AMERICAN UNIVERSITY OF BEIRUT

EXPOSURE TO MYCOTOXINS FROM THE CONSUMPTION OF CORN-BASED BREAKFAST CEREALS IN THE UAE

by
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A thesis
submitted in partial fulfillment of the requirements
for the degree of Master of Science
to the Department of Nutrition and Food Sciences
of the Faculty of Agricultural and Food Sciences
at the American University of Beirut

Beirut, Lebanon
April 2024

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Date of thesis defense: April 25, 2024

ACKNOWLEDGEMENTS

I express my deepest gratitude to my professor and advisor, Dr. Mohamad Abiad, Dr. Christelle Iskandar, and Dr. Ali Chalak, for their guidance and cooperation throughout the project.

My gratitude is also extended to Dr. Hussein Hassan, Associate Professor of Food Science and Technology at the Lebanese American University, for his unwavering support as a committee member. Your encouragement and guidance were instrumental throughout the entire journey. Thank you for your continuous support and motivation, which was crucial in successfully completing this thesis.

I also thank the Lebanese American University (LAU) for providing the necessary resources and facilities to conduct this research. Additionally, I would like to thank Dr. Nisreen Alwan for her contribution to this project. Her insights and assistance were valuable and greatly appreciated. Thank you for your support.

Lastly, I am profoundly grateful to my beloved family – my parents, brother, and fiancé – as well as my friends for their unwavering love and encouragement. Thank you for believing in me and supporting me through everything.

ABSTRACT
OF THE THESIS OF

Karen Bassam Zgheib for Master of Science
Major: Food Technology

Title: Exposure to Mycotoxins from the Consumption of Corn-Based Breakfast Cereals in the UAE

Corn-based breakfast cereals, known as cornflakes, have become a common breakfast choice worldwide, recognized for their convenience and versatility. However, mycotoxins can contaminate these products, potentially adversely affecting human health. This study assessed the occurrence of five mycotoxins (AFB1, OTA, DON, ZEA, and FUM) in all cornflake Stock Keeping Units (SKUs) marketed in the UAE. It also evaluated the effect of different independent variables, including country of origin, temperature on production day, storage time, and presence of chocolate, bran, and nut ingredients. It then estimated the exposure levels of the UAE population to these mycotoxins, along with associated risk factors such as HQ, MOE, liver cancer cases, and kidney disease risk. A total of 76 distinct cornflake SKUs were identified for testing using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. The mean concentrations of AFB1, OTA, ZEA, FUM, and DON among positive samples were 2.0, 1.0, 10.14, 584.9, and 90.6 μg/kg, respectively. With the exception of AFB1, the average levels of all mycotoxins in the cornflake samples remained below the established EU limits. Among positive samples, 4 (5.3%), 1 (1.3%), 1 (1.3%), and 1 (1.3%) SKUs exceeded EU limits for AFB1, OTA, FUM, and ZEA, respectively. The country of origin (developing vs. developed countries) exhibited a significant effect on AFB1 presence in cornflakes ($p < 0.0001$), with higher proportions of positive SKUs in developing countries. Furthermore, higher temperature on the production day was associated with significantly higher AFB1 occurrence ($p=0.009$). Moreover, the presence of chocolate ingredients demonstrated a slight effect on AFB1 ($p=0.05$) and a more pronounced effect on OTA ($p=0.002$), with higher percentages observed in SKUs containing chocolate. Overall, the risk of mycotoxin exposure was low, with HQ values below 1 for all toxins. While daily consumers faced higher risks for some mycotoxins, regular consumers had minimal risk. Liver cancer risk was minimal for both groups (1 liver cancer case and less than 1 case per 1,000,000 people per year, respectively). Similarly, OTA exposures were below the threshold. Besides, the necessary daily intake of cornflakes to reach the TDI (Tolerable Daily Intake) for each mycotoxin is very high across all age groups. However, the observed consumption among children, adolescents and adults remains notably below these thresholds for all mycotoxins, suggesting a safe level of cornflake consumption. Future research should investigate the co-occurrence of mycotoxins and the effect of combined mycotoxin exposures. Routine monitoring is a must to track emerging brands entering the market and to uphold food safety standards, thereby safeguarding public health.

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ABBREVIATIONS

AFB1: Aflatoxin B1
AFM1: Aflatoxin M1
FUM: Fumonisns
ZEA / ZEN: Zearalenone
ARfD: Acute Reference Dose
A_W: Water activity
BMDL10: Benchmark Dose Lower Confidence Limit
BW: Body Weight
DI: Daily Intake
DON: Deoxynivalenol
EDI: Estimated Daily Intake
EFSA: European Food Safety Authority
ELISA: Enzyme-Linked Immunosorbent Assay
EU: European Union
FAO: Food and Agriculture Organization
FFQ: Food Frequency Questionnaire
FSMS: Food Safety Management System
GCC: Gulf Cooperation Council
HACCP: Hazard Analysis and Critical Control Point
HCC: Hepatocellular Carcinoma.
HPLC: High-Performance Liquid Chromatography
HPLC-FD: High-Performance Liquid Chromatography with Fluorescence Detection
HPLC-UV: High-Performance Liquid Chromatography with Ultra-Violet spectroscopy
HQ: Hazard Quotient
IAC: Immunoaffinity Chromatography
IARC: International Agency for Research on Cancer
JECFA: Joint FAO/WHO Expert Committee on Food Additives
LC-FD: Liquid Chromatography with Fluorescence Detection
LC-MS/MS: Liquid Chromatography-Tandem Mass Spectrometry
MC: Mean Concentration
MOCCAE: Ministry of Climate Change and Environment
MoE: Margin of Exposure
MoPH: Ministry of Public Health.
ng: nanogram
OTA: Ochratoxin A
OTB: Ochratoxin B
PCR: Polymerase Chain Reaction
PMTDI: Provisional Maximum Tolerable Daily Intake
PTWI: Provisional Tolerable Weekly Intake
RASFF: Rapid Alert System for Food and Feed
SKUs: Stock Keeping Units
SPE: Solid-Phase Extraction
TDI: Tolerable Daily Intake
UAE: United Arab Emirates
WHO: World Health Organization
µl: microliter

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

The United Arab Emirates (UAE) is committed to upholding its status as a global leader across various sectors, including food safety and quality assurance, as mandated by Food Law No. 2 (2008). With substantial imports of various food items, including corn-based breakfast cereals, which often undergo extended storage periods before consumption, ensuring rigorous food control standards is crucial to safeguarding the integrity and safety of the food supply chain. Moreover, the UAE's predominantly hot and humid desert climate, prevalent for about eight months each year, creates favorable conditions for microbial contamination of stored food items, as highlighted by Regulation No. 6 (2010) on Food Hygiene throughout the Food Chain and UAE climate records. Hence, microbial contamination, particularly due to mycotoxins, emerges as a primary concern in food safety, given its potential to compromise national food reserves and public health. Consequently, legislative measures and regulatory guidelines have become essential in enhancing food storage and consumption practices, promoting consumer confidence, and upholding safety standards (Food Law No. 2, 2008; Regulation No. 6 of 2010 on Food Hygiene throughout the Food Chain).

Grains, including rice, wheat, and corn, are the primary sources of energy and carbohydrates and inherently harbor contaminants (Heshmati et al., 2017). These contaminants are predominantly comprised of microbiological agents, heavy metals, and process-related pollutants that may also be present. Mycotoxins pose significant toxicity risks among these contaminants and are commonly found in various grain products (Thielecke & Nugent, 2018; Eskola et al., 2019). Cornflakes, in particular, have become a popular breakfast choice among individuals worldwide, including residents of the UAE. Although traditional breakfast choices such as bread and traditional Arabic dishes remain important, there has been a gradual transition towards ready-to-eat breakfast cereals, particularly among the younger population. In the UAE, the consumption of cornflakes is influenced by several factors, including urbanization, convenience, versatility, and evolving dietary patterns driven by busy lifestyles. In such a fast-paced society, where time is often limited, ready-to-eat breakfast options like cornflakes provide a quick and convenient solution for individuals and families (*UAE Breakfast Cereals Market 2024-2032: Size, share, growth* 2024). Furthermore, the globalization of food culture has facilitated the adoption of Western breakfast staples, such as cornflakes, among UAE residents (Al Sabbah et al., 2023; Ali et al., 2022; Ng et al., 2011). Additionally, effective marketing strategies employed by cereal manufacturers, along with the perceived health benefits of fortified cereals, contribute to the widespread popularity of cornflakes in the UAE population (Benvenuto, 2018; Parra-Murillo et al., 2021; *UAE Breakfast Cereals Market 2024-2032: Size, share, growth* 2024).

In light of these considerations, examining the presence and exposure to mycotoxins in corn-based breakfast cereals in the UAE's affluent society is imperative. This investigation aims to elucidate potential health hazards and guide policy interventions to improve food safety standards.

