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Bioaccumulation and Consumption Safety of a Sea Food, Gastropod Mollusc (*Thais coronata*): Polycyclic Aromatic Hydrocarbon (PAH) Perspective

***[1]MOSLEN, M; [1]ANIEKAN, I; [1]ONWUTEAKA, J; [2]MIEBAKA, CA**

*[*1]Department of Animal and Environmental Biology, Rivers State University, Port Harcourt, Nigeria*
[2]Institute of Pollution Studies, Rivers State University, Port Harcourt, Nigeria.
**Corresponding Author Email address: moslen4c@yahoo.com; moslen.miebaka@ust.edu.ng*

ABSTRACT: Seafood and other environmental matrices are major entry route of harmful pollutants into humans due to constant contamination of the aquatic environment hence requires monitoring. This study scientifically explored the hypothesis that benthic gastropods bioaccumulate harmful pollutants that pose health risk to consumers of the sea food. Fifty samples of *Thais coronata* were collected per site from major regional fish landings. Two hundred samples were collected monthly for eight months to determine the concentrations of polycyclic aromatic hydrocarbon-PAH and possible health risks due to consumption using regulatory limits for guidance. Total PAH concentrations (µg/kg) varied between 65.68–173.52 suggesting differences in consumed concentrations at different times. The concentration of individual PAH congeners (1.376 ± 0.07-40.356±2.21 µg/kg) and PAH4 were below the European Union maximum limits. The Daily Dietary Intake values ranged from 0.075-2.212 µg/kg for individual PAH congeners while that of PAH4 was 1.359 µg/kg and were all below their respective reference oral doses. The carcinogenic potencies of the PAH congeners ranged from 0.001-143.389, the carcinogenic toxic equivalents (TEQs) of all PAH congeners was 0.1522 while that of PAH4 was 0.0044.The Excess cancer risk (ECR) value (10^{-8}–10^{-5}) was within USEPA guideline of 10^{-6} while the margin of exposure (MOE) of individual PAHs and PAH4 were higher than critical border line of 10,000 given by European Food Safety Authority but diagnostic ratio suggested PAH sources of pyrogenic origin in samples monitored. The study concluded low health risk for consumers of the shellfish (*Thais coronata*) in the study region but with recommendation advisory for regular monitoring to observe changes.

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The European Union had strongly recommended constant measurement of PAHs as wide as possible in food products in order to obtain data on the occurrence and specific concentrations in a variety of matrices (Wenz, *et al.,* 2006). According to Bandowe *et al*., (2014) and Conte *et al*., (2016) polycyclic aromatic hydrocarbons (PAHs) are semi volatile organic compounds made up of two or more bonded aromatic rings in linear, angular or grouped pattern. The bioaccumulation of PAHs and its toxic effects with regards to marine organisms and onward transmission to humans via the food chain is a major health concern due to negative effects (Moslen *et al*., (2019). The origin of PAHs into the environment is both natural and anthropogenic. Major sources of PAH into the environment are mainly pyrogenic (including incomplete burning of coal, oil, gas, wood, garbage or other organic substances) and petrogenic inputs (Domingo and Nadal, 2015). PAHs are also classed as environmentally harmful pollutants (Nakata *et al*., 2014) and are widely detected in the aquatic environment, including water, sediment, fish, benthic invertebrates, sea birds, and sea mammals (Honda *et al*., 2018). Contaminated sediments constitute major source of pollution in estuaries and coastal delta systems because it becomes a sink for different organic and inorganic contaminants (Moslen and Ekweozor, 2016) which could further severely affect benthic community structure of the ecosystem (Daka and Moslen, 2013). *Thais coronata* is a species of sea snail. It is a marine benthic gastropod mollusc in the family Muricidae (the murex or rock snails) (WRMS, 2010). It is a shell fish of high economic and commercial value, consumed for its rich protein and vitamins content in southern Nigeria. In the aquatic ecosystem, benthic organisms are constantly exposed to environmentally harmful pollutants like PAHs particularly, in the Niger Delta region of Nigeria where oil and gas exploration and exploitation had led to the release of hydrocarbons into the aquatic environment. Indiscriminate feeding of fish and benthic organisms lead to bioaccumulation of

