Toxicological Risk Associated with Consumption of Rice Sold in Uganda

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ABSTRACT

Previous studies have reported the presence of aflatoxins (AFB1, AFB2, AFG1 and AFG2), heavy metals (As, Cd and Pb) and organochlorine pesticide residues (OCPs) in rice sold in Uganda. However, the potential health effects associated with consumption of rice have not been evaluated. The aim of this study was to evaluate the health risk of consuming rice sold in Uganda. A total of 45 packed and 30 open traded rice samples were randomly collected from retail outlets in the main rice trading areas of Uganda. Rice was analysed for AFB1, AFB2, AFG1 and AFG2, As, Cd, Pb and OCPs using AOAC standard methods. Dietary exposure of the consumers to contaminants was assessed using the estimated daily intake (EDI). The Hazard index (HI) and the incremental lifetime cancer risk (ILCR) were determined to define the non-carcinogenic and carcinogenic risk from contaminants, respectively. The values obtained for the ILCR for Arsenic detected in rice were 1.14E-2 and 7.28E-2 for adults and infants, respectively. The ILCR of all detected OCPs in adults and infants were higher than 1.0E-2, in both open traded and packed rice. This study established a potential carcinogenic risk from consumption of rice sold in Uganda in both infants and adults at the current level of contamination and consumption rate. Establishment of a monitoring system along the entire rice value chain; enforced by a national regulatory body can provide timely feedback on the levels of contamination and the progress in reducing the contamination burden.

Keywords: rice; aflatoxins; heavy metals; organochlorine pesticide residues; toxicological risk

INTRODUCTION

The global importance of rice as a staple food crop cannot be overstated. Rice is a major food crop for more than 60% of the world’s population (FAO, 2021). Food safety has become an essential requirement worldwide because it influences human health (Sataloff et al., 2017). Foodborne diseases kill an estimated 420,000 people annually, 125,000 of them being children under 5 years of age (WHO, 2015). Ascertaining food safety necessitates predicting the potential health risk associated with exposure to contaminants in food.

Several studies have identified aflatoxins, heavy metals and OCPs as the main contaminants along the rice value chain (Chaiyarat et al., 2015, Simon, 2016, Kong et al., 2018, Korley-Kortei et al., 2019). Aflatoxins (B1, B2, G1 and G2) are the most common aflatoxins in rice (Al-Zareyli & Saleh, 2019; Benkerroum, 2020). Aflatoxosis effects depend on many factors, especially the aflatoxin contamination level, toxicity of the aflatoxin and age of the individual (Benkerroum, 2020). Primary aflatoxosis causes haemorrhage, acute liver damage, oedema, impaired digestion and death (Sarma et al., 2017). Chronic aflatoxosis results into teratogenic, mutagenic and carcinogenic effect (Bbosa et al., 2013).

Due to increasing industrialization and urbanization, toxic elements such as arsenic (As), cadmium (Cd), and lead (Pb), originating mainly from mining, industrial processes, chemical fertilizers and atmospheric deposition; have become a major threat to food safety and human health (Kong et al., 2018; Wu et al., 2014; Mary et al., 2018, Wu et al., 2018; Otitoju et al., 2019). As, Cd and Pb pose food safety risks because they rapidly spread out at different levels in the rice value chain through bioaccumulation (Emumejaye, 2014). As causes injury to the pancreatic beta cells, apoptosis and may result in insulin dependent diabetes mellitus (Simon, 2016). Exposure to Cd may cause nephritis, kidney damage, hypertension, anaemia, and osteoporosis (Hoffman, 2015; Satarug, 2018). Pb can cause bone pain, nerve damage, abortion and anemia (Obeng-gyasi, 2019).

Worldwide, people are exposed to pesticide residues through consumption of food (Khan & Rahman, 2017). Dietary exposure to pesticides is a function of the type, level of pesticide residues and rate of consumption of that food (Zarn & Brien, 2017). Human health effects associated with pesticide residues exposure include headache, dizziness, skin irritation, neurotoxicity, breathing difficulties, cancer and death (Evangelou et al., 2016; Guyton et al., 2015; Lesa et al., 2017).
HQ, HI and ILCR factor are commonly used as indicator for food safety (Ishikawa, et al., 2016; Gide et al., 2020; Kong et al., 2018; RAIS, 2019,) but these have not been determined for rice sold in Uganda. Therefore, the aim of this study was to assess the potential human health risks associated with consumption of packed and open traded rice sold in Uganda.