1.1. Mycotoxins in Cereals

Cereals and cereal-based products, such as maize, wheat, and barley, are essential sources of energy and nutrients. However, despite their nutritional value, these grains are highly susceptible to fungal contamination (Lee & Ryu, 2017; Nematollahi et al., 2019; Amirahmadi et al., 2018; Heshmati et al., 2017; Kyei, Boakye, & Gabrysch, 2020). Favorable environmental conditions can foster fungal growth in various agricultural commodities, including cereals, nuts, spices, soybeans, and coffee beans, producing mycotoxins. These toxic secondary metabolites are produced by various fungi species, belonging essentially to *Aspergillus*, *Penicillium*, and *Fusarium* genera. (Khaneghah et al., 2018b; Majeed et al., 2018; Yu, 2023). In particular, Aflatoxins (AFB1, B2, G1, and G2), Ochratoxin A (OTA), trichothecenes (including Deoxynivalenol (DON)), Fumonisins (FB1, FB2, and FB3), and Zearalenone (ZEA) are among the most prevalent mycotoxins in cereals (Khaneghah et al., 2020; Malachová et al., 2018; Yu & Pedroso 2023). Their presence can occur at various stages: while in the field, during harvesting, post-harvest handling, processing of agricultural commodities, and in storage (Amirahmadi et al., 2018; Campagnolo et al., 2016; El-Sayed et al., 2022). Given their inherent toxicity and persistence, mycotoxins present some serious health threats and economic implications, necessitating thorough understanding and management to ensure food safety (Khaneghah et al., 2018a; Khaneghah et al., 2018c; Sikandar et al., 2022).

Table 1 presents an overview of the major mycotoxins with their corresponding IARC classification, toxigenic producing-fungi, some commonly contaminated food commodities, main toxic effects, and diseases, along with the US FDA and EU regulatory limits for mycotoxin levels in food and animal feed (Alshannaq & Yu, 2017; Amirahmadi et al., 2018; Duarte et al., 2019; EFSA, 2020; EFSA 2017; EFSA, 2006; El-Sayed et al., 2022; Ji et al., 2022; Kabak, 2021; Martinez-Miranda, 2019; Osaili et al., 2022; Saha Turna & Wu, 2021; Schrenk et al., 2020; Sowley, 2016; Zapaśnik et al., 2021).

Table 1 Major mycotoxins, their characteristics, and limits

Mycotoxin	**IARC classification**	**Fungal Species**	**Food commodity**	**Toxic effects/ diseases**	**US FDA (μg/kg)**	**EU (μg/kg)**	**CODEX (μg/kg)**
AFB1, AFB2, AFG1, AFG2	Group 1: Carcinogenic to humans with sufficient evidence	*Aspergillus flavus, Aspergillus parasiticus*	Maize, wheat, rice, nuts	Carcinogenic, hepatotoxic, teratogenic, mutagenic immune suppressive effects, hepatitis, hepatocellular carcinoma	20 for total	2 for AFB1 20 for total Aflatoxins	10
OTA	Group 2B: possibly carcinogenic	*Aspergillus ochraceus, Penicillium verrucosum, Aspergillus carbonarius*	Cereals, coffee, beans, peas, wine, grape juice, dried vine fruit, cocoa	Carcinogenic, nephrotoxic, hepatotoxic, immunotoxicity, genotoxic, teratogenic, embryotoxic, Balkan Endemic Nephropathy	Not set	3	5
FB1, FB2, FB3	Group 2B: possibly carcinogenic	*Fusarium verticillioides, Fusarium proliferatum*	Maize, wheat, sorghum, asparagus	Encephalomalacia, pulmonary edema, carcinogenic, neurotoxic, liver /kidney damage, heart failure,	2000–4000	200–1000	2000

				esophageal cancer			
DON	Group 3: not classifiable; not enough evidence to classify as a carcinogen	*Fusarium graminearum*, *Fusarium culmorum*	Maize, wheat, oats, legumes, fruits, vegetables	Nausea, vomiting, diarrhea, abdominal pain, headaches, dizziness, fever, immune suppression	1000	750	1000
ZEN	Group 3: not classifiable; not enough evidence to classify as a carcinogen	*Fusarium graminearum*, *Fusarium culmorum*	Maize, wheat, barley, oats, sorghum, rye	Carcinogenic, teratogenic, hormonal imbalance, estrogenic effect, reproductive disorders, infertility	Not set	50	-

1.1.1. Economic Impact of Mycotoxins

Cereal grains serve as fundamental staple crops essential for human and livestock diets, with their role in food and feed production expected to increase substantially in the upcoming years (Eskola et al., 2019). Over recent decades, the global food and feed trade has expanded significantly, with rice, wheat, and corn collectively providing approximately 50% of the world's caloric intake (Oliveira et al., 2017). However, mycotoxins, globally prevalent in cereals and cereal-based products, pose significant risks to human health, animal welfare, productivity, and international trade (Eskola et al., 2019; Pitt & Miller, 2017; Sarmast et al., 2021; Wu & Mitchell, 2016). According to the Food and Agricultural Organization (FAO), approximately 25% of global food crops are contaminated by mycotoxins annually, resulting in significant agricultural and economic losses amounting to billions of dollars (Alshannaq & Yu, 2017; FAO, 2013; Neme & Mohammed, 2017; Thielecke and Nugent, 2018). This underscores the urgent need for research and resource allocation to control and mitigate the presence of mycotoxins in food systems. Additionally, according to the Rapid Alert System for Food and Feed (RASFF) of the European Union (EU), mycotoxins are ranked second in terms of the total number of hazard notifications (RASFF, 2018).

1.1.2. Factors Leading to Mycotoxin Production

Mycotoxigenic fungi are prevalent pathogens in agricultural regions worldwide, capable of invading a wide range of crops and exhibiting considerable diversity, allowing for mycotoxin production under various environmental conditions. Numerous factors influence fungal growth and mycotoxin production, and contamination with these toxins can occur at different stages of the food chain, starting in the field and potentially increasing during subsequent processes such as harvesting, drying, and storage. However, the presence of fungi does not always lead to subsequent mycotoxin contamination, as the conditions necessary for mycotoxin production are distinct and may differ from those conducive to fungal growth. Similarly, eliminating fungi from food does not guarantee the absence of mycotoxins due to their resilient chemical properties and heat tolerance (Godswill Awuchi et al., 2022; Kochiieru et al., 2020). The conditions that lead to mycotoxin production are influenced by various factors such as temperature, water activity (a_w), humidity, pH levels, type of fungi (fungal strain), and the substrate they grow on (Daou et al., 2021; Sarmast et al. 2021). Therefore, to prevent the release of mycotoxins, it is essential to adhere to stringent food safety and quality control measures, encompassing good agricultural and manufacturing practices (Ünüsan, 2019).

1.1.3. Detection Methods

Various methods have emerged for mycotoxin detection and quantification, addressing the need for accurate and efficient screening in diverse matrices. Immunoassay-based techniques, such as the Enzyme-linked Immunosorbent Assay (ELISA), utilize antibodies' specificity and offer rapid and cost-effective detection, making them suitable for high-throughput screening (Liew & Sabran, 2022). Besides, chromatographic techniques, such as High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), stand out for their high sensitivity and capability for simultaneous detection of multiple mycotoxins, essential for comprehensive analysis (Smaoui et al., 2020). Furthermore, molecular methods, particularly Polymerase Chain Reaction (PCR) assays, enable specific detection of mycotoxin-producing fungal species, enhancing targeted surveillance efforts (Rahman et al., 2020). Emerging technologies, including biosensors and nanotechnology-based approaches, hold promise for real-time monitoring and portability, addressing the need for on-site detection in various settings (Gong et al., 2023; Zhang et al., 2023).

On the other hand, the analytical methods for modified mycotoxins face several challenges. Direct strategies, such as Immunoaffinity Chromatography (IAC) and Solid-Phase Extraction (SPE) columns, are deficient in standards and purification products tailored for modified mycotoxins. Similarly, indirect strategies lack chemical reagents or enzymes capable of efficiently converting modified mycotoxins into free mycotoxins. Thus, developing sufficiently sensitive analytical methods for modified mycotoxins remains a future endeavor, crucial for enhancing early detection, consumer warning systems, and safety evaluations concerning modified mycotoxins (Tan et al., 2023).

1.1.4. Occurrence and Dietary Exposure to Mycotoxins from Cereals

Given the substantial public health risks linked to mycotoxins, extensive research has been conducted globally, examining the levels of these toxins in cereals and cereal products using various techniques. **Table 2** provides a comprehensive overview of the findings from numerous studies on mycotoxin occurrence and exposure.