**Corresponding Author Email address: moslen4c@yahoo.com; moslen.miebaka@ust.edu.ng*

pollutants in their tissues and gradual buildup of these pollutants in biota over time, could have harmful effect on humans who consume fish as their source of protein (Moslen *et al*., 2017). *Thais coronata* is one of such filter-feeding benthic gastropods capable of pollutant bioaccumulations and satisfies biomonitoring conditions like other aquatic gastropods. It therefore, becomes imperative to regularly monitor bioaccumulation of such harmful substances in marine benthic biota and further assess health risk hazard associated with consumption particularly, in areas where such data and information is lacking. This study therefore, seeks to fill the information gap in bioaccumulation of PAH and health risk concerns of consuming contaminated aquatic gastropod like *Thais coronata*.

MATERIALS AND METHODS

Collection of samples: Aquatic gastropod (*Thais coronata*) samples were freshly collected from regional fish landing ports (sites) located in southern Nigeria (Fig. 1). Samples were collected in two parts of fifty (50) per site (quadruplicate) irrespective of size and sex, making a total of 200 samples per month for the two landing ports. Sampling frequency was on a monthly basis for eight months (October 2018 – May 2019). Incremental sampling was done to ensure aggregate samples representative of lots or sub-lots for laboratory analysis (FSAI, 2015). The samples were wrapped in well labelled aluminum foils preserved in ice-chests and transported to the laboratory for analysis.

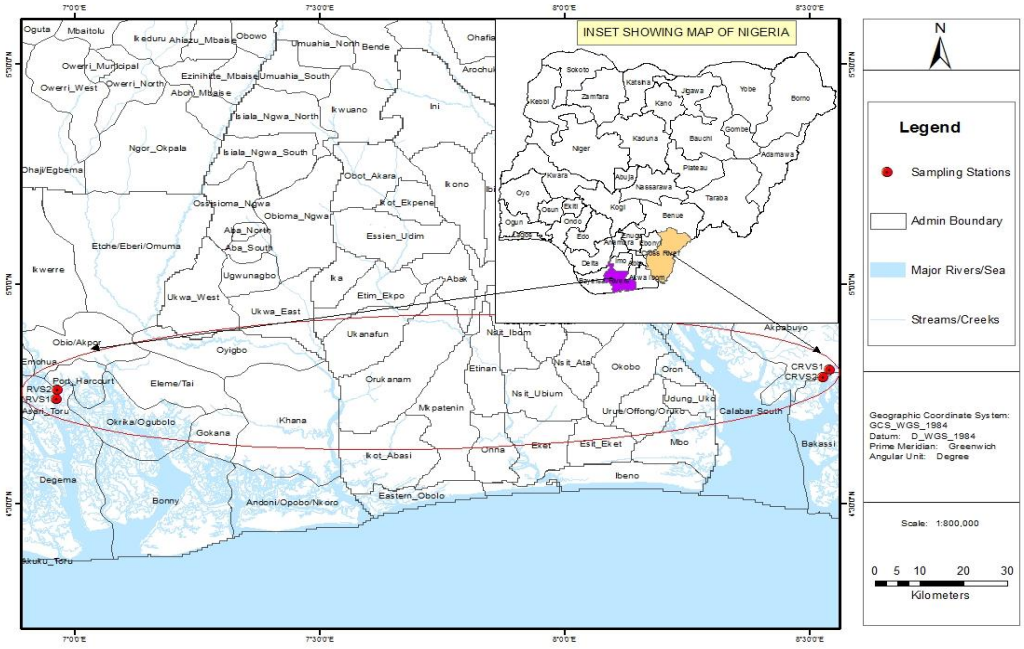

Fig. 1: Inset map (Nigeria) showing study area/region

Extraction and analysis: The extraction of PAH was achieved by the methods of (Pena *et al.*, 2006). Gastropod samples (*Thais coronata*) were properly homogenized after extraction and oven drying. Anhydrous Na_2SO_4 was carefully mixed with 10 g of sample. Twenty (20) ml of dichloromethane was then added to the sample, covered with aluminum foil to avoid evaporation. Supernatant was then separated from extracts by sonication and concentrated using an evaporator. The extract was cleaned using a chromatographic column packed with 1 cm glass wool at the base. It was pre-eluted with twenty (20) ml dichloromethane after adding two (2) gram silica gel with 1cm Na_2SO_4 (anhydrous). Extracts were put in three (3) ml vials before gas chromatographic analysis.

