Exposure to multiple mycotoxins in domestic and imported rice commercially traded in Tehran and possible risk to public health

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ABSTRACT

Mycotoxins are secondary fungi metabolites that induce acute and chronic toxic effects in humans and animals. In the present study, nine mycotoxins including aflatoxins (AFB$_1$, AFB$_2$, AFG$_1$ and AFG$_2$), fumonisins (FB$_1$ and FB$_2$), Ochratoxin A (OTA), deoxynivalenol (DON), and zearalenone (ZEN) were determined in one hundred rice samples collected from Tehran using high performance liquid chromatography (HPLC) with fluorescence or photodiode array detector. In addition, possible risk to public health was investigated by assessing dietary exposure through rice consumption, the margin of exposure (MOE), respective risk of cancer and hazard index (HI) of the monitored mycotoxins in children and adults. The higher mean levels were determined for DON (102.22 µg.Kg^{-1}), followed by FB$_1$ (85.00 µg.Kg^{-1}). For the rests of mycotoxins the levels did not exceed 20 µg. Kg^{-1}. The estimated AFB$_1$ intake for the adults and children through rice consumption exceeds the safe levels established for both carriers and non-carriers of hepatitis B virus. The mean and median determined exposure levels of OTA, DON ZEN and FB$_1$, were found lower than the Provisional Maximum Tolerable Daily Intake (PMTDI) value for both adults and children of Tehran that consuming domestic and imported rice. The mean HI for adults and median HI for adults and children were below one, and mean HI for children was close to one. All the mean, median and maximum MoE values were <10,000 in adults and children, indicating a risk due to AFB$_1$ exposure through rice consumption in Tehran. In addition, the calculated mean cancer risk in adult and child populations of Tehran were 0.27 and 0.64 cases per year per 10^5 individuals, respectively, that shows population in Tehran could be at risk of cancer due to AFB$_1$ exposure through rice consumption as calculated. So further studies are necessary for the monitoring mycotoxins in rice and different food products as well as estimating average dietary exposure and cumulative exposure assessment of mycotoxins for main foods in IR Iran.

1. Introduction

A food contaminant can be biological, chemical or physical in nature, with the former being more common [1,2]. The list of food contamination challenges is very long and keeps growing [3–8].

Mycotoxins are food contaminants with toxic secondary metabolites, and they are produced by filamentous fungi belonging to the molds.

They have great importance in the health of humans and animals, being the cause of acute and chronic diseases [9–11].

Around 300 different mycotoxins have been defined that are produced by about 200 different fungal species. However, there are only 20 mycotoxins that regularly occur in foodstuffs and feedstuffs at concentrations likely to pose a health hazard for animals and people consuming the said products [12]. The commonly known and posing human health

hazards mycotoxins can be categorized into *Aspergillus* mycotoxins (e.g. aflatoxins (AFs)), *Penicillium* mycotoxins (e.g. ochratoxin A (OTA), citrinin) and *Fusarium* mycotoxins (e.g. fumonisins, trichothecenes, zearalenone (ZEN), nivalenol, deoxynivalenol (DON), T-2 toxin, enniatins and beauvericin) [9,13–15].

Nowadays, combined toxicity is gaining increased relevance [16,17]. Exposure to several classes of mycotoxins could possibly result in an additive effect, not excluding also a possible synergistic interaction [18].

