Heavy Metals and Polycyclic Aromatic Hydrocarbons in Commonly Consumed Crayfish in Nigeria and Health Risk Implications

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Heavy metals and polycyclic aromatic hydrocarbons (PAHs) are common environmental pollutants. Its increased presence in the aquatic environment has raised serious concerns about its effect on aquatic life and by extension, a man who is at the apex of the food chain. This research investigated the concentrations of selected heavy metals (Lead, Nickel, Cadmium and Chromium) and PAHs in two species of commonly consumed crayfish in Nigeria and their health risk implications. Wet digestion procedure and atomic absorption spectrometry were used for heavy metal determination while the United States environmental protection agency (USEPA) test method for evaluation of solid waste was used for PAHs analysis. Results showed that Pb, Ni and Cd in both species of crayfish were above tolerable limits. The mean level for Ni in *Litopenaeus setiferus* was 3.063±0.116mg/kg and 5.157±1.676mg/kg in *Litopenaeus stylirostris*. The mean value for Cd in *L. setiferus* was 0.833±0.070 mg/kg and 0.600±0.025 mg/kg for *L. stylirostris*. The mean value for Pb in *L. setiferus* was 4.550±0.656mg/kg and 3.643±0.486 mg/kg in *L. stylirostris*. Mean values for Cr in *L. setiferus* was 0.087±0.082 mg/kg and 0.000 mg/kg in *L. stylirostris*. Mean PAHs concentrations in *L. setiferus* and *L. stylirostris* were 0.0036±0.002 ppm and 0.0083±0.004 ppm respectively. Health risk assessment revealed an increasing health risk due to the...
consumption of both species of crayfish. Results for PAHs in both species of crayfish showed compliance with set limits. Its presence in fish food however suggests possible health concerns especially with regards to their carcinogenic tendencies. Anthropogenic activities should be closely monitored as bioaccumulation along the food chain is implicated.

Keywords: Heavy metals; PAHs; L. setiferus; L. stylirostris; health risk assessment.

1. INTRODUCTION

The use of heavy metals in industry, agriculture, medicine and technology has led to their abundance in nature, raising concerns about their effects on man and the environment [1]. Heavy metals presence in the aquatic environment has raised concerns about their effect on plant and animal life as metals such as Lead and Cadmium are toxic even in trace levels [2]. Although their adverse effects have been known for a long time, human exposure to these heavy metals continues and is on the rise in less developed countries. For example, tetraethyl lead remains a common additive to petrol. This use has decreased in developed countries [3]. Heavy metals released from domestic, industrial and other human activities can contaminate the aquatic environment, affecting the ecological balance of the aquatic species. Heavy metals are not eliminated from the aquatic environment by natural methods as such it has become a major health challenge in persons who eat sea foods [4]. More so, metals tend to accumulate in different organs of aquatic organisms especially fish [5]. Therefore, levels in fish is generally a reflection of the levels in sediments and water of the aquatic environment in which they are found [6]. Humans are exposed to heavy metals through air, food, water, industrial effluents (Jan et al., 2015). Although heavy metals are persistent organic pollutants (Jan et al., 2015), they are known to play pivotal roles in this present day industrialized human society [7]. Furthermore, metals like Iron, Zinc, Calcium, have been reported to be of bio importance to man [8]. These heavy metals though essential for the maintenance of body metabolism, become toxic at higher concentrations [4] Heavy metal toxicity can result to a variety of diseases that can be physiologically observed as signs and symptoms. Symptoms observed may specifically point to particular heavy metal toxicity. Signs and symptoms of the most toxic heavy metals include+ anemia, hair loss, hypertension with renal dysfunction, delayed mental development in children, headache, and loss of nails [1]. Researches have shown that heavy metals change the functioning of vital organs such as the kidney, brain, lungs, liver. Long term bodily exposure to heavy metals can result to a progressive degeneration of muscular, physical and neurological processes which bear resemblance to diseases like Parkinson’s disease, muscular dystrophy and Alzheimer’s disease [3]. Aquatic organisms such as fish have the potential for contamination and accumulation of various toxic chemicals like heavy metals, it has become a potential public health concern as consumption of fish containing high concentrations of heavy metals can result in human health problems [9].