Gas chromatographic (GC) analysis: Gas Chromatographic analysis was done with GC model: HP5890 Series II GC-FID for the PAH congeners as described (Tongo *et al.*, 2017). The initial temperature of the GC was put at 60^{o}C for two minutes and inclined at 25^{o}C per minute and then increased to 300^{o}C for five minutes before letting it to stay for fifteen minutes. The injection port temperature was placed at 250°C with a microlitre splitless injection mode, while the injection port of the flame ionization detector (FID) was kept at 300°C. Fifteen important PAHs (Naphthalene -NaP, Acenaphthylene -AcPY, Acenaphthene -AcP, Fluorine-Flu, Phenanthrene-Phe, Anthracene-Ant, Fluoranthene -FL, Pyrene-Pyr,

Benzo [a] anthracene-BaA, Chrysene-Chr, Benzo [b] fluoranthene-BbFL, Benzo [k] fluoranthene-BkFL, Benzo [a] pyrene-BaP, Indeno [1, 2, 3-cd] pyrene-Ind, Dibenzo [a, h] anthracene-DBA and Benzo [g, h, i] perylene-BP) were noted for the analysis. Comparison of the holding time of standards were done with that of extracts with individual examination of PAHs used for identification and quantization of different components recorded. The non-carcinogenic congeners assessed were Nap, AcPY, AcP, Flu, Phe, Ant, FL, Pyr while the carcinogenic ones were BaA, Chr, BkFL, BaP, BbFL, Ind, DBA and BP (USEPA, 1993). The limit of detection (LOD) was $0.0001 \mu g kg^{-1}$. Recovery method involved use of surrogate standard and recovery rate was done by spiking of sample with known concentration of the surrogate. The concentration of the surrogate and the other samples was given by injection into the GC (Inengite *et al.,* 2010). Analytical standards and reference used comply with those of (Wickliffe *et al*., 2018; Inengite *et al*., 2010).

Exposure assessment: Evaluation of the Dietary Daily Intake (DDI) by consuming PAHs contaminated seafood (*Thais coronata*) was done for adult population using equation. 1 (Halek *et al.,* 2007). The average adult weight in Nigeria was taken as 70 kg (Tongo *et al.,* 2017) obtained from data of the Food and Agriculture Organization (FAO, 2014) on fishery and aquaculture statistics (Tongo *et al.,* 2018).

The Dietary Daily Intake (DDI)- ng/day) = Ci × IFR eqn 1

Where Ci=concentration of PAH in bivalve samples and IFR=fish ingestion rate (IFR) (EFSA, 2005)

Health Risk Assessment: The following indices were used for health risk assessment due to exposure by consuming PAH contaminated sea food (*Thais coronata*). Evaluation of individual PAH carcinogenic potencies, carcinogenic toxic equivalents (TEQs), PAH4 (sum of BaA, Chr, BbFL, and BaP). The excess cancer risk (ECR) and Margin of exposure (MOE) were also assessed and compared with regulatory limits where applicable.

Carcinogenic potencies of individual PAHs (B(A)Pteq) = Ci ×TEFi (Tongo *et al*., 2018) – eqn 2

TEFi = toxicity equivalency factor as used by Nisbet and LaGoy, (1992)

Carcinogenic toxic equivalents (TEQs) = ΣB (A)Pteq (Szewczynska *et al.,* 2013)

To calculate PAH4, it is the summation of BaA, Chr, BbFl and BaP ((FSAI, 2015) as used in equation 3.