Table 2 Comparative analysis of mycotoxin occurrence and exposure levels in cereal products from various countries

Country	**Sample**	**Mycotoxins**	**Analytical method**	**Total samples (N) Positive samples**	**Levels range of positive samples (mean) (μg/kg)**	**N (%) of samples exceeding EU limit**	**Exposure level (ng /kg body weight/ da y) (mean)**	**Reference**
Lebanon	Cornflakes	AFB1	ELISA	N=35 3	1.1 - 2.9	3 (9)	-	(Hassan et al., 2023)
		OTA		6	3.3 – 8.6	6 (17)		
		DON		21	100.9 – 3750	21 (60)		
		ZEA		2	10.4 - 326.3	2 (6)		
		FUM		5	30 - 6285	5 (14)		
UAE	Rice	AFB1	ELISA	N=128				
				48	1 – 4.69	10 (20.8)	0.52 - 13.6	Alwan et al. 2022)
Lebanon	Rice	AFB1	ELISA	N= 105 105	0.5	1 (1)	0.16	Hassan et al. (2022b)
Lebanon				105	1.29	1 (1)	1.27	

UAE	Rice	OTA	ELISA	127	1.4	2 (1.6)	1.42	Hassan et.al (2022a)
Serbia (In 2015)	corn		LC-MS/MS	N=51			-	Kos et al. (2020)
		AFB1		46	0.4–41 (8)	21 (41)		
		OTA		9	0.5–27 (6)	8 (16)		
		FB1		51	192–4253 (1905)	6 (12)		
Serbia (in 2012)	Breakfast cereals		HPLC-FLD	N=82	0.07–11.81		0.18	Torović et al. (2017)
		OTA		17	(1.76)	(3.6)		
Serbia (in 2015)				N=54				
		OTA		7	0.09–2.33 (0.48)	(0)	0.03	
		AFB1		6	0.06–0.15 (0.10)	(0)	0.006	
Pakistan	Breakfast cereals		HPLC-FLD	N= 237			-	Iqbal et al. (2014)
		OTA		113	2.22	70 (30)		
		AFB1		98	1.32	38 (16)		
		ZEN		125	9.91	19 (8)		
Pakistan	Cornflakes	AFB1	HPLC-UV	10	9.8–29.7	(60)	-	Firdous et al. (2014)
Kenya	Corn	AFB1	HPLC-FLD	N=165		(95.3% > FDA limit)	4.3–554	Nabwire et al. (2019)
				165	1.69-403 (76.2)			
Qatar	Corn		ELISA	N=10			-	Hassan et al. (2018)
		AFB1		7	ND-120 (33)	(25)		
		OTA		4	ND-350 (181)	(50)		
Iran	Corn		HPLC	N=188	79-2232	-	-	Fallahi et al. (2019)
		FB1		182				
Egypt	Corn	AFB1	HPLC–FLD	N=61 15	0.2-44.9	1 (1.6%)	-	Abdallah et.al (2019)
Turkey	Corn		HPLC-PDA HPLC-FLD	N=15				Golge and Kabak (2020)
		DON		2	313-331 (49)	0	-	
		ZEN		3	18-337 (28)	0		
Greece	Corn	AFB1 DON OTA ZEN	HPLC ELISA	N=6			-	Skendi et al. (2019)
				6	0.5-8.5 (1.02)	1		
				4	ND-493 (123)	0		
				2	ND-0.7 (ND)	0		

				6	0.8-6.4 (4.05)	0		
Iran	Rice	AFB1	IAC, HPLC-FD	N= 18	1.17 - 30.63	3 (13.6)	2.29	Yazdanpanah et al., (2013)
Portugal	Breakfast cereals		HPLC-FD	N=26				Assunção et.al (2015)
		AFB1		-	0.028	0	0.012	
		OTA			0.026	0	0.011	
		FB1			13	0	5.461	
		DON			59	0	24.827	

1.1.5. Policies and Regulations

Policies and regulations in the United Arab Emirates (UAE) regarding mycotoxins in food, particularly cereals, are essential for ensuring food safety and protecting public health. The UAE follows international standards and guidelines established by organizations such as the Codex Alimentarius Commission, the European Union (EU), and the Gulf Cooperation Council (GCC) Standardization Organization. These regulations cover not only mycotoxins but also additives, chemicals, and other contaminants present in food products, ensuring comprehensive safety measures across the entire food supply chain (Al-Ghamdi et al., 2020; Codex Alimentarius Commission, Gulf Cooperation Council Standardization Organization). In particular, these regulations often include maximum permitted levels (MPLs) for mycotoxins in various food items, including cereals, to safeguard consumer health. Additionally, the UAE's Ministry of Climate Change and Environment (MOCCAE) plays a crucial role in monitoring and enforcing these regulations to prevent the presence of harmful mycotoxins and other food-related hazards. Regular surveillance, testing, and enforcement measures are implemented to ensure compliance with established standards and to mitigate the risks associated with mycotoxin contamination in cereals and other food commodities (*Food safety: The official portal of the UAE government* 2024).

1.2. Consumer Behaviors and Demographic Diversity in the UAE

The UAE's population has experienced remarkable growth, primarily in cities like Abu Dhabi and Dubai. As of January 2023, the population was estimated to be 9.48 million, with a median age of 33.5 years (Data Reports, 2023). The largest age group falls between 25 – 44 years, reflecting the dynamic demographics of the country. Notably, Emiratis make up only 12% of the total population, with the majority of immigrants from different parts of the world. This diversity brings varied income levels and cultural backgrounds to the consumer population in the UAE. Thus, understanding consumer behaviors and market dynamics in the UAE is crucial due to the diverse population representing various cultures and backgrounds. Cultural heritage, socioeconomic status, and lifestyle preferences significantly influence food choices among UAE residents (Al-Khudairy et al., 2019; Al-Dabbagh et al., 2020). Similarly, Khan et al. (2018) underscored the impact of socioeconomic status on dietary habits and emphasized the need for tailored interventions to address disparities in food access and consumption. In addition, food preferences in the UAE vary, with Emiratis favoring traditional Gulf dishes, while the diverse population drives demand for international foods.

1.3. Objective and Significance

1.3.1. Gaps in the Literature

Until now, no study has been performed in the United Arab Emirates (UAE) to assess the safety of cornflakes marketed in the country, particularly concerning their mycotoxin content and the associated exposure risks.

1.3.2. Objective

This study aims to assess the mycotoxin content of different cornflake SKUs available in the UAE market and to evaluate the UAE population's exposure to these mycotoxins through cornflake consumption. A risk assessment will be conducted using Hazard Quotient (HQ) and Margin of Exposure (MOE) methodologies, focusing on assessing the liver cancer risk associated with AFB1 exposure and kidney disease risk linked with OTA intakes. Additionally, this study will investigate the impact of various factors on the presence of mycotoxins in cornflakes. These variables include country of origin (developing vs. developed), the temperature on the production day, storage time, and the presence of bran, chocolate, and nut ingredients. Understanding how these factors influence mycotoxin contamination levels is essential for developing targeted interventions and strategies to mitigate risks associated with cornflake consumption in the UAE.

By providing insights into the levels of mycotoxin contamination, assessing associated health risks, and the factors that can increase mycotoxin presence, this study seeks to ensure safer, healthier, and more informed food consumption practices in the UAE. Furthermore, the findings will serve as a basis for informing future research, public health recommendations, and potential regulatory actions to enhance food safety and protect public health in the UAE.

CHAPTER 2

MATERIALS AND METHODOLOGY

2.1. Sample Collection

The biggest retail markets in the UAE were screened, namely Lulu Supermarket and Carrefour. 76 cornflake Stock Keeping Units (SKUs) were identified and collected for analysis in December 2023. This diverse set of samples encompassed various brands, flavors, and countries of origin, ensuring a representative and comprehensive assessment of cornflake products available in the UAE market. The samples, imported from the UAE to Lebanon, were subsequently transported to the Lebanese American University of Beirut (LAU Beirut) and stored in a refrigerator within the Nutrition Laboratory until the assessment day.

2.2. Sample Preparation and Analysis

The sample preparation and analysis took place during February and March 2024. The cornflake samples were ground using a blender and thoroughly mixed before proceeding with the extraction process. Mycotoxin determination was performed in duplicates for all samples and in triplicate for positive samples using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. Specifically, the RIDASCREEN AFB1 30/15 (R1211), OTA 30/15 (R1312), ZEA (R1401), FUM (R3401), and DON (R5906) test kits from R-biopharm, Germany, were utilized. Each test kit contains 96 wells, and the analysis was conducted following the step-by-step instructions outlined in the kit manuals.

After grinding, 5 grams were weighed from each sample, followed by the addition of 25 ml of 70% methanol for AFB1, ZEA, and FUM; 25 ml of diluted ECO extractor for OTA; and 25 ml of distilled water for DON during the extraction process. The containers were vigorously shaken using a vortex for 5 minutes and then centrifuged for 10 minutes at 3500 g at room temperature. Subsequently, 1 ml of the separated solution was diluted with 1 ml of distilled or deionized water for AFB1 and FUM, while OTA and ZEA were diluted with a sample dilution buffer. No dilution was necessary for DON. During testing, 50 µl of the diluted solution was used per well.

As per the kit manual, a sufficient number of wells were placed into the microwell holder to accommodate all standards and samples, each to be run in duplicate. Standard and sample positions were carefully recorded. Subsequently, 50 µl of either the standard or prepared sample was pipetted into separate wells, with a new pipette tip used for each standard or sample. Following this, 50 µl of enzyme conjugate was added to the bottom of each well, along with 50 µl of antibody solution for AFB1, DON, and FUM, or the diluted enzyme conjugate for OTA and ZEA. The plate was manually shaken to ensure thorough mixing of all added reagents before being incubated for 30 minutes (except for ZEA, which was incubated for 2 hours) at room temperature (20 - 25 °C) and in the dark. After incubation, the liquid from the wells was discarded, and the microwell holder was tapped upside down on a clean paper towel to remove any residual liquid. The wells were washed thrice with 250 µl of washing buffer each time. Following the washing steps, 100 µl of substrate/chromogen was added to each well, and the plate was manually shaken and incubated for 15 minutes (or 30 minutes for ZEA) at room temperature in the dark. Finally, 100 µl of stop solution was added to each well, and the plate was shaken manually again.