Polycyclic aromatic hydrocarbons (PAHs) are colourless organic compounds that consist of three or more benzene rings fused in linear, cluster or angular arrangements [10, 11]. They are naturally present in the environment and are classified as persistent organic pollutants because they resist degradation and can remain in the environment for long periods [12,10]. PAHs are formed during biological processes and as products of incomplete combustion from natural or manmade combustion sources. However, incomplete combustion is the single highest contributor of PAHs to the environment [13,10]. PAHs may be formed in food during processing and domestic food preparation such as barbecuing, smoking, drying, roasting, baking, frying or grilling. Some marine organisms, are known to absorb and accumulate PAHs from contaminated water [14]. PAHs enter into the aquatic environment from anthropogenic sources such as waste water, industrial effluents and leaching from soil contaminated with PAHs [15, 16]. PAHs have a high affinity for stable particles such as sediments in the aquatic environment. PAHs that attach to sediments are ultimately released into the aquatic medium and become available to aquatic life [15]. Furthermore, the chemical stability and lipophilicity of PAHs [17] enables their build up in fatty tissues of fishes after intake [18]. This accumulation eventually results in the alteration of the biochemical and physiological response of fish [19, 20]. Over 50% of the nonsmoking human population come in contact with PAHs through their diet, by inhalation, through skin contact [16]. Long term
human exposure to PAHs may result in the reduction of immune function, liver, kidney damage, premature delivery in pregnant women, low birth weight in babies [21, 22]. Since human exposure to environmental contaminants such as PAH and heavy metals occur basically from eating food contaminated with these chemicals [23], it is therefore, crucial to investigate the levels of heavy metal and PAH in crayfish sold in markets in Rivers State, Nigeria.

This study aimed to investigate heavy metals and polycyclic aromatic hydrocarbons in commonly consumed crayfish in Nigeria and their health risks implications.

2. MATERIALS AND METHODS

2.1 Study Area

This study covered three markets in Port Harcourt metropolis which are: Choba market which is located at latitude 4° 52’ 20” N and longitude 6° 54’ 48” E, Mile one market with latitude 4° 47’ 50” N and longitude 6° 59’ 57” E and Creek road market, located at latitude 4° 45’ 38” and longitude 7° 1’ 47” E. Choba market is located in choba community, obio-Akpor Local Government area of Rivers State, Mile One market is located in Rumuwoji community, Diobu, Port Harcourt. It is a central market that hosts buyers and sellers from different areas of Port Harcourt for business transactions (Okeji et al., 2016). Creek Road market is also known as Borokiri market is located in the old Port Harcourt town.

2.2 Sample Collection

Dried samples of Litopenaeus setiferus (white crayfish) and Litopenaeus stylirostris (native crayfish) were bought from three random sellers in each of the three markets in Port Harcourt. They were kept in plastic bags, labelled accordingly and sent to the laboratory the same day for analysis.

2.3 Sample Preparation for Heavy Metal Determination in Crayfish

Nitric-Perchloric Acid digestion was performed following the procedure recommended by the AOAC [24].

1 g of crayfish was oven-dried at 60°C then put into a 250 ml digestion tube. 10 ml of concentrated HNO3 was added. The contents were mixed and were gently heated at a low to medium heat for 30-45 minutes to allow for the oxidation of all easily oxidizable matter. The mixture was allowed to cool then 5 ml of 70% HClO4 was added and the mixture was boiled gently until dense white fumes appeared.

After cooling, 20 ml of distilled water was added and the mixture was boiled again to release any fumes. The mixture was allowed to cool, then it was filtered completely and transferred quantitatively into a 25 ml volumetric flask by adding distilled water. (Use whatman No.42 filter paper and <0.45µm Millipore filter paper). Heavy metals were determined using atomic absorption spectrometry. (GBC Scientific Equipment, Australia, 2)

Plate 1. Dry sample of Litopenaeus stylirostris  Plate 2. Dry sample of Litopenaeus setiferus
2.4 PAH Analysis for Samples of Crayfish

USEPA Test method 1996 for the evaluation of solid waste was used for the analysis of PAH.

2.5 Health Risk Assessment of Heavy Metals

Several toxicological indices as mentioned below were used to estimate Health Risk Assessment via consumption of the seafood samples.

