Human health risks associated with residual pesticide levels in edible tissues of slaughtered cattle in Benin City, Southern Nigeria

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Pesticide residues in meat is of growing concern due to possible adverse effects on humans. Pesticide levels were assessed in five edible cattle parts: muscle, liver, kidney and tongue tissues to determine human health risk associated with consumption of these tissues. Health risk estimates were analysed using estimated daily intake (EDI), hazard quotient (HQ) and hazard index (HI) for two (2) age/weight categories: 1–11 years/30 kg for children while 70 kg was used for adult. Risks were categorized for non-carcinogenic and carcinogenic health effects and measured at the average, maximum, 50th and 95th percentiles of the measured exposure concentrations (MEC). Total pesticide residues ranged from 2.38 to 3.86 μg/kg (muscle), 3.58 to 6.3 μg/kg (liver), 1.87 to 4.59 μg/kg (kidney) and 2.54 to 4.35 μg/kg (tongue). Residual pesticide concentrations in the tissues were in the order: liver > tongue > muscle > kidney. The concentrations of all the assessed pesticides observed in the tissues were however lower than the recommended maximum residual limits (MRLs). Human health risk estimations for the children showed EDI values for heptachlor epoxide, aldrin and dieldrin exceeding threshold values. Non-cancer risk posed to children on consumption of contaminated cattle parts showed HQ values for heptachlor epoxide, aldrin, dieldrin and HI values for organochlorines exceeding 1. indicating the possibility of non-carcinogenic health risks to consumers especially children from consumption of cattle meat from the selected abattoirs.

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1. Introduction

The drive to boost food security and eradicate crop pests in Nigeria has resulted to the accumulation of obsolete and toxic pesticides in the environment [88]. Although pesticides have become widely accepted as a fundamental part of modern agriculture for the control of insects, weed and crop diseases, there is however growing concern on their toxicity to non-target organisms. Pesticide residues in food items especially in different meat parts are of growing concern due to possible adverse effects on humans [57]. Health effects of pesticides to humans have been documented [53,52,57,92,6,104,58,79,28,11,26,63,21].

Dietary intake constitutes the most common and principal pathway of exposure to pesticides [73], which can be through consumption of contaminated meat and meat products. The contamination of food especially meat and meat products has become a topical issue of considerable concern globally [80,57]. Grazing animals are exposed to pesticides through direct treatment with pesticides, inhalation of pesticide contaminated air or through ingestion of contaminated soils and fodder Ross, 2005, [47]. In Nigeria, cattle graze freely and are predisposed to high levels of contaminants in the environment. These pesticides being lipophilic, accumulate in organs and other fatty tissues, thus providing a major route for human exposure when these fatty parts are consumed as food.

Cattle meat constitute a significant part of most diet in Nigeria, since it is a rich source of protein and nutrients. In fact, Nigeria has a high per-capita consumption rate for meat of 8.6 g/person/day [35]. Different parts of cattle (muscle, liver, kidney, tongue, skin) serve as delicacies in most diets in Nigeria, since it is common and can be eaten in different forms. Concerns however has been raised over the accumulation of pesticides in tissues of livestock animals that serve as food especially cattle [70,76]. Although residual levels of pesticides in meat and meat products have been documented [62,56,105,36,97,5], information concerning human health risk associated with consumption of meat contaminated with pesticide residues is scarce [76].
The study was therefore carried out to assess the possible human health risk associated with consumption of contaminated edible parts of cattle from abattoirs in Benin City, Southern Nigeria.

2. Methods and materials

2.1. Sampling

2.1.1. Sample stations

The sample stations were Ewah (6° 21’ 03” N and 5° 38’ 38” E), Oluku (6° 28’ 13” N and 5° 10’ 30” E), and Oni (6° 21’ 03” N and 5° 38’ 37” E) abattoirs located in Benin City, Southern Nigeria (Fig. 1). These abattoirs were chosen because they serve most markets in Benin City. Samples were collected from April–June, 2013.

2.1.2. Sample collection

All experiments using animal specimens were performed according to the guidelines of the Committee of Animal Care and Use, University of Benin.

A total of 120 samples of cattle muscle, liver, kidney and tongue samples (24 samples each) were collected from the three abattoirs. Samples were wrapped in polythene bags, labeled, placed on ice and sent to the laboratory. Samples were then stored at -20°C until analysis.

2.2. Sample preparation, extraction, cleanup and analysis

Pesticide preparation, extraction, clean-up and analysis were performed according to standard procedures [67,76,3,77,89]. Samples were each weighed and homogenized using a meat blender. Ten (10) g of the homogenized samples was placed into a beaker containing 30 g anhydrous sodium sulfate and thoroughly mixed. This mixture was then placed in a soxhlet extractor. Extraction was done using 150 ml of acetone-hexane mixture. The extracts were filtered and concentration till dryness was done at 40°C using a rotary evaporator.

Clean-up of the sample was done by transferring the extracts into a chromatographic column which contained 1 g activated florisil (60–100mesh) and 1 g layer of anhydrous sodium sulfate.
to absorb moisture. Ten (10) ml of hexane was used to condition the column and then 1 ml each of the sample blanks was transferred into the column. Preceding clean-up, the sample extract was reduced and subjected to clean up. The column was eluted with 10 ml of hexane at a rate of 1–2 ml/min. The eluate was concentrated to dryness using a rotary evaporator at a temperature of 40 °C. One (1) ml ethyl acetate was used to dissolve the residue and it was them transferred into 2 ml injection vials for analysis with electron capture gas chromatography. A total of sixteen target pesticides (α- HCH, β-HCH, γ-HCH, glyphosate, heptachlor, heptachlor epoxide, atrazine, endosulfan I, endosulfan II, endosulfan aldehyde, endosulfan sulphate, aldrin, dieldrin, endrin, carbofuran and dichlorodiphenyl dichloroethane (DDT)) were assessed.

