

# Human Health Risk of Glyphosate Residues in Rice (*Oryza sativa*) and Beans (*Phaseolus vulgaris*) In Gombe State, Nigeria in an Era of COVID-19 Pandemic

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## ABSTRACT

Samples of beans and rice were collected from five Local Government Areas (LGAs) of Gombe State for the quantitative determination of glyphosate residues. The residue extraction was performed using acetonitrile/water (50/50%) solution and the extracts were injected into the injection block of gas chromatography in tandem with a mass spectrophotometer for separation and quantification. The concentrations of glyphosate in bean samples ranged between 0.0164 mg/kg and 0.0508 mg/kg higher than the residues found in rice samples between 0.0175 mg/kg and 0.0434 mg/kg. The highest concentration of glyphosate residues in beans was 0.0508±0.01 mg/kg from Gombe LGA while the rice sample from Akko LGA was highest with 0.0434±0.02 mg/kg. Both samples in all the LGAs were found to be lower than their respective Maximum Residual Limits (MRLs) and lower than the Acceptable Daily Intake (ADI) of 0.5mg/kg/day. Health Index, HI < 1 was obtained in all samples; indicating that the residues in the grains do not pose any threat to the exposed population. However, the Cancer Risk Index, CRI for children were 7.32x10<sup>-4</sup> and 4.090x10<sup>-4</sup> while those for adults were 9.140x10<sup>-2</sup> and 5.120x10<sup>-4</sup> for beans and rice respectively. The TCRI for children and adults were 1.140x10<sup>-3</sup> and 6.030x10<sup>-4</sup> respectively. These are indications that the exposed population is at high risk, with the children more susceptible, probably due to low body weight. The study, therefore, suggests strict monitoring of the use of glyphosate-based herbicides to curtail or minimize the risk of possible bioaccumulation in the body and also calls on further studies on human urine and blood samples of farmers within the study area for further toxicological studies.

## INTRODUCTION

Glyphosate (N- phosphonomethyl glycine) is an organophosphorus-based herbicide applied to combat a wide range of unwanted weeds. Glyphosate has been approved for use in over 130 countries and sold under the trade name Roundup [1]. It acts by blocking the 5- enolpyruvylshikimate-3-phosphate synthase (EPSPS) pathway. This pathway produces phenylalanine, tyrosine and tryptophan which are precursors of varieties of plant growth, defence and other plant development or regulatory compounds [2]. The consequence of the action of glyphosate is that it causes starvation of the plant, hence, the plant suffers chlorosis and necrosis; and eventually dies [3]. It is

absorbed through foliage, minimally through roots and transported to growing points.

Grains are common food items that every human being and most animals depend on. They constitute staple food for the average Nigerian. They include maize, millet, sorghum, wheat, rice, barley, soya beans, groundnut etc. [4]. Rice is one of the important food items in Nigeria [5]. According to a report by statistica.com, milled rice production was estimated to be over 5 million metric tons between 2012 and 2020 and has increased tremendously since then. Rice is rich in starch, an excellent source of energy, iron and some proteins. Beans are an important source of diet serving consumption needs and quality fodder for livestock [6]. It is the 4<sup>th</sup> most important source of protein in

Nigeria and other parts of the world. It is a vital staple food in Nigeria and other parts of sub-Saharan Africa for more than 70 million people. Nigeria is the largest producer in Africa but 4<sup>th</sup> in the world after India, Canada and Burma. In Nigeria, North-East is among the largest producer and Gombe State is one of the six producer states [7] (Femi, 2021). According to [8], beans contain protein, folate, and antioxidants, reduce the risk of cancer, heart disease, and diabetes and help glucose metabolism, prevent fatty liver and control appetite. The World Health Organization (WHO)'s Global Environmental Monitoring System (GEMS) presented 0.027kg/day as the bean consumption rate in Nigeria [9].

Despite glyphosate high efficacy, research revealed that glyphosate leaves residues in crops which are oncogenic, mutagenic, teratogenic and probably carcinogenic [10]; hence, the need to investigate the grains for residues of glyphosate to ascertain that their presence is within safe levels. The purpose of the risk characterization is to present the public with a synopsis and synthesis of all data that should contribute to the conclusion with regard to the nature and extent of the risk.