2.6 Estimated Daily Intake of Metals (EDI)

The method previously described by Gebeyehu et al. [26] was employed for the estimated daily intake of heavy metals. Estimated Daily Intake (EDI) = \( \frac{EF \times LF \times C \times IR \times FC}{BW \times AT} \times 10^{-3} \) (1)

Where EF (exposure frequency) = 365 days/year [26,27,28, 29];
IR (rate of ingestion) = 100 g/day [30, 31]
LF (life expectancy of Nigeria or exposure period Iwegbue et al. [26,27]
BW (body weight) = 70g for adult [30, 32, 31]
AT (average time) = 365 days/ year [32, 26,27]
FC (factor of conversion) = 0.085 Gebeyehu et al. [25]
C (concentration of the fish food).

2.7 Non-Carcinogenic Health Risk

2.7.1 Target hazard quotient

The hazard quotient is the ratio between exposure and reference dose and was calculated based on the method described by Chien et al. [33].

Hazard quotient (TH) = \( \frac{EDI}{RfD} \) (2)

Where EDI is the estimated daily intake; and RfD is the reference dose of the individual metals, thus Cd (0.001), [27, 28]. Pb (0.004), [27, 28]. Cr (0.003) [27,28] Ni (0.02) [27,28]. When HQ is < 1 adverse health effect is unlikely to be observed, but when HQ > 1, there is a tendency that adverse effects are likely to be created or observed [29, 34].

2.7.2 Hazard index

The hazard index was calculated as the summation of all hazard quotients as previously applied by authors [35, 29].

Hazard index (HI) = \( \sum HQ \) 3

Like hazard quotients, HI exceeding 1 suggests adverse health effects, while HI<1 suggesting no apparent health effect [25].

2.8 Carcinogenic Risk

The method previously described by [36] and applied by [25], [29] was employed for the calculation of carcinogenic risk.
Carcinogenic risk = EDI × CSF

Where,
EDI is the estimated daily intake of each heavy metal (mg/kg/day)

CSF (carcinogenic slope factor) for Pb is 0.0085 mg/kg/day [37], 0.38 mg/kg/day for Cd (Yang et al., 2018), 1.7 mg/kg/day for Ni [38] and 0.5 mg/kg/day for Cr (Zeng et al., 2015; [25]). The allowable predicted lifetime risks for carcinogens is $10^{-6}$ to $10^{-4}$ [35, 29].

2.9 Statistical Analysis

Statistical package for Social Science (SPSS version 20) was used to carry out the statistical analysis. The data were expressed as mean ± standard error. One-way analysis of variance (ANOVA) was carried out at $p < 0.05$, and Tukey HSD (Honestly Significant Difference) was used to discern the source of the observed difference. Student t-test was used to show significant deviation for PAHs between the two species of fish food under study ($p = 0.05$).

3. RESULTS

3.1 Heavy Metal Levels in *Litopenaeus setiferus* and *Litopenaeus stylirostris*

The values of heavy metal concentration in the two species of crayfish studied is presented in Figs. 2 and 3. Ni ranged from 2.573 mgkg$^{-1}$ (Creek road market) to 3.063 mgkg$^{-1}$ (mile one market) for *L. setiferus*, and 2.007 mgkg$^{-1}$ (Creek road market) to 5.157 mgkg$^{-1}$ (Choba market) for *L. stylirostris*. Cd ranged from 0.357 mgkg$^{-1}$ (Creek road market) to 0.833 mgkg$^{-1}$ (Choba market) for *L. setiferus*, and 0.450 mgkg$^{-1}$ (Creek road market) to 0.600 mgkg$^{-1}$ (Choba market) for *L. stylirostris*. Pb ranged from 3.330 mgkg$^{-1}$ (Mile one market) to 4.550 mgkg$^{-1}$ (Choba market) to 2.787 mgkg$^{-1}$ (Creek road market) to 3.643 mgkg$^{-1}$ (Choba market) for *L. stylirostris*. Cr in this study was 0.087 mgkg$^{-1}$ at Choba market, while the other market locations recorded Cr concentrations as 0.000 mgkg$^{-1}$ (not detected) for both studied species.
3.2 Estimated Daily Intake (EDI) in *Litopenaeus setiferus* and *Litopenaeus stylirostris* for Consumers (mg/kg/day/body weight)