Gas chromatography analysis was carried out using a Hewlett-Packard (hp) 5890 Series II, equipped with Ni electron capture detector (ECD). Carrier and make up gas was nitrogen at a flow rate of 1.0 and 29 ml/min respectively. The temperature of the injector was held at 250 °C, oven temperature was 100 °C and Electron Capture Detector was set at 300 °C. The injection volume of the gas chromatograph was 1.0 μL. The extraction and clean-up methods efficiency was assessed by comparing the retention time of standards and those from the extracts. Concentrations were obtained using multi-level calibration curves and residual concentrations in each sample were reported in μg/kg lipid.

The lipid content was determined according to standard procedures described by Pardio [76]. The lipid content was used for the estimation of pesticide residual concentration in the tissues.

2.3. Quality control and quality assurance

All analytes were subjected to stringent quality control methods. Before sample analysis, the instruments were calibrated with calibration standards. The target analytes were identified and quantified by comparing the retention times and peak area of the sample with those of the calibrated internal standards (reference standards). The detection of linearity was assessed using linear regression analysis of multi-level calibration curves for each analyte, while the limit of detection (LOD) and limit of quantification (LOQ) were calculated from the curve obtained from the recovery studies. The correlation coefficient (r) of the standard calibration curves ranged from 0.9613 to 0.9994, while the LOD and LOQ ranged from 0.0001 to 0.001 μg/kg and 0.0001 to 0.01 μg/kg respectively. The average recoveries of the target analytes ranged from 70 to 99%
with relative standard deviation (RSD) being less than 10%, which is in good agreement with certified values [93].

2.4. Human health risk assessment

Health risk estimates for pesticide residues in cattle tissues was computed using three basic standard indices: the Estimated Average Daily Intake (EADI), the Hazard Quotient (HQ) and the Hazards index (HI) [33]. Risks were categorized into risk for average, maximum, 50th and 95th percentiles of the measured exposure concentrations (MEC) of pesticide residues.

The Estimated Average Daily Intake (EADI) was calculated by multiplying the residual pesticide concentration in the cattle tissue (mg/kg) by the meat consumption rate (kg/day) in Nigeria, 8.6 kg [35] and dividing the result by the body weight [33]. Two hypothetical age/weight categories were used, 1–11 years/30 kg was assumed as age/weight for children while 70 kg was used for the adult category. The EDI was expressed as mg/kg/day. The EDIs was compared with already established acceptable daily intake (ADI) by USEPA Integrated Risk Information System [99] to assess long-term risk from exposure to pesticide residues through meat consumption since the ADI is based on exposure over a lifetime [76, 95, 34].

The Hazard Quotient (HQ) was used to assess risk associated with non-carcinogenic and carcinogenic health effect. For non-carcinogenic health effects the HQ was obtained by dividing the EDI by the ADI [106] while for the carcinogenic effect, the HQ was calculated by multiplying the EDI by the cancer slope factor (CSF) [100, 15]. The HQ for non-cancer and cancer risk were estimated for the average, maximum, 50th and 95th percentiles of the measured exposure concentrations (MEC) of pesticide residues in each tissue to assess the risk posed to human health on consumption of contaminated cattle tissue.

The Hazards index (HI) was used to assess risk from different exposure pathways. That is assessing risk of pesticide mixtures belonging to the same chemical group. The HI was expressed as the total exposure risk for a given pathway and calculated as the sum of the HQs (HI = EDI/ADI) for each exposure pathway [78]. Two HI’s for all exposure routes were calculated, to assess total exposure for both non-carcinogenic and carcinogenic health effects for all pesticide groups.

2.5. Statistical analysis

Data for each station were summarized separately using descriptive statistics (Microsoft Excel 7.0 program). Statistical differences between the different target pesticides in the various meat parts were evaluated using Analysis of variance (ANOVA), SPSS 15 software and p < 0.05 was considered significant. Multiple bar graphs, box plots and pie graphs were used to represent assessment endpoints.

3. Results and discussion

3.1. Contamination profile of pesticide residues in cattle tissues

The use of pesticides in modern agriculture is extremely essential, however, pesticide contamination in edible tissues of cattle is of growing concern because of their accumulative properties and health risks posed to man and animals [57]. Since majority of pesticides are lipophilic, the lipid content was used for the estimation of pesticide residual concentration in the tissues. Mean percentage concentration of lipids in tissue samples were 3.6% (muscle), 3.5% (liver), 2.3% (kidney) and 3.8% (tongue). The observed lipid concentration is similar to previous studies [76]. However, very limited information on percentage concentration of lipid in tongue tissue are available to compare with this study. Varying levels of pesticide contamination in edible parts of cattle from selected abattoirs in Benin City, Nigeria were observed. The concentrations of total pesticide residues (µg/kg) from the three abattoirs ranged from 2.38 to 3.86 (muscle), 3.58 to 6.3 (liver), 1.87 to 4.59 (kidney) and 2.54 to 4.35 (tongue) (Table 1).  

3.2. Concentrations of pesticide residues in cattle tissues

The mean concentrations of pesticide residues (µg/kg) in the muscle, liver, kidney and tongue tissues are presented in Table 1.