The North-Eastern sub-region to which Gombe State belongs has been devastatingly plundered and dilapidated by socio-political crises ranging from insurgency, communal strife, banditry as well as environmental hazards resulting from pollution, desertification and soil erosion such that further harm to the environment/inhabitants could push it to the precipice. The mainstay of the people of Gombe State is the cultivation of grains and legumes that serve as staple food and sources of income for day-to-day survival. To be able to meet these cardinal daily requirements therefore, modern technology through the use of soil additives is applied. The heavy reliance on the weed killer, glyphosate is not without its residual consequences, hence the need to investigate the output of their application for remnants of glyphosate herbicides.

Consequent to their use, traces of the residues of glyphosate have been found in different food items. According to [11], glyphosate is ubiquitous and is found in water bodies, food, dairy and sanitary products, poultry feeds as well as environmental samples. In a research carried out by [12], South African wheat samples showed an average of 2.1 mg/kg of glyphosate residues. The residues have been linked to many life-threatening diseases that have been well documented.

Gombe State was created out of Bauchi State on 1<sup>st</sup> October 1996 by the Late General Sani Abacha's Administration. It has a population of 2.4 million (2,365,040) by the 2006 census figure and a Land Area of 20265 sqm (16,639km<sup>2</sup>) projected at a growth rate of 3.3%. Gombe State is one of the 36 states of the Federal Republic of Nigeria, located in the centre of the northeast of the country on latitude 9°30' and 12°30'N. Longitude 8°5' and 11°45' E. It is bordering six other North-Eastern States of Nigeria. There are 11 Local Government Areas in Gombe State [13]. The State capital is located in Gombe town founded by Buba Yero (Abubakar) in 1804. The major townships are Gombe the capital, Bajoga, Billiri, Kaltungo, Kumo, Dukku and Deba [13].

Glyphosate (N-phosphonic methyl glycine,) is an organophosphorus-based herbicide that is a non-selective pesticide applied for the control of a broad spectrum of weeds. It is one of the most patronized herbicides because of its efficacy; accounting for 56% of use globally [14] specifically targeted at genetically modified organisms (GMO) or transgenic plants. Glyphosate is applied as a plant growth regulator [15] while its application on non-GMO crops is for desiccation [3].

Despite the global acceptance, it has been posited in many studies as exhibiting both acute and chronic toxicity; toxic and carcinogenic [15] in flora, fauna and humans. According to [11], glyphosate is ubiquitous and is found in water bodies, food, dairy and sanitary products, poultry feeds as well as environmental samples. Glyphosate was found between 0.88-2.77 mg/kg in wheat samples in South Africa [16]. Soil samples from Egypt as reported by [17] recorded between 0.08-9.68mg/kg. In Italy, maize samples showed the presence of glyphosate residues of 0.14mg/kg as submitted [18]. Soybeans samples for Manhattan, USA had between 0.48-8.88 mg/kg [19], while [20] reported glyphosate residues in samples taken from Retail Markets in Canada.

Traces of glyphosate have been found in bread flour and maize meal due to its wide usage in South Africa [15]. Glyphosate residue was reported to have caused the deaths of fish at a concentration of 0.004 ml/l in Kano, Nigeria [21]. The most common routes of exposure of the residues are by ingestion of contaminated food, inhalation and dermal exposure, but the ingestion pathway may be five orders of magnitude higher than the other routes and hence, the study adopted the ingestion pathway [22].

Furthermore, researchers of the current COVID-19 pandemic submitted that older adults (60 years and more), immune-compromised or immune-suppressed patients face higher risks of COVID-19 [23]. It will be inappropriate to allow an individual to be unduly exposed to a 'probably carcinogenic' compound, glyphosate in the name of improving crop yield. The objective of the study is to determine the quantity of glyphosate residue in the beans and rice samples and to evaluate the health risk potentials of the residues. In addition, another aim is to estimate the non-carcinogenic and carcinogenic risk for different exposure pathways for the residues,

## MATERIALS AND METHOD

### Chemicals, reagents and instrument

The solvents used are of analytical grade. The analytical standard of glyphosate (purity 99.9%) was sourced by Monsanto Company. Gas chromatography-mass spectrophotometer (MSD 5975C) work was carried out on an Agilent GC 7890A [2, 24].

