

ASSESSING HUMAN HEALTH RISKS OF POLYCYCLIC AROMATIC HYDROCARBONS IN SMOKED FISH FROM TOGO

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Article Info

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Abstract

The ancient art of smoking, a culinary tradition passed down through generations, has been an integral food preservation method across diverse regions globally. This technique, often coupled with processes like cooking, drying, and salting, not only enhances preservation through its dehydrating, bactericidal, and antioxidant attributes, but also imparts distinctive flavor and hue to food products. Fish smoking, a time-honored preservation practice, remains prevalent, particularly in developing nations where it plays a crucial role in sustaining traditional culinary heritage and ensuring food security.

1. Introduction

Smoking activities are known and have been transmitted from generations to generations in many regions of the world as processing practice to preserve food products (meats, fish or cheeses)(Rivier et al., 2009). It is often combined with cooking, drying, salting. Smoking improves preservation due to its dehydrating, bactericidal, and antioxidant properties, but also flavoring and coloring functions of food(Rivier et al., 2009; Škaljac et al., 2018). Smoking fish is a traditional preservation technique which is still widely practiced in developing countries(Adeyeye and Oyewole, 2016a).

In West African countries, smoking activities are very developed in artisanal fishery sector where, 40%of the products are smoked before consumption(FAO, 2009a).In the coastal area, fish processing is an important part in the local economy and can be considered as good-paying permanent job (Adeyeye and Oyewole, 2016b; Nyebe et al., 2014). The proportion of animal protein from fish is extremely high in the West African coastal region: 47% in Senegal, 62% in Gambia and 63% in Sierra Leone and Ghana(Béné and Heck, 2005). This source of protein is more available in smoked fish form rural and urban low-income households for whom the price of meat remains more expensive(FAO, 2009b).

In Togo, 41% of fish provided from artisanal sector are smoked(Ali et al., 2014).A large part of the imported fish (51706.59 tons in 2012) is also sold on the market in smoked form(Lare, 2016). Today, it is clearly established that smoke contains Polycyclic Aromatic Hydrocarbons (PAHs), known for several decades for their carcinogenicity effect on humans (Doornaert et al., 2003). In 2002 the Rapid Alert System of European Commission rejected smoked fish containing high levels of PAHs from Togo(CE, 2009; Hirsch, 2003). In 2019,

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it was the high level of PAHs in Togo's red palm oil that have been detected and rejected by the same commission(RASFF, 2019).

PAHs are organic compounds and have two or more fused aromatic rings and are known as a potential health hazard(Auby et al., 2013; Puljić et al., 2019). There are 16 PAHs identified by the U.S. Environmental Protection Agency(Nisbet et al., 1984). The seven (7) most important probably recognized as carcinogenic for humans are: benz[a]anthracene, (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (IcP), dibenz[a,h]anthracene (DhA). Even though they have natural and anthropogenic origin, PAHs can contaminate food in many ways through pyrolysis and incomplete combustion of organic matter like wood during the process of smoking(Doornaert et al., 2003).

In 2008 the European food safety authority (EFSA)(Larsen, 2008) identified the carcinogenic properties for 4PAHs(benzo[a]pyrene (BaP), benz[a]anthracene, (BaA), benzo[b]fluoranthene (BbF) and chrysene (CHR))and define a reference for their determination in smoked food. The most widely investigated PAH is BaP and its limit in traditionally smoked fishery products is set at 5.0 µg/kg by European Commission Regulation(Junker, 2014). The maximum level of the sum of the concentrations of the 4PAHs is limited to 30.0 µg/kg by the same commission.

The Population is generally exposed to PAHs by oral, pulmonary and cutaneous routes. The major source of human's exposure to PAHs is food. PAHs are formed during different manners of food cooking and the consumption of fruits or vegetables contaminated by atmospheric pollution (Doornaert et al., 2003; Moriarty, 1988). The dose-response relationship for oral exposure to a mixture of 4PAHs can be assessed on a substance basis. This approach uses the standardized toxic equivalence factor (TEF) that allows to assess the risk induced by PAHs in all kinds of mixtures in a target population(Doornaert et al., 2003). The objective of this study was to determine the level of 4PAHs in smoked fishes from 3 markets in Togo and assess the health risk related to these 4PAHs through smoked fish consumption.