3.2.1. Hexachlorocyclohexanes (HCHs)

The concentrations of total HCHs (ΣHCHs) in cattle muscle, liver, kidney and tongue tissues are presented in Table 1 and further illustrated in Fig. 2. The highest concentrations of ΣHCHs was found in the tongue (1.20 ± 0.15 µg/kg), although concentrations were not statistically significant (p > 0.05, F = 0.02) across the tissues. The result of this study is comparable with findings of [60], who reported higher concentrations of ΣHCHs in tongue tissues of bufalos from slaughter houses in Egypt. The observed levels of ΣHCHs in the tissues were lower than concentrations in reported studies on various parts of food animals [90, 50, 49, 71, 78, 99, 11]. Although limited studies exist on pesticide concentrations in parts of food animals in Nigeria, reported studies by [74] on organochlorine pesticide residues in animal food products from Nigeria, revealed higher levels of HCH isomer (α, β, γ-HCH) concentrations. Comparing with recent studies, the mean levels observed in this study were higher than concentrations reported by [75, 60], who worked on organochlorine pesticide residues in bovine and buffalo tissues respectively. Higher values observed in this study indicate an upsurge in the use of technical HCH and Lindane on pastures in the region. Among the assessed isomers of HCHs, α-HCH dominated (Figs. 3 and 4). Concentrations of α-HCH in the liver were higher than other tissues, being 1.1 times higher than concentrations in the muscle and kidney while it was 1.5 times higher than concentrations in the tongue. The differences in tissue concentrations were however not statistically significant (P > 0.05, F = 3.81). Higher concentrations in the liver may be attributed to the fact that the liver is the primary site of detoxification of xenobiotics [42, 54]. Similar concentrations was reported by [38, 61] who reported α-HCH concentrations in adipose tissues (0.7 µg/kg lipid) and fat (0.6 µg/kg fat) of cattle from Sweden and Sene-Gambian region respectively. Higher concentrations (53 µg/kg fat basis) was reported in Jordan, who assessed organochlorine pesticide residues in meat. The high prevalence of α-HCH in the tissues assessed compared to the other isomers may be attributed to the high volatility of this isomer which increases uptake via deposition or sorption from the atmosphere onto pasture surface which in turn is consumed by cattle or by direct treatment with pesticides [76].

β-HCH is the most stable HCH isomer, it has slow elimination rate and thus has the tendency to accumulate in animal tissues over time as compared to other isomers [76, 25]. Concentrations observed in this study were however the lowest amongst the assessed HCH isomers. The highest mean levels of β-HCH were observed in the tongue tissues (0.2417 µg/kg) being 2.8 times higher than concentrations in the muscle (0.0875 µg/kg), 3 times higher than concentrations in the liver (0.0792 µg/kg) and 2.5 times higher than levels in the kidney (0.0958 µg/kg) (Table 1). Percentage concentration of β-HCH was also highest in the tongue tissues (20.1%) (Fig. 4), however, the differences in tissue concentrations between the tissues were not significant (P > 0.05, F = 0.75). Higher concentrations in the tongue may be attributed to the fact that β-HCH is known to have higher affinity for fats (lipids) [76], hence increased accumulation in the tongue tissue which was more fatty than the other tissues. β-HCH concentrations in the tongue is
Table 1
Mean and range of pesticide residues in muscle, liver and tongue of cattle from selected abattoirs in Benin City, Nigeria with recommended maximum residual levels (MRL).

| Pesticide(μg/kg) | Muscle (Mean ± SD, Range) | Liver (Mean ± SD, Range) | Kidney (Mean ± SD, Range) | Tongue (Mean ± SD, Range) | MRL (JFCRF) | MRL (EU) |
|------------------|--------------------------|--------------------------|---------------------------|---------------------------|-------------|----------|
| Organochlorine   |                          |                          |                           |                           |             |          |
| α-HCH            | 0.71±0.822 (0.10–1.65)   | 0.77±0.369 (0.41–1.15)   | 0.71±0.587 (0.29–1.30)    | 0.53±0.404 (0.18–1.03)    | NA          | 200      |
| β-HCH            | 0.08±0.696 (0.03–0.16)   | 0.07±0.882 (0.03–0.18)   | 0.09±0.167 (0.0–0.29)     | 0.24±0.138 (0.1–0.38)     | NA          | 100      |
| γ-HCH (Lindane)  | 0.33±0.025 (0.33–0.35)   | 0.25±0.109 (0.18–0.38)   | 0.21±0.258 (0.0–0.50)     | 0.42±0.139 (0.3–0.58)     | 1000 (20-Muscle) | 20      |
| Σ HCH            | 1.14±0.317 (0.001–0.002) | 1.10±0.360 (0.001–0.002) | 1.03±0.331 (0.001–0.002) | 1.20±0.147 (0.001–0.002)  | NA          |          |
| Heptachlor        | 0.18±0.063 (0.13–0.250)  | 0.40±0.260 (0.20–0.70)   | 0.29±0.094 (0.20–0.39)    | 0.317±0.138 (0.23–0.48)   | 200         |          |
| Heptachlor epoxide| 0.12±0.066 (0.05–0.18)   | 0.04±0.079 (0.0–0.14)    | 0.12±0.106 (0.003–0.24)   | 0.28±0.104 (0.2–0.4)      | 200         |          |
| Aldrin            | 0.23±0.147 (0.13–0.40)   | 0.04±0.445 (0.19–1.08)   | 0.17±0.064 (1.10–2.3)     | 0.287±0.213 (0.08–0.50)   | 200         |          |
| Dieldrin          | 0.40±0.199 (1.14–0.53)   | 0.80±0.350 (0.45–1.15)   | 0.13±0.051 (0.09–0.19)    | 0.250±0.331 (0–0.7)       | 200         |          |
| Endrin            | 0.08±0.063 (0.03–0.15)   | 0.43±0.399 (0.10–0.88)   | 0.154±0.019 (0.14–0.18)   | 0.400±0.33 (0.18–0.78)    | 50          | 50       |
| Endosulfan I      | 0.35±0.211 (0.11–0.50)   | 0.38±0.101 (0.33–0.50)   | 0.238±0.121 (0.15–0.38)   | 0.225±0.205 (0.005–0.45)  | 200 (100-Muscle) | 50      |
| Endosulfan II     | 0.20±0.040 (0.18–0.25)   | 0.31±0.098 (0.25–0.43)   | 0.21±0.055 (0.18–0.28)    | 0.058±0.080 (0–0.15)      | 200 (100-Muscle) | 50      |
| Endosulfan aldehyde| 0.00±0.007 (0–0.13)     | 0.33±0.232 (0.18–0.60)   | 0.06±0.057 (0.01–0.13)    | 0.04±0.072 (0–0.13)       | 200         | 50       |
| Endosulfan sulfate| 0.01±0.029 (0)          | 0.06±0.052 (0.03–0.13)   | 0.08±0.134 (0.0–0.24)     | 0±0                      | 0          | 200      |
| Σ Endosulfan      | 0.57±0.188 (0.36–0.70)   | 0.85±0.416 (0.83–1.58)   | 0.59±0.029 (0.56–0.61)    | 0.003±0.001 (0–0.01)      | 200         | 50       |
| DDT               | 0.01±0.029 (0)           | 0.05±0.094 (0–0.16)      | 0.05±0.094 (0–0.16)       | 0.05±0.014 (0.05–0.075)   | 2000 (1000-Muscle) | NA      |
| Organophosphate   |                          |                          |                           |                           |             |          |
| Glyphosate        | 0.71±0.083 (0.13–0.28)   | 0.09±0.159 (0–0.28)      | 0.10±0.121 (0–0.24)       | 0.275±0.066 (0.2–0.33)    | 2000 (100-Muscle) | 50      |
| Trazine           |                          |                          |                           |                           |             |          |
| Atrazine          | 0.10±0.101 (0–2)         | 0.12±0.141 (0–0.28)      | 0.10±0.054 (0.05–0.14)    | 0.117±0.052 (0.08–0.18)   | 60 (20-Muscle) | NA      |
| Carbamate         |                          |                          |                           |                           |             |          |
| Carbosuran        | 0.05±0.025 (0.03–0.08)   | 0.17±0.083 (0.10–0.26)   | 0.04±0.044 (0–0.09)       | 0.91±0.153 (0.03–0.33)    | 50          | 10       |
| Σ Pesticide residues | 3.00±0.770 (2.38–3.86) | 1.65±0 (0.004–0.0063) | 0.0028 | 0.0019–0.0046 | 0.0037 | 0.0025–0.0044 |