The absorbance of the samples was measured at 450 nm within 10 minutes of adding the stop solution using a microtiter plate spectrophotometer. Mycotoxin concentration estimation relied on constructing a standard curve based on the absorbance of known concentrations of mycotoxin standards (for example, 0, 1, 5, 10, 20, and 50 µg/kg in the case of AFB1). The concentration of mycotoxin in each sample was then determined by reading the corresponding absorbance from the calibration curve. The results were analyzed using RIDA®SOFT Win (Art. No. Z9999) software, which evaluates the RIDASCREEN® enzyme immunoassays. Detection limits provided by the kit manufacturer for AFB1, OTA, ZEA, FUM, and DON were 1 µg/kg, 0.5 µg/kg, 1750 ng/kg, 25.5 µg/kg, and 18.5 µg/kg, respectively.

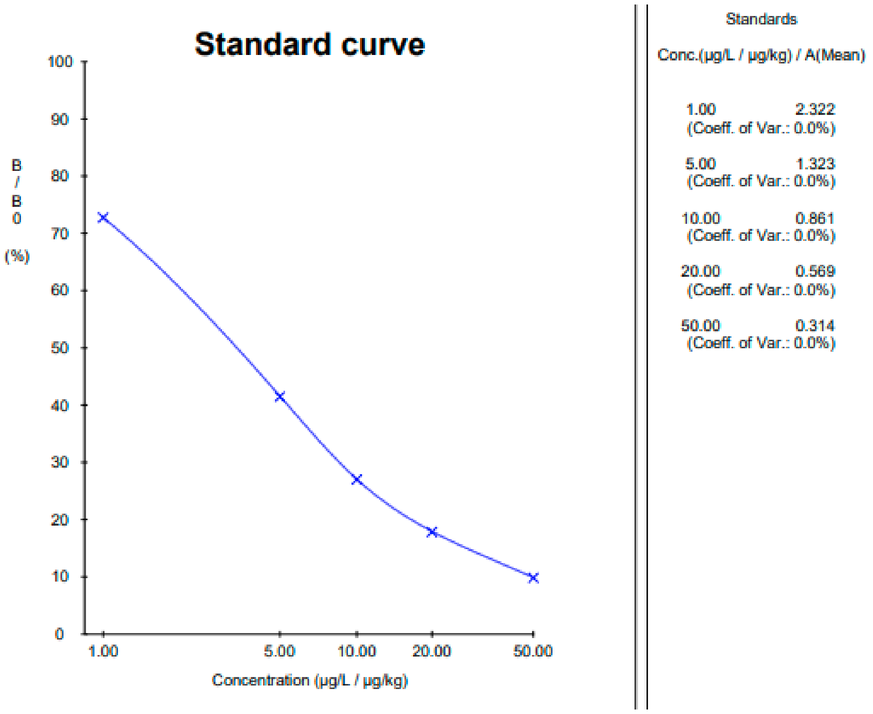

Figure 1 AFB1 standard curve (Kit1)

2.4. Exposure Determination from the Consumption of Cornflakes

To estimate the exposure of UAE residents to mycotoxins from cornflake consumption, food frequency questionnaires (FFQs) were administered to 400 adults, selected through convenience sampling. Among the participants, 82 were daily consumers (consume cornflakes daily), 175 were regular consumers (consume occasionally), and 143 reported rarely or never consuming cornflakes. The average daily consumption of cornflakes in the UAE was obtained from an ongoing total diet study conducted by Abu Dhabi University using FFQs. According to this study, the average daily consumption of cornflakes in the UAE was found to be 70 g/day for individuals who consumed cornflakes daily, 1.5 g/day among those who consumed cornflakes regularly, and 0.25 g/day for individuals who rarely or never consumed cornflakes. The average body weight was 77 kg among participants, obtained from the FFQs.

The dietary exposure / Estimated Daily Intake (EDI) was determined by multiplying cornflake's mean mycotoxin contamination level by the average dietary intake of that food item obtained in the current study and then dividing by the average participants' body weight. This process was outlined by Hoteit et al. (2024) and is represented by this equation:

$$Estimated\ Daily\ Intake\ (EDI)(ng/kg\ bw/day) = \frac{Mean\ Concentration\ (MC)\ (ng/kg) * \ Daily\ Intake\ (DI)\ (kg/day)}{Average\ Body\ weight\ (kg)}$$

2.5. Risk Assessment

The risk assessment was conducted for daily and regular consumers using the Hazard Quotient (HQ) and Margin of Exposure (MoE). Liver cancer and kidney disease risks were also calculated.

2.5.1. Hazard Quotient (HQ) Calculation

The hazard quotient (HQ) represents the ratio of potential exposure to a substance (EDI) to the level at which no adverse effect is expected, such as the tolerable daily intake (TDI) or acute reference dose (ARfD) (EFSA, 2013). The TDI for the five mycotoxins are listed in **Table 3**. This HQ calculation is expressed by the equation:

$$HQ = \frac{EDI(ng/kgBW/day)}{TDI\ (ng/kgBW/day)}$$

Table 3 Tolerable daily intake of mycotoxins

Mycotoxins	Tolerable daily intake (TDI) (µg/ kg bw/day)	Reference
AFB1	0.017–0.082	Turna & Wu, 2022
OTA	0.018	EFSA, 2006
DON	1.0	EFSA, 2017
FUM	1.0	EFSA, 2018
ZEA	0.25	EFSA, 2014

A Hazard Quotient (HQ) below 1, indicates acceptable or tolerable exposure, implying little to no risk. Conversely, a value exceeding 1 suggests an unacceptable level of exposure, posing a potential health risk (EFSA, 2013; Hoteit et.al 2024).

2.5.2. Margin of Exposure (MoE) Calculation

Considering the carcinogenic potential of various mycotoxins, the Margin of Exposure (MoE) was calculated for each mycotoxin as a ratio of the Benchmark Dose Lower Confidence Limit (BMDL10) to the level of exposure (EDI). The BMDL10 for the different mycotoxins is presented in **Table 4**. The MoE serves as an indicator of risk level, where a MoE value greater than or equal to 10,000 suggests low public health concern, while a MoE value below 10,000 indicates high public health concern (EFSA, 2013; Franco et al., 2019; Hobé et al., 2023; Hoteit et al., 2024; Zhang et al., 2023).

$$MoE = \frac{BMDL10\ (ng/kgBW/day)}{EDI\ (ng/kgBW/day)}$$

Besides, as ZEA is categorized as a non-genotoxic carcinogen, a threshold dose exists below which no adverse effects are observed, according to JECFA (2007). Consequently, comparing estimated ZEA intakes with the Provisional Maximum Tolerable Daily Intake (PMTDI) was utilized to assess potential health risks. Exceeding the tolerable intakes established by JECFA may indicate a health risk. The Joint Committee FAO/WHO has established a PMTDI for ZEA at 0.25 µg/kg of body weight (JECFA, 2014).

Table 4 BMDL10 of mycotoxins

Mycotoxins	**BMDL10 (µg/ kg bw/day)**	**Reference**
AFB1	0.4	(EFSA, 2020)
OTA	Critical neoplastic[1] effects: 14.5 Chronic non-neoplastic[2] effects: 4.73	(EFSA, 2020)
DON	210	(EFSA, 2017)
FUM	100	EFSA (2018); Zentai et.al (2019)

[1] MOE below 200 indicates a public health concern for non-neoplastic effects.
[2] MOE below 10,000 indicates a public health concern for neoplastic effects.

2.5.3 Liver Cancer Risk from AFB1

The JECFA has indicated that even minimal exposure to AFB1, as low as 1 ng/kg body weight/day, can elevate the risk of liver cancer. Consequently, it is suggested that in non-European countries, the ingestion of 1 ng/kg body weight/day of AFB1 can lead to an incidence of 0.083 cases of liver cancer per year per 100,000 individuals (Franco et al., 2019; Hoteit et al. 2024). To determine the liver cancer risk based on the daily exposure to AFB1 (ng/kg body weight/day) from cornflakes, the following calculation is proposed, as outlined by JECFA:

$$Liver\ cancer\ risk\ from\ AFB1 = \frac{Exposure\ of\ AFB1\ (ng/kg\ bw/day)\ \times\ 0.083\ cancer\ cases/100{,}000\ persons}{1\ (ng/kg\ bw/day)}$$

2.5.3 Kidney Disease Risk from OTA

To assess the risk of kidney disease, the weekly exposure to OTA was computed and compared to the provisional tolerable weekly intake (PTWI) established by JECFA at 100 ng/kg bw, which was considered in our analysis.

2.5.4 Estimated Daily Consumption of Cornflakes by Age Group

The estimated daily consumption of cornflakes by age group was calculated to assess the potential risk of mycotoxin exposure associated with varying consumption levels. This analysis aimed to determine the maximum consumption threshold above which a significant risk of mycotoxin exposure could occur. The average body weight for adults was obtained from the FFQ. As for children, average body weights were determined by averaging respective weights for girls and boys from the following age ranges: 2 to 5 years, 5 to 12 years, and 12 to 18 years. These weight values were obtained from the CDC (Centers for Disease Control and Prevention).

2.6. Statistical Analysis

The mycotoxin concentration was determined as a mean of 2 replicate measures for all samples and triplicates for positive samples. The data was coded and entered into Excel before being transferred to STATA/SE 18.0 for subsequent analysis. Continuous variables are presented as means and standard deviations, whereas categorical variables are expressed as percentages.

