EatSafe: Evidence and Action Towards Safe, Nutritious Food

Food Safety Hazards and Risk Associated with Select Nutritious Foods

Assessment from Traditional Markets in North-Western Nigeria

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This EatSafe report presents evidence that will engage and empower consumers and market actors to obtain safe nutritious food. It will be used to design and test consumer-centered food safety interventions in traditional markets through the EatSafe program.

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TABLE OF CONTENTS

ACRONYMS AND ABBREVIATIONS **5**
EXECUTIVE SUMMARY **6**
1. INTRODUCTION **8**
1.1. BACTERIAL HAZARDS 9
1.2. CHEMICAL HAZARDS 9
2. RISK ASSESSMENT OVERVIEW AND APPROACH **10**
2.1. OBJECTIVE 10
2.2. KEY COMMODITIES 10
2.3. RA PROCESS 11
3. FIELD DATA COLLECTION **13**
3.1. METHODOLOGY 13
3.2. RESULTS 13
3.2.1. RECONNAISSANCE STUDY 13
3.2.2. CONSUMER SURVEY 14
3.2.3. MICROBIAL FOOD SAMPLING ANALYSES 15
4. SALMONELLA RISK ASSESSMENT **17**
4.1. METHODOLOGY 17
4.1.1. EXPOSURE ASSESSMENT APPROACH 18
4.1.2. MICROBIAL RISK ASSESSMENT MODEL 19
4.1.3. MODEL PARAMETER ESTIMATES 19
4.2. RESULTS 22
5. AFLATOXIN RISK ASSESSMENT **23**
5.1. METHODOLOGY 23
5.2. MODELLING APPROACH 23
5.3. RESULTS 24
5.3.1. DIETARY EXPOSURE ESTIMATES 24
5.3.2. RISK RANKING 24
6. DISCUSSION **25**
7. CONCLUSIONS **33**
8. REFERENCES **36**
9. APPENDICES **46**
9.1. APPENDIX 1. DESCRIPTIVE ANALYSES OF EATSAFE'S KEY COMMODITIES IN NGIERA 46
9.1.1. MAIZE 46
9.1.2. FISH 47
9.1.3. RICE 48
9.1.4. COWPEAS 49
9.1.5. SOYBEANS 50
9.1.6. FRESH VEGETABLES 51

9.1.7. BEEF ... 52
9.1.8. CRITICAL CONTROL POINTS ALONG VALUE CHAINS ... 53
9.2. APPENDIX 2. OTHER HAZARDS IDENTIFIED IN KEY COMMODITIEIS ... 54
9.3. APPENDIX 3. DETAILED METHODS ON FIELD DATA COLLECTION ACTIVITIES ... 56
9.4. APPENDIX 4. INPUT VARIABLES AND PARMAETERS IN THE SALMONELLA RA MODEL ... 58
9.5. APPENDIX 5. PROBABILITY OF ILLNESS FROM CONSUMING ONE PORTION OF STUDIED FOOD PRODUCTS ... 60
9.6. APPENDIX 6. AFLATOXIN RA METHODOLOGY ... 64

LIST OF FIGURES, TABLES, AND BOXES

Table 1. Foods Selected for the Risk Assessments, by Commodity ... 11
Table 2. Consumer Survey Results: Raw Food Purchased ... 15
Table 3. Samples Positive for Salmonella from the Three Study Markets ... 16
Table 4. Data Availability on Salmonella in the Literature ... 18
Table 5. Storage Practices, Consumption Data, and Concentration Data ... 21
Table 6. Microbial Risk Ranking of Common Food Products Sold in Target Markets ... 22

Figure 1. Schematic Representation of Risk Assessment Design Formative Stages ... 12
Figure 2. Salmonella Transmission Model from Retail to Consumption ... 17
Figure 3. Liver Cancer Risk Model, based on Dietary Aflatoxin Exposure ... 24
Figure 4. (A) Estimated Daily Intake of Aflatoxin; (B) Estimated Annual Liver Cancer Cases, both by Commodity ... 25

ACRONYMS AND ABBREVIATIONS

CCP	Critical Control Point
cfu	Colony-forming unit
CI	Confidence interval
DALY	Disability-adjusted life years
EDI	Estimated Daily Intake
FBD	Foodborne diseases
FERG	Foodborne Disease Burden Epidemiology Reference Group
GAIN	Global Alliance for Improved Nutrition
HCC	Hepatocellular carcinoma (i.e., liver cancer)
ILRI	International Livestock Research Institute
HH	Household
LMICs	Low- and middle-income counties
MPN	Most Probable Number
PMTDI	Provisional Maximum Tolerable Daily Intake
RA	Quantitative risk assessment
RTE	Ready-to-eat
SD	Standard deviation
TBC	Total Bacteria Count

EXECUTIVE SUMMARY

Across Africa, multiple chemical, biological, and physical hazards have been identified in both fresh commodities and ready-to-eat foods. In Nigeria, a country with a rapidly increasing population and demand for food, foodborne diseases (that is, diseases transmissible through food), are a leading cause of illness. Past research on the prevalence of foodborne hazards shows high levels of several contaminants in foods sold within traditional markets in Nigeria. However, assessing the prevalence of a hazard in food is not enough to determine the risk it poses to the consumer: while a hazard is something that can cause harm, risk is a combination of the extent of the harm and the likelihood of it occurring. Hazard levels may be high but risks low and vice versa. This study used a risk-based approach to better understand the risk posed by key hazards frequently found in foods sold in traditional markets in Nigeria, and to help identify appropriate mitigation strategies to reduce foodborne disease.

The USAID-funded EatSafe (Evidence and Action Towards Safe, Nutritious Food) Nigeria project aims to generate the evidence and knowledge to engage consumers to demand safer foods in traditional markets in Nigeria. EatSafe focuses on fresh commodities that include Feed the Future priority commodities for Nigeria, as well as others that were deemed relevant in the context of this assessment (i.e., fresh vegetables and beef). Selected ready-to-eat (RTE) foods – including rice, beef, fish, roast corn, moi moi (steamed pudding made from cowpea), and awara (fried fermented soybean cake) – were included in this report as they are commonly available in traditional markets, represent a high-risk scenario for some commodities (such as grains) compared to their fresh/raw forms, and are prepared from fresh foods also sold at the markets.

This report includes results from three studies conducted in three traditional markets from September 2020 to August 2021 in a city of North-Western, Nigeria, including:

- **Field data collection**, including a reconnaissance study, a food sampling and analysis campaign, and survey of 400 consumers to understand handling and preparation practices;
- **Microbial (*Salmonella*) risk assessment (RA)**, to assess the relative exposure and risk of illness from consuming home-prepared beef, fish, tomatoes, green leafy vegetables, and seven select RTE foods; and
- **Chemical (aflatoxin) RA**, to assess the relative exposure and risk of illness from consuming commodities that are susceptible to aflatoxin contamination, including maize, rice, cowpeas, and soybeans.

EatSafe targeted three traditional markets in a city in North-Western, Nigeria. These markets are outdoors and, except for one, do not have access to free running water or clear boundary walls. Vendors of fresh commodities usually had fixed stalls, while vendors of RTE commodities tended to be mobile. In all the markets, infrastructure, waste disposal, and hygiene were found to be very poor.

Findings from the field data collection highlight the importance of both fresh commodities and RTE foods in the study markets. Nearly half of consumers surveyed purchased only raw products when visiting the markets, and another 9% purchased both raw and RTE. Thus, to conduct the microbial risk assessment, which covers food from the market as it is consumed, both raw and RTE products were included.

The *Salmonella* RA found that consumers could be exposed to a very high risk of illness for all studied foods in the three target markets. Consuming a portion of cooked beef, fish, awara, or fresh tomato carried an average 1% to 5% risk of contracting salmonellosis and becoming ill. These risk levels are considered very high. Risk estimates were highest for RTE fish (nearly 10% chance of illness), and moi moi, RTE rice, and RTE beef all had greater than 5% chance of illness. *Salmonella* was detected in over one-third (37%) of tomato samples collected at the market, and the literature identified a prevalence of 11% in fresh fish samples and 3% in fresh beef samples. RTE foods had the greatest risk of illness, all ranking in the top five of the eight commodities studied. Tomato, fresh beef, and fresh fish carried the lowest mean probability of illness, at 0.4%, 0.2%, and 0.05%, respectively.

The aflatoxin RA – using previously published data on contamination levels in Nigeria – found the mean risk of liver cancer from consuming maize, rice, cowpea, and soybean sold in Nigerian markets varied from a high of 0.015% for maize to a low of 0.001% for soybean. The levels documented in the literature are well above the recommended ceiling for daily exposure.

The results of the three studies presented in this report suggest that reducing the risk of foodborne hazards at the market is critical, achievable, and may result in significant public health improvements. As Nigeria enters Phase II of the EatSafe program, these findings are foundational to the design of consumer-facing food safety interventions.

1. INTRODUCTION

With an estimated 600 million cases of foodborne disease (FBD) annually (1), unsafe food is a threat to human health and economies globally. Ensuring food safety is both a public health priority and an essential step to achieving food and nutrition security (2). The vast majority of the FBD burden falls on low- and middle-income countries (LMICs), which lack regulatory structures, including food control and surveillance systems, regulations, that challenge the application of a risk-based approach to food safety (2,3).

The Foodborne Disease Burden Epidemiology Reference Group (FERG), a working group of the World Health Organization, estimates the AFR-D region, which includes Nigeria, as having the highest FBD burden globally (4,5). The burden associated with FBD for this subregion, measured in disability-adjusted-life years (DALYs), is estimated at one DALY (range 0.5-2.3) per 100 population (4), an exceptionally high burden given that one DALY represents one year of full health lost (1).

Feed the Future's EatSafe (Evidence and Action Towards Safe, Nutritious Food) aims to improve food safety in LMICs, with a specific focus on nutritious foods sold in traditional markets. Traditional markets supply the majority of fresh and ready-to-eat (RTE) foods to local communities (6), though poor infrastructure and limited regulations heighten the risk of FBD and thus represent an important intervention point (7). In Nigeria, EatSafe operates in the North-Western region, and focuses on seven Key Commodities: rice, cowpea (moi moi), maize, soybean (awara), beef, fish, and fresh vegetables.

As part of EatSafe's Phase I (Formative Research) in Nigeria, EatSafe consortium partner International Livestock Research Institute (ILRI) led the risk assessment (RA) process documented in this report. As gold standard for evaluating FBD public health concerns, a RA calculates an estimate on the probability, extent, and uncertainty of harm occurring at a given exposure (8,9). RAs are increasingly used to determine policy options, inform interventions to protect public health and the environment, identify supply chain stages where risk can be controlled, and provide the basis for risk communication and stakeholder participation in solution design (9). EatSafe's RA process in Nigeria included three studies documented in this report:

- **Field data collection,** to provide foundational inputs into the RAs, including a reconnaissance study, food sampling, and survey of 400 consumers (Section 3);
- **Microbial (*Salmonella*) RA,** to assess the relative exposure and risk of illness from consuming home-prepared beef, fish, tomatoes, green leafy vegetables, and seven select RTE foods (Section 4); and
- **Chemical (aflatoxin) RA,** to assess the relative exposure and risk of illness from consuming commodities that are susceptible to aflatoxin contamination, including maize, rice, cowpeas, and soybeans. (Section 5).

Salmonella, a microbial zoonotic pathogen, and aflatoxin, a chemical contaminant, represent diverse categories of foodborne hazards that can cause disease in humans. Eliminating these hazards through each stage of the food supply chains including those of EatSafe's Key Commodities, represent key intervention points to improve food safety in LMICs. Appendix 1 contains descriptive analyses of the seven key value chains with a farm-to-fork focus, aiding EatSafe's understanding of critical control points along the value chain and contextualizing the RA for both hazards.

1.1. BACTERIAL HAZARDS

Specific food hazards are often linked with specific value chains, however, across all LMICs, bacterial hazards affect multiple value chains, often simultaneously (10). About 90% of FBDs are believed to be caused by species of *Staphylococcus, Salmonella, Clostridium, Campylobacter, Listeria, Vibrio, Bacillus*, and *Escherichia* (10).

In the African sub-region that contains Nigeria, *Salmonella* infections are estimated to be 0.3 DALYs per 100 people, the highest of all FBD in this region (11). *Salmonella* is highly virulent and as little as 1 to 10 cells can cause salmonellosis (12), with more vulnerable populations at the highest risk (13). Even in healthy people, low exposure can lead to severe gastrointestinal illness (13). Further, previous studies show high prevalence of *Salmonella* in fresh and RTE meats sold in Nigerian traditional markets (14,15). EatSafe chose *Salmonella* as a priority hazard for risk assessment given:

- The highly virulent nature of this bacteria and the extent of its morbidity and mortality when contaminated products are consumed (16);
- The ability of *Salmonella* to survive on multiple foods, for long periods of time (17), making it highly representative for microbial FBD transmission; and
- The high health burden associated with salmonellosis in the WHO subregion of Africa which includes Nigeria (16,18).

1.2. CHEMICAL HAZARDS

Foodborne chemical hazards also cause considerable health burdens in Nigeria. For instance, the FERG study found seven chemical hazards were responsible for 13% of the FBD burden in the African sub-region (1). Of this burden, 78% was due to two heavy metals (lead and methyl mercury) and 14% due to aflatoxins. The health burden from aflatoxin is estimated as 28 (7-78) DALYs/100,000 people/year in the AFR-D region, which includes Nigeria, the highest in the world (4).

Aflatoxins are natural toxins produced by fungi of the genus *Aspergillus*. Aflatoxin can contaminate staple crops, and when consumed by animals, can be transferred to meat, organs, and other products. EatSafe chose aflatoxin as a priority hazard due to the:

- Causal associations between dietary aflatoxin exposure and hepatocellular carcinoma (i.e., HCC, liver cancer) and correlational association between dietary aflatoxin exposure and growth impairment (i.e., stunting) (17);
- Widespread consumption of aflatoxin-susceptible commodities, such as maize, in North-Western Nigeria (19); and
- Known prevalence and magnitude of aflatoxin contamination in local food systems and commodities, as well as abundance of data due to a long history of mitigation efforts (20,21).

2. RISK ASSESSMENT OVERVIEW AND APPROACH

2.1. OBJECTIVE

While bacterial pathogens such as *Salmonella*, and chemical hazards such as aflatoxins, are prevalent in foods consumed in Nigeria, the risk of illness due to ingesting these foods is not well understood.

EatSafe's research question for the microbial (*Salmonella*) RA was:

"What is the relative risk of becoming ill with salmonellosis from eating food made from seven commodities purchased in traditional markets in North-Western Nigeria?

EatSafe's research question for the chemical (aflatoxin) RA was:

"What is the relative risk of being exposed to aflatoxins and developing liver cancer from long-term consumption of foods made from seven key commodities sold in Nigeria?

2.2. KEY COMMODITIES

EatSafe focused on fresh commodities that include Feed the Future priority commodities for Nigeria, as well as other, ready-to-eat foods (RTEs) that were deemed relevant in the context of the RA.[1] Collectively, this report refers to both the seven categories of RTE and fresh foods as EatSafe's Key Commodities in Nigeria. EatSafe selected at least one product per commodity, either in its fresh or RTE form, for the microbial RA (**Table 1**). EatSafe selected these particular products in consultation with local partners because they i) are the most common RTE preparations of the key commodity; and ii) have higher expected higher microbial risk compared to other preparations of the same commodity.

[1] Selected RTE foods were included as they are commonly available in traditional markets, could pose significant public health risks, and are frequently prepared from fresh foods also sold at the markets.

In the chemical RA, only grains (i.e., maize, rice, cowpea, and soybean) were included given the low risk of aflatoxin exposure associated with consumption of beef, fish, and vegetables. Common local preparations (not necessarily RTE foods sold at the marketplace) of each grain were selected to construct the aflatoxin exposure models.

Table 1. *Foods Selected for the Risk Assessments, by Commodity*

COMMODITY	SALMONELLA RA	AFLATOXIN RA
BEEF	• Fresh beef [1]	N/A
	• RTE Beef	
VEGETABLES	• Tomatoes	
FISH	• Fresh fish [1]	
	• RTE fish	
GRAINS	• Roast corn	• Tuwo masara (stiff maize porridge)
	• RTE rice [2]	• Cooked rice [3]
	• Awara (soybean)	• Awara (soybean)
	• Moi moi (cowpea)	• Moi moi

[1] Purchased fresh or raw from the market and then cooked at home.

[2] RTE rice denotes rice-based foods purchased already cooked at markets.

