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Original Article

Aflatoxin B1 Contamination and Dietary Risk Assessment in Commonly Consumed Smoke-Dried Fish Species in Minna, Niger State, Nigeria

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ABSTRACT

The contamination of smoke-dried fish with Aflatoxin B1 (AFB1) poses a significant yet under-investigated food safety threat in Nigeria. This study assessed the levels of AFB1 and evaluated the associated dietary risk among consumers of commonly consumed smoke-dried fish species in Minna, Niger State. A total of 24 smoke-dried fish samples comprising *Clarias gariepinus, Protopterus annectens, Gymnarchus niloticus,* and *Synodontis membranaceus* were collected from three major markets in Minna metropolis: Kure, Mobil, and Kasuwa-Gwari between July and August 2025. AFB1 was quantified using a validated indirect Enzyme-Linked Immunosorbent Assay (ELISA). Dietary risk was evaluated deterministically and probabilistically using the Estimated Daily Intake (EDI) and Margin of Exposure (MOE) methods, with consumption data derived from a structured survey of 84 respondents. The results indicated that AFB1 was detected in all 24 samples (100% prevalence), with concentrations ranging from 0.38 to 6.05 µg/kg (overall mean: 2.95 µg/kg). *Clarias gariepinus* recorded the highest mean AFB1 concentration (5.42 $\pm$ 0.47 µg/kg), while *Synodontis membranaceus* had the lowest (0.69 $\pm$ 0.27 µg/kg). Market level differences were not statistically significant. The deterministic MOE was 27.78, and the Monte Carlo-simulated median MOE was 35.47, both substantially below the regulatory concern threshold of 10,000 for genotoxic carcinogens. Furthermore, 88.3% of simulated population-level MOE values fell below 100, indicating a high probability of public health concern. These findings demonstrate widespread AFB1 contamination across fish species and market locations in Minna and signal an elevated carcinogenic risk for regular consumers. Urgent regulatory intervention, improved smoke-drying practices, proper storage and routine surveillance are recommended.

Keywords: Aflatoxin B1, smoke-dried fish, ELISA, dietary risk assessment, Margin of Exposure

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INTRODUCTION

Fish is a critical source of dietary protein across sub-Saharan Africa, particularly in Nigeria, where it contributes substantially to household nutrition, food security, and economic livelihoods [1]. Smoke-drying is the most widely practiced post-harvest preservation method in the region, valued for its low cost, cultural acceptance, and ability to extend shelf life. However, artisanal smoke-drying is frequently conducted in open, unregulated environments without standardised hygiene protocols, rendering the product vulnerable to fungal colonisation and subsequent mycotoxin contamination [2, 3].

Mycotoxins are toxic secondary metabolites produced by filamentous fungi, principally *Aspergillus*, *Penicillium*, and *Fusarium* species under warm, humid conditions characteristic of tropical climates. Among these, Aflatoxin B1 (AFB1) is the most toxicologically significant, classified as a Group 1 human carcinogen by the International Agency for Research on Cancer [4]. AFB1 is a potent hepatocarcinogen that forms DNA adducts, induces oxidative stress, and causes immunosuppression, with chronic exposure strongly associated with hepatocellular carcinoma especially in populations co-infected with hepatitis B virus [5, 6].

Fish may be contaminated with AFB1 at multiple stages: through ingestion of contaminated aquafeed, direct fungal colonisation during processing, and exposure to mycotoxin-laden environments during open-air smoke-drying and market storage. Toxigenic fungi such as *Aspergillus flavus* and *Aspergillus parasiticus* thrive under the inadequate drying temperatures, high residual moisture, and humid storage conditions commonly found in informal Nigerian fish markets [7, 8].

This study aimed to detect and quantify AFB1 concentrations in four commonly consumed smoke-dried fish species from three major markets in Minna metropolis; and evaluate the dietary risk posed by AFB1 exposure to local consumers using deterministic and probabilistic risk assessment approaches. The findings are expected to provide baseline data for food safety policy, inform targeted surveillance programmes, and support evidence-based interventions to protect public health.

MATERIALS AND METHODS

Study Area

This study was conducted in Minna, the capital city of Niger State, located in North-Central Nigeria at latitude 9°37′N and longitude 6°33′E. Minna experiences a tropical climate with a rainy season from May to October and a dry season from November to April. The city hosts several major fish markets that serve as major distribution points for smoke-dried fish products for urban and peri-urban consumers. Three markets were selected as sampling sites based on their prominence in the local fish trade: Kure Ultra-Modern Market, Kasuwa-Gwari Market, and Mobil Market (Figure 1).