The Estimated Daily Intake of heavy metals in both *Litopenaeus* spp. is presented in Figs. 4 and 5. Ni in *L. setiferus* ranged between 0.0153 mg/kg/day/body weight and 0.0182 mg/kg/day/body weight while that of *L. stylirostris* ranged between 0.0119 mg/kg/day/body weight and 0.0306 mg/kg/day/body weight. Cd in *L. setiferus* ranged between 0.0021 mg/kg/day/body weight and 0.0049 mg/kg/day/body weight while that of *L. stylirostris* ranged between 0.0027 mg/kg/day/body weight and 0.0036 mg/kg/day/body weight. Pb ranged between 0.0198 mg/kg/day/body weight and 0.0270 mg/kg/day/body weight for *L. setiferus*, and between 0.0165 mg/kg/day/body weight and 0.0216 mg/kg/day/body weight for *L. stylirostris*. Cr concentrations in samples purchased from Choba market recorded EDI values of 0.0000 mg/day/kg and 0.0005 mg/day/kg for both species.

3.3 Target Hazard Quotient (THQ) and Hazard Index (HI) of Heavy Metals in *Litopenaeus setiferus* and *Litopenaeus stylirostris* for consumers

The target hazard quotient and hazard index of heavy metals in both *Litopenaeus* spp. is presented in Figs. 6, 7 and 8. THE THQ of Ni ranged from 0.930 to 1.050 for *L. setiferus*, and 1.030 to 2.460 for *L. stylirostris*. Cd ranged from 3.100 to 5.700 for *L. setiferus* and 2.900 to 4.200 for *L. stylirostris*. Pb ranged from 3.100 to 5.700 mg/kg/day/body weight and 0.0270 mg/kg/day/body weight for *L. setiferus*, and between 0.0165 mg/kg/day/body weight and 0.0216 mg/kg/day/body weight for *L. stylirostris*. Cr concentrations in samples purchased from Choba market recorded EDI values of 0.0000 mg/day/kg and 0.0005 mg/day/kg for both species.
for *L. stylirostris*. Pb ranged from 5.725 to 9.675 for *L. setiferus* and 5.800 to 6.600 for *L. stylirostris*. Cr in both *Litopenaeus* spp ranged from 0.0000 to 0.500. Hazard index across the three markets ranged from 11.295 to 15.805 and 10.180 to 12.960 for *L. setiferus* and *L. stylirostris* respectively.

### 3.4 Carcinogenic Risk (CR) of Heavy Metals in *Litopenaeus setiferus* and *Litopenaeus stylirostris*

The carcinogenic risk of heavy metals in both *Litopenaeus* spp is presented in Figs. 9 and 10. The carcinogenic risk for Ni ranged from 3.3E-02 to 3.6E-02 in *L. setiferus* and 3.5E-02 to 8.4E-02 in *L. stylirostris*. Cd ranged from 1.2E-03 to 2.2E-03 in *L. setiferus* and 1.1E-03 to 1.6E-03 in *L. stylirostris*. Pb ranged from 2.0E-04 to 3.3E-04 in *L. setiferus* and 2.0E-04 to 2.2E-04 in *L. stylirostris*. Cr ranged from 7.5E-04 to 0.00000 in *L. setiferus* and 0.00000 for *L. stylirostris*.

### 3.5 Mean Concentration (ppm) of PAH in *Litopenaeus setiferus* and *Litopenaeus stylirostris*

The mean PAH concentrations for both *Litopenaeus* spp is presented in Fig. 11. The mean value for PAH in this study for *Litopenaeus setiferus* was 0.0036±0.002 while *Litopenaeus stylirostris* had a PAH value of 0.0083±0.004.
Fig. 8. Hazard Index (HI) of heavy metals in *Litopenaeus setiferus* and *Litopenaeus stylirostris* for consumers

Fig. 9. Carcinogenic risk of heavy metals in *Litopenaeus setiferus*

Fig. 10. Carcinogenic Risk of Heavy Metals in *Litopenaeus stylirostris*
4. DISCUSSION