Fisher's Exact test was employed to explore the relationships between explanatory (independent) variables (including country of origin, temperature on production day, storage time, presence of bran, chocolate, and nut ingredients) and the presence of mycotoxins, which are treated as dependent variables. Fisher's exact test is well-suited for such scenarios, offering a robust answer for analyzing categorical data and detecting associations between variables, even in cases of a small dataset, particularly when dealing with cells containing low frequencies, which could compromise the reliability of traditional chi-square tests.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Mycotoxin Content in Cornflakes Samples

The incidence of Aflatoxin B1 (AFB1), Ochratoxin A (OTA), Deoxynivalenol (DON), Fumonisin B1 (FB1), and Zearalenone (ZEA) in 76 samples of cornflakes are shown in **Table 5**. In this study, AFB1 was detected in 18 out of 76 samples (23.7%) with a mean concentration of 2.0 ± 0.18 μg/kg. OTA was found in 37 samples (48.7%) with an average concentration of 1.0 ± 0.15 μg/kg, while ZEA was detected in 21 samples (27.6%) with an average concentration of 10.14 ± 0.76 μg/kg. FUM exhibited the lowest occurrence, detected in only 7 samples (9.2%), with a mean concentration of 584.9 ± 38.00 μg/kg. Conversely, DON was the most prevalent mycotoxin, present in 67 samples (88.2%) with a mean concentration of 90.6 ± 14.15 μg/kg. A comparison between these concentrations and the regulatory limits revealed that a small percentage of positive samples exceeded EU limits: 5.3% for AFB1, 1.3% for OTA, FUM, and ZEA each. Except for AFB1, the average levels of all mycotoxins in the cornflake samples remained below EU limits. However, the average concentration of AFB1 was 2 μg/kg, at the borderline of the EU limit. This finding could be concerning, given AFB1's classification as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC), unlike the other four mycotoxins. Furthermore, all mycotoxin averages were below the CODEX and FDA limits, with only one sample exceeding the CODEX limit for FUM.

Compared to other studies assessing mycotoxins, the predominance of DON observed in our study aligns with prior research, suggesting its prevalence as a key concern in cereal products. For instance, Kovač et al. (2021) investigated the presence of various mycotoxins in 118 samples of Croatian cereals. They reported DON detection in 73.7% of Croatian cereal samples (vs.88.2% in our study), highlighting its widespread occurrence and potential risk. Additionally, HT-2 was detected in 45.8% of the samples, FB1 in 43.2%, ZEA in 36.4%, FB2 in 33.9%, and T2 in 16.9%. These results highlight the widespread presence of multiple mycotoxins in Croatian cereals. Another study by Hassan et al. (2023) aimed to assess the presence of mycotoxins in 35 different cornflake SKUs marketed in Lebanon. Similar to our findings, the highest prevalence was observed for DON, with 60% of samples surpassing the EU limit. However, the average concentration of DON was 1206.7 μg/kg, much higher than the average concentration of DON found in our study (90.6 μg/kg). Indeed, their study also indicated a higher percentage of samples exceeding the EU limit as compared to ours. Specifically, 17% of samples exceeded the OTA limit with an average concentration of 1.2 µg/kg, 9% exceeded the AFB1 limit with an average of 1.6 µg/kg, 14% exceeded the FUM limit averaging 774.1 µg/kg, and 6% exceeded the ZEA limit with an average of 15.1 µg/kg. Except for AFB1, the average levels of OTA, FUM, and ZEA in the cornflake samples from their results were higher than in our study. Yet, they remained below the maximum permissible values, aligning with our findings. Notably, in their study, the mean AFB1 level was below the EU limit, contrasting with our findings, where it surpassed the established limit. This comparison underscores the variability in mycotoxin contamination levels observed across different regions and highlights the importance of ongoing monitoring and regulatory efforts to ensure food safety standards are met.

Table 5 Mean, range, and limits for different mycotoxins

Mycotoxins	Mean ± SD	Range	Number of positive samples (%)	EU limit	Codex limit	US FDA limits	Number of samples exceeding EU limit (%)	Number of samples exceeding codex limit (%)	Number of samples exceeding FDA limit (%)
AFB1 (µg/kg)	2.0 ± 0.18	1.0 – 9.6	18 (23.7)	2	10	20	4 (5.3%)	-	-
OTA (µg/kg)	1.0 ± 0.15	0.3 – 3.0	37 (48.7)	3	5	-	1 (1.3%)	-	-
DON (µg/kg)	90.6 ± 14.15	21.9 – 441.9	67 (88.2)	750	1000	1000	-	-	-
FB1 (µg/kg)	584.9 ± 38.00	27.0 – 2830.0	7 (9.2)	1000	2000	2000-4000	1 (1.3%)	1 (1.3%)	-
ZEA (µg/kg)	10.14 ± 0.76	1.757 - 68.37	21 (27.6)	50	-	-	1 (1.3%)	-	-

In another investigation by Elaridi et al. (2019), the presence of mycotoxins, including OTA, OTB, T-2, and HT-2 toxins, was examined in wheat grains (n = 50), wheat flour (n = 50), and bread (n = 37) samples, obtained from major mills in Lebanon using LC-MS/MS. The study revealed low mycotoxins, with wheat grains, wheat flour, and bread samples showing negligible levels of T-2 and HT-2 toxins and OTB. However, only a small subset of wheat flour samples (8%) tested positive for OTA (vs 48.7% in our study), with a concentration mean of 1.9 µg/kg, higher than the average in our findings (1 µg/kg). Notably, one sample surpassed the EU limit of 3 µg/kg for wheat-derived products, while none exceeded the OTA contamination threshold of 5 µg/kg set by the Lebanese Ministry of Public Health (MoPH). The contaminated wheat flour samples originated from various mills in the coastal Beirut area, with the presence of OTA attributed to the hot and humid storage and processing conditions within the mills. The combination of high temperatures and moisture levels promotes fungal growth and mycotoxin production, underscoring the importance of proper storage and processing practices to mitigate the risk of contamination in wheat-derived products.

Furthermore, Alwan et al. (2022) evaluated the levels of AFB1 in rice available in the UAE market. Using ELISA, the study detected AFB1 in 48 (38%) out of 128 rice samples. Among the positive samples, the average contamination level of AFB1 was 1.66 µg/kg (vs. 2 µg/kg in our study). Notably, all samples exhibited contamination levels below the limit set by the Gulf Cooperation Council Standardization Organization (≤5 µg/kg), yet 10 (20.8%) out of the positive samples surpassed the EU limit (vs. 5.3% in our study). A comparable investigation involved 105 rice samples available in the Lebanese market (Hassan et al., 2022a). While all samples tested positive for AFB1, the average concentration was 0.5 µg/kg, which was lower than the AFB1 concentration detected in our cornflake samples. Another study by Hassan et al. (2022b) assessed OTA levels in 105 rice brands available in Lebanon and 127 rice brands from the UAE. Using ELISA, they found that 53% and 58% of samples tested positive for OTA in Lebanon and the UAE, respectively. However, only 1% of samples in Lebanon and 1.6% in the UAE exceeded the EU limit, consistent with our findings. The average OTA concentrations were 1.29 and 1.40 µg/kg in Lebanon and the UAE, respectively, higher than the average OTA concentration in cornflakes observed in our study.

Moreover, in a three-year comprehensive analysis, researchers conducted a global survey to assess the prevalence of mycotoxins in cereals. Among the 41 studies analyzed AFB1, ZEA, and DON exhibited the highest contamination rates (Khodaei et al., 2021). A significant proportion of samples exceeded the EU limits, particularly for AFB1, where 87.5% surpassed the allowed levels, with levels reported as high as 188 µg/kg in Haiti, 76.2 µg/kg in Kenya, and 44 µg/kg in Serbia. Similarly, elevated levels of DON (963 µg/kg) and ZEN (163 µg/kg) were reported in Serbia. Vietnam had the highest Ochratoxin A (OTA) levels at 1662 µg/kg, while Greece reported the lowest at 0.7 µg/kg. Additionally, Italy reported the highest levels of FUMs at 43296 µg/kg. Given that corn is a primary ingredient in various food products, including popcorn, breakfast cereals, flour, and baby food, continuous monitoring of mycotoxin levels in corn is crucial due to potential hazards to the food and feed chain. Similarly, the widespread use of corn in animal feed increases the risk of aflatoxins contaminating milk, underscoring the importance of mycotoxin monitoring (Khodaei et al., 2021). Moreover, Iqbal et al. (2014) and Alim et al. (2018) reported comparable findings concerning the assessment of mycotoxins in cereal-derived products. Their studies revealed that 41% and 52% of the samples tested positive for Aflatoxins (AFs), respectively, with some samples exceeding the EU limits. Iqbal et al. (2014) found that 16% and 8% of the samples were above the EU limits for AFB1 (2 µg/kg) and total AFs (4 µg/kg), respectively, whereas Alim et al. (2018) reported higher numbers, of 22% and 12% for AFB1 and total AFs, respectively. Conversely, our study observed 23.7% positivity for AFB1, aligning closely with Alim et al. (2018). Additionally, 48% and 50% of samples tested positive for OTA in Iqbal et al. (2014) and Alim et al. (2018) studies respectively, with 30% surpassing the maximum OTA level, similar to our findings of 48.7% positivity. Likewise, 53% and 56% of samples were positive for ZEA in the studies mentioned earlier, with 8% and 30% exceeding the maximum level, respectively, compared to our study, where 27.6% tested positive, with only 1.3% exceeding the EU limit. Besides, Palumbo et al. (2019) explained that cereal crops often harbor a range of mycotoxins and fungal byproducts, with wheat and maize showing particularly elevated levels of AFs, FBs, DON, and ZEN compared to other grains. Therefore, it is crucial to continually monitor these prevalent mycotoxins across diverse agricultural products and establish standardized methodologies to obtain precise co-occurrence data. This ensures reliability and consistency, facilitating effective risk assessment to prioritize mycotoxins posing significant health risks to humans and animals.