**METHODOLOGY**

**Study area**

Uganda is a landlocked country in East Africa with an area of 241,038 km². Uganda is divided into four regions, Northern, Central, Eastern and Western, with 112 districts. The study trailed the trade flow routes for rice to identify the main trade areas of rice in Uganda (FEWS NET, 2017).

**Sample acquisition**

In this study, open-traded rice referred to rice sold from an open sack and measured using a cup or a weighing scale. Packed rice referred to rice, which is prepackaged and sold sealed in polythene/polypropylene/jute packages. A total of 75 rice samples, i.e. open-traded (30) and packed (45) were selected randomly from markets and supermarkets in the sampling sites shown on the map (FEWS NET, 2017).

Samples were collected based on production dates and batches within each brand and were representative of all commercially available trade names on the Uganda market. Rice was off-season during the sampling period in some districts hence the limited number of open-traded rice samples picked.

**TABLE 1: Sampling sites and number of samples picked per rice trade area in Uganda**

<table>
<thead>
<tr>
<th>District</th>
<th>Number of open-traded samples</th>
<th>Number of packed samples</th>
<th>Number of samples picked per region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mbale</td>
<td>3</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Iganga</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Jinja</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Mukono</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Kampala</td>
<td>4</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Soroti</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Lira</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Gulu</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Kitgum</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Arua</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Hoima</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Kasese</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Mbarara</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Kabale</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>30</strong></td>
<td><strong>45</strong></td>
<td><strong>75</strong></td>
</tr>
</tbody>
</table>
Analysis of Aflatoxins in Rice

Thin layer chromatography (TLC) was used for the detection of AFs as described in the AOAC official method 975.36 (AOAC, 2000). Grinded rice sample (50g each) was weighed and dispersed in 250 ml of acetone in water (85: 15; v/v). The suspension was blended for 3 min at 5000 rpm using Eberbach 8017 explosion-proof blender (Haverhill–Massachusetts, USA). The blended solution was filtered through Whatman no. 4 filter paper and 150 ml of the extract mixed with 3g of cupric carbonate in a 250ml beaker. The mixture was added to a conical flask containing 170 ml of 0.2 M sodium hydroxide and 30ml of 0.41 M, ferric chloride; mixed vigorously for 10 minutes on a rotator shaker and filtered through Whatman No. 4 filter paper. An aliquot of 250 ml of the filtrate was mixed with 150 ml sulfuric acid (0.03%; v/v) and 10 ml chloroform, shaken for 10 minutes and allowed to settle (24 h). The lower layer of chloroform was transferred into separating funnel containing 1g potassium chloride and 100 ml of 0.2M potassium hydroxide solution, swirled of 30 s, passed through a bed of 1g anhydrous sodium sulfate and recollected into a graduated cylinder. The chloroform extract (8 ml) was evaporated to dryness at 45°C under a gentle stream of nitrogen. The extracts were dissolved in 100µl benzene: acetonitrile (98: 2; v/v) and vortexed for 5 minutes. Finally, spots of 2, 5 and 10 µl of samples and standards were individually applied on the TLC plate. TLC plates were dried and observed under long wavelength UV light (λ = 254 and 366 nm) in an enclosed Camag 2930 UV visualizer (Germany). The concentration (C) of individual AFB1, AFB2, AFG1 or AFG2 in μg/kg was calculated according to equation 1.

\[ C = S \times Y \times VX \times W \]  
\[ \text{Volume (μl) of sample extract} \]
\[ \text{Volume (μl) of final dilution of sample extract} \]
\[ \text{Weight (g) of sample contained in final extract} \]

The concentration of Total AFs was calculated following equation 2.

\[ \text{Total AFs} = \text{Concentration of AFB1} + \text{AFB2} + \text{AFG1} + \text{AFG2} \]  
\[ \text{Equation 2} \]

Aflatoxin health risk assessment

Dietary exposure of the rice consumer population to aflatoxins was assessed by computing the estimated daily intake (EDI) using the mean levels of aflatoxins obtained from rice samples (Taghizadeh et al., 2018). The daily rice consumption in Uganda is estimated at 0.22 kg/person/day (FAOSTAT, 2021). The mean body weight was 72.3 kg and 11.3 kg per adult and infant, respectively (Kirunda, 2017). The EDI of aflatoxins was calculated according to equation 3.

\[ \text{EDI} = \frac{R \times A}{BW} \]  
\[ \text{Equation 3} \]

Where EDI is the estimated daily intake (μg/Kg b.w./day), R is the rice consumption (Kg day⁻¹), A is the mean level of Aflatoxin (μg/Kg) and BW is the average body weight (Kg).

