Assessment of Concentrations and Potential Health Risk of Heavy Metals in Biscuits Commonly Consumed by Primary School Children in Choba, Port Harcourt, Nigeria

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ABSTRACT

Biscuits are widely consumed by primary school children as easy snack especially during break-periods in Nigeria and nowadays heavy metals are omnipresent, hence, metals could be picked up in any of the biscuit manufacturing process flow line such as mixing, cutting, baking and packing and thus becoming a potential public health issue. Therefore, the objective of this paper is to assess the concentrations and potential health risk of heavy metals in biscuits commonly consumed by primary school children in Choba, Port Harcourt, Nigeria. The metal concentrations were determined by Solaar Thermo Elemental Atomic Absorption Spectrophotometer, Model SN-SG 710960 after digesting the biscuit samples using a mixture of 0.1 mL of concentrated HNO_3, 12.9 mL of water, and 2 mL of H_2O_2 at 100^oC temperature. The mean concentrations (, mg/kg) of heavy metals in the biscuits investigated were Fe: 26.33 ± 0.21; 21.56 ± 0.44; 15.76 ± 0.27; Ni: 0.40 ± 0.042; 0.57 ± 0.022; 0.33 ± 0.014 and Pb: 0.0013 ± 0.002; 0.0014 ± 0.003; 0.001 ± 0.002 for biscuit types A, B and C respectively. These concentration of metals in biscuits from this study were compared against WHO/FAO maximum permissible limits, mg/kg (Fe: 426; Ni: 7.9 and Pb: 0.3) set for biscuits. The results indicate that the levels of iron, nickel, and lead detected in all brand of biscuit were significantly below the WHO/FAO limits. The potential health risk of heavy metals in biscuits commonly consumed by primary school children were further appraised by the estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI), and lifetime carcinogenic risk (LCR). Data obtained reveals that the EDIs ranged from 6.25×10^{-7} to 1.6×10^{-2} mg/kg bw/day, the THQs fluctuated between 1.03×10^{-5} to 5.37×10^{-4}, while the HIs varied between 1.42×10^{-3} to 5.39×10^{-4}). Values of HI in all biscuit types investigated are less than unity (HI < 1), suggesting that no possible health risk could be developed from the intake of the biscuit bbrands by primary school children. The values of LCR in all types of biscuits are between 1.87×10^{-4} to 2.84×10^{-9}, which also showed negligible cancer risks. The results demonstrate that iron, nickel, and lead in popular primary school children biscuits in Nigeria poses very pose minimal health risks children. Nevertheless, extended investigation covering more metals and biscuits types would offer additional guarantee of biscuit safety.

Keywords: Biscuits; potential health risk; heavy metals; primary school children; lifetime carcinogenic risk, school snack

INTRODUCTION

According to Arigbede et al (2019), biscuit is a baked, edible, and common flour based food product with key raw materials such as flour, sugar, shortenings, salt, glucose, starch and milk or water for production. However, production of the numerous types of biscuits is depended on the ingredients used and manufacturing process employed. Biscuits are one of the important bakery products in human diet and can be eaten the whole day regardless of time. They are favorite food items for children and are most times granted to them as an expression of love and fondness from friends and family (Iwegbue *et al* (2012). They are also important constituents of Nigerian children's diet in terms of quantity consumed where most of these children eat biscuit on a daily basis. Thus, the chemical contamination of biscuit is possible to affect majority of these children. The total biscuit consumption is estimated to be between 450,000 to 500,000 metric tons.

Food products most often contain small amounts of heavy metals which contribute to dietary intakes and the levels of these metals requires regular observations and control. However, contamination of food products by heavy metals is becoming an unavoidable problem and has been important in extensive research in the last decades (Orecchio and Papuzza 2009). Biscuit is a baked, edible, and common flour-based food product. The key raw materials for production of biscuits are flour, sugar, shortenings, salt, glucose, starch and milk or water. Production of several types of biscuits such as plain, cookies, oates, cream crackers, pop tarts, short bread, digestives, biscotti, shortcakes, ginger nut, cabin, rich tea, wafers depend on the ingredients used and manufacturing process employed (Olayemi *et al.*, 2019). Biscuits are one of the important bakery products in human diet and can be eaten the whole day regardless of time. They are favorite food items for children and are most times granted to them as an expression of love and fondness from friends and family (Iwegbue, 2012). They are also important constituents of Nigerian children's diet in terms of quantity consumed where most of these children eat biscuit on a daily basis. Thus, the chemical contamination of biscuit is possible to affect majority of these children. The total biscuit consumption is estimated to be between 450,000 to 500,000 metric tons (Olayemi *et al.*, 2019).