3.2 Effect of Different Variables on Mycotoxin Presence in Cornflakes

The results of the analysis evaluating the effect of different variables on the presence of mycotoxins in cornflakes are presented in **Table 6**. All data are presented as the percentage of mycotoxin-positive samples for each variable category and the corresponding p-values. A p-value of less than 0.05, indicated by "*", signifies a statistically significant association between each variable and the presence of each mycotoxin. Meanwhile, a p-value less than 0.001, denoted by "**", indicates an even stronger level of statistical significance in this association.

Table 6 Percentage Distribution of Mycotoxin Occurrence According to Various Variables

Variable		AFB1	OTA	DON	ZEA	FUM
Country of origin	**P-value**	**< 0.001****	**0.303**	**1**	**0.701**	**0.589**
	Developing	77.78	66.67	88.89	33.33	0
	Developed	16.42	46.27	88.06	26.87	10.45
Temperature on production day	**P-value**	**0.009***	**0.186**	**1**	**0.717**	**0.584**
	25-30	60	70	90	20	0
	below 25	18.18	45.45	87.88	28.79	10.61
Storage time (months)	**P-value**	**0.81**	**0.428**	**0.85**	**1**	**0.22**
	< 5 months	33.33	50	100	33.33	0
	5 - 10 months	23.64	52.73	85.45	27.27	7.27
	> 10 months	20	33.33	93.33	26.67	20
Presence of bran ingredient	**P-value**	**0.236**	**1**	**1**	**1**	**1**
	yes	50	50	100	25	0
	no	22.22	48.61	87.5	27.78	9.72
Presence of chocolate ingredient	**P-value**	**0.05***	**0.002***	**1**	**1**	**0.037***
	yes	35.48	70.97	87.1	29.03	0
	no	15.56	33.33	88.89	26.67	15.56
Presence of nuts ingredient	**P-value**	**0.463**	**0.756**	**0.339**	**1**	**0.588**
	yes	33.33	41.67	100	25	0
	no	21.88	50	85.94	28.12	10.94

Data are percentages (n=76); * P ≤ 0.05; ** P < 0.001 (Fisher's Exact test); < : less than; >: more than

As shown in **Table 6**, the country of origin, distinguishing between developing and developed nations, significantly impacted AFB1 occurrence in cornflakes ($p < 0.0001$), with a markedly higher proportion of positive SKUs detected in developing countries (77.78%) compared to developed ones (16.42%). Similarly, a trend of higher percentages of positive samples for OTA, DON, and ZEA was observed in developing countries, although statistical significance was not attained. In contrast, Alwan et al. (2022) reported no significant influence of the country of origin on AFB1 exposure in rice in the UAE. Similarly, Hassan et al. (2022a) observed no significant difference between rice brands packed in developing countries compared to those in developed countries. Moreover, Hassan et al. (2022b) observed that rice from developing countries of origin (India, Pakistan, Thailand, China) exhibited higher OTA levels compared to those from developed countries (the United States, Italy). However, the difference was not statistically significant. Conversely, Hassan et al. (2023), in their assessment of mycotoxin occurrence in cornflakes sold in Lebanon, identified a significant effect of country of origin (Lebanon vs. others: Ukraine, France, Turkey, Poland, UK) on AFB1 and FUM levels, but not on OTA, ZEA, and DON. Additionally, Yu and Pedroso (2023) conducted a review to provide updated insights into the occurrence and co-occurrence of mycotoxins in cereal grains and cereal-derived food and feed products, along with their health impacts on humans, livestock animals, and pets. They highlighted that the prevalence and concentration of mycotoxins in finished cereal-based food products are higher in developing countries than developed ones, consistent with our findings. Consequently, individuals residing in developing regions such as Asia and Africa face a greater risk of exposure to elevated levels of mycotoxins than those in developed nations. The lower levels of mycotoxins observed in processed foods in developed countries can be attributed to the relatively lower mycotoxin contents in unprocessed cereal grains, more stringent control over storage conditions, and the implementation of regulatory measures. This disparity also explains why cases of human mycotoxicosis are rare in Western countries but comparatively more frequent in developing regions.

Moreover, a significant association was observed in this study between the temperature on the production day and AFB1 occurrence ($p=0.009$). We initially retrieved the temperature as indicated on the package to determine the temperature on the production day for each cornflake SKU. Subsequently, we cross-referenced this information with data from weather websites, considering the specific country of origin and the city where production occurred. Subsequently, the averages of the recorded high and low temperatures were calculated to obtain a representative value. Specifically, a higher percentage of positive samples was observed when the temperature exceeded 25°C; 60% of samples tested positive when exposed to temperatures ranging between 25-30°C, compared to 18% for temperatures below 25°C. Although a similar trend was observed for OTA and DON, statistical significance was not observed. This can be explained by the fact that higher temperatures can encourage the growth of toxigenic fungi, potentially increasing the risk of mycotoxin production in food products. Similarly, Alwan et al. (2022) noted that samples packed during the spring/summer seasons, characterized by higher temperatures, exhibited higher contamination levels of AFB1 compared to those packed during the Fall/Winter seasons, marked by cooler temperatures. However, this trend was not statistically significant. This indicated that the consistent implementation of appropriate storage measures, regardless of the season in which the products were packed, may account for this observation. Indeed, the UAE's desert climate, characterized by warm temperatures, creates favorable conditions for the proliferation of aflatoxigenic fungi during storage, particularly in the dry and hot summers lasting from April to September. In addition, Hassan et al. (2022b) also investigated the seasonal impact of rice packing in Lebanon on AFB1 levels in rice. Despite Lebanon's recognized high temperature and humidity levels during the spring and summer seasons, which facilitate mold growth and, consequently, AFB1 contamination in food, no significant difference was found between rice brands packed in the fall and winter compared to those packed in the spring and summer.

Furthermore, the influence of storage duration from purchase to the initial assessment was investigated. The results showed no significant effect across different storage periods. Notably, the cornflake samples underwent post-production storage in the UAE, followed by shipment to Lebanon and subsequent storage in the laboratory under refrigeration. This meticulous handling underscores the implementation of effective storage practices, which likely prevented the proliferation of mycotoxin-producing fungi and diminished the risk of mycotoxin contamination during storage and transportation processes. Investigations by Alwan et al. (2022) and Hassan et al. (2022b) into the storage time between packing and purchasing revealed no significant impact on AFB1 levels in rice in the UAE and Lebanon, respectively, suggesting proper storage practices. However, Hassan et al. (2022a) observed higher OTA levels with increased storage time, although significance was only seen in the UAE rice samples. This may be caused by poor barrier properties of the rice packages and suboptimal storage conditions in some retailers. This was emphasized by Hassan et al. (2022), who evaluated the incidence of AFB1 and OTA in thyme and thyme-based products, where they highlighted the importance of implementing food safety management systems (FSMS), monitoring storage conditions, and adhering to good hygienic and manufacturing practices in food products. These measures are essential to safeguarding the microbiological quality of products and preventing mold contamination. Another investigation conducted by Karam et al. (2021) evaluated the microbiological quality of 13 commonly used spices and dried herbs available in Lebanon. Their analysis revealed an order of microbiological quality, with imported brands exhibiting the highest levels, followed by locally packaged products from companies with FSMS, locally packaged items from companies without FSMS, and finally, unpackaged samples. Species and dried herbs sold in open containers were particularly susceptible to microbial contamination. Additionally, the study underlines the necessity for implementing FSMS, adhering to good hygienic and manufacturing practices, conducting routine inspections, and testing products at all stages of production to ensure microbial safety.

On the other hand, various attributes of cornflakes were also examined in this study, such as the inclusion of bran, chocolate, and nuts. First, it was noted that incorporating bran resulted in a higher percentage of positive samples for OTA, DON, and AFB1, yet these outcomes did not attain statistical significance. This trend aligns with previous research findings. Importantly, bran is an inherent component of all whole grains, which may contribute to the observed effects on mycotoxin levels. Therefore, our results will be compared with studies investigating mycotoxin presence in whole grains versus refined grains for further insights. For instance, Gómez et al. (2023) investigated the impact of flour type (white or whole-grain) and processing method (direct or par-baked) on the mycotoxin levels in bread in Spain. Industrial bread exhibited notably higher levels of DON than artisanal bread, with a threefold increase, consistent with our findings. Additionally, a significant difference was evident in the levels of OTA, particularly in whole-grain bread and more prominent in industrial settings. Despite these variations, bread made with whole wheat flour exhibited negligible differences from bread made with white flour regarding average mycotoxin content. Similarly, other research investigations have also indicated elevated levels of DON in the bran or outer layers of grains in contrast to white flour or whole-grain flours compared to their white counterparts (Savi et al., 2016; Sovrani et al., 2012; Tibola et al., 2015). A similar pattern was also noted for OTA concentrations (Peng et al., 2015). The higher mycotoxin contamination observed in whole grain or bran compared to white flour can be attributed to several factors. First, mycotoxins tend to accumulate in the outer layers of grains, such as the bran, where they are more likely to be concentrated. Second, these outer layers are retained during the processing of whole grains, increasing the likelihood of mycotoxin presence in the final product. Third, bran, which contains higher levels of nutrients and moisture, can provide a favorable environment for fungal growth and mycotoxin production. As a result, whole grain or bran-based products may exhibit higher levels of mycotoxin contamination compared to those made from refined white flour (Thielecke & Nugent, 2018; Wang et al., 2020).