### Sample and Sampling

The samples, rice and beans were collected from 5 locations in the 5 Local Government Areas (LGAs) headquarters of the Gombe State for convenience. The collection was made in such a manner that the samples obtained direct produce from the particular locality and were not transported farm produce from elsewhere. The 5g of each of the samples were collected in polyethylene bags, labelled and sealed properly [2, 24].

### Sample Treatment

The samples were separated from every form of impurity such as stones, sand and other unwanted particles or debris. They were kept in an oven of forced air circulation at 35°C for 12 hours or air-dried. The samples were thereafter homogenized in a blender into a fine powder. The powders of each of the samples were passed through a sieve of 2mm [24]. The sieved powders were divided into three equal parts, which constitute three different replications subjected to extraction. 3 replicates of 100 mg each of the samples were extracted using 10ml acidified acetonitrile-water (50:50) (v/v) with 1% Formic acid (v/v) in an ultrasonic bath at an ultrasonic frequency of 42 kHz for 30 minutes in a 15ml centrifuge tube [2, 24].

Each replicate was centrifuged at 4000 rpm for 10 minutes at 20°C and filtered in a Millex HV 0.45 µm with a membrane Dura pore 13 mm. The filtrate was taken for quantification in a Gas Chromatography and Mass Spectrophotometer (GC/MS) [2, 24] to determine the concentrations of glyphosate in the samples.

### Health Risk Indices

Health risk is a measure of the tendency of harmful effects on human health as a result of exposure to pollutants from the environment. The risk assessment is undertaken to predict the carcinogenic and non-carcinogenic risks that may arise as a consequence of exposure of both adults and children to glyphosate or glyphosate-based herbicides living within the study area. This is achievable by the incorporation and intergradations of all possible pathways through which the population is likely to get infested to enable the quantitative determination of the health hazard. This study is adopting the health risk assessment model developed by the United States Environmental Protection Agency (U.S. EPA) to ascertain the human risk of exposure to glyphosate. The model involves a series of steps which includes hazard identification, dose-response assessment, exposure assessment, and risk characterization [25].

### Target Hazard Quotient (THQ)

THQ is used to analyse the potential non-carcinogenic effect of glyphosate in the grain samples by relating the estimated Average Daily Intake, ADI of each of the samples with the Reference dose, RfD or Chronic reference dose for glyphosate (1.75 mg/kg/day) [26] by U.S EPA. However, the European Union (EU) ADI value is pegged at 0.3 mg/kg/day. The Health Index (HI) is expressed as the sum of the THQ as described in the equation above to describe the cumulative non-carcinogenic effect. THQ is calculated using equation (3) In this study, a human could be exposed to glyphosate molecules by the diet of the grains (oral ingestion pathway) from glyphosate-treated crops. Glyphosate exposure occurs through the dietary consumption of glyphosate residues in food and dirty water [27]. The hazard risk assessment involves the calculation of the Acceptable Daily Intake (ADI) or Lifetime Average Daily Dose (LADD) (mg/kg/d). The equation is as shown below:

$$\text{Oral intake of crop (CDI}_{\text{crop}}) \text{ (mg/kg/day)} = \frac{C_{\text{crop}} \times IR \times EF \times ED}{BW \times AT} \quad (\text{Eqn. 1})$$

$$AD_{\text{inh}} = \frac{C_{\text{plant}} \times IR \times EF \times ED}{BW \times AT} \quad (\text{Eqn. 2})$$

$$\text{Target Hazard Quotient (THQ)} = \frac{LADD \text{ (mg.kg}^{-1}.\text{day}^{-1})}{RfD \text{ (mg.kg}^{-1}.\text{day}^{-1})} \quad (\text{Eqn. 3})$$

$$\text{Health Index (HI)} = \sum THQ \quad (\text{Eqn. 4})$$

$$\text{Cancer risk} = \frac{ED \times EF \times SF \times ED}{70 \text{ years (life time)}} \quad (\text{Eqn. 5})$$