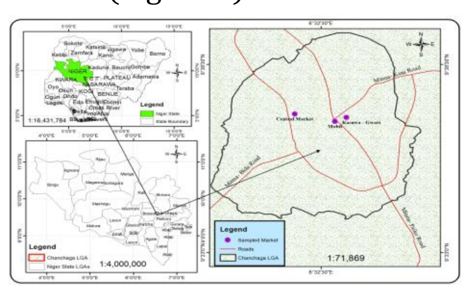

Figure 1. Map of Minna indicates the sampled markets.

Study Design

A cross-sectional food safety survey design was employed. Smoke-dried fish samples were systematically collected from the three selected markets. A structured consumer survey was administered to 84 respondents to obtain data on fish consumption frequency, daily intake quantities, and body weight parameters required for dietary risk estimation. Sampling was conducted between July and August 2025, during the rainy season, when ambient humidity is at its highest and the risk of fungal proliferation is most pronounced.

Sample Collection

A total of 28 smoke-dried fish samples were randomly purchased from the three selected markets. From these, 24 samples representing four species, *Clarias gariepinus* (African catfish), *Protopterus annectens* (African lungfish), *Gymnarchus niloticus* (African knifefish), and *Synodontis membranaceus* (upside-down catfish) were selected for laboratory analysis, with two replicates per species per market (n = 2 per site per species). Samples were collected and immediately sealed in sterile zip-lock polyethylene bags, lablled, and transferred to the Laboratory, to prevent further fungal growth or toxin degradation [9, 10].

Ethical Consideration

All samples were purchased from open markets as commercially available food products. No human subjects were enrolled in any experimental intervention. The consumer survey was conducted in full accordance with the ethical principles of voluntary participation and informed consent. Sample handling and solvent waste disposal were carried out in accordance with the biosafety and environmental safety policies of the Federal University of Technology, Minna.

AFB1 Quantification by ELISA

Aflatoxin B1 was quantified using a validated indirect competitive Enzyme-Linked Immunosorbent Assay (ELISA). The assay employed an AFB1–BSA conjugate (1.5 µL stock diluted in 15 mL carbonate buffer) coated onto microplate wells at 37°C for one hour. After blocking with 0.2% bovine serum albumin (BSA), antiserum solution (1.67 µL diluted in 10 mL 0.2% BSA) was applied, followed by a goat anti-rabbit secondary antibody. Para-nitrophenyl phosphate substrate was used for colour development and absorbance measured at 450 nm on a microplate reader. AFB1 concentrations were calculated from a standard curve generated using serial dilutions of a certified AFB1 standard, and results reported in µg/kg wet weight. All samples were analysed in duplicate [11].

Dietary Risk Assessment

Dietary risk was assessed using the Estimated Daily Intake (EDI) and Margin of Exposure (MOE) approaches. The EDI was calculated deterministically as:

$$\mathbf{EDI = C \times IR / BW}$$

Where C is the mean measured AFB1 concentration (µg/kg), IR is the mean daily fish intake rate (kg/day) derived from the consumer survey, and BW is the mean adult body weight (kg). The MOE was computed as:

$$\mathbf{MOE = BMDL / EDI}$$

The Benchmark Dose Lower Confidence Limit (BMDL) of 0.17 µg/kg body weight/day, as established by the European Food Safety Authority (EFSA) for AFB1-associated hepatocellular carcinoma, was adopted. An MOE substantially below

10,000 is considered indicative of a public health concern for genotoxic carcinogens. Probabilistic risk assessment was conducted using Monte Carlo simulation (10,000 iterations) to account for variability in exposure parameters across the population [12].

Data Analysis

Data were analysed using Python (version 3.x) with the pandas, scipy.stats, numpy, and matplotlib/seaborn libraries. Prevalence was expressed as the percentage of positive samples. Mean AFB1 concentrations and microbial counts are reported as mean ± standard deviation (SD). Differences among fish species and markets were assessed using one-way analysis of variance (ANOVA) with post-hoc multiple comparison testing; means assigned different superscript letters were considered significantly different at $p < 0.05$.

RESULTS

Prevalence and Levels of AFB1

Aflatoxin B1 was detected in all 24 smoke-dried fish samples (100% prevalence) across all three markets. Concentrations ranged from 0.38 µg/kg (Synodontis membranaceus, Kasuwa-Gwari) to 6.05 µg/kg (*Clarias gariepinus*, Kure), with an overall mean of approximately 2.95 µg/kg. The raw detection values for all samples are presented in Table 1.

Table 1. AFB1 concentrations (µg/kg) in smoke-dried fish samples from three markets in Minna metropolis.