Heavy metals contaminants do not dissolve, and they accumulate in the environment (Ciont *et al.*, 2022). The diverse and emerging food security issues have become a global concern (Rai *et al.*, 2019). Traces of different heavy metals were found in food (vegetables, meat, fish, milk, etc.) due to their mobility and bioaccumulation in water sources. By whatever means heavy metals enter food and beverages, once they enter the body, they are oxidized and form stable bonds to enzymes or protein molecules. This results in dysfunction, abnormalities, or even damage (Goswami and Baroliya 2023). Nowadays, there is a growing preoccupation regarding the heavy metal contents in food and beverages and their subsequent consequences for the daily diet and human life chain safety.

Daily meals and intermittent intake of snacks constitute daily food intake. Snacking can effectively contribute to daily nutrient intake and energy requirements; however, the quality and type of this contribution differs from person to person due to factors such as their socio-cultural context, body weight, age and gender. Snacking is known to influence the development of hyperphagia, a health condition which involves compulsive overeating over a long period of time (Bellisle *et al.*, 2003; Iweala *et al.*, 2014). These foods, however, may contain both essential and toxic metals at various concentrations (Radwan and Salama 2006; Hezbullah *et al.*, 2016). Countries around the world have adopted a dietary guideline which involves the inclusion of a variety of food in the diet for the purpose of achieving nutrient adequacy.

Biscuits, like many other processed snacks, can be harmful for children for several reasons: High in sugar: Many biscuits contain high levels of added sugar, which can contribute to the development of tooth decay, weight gain, and increase the risk of type 2 diabetes. Since biscuits is loved by children and also one of the most highly consumed snacks by primary school children in Nigeria, the profiling of heavy metals in biscuits and the possible health risk assessment on the selected biscuits samples is imperative. Hence, the , the objective of this paper is to assess the concentrations and potential health risk of heavy metals in biscuits commonly consumed by primary school children in Choba, Port Harcourt, Nigeria with a view to providing information on the concentration profiles and dietary intakes of heavy metals, and the life-long health risks associated with the eating of biscuits.

MATERIALS AND METHOD

Description of Study Area

Choba, Port Harcourt is a neighbourhood in Port Harcourt, Rivers State, Nigeria. It is located 16 km (9.9 mi) to the northwest of the Port Harcourt central business district, on the eastern bank of the New Calabar River. In 1975 the University of Port Harcourt was established and situated in Choba. There is also the University of Port Harcourt Teaching Hospital, which is also located at Choba. Consequently, there are several privately and Government owned Primary Schools in Choba.