Where,

- ADI = Average Daily Intake (mg/kg/day),
- C<sub>crop</sub> = crop/grain concentration of glyphosate ( mg/kg),
- IR = ingestion rate,
- EF = Exposure frequency (day/year),
- AT= average exposure (day),
- ED = exposure duration (year),
- RfD = Chronic Reference Dose (mg/kg/day),
- RF = Risk Factor
- SF = cancer slope factor and
- LADD=Lifetime Average Daily Dose (mg/kg/d)

## RESULTS

A food combination that occupies a vital role in the list of African food delicacies because of the nutrients it is known to contain and the palatable taste it exhibits coupled with the enticing flavour it exudes. Gas chromatography analysis was performed on the samples of rice and beans collected from 5 LGAs of the state and the results are as shown in **Table 1.** and **Figs. 1, 2** and **3.** Beans have a higher concentration than the rice samples with average values of 0.0355 and 0.0310 mg/kg, respectively.

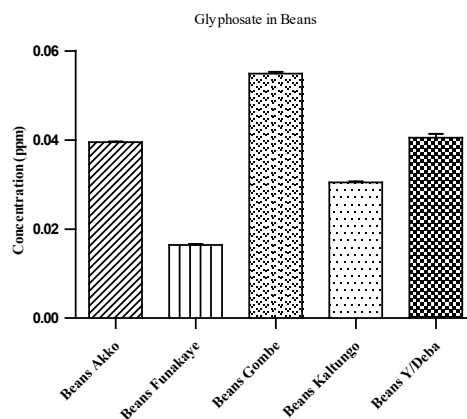
**Table 1.** Represents the concentrations, average concentrations, MRIs and ADI of rice and beans samples from the various LGAs of Gombe State.

Local Government Area	Beans (mg/kg)	Rice (mg/kg)
Akko	0.0395±0.02	0.0434±0.02
Funakaye	0.0164±0.03	0.0323±0.02
Gombe	0.0508±0.01	0.0256±0.01
Kaltungo	0.0305±0.03	0.0175±0.03
Yamaltu-Deba	0.0405±0.01	0.0361±0.01
Average Conc.	0.0355	0.0310
Maximum Res. Limit (MRLs) (USEPA, 2017)	40	0.1
Acceptable Daily Intake (ADI) (mg/kg) (EFSA, 2015)	0.5 (glyphosate)	0.5 (glyphosate)

The result for the Acceptable Daily intake (ADI) is shown in **Table 2.** ADI measures the amount of a substance (originally applied for a food additive, later for a residue of a drug or pesticide) in food or drinking water that can be ingested (orally) daily over a lifetime without an appreciable health risk. The calculated Target hazard Quotient (THQ) and the Health Index (HI) which is a measure of the tendency of harmful effects to human health as a result of exposure to pollutants from the environment and the Cancer Risk Index (CRI) were also determined (**Table 2**).

**Table 2.** Representing the Health Risk Characterization for carcinogenic and non-carcinogenic risk analyses results.

Parameters	Beans (Children)	Rice (children)	Beans (Adults)	Rice (Adults)
ADI (mg/kg)	4.33×10 <sup>-1</sup>	2.42×10 <sup>-1</sup>	5.41×10 <sup>-2</sup>	3.03×10 <sup>-2</sup>
%ADI	24.74	13.83	3.09	1.73
THQ	2.50×10 <sup>-1</sup>	1.35×10 <sup>-1</sup>	3.10×10 <sup>-2</sup>	1.73×10 <sup>-2</sup>
CRI	7.32×10 <sup>-1</sup>	4.09×10 <sup>-4</sup>	9.14×10 <sup>-5</sup>	5.12×10 <sup>-4</sup>
HI (∑THQ)	5.60×10 <sup>-1</sup>		4.83×10 <sup>-2</sup>	
TCRI	1.14×10 <sup>-3</sup>		6.03×10 <sup>-4</sup>	



**Fig. 1.** Graphical representation of the relative abundance of glyphosate residues in beans sample collected from various Local Government Areas.

