

# Benzo [a] Pyrene- carcinogenic and mutagenic equivalents of fingerlings of *Clarias gariepinus* (Burchell, 1822) exposed to water soluble fractions of Nigerian Bonny Light crude oil BLCO

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Research

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## ABSTRACT

The toxicity and PAH of *C.gariepinus* (0.9±0.01g) to 1, 3, 6, 9, 12 and 0.0 ml/L of BLCO oil was determined. There was no mortality in the control but 10, 20, 50 and 60% occurred in crude oil after 96 h exposure. The logarithmic probit line and  $R^2: y = 1.372x + 3.724$ ,  $R^2 = 0.997$  and 96-h  $LC_{50}$ : 9.6 ml/L represented toxicity value for crude oil. Total PAH in exposed fish gave a value of 99.68±4.81 ng/μL. The lowest value of 0.061(0%) Nap to the highest value of 384.68 (84%) in DahA for crude oil was determined but mean  $\sum$  16PAH gave 6% compared to the 11% that of its mean  $\sum$  8PAH. The least observed difference LOD of PAH among 8PAHs was 0.02ng/μL and mean TEQ and MEQ gave 254.67 and 44.84 both corresponding to 11%. BaP-TEQ in BLCO ranged from 0.05(0%) in Chry to 1923(84%) in DahA. while BaP-MEQ ranged 0.09 (0%) Chry -119.25 (30%) DahA. The respective Benzo [a] Pyrene carcinogenic and mutagenic equivalents of *Clarias gariepinus* to BLCO was 2 and 9% respectively. In conclusion, the 96h median lethal concentration of BLCO to *C.gariepinus* fingerlings was 9.6 ml/L in which its mean  $\sum$  16PAH gave 6% compared to 11% that of mean 8PAH. Mean TEQ and MEQ in 8PAH both corresponded to 11% however BaP-TEQ and BaP-MEQ in BLCO ranged respectively from 0% in Chry to 84 and 30% in DahA. The respective Benzo [a] Pyrene carcinogenic and mutagenic equivalents of *Clarias gariepinus* to BLCO was 2 and 9%.

Key words: Toxic equivalent factor, carcinogenicity, mutagen, PAH, bonny light crude oil

## 1. INTRODUCTION

Fishes are good indicators of pollution due to the lipophilic nature and high chemical stability of PAHs which accumulate in the fatty tissues of fish after an uptake from both inland and coastal waters [11, 10, 6]. Two broad groups exist based on their physical and biological properties including, high molecular weight (HMW) and low molecular weight (LMW) PAHs. The HMW PAHs consists of 4-6 aromatic rings and are less readily bio-degraded by indigenous microorganisms, hence can persist in the aqueous environment by bio-accumulating in aquatic organisms like fish and mussels and are more carcinogenic. The LMW PAHs consists of 2-3 aromatic rings and although less carcinogenic, also pose toxic effect to many aquatic organisms [2]. The concentrations of petroleum products toxic to aquatic organisms depend on the type and hydrocarbon constituents, as well as the species involved [14, 13]. Estimated concentrations of petroleum toxic to fish eggs and fingerlings to be 0.5-10 mg/L Benzo (a) pyrene binds to DNA to cause cancer and is frequently used as a marker for carcinogenic disorders and may provide the basis for predicting the impact of exposures of PAH to *C. gariepinus* fingerlings [20]. BaP-TEQ (carcinogenic equivalents and BaP- MEQ (mutagenic equivalents are measure for sum of total 8 number of particulate PAHs ( $\Sigma 8\text{PAH}$ ), having molecular weight greater than 228 grams.  $\Sigma 8\text{PAH}$  includes benzo (a) pyrene (BaP), benz (a) anthracene (BaA), chrysene/iso-chrysene (CHR), benz (b) fluoranthene (BbF), benzo (k) fluoranthene (BkF), indo (123-cd) pyrene (IP), Dibenz (a,h) anthracene (DahA) and benzo(ghi) pyrene (BghiP) [17, 4, 5]. The hardy nature and possession of accessory air-breathing organs enable *C. gariepinus* to tolerate adverse aquatic conditions [23]. Nonetheless, *Clarias gariepinus* fingerlings are very delicate and sensitive to aquatic pollutants including crude oil and other petroleum products. The aim of the study was undertaken to determine the toxicity, PAH and carcinogen and mutagen equivalent levels of Nigerian- petroleum crude oil on *C. gariepinus* fingerlings.