NA, Not Available for sample type assessed.
MRL adapted from Japan Food Chemical Research Foundation (FECRF) [44] and European Union (EU) [29].
comparable with the findings of [61], who reported β-HCH concentrations (0.2 μg/kg fat) in cattle fat samples from butchers and abattoirs in Sene-Gambian region. The mean levels in liver tissues obtained in this study were however lower than levels previously reported by [19] in liver tissues of pork from Romania.

The concentration of γ-HCH (Lindane) in cattle tissues was in the sequence tongue > muscle > liver > kidney (Table 1). Levels were similar to what was obtained for β-HCH isomer in which the tongue had the highest concentration. γ-HCH and β-HCH isomers are known for their lipophilicity [76] hence higher concentration in the tongue tissues could be attributed to the fact that the tongue had higher concentrations of fat (3.8% fat) compared to the other tissues. Mean levels in the tongue (0.4250 μg/kg) was 1.3 times higher than concentrations in the muscle (0.3375 μg/kg), 1.7 times higher than concentrations in the liver (0.2500 μg/kg) and 2 times higher than levels in the kidney (0.2125 μg/kg) (Table 1). The differences in tissue concentrations were however not significant (P > 0.05, F = 1.92). The presence of γ-HCH in the assessed tissues is apparently due to the continuous use of lindane in this region. [74], reported higher levels of γ-HCH in muscle (14.0 μg/kg lipid), liver (30.0 μg/kg lipid), kidney (25.0 μg/kg lipid) and issues from cattle in South Western Nigeria. High concentrations of γ-HCH in various parts of food animals has been reported [61,19,23,83,2].

α-HCH/γ-HCH ratios below 1 are often used as indicators of fresh input of γ-HCH into the environment [55,76]. In this study the mean α-HCH/γ-HCH ratios in all the tissues assessed were above 1, indicating that there was no fresh input of γ-HCH into the environment. There was also no statistical significance (p > 0.05, F = 0.32) in the α-HCH/γ-HCH ratios between the tissues. The concentrations of γ-HCH observed in the muscle, liver, kidney and tongue tissues were below the MRL recommended by the Japan...
Food Chemical Research Foundation [44] and the European Union EU [29].

3.2.2. Heptachlor and heptachlor epoxide

Residual concentrations of heptachlor and heptachlor epoxide in cattle muscle, liver, kidney and tongue tissues are presented in Table 1. It was observed that mean levels were higher in the liver tissues, however concentrations in the tissues were not significantly different ($p > 0.05, F = 0.23$). Heptachlor binds to soil particles and migrates very slowly [32,13] and thus could be taken in by cattle during feeding. Food is considered to be the major source of exposure to heptachlor [31,32]. The relatively high concentration observed in this study may be attributed to its persistence in the environment. Similar studies on the concentration of heptachlor residues in Grasscutter tissues [32] showed higher concentrations in muscle (0.695 μg/kg wet) and kidney (0.403 μg/kg wet), while [12] reported higher concentrations (1.391 μg/kg wet) in game meat from Ghana. Concentrations observed in this study did not however exceed the MRLs of heptachlor in the different tissues.