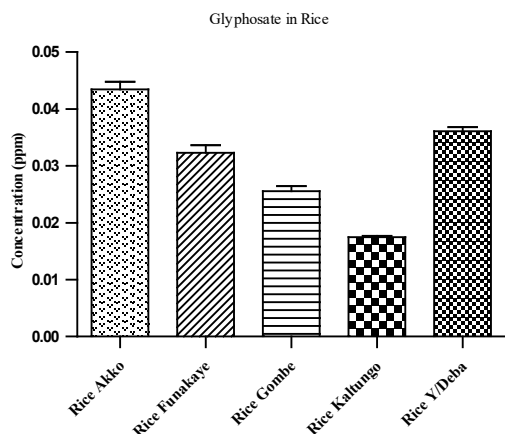


Fig. 2. Graphical representation of the relative abundance of glyphosate residues in rice sample collected from various Local Government Areas.

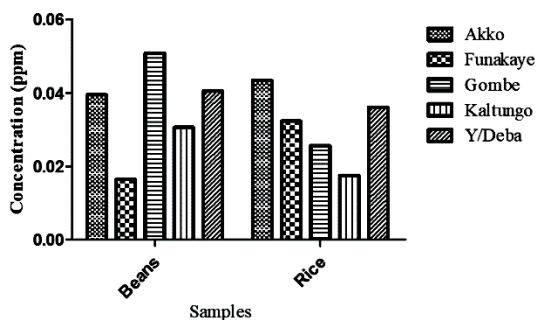


Fig. 3. A bar chart representation of the relative abundance of the samples according to their respective LGAs.

## DISCUSSION

Samples of rice and beans, a food combination that occupies a vital role in the list of African food delicacies because of the nutrients it is known to contain and the palatable taste it exhibits coupled with the enticing flavour it exudes. Gas chromatography analysis was performed on the samples carefully collected from 5 LGAs of the state. The higher concentration in beans than in the rice samples is in agreement with the submission of the European Food Safety Authority [28] that reported 0.04 mg/kg of glyphosate in cereals and grains taken from Norway and Iceland. These results are higher than a previous study [29], which reported a range of 0.001-0.0124 mg/kg for grains from Swiss and [30] reported 0.0058-0.098 mg/kg in barley and 0.006-3.1mg/kg in soybeans from Canada.

The result of this study is however, much lower than those of [12] report of 2.1 mg/kg of glyphosate in wheat sampled from South Africa. The reason for the higher value may be attributed to the morphology and physiology of the plants. The beans plant is a leguminous crawling or climbing plant whose fruits are closer to the ground than those of the rice plant, a cereal with an upright stem, growing against the force of gravity. This helps the beans' translocations of nutrients as well as pesticide residues through its shorter and often narrower stems and accumulates them at the storage sites, the fruits/grain. The rice plant elongated upright stem that only branches to leaves with cylindrical shapes with the flow of nutrients/pesticide residues that may be resisted or impeded by the gravitational force against which the flow of input takes place.

The ranking order in terms of the concentrations of glyphosate residues in the bean's samples revealed as follows according to the LGAs: Gombe>Yamaltu-Deba>Akko>Kaltungo>Funakaye. This order may be added to many factors ranging from geo-political, economic and proximity to the centre, Gombe Township. Gombe, Yamaltu-Deba and Akko LGAs all belong to the same Central Senatorial District of the state, while Kaltungo and Funakaye LGAs are in the South and North Senatorial Districts respectively. Gombe LGA is the state capital and the hub of socio-political and economic activities of the state. Most of the farmers in Gombe LGA and its nearest neighbours; Yamaltu-Deba and Akko LGAs are civil servants and non-civil servants such as artisanship, commercial transportation, trading and the like to make extra cash. These categories of farmers rely on the use of chemical herbicides such as glyphosate to get rid of weeds to boost crop yield. They find the cultural method abhorring, strenuous, and time and energy-sapping. The other runners-up such as Yamaltu-Deba and Akko LGAs are only a stone's throw from the centre which is very much accessible due to proximity and a good road network that encourages access to glyphosate at a lower cost.

Unlike the LGAs in the Central Senatorial District, Kaltungo and Funakaye LGAs are farther from the centre and therefore, do not share the same potential in terms of proximity, accessibility, the population size of farmers and economic power to purchase the herbicide and engage hired hands to apply the herbicide which in itself is a compelling function of misuse, abuse and unnecessary use of the chemical that [22] asserted are the reasons for high concentrations of residues in food samples. The result of this study is compared to an international regulatory agency; the United States Environmental Protection Agency [31] that provided the Maximum Residual Limits (MRLs) for beans and rice.