Market	Fish Species	Sample A (µg/kg)	Sample B (µg/kg)
Kure	*Protopterus annectens*	2.11	2.27
‘	*Synodontis membranaceus*	1.00	0.95
‘	*Clarias gariepinus*	6.00	6.05
‘	*Gymnarchus niloticus*	2.98	3.00
Mobil	*Protopterus annectens*	2.70	2.63
‘	*Synodontis membranaceus*	0.66	0.77
‘	*Clarias gariepinus*	5.22	5.18
‘	*Gymnarchus niloticus*	3.05	3.12
Kasuwa-Gwari	*Protopterus annectens*	2.79	2.90
‘	*Synodontis membranaceus*	0.38	0.40
‘	*Clarias gariepinus*	5.09	5.00
‘	*Gymnarchus niloticus*	3.20	3.27

Mean AFB1 Concentration by Species and Market

Significant species-level differences in mean AFB1 concentrations were observed ($p < 0.05$). *Clarias gariepinus* recorded the highest mean concentration (5.42 ± 0.47 µg/kg), followed by *Gymnarchus niloticus* (3.10 ± 0.12 µg/kg), *Protopterus annectens* (2.57 ± 0.31 µg/kg), and *Synodontis membranaceus* (0.69 ± 0.27 µg/kg) (Table 2). Notably, the mean AFB1 level in *Clarias gariepinus* exceeded the NAFDAC maximum limit of 10 µg/kg for total aflatoxins, and the European Union limit of 2 µg/kg for AFB1 in ready-to-eat foods.

Table 2. Mean AFB1 concentrations (μg/kg) by smoke-dried fish species from Minna markets.

Fish Species	Mean AFB1 ± SD (μg/kg)
Clarias gariepinus	5.42 ± 0.47[a]
Gymnarchus niloticus	3.10 ± 0.12[ab]
Protopterus annectens	2.57 ± 0.31[bc]
Synodontis membranaceus	0.69 ± 0.27[c]

Different superscript letters indicate significant differences among means ($p < 0.05$).

Market-level comparisons revealed no statistically significant differences ($p > 0.05$). Mean AFB1 concentrations were 3.04 ± 1.99 μg/kg at Kure, 2.92 ± 1.70 μg/kg at Mobil, and 2.88 ± 1.77 μg/kg at Kasuwa-Gwari (Table 3).

Table 3. Mean AFB1 concentrations (μg/kg) by market location in Minna, Niger State.

Market	Mean AFB1 ± SD (μg/kg)
Kure	3.04 ± 1.99[a]
Mobil	2.92 ± 1.70[a]
Kasuwa-Gwari	2.88 ± 1.77[a]

Means sharing the same superscript letter are not significantly different ($p > 0.05$).

Consumer Survey and Dietary Exposure Parameters

The consumer survey (n = 84) revealed that most respondents were female (62.8%), aged 31–40 years and possessed tertiary-level education (77.0%). Most participants had over 10 years of fish consumption experience. The most common body weight category was 61–70 kg (51%), with a derived mean body weight of 70 kg. Fish was most frequently consumed 2–3 times per week (approximately 30 respondents), with an average daily intake of 145 g. Catfish (*Clarias gariepinus*) was the most consumed species (36.8%). The majority sourced their fish from local markets (70 respondents), with limited access to cold storage reported by 34.1% of participants.

Dietary Risk Assessment

Using the mean AFB1 concentration of 2.947 μg/kg, an average daily fish intake of 145 g (0.145 kg), and a mean body weight of 70 kg, the deterministic EDI was calculated as 0.0061 μg/kg body weight/day, yielding a deterministic MOE of 27.78. Both the EDI and MOE signal a level of dietary exposure warranting public health concern (Table 4).

Table 4. Deterministic AFB1 dietary risk parameters for smoke-dried fish consumers in Minna.

Parameter	Value
BMDL (μg/kg bw/day)	0.17
Mean AFB1 concentration (μg/kg)	2.947
Mean daily intake (kg/day)	0.145
Mean body weight (kg)	70
EDI (μg/kg bw/day)	0.00612
Deterministic MOE	27.78

BMDL: Benchmark Dose Lower Confidence Limit; EDI: Estimated Daily Intake; MOE: Margin of Exposure.

Monte Carlo Simulation

Probabilistic risk assessment via Monte Carlo simulation (10,000 iterations) produced a simulated mean EDI of 0.00691 μg/kg body weight/day and a median simulated MOE (P50) of 35.47. The 95th percentile (P95) MOE was 143.38. Critically, 88.3% of the population-level MOE distribution fell below 100, and 100% of simulated MOE values fell below 1,000, the threshold below which substantial concern for genotoxic carcinogens is conventionally indicated (Table 5).

Table 5. Monte Carlo simulation results for AFB1 Margin of Exposure.