PAH4 Index (PAH4) = $\sum BaA + BbFL + Chr + BaP$ - eqn (3)

The excess cancer risk was evaluated using equation 4 (Xia *et al*., 2010).

Excess Cancer Risk (ECR) = $\frac{\sum Q\ X\ B\ (A)\ PTeq\ X\ IFR\ X\ ED}{BW\ X\ ATn}$ eqn (4)

Where Carcinogenic potency of BaP (Q) mg kg^{-1} d^{-1} (7.3) (Ding, 2012)

Exposure Duration (ED) = 30 years (Qu *et al*., 2015)

Adult body weight (BW) = 70 kg (Tongo *et al*., 2105)

Average life span (ATn) = 8760 days (Huang *et al*., 2014)

Evaluation of Margin of Exposure (MOE): MOE was also evaluated as an acceptable method of risk assessment approved by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA 2005; FAO/WHO, 2005) as used in equation 5

MOE=BMD10 x BW/E - eqn (5)

Where BMDL10 =70 µg/kg bw/day (for BaP) or 340 µg/kg bw/day (for PAH4) (EFSA, 2008).

E=DDI=Dietary Daily Intake (DDI) (Tongo *et al.,* 2018)

BW=Adult body weight (BW) - 70 kg (Tongo *et al.,* 2015)

Evaluation of PAH sources: The Ant/Ant+Phen, Flu/Flu+Py (Brandi *et al*., 2007), BaA/(BaA+Chry) (Nyarko *et al*., 2011) and LMW/HMW (Nasher *et al.,* 2013) ratios were used for evaluation of possible sources of PAH in gastropod samples.

RESULT AND DISCUSSION

Concentration of PAH in Gastropod Samples Examined: The concentration of total PAH for each month was assessed and presented in Fig. 2. Total PAH concentrations (µg/kg) varied across the study period with values ranging from 65.68 – 173.52 suggesting that consumers of the gastropod (*Thais coronata*) were exposed to varying degrees of PAH at different times. The high molecular weight PAH

congeners were generally dominant relative the low molecular weight components. The value of the current study was generally higher than those reported for marine bivalves (3.26 to 64.45 ng/g) and gastropods (4.81 to 23.8 ng/g) (Pham *et al.*, 2019) but lower than total PAH concentrations (634.7, 476.7, 129.5, 452.5 and 415.0 ng g^{-1}) reported (Safahieh *et al.*, 2011) in ark clams at different sites.

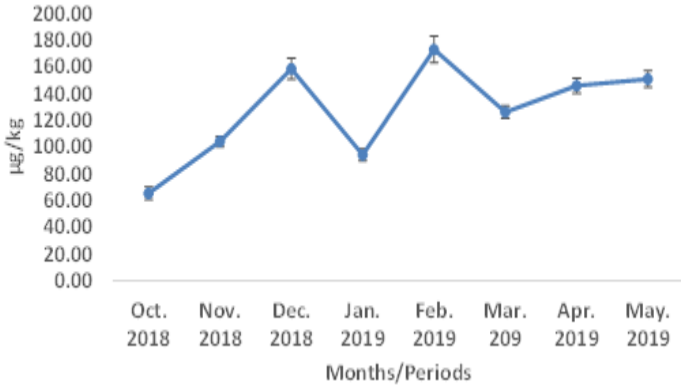

Fig.2: Temporal variation of total PAH concentrations for the study period

Individual PAH congeners also showed variations during the study period with concentrations (µg/kg) ranging from 1.376 ± 0.07 - 40.356 ± 2.21(Table 1). Indeno (1, 2, 3-cd) pyrene (Ind) had the highest mean concentration followed by Dibenzo (a, h) anthracene (DBA) while Phenanthrene (Phe) had the lowest concentration. This corroborates the report of (Nwaichi and Ntorgbo, 2016) who also reported Ind as having the highest mean concentration (171.000 ± 0.430 g kg^{-1}) during their evaluation of shell fish obtained from the Niger delta region. The mean concentration of BbFL of this study was lower than the European Union (EU) limit of 12.000 µg kg^{-1} while that of Ind exceeded the EU limit of 30.0 µg kg^{-1}. Analysis of variance (ANOVA) (Table 2) indicated that variations observed were significantly different for the following PAH congeners between the study periods: Anthracene ($p<0.05$): Dec<Apr=May=Feb=Mar<Jan=Nov=Oct; Benzo (a) anthracene ($p<0.01$): Oct<Nov=May<Dec=Mar=Apr=Jan=Feb; Benzo (k) fluoranthene ($p<0.05$): Nov<Feb=Oct=Dec= May<Mar=Apr=Jan; Dibenzo (a, h) anthracene ($p<0.05$): Dec<May= Apr=Mar=Feb =Nov<Jan=Oct.