## 2. MATERIALS AND METHODS

### 2.1. Experimental fish and petroleum

A total of seven hundred and twenty (180) fingerlings of African catfish (mean weight  $0.96 \pm 0.1\text{g}$ ) were obtained from local outskirts in Enugu Nigeria and transported to Fisheries Laboratory of the Department of

Animal/Fisheries Science and Management, Enugu State University of Science and Technology ESUT, Enugu Nigeria. They were held in four fiber reinforced plastic (FRP) tanks, containing 320 L of de-chlorinated tap water. Aeration was provided to all tanks round the clock in order to maintain dissolved oxygen contents. Before the commencement of the study, the fish were acclimatized for two weeks and were fed with commercial fish diet composed of 40% crude protein. The faecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. Petroleum (crude oil) was obtained from Nigerian National Petroleum Cooperation Enugu. The water soluble fraction WSF was prepared following the method of [28], which involved 20-h mixing of 10:1 clean water to petroleum with a rotator magnetic stirring rod, separated layers after resting for 12-hrs with separating flask before storing as stock solution in corked 50L plastic gallons. Ethical clearance from Enugu State University of Science and Technology Committee on Experimental Animal Care was obtained and followed.

### 2.2. Acute toxicity test

Toxicity of crude oil to *C. gariepinus* was carried out according to the OECD guideline for testing of chemicals No. 203 in a semi-static renewal system by using 200 L capacity glass aquaria. Thirty (30) fish per treatment were randomly exposed to 6 experimental treatments (1, 3, 6, 9, 12 and 0 ml/L of water soluble fractions WSF which served as the control in triplicate to determine 96h  $\text{LC}_{50}$  using the probit analysis proposed by [7, 26] and polycyclic aromatic hydrocarbons (PAH) in exposed fish [14]. The exposure was renewed each day and was analyzed using LC-MS/MS to ensure the agreement between nominal and actual concentrations of the petroleum in the aquaria. The experiment was conducted under the natural photoperiod of 12:12 light-dark cycle. The physico-chemical parameters of the test water were analyzed daily, using standard methods [1] and were recorded (dissolved oxygen  $7.50 \pm 0.45 \text{ mg L}^{-1}$ , temperature  $27.75 \pm 0.5 \text{ }^{\circ}\text{C}$ , pH  $7.8 \pm 0.13$  and free carbon dioxide  $4.28 \pm 0.6 \text{ mg L}^{-1}$ ). The test fish of 9 and 12 ml/L were sampled to determine  $\Sigma 16\text{PAH}$ ,  $\Sigma 8\text{PAH}$ , TEQ and MEQ. A portion of the sample using the GC-MC was taken for extraction and analysis of PAH [29, 19].

### 2.3 PAH extraction

The method described by [26,12,15] with slight modification for extraction and dosing of PAHs was employed.

The toxic equivalent factors ( $BaP_{TEF}$ ) and mutagenic equivalent factors ( $BaP_{MEF}$ ) relating the carcinogenic mutagenic potency of individual PAH to BaP have been used [19, 25, 5]. The BaP carcinogenic equivalent ( $BaP_{TEQ}$ ) and BaP mutagenic equivalent ( $BaP_{MEQ}$ ) for the individual PAHs was calculated:  $BaP_{TEQ} = \sum C_i \times BaP_{TEF}$ ;  $BaP_{MEQ} = \sum C_i \times BaP_{MEF}$ , where  $C_i$  = concentration of PAHs.