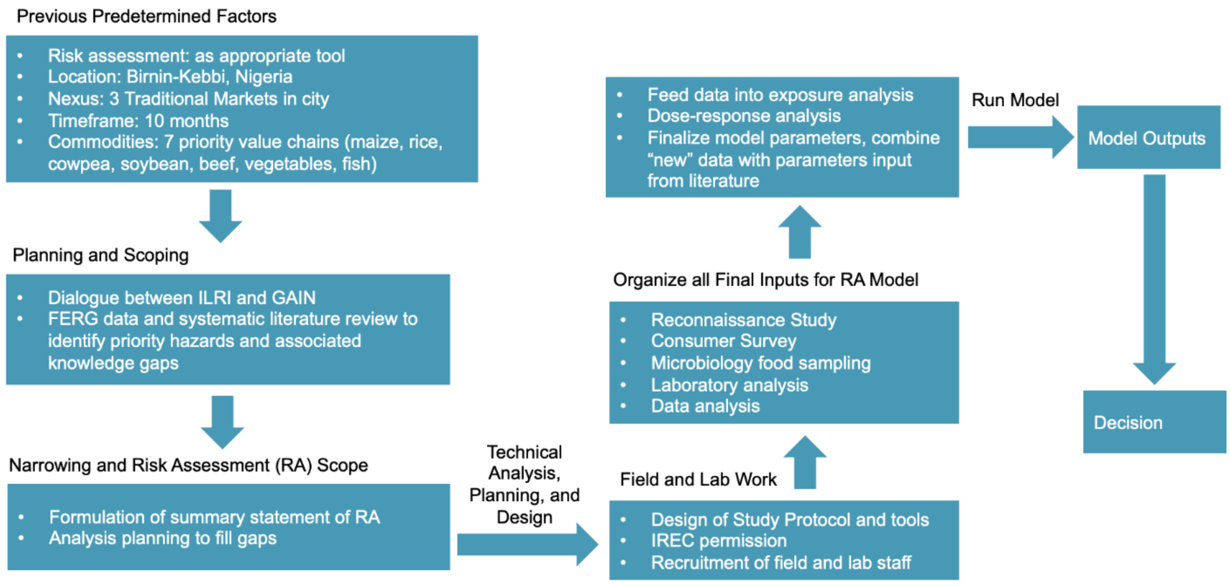
[3] Cooked rice denotes a general category, encompassing all forms or recipes. It is independent from where the food is prepared or consumed.

2.3. RA PROCESS

EatSafe followed a multi-step process for the RA, with all steps contributing to the input parameters that built the final RA model (**Figure 1**; (22). All study protocols and tools were approved by the local Institutional Review Board (Institutional Research and Ethics Committee).

EatSafe considered several predetermined factors (e.g., location of the study site, the three markets as the node of interest, and focus on the seven EatSafe Key Commodities in Nigeria). In the planning and scoping stages, EatSafe identified a range of hazards in foods consumed in Nigeria, as described in Appendix 2, ultimately selecting *Salmonella* and aflatoxin as the target hazards (see Section 1 above for justification). Included in this step were literature reviews to identify existing data sources as well as data gaps. In the technical analysis, planning, and design stage, EatSafe found that while the literature identified hazard prevalence of aflatoxin for some foods, important data on consumption patterns and *Salmonella* exposure levels – two inputs essential for the RA model – were not available, and thus field work was required to address those knowledge gaps.

Figure 1. *Schematic Representation of Risk Assessment Design Formative Stages*



EatSafe then conducted an *exposure assessment* that determined the amount of *Salmonella* and aflatoxin present in foods consumed in Nigeria (i.e., the dose of the hazard ingested) by examining the growth or decline of hazard levels during handling and preparation processes. Next, EatSafe identified the *dose-response* by estimating the mathematical relationship between the dose of hazard ingested, over a meaningful exposure window, and its health impact (e.g., infection or symptomatic illness).

Last, EatSafe input the results of the exposure assessment into the dose-response relationships, accounting for variability and uncertainty as appropriate, and a calculated estimate of risk.

3. FIELD DATA COLLECTION

3.1. METHODOLOGY

EatSafe designed field data collection using a mixed-method approach to fill key data gaps on hazard occurrence in the Key Commodities, as well as obtain local data to input into the RA. New data were collected only where it was lacking in the recent literature – specifically, EatSafe collected data from the target markets on awara (soybean), roast corn, and tomatoes, while data on beef, fish, rice, and cowpea were obtained through the literature.

Overall, EatSafe conducted three field activities:

- **Reconnaissance study,** to understand the layout and main features of the market, and inform design of the consumer survey and microbiological sampling.
- **Consumer survey** to gain insights into consumption frequency, volume, and behaviors (i.e., storage time, cooking) – all critical inputs to the exposure model.
- **Microbiological food sampling** and laboratory analysis to collect the microbial data, which were then used to estimate total bacteria counts (TBC) and as inputs into the RA model.

Appendix 3 contains methods on the consumer survey and food sampling activities.

3.2. RESULTS

3.2.1. RECONNAISSANCE STUDY

As a preliminary activity to understand key features of the study markets that took place over one week during February 2021, the reconnaissance study built a picture of how the three target markets functioned at a structural and social level. It also proved beneficial in building sustainable working relationships with key market stakeholders. Key findings include:

- All three markets operate out of doors. One market was significantly larger in size with more modern vending structures than the other two.
- For all three markets, Friday is the busiest trading day of the week with afternoons, between 2 – 6 PM, being the busiest time of day.
- Only one market has access to running water. A different market has a borehole, but it is not functioning.
- Maps of the three markets were drawn showing stall layout, main walkways, entry/exit points, toilets, water sources as well as important hubs such as bus stations and hospitals surrounding the marketplaces.

- All three markets sell a wide variety of food products, encompassing EatSafe's seven Key Commodities in Nigeria.
- Vendors of similar commodities seem clustered in different sections of the market.
- One market has boundary walls, with gates that can be locked at night. The other two markets do not have boundary walls and are not secured at night.
- Certain products are only sold at certain times of the day in all three markets. For example, awara is only sold in the evening hours.
- The presence of unofficial vendors who may not formally own a stall but move throughout the marketplace was present in all three markets.
- The average daily ambient temperature recorded was 43^{o} C during the visit. Later field work confirmed these estimates, of 31-35 ^{o} C.

3.2.2. CONSUMER SURVEY

EatSafe designed the survey following the reconnaissance study to address data gaps in variables necessary for the RA model. For example, the consumer survey supplemented the lack of data identified during the literature review on consumer consumption of EatSafe's seven Key Commodities in Nigeria.

EatSafe deployed the consumer survey and collected food samples during two visits to the study markets in April and June 2021. Across the two visits, a total of 500 consumers were interviewed at the point of purchase.[2] Approximately 60% of the consumers were male, while the remaining were females. On average, consumers were 31-35 years old. Most female consumers reported having salaried employment, while male consumers reported employment in the trading business.

About half of surveyed consumers purchased raw products, while slightly less (44%) purchased RTE products, and the remaining 9% of purchased both. Men reported purchasing more RTE products, while women reported purchasing more raw products.

Raw Commodities: Results of the survey indicate 188 consumers purchased raw products (fresh beef, fish, and tomatoes). The majority (89%) were bought to be prepared and consumed in the home, primarily in a stew. **Table 2** outlines the results of raw food purchasing and consumption. No consumer reported using any form of temperature control after purchase when transporting food from the market to the place

[2] In April, 400 consumers were surveyed, while 100 consumers were surveyed in June. In April, the first day of the week-long visit yielded the greatest number of consumer respondents across the three markets, 250 of the 400. This was expected, since Fridays had previously been identified during the reconnaissance study as being the busiest day.

of consumption. In storing raw foods, only 23% of consumers used refrigeration in the home, while the remaining consumers reported keeping products at room temperature.

Table 2. *Consumer Survey Results: Raw Food Purchased*

CONSUMERS, # (%)	PURCHASE FREQUENCY	# HH MEMBERS	HOME STORAGE		ELAPSED TIME [1]
			Refrig. temp.	Ambient temp.	
Tomatoes 167/188 **(89%)**	Daily	6	19	125	141 min
Beef 68/188 **(36%)**	Twice weekly	5	14	45	100 min
Fish 39/188 **(21%)**	Twice weekly	5	6	23	83 min

[1] Average elapsed time between purchase and preparation

RTE Foods: Of the seven reported RTE products purchased by consumers, most purchased RTE rice, followed by RTE fish and beef (each ~20%). About two-thirds of consumers purchased the RTE foods for immediate, individual consumption, while the remaining 22% were bought for consumption with their household at a later time.

The average time between purchase and consumption for RTE products consumed later was 60 minutes. When asked if they alter the RTE products they purchased prior to consumption, only 17% of consumers reported they would alter the RTE products prior to consumption (e.g., by further processing or reheating).

3.2.3. MICROBIAL FOOD SAMPLING ANALYSES

EatSafe collected a total of 279 food samples over the three days of the first field visit. The ensuing laboratory analysis included total bacterial counts (TBC) estimation, as well as *Salmonella* isolation, identification, and concentration estimation. *Salmonella* prevalence on all commodities sampled from the three markets ranged from 20% to 38% (**Table 3**). Across all markets, *Salmonella* was isolated from 72 of the 279 samples (26%). In 40 samples, *Salmonella* could be clearly identified from colony morphology; the other 32 samples were "*Salmonella* suspects" whose classification as *Salmonella* was confirmed via further molecular testing. *Salmonella* was isolated in 37% of fresh tomato samples and 10% awara samples.

The majority of samples from all foods, 89% (n=249), had a TBC of more than 500 $\times 10^9$ cfu/g. For fresh tomatoes, this percent was 80%. The proportion of high-TBC samples was high across the three markets, ranging from 78% to 93%. This extremely high count shows that the food samples were heavily contaminated, suggesting that the samples harbored more bacteria – of all kinds – than would be expected in foods handled hygienically. While the exact point of contamination in the supply chain is unknown, handling at markets are a likely contamination point. This hypothesis is supported by the finding that almost all awara samples (95%) had TBC >500 cfu/g. Awara sold at markets is an RTE food, and high levels of contamination could only be a result of either improper cooking or unsafe handling after cooking.

Table 3. *Samples Positive for Salmonella from the Three Study Markets*

MARKET	POSITIVE SAMPLES							
	TOMATO		AWARA		ROAST CORN		TOTAL	
	#	%	#	%	#	%	#	%
Market #1	32/110	29%	2/44	5%	0/8	0%	34/162	**21%**
Market #2	13/27	48%	1/35	3%	-	-	14/62	**23%**
Market #3	12/17	71%	8/37	22%	0/1	0%	22/55	**38%**
TOTAL	57/154	**37%**	11/116	**10%**	0/9	**0%**	70/279	**25%**

The Most Probable Number (MPN) of *Salmonella* was determined in 40 of 72 samples. The MPN ranged from 30 to 11,000 cells per gram (cfu/g), though some differences between the markets were observed.[3] While there is no established acceptable threshold for *Salmonella* concentrations, these concentration levels are considered likely to cause illness, if present in average portions of the considered foods. For reference, based on the dose-response relationship used in this study (23,24), a dose (where dose = concentration × portion size) of 36 cfu – which is reached and surpassed with these concentrations – translates into a probability of illness of approximately 50% (i.e., a person would become ill, on average, every two times that one consumes a food, if no heating or other *Salmonella* reduction measure were implemented before consumption).

[3] The MPN ranged from 30 to 640 cfu/g) in Market #1, 30 to 11,000 cfu/g in Market #2, and 30 to 1,500 cfu/g in Market #3.

4. SALMONELLA RISK ASSESSMENT

This section includes the methodology and results for the Salmonella RA.

4.1. METHODOLOGY

A transmission model, valid for all commodities, was designed to visualize potential contamination pathways for the selected raw and RTE food products, from purchase at the market to consumption, and the associated health outcomes (**Figure 2**). EatSafe then developed a mathematical model to represent the transmission model and was customized to each of the foods considered. This model "follows" portions of the food and the associated pathogen loads through the relevant stages of the food chain, starting at the retail phase and ending with the estimated number of human cases of illness. EatSafe determined that spreadsheet-based open-source model '*Swift QMRA*' or sQMRA (25), coupled with the Monte Carlo-based software @Risk was the appropriate tool for the purpose of rapidly screening the relative risk of multiple foods relevant to EatSafe's goals.[4]

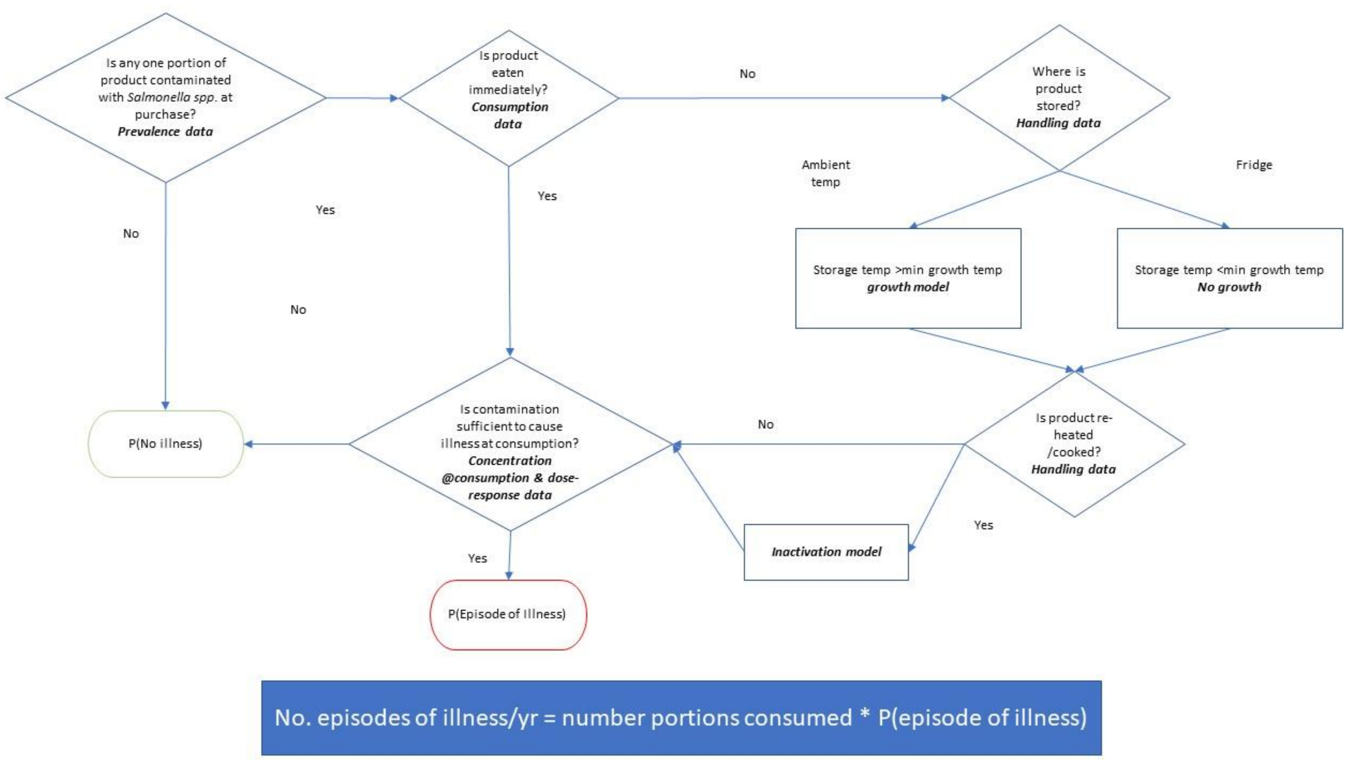


Figure 2. *Salmonella Transmission Model from Retail to Consumption*

[4] The Monte Carlo algorithm accounts for variability in all variables and parameters. The sQMRA tool provides a simplified model to quickly obtain relative public health risks and identify higher-risk foods, which can then be evaluated in a more extensive custom RA (25).

4.1.1. EXPOSURE ASSESSMENT APPROACH

EatSafe designed an exposure assessment study to estimate the average number of *Salmonella* c/p/d (cells/person/day) to which a consumer is exposed for each key commodity. An exposure assessment requires data on practices of consumers as well as the frequency and intake of potentially contaminated products (26). In the equation below, EatSafe sought to understand four components of exposure:

$$\text{Exposure} = \text{Frequency} \cdot \text{Ingestion} \cdot \text{Prevalence} \cdot \text{Concentration}$$

In this equation, *frequency* is the number of ingestion events per day (events/person/day); Ingestion is the volume or mass consumed per person per ingestion event (volume/event); Prevalence is the probability that the ingested item is contaminated with *Salmonella;* and *Concentration* is the number of *Salmonella* cells per volume or mass when the exposure matrix is contaminated (cells/volume).

EatSafe first identified information from the literature; where not readily available, EatSafe conducted field work to generate data needed for the RA model (**Table 4**).

Table 4. *Data Availability on Salmonella in the Literature*

Key Commodity	Is data *on Salmonella* available in the literature?			Data Collection Methods [1]
	Prevalence and Concentration	**Consumption (Frequency)**	**Temperature and Time**	
BEEF				
Fresh Beef	Yes	No	No	*S, T*
RTE Beef	Yes	No	No	*S, T*
FRESH VEGETABLES				
Fresh Tomato	No	No	No	*S, T, F*
FISH				
Fresh fish	Yes	No	No	*S, T*
RTE fish	Yes	No	No	*S, T*
GRAIN				
RTE rice	Yes	No	No	*S, T*
Cowpea - Moi-moi	Yes	No	No	*S, T*
Soybean - Awara	No	No	No	*S, T, F*
Maize - Roast corn	No	No	No	*S, T*

[1] *S = Consumer survey; T = Temperature recording; F = Food Sampling*

4.1.2. MICROBIAL RISK ASSESSMENT MODEL

Input variables and parameters, and how they were represented in the model and linked to each other, is described in Appendix 4. The concentration of *Salmonella* was calculated by accounting for i) *Salmonella* growth or reduction, based on storage, cooking, and handling practices post-purchase (described in terms of temperature and time); and ii) consumption amounts (i.e., portion size).[5] The exposure was expressed in terms of the number of cells ingested with each portion of food, calculated by multiplying the concentration by the portion size. The probability of illness following consumption was determined using an established dose-response equation.