Non-carcinogenic risk was assessed using the hazard index as the indicator (Ishikawa et al., 2016). The Hazard Index (HI) was calculated following equation 4.

\[ HI = \sum_{EDI/TD_{50}}^{1} \times \frac{50,000}{TD_{50}} \]  
\[ \text{Equation 4} \]

Where TD50 is the dose (μg/kg /body weight/day) required to induce tumors in half of test animals that would have remained tumor-free at zero dose. TD50 of total aflatoxin=1.3μg/kg and 50,000 is a safety factor as described by Ishikawa et al., (2016) and Ismail et al., (2016).

Carcinogenic risk is the incremental probability of an individual developing any kind of cancer in a lifetime because of exposure to carcinogens (US EPA, 2012). Co-exposure to hepatitis B viruses has a strong influence on the carcinogenic risk of aflatoxins to humans. In epidemiological studies, there is an interaction between aflatoxin exposure and hepatitis B infection, and individuals positive for hepatitis B surface antigen (HBAg) have a multiplicative risk for cancer when presented together with aflatoxin exposure (JECFA, 2018). Following the Joint FAO/WHO Expert Committee on Food Additives model, the population risk for cancer is estimated to be 10% for carriers of hepatitis B in Uganda (WHO, 2020). This implies that 90% are not carriers of hepatitis B. The potencies of hepatitis B virus (HBV) infection (HBAg⁻) and HBV non-infection (HBAg⁺) values are 0.3 and 0.01 cancer cases/year/100,000 subjects, respectively (JECFA, 2018). JECFA (FAO/WHO, 2018) concluded at its 83rd meeting that the prospective Chinese study by (Yeh et al., 1989) which demonstrated a close to linear relationship between aflatoxin exposure and mortality from hepatocellular carcinoma, was still the pivotal study for the carcinogenic risk assessment of aflatoxins. The estimated population at risk of cancer from aflatoxins in Uganda was determined using equation 5.

\[ PR = (0.01 \times 90\%) + (0.30 \times 10\%) \times EDI \]  
\[ \text{Equation 5} \]  
\[ \text{(JECFA, 2018)} \]

Where PR is the population at risk of cancer due to exposure to aflatoxins from rice sold in Uganda (cases/year/100,000 people).

Analysis of heavy metals in rice

The analysis of heavy metals was done using an Atomic absorption spectrophotometer (Model Varian Spectra AA 250 plus) following the method AOAC 999.10 (AOAC, 2010). A rice sample of 2g each was placed in a conical flask. The samples were first digested with 10ml HNO3 at a temperature of 65°C for 20 min and then with 5ml HClO4 at a temperature of 65°C for 20 min and subsequently raising the temperature to 195°C until the volume was clear and near to dry. The solution obtained thereafter was diluted with 20 ml of 20% H2SO4 and filtered. The filtrate obtained was transferred to a 100 ml conical flask, and further diluted with distilled deionized water to the 50 ml mark. The digested samples were analysed of heavy metals with using the atomic absorption spectrophotometer (Model Varian Spectra AA 250 plus). The atomization temperatures of As, Cd and Pb were 2600°C, 1800°C and 2100°C and were determined at wavelength of 193.7nm 228.8nm and 283.3nm, with lamp currents of 9.0mA, 4.0mA and 9 mA respectively using acetylene as the fuel and air as the support gas.
Heavy metal health risk assessment

The heavy metal health risk for adult and infants was considered separately since each exposure pathway changes with age (Almosnino et al., 2019). Dietary exposure of the rice consumer population to heavy metals was assessed by computing the estimated daily intake (EDI) following equation 6.

\[
EDI = \frac{R \times HM}{W} \quad \text{Equation 6 (Sappington et al., 2017)}
\]

Where \( R \) is the daily rice consumption (Kg day\(^{-1}\)), \( HM \) is concentration of heavy metals (mg kg\(^{-1}\)) and \( W \) is the average body weight (kg).