Aflatoxins B_1 (AFB$_1$), B_2 (AFB$_2$), G_1 (AFG$_1$), G_2 (AFG$_2$), and M_1 (AFM$_1$) were classified as carcinogenic to humans (Group 1) [19]. They can be regarded as the most important mycotoxins owing to their genotoxic and carcinogenic properties. AFB$_1$ is the most potent followed by AFG$_1$ and AFM$_1$. The chronic AF-exposure induces liver cancer, particularly in conjunction with chronic hepatitis B virus infection, growth impairment in humans, while high exposures cause acute symptoms and episodic poisoning outbreaks, even death [9,20–22]. OTA has been demonstrated to be nephrotoxicity and renal cancer. It was classified as group 2B (Possibly carcinogenic to humans) (World Health Organization and Cancer, 1993). OTA can pass over the placental barrier and fetal blood and that it is released into breast milk [23,24]. Estrogenic effect which has been created in children with precocious sexual development who were exposed to contaminated food is the most important toxic effect of the ZEN family [25]. ZEN was classified as group 3 (Not classifiable as to its carcinogenicity to humans) [26]. Fumonisins have been indicated to cause neoplasiain in humans they are related with neoplasia, stunting of children, and blocked folic acid absorption and its consequence is folic acid deficiency that associated with birth defects [27]. FB$_1$ was classified as possibly carcinogenic to humans (Group 2B) [28]. DON is ribotoxic and causes cellular affection by binding to ribosomes and modulation of gene expression, leading to inhibition of protein synthesis. The main objects of DON are gastrointestinal tract, and immune, reproductive, endocrine, and nervous systems [29,30]. DON was classified as group 3 (Not classifiable as to its carcinogenicity to humans) [26].

Mycotoxins are consumed by humans through contaminated plant origin food (cereal grains) or through the intake of animal origin food (meat, milk, and egg) [31]. Cereals and different types of nuts and their products are the main exposure sources of mycotoxins [20].

Around the world, rice, wheat, and maize, and to a lesser extent, sorghum and millets are important staples critical to daily survival of billions of people. Rice is the single most important source of calories for humans. Rice contributes approximately 21 % of world per capita caloric intake, and 27 % of per capita calories in the developing countries [32]. Rice is one of the main crops in Iran with mean consumption of 110 g per person per day. [33]. Most studies in IR Iran focused on single group of mycotoxins such as total aflatoxins (AFT), OTA, or ZEN, but not multiple mycotoxins exposure [34–36].

Exposure assessment is an essential step of risk assessment, a tool for risk managers involved in food safety [37]. The exposure assessment methodologies include point estimates of dietary exposure, deterministic and probabilistic approaches. The exposure is calculated as the product of food consumption and the levels of the substance in question determined [37].

2. Materials and methods

In the present study, nine mycotoxins including AFB$_1$, AFB$_2$, AFG$_1$, AFG$_2$, FB$_1$, FB$_2$, OTA, DON and ZEN were analyzed using HPLC method in imported and domestic rice samples collected from Tehran markets. This study reports for the first time the exposure to multiple mycotoxins through consumption of imported and domestic rice and the possible risk associated thereof in adults and children in Tehran (Tables 5–8).

Two approaches are applied:

1 Comparing the determined exposure levels with Provisional Maximum Tolerable Daily Intake (PMTDI) and

2 Calculation of Hazard Quotient (HQ) and Hazard Index (HI).

In addition, for the aflatoxins that are associated in the literature with genotoxic and carcinogenic hazards, two more indices are calculated: the margin of exposure (MOE), which is the ratio between the reference point derived from animal studies and the estimated human intakes, and the cancer risk taking also into consideration the prevalence of Hepatitis B Virus (HBV) [38–40].

2.1. Sample collection

A total of 100 packaged rice samples were obtained from three retail markets in Tehran, IR Iran. Sampling plan was performed according to the guideline of ISO 24,333 [41]. The samples were categorised as domestic (80) and imported (20) based on the brand names (Table 1). A 1000 g subsample was then milled (Laboratory Mill 3100; Perten, Sweden) with a 0.8 mm screen and collected in sealed plastic bags (200 g), then stored in a freezer at $-80\ ^\circ$C until analysis.

2.2. Chemicals and reagents

The standards of AFs (B_1, B_2, G_1 and G_2), fumonisin B_1 (FB$_1$), fumonisin B_2 (FB$_2$), DON, OTA and ZEN were supplied from Sigma-Aldrich (St. Louis, MO, USA). All standard solutions were prepared in methanol and kept in dark glass at $-20\ ^\circ$C and were brought to room temperature before analysis. All used solvents were analytical reagent grade and obtained from Merck Company (Darmstadt, Germany). The immunoaffinity columns (IAC) for AFT (B_1, B_2, G_1 and G_2), fumonisins (FB$_1$ and FB$_2$), OTA and ZEN were purchased from Libios (France).

