetrically according to the method of oven drying to constant weight (VICH GL, 2003).

2.4 Determination of PAHs

After validating the method used to determine PAHs according to ISO 15753-2004 standard (**7**), the extraction then the dosage of PAHs were carried out. A test sample of 2.5 g of fish flesh previously lyophilised was placed in a centrifuge tube then 10 mL of a acetonitrile/acetone mixture (V/V; 60/40) was added. The mixture obtained was vortexed for 30 seconds and sonicated for 5 minutes before being centrifuged at 4,000 revolutions per minute for 5 minutes. The upper phase was taken and transferred into a tared conical tube and the solvent was evaporated with a rotary evaporator at 35°C. The extraction was repeated twice with 10 mL of an acetonitrile/acetone mixture. The extract was then purified on C18 cross-linked cartridges (Waters SEP Pack). Then, 2 mL of the acetonitrile/acetone mixture was placed in a conical tube containing the vortexed extract for 15 seconds then centrifuged for 30 seconds. The upper phase was transferred into a tube and the operation was repeated twice. The different supernatants were transferred to a C18 cartridge previously conditioned with 12 mL of methanol and 12 mL of acetonitrile. The elution was conducted with 5 mL of the acetonitrile/acetone mixture at atmospheric pressure, then the eluate was concentrated in a rotary evaporator (35°C) at 50 mg. The extract purified was taken into 1 mL of hexane. The tube was crimped and stored at -18°C before analysis.

The validation of the method used to determine BaP focused on the tests of repeatability, of reproducibility, of trueness, of detection and quantification limits and of linearity ; the last one was tested between 0 and 10 µg/L with 5 calibration points (0 µg/L, 2.5 µg/L, 5 µg/L, 7.5 µg/L et 10lµg/L). The BaP concentrations were obtained from the calibration curve with a Pearson correlation coefficient $R^2 = 0.9995$.

The interpretation of the results on hydrocarbons was based on the Commission regulation (EC) N°1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

2.5 Estimate of Intakes

According to the definition of the codex Alimentarius, the estimate of the exposure is the assessment of the quantitative exposure of the probable ingestion of chemical dangers through foods (**8**). The BaP intake was estimated from the data of fish consumption in Côte d'Ivoire (**9**) and **(10)**. The flesh of fish consumed by an Ivorian adult of 60 kg is estimated at 43.8 g/day/person (**9**). So, the BaP average intake of an Ivorian adult consuming fish flesh and the minimum and maximum BaP intakes were calculated as described by **(11)**. The estimated values of the exposure were compared to the Toxicity Value Reference (TRV), 5 ng/kg BW/d, set by the Netherlands National Institute for Public Health and the Environment (RIVM: Rijksinstituut voor Volksgezondheid) **(1).**

Intake of BaP (µg/j) = $[BaP_i]$ x (Qi) amount of food ingested (kg/d)
[BaP] = concentration of BaP found in fish flesh (µg/kg)
(Q_i) = amount of food ingested (kg/d) = 0.00073

2.6 Statistical Analysis

The average concentrations of parameters (moisture, fat, BaP) were calculated with their standard deviation. The statistical processing was made with SPSS 12.0 software and the significance threshold was set at 0.05. The comparisons between the different parameters were made with ADE4 software.

3. Results

3.1 Changes in the moisture content of fresh and smoked fish according to the smoking area

Figure 1 illustrates the changes in the fish moisture content according to their condition and the smoking site. The final water levels vary from one site to another and are between [30.04% – 55.34%] while the initial average level is 66.24 %.