On the other hand, heptachlor epoxide occurred less frequently and at a lower levels compared to heptachlor. Similar results were observed by Ahmad, who reported higher concentrations of heptachlor than heptachlor epoxide in meat samples from Jordan. The highest concentration of heptachlor epoxide was observed in the tongue (0.2833 μg/kg) and it was 2.3 times higher than that in the muscle, 6.2 times higher than concentration in the liver and 2.2 times higher than that in the kidney. Variations in tissue concentrations were however not significant ($p > 0.05, F = 1.78$). Heptachlor epoxide is very resistant to chemical or biological changes in soil [32] and can subsequently be taken up by cattle with food. The obtained data of heptachlor epoxide concentrations were lower than those recorded for meat samples in Jordan. The observed concentrations were also lower than the MRLs for the assessed tissues (Table 1).

3.3.3. Aldrin, dieldrin and endrin

Aldrin, dieldrin and endrin are known to be closely related organochlorine pesticides that are extremely persistent in the environment [72]. Despite the ban of these organochlorines [1], residual concentrations have been reported in tissues of food animals [62,56,105,36,97,103]. Concentrations of aldrin, dieldrin and endrin varied in the tissues: Muscle = Dieldrin > Aldrin > Endrin. Liver = Dieldrin > Endrin > Aldrin; Kidney = Aldrin > Endrin > Dieldrin; Tongue = Endrin > Aldrin > Dieldrin.
Enhanced levels of dieldrin and endrin residues were observed in the liver while concentrations of aldrin were lowest in the liver (0.0458 μg/kg) compared to dieldrin and endrin (Table 1). Tissues concentrations of aldrin were generally lower than dieldrin concentrations in the muscle and liver tissues, being 2 and 18 times lower respectively. Lower concentrations of aldrin in relation to dieldrin could be attributed to the fact that aldrin rapidly metabolizes to dieldrin in a wide range of organisms [13,46] while higher concentrations of aldrin found in the kidney and tongue in relation to dieldrin could be attributed to recent uptake by the cattle from the environment [13]. However variation in the pesticide concentrations between the tissues were not significantly different (p > 0.05).

Concentrations of aldrin and endrin observed in this study were lower than the mean values of 0.058 and 0.127 mg/kg reported respectively for Aldrin and endrin by [1], who assessed OCPs residues in cow milk from Sohag and Qena governorates. Higher values of dieldrin and endrin were also reported by [3], in bovine muscles in Egypt while a similar value (0.174 μg/kg) was reported for aldrin. In the same study, higher values were reported for aldrin and endrin in chicken muscles while a lower value (0.259 μg/kg) was reported for dieldrin. In addition, higher concentrations were reported for aldrin in meat, Grasscutter tissues [13] and Game meat [12]. Lower concentrations of dieldrin and endrin were reported in Grasscutter tissues [13] and Game meat from Ghana [12].

The levels of these OCPs observed in this study did not exceed the MRLs estab-
lished by the Japan Food Chemical Research Foundation [44] and the European Union [29] (Table 1) for pesticide residues in cattle muscle, liver, kidney and edible offal (tongue).

The presence of these banned organochlorines in the assessed tissue of cattle, calls for concern as cattle meat constitutes a major part of most diet in Nigeria. These pesticides are known to be toxic [102,37] and can biomagnify along the food chains, in which humans are at the top position.

3.3.4. Endosulfan and metabolites

Endosulfan (alpha (I), beta (II)-isomer and the sulfate and aldehyde derivative) was present in the assessed tissues. The concentrations of total endosulfan (Σ endosulfan) in cattle muscle, liver, kidney and tongue tissues are presented in Fig. 2. The highest concentrations of Σ endosulfan was found in the liver (0.856 ± 0.416 μg/kg), although concentrations were no statistically significant (p > 0.05, F = 0.315) across the tissues. Higher concentrations of endosulfan was reported in bovine fat [75] and cattle meat [65]. Varying levels of endosulfan concentrations have been reported in cow milk Cisato et al., human tissues and cord blood samples [18], adipose tissue [5] and human breast milk samples [16,84,81].

The concentrations of the isomer endosulfan I were generally higher than the other metabolites in all the tissues assessed (Table 1). Higher concentrations of endosulfan I could be attributed to the fact that technical endosulfan contains a higher ratio of endosulfan I than endosulfan II, endosulfan I is also a more stable isomer [86,87]. The observed concentrations in this study were lower than concentrations of endosulfan I reported in Grasscutter tissues [13] while higher concentrations was reported in game meat [12] and it was not detected in meat (lamb and beef) from Jordan. High levels of endosulfan II in tissues of imported bovine and chicken samples have also been reported [3]. Mean values of endosulfan aldehyde and endosulfan sulphate in the assessed tissues were generally lower than the isomers (endosulfan I and II). Higher concentrations were however reported in Grasscutter tissues [13] fat and lean beef [23]. Although endosulfan has been banned, its prevalence in this study could be attributed to continuous use of the pesticide. Concentrations were however lower than the established MRLs [43,44].

Fig. 8. (a–d) Hazard quotient for cancer risk assessment based on the average, maximum, 50TH and 95TH Percentile concentrations for children (1–11/30kg) category.
3.3.5. DDT

Only the parent DDT compound was assessed, other DDT metabolites were not considered in the target pesticide standard mixture. The distribution pattern was in the sequence Tongue > Liver = Kidney > Muscle. Similar higher concentrations was reported in tongue samples of buffalo from Egypt with mean concentration of 62.83 ng/g lipid weight [60]. Much higher concentrations of DDT has been reported in milk [51], meat [85,2], liver, kidney and muscle tissues [76]. Concentrations observed in this study were however lower than what was obtained in liver, kidney and tongue tissues of buffalo from Egypt [60]. Although the use of DDT has been banned in Nigeria, residual concentrations in tissues of cattle observed in this study could be attributed to its persistence in the environment. The reported concentrations of DDT in this study were however lower than the recommended MRLs [44] for DDT in cattle tissues (Table 1).