2. Material and methods

2.1 Samples collection

Smoked fish samples were collected in three (3) markets from Maritime and Plateaux regions in Togo. Samples were purchased from wholesalers at three (3) markets and were mixed to make a single homogeneous sample per market. The sampling was done in 2 markets in Maritime's region. Adawlato located at 6°07 latitude and 1°13 longitude and Adidogomé located at 6°12 latitude and 1°08 longitude. In Plateaux region, the sampling was done in Atakpamé market located at 7°31 latitude and 1°70 longitude of GPS. At Adawlato market, two samples were collected (ADAW1 and ADAW2). The fish samples named ADAW2 provided from industrial fishery and are composed of mackerel (*Scomber japonicus*), horse mackerel (*Trachurus* sp.) and damselfish (*Chrysiptera parasema*) traditionally smoked in Togo. The remaining samples from Atakpamé (ATAK), adidogomé (ADID) and Adawlato 1 (ADAW1) are composed of anchovies (*Engraulis encrasicolus*) and sardinella (*Sardinella maderensis* and *Sardinella aurita*). Samples were stored in carrier bags with seals and were transported to Central Laboratory for Analysis and Testing(LCAE), Tunis (Tunisia) where the analyses were performed.

2.2 Chemical products

Four (4) PAH standards certified reference material of benz[a]anthracene (B[a]A), chrysene (CHRY), benzo[b]fluoranthene (B[b]F) and benzo[a]pyrene (B[a]P) were purchased from Sigma-Aldrich. Hexane was

obtained from Sigma-Aldrich and dichloromethane, Acetonitrile (99.9%) from VWR chemical., Sodium chloride (NaCl) and Magnesium Sulfate (MgSO₄) were obtained from Agilent technologies

2.3 Equipment

Fixed speed vortex mixer (Isolablaborgeräte GmbH) was used for the shaking of the tubes. Hettich® Universal 320R centrifuge used, was purchased from Sigma-Aldrich. The QuEChERS kits were used for the extraction and cleanup of smoked fish samples, Conical tube (50 ml) was used for the extraction and high performance liquid chromatography (HPLC) series 1200 were purchased from Agilent technologies.

2.4 Laboratory Analysis

At the laboratory, the samples were cleaned, the head and scales were removed and the fish were ground with a blender to homogenize in order to ensure analytical test portion representing whole samples. The ground fishes were kept in the freezer to avoid deterioration until the day of analysis. For the analysis, the ground fishes were taken out from the freezer and purified by QuEChERS method called QuEChERS dispersive solid phase extraction (dSPE). This method offers a fast, efficient, and accurate determination of PAHs in smoked fish samples. About 2 to 3 g smoked fish sample was put in a centrifuge tube (50 ml) and spiked with d-PAHs, mixed well, and left for 30 min at laboratory temperature. After adding water and leaving it to homogenize, acetonitrile was added, and mixed vigorously for 1 min on the vortex. Original Agilent Bond Elu QuEChERS extraction salt was added to the tube and the tube was shaken immediately for 1 min on vortex.

The content was then centrifuged for 5 min at 4000 rpm. Finally, the extract was transferred into appropriate tubes or vials and the analysis was performed on high performance liquid chromatography (HPLC) brand Agilent technologies series 1200.

2.5 Quality Assurance

The analytical method was accredited in accordance with ISO 17025:2017. The validation plan included determination of linearity, reproducibility, precision, accuracy, limit of quantification (LOQ) ≤ 0.9 ppb and limit of detection (LOD). Method precision was evaluated by repeatability, using blank smoked fish fortified or doped sample with 100 ppb PAH. Accuracy was calculated by recoveries ranged from 50% to 120%. The corrected concentration is equal to the concentration calculated after integration with high performance liquid chromatography (HPLC) on the PAH recovery: $[\text{PAH}]_C = [\text{PAH}]_R \times [\text{PAH}]_{\text{rec}}$ where $[\text{PAH}]_C$ = PAH corrected; $[\text{PAH}]_R$ = PAH read; $[\text{PAH}]_{\text{rec}}$ = Recovery rate of PAH.