The non-carcinogenic health risk was assessed using hazard quotients (HQ) and hazard indices (HI). The HQ was calculated following equation 7.

\[
HQ = \frac{EDI}{RfD} \quad \text{Equation 7 (Sappington et al., 2017)}
\]

Where \( EDI \) represents estimated daily intake (µg/Kg b.w./day) and \( RfD \) is the reference dose (µg /Kg. day).

### TABLE 2: Reference dose and cancer slope factors of the heavy metals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>As</th>
<th>Cd</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>RfD (mg/kg/day)</td>
<td>0.0003</td>
<td>0.001</td>
<td>0.02</td>
</tr>
<tr>
<td>CSF (mg/kg/day)-1</td>
<td>1.5</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

N/A means not available. Reference: (Sappington et al., 2017; WHO, 2011)

To assess the overall potential non-carcinogenic risk posed by all 3 heavy metal, the hazard index (HI), which is the sum of the HQ values of the individual metals, was calculated according to equation 8 (RAIS, 2020).

\[
HI = \sum_{i=1}^{n} HQ_i \quad \text{Equation 8 (Sappington et al., 2017)}
\]

Carcinogenic health risk was assessed using the incremental lifetime cancer risk (ILCR) value of the carcinogenic heavy metals according to equation 9.

\[
ILCR = CDI \times CSF \quad \text{Equation 9}
\]

### TABLE 3: Constant parameters used in the determination of heavy metal carcinogenic risk

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF(r) (days)</td>
<td>365.0</td>
</tr>
<tr>
<td>AT (years)</td>
<td>70.0</td>
</tr>
<tr>
<td>ED(t) (years)</td>
<td>61.5</td>
</tr>
</tbody>
</table>

Reference: (US EPA, 2021)

Analysis of organochlorine pesticide residues in rice

The extraction procedure for organochlorine pesticide residues was done according to methods described by AOAC official method 2007.01 (AOAC, 2007). Gas chromatographic analysis was done using a Varian CP-3800 gas chromatograph (Varian Associates Inc. USA) equipped with 62Ni electron capture detector. A volume of about 1 µL of the extract was injected and the separation was performed on a fused silica gel capillary column. The carrier and make up gas were nitrogen at 35°C blown in at a flow rate of 1.0 and 29 ml/min respectively. The injector and detector temperatures were 270°C and 300°C respectively. The column oven temperature was programmed as follows: 80°C for 1 min to 18°C at 25°C/min and up to 300°C at 5°C/min held for 1 min.

Dietary exposure was calculated as the estimated average daily intake (EADI) and was determined according to equation 11:

\[
EADI = \frac{C \times CR \times BW}{BW} \quad \text{Equation 11 (US EPA, 2012)}
\]

Where, EADI is the amount of daily pesticide residue that enters the body (µg/Kg/day). C is the mean concentration of residual pesticide (µg/Kg). CR is the daily consumption rate (Kg/day) and BW is the average body weight of an individual (Kg).

The non-cancer risk of pesticide exposure was determined as the hazard quotients (HQ) and hazard index (HI) (RAIS, 2020). Hazard quotients were obtained according to equation 12.

\[
HQ = \frac{EADI}{RfD} \quad \text{Equation 12 (RAIS, 2020)}
\]

Organochlorine pesticide residues health risk assessment

To assess the risk of OCPs contained in open traded and packed rice from the Ugandan market on consumers, the guidelines for pesticide exposure assessments were used (US EPA, 2012).
Where, HQ is the hazard quotient and RfD is the reference dose (mg /Kg-day) of each pesticide, exposure to which is likely to be without an appreciable risk of deleterious effects.

Hazard index is used to assess the non-carcinogenic risk involved in exposure to mixtures of the detected pesticides belonging to organochlorine chemical group (Echodu et al., 2019)(RAIS, 2020). The hazard index (HI) was obtained following equation 13.

\[ HI = \sum_{i} HQ_i \]  

Equation 13 (RAIS, 2020)

The incremental lifetime cancer risk of rice consumers caused by a single OCP through oral intake was calculated based on equation 14 (Echodu et al., 2019)(RAIS, 2020).

\[ ILCR_i = EADi \times SFi \]  

Equation 14 (RAIS, 2020)

Where, ILCR is the incremental lifetime cancer risk caused by the ith OCP. SF is the cancer slope factor for the specific exposure pathway of the ith OCP (mg/kg-day).

The total incremental lifetime cancer risk of all detected OCPs was calculated based on Equation 15 (RAIS, 2020).

\[ TILCR_i = \sum_{i} ILCR_i \]  

Equation 15

Where, TILCR is the total incremental lifetime cancer risk caused by detected OCPs.