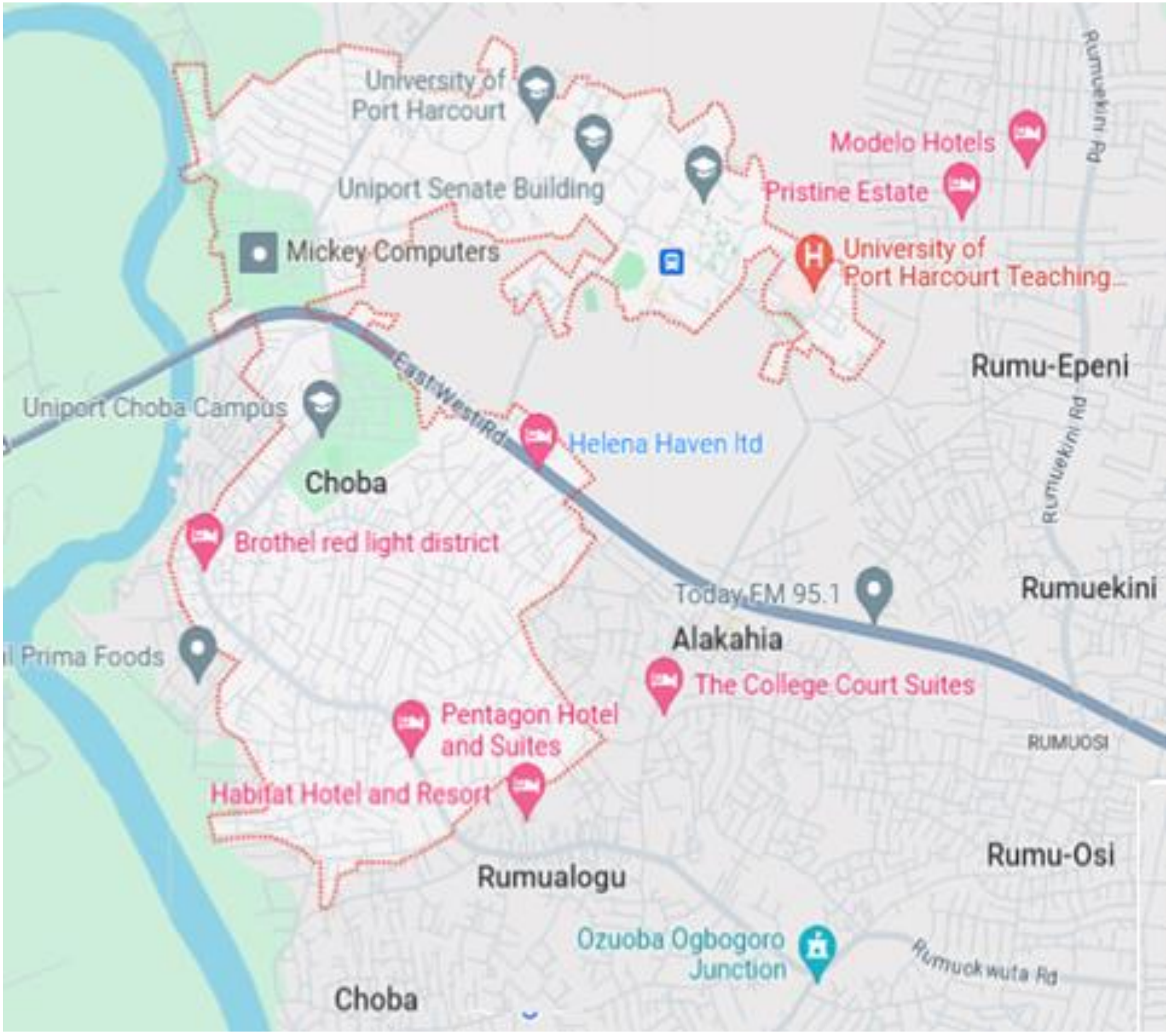

Figure 1. Map of the Study Area Source: Google map (https://www.google.com/maps/place/Choba)

Sample Selection and Collection

An on the spot surveillance was carried out for a period of one-term consisting of 15 weeks in the private and government owned primary schools to ascertain the type of biscuits commonly provided to the primary school children by their parents as snacks. Three different brands of biscuits commonly consumed by the primary school children and easily commercially available ones were purchased from various retail shops and supermarkets in Choba, Rumuoalogu and Alakahia Nigeria. Collecting the biscuits as based on batch numbers and date of manufacture. A total of 3 brands of biscuit samples with three different batch numbers and different dates of manufacture were taken together and were labeled as Biscuit Type A, Biscuit Type B and Biscuit Type C.

Sample Homogenization: The biscuit samples were cut open from their polyethylene wrappers. Then the biscuit samples were dried in an oven at 60ºC approximately 30 min for complete crispiness of samples. Each sample was ground to fine powder using a pre-cleaned agate mortar and pestle.