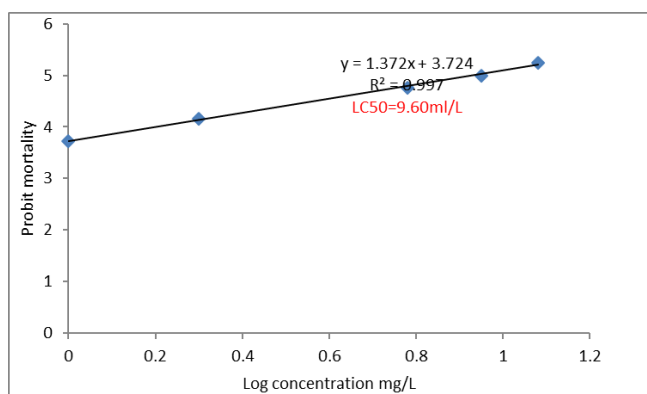
### 2.4. Statistical Analysis

Data obtained were expressed as standard mean  $\pm$  standard error of mean and analyzed using the statistical package SPSS 20.0 computer program (SPSS Inc. Chicago Illinois, USA). Figure of toxicity was done with logarithmic probit line graph while PAH and equivalent values of Bap were done on bar and pie charts.

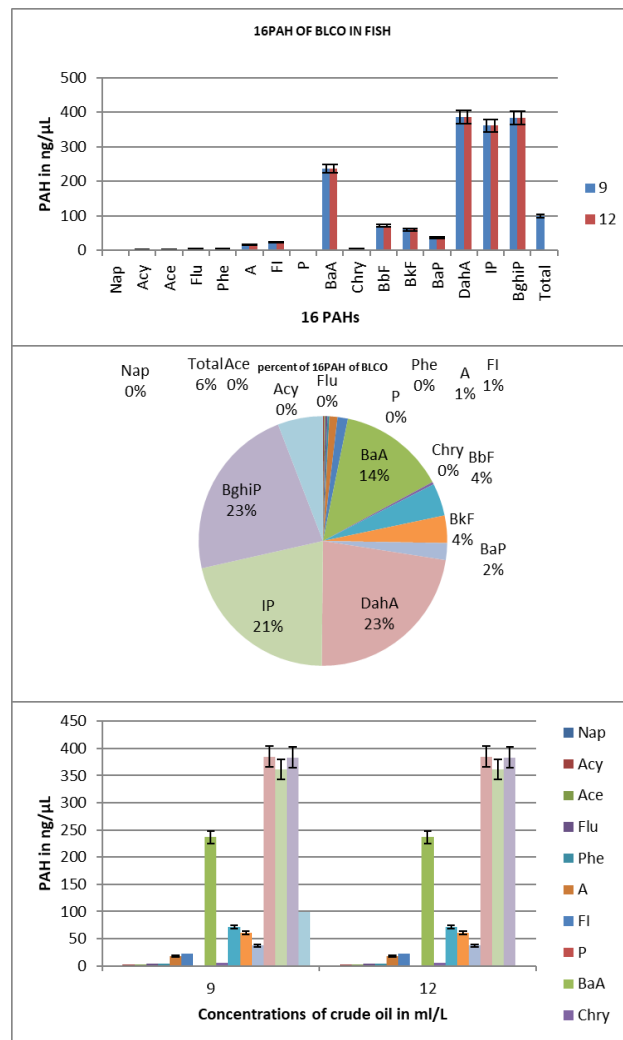
## 3. RESULTS

### 3.1 Mortality and Logarithmic probit line for 96-h $LC_{50}$ of petroleum products to *C. gariepinus*

Figure 1 gave mortality values of fish to various concentrations (0, 1, 3, 6, 9 and 12 ml/L. There was no mortality in the control but 10, 20, 50 and 60% for crude oil occurred respectively in 1, 3, 6, 9 and 12 ml/L after 96-hour exposure. Figure 1 gave the logarithmic probit lines and  $R^2$ :  $y = 1.372x + 3.724$ ,  $R^2 = 0.997$  and 96-h  $LC_{50}$ : 9.6 ml/L for crude oil to *C. gariepinus*.



**Figure 1.** Logarithmic probit line for determination of 96-h  $LC_{50}$  crude oil to *C. gariepinus*



**Figure 2.** 16PAHs in fish exposed to 9 and 12ml/L of BLCO

Different superscripts in a row indicate significant difference between means (ANOVA,  $P < 0.05$ )

KEY: Nap = Naphthalene, Acy= Acenaphthylene, Ace= Acenaphthene Flu= Fluorene, Phe= Phenanthrene, A=Anthracene, FI=Fluoranthene P = Pyrene BaA=Benz [a] anthracene, Chry = Chrysene, BbF= Benzo [b] fluoranthene, BkF=Benzo [k] fluoranthene, BaP = Benzo [a] pyrene DahA = Dibenz [ah] anthracene IP = Indeno [123-cd] pyrene, B[ghi] P = Benzo [ghi] pyrene, CO=Crude oil, P= Petrol, K= kerosene, D= Diesel, pp=petroleum products, c= concentration in ml/L, nd= not detected.