**TABLE 4:** Constant parameters used in the determination of OCP carcinogenic risk

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Heptachlor</th>
<th>Heptachlor epoxide</th>
<th>Endrin</th>
<th>Endrin aldehyde</th>
<th>Endosulfan I</th>
<th>Endosulfan II</th>
<th>Endosulfan sulphate</th>
<th>Aldrin</th>
<th>4,4-DDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>RfD (mg/kg/day)</td>
<td>0.50</td>
<td>0.01</td>
<td>0.03</td>
<td>0.03</td>
<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
<td>0.03</td>
<td>0.50</td>
</tr>
<tr>
<td>CSF (mg/kg/day)-1</td>
<td>4.5</td>
<td>9.1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>17</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Data analysis**

Collected data was subjected to one-way analysis of variance (ANOVA) for comparison using the SPSS software (Version 15, SPSS Inc, Chicago, USA). Results were expressed as mean ± standard deviation and variations were considered significant when P < 0.05. Analysis of differences in means of packed and open traded rice samples was done using student’s t-test at a 5% level of significance.

**RESULTS AND DISCUSSION**

**Incidence of aflatoxins in rice sold in Uganda.**

Results of the incidence of AFs in open traded and packed rice samples are shown in Figure 2 and 3. The figures show the type, occurrence and levels of the AFs in open traded and packed rice samples on the Ugandan market.

**FIGURE 2:** Concentration of aflatoxins in open traded rice samples sold in Uganda
All open traded rice samples had detectable levels of total aflatoxins ranging from 0.02 to 14.86 μg/kg (Figure 2). The levels of total aflatoxins reported in packed rice samples ranged from non-detectable to 2.12 μg/kg (Figure 3). Open traded rice samples reported a mean AF concentration of 3.87 ± 4.82 μg/kg, which was significantly (p<0.05) higher than that of packed samples (0.42 ± 0.53 μg/kg).

**Aflatoxin concentration in packed rice samples (μg/kg)**

<table>
<thead>
<tr>
<th>Packed rice samples</th>
<th>Total Aflatoxins (μg/kg)</th>
<th>Aflatoxin B1 (μg/kg)</th>
<th>Aflatoxin B2 (μg/kg)</th>
<th>Aflatoxin G1 (μg/kg)</th>
<th>Aflatoxin G2 (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| AFB1 levels for open traded and packed rice ranged from non-detectable levels to 5.79 μg/kg and 0.79 μg/kg, respectively. AFB2 was detected in some open traded rice samples but not in packed rice. AFB2 levels for open traded rice samples ranged from non-detectable levels to 0.83 μg/kg. AFG1 and AFG2 were not detected in both open traded and packed rice samples.

**FIGURE 3:** Concentration of aflatoxins in packed rice samples sold in Ugandan

**Toxicological risk assessment of aflatoxins detected in rice sold in Uganda**

Results for the health risk associated with aflatoxins in rice sold in Uganda are presented in Table 5. The hazard index (HI) of rice in adults was 1.89E-9 and 1.95E-9 μg/Kg bw/day for open-traded and packed rice, respectively. The HI of rice in infants was 1.21E-7 and 1.25E-8 μg/Kg bw/day for open traded and packed rice, respectively. In general, it is accepted that an HI≤1 indicates non-significant health risk, HI of 1.1 - 10 reflects a moderate risk while HI>10 indicates high risk (Ogunkunle & Fatoba, 2013). HI values from the consumption of open traded and packed rice obtained in this study were less than one. The HI values in this research imply that intake of rice from the Ugandan market will not pose any non-carcinogenic AF related toxicological risk to both infants and adults. However, although low levels of aflatoxin exposure may not result into immediate observable effects, repeated exposure to multiple aflatoxins over a long period of time may result into detrimental health consequences (Echodu et al., 2019).