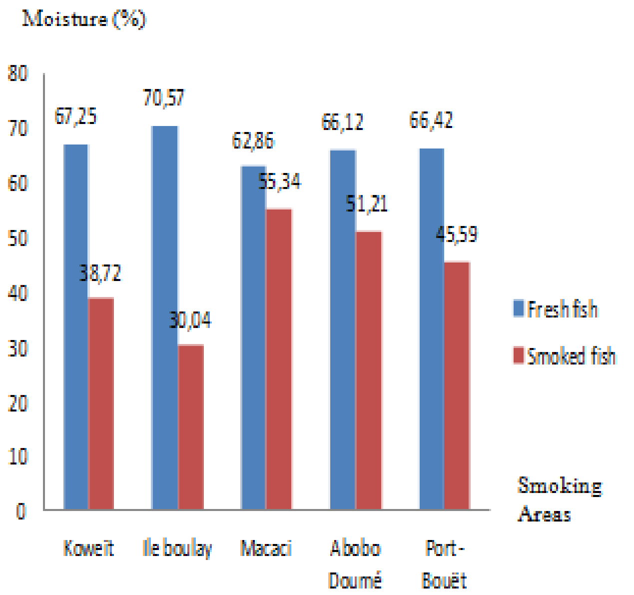

Figure 1: Changes in fish moisture content during smoking

3.2 Changes in fat content of fresh and smoked fish according to the smoking area

The average fat content in fish used for smoking is 18.82%. This content diminishes with processing to reach an average value of 15.29 %. According to smoking sites, these values are heterogeneous with a maximum value of 21.2% at Koweït and a minimum value of 3.42% at Abobo Doumé (**figure 2**).

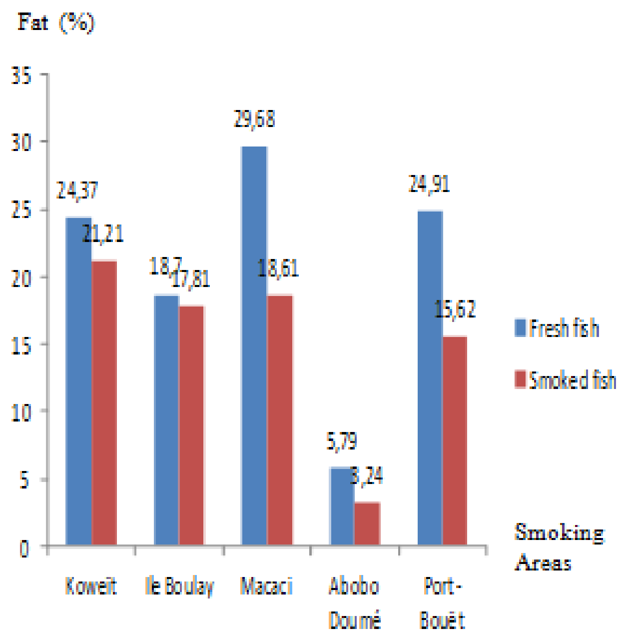

Figure 2: Changes in fish fat content during smoking

3.3 Changes in BaP concentration of fresh and smoked fish according to the smoking sites

From the validation of the method for determining BaP (**7**), the concentrations have been measured. In table 3, the levels of contamination in fresh and smoked fish according to the smoking sites are shown. The average concentration of BaP found in fish before smoking is 14.65 ± 4.92 µg/kg. This average goes up to 64.97 ± 30.80 µg/kg in the flesh of smoked fish. These averages vary after smoking, according to the smoking site. The highest average level is found in smoked fish collected at Port-Bouët (101.64µg/kg) and the lowest average level is observed at Macaci (24.76 µg/kg). Whether fish are processed or not, the concentrations are all superior to the maximum levels defined by differenst regulations.

Table 2: Concentrations of BaP measured in fish according to the smoking sites (µg/kg)

Smoking areas	*Fish before smoking*		*Fish after smoking*	
	Average	Min – Max	Average	Min - Max
Abobo Doumé	12.31±9.90	[0.93-47.92]	55.62±32.41	[13.28-136.2]
Macaci	12.26±5,86	[4.84 – 23.49]	24.76±14,48	[6.73 – 59.24]
Koweït	16.08±18.62	[0.13-70.96]	53.20±58.76	[16.67–173.87]
Port – Bouët	10.04±19.03	[0.13-70.96]	101.64±128.9	[0.65 – 510.3]
Ile Boulay	22.57±24.82	[0.92 – 71.26]	89,65±78,13	[33.17–320.88]
AC	14.65±4.92	[10.04– 22.57]	64.97±30.80	[24.76–101.64]
Max level set by IMO	2		5	

AC= Average Concentration; Max= maximum; IMO= Ivoirian Ministerial Order (No 065/2010/MIPARH)

3.4 Comparison of parameters (moisture, fat, BaP) between fresh and smoked fish

Comparison of parameters (moisture, fat, BaP) between fresh and smoked fish is represented in **figure 3**. The two ellipses overlap in an area, which shows the absence of significant difference in the parameters studied between fresh and smoked fish. As to the individual representation of variables **(figure 4)**, the values of moisture and BaP show a significant difference between fresh and smoked fish of at least 5%.