3.3.6. Glyphosate

Glyphosate is a common herbicide used in agriculture that readily and permanently binds to soil particles and remains in the upper few centimetres of soil [20], which inevitably is consumed by cattle from soil. There are however only a few studies available on the levels of glyphosate pesticides in parts of food animals. This may be attributed to the fact that US EPA [101] classifies glyphosate in Toxicity Category III, that is, it is non-carcinogenic to humans. In spite of this, significant poisoning effects caused by both intentional and accidental exposure to glyphosate have been recorded in humans and laboratory animals [20]. Glyphosate residues in animal feeds

![Diagram](image-url)
from pre-harvest glyphosate treatment of cereals may result in residual concentrations in meat, milk and eggs [44]. Results of this study revealed varying levels of glyphosate in cattle tissues with the highest level of glyphosate observed in tongue tissues (Table 1). The observed mean values were however not significantly different across the tissues ($p > 0.05, \, F = 2.09$). Higher levels in the tongue may be attributed to the fact that the tongue is the first point of contact with pesticides and the first organ of defense against xenobiotic exposure [60]. Concentrations of glyphosate observed in the tissues were however below the MRL [43,44] (Table 1).

3.3.7. Atrazine

Atrazine levels varied nominally ($p > 0.05, \, F = 4.0$) across the tissues assessed. Atrazine is one of the most widely used herbicide globally [69,96], commonly used in Nigeria for the control of weeds in most farms [30]. Despite its intensive use, atrazine has been implicated in a number of health effects [22,96,41,30]. High concentrations of atrazine in serum and urine samples of cattle were observed by [77], with concentrations of 0.739 and 1.389 $\mu$g/l respectively. In the present study higher concentration of atrazine observed in the liver could be attributed to the liver being the primary site of detoxification [54]. Concentrations were lower than the recommended MRLs [44].

3.3.8. Carbofuran

Carbofuran, a carbamate pesticides is used extensively in modern agriculture for the control of insect pests. Serious public concerns regarding environmental and food safety has however been raised [64]. The concentrations of carbofuran in the tongue were relatively higher compared with those of the muscle, liver and
3.3. Spatial variation of pesticide residues in the different cattle tissues

The concentration of pesticide residues in cattle tissues from Ewah, Oluku and Oni abattoirs is presented in Fig. 3a-d. The highest level of total pesticide residues was recorded in the liver at Ewah abattoir with a value of 6.30 μg/kg and the lowest level was recorded in the kidney at Oluku abattoir with a value of 1.87 μg/kg. Generally, Oni abattoir had the highest residual pesticide concentrations in all the tissues assessed except in the liver tissues where Ewah abattoir had the highest residual concentration (6.30 μg/kg). Concentrations in tissues were however not significantly different (p > 0.05) between the abattoirs except for kidney tissues, in which concentration at Oni abattoir was significantly (p < 0.05, F = 3.45) higher than Ewah and Oluku abattoir. The results suggest that cattle meat and tissues from the respective abattoirs sold in markets in Benin City come from the same source. This raises concern over the consequences that might result from accumulation of these pesticides in cattle tissues consumed as meat in Benin City, as their accu-
Cumulative properties could pose health risks to man and animals [57].

3.4. Incidence of pesticide contamination

α–HCH was observed to have the highest prevalence among all the pesticide assessed in all the tissues except for liver tissues (Table 1, Fig. 4a–d), where dieldrin (16.3%) had the highest occurrence. The high prevalence of α-HCH in the tissues assessed compared with the other pesticides may be attributed to its high volatility which increases uptake via deposition from the atmosphere onto pasture surface which in turn are consumed by cattle [76].

3.5. Variation in pesticide concentration in cattle tissues

The results reflects different levels of pesticide residues in the muscle, liver, kidney and tongue samples of cattle. Residual pesticide concentrations in the tissues was in the order: Liver > Tongue > Muscle > Kidney (Fig. 5). The difference in the levels of pesticides in the tissues of cattle may be attributed to the levels of contaminants in pastures where these animals graze/drink, the type of husbandry practices, the physical and chemical properties of the pesticide and also the location of the slaughter houses or abattoirs (proximity to pesticide contamination) [40]. The liver was observed to have the highest residual pesticide concentration compared to the other tissues, concentrations were however not
3.6. Human health risk assessment

3.6.1. Maximum residual level (MRL)

Pesticide residues in cattle tissues were compared with maximum residual level (MRL) (Table 1) established by the Japan Food Chemical Research Foundation [44] and the European Union [29]. Mean concentrations of pesticide residues in all the tissues assessed were however lower than the MRLs. Although mean pesticide residues in the cattle tissues were lower than the MRL, the persistent and bio-accumulative nature of pesticides is of great concern because of the possible build up to toxic levels [45].

3.6.2. Estimated daily intake (EDI)

The Estimated daily intake (EDI) of pesticide residues in cattle tissues are presented in Tables 2–5. Contribution to dietary intake of pesticide residues from consuming cattle tissues was in the order Liver > Tongue > Muscle > Kidney for both children and adults.