Percentile / Parameter	MOE Value
1st Percentile (P1)	4.81
5th Percentile (P5)	8.64
50th Percentile / Median (P50)	35.47
95th Percentile (P95)	143.38
Probability MOE < 100	88.3%
Probability MOE < 1,000	100%
Simulated Mean EDI (µg/kg bw/day)	0.00691

DISCUSSION

The prevalence of AFB1 (100% of samples) across all fish species and markets represents a striking finding that underscores the pervasive nature of aflatoxin contamination in smoke-dried fish sold in Minna. Similar study documented significant AFB1 levels in Kenya dried fish products, attributing contamination to poor post-harvest infrastructure and high ambient humidity [9]. Excessive aflatoxin concentrations in smoked fish from Ilorin, Nigeria was also reported [2], with report of AFB1 co-contamination with ochratoxin A in Egyptian fish markets [13]. The present findings align with this regional pattern and extend it to a previously understudied geographical area.

The species-specific variation in AFB1 levels is a notable observation. *Clarias gariepinus* exhibited the highest means AFB1 concentration (5.42 ± 0.47 µg/kg), which may be explained by its relatively high lipid content, a physicochemical property that enhances the bioavailability and tissue retention of lipophilic compounds such as aflatoxins. As an omnivorous, bottom-feeding species, *C. gariepinus* is also more likely to ingest contaminated organic material and feed residues, increasing its toxin burden [14, 15]. Similar study reported significantly higher aflatoxin accumulation in catfish compared to other species in aquaculture systems [16]. The markedly lower AFB1 levels in *Synodontis membranaceus* may reflect differences in feeding ecology, metabolic detoxification capacity, or lower lipid affinity.

The mean AFB1 concentration of approximately 2.95 µg/kg falls within the upper range reported in recent comparative literature. Adetunjii *et al.* [17], reported AFB1 levels of 0.3-6.7 µg/kg in fish and meat products, with a mean of approximately 2.6 µg/kg, closely paralleling the present findings. Ezekiel *et al.* [18], observed AFB1 levels of 0.5–5.0 µg/kg in animal-derived foods including fish, with *Aspergillus flavus* identified as the dominant contaminating species. While the overall mean concentration did not exceed the NAFDAC regulatory limit of 10 µg/kg for total aflatoxins, the mean AFB1 level in *Clarias gariepinus* (5.42 µg/kg) exceeded the European Union limit of 2 µg/kg for AFB1 alone in ready-to-eat foods, indicating a significant regulatory concern for that species.

The absence of statistically significant market-level differences in AFB1 concentration ($p > 0.05$) suggests that contamination is driven primarily by species-level biological factors and post-harvest handling conditions common to all three markets, rather than location-specific factors. This implies that interventions must target fish processing and storage practices system-wide, rather than focusing on individual market sites.

The deterministic MOE of 27.78 and the Monte Carlo-simulated median MOE of 35.47 are both in orders of magnitude below the regulatory concern threshold of 10,000 for genotoxic carcinogens, probably indicating that current levels of dietary AFB1 exposure from smoke-dried fish consumption in Minna does not represent a substantial carcinogenic risk. These findings are consistent with those of Deng *et al*. [19], who reported population-level MOE values well below 100 for dietary AFB1 exposure in high-risk regions, and with European Food Safety Authority benchmarks indicating elevated risk at mean AFB1 exposures generating MOE values below 1,000 [20].. The Monte Carlo simulation further revealed that 88.3% of the population-level MOE distribution fell below 100, a threshold often used as an indicative lower bound for substantial concern and that the probability of the MOE remaining below 1,000 was 100%, placing the entire consumer population at appreciable carcinogenic risk.

The elevated risk in this population is compounded by the high frequency of fish consumption (predominantly 2–3 times per week, with some daily consumers), the predominance of *C. gariepinus*, the most heavily contaminated species in local diets (36.8% of respondents), and the lack of access to cold storage reported by over a third of survey participants. These factors collectively maintain conditions conducive to ongoing fungal growth and toxin accumulation throughout the supply chain. The findings support the need for immediate, multi-level intervention encompassing improved drying technologies, standardized hygiene protocols, routine market surveillance, and consumer education.

CONCLUSION

This study provides evidence of widespread AFB1 contamination in smoke-dried fish sold in Minna, Niger State. The 100% prevalence of AFB1 across all four fish species and three major markets, combined with species-specific contamination levels and a dietary risk assessment demonstrating MOE values far below regulatory safety thresholds, collectively indicate that regular consumption of smoke-dried fish in the study area poses an appreciable and clinically relevant carcinogenic risk to consumers. *Clarias gariepinus*, the most widely consumed species, carries the highest contamination burden and thus constitutes the greatest risk. These findings establish important baseline data for mycotoxin surveillance in the region and have direct implications for food safety policy.

DECLARATION

This research was originally carried out by the authors and was not copied from anywhere.

AUTHORS' CONTRIBUTIONS

RED conceptualized and designed the study, ASB and SF participated in sample collection and data analysis which was reviewed and interpreted by SPO. RED prepared the first draft of the manuscript, reviewed by SPO and ASB. All authors contributed to the development of the final manuscript and approved its submission.

CONFLICT OF INTEREST

The author declares no conflict of interest in relation to this work.

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