Sample Digestion

A 0.2 g of powdered biscuit sample of each of the thirty biscuit samples was transferred into a Teflon beaker, followed by addition of a mixture of 0.1 mL of concentrated HNO_3, 12.9 mL of water, and 2 mL of H_2O_2 and heated in a fume chamber for 2 hours at 95 ^{o}C. The digested aliquot was withdrawn from the hot plate and was cooled to room temperature, poured into 25 mL plastic vial and made up to the mark with distilled deionized water. The concentrations of Fe, Ni and Pb in digested sample solution were determined using Solaar Thermo Elemental Atomic Absorption Spectrophotometer, Model SN-SG 710960.

Quality Control

The quality control was performed in order to determine the reliability and accuracy of results. The quality control approaches included the use of sample replicates, analytical blanks, calibration and recovery analysis. Analytical blanks were also prepared in the same manner excluding the sample for metal determination.

Potential Health Risk Assessment

Estimated Daily Intake (EDI)

Since biscuits samples are simple a consumable products an estimated daily intake of the sample has to be determine using the following formula (Equation 1)

$$EDI = \frac{Cm\ x\ IR}{BW} \qquad (1)$$

Where Cm represent Concentration of heavy metals in selected biscuits brands in (mg/kg), IR the ingestion rate of heavy metals in (kg/person/day) and BW the average body weight of individuals for both Adult and Children consuming these biscuits.

Target Hazard Quotient (THQ)

This is used to estimate non-carcinogenic risk level of heavy metals in a food sample where THQ <1 it signifies no sign of any health challenges pose by the heavy metals present in the sample while THQ > 1 signifies possible life threat when the sample is consumed (USEPA 2016). Equation 2 is employed to estimate the THQ.

$$THQ = \frac{EDI * EF * ED}{AT * RFD} x\ 10^{-3} \qquad (2)$$

Target Hazard quotient (THQ) was calculated from the ratio of EDI to the oral reference dose (RfD), where, ED is the exposure duration (6 years), EP is exposure frequency (365 days/year); AT is the average time for non-carcinogens (ED × EP). A reference dose (RfD) is an approved amount of heavy metal that can be ingested daily over a lifetime without appreciable risk to human health it differs from metal-to-metal nickel 0.02 mg/kg/day, lead 0.004 mg/kg/day and Iron 0.007 mg/kg/day (USEPA 2007; USEPA 2011).

Hazard Index

Hazard Index (HI) is the combination of individual THQs to form the hazard index (HI) it is required for the risk assessment for the presence of heavy metals in the biscuit samples is presented in equation 3..

$$\boldsymbol{HI} = \sum THQFe + THQNi + THQPb \quad (3)$$

The values of HI are also used to show the level of non-carcinogenic effect of heavy metals present in a sample if HI < 1 it indicates no possible health risk to be acquired from the Intake of the biscuits but if HI > 1 it signifies possible health risk to be gotten on the long run.

Incremental lifetime carcinogenic risk (ILCR)

Carcinogenic risk (CR) specifies an increase in the possibility of an individual of developing cancer over a lifetime owing to exposure to a potential carcinogen. The possibility of cancer risks in the studied biscuit samples through the intake of carcinogenic heavy metals was estimated using the Incremental Lifetime Cancer Risk (ILCR). Incremental Lifetime Cancer risk to individual metal was obtained using cancer slope factor (CSF), which is the risk produced over a lifetime exposure of 1 mg/kg bw/day and it is specific to a particular contaminant (Bamuwamye *et al.,* 2015). The carcinogenic risk of both Pb and Ni were calculated. The equation that was applied for estimation of the cancer risk is as follows in equation 4 (Liu et al, 2013):

$$\mathbf{ILCR = CSF \times EDI} \quad (4)$$

Where, ILCR is the Incremental Lifetime Cancer Risk (limitless), CSF is the carcinogenic slope factor for Pb and Ni as 0.0085 mg/ kg/day and 0.84 mg/kg/day, respectively and EDI is the estimated daily intake of individual metals (µg/kg/bw/day).