Besides, the presence of mycotoxins may be influenced by including chocolate ingredients. In our study, chocolate showed a slight effect on AFB1 ($p=0.05$) and a more pronounced and statistically significant effect on OTA ($p=0.002$), with higher percentages observed in SKUs containing chocolate (35.5% vs 15.6% for AFB1 and 70.97% vs 33.3% for OTA). This observation is consistent with the findings of Martins et al. (2018), who reported that 89% of breakfast cereals containing chocolate contained OTA. Moreover, in chocolate-containing breakfast cereals, DON and ZEA were detected in 62% and 73% of samples, respectively. This is particularly concerning as these products are predominantly consumed by children, who are known to be more susceptible to the adverse effects of mycotoxins. Chocolate products generally have a low water content, insufficient to support microbial growth and mycotoxin production. Thus, the presence of mycotoxins may have occurred due to the proliferation of toxigenic fungi in previous processing steps of the raw material (Copetti et al., 2012).

As for nuts, their presence in the ingredients increased the percentage of positive samples for AFB1 and DON, although this result did not reach statistical significance. In a study by Osaili et al. (2023) from 2017 to 2021, the prevalence of aflatoxins in nuts and nut products imported into the UAE from 57 countries was examined. Their findings revealed aflatoxin levels exceeding Maximum Residue Limits (MRLs) in imports from developing and developed nations, indicating a widespread global risk. Approximately 8.6% of imported nut samples were rejected, with an overall mean aflatoxin level of 77.1. Non-conformity was observed in samples from 32 countries, with mean aflatoxin values ranging from 81.0 to 92.7 μg/kg in pistachios, peanuts, and mixed nuts. Notably, a significant difference ($p < 0.05$) was noted in mean aflatoxin levels between peanut butter samples (29.3 μg/kg) and other nut types. Furthermore, processing methods significantly impacted aflatoxin levels, with ground samples exhibiting the highest mean level at 158.9 μg/kg. Additionally, packaging material played a role in AFB1 levels in nuts, with nuts packaged in fabric containers exhibiting the highest mean aflatoxin levels at 108.1 μg/kg, while nuts packaged in glass containers had the lowest mean level at 29.7 μg/kg. Hence, the incorporation of nuts in cereal products can introduce mycotoxins, such as AFB1, due to potential contamination of the nuts themselves. Numerous studies have demonstrated that nuts, like other agricultural products, are susceptible to fungal contamination and subsequent mycotoxin production under certain conditions. If contaminated nuts are utilized as ingredients in cereal manufacturing processes, the mycotoxins present in the nuts can transfer to the cereal matrix, indirectly contributing to cereal contamination. This emphasizes the critical importance of ensuring the safety and quality of all ingredients used in cereal production to decrease the risk of mycotoxin contamination in the final product. A review by Owolabi et al. (2023) examining a decade of data from the Rapid Alert System for Food and Feed (RASFF) revealed increased mycotoxin contamination in nuts and nut products, particularly those destined for EU countries. From 2011 to 2021, 63% of mycotoxin-related RASFF notifications were linked to nuts, nut products, and seeds, with aflatoxins (AFs) responsible for 95% of these notifications. Ground nuts, notably from China, were the most frequently affected. These findings underscored the significant incidence of border rejections for contaminated nut products entering EU countries, advocating for enhanced quality control measures.

3.3 Exposure and Risk Assessment

The Estimated Daily Intake (EDI), Hazard Quotient (HQ), Margin of Exposure (MoE), as well as the risks of liver cancer associated with AFB1 consumption and kidney disease linked to OTA intake were calculated for both daily and regular consumers. The summarized findings from EDI, HQ, and MoE are presented in **Table 7.** For daily consumers of cornflakes, the average intake was 70 g per day based on the FFQ results reported by Abu Dhabi University. The estimated daily intakes (EDIs) of AFB1, OTA, DON, ZEN, and FUM were calculated at 1.8, 0.91, 82.36, 9.22, and 531.7 ng/kg BW/day, respectively. Conversely, regular consumers, with an average daily intake of 1.5 g, exhibited lower EDIs of 0.039, 0.019, 1.76, 0.2, and 11.39 ng/kg BW/day for AFB1, OTA, DON, ZEN, and FUM, respectively. These findings suggest variations in mycotoxin exposure levels among different consumer groups, emphasizing the importance of monitoring dietary intake. Furthermore, it is noteworthy that higher consumption levels correlate with elevated Estimated Daily Intake (EDI) of mycotoxins.

Besides, the HQ values for the exposure to all mycotoxins, encompassing daily and regular consumers, were below 1. This suggests that the exposure levels to these mycotoxins from the consumption of cornflakes are relatively low compared to their respective reference doses, indicating a low risk associated with this exposure.

Moreover, for daily consumers, the Margin of Exposure (MoE) to AFB1, DON, and FUM showed values below 10,000, indicating a potentially elevated risk. As for regular consumers, the MoE values for all mycotoxins, except FUM, exceeded 10,000, suggesting a lower risk than for daily consumers.

As for ZEA, categorized as a non-genotoxic carcinogen, the EDI was compared to the provisional maximum tolerable daily intake (PMTDI) of 0.25 µg/kg BW (JECFA, 2014). The results revealed that the EDI remained below this threshold for daily and regular consumers.

Table 7 EDI, HQ, and MoE for different mycotoxins

		AFB1	**OTA**	**DON**	**ZEN**	**FUM**
Daily consumers	**EDI** (ng/kg bw/day)	1.8	0.91	82.36	9.22	531.7
	HQ	0.02-0.11	0.05	0.082	0.037	0.53
	MoE	222.22	**neo**: 15934 **non-neo**: 5197.8	2549.8	-	188
Regular consumers	**EDI** (ng/kg bw/day)	0.039	0.019	1.76	0.2	11.39
	HQ	0.0005-0.002	0.001	0.00176	0.0008	0.0114
	MoE	10256.4	**neo:** 763157.9 **non-neo**: 248947.4	119318	-	8779.6

EDI: Estimated Daily Intake; HQ: Hazard Quotient; MOE: Margin of Exposure; bw: body weight.

A recent study by Hoteit et al. (2024) investigated the dietary exposure of Lebanese adults to multi-mycotoxins (AFB1, AFM1, OTA, OTB, DON, T-2, and HT-2) and evaluated their associated health risks in cereals. Their findings revealed that pasta exhibited the lowest AFB1 concentration (0.005 µg/kg), while rice showed the highest (0.5 µg/kg). Moreover, within this food group, the total exposure to AFB1 was 0.86 ng/kg bw/day, with cornflakes contributing the least to exposure (0.0003 ng/kg bw/day) and rice contributing the most (0.48 ng/kg bw/day). This indicates that the exposure to mycotoxins from the consumption of cornflakes in their study was lower than the ones found in our study for both daily and regular consumers (1.8 ng/kg bw/day and 0.039 ng/kg bw/day, respectively). Moreover, in their study, the MoE to AFB1 for most cereals exceeded 10,000, except for rice (827.90) and bulgur (4868.50). In contrast, our findings indicated that for daily consumers, the MoE for AFB1 was below 10,000 (MoE=222).

Regarding OTA, Hoteit et al. (2024) reported total exposure of 3.29 ng/kg bw/day, with bread contributing the highest amount at 1.18 ng/kg bw/day, while cornflakes contributed the least at 0.0006 ng/kg bw/day. In our study, the OTA exposure from cornflake consumption was higher, at 0.91 ng/kg BW/day for daily consumers and 0.019 ng/kg BW/day for regular consumers. Their findings indicated that all MOE non-neo and MOE neo values were above 200 and 10,000, respectively, which aligned with our results. As for DON, Hoteit et al. (2024) reported an exposure of 0.11 ng/kg bw/day from cornflakes, 332.18 ng/kg bw/day from bread, and a total exposure of 347.05 ng/kg bw/day. In comparison, our study found exposure levels of 82.36 ng/kg bw/day for daily consumers and 1.76 ng/kg bw/day for regular consumers from the consumption of cornflakes, both higher than the exposure reported by Hoteit et al. (2024) from cornflake consumption. Although all MOE values in their study were above 10,000, exceptions were noted for bread and manakeesh, with values of 632.18

and 6879.87, respectively. Notably, in our study, the MOE for DON for daily consumers was below 10,000, indicating a potential risk (2549.8). Lastly, the Hazard Quotient (HQ) associated with the multi-mycotoxins analyzed in their study indicated that all cereal and cereal products examined had values below 1, aligning with our findings.

Furthermore, Andrade et al. (2018) studied mycotoxins and their estimated daily intake in popcorn and cornflakes in Brazil. They observed that, based on the upper bound perspective, where the upper limit was the limit of detection (LOD), the total mean intake for fumonisins and T-2 toxin from consuming cornflakes were 0.01 and 0.02 ng kg/bw/day, respectively. In comparison to our results, the estimated daily intakes (EDI) of FUM for both daily and regular consumers were higher than that reported in their study (531.7 ng/kg bw/day and 11.39 ng/kg bw/day, respectively). In addition, their findings suggested that heavy consumers of cornflakes also encountered minimal risk, as the concentration of FUM in commercial samples was generally low, with a maximum of 13 ng/kg bw/day. This closely aligns with the estimated daily intake (EDI) of FUM for regular consumers identified in our study (11.39 ng/kg bw/day).