2.6 Estimation of daily dose intake (DDI) relative to PAHs content in smoked fish

According to the study of Lare (2016), the average consumption of processed fish (smoked and fried) in Togo is around 15.60 kg per person per year (which corresponds approximately to 0.042 kg per day). In 2018 the complementary study of GIZ (2018) has shown that 80 to 95% of the fish are consumed in smoked form. The daily consumption of smoked fish was evaluated to be 40 g in this study. The average of adult body weights was estimated at 60 kg (Storelli, 2008). The daily dose intake (DDI) of PAHs through smoked fish consumption was calculated by multiplying the content of PAHs by the daily quantity of smoked fish consumed per person in Togo.

$$\text{Daily dose intake or DDI} = \frac{C \times A}{BW}$$

C = Content of PAH; A = Quantity of smoked fish consumed per day; BW = Body weight of adult person.

2.7 Estimation of toxic potential relating to benzo [a] pyrene

Benzo[a]pyrene (BaP) is PAH that has been well characterized as the most potent carcinogenic. It is a reference from which the toxic potential of other PAHs will be established. A toxic equivalence factor (TEF) is an estimation of the relative toxicity of each PAH fraction compared to Benzo[a]pyrene (Hafiz et al., 2019). The DDI of each PAH is multiplied by its TEF (Doornaert et al., 2003; Nisbet and Lagoy, 1992). The toxic equivalence quantity (TEQ) or $\sum \text{BaP}_{\text{eqi4PAHs}}$ is obtained by adding the values of toxic potential of 4 PAHs (benzo[a]pyrene (BaP), benz[a]anthracene, (BaA), benzo[b]fluoranthene (BbF) and chrysene (CHR)). TEQ is calculated for quantifying the toxic potential of 4 PAHs and further assessing the health risk of human exposure to PAHs (Wang et al., 2018).

2.8 Assessment of lifetime excess cancer risk by oral route (CR_{oral})

The lifetime excess cancer risk (CR_{oral}) was calculated for the reference substance that is BaP. The lifetime excess cancer risk CR_{BaP} is an acceptable daily intake of BaP that would increase carcinogenic risk to 1×10^{-6} over 70-year life of an adult (Doornaert et al., 2003; Santodonato, 1981).

The lifetime excess cancer risk oral for BaP (CR_{BaP}), proposed by INERIS (Doornaert et al., 2003), is established by RIVM (Baars et al., 2001) and recommended by AFSSA (AFSSA, 2003) in 2003. It was determined by the cancer-causing ability of BaP for ingestion at $0.2 \text{ (mg/kg/day)}^{-1}$. The dose-response induced by 4 PAHs ($\text{CR}_{4\text{PAHs}}$) was evaluated by multiplying the toxic equivalence quantity (TEQ) by BaP lifetime excess cancer risk (CR_{BaP}).

$$\text{CR}_{4\text{PAHs}} = \sum (4\text{TEQ}) \times \text{CR}_{\text{BaP}}$$

3. Results

3.1 PAHs content in smoked fishes

The content of benzo[a]pyrene (BaP), benz[a]anthracene, (BaA), benzo[b]fluoranthene (BbF) and chrysene (CHR) in all samples were higher than the maximum limits (ML) values set by European commission ($30.0 \mu\text{g/kg}$) (Juncker, 2014). Table 1 shows that the highest content of 4 PAHs was observed in Atakpame market (ATAK) where the concentration was 6 times higher than the maximum limit (ML) of BaP value. The lowest content was observed in ADAW2 ($35 \mu\text{g/kg}$). The content of BaP has the same trend in all samples and increased 5 to 9 times than ML BaP ($5.0 \mu\text{g/kg}$) except ADAW2 that has a content inferior ($2.24 \mu\text{g/kg}$) to ML value but it is the only one that contains Chrysene ($21.91 \mu\text{g/kg}$).