Acceptable risk levels for carcinogens range from 10^{-4} (risk of developing cancer over a human lifetime is 1 in 10,000) to 10^{-6} (risk of developing cancer over a human lifetime is 1 in 1,000,000). This means that carcinogenic risk index between 10^{-6} and 10^{-4} indicates an interval of allowable predicted lifetime risks for cancer causing agents. Hence, contaminants having the risk factors less than 10^{-6} may not be treated as contaminants for further concern. The cumulative cancer risk as a result of exposure to multiple carcinogenic trace metals due to consumption of a particular brand of biscuit was assumed to be the sum of the individual trace metal increment risks and calculated by the equation 5 (Liu et al, 2013)

$$ILCR = CSF \times EDI \qquad (5)$$

Where, CSF is the carcinogenic slope factor for Lead and nickel is 0.0085 and 1.7mg /kg-1 day-1 USEPA 2004, respectively, and EDI is the estimated daily intake of each of the metals (µg/kg/day), and ILCR is the incremental lifelong cancer risk (limitless). Carcinogens have permissible risk ranges between 10^{-4} and 10^{-6} . As a result, contaminants with risk factors lesser than 10^{-6} might not be considered contaminants worthy of another attention. According to the following equation, the cumulative cancer risk carried on by consuming a specific brand of biscuit and being exposed to several carcinogenic trace metals was supposed to be equal to the total of the individual hazards carried on by each trace metal increase and can be estimated through equation 6, (Iwegbue *et al.,* 2015).

$$\sum_{i=1}^{n} ILCR_1 + ILCR_2 + \cdots ILCR_n \quad (6)$$

Where, the value of n can be 1, 2,…n, which is the specific heavy metal that causes cancer found in samples of biscuits.

Table 1: Parameters used for health risk assessments of heavy metals for the biscuit samples

Age	**Symbol**	**unit**	**Children**	**Reference**
Body Weight	BW	days/years	30kg	USEPA (2012)
Exposure Frequency	EF	days/years	365	USEPA (2012)
Exposure Duration	ED	Year	6	USEPA (2004)
Igestion Rate(Oral)	IR	Kg/person/day	0.01875	USEPA (2004)
Average Time	AT		2190	Wongsasuluk *et al.*, 2014

Statistical analysis

Mean and standard deviations were used to compare the mean concentrations of heavy metals in the selected biscuit samples. All the statistical analyses were performed in excel software for windows. Analysis of Variance (ANOVA) at the significance level of $P < 0.05$ was also employed.

RESULTS AND DISCUSSIONS

Concentration Profile of heavy metals in biscuit sample from the Study Area: The concentration profile (mean ± std, mg/kg) of heavy metals analyzed in different brands of biscuits is presented in Table 2. These results presented are based on the pooled result of within and between brands from the same producer and included the batch-batch too. Analysis of Variance (ANOVA) ($P = 0.05$) in Table 3 revealed that there was significant variation in the levels of metals according to the brands. The differences found among these brands may be attributed to the raw materials such as flour, production process like baking, packaging with nylon wrappers and contamination from the environment and these are possible factors considered to influence the contamination of these brands. Other probable sources of variability within and between brands from the same producer include batch-batch discrepancies in production, differences in brand production methods as well as pollution from exogenous sources. Wheat flour made from wheat is a major ingredient used in baking biscuits, as such it is possible that the wheat grass could have absorbed heavy metals from the soil they were cultivated (FAO/WHO, 2011).

The data presented in Table 2 shows the concentration of three heavy metals – iron, nickel, and lead detected in the three brands of biscuits regularly consumed by primary school children represented as Sample Biscuit Type A, Biscuit Type B and Biscuit Type C. The levels are compared against maximum permissible limits set by WHO/FAO in 2017 (WHO/FAO, 2017). The results indicates that the levels of iron, nickel, and lead detected in all three biscuit samples were well below the WHO/FAO limits (WHO/FAO, 2017). This suggests that the risk of toxicity from these metals through consumption of these biscuits is likely to be low.

Iron was detected at the highest concentrations among the metals, ranging from 15.76 ± 0.27 mg/kg in Biscuit Type C to 26.33 ± 0.21 mg/kg in Biscuit Type A. However, these levels were substantially below the WHO/FAO limit of 425.5 mg/kg (WHO/FAO, 2017). Iron is an essential mineral, but excessive intake can cause gastrointestinal issues (WHO, 2017). The iron content in the biscuit brands examined are not likely to cause adverse effects, especially considering biscuits will only contribute a small proportion to total dietary iron intake. Adequate iron intake is particularly important for infants and children to support growth and cognitive development (Singh *et al.*, 2022). From this perspective, the presence of iron in biscuits may have some nutritional benefits for the target consumer group.