Comparing the EDI with the recommended ADIs to predict exposure to pesticide residues from tissue consumption, showed EDI values for aldrin exceeding threshold values in all the assessed tissues and for all exposure concentrations for children and adult (Tables 2–5), except for muscle tissues were the EDI value only exceeded threshold value for the maximum exposure concentration for the adult category (Table 2). Heptachlor epoxide exceeded threshold values for children in the muscle and tongue tissues for all exposure categories (Table 2 and 5), while it exceeded threshold values in the maximum and 95th percentile concentrations for liver.
Table 2
Estimated daily intake (EDI) of pesticide residues in cattle muscle.

| Muscle | Pesticide | EDI (mg/kg/day) | ADI | ADI source | Risk for average exposure | Risk for maximum exposure | Risk for 50th percentile conc. | Risk for 95th percentile conc. |
|--------|-----------|-----------------|-----|------------|--------------------------|--------------------------|-----------------------------|-----------------------------|
|        |           |                 |     |            | 1–11 years/30 kg         | 70 kg/adult              | 1–11 years/30 kg           | 70 kg/adult                  |
|        |           |                 |     |            | 1–11 years/30 kg         | 70 kg/adult              | 1–11 years/30 kg           | 70 kg/adult                  |
|        |           |                 |     |            | 1–11 years/30 kg         | 70 kg/adult              | 1–11 years/30 kg           | 70 kg/adult                  |

- ATSDR-Agency for TOXIC Substances and Disease Registry IRIA-USEPA Integrated Risk Information System.
- ADI > EDI.

Table 3
Estimated daily intake (EDI) of pesticide residues in cattle liver.

| Liver | Pesticide | EDI (mg/kg/day) | ADI | ADI source | Risk for average exposure | Risk for Maximum Exposure | Risk for 50th Percentile Conc. | Risk for 95th Percentile Conc. |
|-------|-----------|-----------------|-----|------------|--------------------------|--------------------------|-----------------------------|-----------------------------|
|       |           |                 |     |            | 1–11 years/30 kg         | 70 kg/adult              | 1–11 years/30 kg           | 70 kg/adult                  |
|       |           |                 |     |            | 1–11 years/30 kg         | 70 kg/adult              | 1–11 years/30 kg           | 70 kg/adult                  |
|       |           |                 |     |            | 1–11 years/30 kg         | 70 kg/adult              | 1–11 years/30 kg           | 70 kg/adult                  |

- ATSDR-Agency for TOXIC Substances and Disease Registry IRIA-USEPA Integrated Risk Information System.
- ADI > EDI.
| Pesticide       | EDI(mg/kg/day) | Risk for average exposure 1-11years/30kg | Risk for maximum exposure 1-11years/30kg | Risk for 50th percentile conc. 1-11years/30kg | Risk for 95th percentile conc. 1-11years/30kg |
|----------------|----------------|------------------------------------------|------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| α-HCH          | 8.00E-03       | ATSDR                                    |                                          |                                               |                                               |
| γ-HCH          | 3.00E-04       | IRIS                                     |                                          |                                               |                                               |
| β-HCH          | NA             | NA                                       |                                          |                                               |                                               |
| Heptaclor      | 5.00E-04       | IRIS                                     |                                          |                                               |                                               |
| Heptaclor epoxide | 1.30E-05     | IRIS                                     |                                          |                                               |                                               |
| Aldrin         | 3.00E-05       | IRIS                                     |                                          |                                               |                                               |
| Endosulfan I   | 6.00E-03       | IRIS                                     |                                          |                                               |                                               |
| Endosulfan II  | 6.00E-03       | IRIS                                     |                                          |                                               |                                               |
| Endosulfan aldehyde | 6.00E-03      | IRIS                                     |                                          |                                               |                                               |
| Endosulfan sulfate | 6.00E-03     | IRIS                                     |                                          |                                               |                                               |
| Diedrin        | 5.00E-05       | IRIS                                     |                                          |                                               |                                               |
| Endrin         | 3.00E-04       | IRIS                                     |                                          |                                               |                                               |
| DDT            | 5.00E-04       | IRIS                                     |                                          |                                               |                                               |
| Glyphosate     | 1.00E-01       | IRIS                                     |                                          |                                               |                                               |
| Carbofuran     | 5.00E-03       | IRIS                                     |                                          |                                               |                                               |
| Atrazine       | 3.50E-02       | IRIS                                     |                                          |                                               |                                               |

ATSDR-Agency for TOXIC Substances and Disease Registry IRIA-USEPA Integrated Risk Information System.

EDI > ADI.

| Pesticide       | EDI(mg/kg/day) | Risk for average exposure 1-11years/30kg | Risk for maximum exposure 1-11years/30kg | Risk for 50th percentile conc. 1-11years/30kg | Risk for 95th percentile conc. 1-11years/30kg |
|----------------|----------------|------------------------------------------|------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| α-HCH          | 8.00E-03       | ATSDR                                    |                                          |                                               |                                               |
| γ-HCH          | 3.00E-04       | IRIS                                     |                                          |                                               |                                               |
| β-HCH          | NA             | NA                                       |                                          |                                               |                                               |
| Heptaclor      | 5.00E-04       | IRIS                                     |                                          |                                               |                                               |
| Heptaclor epoxide | 1.30E-05     | IRIS                                     |                                          |                                               |                                               |
| Aldrin         | 3.00E-05       | IRIS                                     |                                          |                                               |                                               |
| Endosulfan I   | 6.00E-03       | IRIS                                     |                                          |                                               |                                               |
| Endosulfan II  | 6.00E-03       | IRIS                                     |                                          |                                               |                                               |
| Endosulfan aldehyde | 6.00E-03      | IRIS                                     |                                          |                                               |                                               |
| Endosulfan sulfate | 6.00E-03     | IRIS                                     |                                          |                                               |                                               |
| Diedrin        | 5.00E-05       | IRIS                                     |                                          |                                               |                                               |
| Endrin         | 3.00E-04       | IRIS                                     |                                          |                                               |                                               |
| DDT            | 5.00E-04       | IRIS                                     |                                          |                                               |                                               |
| Glyphosate     | 1.00E-01       | IRIS                                     |                                          |                                               |                                               |
| Carbofuran     | 5.00E-03       | IRIS                                     |                                          |                                               |                                               |
| Atrazine       | 3.50E-02       | IRIS                                     |                                          |                                               |                                               |

ATSDR-Agency for TOXIC Substances and Disease Registry IRIA-USEPA Integrated Risk Information System.