EatSafe used the 'Auto' function in @Risk, an add-on to Microsoft Excel, which runs sufficient iterations to a maximum of 50,000 until all input parameters converged.[6] EatSafe also used a sensitivity analysis to determine how input parameters influenced the main output: the probability of illness from consumption of each product.

4.1.3. MODEL PARAMETER ESTIMATES

EatSafe summarized all the data from the literature and field work to provide input variables and model parameters, as well as their statistical distributions, for all steps in the RA models for each product. Results provide point estimates for mean c/p/d (cells/per person/daily) with accompanying ranges based on probability intervals that reflect the variability and uncertainty of the underlying data. These estimates allow to rank the transmission routes from highest to lowest mean risk. In each step of the RA model, parameters were calculated to answer the following questions:

- Given the overall proportion of portions found contaminated, what is the probability that one specific portion is contaminated?
- For what time and temperature is the product stored, from purchase at the market to consumption? (How much bacterial growth, if any, can occur during storage?)
- Is the product cooked prior to consumption? (How much bacterial reduction can occur, due to cooking?)
- What is the bacterial dose ingested while eating one portion of the product?
- What is the P(illness) from eating one portion of product?

In **Table 5,** EatSafe summarizes the consolidated from both the literature and field results used as input parameters in the model, including storage practices, consumption data, and concentration data. Other input parameters, including cooking and storage practices,

[5] Because EatSafe is focused on markets, cross-contamination at the household level was not explored. However, because the RA model will inform which products need risk mitigation at the market, the risk of bringing contaminated products or introducing cross-contamination into the home may fall.

[6] EatSafe used the default settings of 3% tolerance and 95% confidence intervals (i.e., when there is a 95% probability that the mean of the tested output is within +/- 3% of its "true" expected value, which includes accumulated data from the iterations already run).

indicated that the majority of all products were stored at ambient temperature. All fresh beef, fish, and tomato were cooked after storage. Less than 25% of consumers reported cooking RTE fish. The remainder of the Key Commodities were not cooked after storage.

Table 5. *Storage Practices, Consumption Data, and Concentration Data*

PRODUCT	STORAGE PRACTICES				CONSUMPTION DATA				CONCENTRATION DATA			
	AMBIENT TEMP		FRIDGE TEMP		% OF POP [2]	PORTION (g) [3]		REF	SAMPLES		DATA [4]	REF
	%	HOURS (MAX) [1]	%	HOURS (MAX) [1]		MONTH	MEAN (SD)		#	%		
Fresh Beef	76%	1.67 (5)	24%	1.42 (5)	39%	8	213 (200)	(15,27,28)	3/105	3%	8.1 (8.1)	(15,27–29)
RTE Beef	100%	0.10 (2)	0%	N/A	20%	28	117 (101)	(27,29,30)	21/74	28%	4.1 (0.7)	(31)
Fresh Fish	95%	1.31 (3)	5%	0.5 (0.5)	21%	8	91 (59)	(30,32,33)	32/295	11%	2.1 (1.3)	(30,32,34)
RTE Fish	95%	1.10 (8)	5%	4 (5)	24%	8	109 (73)	(35,36)	3/48	6%	5.4 (5.1)	(35,36)
RTE Rice	97%	0.13 (3)	3%	1 (1.75)	63%	28	345 (199)	(35,37)	3/24	13%	4.8 (0.6)	(35)
Moi Moi (cowpea)	66%	0 (0)	33%	4 (4)	2%	28	833 (288)	(35)	3/24	13%	4.9 (0.7)	(35)
Awara (soybean)	100%	0.00 (0.05)	0%	N/A	16%	8	94 (44)	(38)	11/116	9%	1.7 (1.3)	Fieldwork
Roast corn	100%	0.43 (2)	0%	N/A	20%	N/A	N/A	Fieldwork	0/9	0%	NA	Fieldwork
Tomato	86%	2.07 (6)	14%	1.6 (7)	89%	28	249 (172)	(39,40)	57/154	37%	1.9 (1.8)	Fieldwork

[1] Refers to mean hours stored. Storage times considers the time to first preparation or consumption but not additional storage of "leftovers." The category stored in a "water bag" added to "fridge" category.

[2] Derived from consumer survey data . Portion size was calculated as the total purchase divided by number of portions (number eating x no. meals). Outliers were removed (beef: 3 records removed >1kg, fish: 3 records removed >500g, tomatoes: 6 records >1kg).

[3] The number and proportion of consumers that consume a specific food are provided for context, based on urban area population estimates. These values are not used in the risk calculations.

[4] Mean concentration of *Salmonella*, in units of Log cfu/g (i.e. decimal logarithms)

4.2. RESULTS

EatSafe ran risk models separately for each Key Commodity to estimate individual estimates as well as relative ranking (**Table 6**). All commodities included in this study, however, were found to have mean concentration of Salmonella at levels very likely to result in illness, indicating the poor standard of food safety across commodities in this market and a potentially high risk posed to consumers**.** The detailed model outcomes for each product listed in the risk ranking can be found in Appendix 5, expressed in terms of probability of illness per portion consumed.

Table 6. *Microbial Risk Ranking of Common Food Products Sold in Target Markets*

PRODUCT	MEAN PROBABILITY OF ILLNESS (95% CI) [1]
1. RTE Beef	**2.9%** (0.02% - 3.5%)
2. Moi Moi	**1.7%** (1.0% - 2.0%)
3. RTE Rice	**1.4%** (0.9% - 1.7%)
4. RTE Fish	**0.7%** (0.4% - 0.8%)
5. Awara	**0.7%** (0.3% - 0.7%)
6. Tomato	**0.4%** (0.02% - 0.5%)
7. Fresh Beef	**0.2%** (0.2% - 0.3%)
8. Fresh Fish	**0.05%** (0.0% - 0.08%)

[1] Values in this column indicate the average probability of illness following consumption of a food portion (i.e., 2,900 illness cases per 100,000 exposure events, or 2.9% of exposure events). Although national standards are not available for *Salmonella* for these commodities, salmonellosis can occur from exposure to as low as 1 cell and international standards generally consider any detection to be potentially hazardous to health.

While multiple assumptions were made during the development of the model, these outputs allow EatSafe to estimate which food tends to pose the most risk from a food safety perspective. The four foods that pose the most risk are all RTE foods, highlighting the need for market-based interventions to focus on these products if the risk of FBD is to be mitigated within the market.

5. AFLATOXIN RISK ASSESSMENT

This section includes the methodology and results for the aflatoxin RA.

5.1. METHODOLOGY

EatSafe conducted a review of peer-reviewed scholarly literature in Web of Science and Google Scholar to characterize available data on aflatoxin contamination in Nigerian maize, cowpea, soybean, rice, and other food products derived from them. These data were used as input in the RA model. Contamination data included in the model are specific to Nigeria, but not specific to the state or specific study ,rakers in which EatSafe operates. Appendix 6 contains detailed methods for the aflatoxin RA.

The search yielded 189 total studies, of which 29 (15%) were eligible for inclusion in the risk analysis. Most studies (n=15) examined maize, followed by rice (n=10), cowpea (n=4),[7] and soybean (n=3). Prevalence data (% positive samples) were available for 80% of reported batches in the 29 studies, which were then included in the RA model (**Figure A1** in Appendix 6).

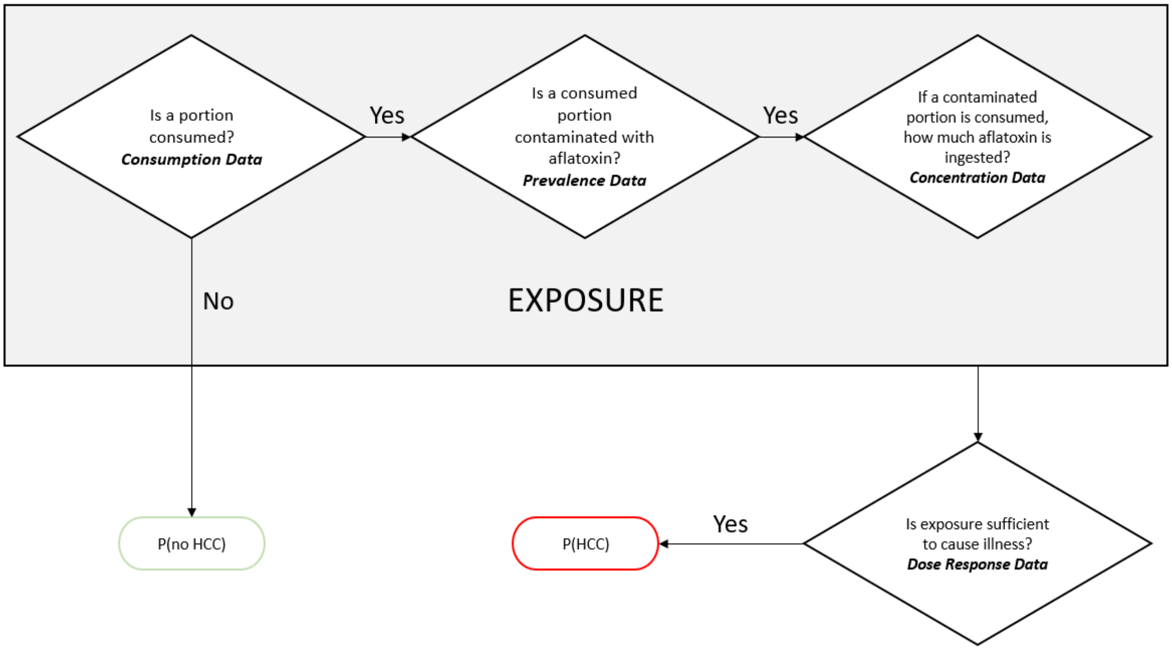
5.2. MODELLING APPROACH

EatSafe developed an RA model to estimate exposure to total aflatoxin associated with maize food consumption, as well as the risk of liver cancer (hepatocellular carcinoma, "HCC"; **Figure 3**). The model considers a "market to table" scenario to assess absolute and relative exposure associated with the commodity. Key inputs into the model include consumption data, aflatoxin prevalence, and concentration in each commodity at the point of purchase – all derived from the literature. The model follows portions of maize through home handling, preparation, and consumption.

EatSafe developed estimated daily intake (EDI) probabilities based on the distributions of aflatoxin concentrations, commodity consumption, and adult body weight for the four commodities. Once EDIs were developed, EatSafe compared its estimates with the proposed provisional maximum tolerable daily intake level (PMTDI) of 1 ng $kg_{\_bw}^{-1}$ day^{-1}, which is used as a threshold for judging risk associated with aflatoxin exposures (42).[8]

[7] One included study reported aflatoxin contamination in cowpea in the neighbouring country of Benin (41); this study was included given that few Nigeria-specific studies of aflatoxin in cowpea were identified.
[8] Given that aflatoxin is a carcinogenic substance that is considered harmful at any dose, there is no tolerable daily intake (TDI) level.

Figure 3. Liver Cancer Risk Model, based on Dietary Aflatoxin Exposure



5.3. RESULTS

5.3.1. DIETARY EXPOSURE ESTIMATES

The commodity with the highest estimated EDI in the exposure model was maize, with a value of 29.7 ng $kg_{\_bw}^{-1}$ day^{-1}, substantially higher than the PMTDI level (**Figure 4a**). The estimate of EDI in the rice and cowpea models also exceeded the PMTDI level, but more modestly, with estimates of 8.88 ng $kg_{\_bw}^{-1}$ day^{-1} and 3.14 ng $kg_{\_bw}^{-1}$ day^{-1}, respectively. Soybean EDI estimates were much less than the other commodities, with an EDI of 0.28 ng $kg_{\_bw}^{-1}$ day^{-1}, below the PMTDI level.

5.3.2. RISK RANKING

The mean risk of liver cancer attributable to consumption of maize, rice, cowpea, and soybean in the target population was estimated as 1.44, 0.429, 0.152, and 0.013 liver cancer cases per year/100,000 population, respectively (**Figure 4b**), with relatively high variability. The relative ranking of these products is consistent with existing evidence that grains, and maize in particular, are more susceptible to aflatoxin accumulation than pulses. For both maize and rice in the study area, the higher estimated risk levels are reflective of both high consumption and contamination levels, whereas cowpea and soybean, despite moderate consumption levels, harbor low concentrations of aflatoxin and therefore pose lower risk of aflatoxin-associated liver cancer.

EatSafe's estimates for liver cancer attributable to aflatoxin-contaminated maize are below levels previously reported for infants and children in Nigeria (43), but higher than those from a European population with lower consumption and contamination (44). EatSafe's estimates of maize-associated liver cancer risk are similar to estimates of aflatoxin exposure via groundnuts in a Nigerian population (45). Other estimates found that liver cancer incidence rate, from all causes, is 2.6 cases per 100,000 population (46); thus, EatSafe's findings suggest that a substantial proportion of total liver cancer incidence may be attributable to dietary aflatoxin exposure via maize consumption.

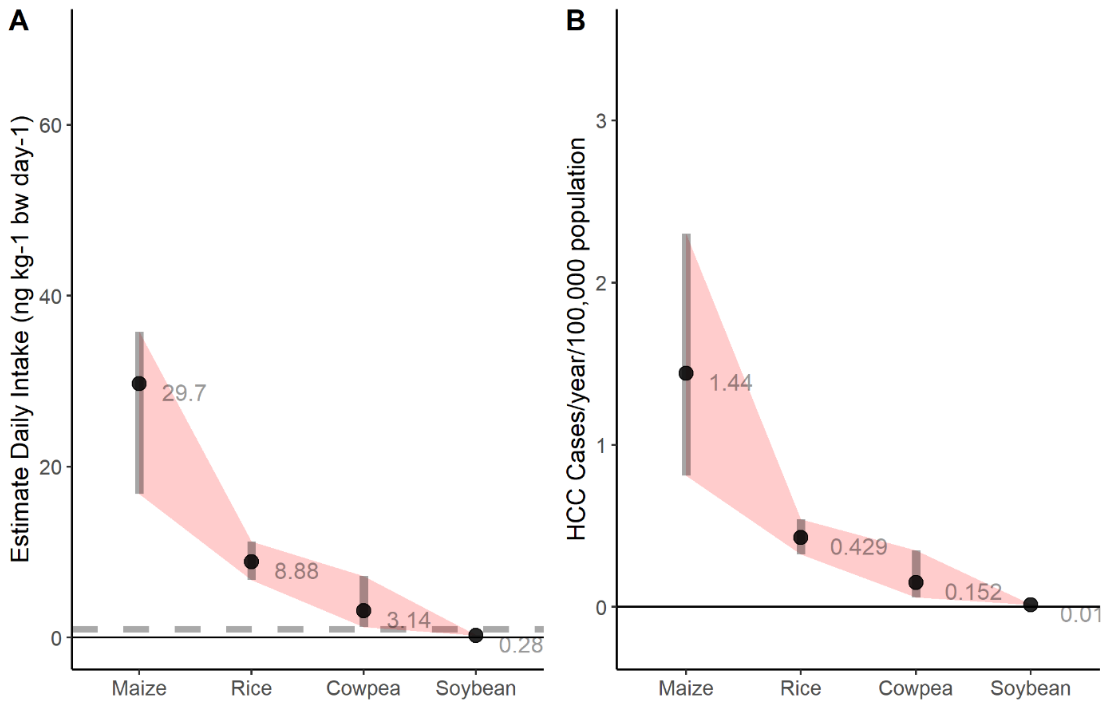


Figure 4. *(A) Estimated Daily Intake of Aflatoxin; (B) Estimated Annual Liver Cancer Cases, both by Commodity* [9]

6. DISCUSSION

Quantifying risks is important in understanding the human health impacts associated with the consumption of unsafe foods. Prior to this EatSafe risk assessment effort, risk-based approaches to quantify foodborne disease risk in Nigeria were very limited, especially in the context of traditional food markets. Overall, the core novel contributions of this effort lie in:

- The targeted collection of novel field data specific to traditional markets in North-Western, Nigeria and tailored to risk assessment needs;

[9] Points represent the estimated mean. Bars represent the 95% credible interval around the estimate. The hashed line in both panels represents the PMTDI level for aflatoxin (1 ng kg_{bw}^{-1} day^{-1}).

- The synthesis of a large body of research from the existing literature, and its translation into inputs needed to derive risk estimates;
- The development of a suite of simple exposure and risk models that could be applied to other contexts;
- Risk estimates for seven key commodities sold in Nigerian traditional markets in for two key foodborne hazards (*Salmonella* and aflatoxins);
- A risk-based ranking of these commodities, which was possible due to the common risk assessment approach adopted across the considered foods.

A risk-based approach to informing intervention design is well supported as a concept, but often not implemented due in part to the resource-intensive nature of risk assessments. Many studies measure hazard occurrence; this is necessary information, but often not sufficient to understand risk and how to mitigate it. This study provides an approach that goes beyond hazard measurement and combines key components needed to assess risk, in a targeted and cost-effective manner. At the same time, as the first effort to describe foodborne risks from foods purchased in traditional markets, this work is also based on assumptions and incomplete data. As such, models and results should be considered as a first iteration of the assessment, yielding evidence fit for the purpose of EatSafe's formative research, and amenable to further refinements possibly including other hazards or foods.