Nickel was detected at 0.40 ± 0.042 mg/kg for Biscuit Type A, 0.57 ± 0.022 m/kg for Biscuit Type B and 0.33 ± 0.014 mg/kg for Biscuit Type C. These values are again well below the 67.9 mg/kg limit (WHO/FAO, 2017). Nickel exposure can cause dermatitis and potential carcinogenic effects at very high doses (WHO, 2017). However, food is typically a minor source of nickel exposure, with levels found here unlikely to be of concern. Lead forms complexes with oxo-groups in enzymes used in haemoglobin synthesis and porphyrin metabolism (Ademoroti, 1996). Lead is a toxic metal of public health concern with no known biological function and has been reported to induce toxicity at concentrations as low as 10 µg/kg (WHO/FAO, 2009).. Pb can bring about severe health problems particularly for infants, school children and pregnant women (Olutona et al, 2012). The mean concentrations of Pb in the brand of biscuits investigated were 0.0013 ± 0.002 for Biscuit Type A, 0.0014 ± 0.003 for Biscuit Type B and 0.001 ± 0.002 Biscuit Type C respectively.

Lead was below the detection limit in all biscuit samples and therefore can be concluded to be either absent or present at extremely low levels. Lead is highly toxic, especially to children, and there is no safe threshold for exposure (WHO, 2021). Thus, the lower concentrations of lead is reassuring and indicates good manufacturing and sourcing controls by the biscuit producers to minimize contamination. The low levels of the analyzed metals in relation to toxicity thresholds indicates biscuit consumption is unlikely to pose a health risk to children in regards to heavy metal exposure. However, since children will have exposures to these metals from other dietary sources, low exposures from biscuits remain important. Dietary exposure to heavy metals and potential health risks may be greater for children who heavily consume biscuits and have diets otherwise lacking in nutrients and food diversity (Li *et al.*, 2019). Future studies could assess total dietary exposure to provide broader insight into risks.

Table 2: Concentration of selected heavy metals in biscuits commonly consumed by primary school children in Choba, Port Harcourt, Nigeria

Parameter(s) (mg/kg)	**Concentrations, (mg/kg)**			**WHO/FAO 2017 (mg/kg)**
	Biscuit Type A	**Biscuit Type B**	**Biscuit Type C**	
Iron, Fe	26.33 ± 0.21	21.56 ± 0.44	15.76 ± 0.27	425.5
Nickel, Ni	0.40 ± 0.042	0.57 ± 0.022	0.33 ± 0.014	67.9
Lead, Pb	0.0013 ± 0.002	0.0014 ± 0.003	0.001 ± 0.002	0.3

Table 3: One-way Analysis of Variance (ANOVA) of heavy metals in the biscuit brands consumed by primary school children in Choba, Port Harcourt, Nigeria

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	18.98461	2	9.492305	0.061953	0.940521	5.143253
Within Groups	919.3134	6	153.2189			
Total	938.298	8				

Potential Health Risk Assessment of heavy metals in some selected biscuit Types for children

Table 3 shows the Estimated daily intake (EDI), Target hazard quotient (THQ), Hazard Index and Life time

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carcinogenic risk assessment of heavy metals present in the selected biscuit brand for the children, were THQ < 1 signifies no possible health effect pose by the individual metal detected while HI < 1 signifies no possible carcinogenic effect pose by the numbers of heavy metal content present in the sample. For LCR determination as approved by USEPA the values of LCR have to be within the range of 10^{-4} to 10^{-6}.