3.2 PAHs in exposed fish to petroleum

Total PAHs values in ng/μL (figure 2) of petroleum products in exposed fish ranged from highest value of 99.68±4.81 ng/μL crude oil. The lowest value of 0.061 was shown in Nap while highest value was 384.68 in DahA >383.47 B[ghi]P > 361.38 IP >236.41BaA >71.59 BbF>60.17 BkF >37.17 BaP> 22.82FI> 17.72A >5.33Chry >4.56 Flu> 4.35 Phe>2.79 Acy>2.40Ace >0.06Nap for crude oil. The mean  $\sum_{16}$ PAH gave 6% compared to the 11% that of its mean  $\sum_{8}$ PAH.

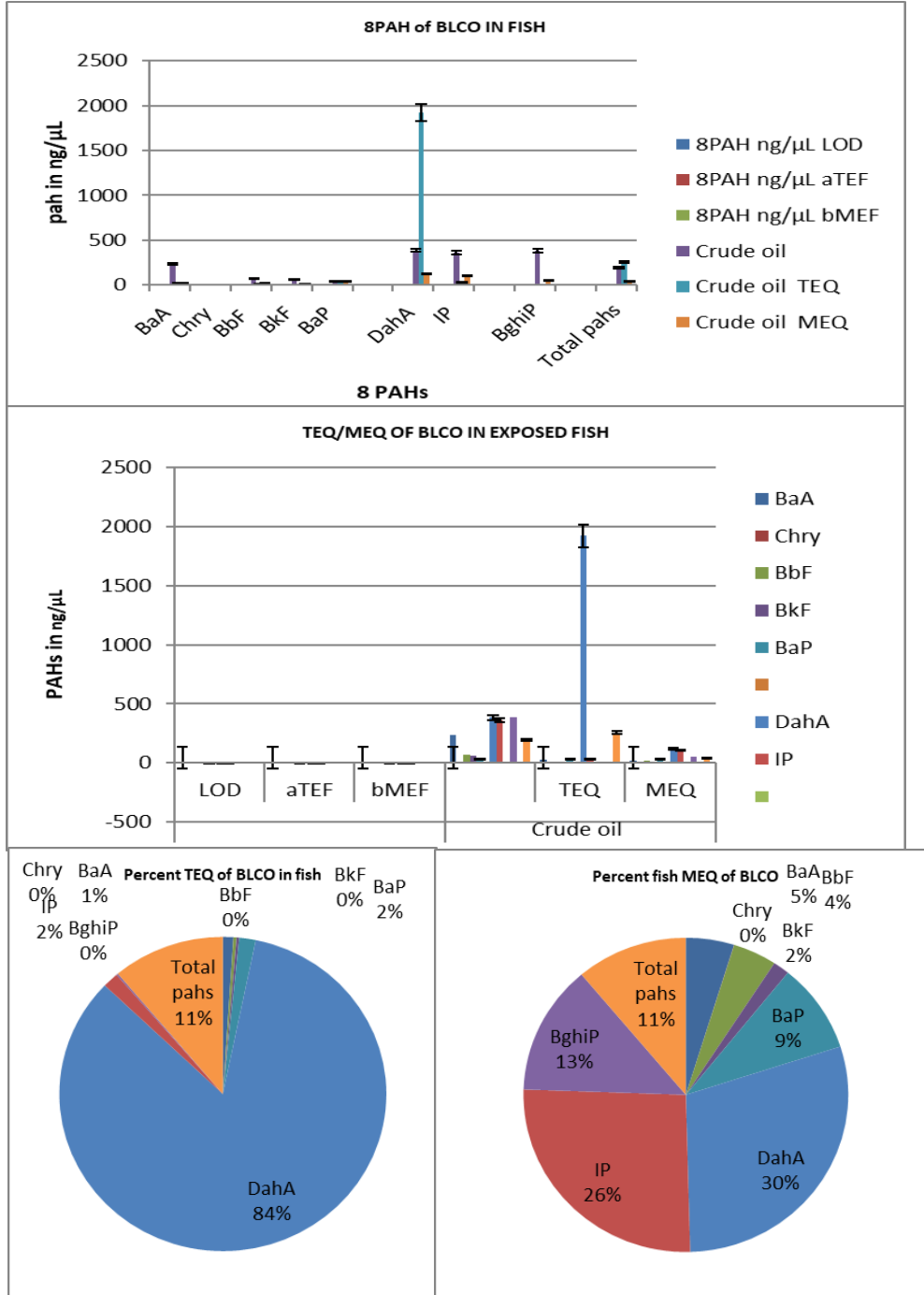


Figure 3. Bap-TEQ and Bap- MEQ of crude oil

TEF\*: toxic equivalency factors for cancer potency relative to BaP (Nisbet and LaGoy et al. 1992)

MEF\*: mutagenic potency factor relative to BaP (Durant et al. 1996 and 1999)

### 3.3 Carcinogenicity and mutagenicity equivalents

The least observed difference LOD of PAH among 8PAHs was 0.02ng/μL and mean TEQ and MEQ gave 254.67 and 44.84 both corresponding to 11%. BaP-TEQ in BLCO ranged from 0.05(0%) in Chry to 1923(84%) in DahA. Similarly, BaP-MEQ ranged 0.09 (0%) Chry -119.25 (30%) DahA. The respective Benzo [a] Pyrene carcinogenic and mutagenic equivalents of *Clarias gariepinus* to BLCO was 2 and 9% respectively.

### 4. DISCUSSION

The observed value of 9.6ml/L as 96h LC<sub>50</sub> in this study is closely related to the value of 9.35 ml/L obtained in the same species exposed also to WSF of BLCO [16], but is higher than 1.069 ml/L reported for *Oreochromis niloticus* juveniles exposed to Quai-boe light crude oil [27]. It was however lower than the range value of 32-88 ml/L for Australian crude oil to *Menidia beryllina* juvenile fish [18]. The impact of petroleum water soluble fraction previously under-reported has in recent times posed critical health concerns to aquatic biota, especially fish [8, 24]. The foregoing gave an indication that