2.3. Sample preparation

The extraction procedure for each mycotoxin is as follows:

Aflatoxins: Ten grams of samples was shaken at high speed for 3 min with 100 mL of methanol: H2O (80:20 v/v) as extraction solvent. After filtration, 7 mL of the filtrated extract was added to 35 mL of phosphate buffer saline (PBS). Thirty-six millilitre of the sample extract was passed through the IAC (1 drops/second). Finally, the column was washed with 15 mL PBS. For elution of AFs from the column, a portion of 0.5 mL HPLC grade methanol was passed through the column followed by an additional portion of 0.75 mL of the same solvent one minute afterward. HPLC grade water was added to the eluent to the volume of 3 mL and 100 µL of the final solution was injected into HPLC [42].

Ochratoxin A: Ten grams of samples was blended for 3 min with 1 g of sodium chloride, 300 mg of NaHCO3 and 100 mL of methanol: H2O (80:20 v/v). After filtration, 7 mL of the filtrated extract was added to 35 mL of PBS. The sample extract (36 mL) was passed through the column (1 drops/second). The column was washed with 15 mL PBS. For elution of OTA from the column, a portion of 0.5 mL HPLC grade methanol was

Table 1
The type and origin of rice samples collected from Tehran markets.

Iranian rice			Imported rice		
Name	Region	No. of sample	Name	Region	No. of sample
Neda rice	Mazandaran	8	Basmati Super kernel rice	Pakistan	6
Tarom rice	Gilan	20	Fortified Basmati rice	India	2
Tarom rice	Mazandaran	6			
Hashemi rice	Gilan	16	Super kernel sella rice	Thailand	6
Hashemi	Mazandaran	14			
Ali kazemi rice	Gilan	4	Texmati rice	India	4
Pardis rice	Mixed	4	Thai rice	Thailand	2
Domsia	Mazandaran	8			

passed through the column followed by an additional portion of 0.75 mL of the same solvent one minute afterward. Finally, the eluent was added to the 1.75 mL of water containing 2 % acetic acid and then 100 μL of the final solution was injected into HPLC [43].

Zearalenone: ZEN content of samples was detected according to the method of ISO 15850:2010. Twenty-five grams of rice samples were extracted for 3 min with acetonitrile-water (75:25, v/v). After filtering, 12 mL of the filtrate was diluted with 88 PBS and fifty mL of extract was passed through IAC at a flow rate of 1 drops/second. Finally, the column was rinsed with 15 mL PBS e and dried with a gentle vacuum. For ZEA elution from the column, a portion of 0.5 mL HPLC grade methanol was passed through the column followed by an additional portion of 1 mL of the same solvent one minute afterward, and then the eluent diluted with 1500 μL deionized water. Finally, 100 μL of the final solution was injected into HPLC [44].

Deoxynivalenone: DON content of rice samples was determined using the method of ISO 15891:2010. To determine the DON content of rice, 10 g of rice samples were extracted with acetonitrile-water (84:16, v/v) with a high speed blender for 3 min. After filtration, 2 mL of the extract was passed through the IAC. The column was washed with 15 mL PBS and dried under vacuum. Finally, DON was eluted from IAC with 1 mL HPLC grade acetonitrile followed by an additional portion of 1 mL of the same solvent one minute afterward. The eluate was dried at 40 °C, reconstituted with 500 μL water-methanol (90.5:9.5, v/v), and then 100 μL injected into the HPLC [45].

Fumonisins: FB_1 and FB_2 were extracted and cleaned up using the method of Visconti et al. (2010) with some modification [46]. Samples (25 g) were extracted in a blender jar by adding 2.5 g NaCl, 125 mL methanol:acetonitrile:water (25:25:50, v/v/v) and blending at high speed for 5 min. Extracts were filtered and 10 mL aliquots diluted with 40 mL PBS and mixed well. Forty mL of diluted extract was passed through IAC at a rate of 1 drops/second. The columns were then washed with 10 mL PBS at the same rate until air came through columns. Fumonisins were eluted from the column with 750 μL HPLC grade methanol followed by an additional portion of 750 μL of the same solvent one minute afterward. Residues were diluted in 1500 μL of HPLC grade water, then 500 μL of this solution was mixed with 500 μLO-phthaldialdehyde (OPA) reagent, and finally 200 μL of solution was injected into HPLC exactly 3 min after adding OPA reagent.