The potency of cancer in Uganda was 4.79E-4 and 4.95E-4 adults/year/100,000 for open traded and packed rice, respectively (Table 5). Similarly, 3.07E-2 and 3.17E-3 infants/year/100,000 cases were reported in this study for open traded and packed rice, respectively. Aflatoxigenic contamination in Uganda has been reported in maize (Zea mays L.), sorghum (Sorghum bicolor L.), finger millet (Eleusine coracana), peanuts (Arachis hypogaea L.) cassava (Manihot esculenta) and rice (Oryza sativa) (Echodu et al., 2019; Lukwago et al., 2019; Omera et al., 2020; Taligoola et al., 2010). It is common practice in Uganda to eat these starch-based foods because they are cheap and readily available especially among the poor. This daily consumption rate intern increases chances of daily exposure to high concentrations of aflatoxins, which could explain the increasing risk of dying from liver cancer by 75 years in Uganda being 8.6% as reported by (IARC, 2020).

There are limitations to the method used in this study. The first is the reliance on the slope factors determined by (JECFA, 2018) to apply to increased hepatocellular carcinoma cancer from aflatoxin exposure in HBV− and HBV+ individuals. There is a great deal of uncertainty and variability around these estimates conducted in the JECFA study. These slope factors were based upon a study that assumed a multiplicative interaction of aflatoxin and HBV in causing liver cancer, which appears to be accurate for...
populations in high risk areas such as many parts of Asia and Africa (Liu et al., 2012) but may not be accurate for populations where both risk factors are low. Furthermore, HBV prevalence is likely to decline in the future based on increased HBV vaccination that is ongoing in Uganda. Nonetheless, these results represent a first effort in understanding the extent to which aflatoxin contamination levels in rice would expose the rice consumer population to liver cancer in Uganda. Important to note is that low levels of AF may not result in immediate observable effects but repeated exposures to multiple AFs from different food sources over a long period may result into liver cancer (Korley Kortei et al., 2019).

### TABLE 5: Estimated Daily Intake (EDI) and Hazard Indices (HI) of Aflatoxins in rice.

<table>
<thead>
<tr>
<th>Rice category</th>
<th>Mean total Aflatoxin (µg/kg)</th>
<th>Age</th>
<th>BW (Kg)</th>
<th>EDI (µg/kg/.bw/day)</th>
<th>HI</th>
<th>Population at risk (cancer/year/100,000 people)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open traded rice</td>
<td>3.87&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Adults (18-65 yrs)</td>
<td>72.3</td>
<td>1.23E-4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.89E-9</td>
<td>4.79E-4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Infants (6-59 mths)</td>
<td>11.3</td>
<td>7.88E-3</td>
<td>1.21E-7</td>
<td>3.07E-2</td>
</tr>
<tr>
<td>Packed rice</td>
<td>0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Adults (18-65 yrs)</td>
<td>72.3</td>
<td>1.27E-4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.95E-9</td>
<td>4.95E-4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Infants (6-59 mths)</td>
<td>11.3</td>
<td>8.14E-4</td>
<td>1.25E-8</td>
<td>3.17E-3</td>
</tr>
</tbody>
</table>

(1) yrs means years, mths means months, BW is body weight, EDI is the estimated daily intake, HI is the hazard index.

(2) Results are means ± standard deviation. Means in the same row having the same superscripts (a, b, c) are not significantly different (p>0.05).

(3) PR = (0.01 x 90%) + (0.30 x 10%) x EDI

### Incidence of heavy metal contamination in rice sold in Uganda

Results for the incidence of heavy metal contamination from this study are shown in Table 6. Results (0.11±0.05, 0.07±0.02, 0.38±0.59) µg/kg indicated no significant difference (p>0.05) between the mean values of As, Cd, Pb in open traded and packed rice. As concentration varies according to the soil where the rice was cultivated and the type of rice (Mania et al., 2017). The anaerobic growing conditions of flooded rice paddies allow rice to take up, sequester and accumulate As (Zhao & Wang, 2020). As bioavailability to rice under the flooded conditions is the main reason for an enhanced As accumulation by flooded rice (Honma et al., 2016).

### Toxicological risk assessment of heavy metals detected in rice sold in Uganda

The EDI, HQ, HI and ILCR of rice in adults and infants for commercially available rice are presented in Table 6. Given that the levels of As, Cd and Pb in packed and open traded rice samples were not significantly (p>0.05) different, the means of each heavy metal were calculated and the toxicological risk associated with consumption of rice sold in Uganda in adults and infants evaluated.