Besides, it is important to note that comparing the daily exposure to mycotoxins across different countries requires caution due to various influencing factors. Differences in methodologies used for assessing cereal contamination and variations in cereal consumption among populations can notably impact the results. Moreover, differences in consumption patterns, types of cereals studied, and environmental conditions in each country significantly contribute to determining population exposure levels.

3.3.1 Liver cancer risk based on the overall daily exposure to AFB1

The liver cancer risk associated with AFB1 exposure from cornflake consumption in the UAE was assessed. Notably, the highest risk was observed among daily consumers, with 0.15 additional liver cancer cases / 100,000 persons/year (~1 case /1,000,000 persons /year). For regular consumers, the risk decreased to 0.0032 cases per 100,000 persons per year (less than 1 case /1,000 000 persons /year).

A comparison with the study by Hoteit et al. (2024) revealed that their findings indicated a total AFB1 exposure linked to 0.105 additional liver cancer cases per 100,000 people per year from the consumption of cereals and cereal products in Lebanon. This suggests a comparable risk for daily consumers, as observed in our investigation (0.15 cases per 100,000 persons per year). Another study by Raad et al. (2014) reported a risk of 0.053–0.055 additional liver cancer cases per 100,000 people per year for regular consumers based on a total diet study involving an adult urban Lebanese population. Daou et al. (2021) also found a risk of 0.076 HCC cases /100,000 people/year associated with the consumption of wheat and wheat products in Lebanon. In comparison to these studies, our findings revealed a lower risk of liver cancer associated with AFB1 exposure among regular consumers, with a rate of 0.0032 cases per 100,000 persons per year. Similarly, our findings indicated lower rates than Hassan et al. reported (0.35–0.41 cases of HCC / 100,000 people/year).

3.3.2 Kidney Disease Risk Based on the Overall Weekly Exposure to OTA

The weekly exposure to OTA in our study was 6.37 ng/kg bw for daily consumers and 0.133 ng/kg bw for regular consumers. These values remained below the PTWI set by JECFA for OTA (100 ng/kg bw), indicating a low risk of kidney disease associated with OTA exposure from cornflake consumption. Conversely, in the study by Hoteit et al. (2024) in Lebanon, the overall weekly exposure to OTA was higher, at 28.68 ng/kg bw. Yet, it remained below the established PTWI for OTA across all food groups. These results also indicate a low risk of kidney disease associated with OTA exposure.

3.3.3 Estimated Daily Consumption of Cornflakes by Age Group

The maximum daily intake of cornflakes (in grams) required to reach the Tolerable Daily Intake (TDI) levels for various mycotoxins across different age groups is presented in **Table 8**.

Table 8. Maximum Cornflakes Intake (g/day) reaching TDI of each mycotoxin by Age Group

Age (years)	**Average weight (kg)**	**Maximum Cornflakes Daily Intake (DI) (g/day) reaching TDI of each mycotoxin:**				
		AFB1	**OTA**	**DON**	**FUM**	**ZEA**
2 – 5	14.5	100 – 600	260	160	25	360
5 – 12	25	200 – 1030	450	280	43	620
12 – 18	58.5	500 – 2400	1053	650	100	1440
Adults (> 18)	77	600 – 3160	1390	850	132	1900

TDI: Tolerable Daily Intake

The results showed that for all age groups, cornflakes' daily intake (DI) values would need to be high to reach the TDI for each mycotoxin. However, when considering the average weight and typical portion sizes of children and adolescents, it becomes evident that their cornflake consumption does not approach the high levels necessary to reach the TDI for AFB1, OTA, DON, and ZEA. For FUM, the situation differs due to its concentration in cornflakes exceeding EU and Codex limits in this study. Consequently, the average FUM intake is relatively low compared to the other mycotoxins assessed. For example, in the case of children aged 2 to 5 years, the maximum cornflake intake reaching TDI for FUM is 25g/day, which is notably lower than for other mycotoxins. Specifically, the maximum intake reaching TDI for ZEA in this age group is 360 g/day, 100 – 600 g/day for AFB1, and 360 g/day for OTA.

On the other hand, data from the National Food Consumption Survey (ENCA) provides valuable insights into the typical consumption patterns of children aged 2–5 years and 6–13 years. According to ENCA, these age groups' mean consumption of cornflakes was reported to be 13 g/day and 14 g/day, respectively (Foerster et al., 2022). These values are notably lower than the maximum cornflake intake reaching TDI levels for mycotoxins observed in our study. This indicates that the average consumption of cornflakes among children, as reported in national surveys, remains well below levels of concern regarding mycotoxin exposure.

Furthermore, for adults, the maximum cornflake intake necessary to reach the TDI for mycotoxins is notably higher compared to children and adolescents, given their higher average weight. For example, considering adults, the maximum cornflake intake reaching TDI for AFB1 is 600 – 3,160g/day, whereas for children (2 – 5 years), it ranges from 100 to 600 g/day. Nevertheless, the actual consumption of cornflakes among adults also falls well below these levels, indicating a low risk of mycotoxin exposure from cornflakes consumption across all age groups. This study found that the average consumption of cornflakes among daily consumers was 70g/day and 0.25g/day among regular consumers, significantly below the calculated thresholds for reaching TDI levels of mycotoxins. While the risk of mycotoxin exposure from cornflake consumption is low, continued vigilance and adherence to safety standards are essential to safeguard public health.

3.3.4 Strengths and Limitations

This study is the first in the UAE to comprehensively analyze the levels of the five mycotoxins across all cornflake SKUs available in the country's market. Furthermore, it is the first study to evaluate the estimated daily exposure to AFB1, OTA, DON, ZEA, and FUM from cornflake consumption and assess the associated health risks, providing invaluable insights for public health interventions. Additionally, the study included a large sample size of 76 SKUs, with analyses conducted in duplicate for all samples and in triplicate for positive samples, ensuring heightened accuracy and reliability of the findings.

While our study offers valuable insights, it is essential to acknowledge its limitations for future research. Firstly, the adults surveyed using the FFQ were chosen via convenient sampling, potentially limiting the representativeness of UAE consumption patterns and the generalizability of the findings. Additionally, the FFQ itself poses limitations, including self-reporting bias. Furthermore, it is worth noting that the FFQs were exclusively administered to adults over 18 years old, overlooking that children are among the highest consumers of cornflakes.

CHAPTER 4

CONCLUSION AND FUTURE DIRECTIONS

In conclusion, cornflakes, a common breakfast staple, are susceptible to mycotoxin contamination, reflecting a broader issue in cereal products worldwide. This study investigated the presence of AFB1, OTA, DON, ZEA, and FUM in all cornflake SKUs available in the UAE market, its associated health risks, and the influence of various factors on mycotoxin presence. The findings revealed that all mycotoxin averages remained below the EU limits except for AFB1. DON emerged as the most prevalent mycotoxin in the samples but remained within acceptable levels. Moreover, the exposure and risk assessment revealed that the levels of mycotoxins in cornflakes, while present, generally pose a low risk to consumers. The HQ values for all mycotoxins across different consumption categories were below 1, indicating a tolerable exposure. However, for daily consumers, the MoE for AFB1, DON, and FUM fell below 10,000, suggesting a higher risk associated with these mycotoxins. Conversely, MoE values for regular consumers exceeded 10,000 for all mycotoxins except FUM, indicating a low risk. Additionally, the results indicated a low risk of liver cancer associated with AFB1 exposure and a low risk of kidney disease linked to OTA intake. As for children, the daily intake values of cornflakes would need to be high to reach the TDI for each mycotoxin across all age groups. However, the actual consumption among children and adolescents falls significantly below these thresholds for all mycotoxins, suggesting a safe level of cornflake consumption.

In assessing the influence of independent variables on mycotoxin presence in cornflakes, significant effects were observed for AFB1 based on the country of origin (p

< 0.0001), with a higher proportion of positive samples originating from developing countries. Furthermore, the higher temperature on the production day yielded a significant impact on AFB1 presence ($p = 0.009$). Also, including chocolate as an ingredient had a marginally significant effect on AFB1 ($p = 0.05$) and a more pronounced influence on OTA ($p = 0.002$). These findings underscore the importance of considering various factors in mitigating mycotoxin contamination in cornflakes production.

Overall, the findings of this study underscored the stringent food safety practices in the UAE, as evidenced by the majority of cornflake samples meeting EU limits for mycotoxin levels. Additionally, the rigorous enforcement of food safety regulations in the UAE has contributed to a low risk from the exposure to mycotoxins for consumers. However, it is crucial to maintain strict monitoring practices, especially with emerging brands entering the market. This ongoing surveillance is essential to promptly identify any deviations from safety standards and ensure swift corrective actions to protect public health. By continuously monitoring mycotoxin levels, authorities can uphold food safety standards and maintain consumer confidence in the safety and quality of food products available in the UAE market.

In future studies, since most grains contain multiple mycotoxins, it is crucial to investigate the co-occurrence of mycotoxins and the combined effects of co-exposures to several mycotoxins. Additionally, for verification purposes, the results obtained from ELISA could also be compared with HPLC, which is the gold standard method for mycotoxin analysis. Finally, exploring innovative mitigation strategies is also vital for effective mycotoxin control in cereal products.

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