2.4. Liquid chromatography

The quantification of mycotoxins was performed with a Waters 2695 HPLC system (Separation Module Alliance, USA) with UV/Vis detector (Waters 2489) or fluorescence detector (Waters 474). Analytical separation was performed on an Agilent Eclipse XDB C18 (150 mm × 4.6 mm, 5 μm) column using an isocratic mixture of different solutions (Table 2). A Kobra cell (Libois, France) was used for derivatization of AFs with electrochemically generated bromine.

2.5. Method validation

Table 2 shows the HPLC condition for analysis of mycotoxin in rice samples. For method validation, the parameters assessed were linearity, limit of detection (LOD), limit of quantification (LOQ), recovery and precision. For construction of spiked calibration curves, the rice samples spiked at different concentrations of mycotoxins and were prepared in triplicates at three days and then treated according to the procedure described previously.

2.6. Dietary exposure assessment

Dietary exposure was performed using the deterministic approach [37] by combining rice intake and mycotoxin contamination data, according to the following equation (1):

Dietary Exposure (ng/kg bw/day) = Concentration of mycotoxin (ng/kg rice) × Rice intake (kg rice/kg bw/day)

Where body weight 70 kg for adults and 15 kg for children (3–4 years) were used as the average weight. The mean rice consumption for the Iranian population based on the national nutrition survey is 110 g per person per day [33]. Since there is no available data for rice consumption by children in Iran, we assumed rice consumption by children as half of 110 g per person per day.

2.7. Risk characterization

2.7.1. Genotoxic and carcinogenic aflatoxins

The risk characterization of the genotoxic and carcinogenic aflatoxins was performed via both MOE and cancer risk approaches [38,47].

MOEs were calculated (using Eq. 2) by dividing the relevant reference value (e.g. BMDL10 which is 170 ng/kg b.w./day based on the rodent data for AFB_1) [38], by the estimated human intakes:

Margin of exposure = Reference Value (ng/kg bw./day)/Exposure (ng/kg bw/day)   (2)

The cancer risk for AFB_1 (hepatocellular carcinoma, HCC) was calculated by multiplying the probability of cancer (Pcancer) with the estimates of mean, median and maximum AFB1 exposure (Eq. 3) [38,40].

Cancer risk = Pcancer × Exposure (ng/kg b.w/day)   (3)

Table 2
The HPLC condition for determination of nine mycotoxins in rice sample.

Parameter of HPLC condition	Aflatoxins	Ochratoxin A	Zearalenone	Deoxynivalenone	Fumonisins
HPLC condition	Column: C_{18}, mobile phase: methanol; water containing 350 μL of HNO3 (4 M) and 120 mg/L KBr (40:60 v/v), Flow rate: 1.8 mL min^{-1} Fluorescence detector:	Column: C_{18}, mobile phase: Water containing 2 % Acetic acid;(54:46 v/v), Flow rate: 2 mL min^{-1}, Fluorescence detector	Column: C_{18}, mobile phase: Water containing 2 % Acetic acid; (54:46 v/v), Flow rate: 2 mL min^{-1}, Fluorescence detector	Column: C_{18}, mobile phase: water; methanol (90:10), Flow rate: 1.5 mL min^{-1}, Photodiode array detector; at wavelength 218 nm	Column: C_{18}, mobile phase: methanol; phosphodehydrogen sodium 0.1 M (77:23), Flow rate: 1 mL min^1, Fluorescence detector
	$λ_{Ex}$[a] 360 nm $λ_{Em}$[b]:435 nm	$λ_{Ex}$: 333 nm $λ_{Em}$: 460 nm	$λ_{Ex}$: 333 nm $λ_{Em}$: 460 nm		$λ_{Ex}$: 335 nm $λ_{Em}$:440 nm
Derivatization	Post column derivatization using Kobra cell	–	–	–	Pre-column

[a] Ex: Excitation.
[b] Em: Emission.