### TABLE 6: Human toxicological risk associated with heavy metals in rice sold in Uganda

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Mean heavy metal concentration (mg/Kg).</th>
<th>RFD (mg/Kg/day)</th>
<th>Age</th>
<th>BW (Kg)</th>
<th>EDI (µg/kg /bw/day)</th>
<th>HQ</th>
<th>HI</th>
<th>ILCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>0.11±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0E-4</td>
<td>Adults (18-65 yrs)</td>
<td>72.3</td>
<td>3.54E-5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.118&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.14E-2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cd</td>
<td>0.07±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.0E-3</td>
<td>Infants (6-9mths)</td>
<td>11.3</td>
<td>2.27E-4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.756&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.28E-2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pb</td>
<td>0.38±0.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0E-2</td>
<td></td>
<td></td>
<td>8.04E-4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.040&lt;sup&gt;d&lt;/sup&gt;</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

(1) Where As is arsenic, Cd is cadmium, Pb is lead, BW is body weight, EDI is the estimated daily intake, HQ is the hazard quotient, HI is the hazard Index, and ILCR is the incremental life cancer risk. N/A means not applicable. E means exponent.

(2) Results are means ± standard deviation. Means in the same column with the same superscripts (a, b, c,d,e,f) are not significantly different (p>0.05).

(3) Figures in bold are above the recommended acceptable level (USEPA, 2014)
The EDI of As, Cd and Pb in both open traded and packed rice were significantly lower than the reference dose recommended by (Sappington et al., 2017) in both adults and infants. Hazard quotients recorded for the metals ranged from 0.006 to 0.756 (Table 6). The values were below the USEPA permissible limit of 1; implying there is no potential non-carcinogenic toxicological risks associated with As, Cd or Pb from rice in both adults and infants. The results in this study were similar to previous studies by Omar et al., (2015) and Zullaiflee et al., (2019).

Hazard indices recorded for heavy metals in both open traded and packed rice were 0.15 and 0.94 for adults and infants, respectively (Table 6). The results imply that there is no potential non-carcinogenic toxicological risk from the combination of As, Cd and Pb in rice to adults and infants. The values obtained for the ILCR for as through the consumption of rice analyzed in this study were 1.14E-2 and 7.28E-2 for adults and infants, respectively. According to the US EPA, any ILCR value equal to or less than 1.00E-4 is safe and poses no considerable expected carcinogenic toxicological risk to a population (USEPA, 2014). The ILCR for as revealed a potential carcinogenic risk from consumption of open traded and packed rice in both infants and adults at the current level of contamination and consumption rate. The results in this study are similar to the range reported in Monrovia (Gomah et al., 2019) and China (Fu et al., 2015).

### TABLE 7: Human toxicological risk associated with pesticide residues in open traded rice in Uganda

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>RID (μg/kg/day)</th>
<th>RC (mg/kg)</th>
<th>Adults</th>
<th>Infants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>EADI (μg/kg Bw/day)</td>
<td>HQ</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>5.00</td>
<td>0.02a</td>
<td>2.32E-3 a</td>
<td>4.64E-4 a</td>
</tr>
<tr>
<td>Heptachlor epoxide</td>
<td>0.013</td>
<td>0.07a</td>
<td>8.12E-3 a</td>
<td>6.24E-1 d</td>
</tr>
<tr>
<td>Endrin</td>
<td>0.03</td>
<td>0.01a</td>
<td>1.16E-3 a</td>
<td>3.87E-2 c</td>
</tr>
<tr>
<td>Endrin aldehyde</td>
<td>0.03</td>
<td>0.08a</td>
<td>9.28E-3 a</td>
<td>3.09E-1</td>
</tr>
<tr>
<td>Endosulfan I</td>
<td>6.00</td>
<td>0.16b</td>
<td>1.86E-2 b</td>
<td>3.10E-3 b</td>
</tr>
<tr>
<td>Endosulfan II</td>
<td>6.00</td>
<td>0.03a</td>
<td>3.48E-3 a</td>
<td>1.59E-3 b</td>
</tr>
<tr>
<td>Endosulfan sulphate</td>
<td>6.00</td>
<td>0.03a</td>
<td>3.48E-3 a</td>
<td>1.59E-3 b</td>
</tr>
<tr>
<td>Aldrin</td>
<td>0.03</td>
<td>0.03a</td>
<td>3.48E-3 a</td>
<td>1.16E-1 d</td>
</tr>
<tr>
<td>4,4-DDE</td>
<td>0.50</td>
<td>0.03a</td>
<td>3.48E-3 a</td>
<td>6.96E-3 b</td>
</tr>
<tr>
<td>Hazard Index</td>
<td>1.10</td>
<td></td>
<td>1.45E-1</td>
<td>9.37E-1</td>
</tr>
</tbody>
</table>