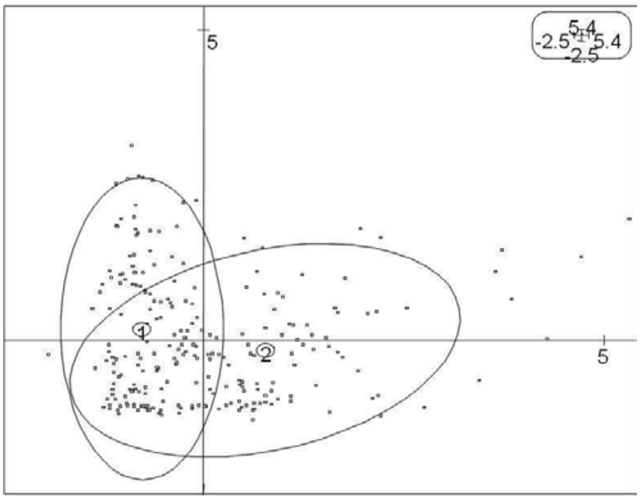

Figure 3: Comparison of parameters studied in fresh and smoked fish (1= fresh fish; 2= smoked fish)

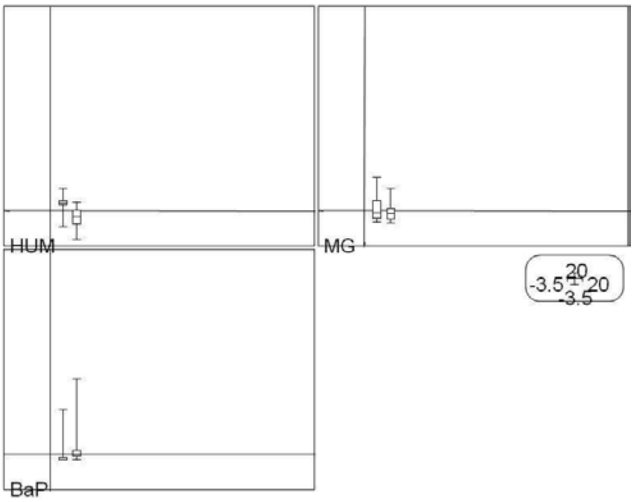

Figure 4: Individual representation of variables in fresh and smoked fish (from left to right: fresh fish; smoked fish)

3.5 Comparison of parameters (moisture, fat, BaP) according to the smoking area

As for the comparison of parameters studied (moisture, fat, BaP) according to the smoking area (**figure 5**), except area n° 4, all the other areas have substantially the same values. Concerning the individual representation of each parameter according to the smoking site (**figure 6**), the values of the parameters studied do not vary with the smoking site.

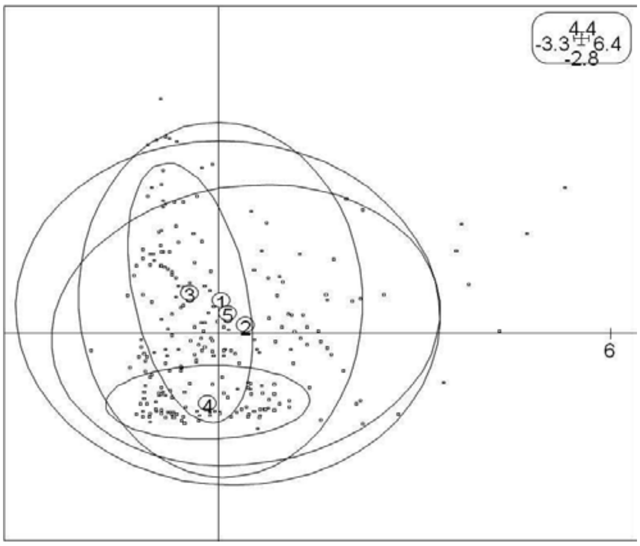

Figure 5: Comparison of parameters studied according to the smoking site (1= Koweït ; 2= Ile Boulay ; 3= Macaci ; 4= Abobo Doumé ; 5= Port-Bouët