Table 1: PAHs content in smoked fish samples

	PAH recovery	ADAW1 ($\mu\text{g/kg}$)	ADAW2 ($\mu\text{g/kg}$)	ADID ($\mu\text{g/kg}$)	ATAK ($\mu\text{g/kg}$)
Benz[a]anthracene (BaA)	95%	28.42	5.29	33.03	45.68
chrysene (CHR)	92%	0	21.91	0	0
Benzo[b]fluoranthene (BbF)	90%	30	5.56	44.44	102.00
Benzo[a]pyrene (BaP)	72%	29.70	2.24	47.22	47.47
Maximum Limit (ML) BaP			5.0 $\mu\text{g/kg}$		
Σ (Somme) 4PAHs	-	88.12	35	124.69	195.15
Maximum Limit (ML) 4PAHs			30,0 $\mu\text{g/kg}$		

ADAW1: Adawlato1 market; ADAW2: Adawlato2 market; ADID: Adidogome market; ATAK: Atakpame market; ML: Maximum Limit

3.2 Estimation of daily dose intake (DDI) of PAHs

The average of DDI of BaP in smoked fish in Togo's market (table 2) was 3 times higher (21.08 ng/kg bw/day) than the median dietary exposure for high consumers evaluated by EFSA (6.5 ng/kg bw/day) (Larsen, 2008). The lowest DDI of BaP was observed at Adawlato2 market (1.4 ng/kg bw/day). The highest DDI of 4PAH was found at Atakpamé market (ATAK) and was 4 times higher than the median dietary exposure evaluated by EFSA (34.5 ng/kg bw/day) (Larsen, 2008).

Table 2: Daily dose intake of PAHs in consumption of smoked fish

	DDI (ng/kg bw/day)			
	ADAW1	ADAW2	ADID	ATAK
Benzo[a]anthracene (BaA)	18.94	3.52	22.02	30.45
Chrysene (CHR)	0	14.60	0	0
Benzo[b]fluoranthene (BbF)	20.00	3.70	29.62	68.00
Benzo[a]pyrene (BaP)	19.80	1.4	31.48	31.64
TDI_{BaP} for high consumer	6.5 ng/kg bw/day			
Σ DDI _{4HAP}	58.74	23.22	83.12	130.09
TDI_{4PAH} for high consumer	34.5 ng/kg bw/day			

DDI: Daily Dose Intake; TDI_{BaP}: Tolerable Dose Intake of BaP; DDI_{4HAP}: Daily Dose Intake for 4PAH; TDI_{4PAH}: Tolerable Dose Intake for 4PAH; bw: body weight; ADAW1: Adawlato1 market; ADAW2: Adawlato2 market; ADID: Adidogome market; ATAK: Atakpame market

3.3 Estimation of toxic potential

The estimation of toxic potential of smoked fishes (table 3) in Togo was ranged from 2.26 to 41.48 ng/kg with an average concentration of 26.01 ng/kg. The TEQ in each market was very close to the value of TEF_{BaP}. The highest concentration of TEQ and TEF_{BaP} was observed in Atakpamé market (41.48 ng/kg; 31.64 ng/kg) and the lowest concentration was observed in the sample of ADAW2 (2.26 ng/kg; 1.4 ng/kg).

Table 3: Toxic equivalence quantity (TEQ) of smoked fish

	TEF	ADAW1 (ng/kg)	ADAW2 (ng/kg)	ADID (ng/kg)	ATAK (ng/kg)
Benz[a]anthracene (BaA)	0.1	1.89	0.35	2.20	3.04
chrysene (CHR)	0.01	0	0.14	0	0
Benzo[b]fluoranthene (BbF)	0.1	2.00	0.37	2.96	6.80
Benzo[a]pyrene (BaP)	1	19.80	1.4	31.48	31.64
TEQ (BaP_{eq4HAP})		23.69	2.26	36.64	41.48

TEF: Toxic Equivalent Factor; TEQ (BaP_{eq4HAP}): Toxic Equivalent Quantity for 4PAH; ADAW1: Adawlato1 market; ADAW2: Adawlato2 market; ADID: Adidogome market; ATAK: Atakpame market

3.4 Assessment of lifetime excess cancer risk CR₀ of PAHs in smoked fish

The estimation of lifetime excess cancer risk by smoked fish consumption in table 4 shows that cancer risk was very high for the population compared to the standard of BaP (1×10^{-6}). The average value of dose response induced by a mixture of 4 PAHs was 5.19×10^{-6} . Only the sample of ADAW2 presented a CR₀ lower than 1 for 10^6 persons as recommended by INERIS (Doornaert et al., 2003).