Across both higher and lower income settings, risk assessment studies are developed by compiling information from a variety of data sources, with each data source contributing in varying degrees to understanding potential public health risks attributable to a disease agent (9). When dealing with limited and often unavailable data for LMICs (47), it is critical to remember the increased uncertainty associated with certain data sources in developing a risk assessment (48). Such limitations must be kept in mind when interpreting results. Nevertheless, the findings in this report strengthen the case for future, more extensive risk-based approaches to be used in resource poor settings to generate deeper insights into food safety risks and to inform decision-making at a public health policy level.

First and foremost, this study documents that concerns about food safety problems originating from foods sold at traditional markets are well grounded. A high prevalence of *Salmonella* was observed in the food products sampled at the study markets, and positive samples most often harbored very high concentrations. While contamination patterns differ significantly by food, in general this statement also holds true when considering prevalence and concentration evidence in the Nigerian literature, for foods not sampled *de novo* in this study. While *Salmonella* can be transferred through a wide range of foods, surfaces, and environmental compartments (water, soil, air), it is a zoonotic pathogen, with a primary reservoir in domestic or wild animals. Since few or no live animals have been observed at the study markets, the contamination of foods such as fresh tomatoes – when observed at high concentrations – could have occurred upstream in the supply chain. However, cross-contamination at the market could also have occurred between animal source foods such as raw meat or fish and plant-derived foods, or via the market environment (e.g., soil, surfaces, tools).

Findings of *Salmonella* on cooked RTE food such as awara also suggest that its contamination could have occurred due to unsafe conditions after cooking, including at the market. While this is not the only possible explanation, it is strong evidence pointing at the role of handling, storage, and environmental hygiene at the market. This line of evidence is also supported by the high total bacterial count (TBC) observed in both fresh and RTE foods sampled in the study markets; again, while contamination of fresh or raw commodities could have occurred anywhere along the supply chain, high bacterial counts post-cooking suggests unsafe handling or storage by the preparer or vendor. Furthermore, environmental conditions observed at the market can be favorable to bacterial growth, which can exacerbate existing contamination.

Salmonella risk estimates across the variety of foods analyzed confirm that foods commonly sold at traditional markets in Nigeria can potentially cause an unacceptably high health burden. Surprisingly, several plant-based foods carried risk of similar magnitude as animal-source foods. In addition, it is possible that *Salmonella* may grow during market storage and handling, so even one cell on a product has the potential to cause illness. It should also be kept in mind that only acute illness was considered in this study; chronic illness and sequelae, if accounted for, would increase burden estimates. For all foods included in the study, estimated risk levels (based on specific model inputs and assumptions) are of magnitudes that could cause large outbreaks. While risk estimates for foods purchased raw and prepared or cooked at home point to the role of both market and home practices, risk estimates for RTE foods more directly reflect risk associated with market practices. The joint consideration of hazard occurrence patterns in the market and risk estimates can provide insights on critical control points where risk can be most effectively mitigated.

The review of aflatoxin levels in Nigerian staple grain and pulse commodities indicated that this hazard is prevalent in the region and can accumulate at levels sufficient to warrant public health concern. As expected, both prevalence and contamination level were highly commodity-dependent, with maize (a very susceptible host crop) showing far higher levels of aflatoxin than the other commodities. Given that maize is an important and widely consumed staple food in the region, high levels of contamination in maize are of great relevance for risk management; this was confirmed in the exposure assessment, with the estimated per capita daily aflatoxin exposure from maize exceeding the PMTDI threshold level by a factor of 30. While high levels of dietary aflatoxin exposure attributable to maize consumption were expected given its high levels of consumption and toxin susceptibility, the finding that exposure attributable to rice consumption also far exceeded the PMTDI level was unexpected. Unlike maize, which accumulates much of its toxin burden before harvest, aflatoxin accumulation in rice primarily occurs post-harvest. Thus, post-harvest nodes of the value chain (including the marketplace) may constitute important control points for aflatoxin management in rice. Exposure levels associated with consumption of cowpea and soybean were found to be much lower, which is consistent with existing evidence that these commodities are not susceptible to high levels of aflatoxin contamination under normal circumstances.

The microbial risk assessment developed in this study is based on data, scenarios, and assumptions that should be carefully validated before estimates can be considered final. For instance, the assessment considers only two simplified consumption scenarios, one for home-prepared and one for RTE foods. The scenarios are well supported by novel context-specific data on time to consumption, storage conditions, type of cooking (if used), and leftover management, collected over different times and days of the week. However, other preparation and consumption patterns may exist, as the consumers polled were observed purchasing at least one of the target foods and may not be representative of the market population as a whole. While portion sizes were well characterized, patterns of consumption over time that could provide insights on repeated exposures were not investigated. Assumptions regarding *Salmonella* growth and decline models are likely those that need to be most carefully validated and refined, as they can significantly impact risk estimates. For these two classes of phenomena, this version of the model can be considered close to a worst-case scenario, as growth was assumed to occur at high rates. In practice, *Salmonella* growth and decline rates are dynamic and very sensitive to parameters of time, food moisture, and temperature; a more detailed knowledge of pre-retail, retail, and home handling would be needed to rigorously identify growth conditions. Also, available *Salmonella* dose-response relationships are derived from outbreak data (i.e., likely representing higher-virulence strains) and are known to overestimate risk. Lastly, the model does not explicitly account for cross-contamination, and this omission may underestimate risk. While the impacts of cross-contamination are largely accounted for in RTE microbial levels as measured, they are not in the model for home-prepared foods. While sampling foods at the point of consumption would provide useful insights, the choice of omitting cross-contamination from the model is justified by its practical intractability, i.e., its inclusion in the model would add significant uncertainty and raise more questions than it would provide answers.

While the aflatoxin risk assessment approach used in this study has yielded important insights regarding the relative risk associated with each of the selected commodities, it represents a highly simplified scenario that does not consider events at pre- and post-market value chain nodes, which may substantially influence the risk of exposure and warrant further investigation. EatSafe's model was designed to estimate risk of exposure associated with foods unaltered from the point of sale at the market. However, the toxin burden at consumption may in many cases be modulated by post-market storage practices and detoxification events (dehulling, sorting, etc.), which EatSafe was unable to account for given the datasets presently available. Since post-market phenomena may lead to either higher (e.g., toxin accumulation in storage) or lower (e.g., toxin reduction via sorting or detoxification) toxin levels, and that these factors are uncharacterized in the study population, EatSafe was unable to determine the extent of over- or under-estimation in this RA. Further iterations of this model accounting for additional variables between purchase and consumption, such as storage, sorting/detoxification, and processing, may yield more precise estimates.

The two hazards considered differ in their amenability to intervention at different points of the value chain. While *Salmonella* must be controlled all along the food chain from farm to fork, for aflatoxin control, the marketplace, in general, is not considered among the most critical nodes for control. Indeed, aflatoxin accumulation in food is more likely to manifest at pre-harvest (in susceptible crops like maize and groundnut) and over the course of storage (pre- and post-market). These stages are the most efficacious targets for mitigating the toxin burden. While the marketplace environment is not well-suited to the prevention of toxin accumulation, it may serve an important role in mitigating exposure by controlling the consumption of contaminated food. This can be achieved through a number of pathways, including, but not limited to: 1) sourcing non-contaminated products (i.e., from verifiably safe producers), 2) cleaning/sorting products prior to sale (e.g., through sorting, processing, etc.), and 3) selling products in forms less vulnerable to post-market toxin accumulation (e.g., dehulled/decorticated, hermetically packaged, etc.). All of these routes to exposure prevention require investment and buy-in from market actors as well as an enabling environment for change, and therefore are inherently linked to consumer demand for safer foods. Understanding consumers' relationships with trusted vendors and the drivers of consumer choice is imperative in reducing the risk from this hazard at the market, especially if 'upstream' value chain interventions are not an option.

Considering both microbial and aflatoxin contamination, the outline of the supply chain for the seven priority commodities (Appendix 1) provides context to better understand how different foods reach the market, and where they can be vulnerable to contamination. For meat and aquaculture fish, the production stage is most often where microbial contamination enters the supply chain. For fresh vegetables, microbial contamination can start at the farm, e.g., via wastewater irrigation and manure application, but can also occur in market and retail environments where both meat and vegetables are in close proximity. Grains and legumes generally do not become contaminated during production, but can be vulnerable to contamination during processing, storage, and retail in bean/kernel or flour form, e.g., from pests or cross-contamination. Their low moisture generally protects them from bacterial growth, compared to other commodities.

There are opportunities for microbial risk control at markets. While this assessment focuses on current risk for consumers, evidence from field data, risk estimates, and knowledge of supply chains can provide insights into risk management options. At markets, risk management for meat and fish can mainly target storage time and conditions to prevent bacterial growth, and storage and handling practices to reduce the likelihood of cross-contamination with surfaces, other foods, or the environment. Importantly, animal source food can be the vehicle introducing contamination into the market, hence cross-contamination control is meant to protect meat, but also protect other foods from becoming contaminated with the pathogens commonly found on meat. The fact that fish are commonly sold alive in traditional food markets is an important leverage point that could protect the food from microbial hazards but could also create new pathways for cross-contamination (e.g., killing and cleaning the fish on site). For fresh vegetables, control of cross-contamination at the market is key (in particular protecting vegetables from contact with animal source foods or contaminated water), as well avoiding conditions favorable to bacterial growth, such as cutting vegetables. For dry commodities such as grains and legumes, protection from cross-contamination is likely the most effective risk control approach at the market.

Overall, key control processes to manage microbial hazard levels -and hence risk- in the market, across commodities, can be classified as: (1) reduction of cross-contamination between foods and the market environment (e.g. soil, air, pest, water); (2) reduction of cross-contamination between different foods, in particular from animal source foods to vegetables, grains, and legumes; (3) avoidance of conditions favorable to bacterial growth, in particular in terms of temperature, moisture level, and storage time; (4) hazard abatement measures, e.g. washing vegetables, which would need careful validation as water is also a vehicle for cross contamination. All these approaches rely heavily on behaviors and practices but are also enabled or constrained by available tools and infrastructure. EatSafe will explore specific measures while developing proposed standards linked to EatSafe interventions (Activities 2.1-2.5).

Both home-prepared and foods purchased as RTE were found to be potentially associated with high risk of salmonellosis. The risk ranking highlights the importance of RTE products as a route of exposure to *Salmonella*. EatSafe found that the risk of becoming ill with salmonellosis could be ten times higher in consumers of RTE fish than in consumers of fish purchased raw from the same markets and subsequently cooked at home. Reasons for this lower risk among consumers of home-cooked fish is likely due to proper cooking prior to consumption at home, unlike the RTE consumers who tend to eat the fish immediately, without re-heating, as observed during the reconnaissance study and verified via the consumer survey. For RTE foods, the same types of risk control approaches mentioned above apply, with cooking often providing a strong opportunity for hazard abatement.

This work did not set out to quantitatively determine risk factors for *Salmonella* contamination in different marketplaces. However, some of the market observations can raise potential hypotheses for risk-enhancing or risk-mitigating practices which can be further explored during intervention design and prototype development. For instance, the markets differed significantly in the level of infrastructure and hygiene services offered, such as potable water, toilets, or waste management. Public water sources were not sampled in this study. Interestingly, only the one market of the three markets with a functioning water supply was found to have the lowest prevalence of *Salmonella* on foods sampled, indicating the possible role that access to clean running water may play in improving hygiene standards. However, understanding how sustainable strategies for long-term improved infrastructure changes can be introduced and maintained at the market level requires a much deeper understanding of the complex governance of this traditional and unregulated sector (6).

High-level information on cooking practices was collected as part of the consumer survey. However, home-based observations were not an aspect of the fieldwork, making the verification of cooking practices beyond the scope of this risk assessment. Similarly, the role of cross contamination within the home was not assessed in this study. For bacterial hazards, cross-contamination in the home (e.g., from raw meat to vegetables) could increase the proportion of contaminated food portions as well as the number of microbial cells on a portion; both would lead to an increase in risk compared to the estimates presented here. Our findings show that there is a higher risk of illness for consumers of RTE products who may not be able to apply any risk mitigation strategies to their food prior to consumption. However, across both RTE and raw products, an assumption can be made that if foods are made safer in the marketplace, then this will be a protective measure for all.

The abundant presence of hawkers (vendors that do not have a fixed stand and move about the market), as found in each of the three field trips during this study, is a factor which needs careful consideration when planning food safety interventions. On the one hand, the mobile nature of these vendors could be viewed as a stumbling block when trying to locate and recruit them for intervention strategies and indeed when trying to carry out follow up studies. They also, on the other hand, provide a unique opportunity, given that if targeted successfully they can disseminate food safety messages far beyond a single marketplace, acting as spreaders of improved food safety standards with a much greater range and impact than vendors located in a stationary food stall in one market. Products sold by these hawkers often tend to be RTE in nature. The RA model shows that RTE products, beef, moi-moi, rice and fish, carry the greatest risk of salmonellosis per portion consumed. The consumer survey also highlights the high consumption rates for RTE products by individual consumers; RTE food is purchased to eat on the go or immediate consumption, it seems to be treated as a convenience food, purchased more so by men than by women. Given the frequent presence of hawkers across the marketplaces, the food safety risk associated with the type of foods they sell, both this cohort of vendors and their RTE food products, merit further investigation in future food safety risk analysis work.

7. CONCLUSIONS

From the findings of this activity, it can be concluded that considerable foodborne hazards are present in foods sold in traditional markets in North-Western, Nigeria. Most importantly, the observed levels of microbial hazards (namely *Salmonella*) can be associated with considerable risk, given the handling, storage, and preparation practices both in the market and in the homes where the foods are consumed. While data and assumptions would need further refinement before these estimates can be validated, this effort provides a detailed synthesis of the best available data on key risk-relevant parameters, and a first picture of relative risk among commodities and foods commonly sold in traditional markets in Nigeria. The Box below identifies key recommendations for future EatSafe studies. Key findings and conclusions from this report include:

- A high prevalence of *Salmonella* was observed in all food products sampled, providing evidence that a significant contamination problem exists in foods sold at the markets, both fresh and ready-to-eat.
- Samples positive for *Salmonella* were most often contaminated at high concentrations, i.e., likely to cause illness if no reduction measures take place before consumption.
- Fresh tomatoes were the commodity most frequently contaminated by *Salmonella* (37% of samples), among those sampled in the study markets.
- Total bacterial counts in the sampled products were also high; while total counts are not directly associated with risk of illness, they can provide an indication of hygiene levels; in this case, total counts may indicate that foods were not handled safely, at or before reaching the market. For RTE cooked foods, high total counts imply that bacteria are present and possibly multiplying in the food after cooking.
- The frequent presence of *Salmonella* on foods not of animal origin indicates that cross-contamination is occurring at one or multiple points in the supply chain, including possibly at the market.
- In the three target markets, prevalence of *Salmonella* on foods sampled varied from 20% to 37%.
- RTE beef was estimated as the food associated with the highest risk of salmonellosis if consumed, among those assessed; beef purchased fresh and prepared at home was associated with a lower risk (by almost 2 Logs).
- Grains and legumes were associated with significant risk of salmonellosis in their RTE form, which indicates recontamination after cooking, possibly including storage and handling conditions at the market; moi-moi made from cowpeas was the food associated with the highest mean risk in this category.

- Vegetables (tomatoes) were also associated with considerable risk, of the same order of magnitude as some animal and legume foods, again highlighting the likelihood of cross-contamination.
- Overall, RTE foods were estimated to be associated with a higher risk of salmonellosis than foods purchased fresh/raw and prepared at home; if confirmed, these findings highlight the importance of vendor practices at the market as well as at the preparation site.
- Aflatoxin intake was generally higher than 1 ng per Kg of body weight per day, except for soybean (awara); maize was the commodity associated with the highest exposure, and hence the highest risk.
- While risk associated with aflatoxin contamination is much lower than risk of salmonellosis, it is still a key contaminant affecting multiple staple foods and thus merits attention. However, there is a limited number of aflatoxin-reducing interventions that can be applied at the market.
- Other hazards not assessed in this study, such as pesticide residues or non-bacterial microbial hazards, also warrant consideration; very little data are available for Nigeria, highlighting the need for investments in data collection that can inform the prioritization of food safety interventions.
- Overall, taken together, these findings indicate a high potential for market-based interventions to be effective in reducing risk, in particular microbial risk, with impacts dependent on the specific commodity/food and on preparation and consumption patterns in the consumer population.