The results revealed that the various concentrations of glyphosate residues are below their respective MRLs. These are a demonstration of the fact that herbicide is applied within the manufacturer's dictate and the exposed population is safe. MRLs are the amounts of pesticide residues that are expected (legally allowed) to remain on food products when a pesticide is used according to the manufacturer's specification (Good Agricultural Practice, GAP), that will not be a concern to human health. The MRLs are not toxicological limits but only legal postulations to ensure safe use/applications of pesticides and therefore, not enough of a criterion to determine safety.

In light of the above, the results of the study are further measured against the Acceptable Daily intake (ADI), which is a measure of the amount of a substance (originally applied for a food additive, later for a residue of a drug or pesticide) in a food or drinking water that can be ingested (orally) daily over a lifetime without an appreciable health risk. The EFSA (2015) set the ADI for glyphosate at 0.5 mg/kg/day. The calculated ADIs for this study as shown in Table 2 are all below the set standard. This is indicative of the safety of the population under study. According to [32] as long as the dietary exposure (estimated Daily Intake (EDI) or Chronic Daily Intake (CDI) is lower than or equal to the toxicological reference values (exposure  $\leq$  the ADI or ARfD) a consumer's health risk can be excluded with a degree of certainty. The ADI and %ADIs are both indicative of absolute safety.

### Non-Carcinogenic Risk

The results of the calculated Target hazard Quotient (THQ) and the Health Index (HI) which is a measure of the tendency of harmful effects to human health as a result of exposure to pollutants from the environment. The results showed that the HI < 1 in both children ( $5.60 \times 10^{-1}$ ) and adults ( $4.83 \times 10^{-2}$ ). According to [33], a THQ or HI < 1 signifies no associated risk; meaning the exposed population is not likely to pose any significant adverse health risk. The above results agree with those of [34] on glyphosate on human health via food contamination; [35] and [36] both on dietary exposure to organochlorine pesticides where they obtained HI < 1. The concern, however, is that the HI values are too close for comfort. The effect of the consumed food items with the glyphosate residues may be additive or synergistic. This means that even pesticides that were detected at safe levels may eventually pose health hazards to humans due to combined and accumulated effects in the body.

### Carcinogenic Risk analysis

The Cancer Risk Index (CRI) revealed that the exposed population falls within the bracket [37] classified as Grade VI (high-risk level) as the CRIs are within  $5.010^{-4}$  -  $10^{-3}$ . This calls for the attention of all and sundry. The TCRI values for children ( $1.14 \times 10^{-3}$ ) and adults ( $6.03 \times 10^{-4}$ ) suggest a much higher risk for children which may be due to their small body weight. These results, however, agree with that of [38] which asserted that the Incremental Lifetime Cancer Risk (ILCR) value of most of the world was higher than  $1.0 \times 10^{-4}$ . It was shown in a case that the CRI is  $3.4 \times 10^{-3}$  for heavy metals in soil, vegetables and rice from the vicinity of Tailoring Pond in China [39].

### CONCLUSION

The concentrations of glyphosate residues in the beans and rice samples collected from five LGAs namely, Akko, Funakaye, Gombe, Kaltungo and Yamaltu-Deba in Gombe State were determined using gas chromatography coupled with a mass spectrophotometer. The results revealed that the amounts of the pesticide are generally below their respective MRLs by USEPA standards and were further found to be lower than the EFSA ADI value of 0.5mg/kg/day for glyphosate. In another analysis using the THQ and HI, all the samples also ranked below 1 (HI < 1). These outcomes are suggestive of the fact that the exposed population is safe and Good Agricultural Practice (GAP) was adopted. The palpable fear, nonetheless, is the possibility of the residues of glyphosate having additive or synergistic effects, leading to bioaccumulation in the body due to the continuous consumption of these food items. In contrast to the above non-cancer risk analyses, the CRI and TCRI values show results that portray the population to be at high risk if steps are not taken to stem the tides of use and applications of glyphosate-based herbicides.

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