Table 1: Summary of Parameters (Mean Concentration, DDI, B (A)Pteq, ECR and MOE, TEQ, Ant/Ant+Phen, Flu/Flu+Py, BaA/(BaA+Chry and LMW/HMW) analysed during the study

PAH Congeners	MEAN ± SE	TEFs	DDI (CixIFR)	B(A)Pteq (Ci x TEFi)	ECR	MOE
Naphthalene	3.165 ± 0.17	0.001	0.173	0.003	6.19 X 10^{-8}	2.82 X 10^{-7}
Acenaphthylene	4.032 ± 0.22	0.001	0.221	0.004	7.89 X 10^{-8}	2.21 X 10^{-7}
Acenaphthene	2.992 ± 0.16	0.001	0.164	0.003	5.86 X 10^{-8}	2.98 X 10^{-7}
Fluorene	2.278 ± 0.12	0.001	0.125	0.002	4.46 X 10^{-8}	3.92 X 10^{-7}
Phenanthrene	1.376 ± 0.07	0.001	0.075	0.001	2.69 X 10^{-8}	6.49 X 10^{-7}
Anthracene	3.739 ± 0.20	0.01	0.205	0.037	7.32 X 10^{-7}	2.39 X 10^{-7}
Fluoranthene	5.065 ± 0.27	0.001	0.278	0.005	9.91 X 10^{-8}	1.76 X 10^{-7}
Pyrene	5.343 ± 0.29	0.001	0.293	0.005	1.05 X 10^{-7}	1.67 X 10^{-7}
Benzo (a) anthracene	13.811 ± 0.75	0.1	0.757	1.381	2.70 X10^{-5}	6.47 X 10^{-6}
Chrysene	2.031 ± 0.11	0.01	0.111	0.020	3.98 X 10^{-7}	4.40 X 10^{-7}
Benzo (b) fluoranthene	2.445 ± 0.13	1	0.134	2.445	4.79 X 10^{-5}	3.65 X 10^{-7}
Benzo (k) fluoranthene	2.594 ± 0.13	0.1	0.142	0.259	5.08 X 10^{-6}	3.44 X 19^{-7}
Benzo (a) pyrene	6.516 ± 0.35	0.1	0.357	0.652	1.28 X10 $^{-5}$	1.37 X 10^{-7}
Indeno (1, 2, 3-cd) pyrene	40.356 ± 2.21	0.1	2.212	4.036	7.90 X 10^{-5}	2.21 X 10^{-6}
Dibenzo (a, h) anthracene	28.677 ± 1.57	5	1.572	143.389	2.81 X 10^{-5}	3.11 X 10^{-6}
PAH4	24.804 ± 1.35		1.359	152.244		1.75 X 10^{-7}
TEQ (All PAH Congeners)	0.1522					
TEQ (PAH4)	0.0044					
Ant/Ant+Phen	1.0013					
Flu/Flu+Py	1.0053					
BaA/(BaA+Chry)	1.0020					
LMW/HMW	0.1645					