Recommendations for Intervention Design and Future Studies under EatSafe

EatSafe aims to generate the evidence and knowledge on leveraging the potential for increased consumer demand for safe food to substantially improve the safety of nutritious foods in traditional market settings. Central to EatSafe's work is understanding (and potentially shaping) the motivations, attitudes, beliefs, and practices of consumers and food vendors. EatSafe should consider the following findings and observations during the design of its interventions:

- The high prevalence of *Salmonella* observed in all food products sampled provides evidence that a significant contamination problem exists in foods sold at the markets, both fresh and ready-to-eat. *Salmonella*, and bacterial pathogens more in general, should be considered as key target for risk reduction interventions.
- Fresh tomatoes, found very likely to harbor *Salmonella*, can be consumed raw. The lack of cooking post-purchase makes this commodity – as well as other vegetables consumed raw – highly vulnerable. Attention to this category of products is warranted.
- Total bacterial counts observed in ready-to-eat foods sold at the market indicate that cross-contamination is likely occurring at the market. Given the known links between bacterial contamination of foods and food handling and hygiene practices, it is likely that improving such practices in markets could significantly reduce foodborne risk.
- Food sold by some vendors harbored *Salmonella*, but food sold by other vendors did not, highlighting differences in the safety of foods sold at different shops. If related to vending practices or other visible cues (including a safety brand), these differences could be leveraged in interventions facilitating how consumer can identify safer shops.
- Most food products were associated with a high potential risk of salmonellosis. While some carry a higher risk than others, interventions would be most effective if considering practices that impact a basket of products instead of a single commodity.
- RTE foods were associated with a similar or higher potential risk of salmonellosis as foods purchased raw and prepared at home. While RTE foods are not directly in scope for EatSafe, they are an important part of the life of traditional markets; interventions should consider the broader context of foods sold at markets, including vending practices and consumers' perceptions that may be relevant to both types of foods.
- The risk associated with aflatoxin contamination is much lower than the risk of salmonellosis; however, it is still an important contaminant affecting multiple staple foods. Most relevantly for EatSafe's purposes, there is a limited number of aflatoxin-reducing interventions that can be applied at the market. Hence it appears warranted to deprioritize this class of hazards as targets for EatSafe's interventions in Nigeria.
- Findings of this study suggest that risk reduction at the market is needed, possible, and can be substantial. Interventions encompassing a range of food handling and hygiene practices are likely to yield public health benefits, and further identifying specific high-risk practices and critical control point in the market can amplify such benefits.

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9. APPENDICES

9.1. APPENDIX 1. DESCRIPTIVE ANALYSES OF EATSAFE'S KEY COMMODITIES IN NGIERA

9.1.1. MAIZE

Production: Nigeria is the second largest maize producer in sub-Saharan Africa, after South Africa (49). Maize is usually grown with other crops such as rice, cowpea, and soybeans, except for farms in Northern areas of the country (50). About half of Nigerian farmers grow maize. The main production season is from May to October in the North of the country, and March to August in the South (50). A second season, which is rain-fed, runs in the South from August to January; in the North, this second season also exists as a result of irrigation (50). The crop matures within 3 to 4 months of planting (51). National production levels are forecasted at 11.1 million metric tons annually (51), though decreased grain due to conflict exacerbated by COVID-19 was anticipated in the 2021/2022 season. Production constraints include levels of education and limited credit options in the North (52), as well as poor soil fertility, drought, and lack of fertilizer, seeds, herbicides, and insecticides (19).

On-farm Storage: Storage facilities are essential, enabling producers to hold produce until prices are higher before the next harvest (53). In most areas of Nigeria, maize is preserved by sun-drying (54), though other storage methods used in the North include rhombuses (raised cylindrical structures of mud or dry grass), and storage on bare floor or roof (54,55). Though these methods are not moisture, rodent, or insect proof, producers are slow to adopt new methods due to high cost and low availability (56).

Trading and Transporting: Farmers most frequently sell to maize traders, which are seen as separate from farmers (57). Traders move maize from the North, where production dominates, to the South, where consumption dominates. The average Northern trader is an educated male who operates a substantial small-medium enterprise (>300,000 USD per annum gross income). Each trader serves around 400 smallholder farmers (directly or via other traders). In the North, 80% of traders operate as "wholesalers" (i.e., taking possession) rather than "brokers" (i.e., moving the maize just for a broker fee without buying and selling it). About 50% of traders buy direct from farmers and around 70% receive some of their maize through "field brokers," who are agents that approach farmers and other traders to buy grain with information on price circulated by fellow traders, transporters, and from local radio and newspapers (58). Contracts are rarely used, as most maize is sold at "spot markets." Most traders rely on third parties for hiring transport (third party logistics). Maize is typically transported in bags labelled with the trader's name, thus introducing traceability (59). Trading is often the grain trader's main occupation (82%) with agriculture as a secondary one. All traders surveyed had worked in this sector for over ten years (19).

Processing: Approximately 40% of the maize produced in Nigeria is processed for animal feed, including fish and poultry feed (60). Growth in these industries have corresponded to higher demand for maize feed (50,53). Processing maize for added value products for human consumption is also common, with most small-scale processors relying on traditional techniques (e.g., pap, roast corn, boiled corn, maize balls and beer) (51).

Marketing: Supermarkets and modern fast retail sell processed maize products (flour, snacks, beer), serving primarily more affluent consumers (61).[10] However, some industrially produced maize products are sold in traditional markets – including unprocessed maize, as well as traditional maize value-added products. At wholesale markets, grain storage structures varied from 0 to 300, with seasonal variation (19).

Consumption: Maize is an important staple for Nigerians; it is consumed as corn flour, confectionery, roast corn, boiled, or prepared as porridge (51). Consumption was forecasted to increase by 2.5% and imports by 100% (from a low base, as Nigeria follows protectionist agricultural policies to encourage domestic production) (51).

9.1.2. FISH

Production and capture: Nigeria is the second largest producer of farmed fish in Africa, after Egypt, and has the largest import market in Africa. Domestic fish supplies are around 1.06 million tons of fish per year, comprising capture fisheries and aquaculture (75% and 25%, respectively) (62). Most aquaculture production is small-scale and land-based, practiced at subsistence levels using reservoirs and undrainable ponds. Catfish is the main species farmed (90%), as it tolerates crowding and a variety of environmental conditions (63). Other types of production, less common, include semi-intensive, intensive, integrated agriculture-aquaculture and sewage-fish (64). Most costs for producers are related to soybean and maize feed (50).

Only a handful of hatcheries service thousands of fish farms, suggesting farmers source fish fry by other means or from other areas. Though there were around 20,000 capture fishers in the state, most fish tend to be mostly bought by traders (65). Most fish in northern Nigeria is consumed fresh, contrasting significant frozen fish consumption in the south of the country (66).

Trading and Transport: Fishers and fish traders operate mostly in the traditional, informal sector. Lack of storage facilities, stable electricity, ice, and water, as well as insufficient credit, inadequate biosecurity, lack of a standard unit of measurement, poor road infrastructure, and lack of stable electricity are among the challenges fish traders face (50,66,67).

Processing: Processing is mainly carried out in the traditional sector, often by women. The most common processing technique is smoking. In Nigeria, most smoking uses traditional drum, box or mud-ovens – though in other contexts, this method has identified possible

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[10] Convenience shops or mini-marts (which are small retail outlets that follow modern practices in premise layout, products, retail methods and stock management), restaurants, hotels, and eateries across Nigeria.

contamination with polycyclic aromatic hydrocarbons. Though improved smoking ovens and dryers have been developed, adoption is low due to low access and high costs (68).

Marketing: Though some farmed fish are sold in supermarkets, most pass through traditional wholesale and retail. Fresh fish comprises a complex marketing chain with six routes to reach consumers (69). Marketing efficiency was high, with most production coming from catfish and tilapia aquaculture, followed by wild catch fish. Catfish are often transported live to markets; it can be kept alive at restaurants until its ready to be prepared (56).

Consumption and Food Safety: Fish comprise around 40% of Nigeria's protein intake, at 13.3 kg/person/per year (64). Domestic fish is either sold fresh or processed. While consumption of catfish is rising throughout Nigeria (70), hazard concentration changes over time, accumulating and varying during seasons (71). There are patterns showing certain heavy metals to bioaccumulation in either fish or shrimp (72). Further, raw fish that has already started to spoil is sold to street vendors and to household consumers at a cheaper price (67), increasing the risk of possible foodborne disease.

9.1.3. RICE

Production: Nigeria is the largest producer and consumer of rice in West Africa, and second in Africa after Egypt. Rice production is estimated at around 4 million tons, with the highest production volume in states in the northwest (60). Rice production throughout the country has dramatically increased since 2010, during a so-called "Rice Revolution." Unlike other States with large-scale government irrigation schemes, increasing use of farmer-based, small pump irrigation schemes and power tillers has driven the increase in the North-Western region, based on self-help efforts by farmers' groups (60). Throughout the country, about 90% of the rice planted area uses seeds sourced by farmers through local open markets and farmer exchanges. In addition to only 10% of potentially irrigable land is irrigated, an underdeveloped input supply limits access to technologies. Rice yield loss from diseases are also quite high, ranging from 11%-43% (73).

Trading and Processing: Paddy rice is either sold directly by farmers at markets, aggregated by middlemen, or collected by cooperative groups. It then enters a processing step where most paddy is parboiled either in the traditional or modern sector. In the former, paddy is soaked for 2-5 days and continuously heated before being spread to dry in the sun. It then passes to small-scale millers where paddy is hulled between two revolving metal blades. In the industrial sector, after parboiling, rice is milled with more sophisticated machinery to remove the husk (resulting in brown rice) and then polished to remove the bran (resulting in white rice). Both types of rice are destoned and packaged. Recently, a third level have been added to the chain, where low grade broken fractions, a by-product of rice milling is converted to rice flour and used for the production of diverse rice-based products (74). Three categories of millers are present in the country, namely, large millers, small millers, and cottage millers. Small- and medium-scale rice millers account for more than 80 percent of the local market. Around 20 large scale millers account for the remainder (74).

Marketing: From the millers, brown and white rice pass to wholesalers and then retailers. More than 90% of domestic rice sales occurs in the traditional market, being sold in loose form, via traditional retailers. Modern retail sells packaged and branded rice, most of which is imported (74). Rice demand has increased rapidly due in part to its ease of preparation, use in RTE foods, and its general availability among food vendors and restaurants located in work places, especially in urban areas (73). One study (73) found that rice vendors are already well-adapted to meeting consumer demand, with vendors reporting strategies to attract customers such as selling long grain or un-adulterated rice, delivering to customer location, or selling on credit.

Consumption: Rice consumption in Nigeria is high and steadily growing, with an estimated 32 kg consumed per person per year. Consumption far exceeds production and so demand is increasingly met by imports which represent about 40% of milled consumption (74). Nigerian consumers prefer the long grain parboiled rice from Thailand and India, with a demand that is expected to remain high (75). Household respondents prefer imported rice to the local rice, because of its higher quality, better taste and rice being free of stones and other debris. Local rice remains cheaper than imported rice. However, there is some optimism that "Rice Revolution" may lead to increased self-sufficiency and even exports. Rice is consumed in various forms including boiled rice, fried rice, jollof rice, coconut rice and *tuwo* (76).

9.1.4. COWPEAS

Production: Nigeria is the largest producer and consumer of cowpea, accounting for about 45% of the world's cowpea production (77). An average of 5Kg cowpea grain is purchased and consumed per household per week, with factors such as income level of consumers, taste of the product, prices of cowpea and that of its substitutes, and population level influencing consumer patterns in Nigeria (78). With regards to production, cowpea is produced by smallholders primarily for human consumption (79), but also for animal feed.

Trading, processing, marketing: Local methods of storing and preserving cowpeas have been described as grossly inadequate and a serious threat to the cowpea supply chain and public health (80). Local methods of storing and preserving cowpeas have been described as grossly inadequate and a serious threat to the cowpea supply chain and public health (81). During storage, if moisture and temperature are favorable to fungal growth, there is potential for aflatoxigenic molds to proliferate and produce toxins, which would increase the levels of the toxin in the commodity. This is true for all commodities affected by aflatoxin contamination, including cowpea. Furthermore, chemical pesticides are often used during cowpea storage to control pests and avoid product loss. Loss during storage has also been mentioned as a concern for cowpea traders in the country primarily due to insect damage. Although the Purdue Improved Cowpea Storage bags reduce the losses, the cost per bag has been a constraint for both producers and traders (81).

Consumption: For human consumption, cowpea is consumed as dry grain or fresh vegetable (82), in traditional dishes, that are either prepared by consumers in their homes, or by women entrepreneurs who trade in street food; in addition cowpea is served in restaurants and fast food outlets, especially in the urban areas of Nigeria (81). Cowpeas can be consumed twice a week or more and usually in the form of bean-staple mixes and for infant feeding, they are highly versatile and can substitute for cereals or be mixed with them to develop a variety of products such as doughnuts, couscous, spaghetti and traditional dishes such as alkaki, furu and moi moi (83). This gives good potential for value addition.

9.1.5. SOYBEANS

Production: Nigeria is the second largest soybean producer in Sub Saharan Africa, after South Africa, though the North-Western region is not a main producer.

Trading, processing, and marketing: Similar to cowpea and maize, soybean is used both in human food production as well as an ingredient in animal feed formulation. No part of the soya plant is wasted, the leaves and husk are used as feed for cattle, poultry and pigs, with Nigerian feed millers and poultry farms being important players (84). Soy cake is the main protein source especially in commercial fish and poultry production, while oil production is carried out extensively by multiple local, regional and national processors (85).

Soybean traders face many challenges including lack of modern storage facilities, high rental cost of stores, risk of adulteration by processors, reduced demand due to animal diseases such as bird flu effecting poultry production, and transport costs. Challenges faced by processors include lack of power supply, lack of storage facilities, and contamination during grinding. The quality of processed products (oil and cake) is determined by the quality of the seeds (soybeans) used in processing and the source of the seeds. Additional constraints include lack of awareness on processing and utilization, limited availability of processing equipment, poor market structure and weak policy support (84).

Consumption: As a human food, soybean is used in the preparation of local recipes among them soy flour, soy bread, soy cake, and soymilk (77). Household processors use produce from their farms and from the local markets, and process these into various products or ingredients for products including: soybean curds (awara), seasoning (dawa dawa), soya soup, doughball snack (fura), steamed dumpling (moi moi); flavored milk drink (fura de nono) and crunchy dessert (alkaki) (84). Several of these were originally made from different products but have been adapted to soybeans. For example, dawa dawa is a seasoning for stews and soups originally made from fermented locust beans; Nigerian women innovated the use of soybeans for this purpose.

Low consumption levels for soybean across the country have been reported with soybeans only occasionally eaten, not even on a weekly basis (86). In a case study in Oyo State, consumption patterns of soybean and soybean products was found to be influenced by socioeconomic factors, with marital status playing a role in household consumption (87). Older data from the Eastern part of the country give an average quantity of soybean consumed in farmers' households as 2.5 kg weekly in January and February after harvesting, but it decreased to an average of 1.5 kg weekly in June during the planting season, per person, the average consumption per capita was about 0.25 kg per weekly, ranging from 0 to 3.5 kg (88).

9.1.6. FRESH VEGETABLES

Production: The key fresh vegetable crops in terms of volume produced in Nigeria are tomatoes, potatoes, okra and onion with a large proportion of the tomato (>40%), peppers (>35%) and onions (>50%) being produced in Kaduna and Kano States (27). Tomatoes are important, accounting for almost one-fifth of vegetable consumption, and often consumed in combination with onions and peppers (89). Tomatoes are commonly grown in 24 out of the 40 states in Nigeria (although Kaduna and Kano States are the highest producing states, as mentioned above). The northern parts of the country supplying the south with produce during the dry season (80% of northern production consumed in the south in this season) and the southern states supply their own market during the rainy season (89). Producers can be small or large scale. Access to quality seeds is a key challenge and most farmers use open pollinated varieties from the traditional sector; hybrid seeds have less than 2% of market share (89).

Trading, processing, marketing: Vegetables are mainly sold through traditional markets (95-99%), with the formal retail channels serving only a very small share of the market (28). Traditional value chains are highly organized. Wholesale traders buy produce from farmers, which they in turn sell to retailers. The largest open markets are in the southern parts of the country. Lagos alone has more than 30 traditional markets that sell vegetables. The retailers sort produce into different quality classes to sell at smaller markets, such as along the road. Processing of vegetables is very limited (90). The retailers sort produce into different quality classes to sell at smaller markets, such as along the road. Processing of vegetables is very limited (90).

Tomatoes are sold 4-5 days after harvest with long distances between production and consumption leading to high post-harvest losses (90). The use of baskets instead of crates for storage and transportation leads to massive spoilage of produce (91). Vegetable value chain actors appear to be well aware of the challenges they face, including limited access to high quality seeds, low knowledge of good agricultural practices, pest and diseases, long distance between producer and consumer, lack of storage, and lack of year-round facilities, to name a few (91).

Consumption: Data regarding consumption of fresh vegetables in Nigeria is scarce but evidence suggests that vegetable intake is insufficient and production cannot meet demand year-round (92). Income is an important factor influencing household decision around consumption of fruits and vegetables as well as location, membership of cooperative group and access to a refrigerator (93). One study found that two-thirds of consumers in Akwa Ibom State (n=50 of 65) bought their vegetables from traditional markets, with the remainder purchasing directly from farmers or street sellers (92).

Monthly income, availability within town/area of residence, price of the item and visual attractiveness were the most important factors that influenced the preference for consumption of fruits and vegetables in this area. Another study (94) shows how an average household in the sample area considered the demand for fresh vegetables to be a luxury good with a higher demand for fresh vegetables in households with younger members, compared to households with older members.

9.1.7. BEEF

Production: Cattle contribute to 12% of the agricultural Gross Domestic Product in Nigeria, generating USD 6.8 billion annually (95). Both the total stock of cattle and total annual cattle slaughtered in Nigeria increased from 13.9 million to 20 million head, and 144,000 head to 321,000 head, respectively, from 1990 to 2017. However, the carcass yield reduced from 141 to 116 kg/animal from 1991 to 2017.