Table 4: Estimation of lifetime excess cancer risk

	ADAW1	ADAW2	ADID	ATAK
TEQ_{4PAHs}	23.69	2.26	36.64	41.48
CR_{4PAHs}	4.73×10^{-6}	0.45×10^{-6}	7.32×10^{-6}	8.29×10^{-6}
CR_{BaP}	1×10^{-6}			

TEQ_{4PAHs}: Toxic Equivalent Quantity for 4PAH; CR_{4PAHs}: lifetime excess cancer risk for 4PAH;

CR_{BaP}: lifetime excess cancer risk of BaP; ADAW2: Adawlato2 market; ADID: Adidogome market; ATAK: Atakpame market

4. Discussion

Benzo[a]pyrene is used as a marker of occurrence and effect of the carcinogenic PAHs in food. Benzo[a]pyrene (BaP), benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF), and chrysene (Chry) are four PAHs that are considered as a reference for the determination of PAHs in food according to EFSA (Larsen, 2008). The presence of these 4PAHs in food is relevant because of their toxicity and their occurrence. The highest contents of 4PAH (88.12 - 195.15 µg/kg) were observed in Atakpamé, Adidogomé and Adawlato1 markets where the samples were composed of anchovies and sardinella that have small size (4 – 9 cm). The traditional smoked fish process leads to dehydrated products that can be preserved for 6-12 months. The highest contents of PAHs have been reported in traditional smoked fish in developing countries as Nigeria, Ivory Coast and Cambodia (Škaljac et al., 2018; Tongo et al., 2017; Traore, 2016). The concentrations of 4PAHs found in Cambodia (Škaljac et al., 2018) and Ivory Coast (Traore, 2016) are 3–6 times more than the concentration found in Atakpamé market which presents the highest content of 4PAHs. Rivier & al (2009) have shown that the traditional smoked fish process with wood gives off much smoke which contaminates food products. The study conducted at CIRAD by the same authors showed that the fishes smoked with wood contained 48 times 4PAHs more than the fishes smoked with coal and sawdust. The contents of PAH in smoked fishes in the present study are very close to the mean concentration of 6PAHs (263 µg/kg) found in smoked and dried fishes exported in France by Togo and Ivory Coast (Hirsch, 2003). The results found in this study confirm Puljić & al (2019) works. They found the similar content of 4PAH in traditional smoked meat in the southern region of Bosnia and Herzegovina (Puljić et al., 2019). The samples of ADAW2 present a content of BaP (1.4 µg/kg) lower than the BaP ML (5.0 µg/kg) and the sum of 4PAH (35 µg/kg) is slightly higher than 4PAH ML (30,0 µg/kg). However, the chrysene (CHR) was detected only in ADW2 samples (21.91 µg/kg). The samples of ADAW2 were composed of imported fish species (Mackerel, Horse mackerel and Damsel fish) smoked traditionally. The imported fishes sold in West Africa coastal countries come mainly from the coasts of the former Soviet Union and European Union which are highly industrialized countries (Weigel, 1999). CHR and BaP are often associated in food and their toxicities have been evaluated just like 4PAHs and 8PAHs (Larsen, 2008). The presence of chrysene may be due to the ubiquitous nature of PAHs that can be found in many compartments of the environment, including the sea water which is the living environment for fish. This corroborates the results found in Ivory Coast in fresh tunas that contain higher concentration of CHR than the BaP, BaA, BbF (Traore, 2016). The content of PAHs in smoked fish from different markets gives the following classification: ATAK > ADID > ADAW1 > ADAW2.

The estimation of the daily dose intake (DDI) shows that the quantities of PAHs consumed by adult population varies from 23.22 to 130.09 ng/kg bw/day for 4PAHs and from 1.4 to 31.64 ng/kg bw/day for BaP. In this study the DDI is compared to the median dietary exposure of higher consumer in European countries (Larsen, 2008). The calculation of daily dose intake is directly related to smoked fish consumption. This estimation did not consider the food preparations with smoked fish which can involve the dilution of PAHs content. Considering the carcinogenic potential of 4HAPs it is convenient to keep the DDI As Low As Reasonably Achievable (ALARA) (Hirsch, 2003). The values found are well above the tolerable dose intake of BaP fixed by EFSA (Larsen, 2008) in European countries for highest consumers which are respectively 6.5 ng/kg bw/day for BaP and 34.5 ng/kg bw/day for 4PAHs. Although the values of DDI are higher than the median dietary exposure in Europe, the daily quantities intake in Togo are lower than the daily dose intake found by Xia & al (2010) for citizen of Taiyuan in China (532.56 ng/d) for adult population.