Cancer probability (Pcancer) [40] deals with the percentage of both carriers (%Pop.HBsAg+ = 0.017) and non-carriers (%Pop.HBsAg$^-$ = 0.983) of HBV infection in the population of Tehran [48], as well as with the carcinogenic potency of AFB$_1$, (0.01 additional cancer cases per 100 000 for chronic hepatitis B virus surface antigen negative (HBsAg$^-$) populations and 0.3 additional cancer cases per 100 000 for HBsAg + populations [47].

Pcancer = (PHBsAg+ × %Pop.HBsAg+) + (PHBsAg- × %Pop.HBsAg-)(4)

2.7.2. Non-genotoxic mycotoxins

For the remaining individual mycotoxins (FB$_1$, OTA, DON and ZEN), the calculated exposure was compared to the dose reference value (PMTDI) in order to calculate the HQ (ratio between exposure and a reference dose). The HI was calculated as the sum of the respective Hazard Quotients (HQs) for the individual mixture components of the same family [49]. If HI > 1, the total concentration (or dose) of mixture components exceeds the level considered to be acceptable [50].

3. Results

3.1. Method performance

Results of the mean recovery and coefficient of variation on all analytes are shown in Table 3. The mean recovery and coefficient of variation ranged 83.00−97.00% and 4.10−8.20%, respectively, which were in the acceptable range of European Commission regulation [39]. LODs and LOQs of the analytical methods used for mycotoxins analysis ranged 0.10−17 ng/mL and 0.30−50 ng/mL, respectively (Table 3).

3.2. Occurrence of mycotoxins in rice

The occurrence of AB$_1$, AFT, OTA, DON, ZEN and FBs in rice samples are shown in Table 4. The findings of this study were compared with the maximum levels established by Iranian National Standards Organization. The maximum levels of AB$_1$, AFT, OTA, DON, ZEN and FBs are 5, 30, 5, 1000, 200 and 1000 μg kg^{-1}, respectively [51].

As Table 4 indicates the percentage of contaminated samples of imported and domestic rice to AB$_1$, AFT, OTA, DON, ZEN and FBs. The level of AB$_1$ and OTA in 16 % and 3% of samples were higher than the maximum limit, respectively. Regarding ZEN, DON and FBs, all samples were contaminated at the levels lower than maximum limit. In our study, among 20 domestic samples and 80 imported samples, 16 % and 3% were contaminated with AFB$_1$ and OTA above the national maximum limits [51], while no AFG$_1$ was detected in samples. Besides AFB$_1$ and ZEN, co-contamination of other mycotoxins such as DON, OTA, and FB$_1$ were detected in rice samples. The results in the present study indicate that the most frequently mycotoxins were AFB$_1$, FB$_1$ and OTA. Additionally, it was found that fifteen and three domestic rice samples were exceeded the maximum limits (MLs) set for AFB$_1$ and OTA respectively (5 mg kg^{-1}). One imported sample exceeded the maximum limits (MLs) for rice set in Iran for AFB$_1$ (5 mg kg^{-1}).

3.3. Dietary exposure assessment

Deterministic dietary exposures (ng/kg b.w./day) through the consumption of domestic and imported rice contaminated by multiple mycotoxins in Tehran shown in Table 5.

In this study, the estimated AFB$_1$ intake for the adults and children through rice consumption was higher than PMTDI for aflatoxin (1.0 ng kg^{-1} of body weight per day for adults and children without hepatitis B virus and 0.4 ng kg^{-1} of body weight per day for adults carrying hepatitis B virus) [52].

Results further revealed that daily dietary exposure of AFT ranged from 1.52–751.74 ng/kg of body weight per day, which exceeds the permissible limit of 1 ng kg^{-1} of body weight per day as defined by the Joint FAO/WHO Expert Committee on Food Additives [53].

Although the mean and median of calculated dietary exposure of OTA for both adults and children consuming domestic and imported rice were found lower than the PMTDI of 17.1 ng/kg b.w./day [54], the maximum level of calculated dietary exposure of OTA for both adults and children of Tehran exceeded the PMTDI of 17.1 ng/kg b.w./day.