Around 70% of the nation's cattle are in northern Nigeria, kept in large herds by semi-sedentary Fulani herders. Historically, Fulani herders moved south in the dry season, but they have recently come into conflict with settled farmers over water resources.

Transport and live animal sale: Cattle are transported from production areas in the North to major consumption centers in the South. They are generally sold in live cattle markets. Sales are often mediated by middlemen (i.e., brokers) who aid buyers or sellers and charge a commission. Butchers scout for inexpensive animals, the old and the sick, for slaughter and distribution of meat. Challenges faced by the meat sector are manipulation by market intermediaries, high cost of transportation, inadequate security within the markets, purchase of stolen animals, and poor infrastructure (95).

Slaughter: Slaughterhouses are located near live cattle markets, characterized by lack of potable waters, proper waste disposal facilities, or sanitary inspectors (96). Essential infrastructure and equipment are lacking and maintenance is inadequate. There is typically no separation between clean and dirty areas and no practice of sanitation systems. Animals are often slaughtered on the floor because of the absence of mechanical or manual hoists: a major source of contamination (33). Water for meat processing is obtained from the same source to which animal wastes have been discharged, resulting in major contamination (33). The abattoir attracts wild and domestic carnivores, rodents, and other insects that serve as vectors of disease transmission to humans. Because meat transport and storage facilities are inadequate and unhygienic, several attempts to upgrade facilities have been unsuccessful, likely due to economic unviability, conflicts of interest, and high cost (97).

Abattoir to consumption: A previous ILRI study estimated that the majority of meat was sold at the meat markets adjacent to slaughterhouses and most beef is consumed in the home (98).

Consumption: Beef is considered an every-day meat whereas pork and poultry more for special occasions. However, domestic production cannot meet increasing demand. Consumer demand for beef in Nigeria is expected to exceed 450,000 tons in 2023, but the domestic industry will only be capable of delivering 370,000 tonnes (99).

9.1.8. CRITICAL CONTROL POINTS ALONG VALUE CHAINS

A critical control point (CCP) is a point within the production and preparation of food at which a control measure may be applied to prevent, reduce, or eliminate a hazard in order to reduce risk. From the descriptions of the value chains, several potential CCPs for microbial hazards can be identified. The critical points will vary depending on the hazard being addressed, as well as the commodity and the specific supply chain. While a detailed characterization of CCP in traditional markets' supply chains was out of scope for this work, EatSafe provides a summary here for context.

Mycotoxins are a concern in many of the cereals used for food and feed. For maize, proper harvesting, transportation, and optimal storage are critical to aflatoxin management. Maize used in flour milling should be adequately dried to ensure production of good quality flour. Aflasafe® technology has been used to address the challenge during production. Adding mycotoxin binders during the production of animal feeds can reduce exposure to humans through animal source foods, especially dairy.

For fresh vegetables, soil and water can be a source of both chemical and microbial contamination (e.g., heavy metals, parasite eggs, pathogenic bacteria), and use of chemicals to control pests or weeds can result in residue levels above acceptable limits. Cross-contamination with microbial hazards can occur at several points in the supply chain of vegetables, including during handling at the market and during home food preparation, especially from animal-source-foods. For instance, unhygienic handling pre- and post-cooking, such as use of same containers and tools for raw meat and vegetable eaten raw or already cooked, can result in bacterial contamination. Cross-contamination is much less of an issue for chemical hazards of non-biological origin.

In animal production, both cattle and fish, inputs especially animal feed contaminants and antibiotics administered to the animals can result in residues in the products. The excessive use of antibiotics in food production can result in antimicrobial resistance in bacteria in animals, humans, and the environment. For food animals, contamination of the meat commonly occurs during slaughter, via cross-contamination between fecal matter and carcasses. Smoking of meat and fish is linked to polycyclic aromatic hydrocarbon contamination and other harmful chemicals. Heavy metal contamination is a concern in the fish, soybean, and rice value chains, in addition to the risks they pose through consumption of vegetables.

9.2. APPENDIX 2. OTHER HAZARDS IDENTIFIED IN KEY COMMODITIEIS

SPECIFIC PRODUCT	HAZARD	REF
VEGETABLES		
Brassica oleracea, *Cochorus olitorius*, *Amaranthus hybridus*	*Salmonella*, *Citrobacter freundii*, *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Alcaligenes* spp., *Escherichia coli*, *Providencia* spp., Vibrio spp., *Pseudomonas* spp.	(56)
Cabbage, carrots, pepper, lettuce, spinach, cucumber, garden egg, tomatoes, beetroot	*Strongyloides* spp., *E*. histolytica, Ascaris, *G.* lamblia, *Trichuris* spp., hookworms	(100)
Fresh vegetables	Intestinal helminths	(59)
	Cryptosporidium oocysts	(101)
	Heavy metals	(102)
Ocimum grattisimum, Cucurbita maxima, Talinum triangulare, Murraya coenigii, Solanum nigrum; Gongronema latifolium, Teifeairia occidentalis	*Entamoeba* spp., *A. lumbricoides*, *Giardia* spp, *Fasciola* spp., *Taenia* spp.	(103)
Amaranths/fluted pumpkin	Pesticide residues	(104)
Telfairia occidentalis; Celosia argentea (sokoyoto)	Pesticide residues	(105)
FISH		
Pond fish	*Salmonella*	(106)
RTE shrimps	*Salmonella*	(107)
	Vibrio spp.	
Pond fish	*E. coli*	(106)
Coastal water bodies	*Salmonella*	(32)
Smoked and fresh fish	*Salmonella*	
Clarias gariepinus, Tilapia zilli	Organochlorine pesticide beta BHC	(108)
Smoked fish samples	Pesticide residue levels (DDT, dichlorvos, Lindane)	(109)
Dam reservoir fish	Organochlorine pesticides	(110)
Pond fish	*Shigella*	(106)
Smoked African chad	*Bacillus* cereus	(111)
MEAT		
Meat/meat pie	*Bacillus cereus*	(111)
	Staphylococcus spp.	(112)
Raw meat, (barbequed, roasted, spiced sundried, shredded fried)	*Salmonella*	(113)
Sausages	Polycyclic aromatic hydrocarbons (Napthalene; acenaphthylene; Fluorene)	(114)

	Heavy metals – arsenic; cobalt; cadmium; lead	
SOYBEAN		
Soybean grains from farmers	Aflatoxins (B1, G1); Ochratoxin; Fumonisin (FB1); Zearalenone	(115)
Soy beans from market traders	Heavy metals and pesticide residues	(116)
Awara	*Stapylocoocus aureus*, *Salmonella*; *E. coli*; *Shigella* spp.; *Pseudomonas* spp.	(117)
MAIZE		
Fermented maize gruel (*ogi*)	Aflatoxins; Zearalenone; Sterigmatocystin; Deoxynivalenol; aflatoxins (B1, B2, G1, G2); fumonisins (B1, B2, B3)	(118,119)
Maize-based breakfasts	Organochlorine pesticides	(120)
RICE		
RTE / cooked rice	*Staphylococcus aureus*, *Brahamella* spp, *Enterobacter aerogenes* and *Salmonella typhi*	(121)
Rice grains	Cadmium and lead levels	(122)
Cooked rice dishes	Heavy metal (Cu, Hg, Cd)	(123)
Fried rice	*Bacillus* cereus	(111)

9.3. APPENDIX 3. DETAILED METHODS ON FIELD DATA COLLECTION ACTIVITIES

An experienced consultant, affiliated with the Federal University of Agriculture, Abeokuta, Nigeria, led the field data collection, with support from EatSafe consortium partners. Data collection for both the consumer survey and microbial sampling (overlapping on same dates) occurred before the start of Ramadan, to avoid biases that may come because of the event. Therefore, date options were identified in mid-April. Consultations with local stakeholders led EatSafe to conduct a second field visit in June (data excluded from this report).

Consumer Survey: The questionnaire aimed to understand consumption behavior, at individual and group levels. EatSafe determined an optimal sample size (n=385 consumers) based on a 95% CI and 5% error margin, for a total of 400.

During the first visit to markets in April, all consumers interviewed were age 18 or older and gave voluntary, informed, and written consent prior to the interview. Enumerators recruited consumers they saw who had visibly purchased food in the markets. Once consumers confirmed that food had been purchased within the given market, the enumerator proceeded with the questionnaire. Interviews were carried out in the local language, Hausa, with enumerators transcribing into English. The enumerator verbally communicated information on the rationale of the study to the consumer at the start of the interview. Consumer survey data was recorded in paper format, which were scanned for subsequent data entry and archival following daily interview completion.[11] An Excel spreadsheet was used for data entry with analysis carried out using R statistical programming.

Nine locally recruited enumerators were trained by ILRI in collaboration with in-country EatSafe project staff. A total of four, two, and three enumerators were located in the three markets, respectively. Allocation of enumerators to specific points varied across the three markets as informed by maps drawn during the participatory mapping exercise during the reconnaissance study as well as the location of stalls selling commodities of interest, and entry and exit points to the markets.

Each enumerator repeated the questionnaire process through out a one-day period until sample number was attained. Criteria for inclusion/exclusion of consumers was that the products they will have purchased will have been purchased from within the three study markets and consumers over the age of 18. Consumer profiles and purchases varied depending on the time of day.

Microbial sampling and laboratory methods: A sample size of 398 (186 for awara, 139 for masa, and 93 for tomatoes) was determined prior to the reconnaissance survey. The estimate was based on prevalence data for *Salmonella* in the commodities: 10% for masa, 14% for

[11] Where converting hard copies of the consumer survey to soft copies and sharing through email given the infrastructural challenges in the markets was not feasible during the three-day visit, the consultant traveled with the hard copies to the ILRI Nigeria office and delivered them there in person. Once delivered, a research associate ensured all data was entered, cleaned, and reviewed for accuracy.

awara, and 5% for tomatoes. These estimates were based on the goal of having a reasonable probability of finding positive samples, given an approximate prevalence derived from other contexts. Calculations were done using Epitools, with a 95% CI.

A list of tomato vendors available in each day of the study was obtained, and samples were taken from each vendor during morning, mid-day and evening). Total samples depended on the number of vendors visiting the markets during specific days. Multiple sampling enabled capturing variability in microbial load data. Awara and bula are mostly sold in the afternoon (from 2-6 PM) and hence, samples were collected in the afternoon. The number of bula samples will depend on the vendors available.

EatSafe developed a three-day food sampling plan prior to engaging vendors in the market. Vendor consent was obtained orally prior to sample purchase. Vendor's participation in the food sampling was voluntary. They were free to leave the sampling process at any stage; their initial inclusion on the first day of sampling did not imply any obligation to remain in the study. Likewise, other vendors who wanted to become involved on days two or three of the sampling process were invited to do so. No identification details from vendors were taken to ensure data cannot be traced back to specific stalls. Where consent was given, tomatoes, awara, roast corn, and bula samples were purchased from vendors at the current market price.

Samples were collected in sterile polythene bags, packed in a container with ice packs and transported to the laboratory at the end of each day ensuring the the cold chain was maintained. All samples were processed within 24 hours after delivery to the laboratory. Trained staff handled the samples, at the field, during transport, and in the lab during analyses. Ambient temperature readings were obtained from Google weather mobile application at mid-day and afternoon on all three sampling days. A mean temperature for mid-day and afternoon was then calculated from these readings. Temperature measurements, in addition to readings taken from meteorological websites, helped inform the bacterial modelling process for the RA to estimate potential bacterial growth post purchase

A laboratory work protocol outlining methodology for sample preparation, bacterial counts, cfu/g calculation, isolation and identification of bacteria was be provided by the in-country partners. An ILRI microbiologist undertook quality assurance of the samples. The analyses included a quantification of microbial load and enumeration of non-typhoid *Salmonella* Antibiogram testing of all isolates from the analyzed products helped determine susceptibility of *Salmonella* isolates to commonly used antibiotics and hence, detect any antimicrobial resistant. For further speciation of the isolates using the MALDI-TOF technique, isolates were firstly be transported from the lab in Abeokuta to ILRI, Nairobi, where analysis will occur. Conventional polymerase chain reaction was run to complement prior testing results to identify resistance genes.

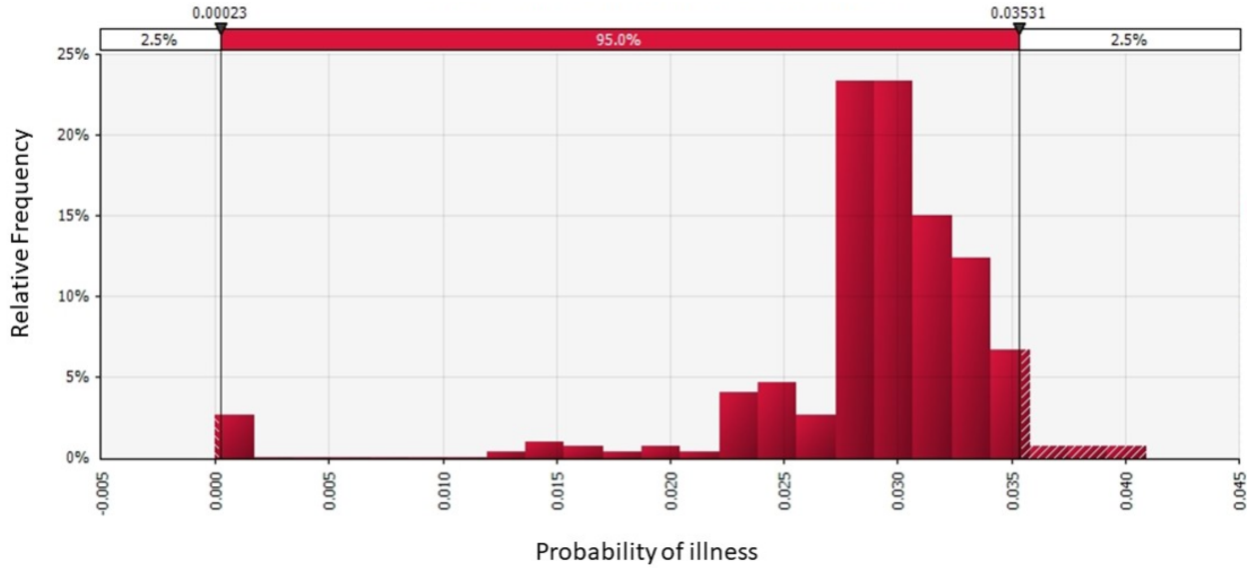
9.4. APPENDIX 4. INPUT VARIABLES AND PARMAETERS IN THE SALMONELLA RA MODEL

<table>
<tr><th>MODEL SECTION</th><th colspan="3">DISTRIBUTION OR EQUATION</th><th>DATA SOURCE(S)</th></tr>
<tr><td>Number of portions of each commodity consumed per year (N)</td><td colspan="3">N = (Frequency of consumption per year) *(Proportion of population consuming*urban area population estimates)</td><td>Fieldwork (consumption data from consumer survey)</td></tr>
<tr><td>Number of portions contaminated with Salmonella in markets (Nr)</td><td colspan="3">Nr = N*Promotion of samples contaminated at retail</td><td>Fieldwork and literature (Prevalence data)</td></tr>
<tr><td>Dose of Salmonella present at retail in markets (dr)</td><td colspan="3">Sampled from a Poisson distribution with mean equal to portion size in g *concentration in log cfu/g</td><td>Fieldwork (portion size from consumer survey); Fieldwork and literature (concentration data)</td></tr>
<tr><td rowspan="6">Dose of Pathogen present after storage (d_{st})</td><td colspan="3">D_{st} is sampled from a Poisson distribution with a mean equal to the product of d_r * Growth factor for Salmonella (G) truncated by the maximum population density MPD=1.0E+09 cfu/g</td><td>(124)</td></tr>
<tr><td colspan="3">Growth during storage is assumed to occur where mean storage temp (t_{st_mean}) > min growth temperature (t_{min} for Salmonella 5.2°C)</td><td>(124)</td></tr>
<tr><td colspan="3">Where growth occurs, G is sampled from Exponential primary growth model with parameters Storage time (t_{st}) and generation time (t_{gen}) of Salmonella) G= $e(\ln^2/t_{gen})*t_{st}$</td><td>(125)</td></tr>
<tr><td colspan="3">Storage time (t_{st}) in hours exponentially distributed with mean = t_{st_mean} and truncated at t_{st_max}</td><td>Fieldwork (t_{st_mean} and t_{st_max} from consumer survey)</td></tr>
<tr><td colspan="3">Generation time (t_{gen}) is described with the temperature model from [76] as a secondary model
t_{gen} (the minimum generation time for Salmonella in chicken = 0.3hr) = t_{gen_min}/ ((T_{st}-T_{min})/(T_{opt}, the optimum growth temperature for Salmonella = 39°C, –T_{min}))</td><td>(124)</td></tr>
<tr><td>Storage temp (T_{st}) assumed to be normally distributed w/ mean = T_{st_mean} and SD T_{st_stdev}</td><td>Fridge T_{st_mean} = 5.99 and T_{st_stdev} = 1.83</td><td>Ambient T_{st_mean} = 32.04 and T_{st_stdev} = 3.5</td><td>Fridge temperature from (126); Ambient temperature from fieldwork</td></tr>
</table>