4.1 Concentration of Heavy Metals in *Litopenaeus setiferus* and *Litopenaeus stylirostris*

It was observed that Nickel (Ni) (Figs. 2 and 3) for both *Litopenaeus* spp in the study area exceeded the permissible limit of 0.5mgkg$^{-1}$ by FAO/WHO [39]. The Ni level in this study was found to be lower than the level reported by Ezemonye et al. [40] on Potential health risk consequences of heavy metal concentrations in surface water, shrimp (*Macrobrachium macrobrachion*) and fish (*Brycinus longipinnis*) from Benin river, Nigeria. The results of this study is however similar to the level of Ni reported by Zodape, [41] on metal contamination in prawns and shrimps from Malad market of Mumbai.

Ni may enter the aquatic environment through direct leaching from rocks [42]. High concentrations of Ni induces stress reaction, which results in reduced immune potential and increased mortality of fishes [43].

Cadmium (Cd) level in Figs. 2 and 3, showed both *L.setiferus* and *L.stylirostris* was higher than 0.50 maximum allowable limit specified by Food and Agricultural Organization/World Health Organization [39, Elnabris et al., 2013], 0.01 mg/kg as specified by World Health Organization (WHO, 2003; Anim-Gyampo et al., 2013; Izah et al., 2016), 0.2 mgkg$^{-1}$ permissible limit by (USEPA 2011). The Cd levels in this study were seen to be higher than values reported by Orajiaka et al. (2020) on bioaccumulation of heavy metals and potential health risk through consumption of sea foods. However, the results of this present study are in close range to the level obtained by Ezemonye et al. [40] who revealed that Cd levels in shrimps (*Macrobrachium macrobrachion*) obtained from the Benin river were above permissible limits.

High concentrations of Cd in the human body has been linked to very serious health conditions such as liver and kidney damage, and bone demineralization (Muhammad et al., 2014; Izah et al., 2016). Studies have shown that exposure to high concentrations of Cd reduces feeding activity which results in the inhibition of growth in fish [44].

Lead (Pb) level in Figs. 2 and 3, showed both *Litopenaeus setiferus* and *Litopenaeus stylirostris* exceeded permissible limit of 0.5mgkg$^{-1}$ specified by FAO/WHO [39], 0.01 mgkg$^{-1}$ limit as specified by World Health Organization (WHO, 2003; Anim-Gyampo et al., 2013; Izah et al., 2016), furthermore, Pb level in *L. setiferus* and *L. stylirostris* across the three markets where either above or within the 4.0mgkg$^{-1}$ permissible limit specified by USEPA (2011) apart from *L. stylirostris* purchased from creek road and mile one markets which were less than the 4.0 permissible limit by (USEPA...
The Pb levels in this study were seen to be lower than values reported by Ezemonye et al. [40] on Potential health risk consequences of heavy metal concentrations in surface water, shrimp (Macrobrachium macrobrachion) and fish (Brycinus longipinnis) from Benin river, Nigeria. The result of this present study is in close range to the results reported by Orajiaka et al. (2020) on bioaccumulation of heavy metals and potential health risk through consumption of sea foods.

Pb exposure accounts for 0.6% of the global burden of diseases, with the highest burden in developing countries. According to WHO, Pb is a toxic metal whose common use has caused extensive environmental contamination and health problems in many parts of the world especially in developing countries [45]. Pb is not useful for fish, and too much amounts can cause decreases in the sustenance of life, and growth rates, as well as development and metabolism, in addition to increased mucus formation in the aquatic species (Orajiaka et al., 2020).

The chromium (Cr) level in Figs. 1 and 2, is below the 0.6 mgkg\(^{-1}\) permissible limit by WHO/FAO (1989), the 8.0 mgkg\(^{-1}\) recommended permissible limit by (USEPA 2011). The Cr level is this study is similar to the report of Omolara et al. [46] on the assessment of heavy metal content in imported and local fish and crustacean species obtained within Lagos metropolis. Cr is one of the world’s highly soluble metal pollutant that has various uses in metals and chemical industries such as stainless steel production and non-iron alloy production (Das & Mishra, 2007). Cr may enter the aquatic environment during weathering of Cr containing rocks, soil leaching (Oliveira, 2012). The Cr level in this study suggests that there may be no acute toxicity associated with Cr as a result of consumption of both Litopenaeus setiferus and Litopenaeus stylirostris from the study area, thereby portending no adverse health effects accrued to high chromium content such as damage of liver and kidney, disorder of the nervous and circulatory system, and respiratory problems such as running nose, and breathing challenges (Muhammad et al., 2014; Izah et al., 2016).