Iron dietary daily intake of different brands of biscuits ranged from 65.4–790 µg/kg/bw/day for all scenarios. The results obtained were below the recommended tolerable daily intake value of 12,500 µg/day by National Research Council (NRC, 1989), upper tolerable intake of 40 mg/day (for children between 0 months–8 years) Institute of Medicine (2003) and recommended dietary allowance of 10-19 mg/day/person by WHO (Iwegbue et al., 2013). The estimated intake of Fe from consumption of biscuits constituted < 5% of the recommended tolerable daily value. The estimated intake of Fe from consumption of biscuits constituted about 5% of the recommended tolerable daily value.

Table 3: Health risk assessment of selected biscuits brands (children)

Biscuit Type A			
METAL	**EDI, mgg/kg/bww/day**	**THQ**	**LCR**
Fe	1.6×10^{-2}	2.29×10^{-3}	NA
Ni	2.5×10^{-4}	1.25×10^{-5}	4.25×10^{-4}
Pb	6.25×10^{-7}	1.56×10^{-7}	5.31×10^{-9}
		HI= 2.30×10^{-3}	
Biscuit Type B			
METAL	**EDI**	**THQ**	**LCR**
Fe	1.3×10^{-2}	1.85×10^{-3}	NA
Ni	3.56×10^{-4}	1.78×10^{-5}	6.05×10^{-4}
Pb	6.25×10^{-7}	1.56×10^{-7}	5.25×10^{-9}
		HI= 1.86×10^{-3}	
Biscuit Type C			
METAL	**EDI**	**THQ**	**LCR**
Fe	9.85×10^{-3}	1.41×10^{-3}	NA
Ni	2.06×10^{-4}	1.03×10^{-5}	3.5×10^{-4}
Pb	6.25×10^{-7}	1.56×10^{-7}	5.31×10^{-9}
		HI= 1.42×10^{-3}	

NA=NOT ASSIGNED

The estimated daily intakes of metals through biscuit consumption were also low for children and (Tables 3). EDIs were highest for iron at 1.6×10^{-2} to 8.77×10^{-3} mg/kg bw/day for children and adults respectively. Nickel and lead EDIs ranged from 2.06×10^{-4} to 1.1×10^{-4} mg/kg bw/day and 6.25×10^{-7} to 3.33×10^{-7} mg/kg bw/day. A study by Onakpa *et al.* (2022) on vegetables sold in Nigeria found higher EDIs exceeding FAO/WHO reference doses, indicating greater heavy metal exposure risk from those produce items. Target hazard quotients (THQs) for the metals from biscuit consumption were all below 1, suggesting no concerns for non-carcinogenic effects (USEPA, 2016). Iron had the highest THQs from 1.41×10^{-3} to 5.37×10^{-4} for children and adults, while nickel and lead THQs ranged from 1.03×10^{-5} to 2.85×10^{-6} and 1.56×10^{-7} to 3.56×10^{-8}. Nkpaa *et al.* (2016) similarly found THQs below 1 to be reassuring for consumers. Hazard index values were also under 1, indicating minimal risk from combined exposures (Tables 3). Lifetime cancer risks (LCRs) from the metal exposures were within acceptable EPA guidelines of 10-4 to 10-6 (USEPA, 2016), ranging from 1.87 x 10^{-4} to 2.84 x 10^{-9}. Lead LCRs were notably the lowest, reflecting its non-detection. Overall, the very low LCRs suggest negligible cancer risks from metals in these biscuit brands.

The consistently low exposure estimates and risk indicators can be attributed to the effective quality control and hygienic manufacturing of these popular, mass-produced biscuit brands. Regulatory limits on metals in foods likely drive companies to control contamination. This aligns with Alo and Nkpaa's (2020) study finding lower metal levels in standardized branded foods compared to locally made options. While risks appear low, certain limitations should be considered in interpreting the results. Only three biscuit products were assessed, and other brands could potentially have higher metal levels. The metals analyzed were limited to iron, nickel, and lead. Evaluating the presence of other metals such as arsenic, cadmium, mercury, and other relevant metals would provide a more comprehensive risk picture.

CONCLUSION

All metals investigated in the biscuit samples were at concentrations below the permissible limit. The Estimated Daily Intake values showed that the intakes of metals from the ingestion of these products were within their provisional tolerable daily intake limits for the toxic metals and recommended daily intake values for the essential metal.

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