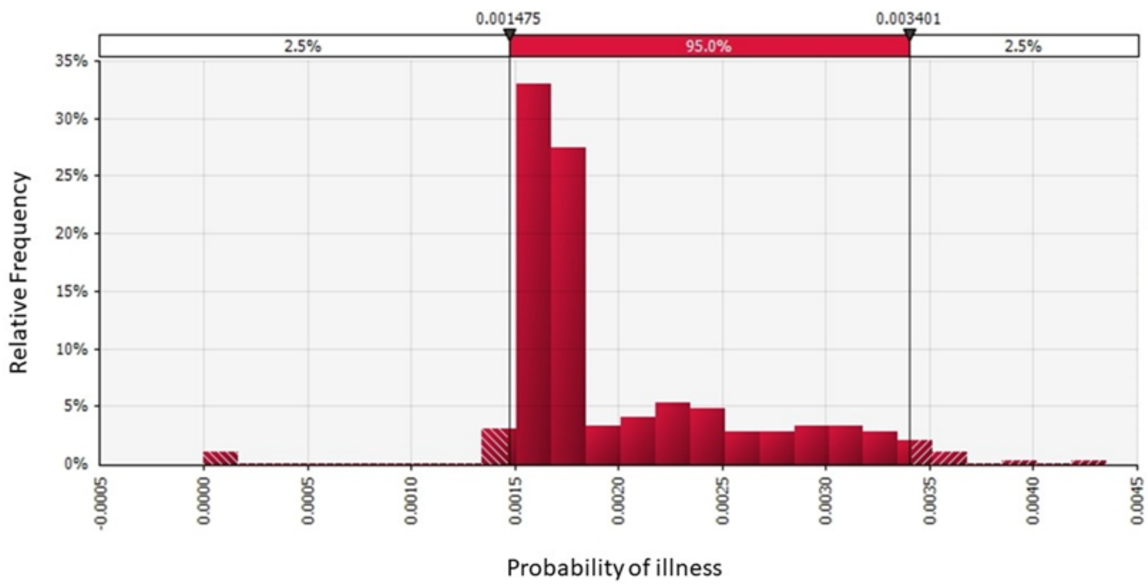
Inactivation during storage	No activation of *Salmonella* is assumed during storage at room/fridge temperature; Probability of survival of one cfu *Salmonella*/day at fridge or room temp. = 1			(127)
Cross-Contamination	Given that EatSafe did not conduct household observations, no cross-contamination is assumed in this work.			
Probability of *Salmonella* survival (P_{he}) in product after cooking of product	P_{he} assumed to be a pert distribution with min (P_{he_min}), most-likely (P_{he_ml}), and maximum (P_{he_max}), survival probability	Done: $P_{he\_min} = 0$ $P_{he\_ml} = 0$ $P_{he\_max} = 0.00041$	Raw: $P_{he\_min} = 1$ $P_{he\_ml} = 1$ $P_{he\_max} = 1$	(25); (128) for done
Proportion of portions cooked or re-heated (S_{pr_done}) or eaten 'raw' ($S_{pr\_raw} = 1 - S_{pr\_done}$)	Due to lack of household observation those who indicate that they cook their purchased product are assumed to have cooked them to "done"			Fieldwork (S_{pr_raw} and S_{pr_done} from consumer survey)
Dose of *Salmonella* present on product after storage & cooking (d_{pr})	d_{pr} is a sample from a Binomial distribution with parameters d_{st}, P_{he}			Calculated
Probability of illness after consumption of product (P_{ill})	Function of ingested dose d_{pr} and probability of illness after ingestion of 1 cfu of *Salmonella*. (P_{ill1}) $P_{ill} = 1 - (1 - P_{ill1})^{dpr}$ where P_{ill1} *is* sampled from a Beta-Binomial dose-response model *Beta* (α=0.00853,β=3.14)			(23,24)