**4.2 Estimated Daily Intake of Heavy Metals in Litopenaeus setiferus and Litopenaeus stylirostris**

The daily intake of metals in the adult population through the consumption of both Litopenaeus spp. (Figs. 4 and 5) was compared to the recommended daily intake level (DIL) of metals and their upper tolerable daily intake (UTDI) set up by the Institute of Medicine for people aged 19 to 79 years [47, 48]. Ni through EDI was lower than TDI and UTDI reported by Food and drug administration, [47,48]. This indicates that there may be no health risk from the ingestion of Ni through the consumption of both Litopenaeus spp. The Pb and Cd through EDI were more than the TDI but below the UTDI, which indicates that the absorption of Pb and Cd into the body system was within the UTDI range. Considerably, there may be no health risk from ingestion of Pb and Cd through the intake of both Litopenaeus spp from the EDI analysis of Pb and Cd. The EDI of Cr was lower than the recommended TDI indicating that there may not be toxicity emanating from ingestion of Cr through both Litopenaeus spp.

**4.3 Target Hazard Quotient (THQ) and Hazard Index (HI) of Heavy Metals in Litopenaeus setiferus and Litopenaeus stylirostris**

The target hazard quotient (THQ) results showed that for Ni (Figs. 6 and 7) the values were greater than 1(>1) except for choba and mile one market locations where the values for L. setiferus were less than 1(<1). In addition, Cd had values exceeding 1 for both species. Pb had THQ values greater than 1(>1) for both species. For Cr, in L. setiferus, the THQ at choba market location was less than 1(<1), while the remaining locations and species recorded a THQ of 0.0000. This result implies that the population in the study area may be exposed to non-carcinogenic health risks due to the ingestion of both Litopenaeus spp. The Hazard Index (HI) values (Fig. 8) for both species shows that there is an indication of aggravated health risk due to the consumption of both L. setiferus and L. stylirostris.

**4.4 Carcinogenic Risk of Heavy Metals in Litopenaeus setiferus and Litopenaeus stylirostris**

The U.S. Environmental Protection Agency reported that 10\(^{-6}\) to 10\(^{-4}\) is the range of allowable lifetime risk expected for carcinogens. Based on the carcinogenic risks for studied heavy metals in both Litopenaeus spp, (Figs. 9 and 10) the values often exceeded the permissible predicted lifetime risks for carcinogens. As such, the risk of developing cancer due to the ingestion of this
fish species (L. setiferus and L. stylirostris), thereby prompting the need to carefully assess anthropogenic activities that may increase the concentration of heavy metals in the fish food within the study area.

4.5 Mean Concentration (ppm) of PAH in Litopenaeus setiferus and Litopenaeus stylirostris

The mean value for PAH in this study (Fig. 11) for L.setiferus was 0.0036± 0.002 while L.stylirostris had a PAH value of 0.0083±0.004. Statistically, there is no significant difference in terms of PAH concentrations between the two species. WHO (2003), recommended a maximum intake of 0.02ppm (human) body weights. The results from this study showed that crayfish, (white and native) purchased from these markets do not pose any health risk as the PAH concentration levels are lower than the WHO recommended standard. However, the occurrence of PAH in fish food suggests possible health concerns, especially with regards to their carcinogenic tendencies (Akpambang et al., 2009).

5. CONCLUSION

Heavy metal concentrations of Pb, Ni, and Cd in both species of crayfish were above WHO/FAO standard except Cr whose concentration was below WHO/FAO standard. This implies that anthropogenic activities around the Port Harcourt metropolis had a negative impact on both species of crayfish and as such pose a risk to the human population that ingest them. Health risk assessment showed an aggravated risk of cancer as well as other symptoms of heavy metal toxicity due ingestion of both species of crayfish. Average PAH levels in both species of crayfish were below WHO standard. However, its presence in fish food suggests possible carcinogenic tendencies.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, this research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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