EDI > ADI.
tissues in children and adults (Table 2). Dieldrin exceeded threshold values in muscle tissues for all exposure categories in children and in the maximum exposure concentration for adults (Table 2), while in kidney and tongue tissues dieldrin exceeded threshold values in the average, maximum and 95th percentile concentrations for children (Table 4 and 5) and in average exposure concentration for children in tongue tissues.

Although residual levels of pesticides in meat and meat products have been documented [68, 62, 56, 59, 105, 36, 97, 5], information concerning dietary intake of pesticide residues from meat are limited [76], [24] estimated dietary intake of γ-HCH and ∑DDT from consumption of meat products (beef, pork, lamb, poultry, cured/processed meats) from different cities in Sweden to be 0.0828 and 0.00949 kg/kg bw/d respectively which were however higher than concentrations observed in this study.

3.6.3. Hazard quotient (HQ)
3.6.3.1. Non-carcinogenic health effects. The HQs for non-carcinogenic health effects for risk of average, maximum, 50th and 95th percentile exposure concentration are presented in Figs. 6 and 7.

3.6.3.1.1. Risk for 1–11 years/30 kg category. The non-cancer risk posed to human health under the age/weight category of 1–11 years/30 kg (children) on consumption of contaminated cattle muscle and liver tissues showed the HQ of heptachlor epoxide, aldrin and dieldrin exceeding 1 in children for all the exposure categories (Fig. 6a,b). For kidney tissues HQ values exceeded 1 for heptachlor epoxide and aldrin for all the exposure concentrations, while dieldrin exceeded 1 in all the exposure categories except for the 50th percentile concentrations. HQ values for heptachlor epoxide and aldrin in tongue tissues exceeded 1 in all the exposure categories (Fig. 6c), while dieldrin exceeded 1 only in the average exposure concentration (Fig. 6d).

3.6.3.1.2. Risk for adult/70 kg category. HQs in muscle tissues for heptachlor epoxide showed values above 1 for all exposure categories except for the 95th percentile concentration, while aldrin and dieldrin exceeded 1 only in the maximum exposure concentration (Fig. 7a). For liver tissues, aldrin and dieldrin exceeded 1 in all the exposure categories while heptachlor epoxide was above the threshold value of 1 for only the maximum and 95th percentile exposure concentration. HQ in kidney tissues showed values for heptachlor epoxide exceeding 1 in all the exposure concentrations except for the average concentration while dieldrin exceeded 1 only for the maximum exposure concentration (Fig. 7c). For tongue tissues, HQ values for heptachlor epoxide and aldrin exceeded 1 in all the exposure concentrations (Fig. 7d).

The exceedance of the target value of 1.0 indicates that average, maximum, 50th and 95th percentile exposure concentrations of these pesticides especially for heptachlor epoxide, aldrin and dieldrin would result in non-carcinogenic health effects from consumption of cattle muscle, liver, kidney and tongue tissues in children and adults. [76] reported that exposure to organochlorine pesticides (OCPs) through consumption of bovine muscle samples from Veracruz, Mexico could result in non-cancer risk.

3.6.3.2. Carcinogenic health effects. The HQs for cancer risk assessment based on the average, maximum, 50th and 95th percentile concentrations showed values for all the pesticides in the different tissues for both children and adult, below 1 (Figs. 8 and 9) indicating that daily intake of the cattle tissues would not pose cancer risk. However prolonged consumption of these pesticides through meat could pose severe health implication to humans especially children [39].

3.6.3.3. Hazard index. Risk assessment of pesticide mixtures by hazard index (HI) for non-cancer risk assessment using average, maximum, 50th and 95th percentile concentrations showed values above 1 for the organochlorines in all the tissues assessed for both the children and adult category (Figs. 10 and 11). Results indicate that there is the possibility of health risk associated with exposure to organochlorines through consumption of cattle meat. The hazard index of organophosphate, carbamate and trazine estimated for both children and adults did not exceed the value of 1.

HI values were however below 1 for cancer risk assessment for all the pesticide groups for children and adults for all exposure concentrations (Figs. 12 and 13), indicating no cancer health risk from ingestion of multiple pesticides contained in edible parts of cattle.

4. Conclusion
The present study has shown the accumulation of pesticide residues in cattle tissues. The concentrations of all the assessed pesticides observed in the muscle, liver, kidney and tongue tissues were however lower than the recommended maximum residual limit (MRL) set by the Japan Food Chemical Research Foundation for pesticide residues in cattle tissues and thus within safe limits. However, human health risk assessment showed EDI estimations for heptachlor epoxide, aldrin and dieldrin exceeding threshold values, indicating possible health hazards for consumers especially children. Results indicate that there is the possibility of non-cancer health risk associated with exposure to the organochlorines (γ-HCH, heptachlor, heptachlor epoxide, aldrin and dieldrin) through consumption of cattle meat from the selected abattoirs. This findings provide preliminary baseline data on human health risks associated with consumption of edible cattle parts (muscle, liver, kidney and tongue) contaminated with pesticide residues in Benin City. Regular monitoring of pesticide residues in meat and meat products is therefore necessary to mitigate the impact of these pesticide on the health of consumers.

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