9.5. APPENDIX 5. PROBABILITY OF ILLNESS FROM CONSUMING ONE PORTION OF STUDIED FOOD PRODUCTS

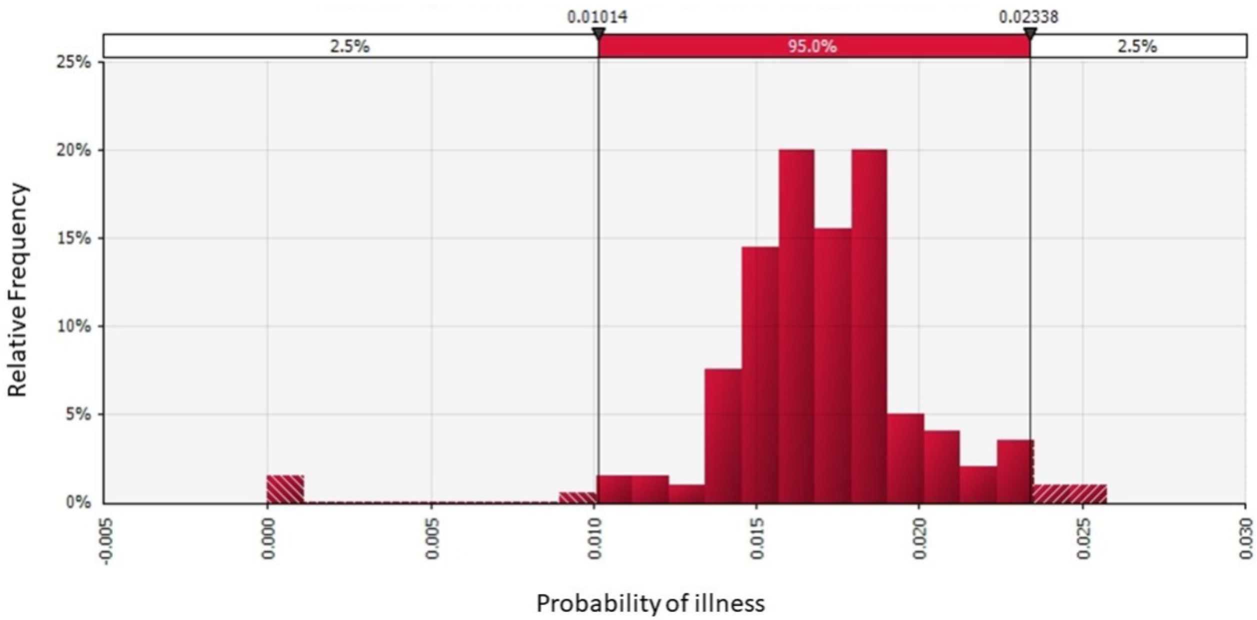
RTE Beef



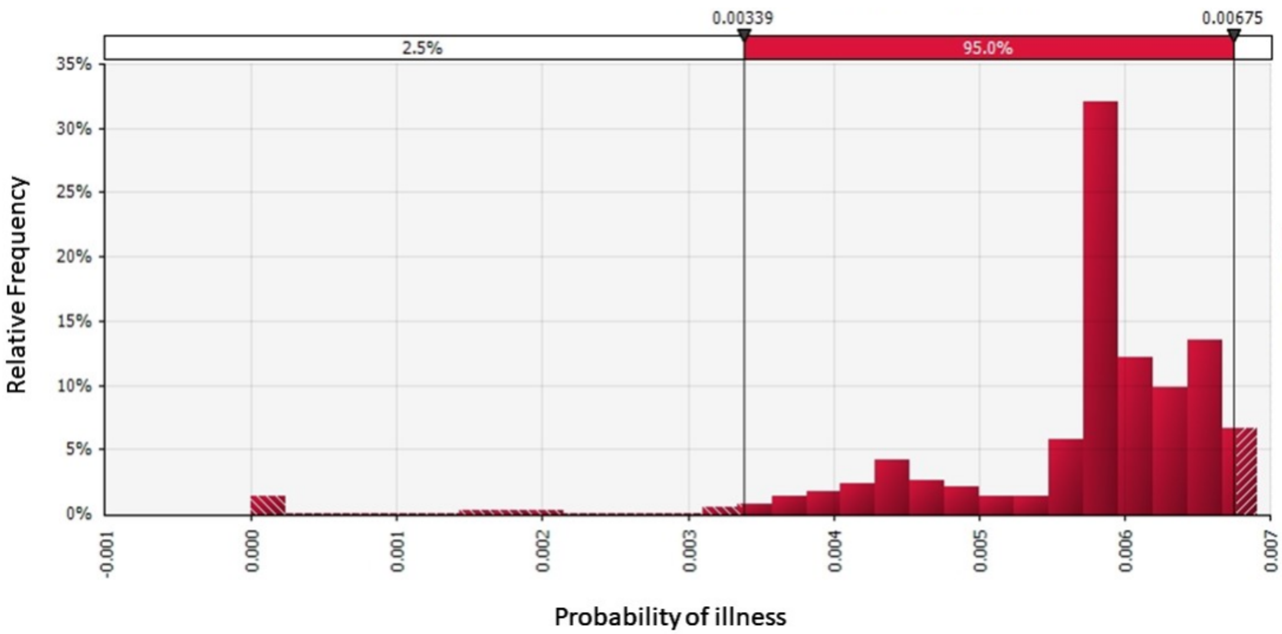
Fresh Beef



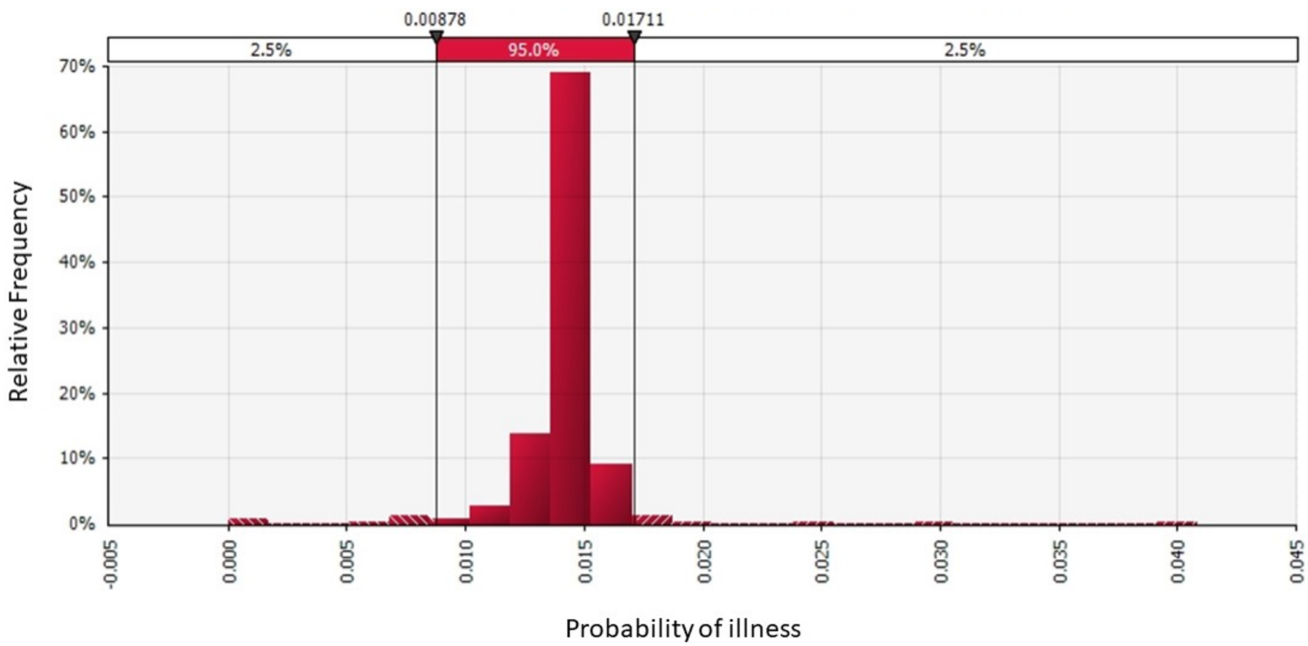
Moi Moi



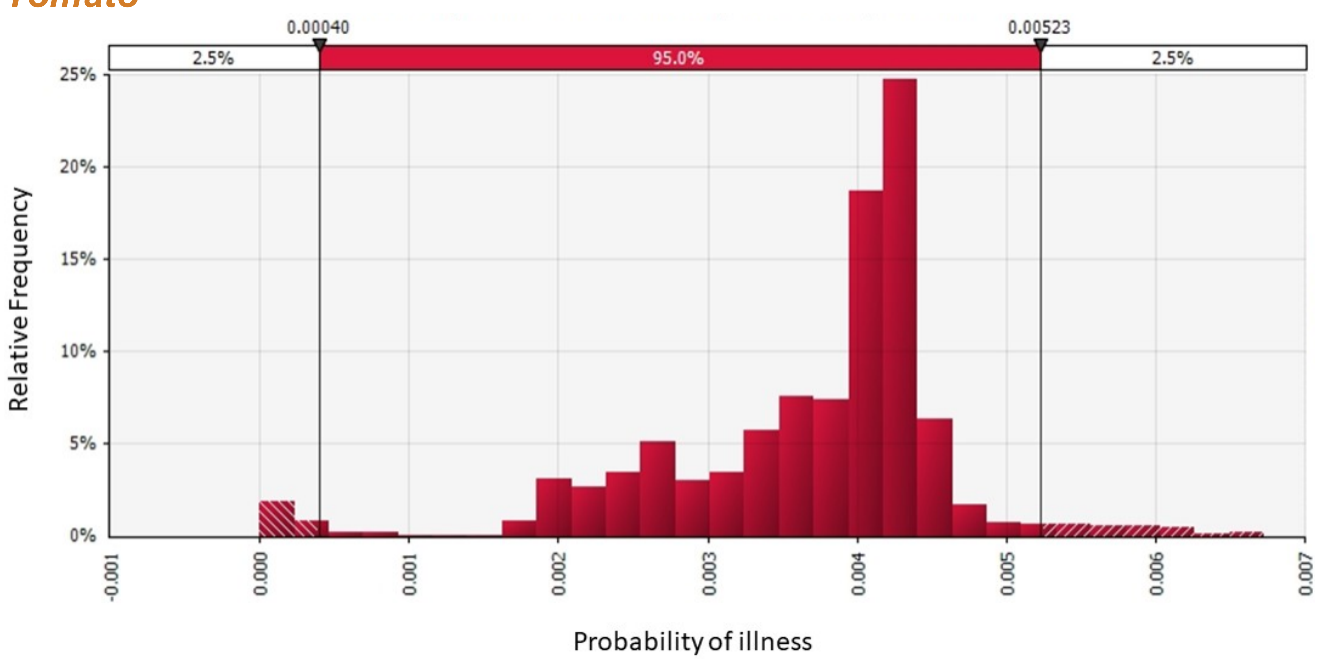
Awara



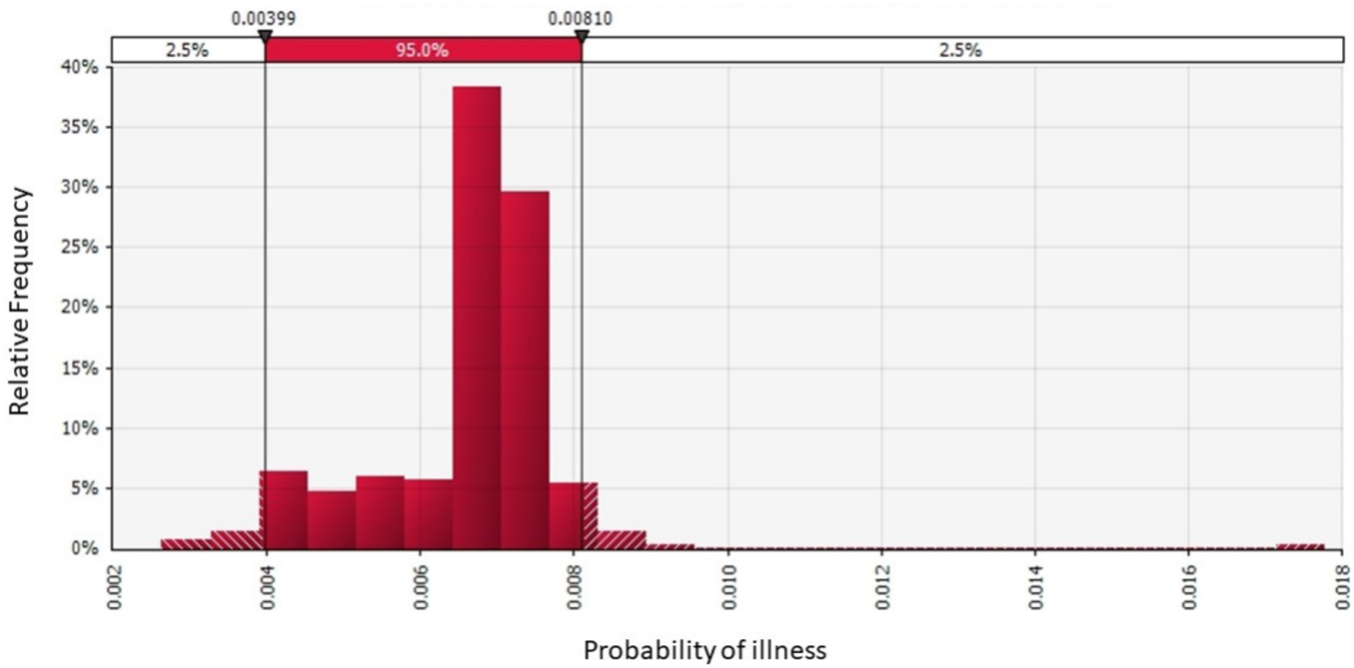
RTE Rice



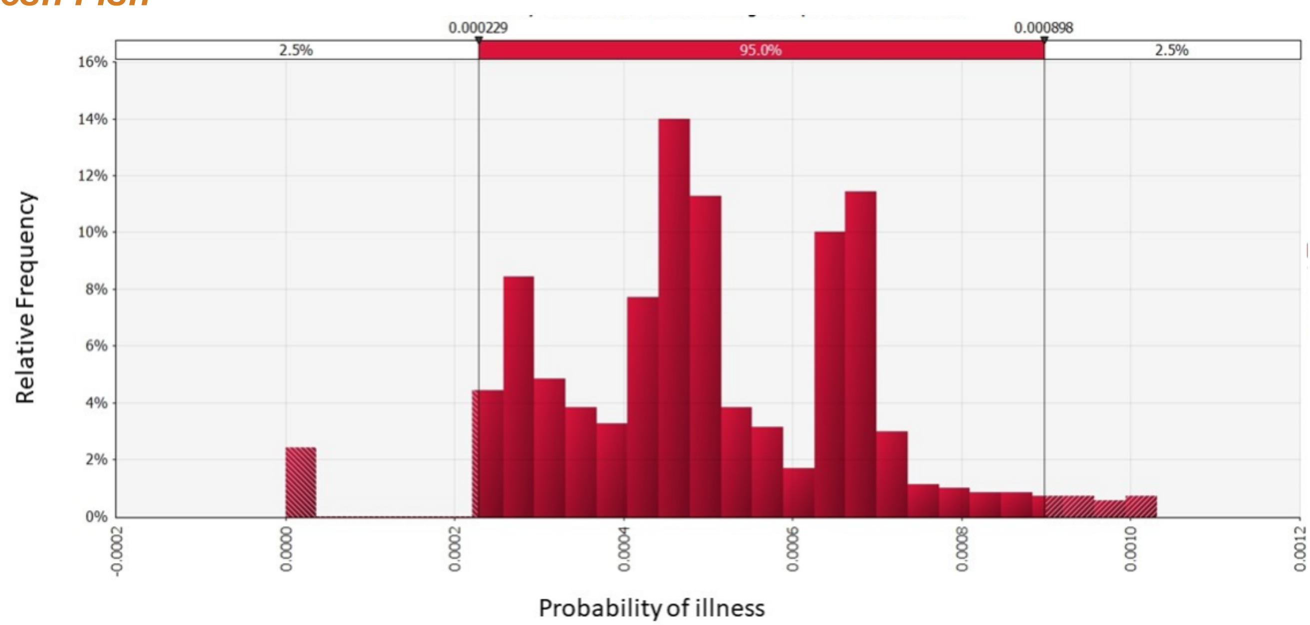
Tomato



RTE Fish



Fresh Fish



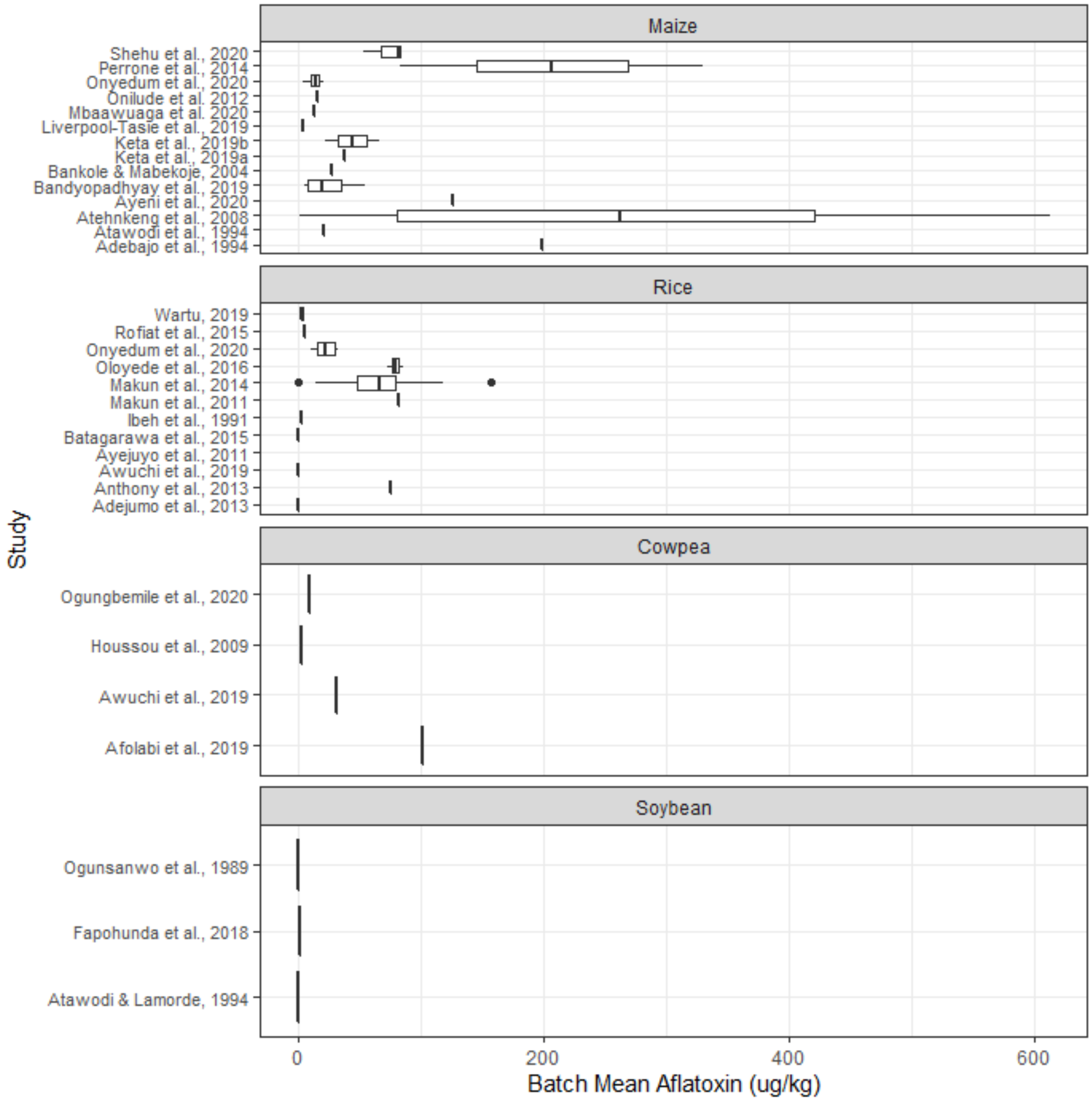
9.6. APPENDIX 6. AFLATOXIN RA METHODOLOGY

Contamination Data: A systematic literature review of peer-reviewed scholarly literature was conducted in Web of Science to characterize available data on aflatoxin contamination in Nigerian maize, cowpea, soybean, rice, and other food products derived therefrom. Each search included three keywords: i) “aflatoxin;” ii) the commodity name; and iii) the geographic focus, “Nigeria.” Eligibility for inclusion was assessed using the following criteria: i) publication date 1980-2020; ii) aflatoxin concentration determined for raw commodity (not cooked or prepared); iii) samples are reasonably intended for human consumption (not for livestock feed, etc.); iv) fungal growth is naturally-occurring, not artificially inoculated; v) aflatoxin is detected in at least one sample (studies with 0% detection were retained for prevalence model, but excluded from contamination model); vi) reported means are of batches of several samples, not replicates of the same pooled sample(s); vii) the number of total samples analysed is reported; and viii) an estimate of batch variance (standard deviation, range, etc.) is reported. Additional studies were identified by the authors via non-systematic searches in Google Scholar using similar keywords.

Data extracted from eligible studies included: mean, standard deviation, median, and range of aflatoxin concentration (µg/kg); study year; country; sub-national division; commodity type; sample source (household, market, or prepared food); metabolite (total aflatoxin or aflatoxin B1); total number of samples; number of positive samples or detection rate; limit of detection. Individual batch means reported within studies were extracted separately, when possible. If no variance or standard deviation was reported, the standard deviation was imputed based on the range of values using an estimator which is reasonably robust for skewed data (129). To be maximally conservative (i.e., to prevent under-estimation), total aflatoxin concentrations were extracted from the studies, when available, as opposed to only aflatoxin B1.

The search yielded 189 total studies, of which 29 (15%) were eligible for inclusion in the RA. Prevalence data (% positive samples) were available for 80% of reported batches in the 29 studies (**Table A1**), which were then included in the RA model.

Table A1. *Aflatoxin Concentrations in Raw Commodity Batches from Included Studies* [12]



Risk Assessment Model: The risk model included three assumptions: i) storage duration of the product is null, (i.e., no fungal growth or additional toxin accumulation occurs before consumption); ii) aflatoxin concentration is stable at normal cooking temperatures (i.e., no concentration reduction occurs during cooking) (130); and iii) the consumer does not take detoxification procedure (i.e., no “inactivation” of the toxin).

[12] Boxplots reflect distributions of multiple sample means reported in a study. Only the mean is shown for studies reporting only a single batch.

The estimated daily exposure (EDI) of aflatoxin was estimated for the study population using an adapted equation (44):

$$EDI\ (ng\ kg^{-1} bw\ day^{-1}) = \frac{C\ \times S\ \times i\ \times f_{cons}}{BW}$$

where C = concentration of aflatoxin per kg sample (μg/kg), S = g consumed/person/day, i = frequency of contamination among portions, f_{cons} = frequency of maize consumption in the population, and BW = body weight (kg). An exposure model to estimate aflatoxin dose ingested was constructed for each commodity using the EDI equation and incorporated into a Monte-Carlo simulation model. The model was coded in the R software. A second-order Monte-Carlo approach was used to account for both variability and uncertainty (100 iterations in both dimensions) in input parameters and implemented using the 'mc2d' package in R.

To account for the fact that not all samples (and, thus, not all portions consumed) are contaminated with detectable aflatoxin levels, the probability of consuming a contaminated portion (i) was approximated by aflatoxin prevalence, or detection rate. Prevalence data reported by the literature were fit to a beta distribution; uncertainty around the parameter estimates was modelled using parametric bootstraps (1000 iterations). In addition to representing the probability of consuming a contaminated portion, the i parameter was used to partially correct for possible overestimation of exposure due to tendency in the literature to report means only for contaminated samples (i.e., excluding non-detects). Due to the small number of studies reporting detection rate for soybean, it was not possible to fit a beta distribution; instead, the mean detection rate reported in the literature (7%) was used.

The empirical distribution of aflatoxin concentrations means across relevant studies was examined and a series of goodness-of-fit tests were performed to select an appropriate probability distribution to describe the observed aflatoxin concentrations (C), for samples where aflatoxin was detected. Concentration estimate data were assumed to be derived using assays of the same sensitivity and precision; in other words, the initial weight of samples collected and analyzed was considered uniform across samples. Given the known skewness of aflatoxin data, three probability distributions suitable for skewed data were considered: gamma, Weibull, and log-normal, in addition to the normal distribution. Q-Q plots, P-P plots, and CDFs were examined for model fit. Three Goodness-of-Fit statistics (namely the Kolmogorov-Smirnov statistic, the Cramer-von Mises statistic, and the Anderson-Darling statistic) were estimated for each of the probability distributions and compared. As a final test of Goodness-of-Fit across the candidate distributions, uncertainty in the parameters of the fitted distributions was estimated using parametric bootstraps (1000 iterations). Probability distributions were then selected for maize, rice, and cowpea concentration models were Weibull, truncated normal, and truncated log-normal, respectively. All distributions were truncated between 0 and the highest observed sample maximum reported in the included studies. Given that there were very few (n=1) observations of detectable aflatoxin in soybean batches in the included studies, it was not possible to compute Goodness-of-Fit statistics for the candidate distributions. Instead, a log-normal distribution (truncated between 0 and the highest observed sample maximum) was assumed and fit to the available parameters. As the one included study reporting detectable aflatoxin in Nigeria reported a sample maximum of only 2 µg/kg, a higher (i.e. more conservative) value of 4 µg/kg, reported in a Rwandan study (131) was used as the upper bound of the truncated distribution.

Two food consumption parameters were included as inputs in the exposure assessment model: 1) the frequency of consumption events (f_{cons}), and 2) the quantity of product consumed per consumption event (S). For all commodities, f_{cons} and S were derived from reported consumption data for key prepared food consumed in the study region. Namely, the local foods selected included *tuwo masara* (masara is "maize" in Hausa), cooked rice, *moi-moi* (cowpea), and *awara* (soybean), each of which is among the most common local preparations of the focus commodity. Empirical distributions were fit to f_{cons} data (consumption events/week) for each commodity, based on values reported in the literature (**Table A1**). To account for the fact that aflatoxin data were reported for raw (unprepared) commodities, mass conversion rates (m) were applied to portion sizes to determine the equivalent of raw commodity consumed per portion of prepared food. Consumption amount S was assumed normally distributed and means (±SD) from prior studies were used to fit a probability distribution truncated at the lower bound of 0 (e.g., no negative consumption).

Table A1. *Summary of Aflatoxin Exposure Model Input Parameters, by Commodity*

VARIABLE	VALUE OR DISTRIBUTION	DATA SOURCES
MAIZE		
Aflatoxin concentration	*~ TWeibull(bootstrap on observed values, 0, 1874)*	Included studies
Contamination frequency	*~ Beta(bootstrap on observed values)*	Included studies
Consumption frequency	*~ Emp({0, 1/7, 1}, {0.104, 0.150, 0.746})*	(132)
Mass conversion rate	$\frac{1\ g\ maize}{5.15\ g\ tuwo\ masara}$	(133)
Consumption level	*~ TN(200*m, 86.6*m, 0, ∞)*	(134)
Mean adult body weight	*~ N(63.7, 10.28)*	(135)
RICE		
Aflatoxin concentration	*~ TN(bootstrap on observed values, 0, 372)*	Included studies
Contamination frequency	*~ Beta(bootstrap on observed values)*	Included studies
Consumption frequency	*~ Emp({0, 2/7, 4/7, 1}, {0.837, 0.0709, 0.0511, 0.0378})*	(136)

Mass conversion rate	$\frac{1\ g\ rice}{3\ g\ cooked\ rice}$	Assumed
Consumption level	*~ TN(420*m, 182.23*m, 0, ∞)*	(134)
Mean adult body weight	*~ N(63.7, 10.28)*	(135)
COWPEA		
Aflatoxin concentration	*~ TlnN(bootstrap on observed values, 0, 200)*	Included studies
Contamination frequency	*~ Emp({1, 0}, {0.367, (1-0.367)})*	Included studies
Consumption frequency	*~ Emp({1/7, 3/7, 5/7, 1}, {0.474, 0.363, 0.009, 0.153})*	(137)
Mass conversion rate	$\frac{1\ g\ cowpea}{5.23\ g\ moimoi}$	(138)
Consumption Level	*~ TN(198.3*m, 122.4*m, 0, ∞)*	(134)
Mean adult body weight	*~ N(63.7, 10.28)*	(135)
SOYBEAN		
Aflatoxin concentration	*~ TlnN(1.88, 0.91, 0, 4)*	Concentration (115); truncation upper bound (131)
Contamination frequency	*~ Emp({1, 0}, {0.073, (1-0.073)})*	Included studies
Consumption frequency	*~ Emp({0, 1/7, 2/7 3/7, 1}, {0.1875, 0.1667, 0.2083, 0.239})*	(139)
Mass conversion rate	$\frac{1\ g\ soybean}{1.927\ g\ awara}$	(140)
Consumption level	*~TN(94*m, 44*m, 0, ∞)*	Fieldwork
Mean adult body weight	*~ N(63.7, 10.28)*	(135)

Previously reported estimates of adult body weight for a rural Nigerian population (135) were used to fit a probability distribution of this parameter for the exposure assessment. Mean (±SD) reported body weight for the general population estimated in that study was 63.17 (±10.28). This parameter was assumed to follow a normal distribution (141).

HCC Risk Characterization: The risk of aflatoxin-associated HCC attributable to consumption of each commodity was estimated based on probability distributions of exposure (i.e., EDI), the constituent aspects thereof, and the carcinogenic potency of aflatoxin. The carcinogenic potency of aflatoxin, defined as the probability of HCC (‘*r*’) per 100,000-individual population was estimated using the equation (44):

$$r = (0.3 \times HBsAG^{+}) + (0.01 \times (1 - HBsAG^{+}))$$

It has been demonstrated that the probability of HCC attributable to aflatoxin exposure is greater for individuals with Hepatitis B surface antigen (HBsAG) positivity by a factor of 30. A HBsAG positivity rate of 13.2% has been reported for the Nigerian population (142). Thus, the carcinogenic potency was estimated as: (0.3*0.132) + (0.01*(1-0.132)) = 0.04828. The risk of disease (HCC cases/year per 100,000 population) was estimated by multiplying the EDI by the carcinogenic potency (44), using the equation:

$$HCC\ Risk = EDI \times r$$