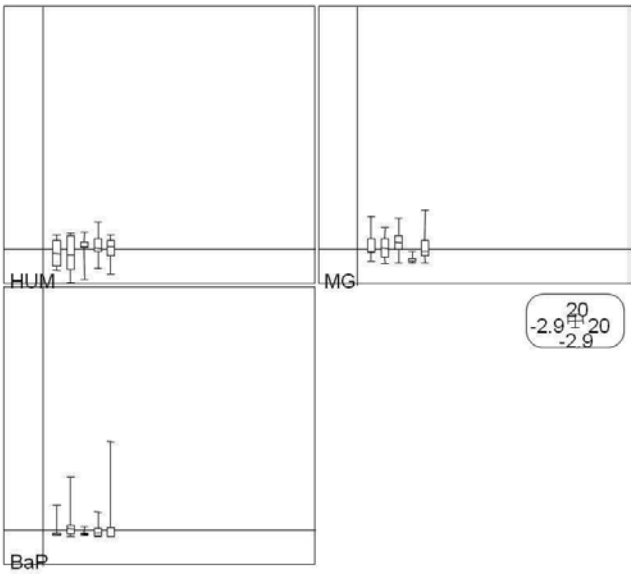

Figure 6: Individual representation of each parameter according to the smoking site (from left to right: Koweït ; Ile Boulay ; Macaci ; Abobo Doumé ; Port-Bouët)

3.6 Influence of moisture and fat on the levels of BaP

The correlation matrix between moisture, fat and BaP (**table 4)** shows that there is significant correlation between moisture and BaP. When the moisture content goes down, the concentrations of BaP in the samples collected increase.

Table 3: Correlation matrix between moisture, fat and BaP in smoked fish

	Moisture	*Fat*	*BaP*
Moisture	1.000000		
Fat	0.026910	1.000000	
BaP	0.341730	0.001020	1.000000

Values in bold show a significant difference (at the 5% level of significance)

3.7 Intakes of Benzo(a)pyrene

The intakes of BaP calculated are presented in **table 5.** These daily intakes were assessed in three categories of individuals:

intake in the occasional consumer (estimated minimum value equal 0.09 ng/kg body weight), intake in the average consumer (estimated average value equal to 9.48 ng/kg body weight) and intake in the large consumer (estimated maximum value equal to 74.5 ng/kg body weight). BaP was found in more than 95 % of the samples tested.

Table 4: Intakes of Benzo(a)pyrene estimated in the Ivorian adult consuming fresh and smoked fish

Fish condition		*Concentration of BaP (µg/kg)*	*Estimated Intake (EI) (ng/kgBW/d)*	*EI/TRV*
Fresh	Min	0.13	0.09	0.018
	Avg	14.65±4.92	10.69±3.59	2.13
	Max	71.26	52.01	10.40
	RV	2	1.46	0.29
Smoked	Min	0.65	0.47	0.09
	Avg	64.97±30.80	47.4±22	9.48
	Max	510.3	372.51	74.50
	RV	5	3.65	0.73

TRV: Toxicity Reference Value = 5 ng/kg body weight/day ; EI: Estimated Intake , Avg: Average; Min : minimal; Max : Maximal

4. Discussion

The results obtained in our study have allowed to show that the methods used to preserve fish through thermal treatment change its chemical composition. In our case, two factors are involved: smoking and flavouring. With the action of flames, the residual water in smoked fish varies between 30 % and 50 %, and the fish is done. The water loss modifies the ideal breeding ground for bacteria responsible for the decrease in the storage time of foods. Traditional smoking of fish is a phenomenon which hinders the growth of bacteria through two actions: dehydration and the antiseptic effect of smoke. The results of this study confirm those of **(12)** and **(13**). The impregnation of smoke in the flesh of the smoked fish gives a characteristic smell to its flesh. But the research of **(14)** shows that the smoke is produced by an indirect combustion process of resinous, wood organic materials with the risk of formation of hydrocarbons as foodstuffs are exposed to products of direct combustion in the presence of open flames. Indeed, the concentration of BaP goes up when moving from fresh fish with an average concentration of 14.65 ±4.92 µg/kg to smoked fish with an average value of 64.97±30.80 µg/kg.

The analysis of the results shows that the smoked fish from the area of Abidjan are contaminated by BaP, a polycyclic aromatic carbon with a proven carcinogenic potential. In **(15)** reported an average level of BaP contamination in smoked fish of about 34.07 µg/kg for a total of 278.22 µg/kg of PAH. That analysis has also revealed that the fresh or frozen raw material was contaminated by BaP before smoking with levels above the regulatory limits. The values found are higher than those of several authors such as **(16)**, **(15)** and **(7)** respectively in fresh fish, smoked fish and smoked meat.

In 2003, AFSSA recommended a guide value about 6 PAHs for 5.5µg/kg of fresh material. In 2012, the research of (**17**) on bush meat highlighted a correlation between BaP and the 6 PAHs (the correlation coefficient was 0.95). The correlation coefficient confirms the role of BaP as marker of the presence of PAHs. The level of BaP contamination in the samples of smoked sardines (64,97 ± 30,80 µg/kg) corresponds to the level of contamination described by AFSSA in 2003 (63 µg/kg) while the BaP values found in the fresh fish used for smoking (14,65±4,92 µg/kg) are at least ten times higher than those described by AFSSA (0.00 – 1.21 µg/kg).