(1) RID= Reference Dose. RC= Residual concentration. HQ= Hazard quotient EADI= Estimated Average ILCR= Daily Intake Toxicological risk Incremental life carcinogenic risk. Bold values are above acceptable safe value

(2) Means in the same column having the same superscripts (a, b, c, d) are not significantly different (p>0.05)

Estimated daily intake (EDI) for heptachlor epoxide (0.05) and endrin aldehyde (0.06) in open traded rice were higher than that of the respective reference dose for infants (Table 7). Consequently, hazard quotients estimated for heptachlor epoxide (4.05) and endrin aldehyde (2.00) were above one (1); suggesting that there is potential non-carcinogenic effects from each of the 2 OCPs in infants. The toxicological risk results from this study supports the findings that the estimated exposure levels are age-dependent given that infants have higher food consumption per kilogram of their body weight and consequently have higher estimated exposure levels (Union, 2019). The hazard indices of open traded rice in both adults (1.10) and infants (7.14) were greater than 1 which suggests potential non-carcinogenic toxicity from the combined OCP consumption. The HI in infants was significantly (p>0.05) higher than that in adults implying that infants are at greater risk of non-carcinogenic effects.
upon consumption of open traded rice compared to the adults. A study on the risk associated with consumption of rice grans, in Punjab Province, Pakistan similarly suggested a potential non-carcinogenic toxicological risk in adults and children (Mumtaz et al., 2015).

The USEPA regards an ILCR in the range of 1.0E-7 to 1.0E-4 as an acceptable carcinogenic risk value (USEPA, 2021). ILCR values above 1.0E-4 are considered as carcinogenic. The ILCR for heptachlor, heptachlor epoxide and Aldrin in open traded rice were higher than 1.0E-4 in both adults and infants (table 7), which implies that the residual concentration detected in open traded rice, is carcinogenic.

### TABLE 8: Human toxicological risk associated with pesticide residues in packed rice in Uganda

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Rfd (µg/kg/day)</th>
<th>RC (mg/kg)</th>
<th>Adults</th>
<th>Infants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EADI</td>
<td>HQ</td>
<td>ILCR</td>
<td>EDI</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>5.00</td>
<td>0.01a</td>
<td>1.16E-3</td>
<td>2.32E-4</td>
</tr>
<tr>
<td>Heptachlor epoxide</td>
<td>0.01</td>
<td>0.01a</td>
<td>1.16E-3</td>
<td>8.92E-2</td>
</tr>
<tr>
<td>Aldrin</td>
<td>0.03</td>
<td>0.01a</td>
<td>1.16E-3</td>
<td>3.87E-2</td>
</tr>
<tr>
<td>Endosulfan I</td>
<td>6.00</td>
<td>0.01a</td>
<td>1.16E-3</td>
<td>1.93E-4</td>
</tr>
<tr>
<td>Endosulfan II</td>
<td>6.00</td>
<td>0.01a</td>
<td>1.16E-3</td>
<td>1.93E-4</td>
</tr>
<tr>
<td>Hazard Index</td>
<td>0.13</td>
<td>3.50E-2b</td>
<td>0.80</td>
<td>2.30E-1b</td>
</tr>
</tbody>
</table>

(1) Rfd= Reference Dose. RC= Residual concentration. HQ= Hazard quotient | EADI= Estimated Average Daily Intake. ILCR is incremental life carcinogenic risk. Bold values are above acceptable value

(2) Results are means ± standard deviation. Means in the same column with the same superscripts (a, b) are not significantly different (p<0.05)

The EADI for both adults and infants in packed rice were lower than the reference dose. The HQ were similarly lower than the acceptable safe limit of 1, in both adults and infants (RAIS, 2020). This implies that consumption of packed rice poses no potential non-carcinogenic risk in adults and infants related to OCPs. The ILCR of all detected OCPs in adults and infants were higher than 1.0E-4, which implies that there is risk of carcinogenic effects from consumption of packed rice.

### CONCLUSION

This study indicated that the incidence of aflatoxin, heavy metal and OCP contamination in rice sold in Uganda is quite common. In order to minimize/prevent contamination. Establishment of a monitoring system along the entire rice value chain; enforced by a national regulatory body can provide timely feedback on the levels of contamination and the progress in reducing the contamination burden.

### CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### REFERENCES