Table 2: summary of ANOVA output with adjusted Ms and F-values

PAH Congeners	Adj MS values	F-values
Naphthalene^{ns}	0.0000630	1.02
Acenaphthylene^{ns}	0.0000335	0.83
Acenaphthene^{ns}	0.0000465	2.02
Fluorene^{ns}	0.0000151	1.50
Phenanthrene^{ns}	0.0000085	1.53
Anthracene*	0.0000858	2.97
Fluoranthene^{ns}	0.0000831	1.65
Pyrene^{ns}	0.0001095	1.71
Benzo (a) anthracene**	0.0018633	4.06
Chrysene	0.0000058	0.82
Benzo (b) fluoranthene*	0.0000144	2.43
Benzo (k) fluoranthene*	0.0000211	2.65
Benzo (a) pyrene^{ns}	0.0000407	2.11
Indeno (1, 2, 3-cd) pyrene^{ns}	0.002290	1.87
Dibenzo (a, h) anthracene*	0.0027517	2.81

*Key: ns = Not significant; * = significant ($p<0.05$); ** = significant ($p<0.01$)*

Other parameters evaluated (Table 1) during the study included daily dietary intake (DDI), potential toxic equivalence (B(A)Pteq) of individual and total PAH congeners, Excessive cancer risk (ECR), Margin of exposure (MOE), mean concentrations and toxic equivalence (TEQ (PAH4) of PAH4 and different source ratios (Ant/Ant+Phen; Flu/Flu+Py; BaA/(BaA+Chry) and LMW/HMW).

The concentration of PAH congeners of the present study was lower than values (1.2 - 2.1 PPB) earlier reported (Wickliffe *et al*., 2018) but in consonance with concentrations (9.49 - 31.23 ng/g) reported by Barlow and Muralidharan, (2012). BaP had been reported as indicator or biomarker for the presence and effect of carcinogenic PAHs in foods by European Union commission (EC, 2006).

Mean concentration (6.516 ± 0.35 µg kg−1) of BaP in this study is below European commission maximum limit of 10 ng/g for PAH congeners in bivalves (fresh weight). Mean BaP value of this study also show variance with that (79.0 ± 30 ng kg^{-1}) reported by Moslen *et al.*, (2019) in a similar study. BaP was concluded to be a human carcinogen with genotoxic effects by the International Agency for Research into Cancer (IARC) in 2012 (FSAI, 2015). European Food Safety Authority (EFSA) however, concluded that only BaP cannot be used to indicate PAH presence in food and recommended the use of PAH4 (the sum of BaP, BaA, BbFL and Chr) (FSAI, 2015). The concentration of PAH4 of the current study was below the EU maximum limit of 30 µg kg^{-1} for PAH4 in the Commission Regulation (EU) No 835/2011 suggesting low health risk.

The presence of PAH compounds in foods will remain a source of concern due to carcinogenic and mutagenic effects. Consumption of contaminated foods is one certain route of exposure hence the assessment of daily dietary intake (DDI) in this study. The DDI values of the present study ranged from 0.075 - 2.212 µg/kg for individual PAH congeners while that of PAH4 was 1.359 µg/kg. The DDI values of the present study falls below the maximum (0.0173 mg/day) reported by Tongo *et al.,* (2017) for smoked fish in the Niger Delta region, apparently due to the higher consumption rate of smoked fish. However, the DDI values of the present study was within the range (0 - 0.0005mg/kg/day) recorded by Tongo *et al.,* (2018) for various shell fish in the study area but lower than the values (0.10 - 2.33 mg/kg body weight/day) observed by Olayinka *et al.,* (2019) in fish and other shell fishes. It is pertinent to state that the DDI values of the present study were generally below the available reference dose (USEPA, 1993) of PAH congeners, implying minimal risk of exposure even at long-term consumption of the gastropod (*Thais coronata*) under investigation. The carcinogenic potencies (B(A)Pteq) of the PAH congeners were also assessed and values obtained ranged from 0.001 - 143.389 for Phenanthrene and Dibenzo (a, h) anthracene respectively while PAH4 had a value of 152.244. The B(A)Pteq values obtained in this study is comparable to values (0.012 to 900.0 ng kg^{-1}) reported by Moslen *et al.,* (2019) in similar study except BaA, BbFL, Ind, and DBA with elevated values. The carcinogenic toxic equivalents (TEQs) of all PAH congeners was 0.1522 while that of PAH4 was 0.0044 in the present study.