petroleum products with high molar mass and greater mean  $\sum$ 8PAH, were more carcinogenic and mutagenic compared to lighter petroleum with lower mean $\sum$ 8PAH. Crude oil has shown the ability to cause cancer and changes in the genetic makeup and may damage the genome materials or disrupt cellular metabolic processes of exposed fish to humans alike that consume them [9]. Recent approaches have centered to identify and quantify PAHs in water, soil and air environment, their emission sources through various methods in order to evaluate their carcinogenic and mutagenic effects to human health [7, 21, 3]. The approaches distinguish anthropogenic multiple releases chiefly from petroleum and other sources [20, 9]. BaP is widely accepted as the indicator for measurement of carcinogenicity, thus BaP-equivalent toxicity for other carcinogenic PAHs has been recommended [31, 2] and evaluated for cancer risk assessment [21, 5, 24, 30]. The result showed that BLCO contained greater percentage mean of 8PAH than 16PAH and was less carcinogenic than mutagenic to exposed group of *C. gariepinus*. There is greater need for further investigation of the

biochemistry and physiology on the mutagenic effects to fish, animals and humans alike that consume them given its high responses on the experimental fish to BLCO in the present research.

### 5. CONCLUSION

The 96h median lethal concentration of BLCO to the experimental fish fingerlings was 9.6 ml/L. The mean  $\sum$  16PAH gave 6% compared to 11% that of its mean 8PAH. Mean TEQ and MEQ in 8PAH both corresponded to 11% however BaP-TEQ in BLCO ranged from 0% in Chry to 84% in DahA while BaP-MEQ ranged from 0% in Chry to 30% in DahA. The respective Benzo [a] Pyrene carcinogenic and mutagenic equivalents of *Clarias gariepinus* to BLCO was 2 and 9%.

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