Toxic equivalence factor (TEF) is an estimate of the relative toxicity of individual PAH fraction compared to Benzo[a]Pyrene as reference. The total PAH concentration is expressed as Benzo[a] Pyrene Equivalents (Hafiz et al., 2019). TEF is a useful tool for the regulation of compounds mixture PAHs that were supposed to have a common mechanism of actions. The use of the concept of toxic equivalence factor (TEF) allows to add the risks related to a carcinogenic co-exposure of a mixture of substances in accordance with the carcinogenic power of a substance of reference known as BaP (Doornaert et al., 2003).

The toxic equivalence factors (TEFs) were used to calculate toxic equivalence quantities (TEQs) of 4PAHs (Wang et al., 2018). The estimation of toxic potency of BaA (0.35-3.4 ng/kg/d) BbF (0.37 - 6.80 ng/kg/d) and CHR (0-0.14 ng/kg/d) in analyzed samples is lower than TEF of BaP (1.4-31.64 ng/kg/d) in the same samples.

The values obtained for BaA, BbF and CHR are lower than those found in smoked fish species in Nigeria Southern markets (Tongo et al., 2017) and the TEQ for 4PAH ranged from 2.26 to 41.48 have the same trend. The values of TEQs of 4PAH in Togo market remain higher than cold smoked mullet fish in Egypt (Hafiz et al., 2019) and marine species in Spain (Llobet et al., 2006).

The dose-response assessment induced by the mixture of 4HAPs was calculated by substances approach that uses the concept of toxic equivalence factor (TEF) and quantifies the carcinogenic potency of the mixture of substances according to the carcinogenic potency of the reference substance which is BaP. The lifetime excess cancer risk by oral route (CR_o) of the mixture is assessed by calculating an equivalent concentration of benzo[a]pyrene in the exposure mixture (Doornaert et al., 2003). The assessment of lifetime excess cancer risk of 4 PAHs (CR_{4PAHs}) reveal a higher cancer risk among Togolese population. The CR_{4PAHs} ranged from 0.45×10^{-6} to 8.29×10^{-6} .

The lowest value (0.45×10^{-6}) is found in ADAW2 samples composed of mackerel, horse mackerel and damselfish fish imported, smoked traditionally. The lifetime excess cancer risk associated to the consumption of imported fish smoked traditionally is 0.45 case per 10^6 people weighing meanly 60 kg during 70-years life. This value is lower than INERIS threshold value that is 1 case for 10^6 people (Doornaert et al., 2003). The consumption of imported fish smoked traditionally does not currently constitute a health risk for people in Togo.

The samples of small size (4-9 cm) fish (ADAW1, ADID, ATAK) composed of anchovies and sardinella contains the highest values (4.73×10^{-6} - 8.29×10^{-6}) of lifetime excess cancer risk of 4PAHs (CR_{4PAHs}). The average value of CR_{4PAHs} of adult population was estimated to be 6.78×10^{-6} for a daily intake of 40 g of small smoked fish. This value is greater than the threshold value (1×10^{-6}). The assessment of lifetime excess cancer risk ranged from 6 to

7 adult for 10⁶ people over 60-year life. The values of CR_{4PAHs} in Togo are greater than the cancer risk observed in Southern Nigeria (Tongo et al., 2017) and Taiyuan in China (Xia et al., 2010). The carcinogenic risk is high for the consumption of small smoked fish by Togolese population.

5. Conclusion

In this study, the content of 4 PAH has been evaluated in traditionally smoked fishes from three (3) markets in Togo. The estimation of the daily dose intake shows values that are higher than European countries tolerable daily dose. The assessment of the lifetime excess cancer risk reveals that the consumption of smoked fish exposes the population to PAH contamination and to a high level of human cancer risk in Togo. However, in Adawlato 2 market the carcinogenic risk is lower because of the industrial nature of the fishes that contain more concentration of chrysene considered to be weak in carcinogenic risk. This study is the first step toward the improvement of the fish smoking technique in order to have safer smoked fish products.

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