The Excess cancer risk (ECR): Dietary exposure by consuming contaminated seafood could also predispose humans to Excess cancer risk (ECR) Moslen *et al.,* (2019). Evaluation of ECR in this study showed values ranging from $10^{-8} – 10^{-5}$. The observed ECR values of this study compared favourably with the acceptable guideline value of 10^{-6} (USEPA, 2001). This guideline limit implies cancer risk level of one in a million ($ECR=10^{-6}$) during a seventy-year period of lifetime, is considered tolerable but a case of an extra lifetime cancer risk of one in ten thousand or greater ($ECR=10^{-4}$) is considered serious (Jing *et al.,* 2013). This is however, also subject to some national and regional standards and legislations. ECR values of this study accords with those earlier reported Tongo *et al.,* (2017) in similar studies

The margin of exposure (MOE) is defined as the ratio of the no-observed-adverse-effect level (NOAEL) or benchmark dose lower confidence limit (BMDL) for the critical effect to the theoretical, predicted or estimated exposure dose or concentration (WHO/IPCS, (2009). It is one of the most credible and practical scientific approach for the formulation of advice since it considers both the dietary exposure and the available data on the dose–response relationship when evaluating genotoxic and carcinogenic substances (Barlow *et al.,* 2006). The extent of the MOE gives an indication of the level of concern, but

not a precise quantification of the risk (Benford *et al*., 2010). The MOE of individual PAH congeners of this study ranged from 1.37 X 10^{-7} - 6.47 X 10^{-6} while that of PAH 4 was 1.75 X 10^{-7}. The MOEs of individual PAHs and PAH4 were higher the critical border line of 10,000 given by EFSA, (2005) signifying low health risk exposure margin for consumers of the gastropod (*Thais coronata*) in the study area. The MOEs of this study were generally lower than figures earlier reported in similar studies (Wu *et al*., 2016). PAH source could be petrogenic or pyrogenic in origin. Certain diagnostic ratios could be used to differentiate the sources of PAHs with respect to their stability, physical and chemical attributes (Nasher, 2013). The Ant/Ant+Phen, Flu/Flu+Py, BaA/(BaA+Chry) and LMW/HMW ratios were applied in this study and the following values (1.0013, 1.0053, 1.0020 and 0.1645) obtained respectively. In the current study, the Ant/Ant+Phen ratio was > 0.1 (Brandli *et al.,* 2007); Flu/Flu+Py ratio was > 0.4 (Brandli *et al.,* 2007), BaA/(BaA+Chry) ratio was > 0.350 (Nyarko *et al.,* 2011) and LMW/HMW ratio was <1 Nasher *et al.,* (2013) suggesting PAH sources of pyrogenic origin in the gastropod samples studied. The BaA/(BaA+Chry) values obtained in this study corresponds to values earlier reported in similar studies (Nwaichi and Ntorgbo, 2016; Nyarko *et al.,* 2011) who also attributed PAH source of their samples to be pyrogenic in origin. Moslen *et al.,* (2019) had linked major anthropogenic activities in the study area to petrogenic and pyrogenic sources including illegal petroleum refining/bunkering activities, burning of confiscated petroleum products on water ways and mangrove environments by security agencies, combustion of tyres/plastics and other organic substances of non-point sources within the Niger Delta region.

Conclusion: Regulatory agencies had recommended regular monitoring to detect PAHs in food due to carcinogenic and genotoxic health concerns posed by such chemicals. This study investigated a commonly consumed commercial seafood (*Thais coronata*) with respect to PAH contamination and health risk. Contamination concentrations in addition to standard health risk indices such as carcinogenic potencies, carcinogenic toxic equivalents, excess cancer risks and margin of exposure were evaluated and compared to available regulatory limits of USEPA and European Union. Concentration of PAH congeners were generally low except for Ind while health risk indices also compared lower than regulatory limits. Standard diagnostic ratios applied also suggested PAH of pyrogenic origin. The study therefore concluded low health risk concern for consumers of seafood (*Thais coronata*) in the study region but recommended constant monitoring to observe concentration changes.

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