The fat content in the samples of fresh sardines used for smoking is between 5 and 30 %, so the fish are fat as described by **(18**) and the processing technology used at the smoking sites of the area of Abidjan is justified by the necessity of avoiding oxidation reactions. But there is little or no relation between the fat content and the level of BaP contamination in smoked fish.

The analysis of the results has also showed a correlation between the moisture content and the concentration of BaP. The more the product is dehydrated, the more it has undergone the action of heat, either in intensity or in duration, as was confirmed by observations at the smoking sites. The results also highlight a certain specificity of production areas. Indeed, the highest concentrations of BaP are found in the smoked fish taken at the smoking sites of Port-Bouët and Ile Boulay, sites well known as fishing villages (**19**). The concentrations of BaP in the samples of smoked fish are all the higher as the activity takes place in enclaves such as Port-Bouët and Ile Boulay where the unsold smoked fish are reheated several times while stocks last. The major concentrations before smoking are found in decreasing order at Ile Boulay (22.57µg/kg), Abobo Doumé, Koweït, Macaci and Port-Bouët (10.04 µg/kg). The variations of BaP concentrations in fresh fish do demonstrate the contamination of aquatic animals is influenced by their environment, as mentioned by **(18).**

The highest concentrations of BaP were found in the finished products with the lowest moisture content, thus in the products most heated. This result could be explained by the fact that operators use, in general, highy varied fuels, ranging from the simple plant material (branches of rubber tree, of mangrove, cassava peelings, etc.) to the animal material (fish scales). All kinds of wood are appropriate except the rubber tree which causes unpleasant flavour when it is used as a combustion agent for the smoking of foodstuffs (**20**). A comparative study of energy sources has shown that the level of BaP contamination changes with the sources of energy when moving from charcoal to sawdust and branches. So, the regular consumption of foodstuffs with high levels of BaP can be really hazardous for health owing to its proven carcinogenic potential.

The concentrations of BaP in fresh fish are seven times the regulatory value (Ivorian Ministerial Order No 065 MIPARH 2010, Commission Regulation (EC) No 1881/2006**),** and after thermal treatment**,** these concentrations are four times as high as the ones before smoking. With regard to the maximum values defined by the Ivorian regulation, smoking increases by 2.5 times at most the initial concentration of BaP. The average concentration of Benzo(a)Pyrene found in *the* flesh of fish remains superior to the maximum limit value set by international authorities (Commission Regulation (EC) No

1881/ 2006**)**. Yet the flesh of fish contributes to the BaP daily intake of different consumers in proportions between 3 % (small consumers) and 14.8% (large consumers). The average intake estimated for fresh and smoked fish is respectively 10.69 and 47.4 ng/kg body weight/d. These intakes are very superior to the levels described by AFSSA in 2003 (0.67-1.83 ng/kg body weight/d). These values don't take other eaten foods into account.

Compared to the Toxicity Reference Value (TRV) established at 5 ng/kg body weight by the Netherlands Institute for Public Health and the Environment (RIVM) (**1**), the intakes estimated are at least three times as high as this reference value and are superior even to the intakes estimated for small consumers. Side effects are likely to occur. Between fresh and smoked condition, there are changes in the fish which may increase the risk factors, and consequently the risks to consumers; the risk factors are, among others, ancestral practices, types of vegetal products and their preparations before use to light fire for smoking. Given the carcinogenic and genetoxic properties of BaP (**1**), the toxicological risk also exists, and even chronic exposures to relatively low concentrations can generate cancer (chronic carcinogenic effect) as the molecules studied are molecules with no threshold effect (**8**).

5. Conclusion

The concentrations of BaP in 95% of the samples tested in fresh and smoked fish are above the maximum value set by the Ivorian and European regulations. The average intake of BaP estimated from the same samples for an adult is at least 3.6 times the Toxicity Reference Value. The risk is thus real if fish is the only animal protein present in consumers' diet. The quality control of fish is thus necessary; hence the interest of this work to assess the levels of contamination of BaP in smoked fish. Therefore, it is important to make the stakeholders of the smoked fish chain (fishermen, smokers, sellers) aware of the strict observance of good practices of heat treatment, train them to implement proper hygiene rules and take appropriate measures to ensure the traceability of smoked fish all along the chain, from the management of the raw material to its release for consumption.

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