There was no health risk associated with DON and ZEN exposure for both adults and children of Tehran that consuming domestic and imported rice at the mean, median and maximum measured contamination levels as none of the exposure levels exceeded the safe health limits for DON (1000 ng/kg b.w./day) (JECFA and Additives, 2010) and ZEN (250 ng/kg b.w./day) [55,47]. All the determined exposure levels of FB$_1$ except maximum dietary exposure level in children that consuming domestic rice, were found lower than the PMTDI value of 2000 ng/kg b. w./ day for FBs [47,56].

3.4. Risk characterization

The measured risk characterization due to AFB$_1$ exposure by domestic and imported rice consumption using the MOE and the liver cancer risk approach are shown in Tables 6 and 7, respectively. The EFSA's scientific committee is considered that in general a MOE of 10,000 or higher, if it is based on the BMDL10 from an animal study, would be of low concern from a public health point of view and might reasonably be considered as a low priority for risk management actions [38].

The MoE values were calculated at exposures of mean, median and maximum AFB$_1$ concentration in adults and children of Tehran. The results showed that, all the mean, median and maximum MoE values were <10,000 in adults and children, indicating a high risk due to AFB$_1$ exposure through rice consumption in Tehran. This is due to high estimated AFB$_1$ intake for the adults and children through rice consumption (higher than PMTDI value of 1 ng/kg b.w./ day). Calculated mean and median MoE were in the following decreasing order: children consuming domestic rice > adults consuming domestic rice > children consuming imported rice > adults consuming imported rice.

As shown in Table 7, the calculated mean cancer risk in adult and child populations of Tehran were 0.27 and 0.64 cases per year per 10^5 individuals, respectively, whereas the median risk of development of liver cancer in adults and children of Tehran were calculated to be 0.06 and 0.13 cancers/year/10^5/ng AFB$_1$/kg b.w./day, respectively.

Also, the results showed that, at maximum, the total liver cancer risk

Table 3
Results of validation assessment of HPLC method developed for determination of nine mycotoxins in rice samples (n = 3).

Parameter of analytical method	Aflatoxins	Ochratoxin A	Zearalenone	Deoxynivalenol	Fumonisins
LR[a]	0.08−7.2 (ng/mL)	0.25−15 (ng/mL)	10−250 (ng/mL)	50−2500 (ng/mL)	0.05−2 (ng/μl)
R^2	0.985−0.999	0.999	0.998	0.999	0.998
LOD (ng/mL)	0.10	0.10	3.33	15.00	17.00
LOQ (ng/mL)	0.30	0.30	10.00	45.00	50.00
Recovery (%)	83.00−97.00	80.00	87.00	76.00	95.00
RSD (%)	4.10−8.20	6.80	7.70	6.70	5.40

[a] LR, linear range.

Table 4
Occurrence of mycotoxins in rice samples collected from retail markets in Tehran.

Type of rice	Mycotoxin	Positive sample[a] (%)	Contamination (µg Kg^{-1})			Maximum limit in ISIRI	No. of sample higher than maximum limit
			Mean ± SD	Median	Max		
Domestic samples (No = 80)	AFB$_1$	34	15.13 ± 33.78	3.81	184.77	5	15
	AFT	32	18.54 ± 38.36	6.05	205.02	30	5
	OTA	58	1.57 ± 6.23	0.26	46.79	5	3
	DON	13	94.64 ± 13.13	92.67	140.51	1000	0
	ZEN	26	<LOQ	–	–	200	0
	FB$_1$	48	88.44 ± 106.01	44.99	608.21	–	0
Imported samples (No = 20)	AFB$_1$	12	1.65 ± 2.18	0.86	7.41	5	1
	AFT	8	1.45 ± 1.57	0.97	5.31	30	0
	OTA	11	0.33 ± 0.35	0.21	1.07	5	0
	DON	3	117.38 ± 24.78	115.02	153.02	1000	0
	ZEN	13	<LOQ	–	–	200	0
	FB$_1$	14	73.24 ± 77.65	42.55	311.74	–	0
Total samples	AFB$_1$	46	11.61 ± 29.56	2.39	184.77	5	16
	AFT	40	15.14 ± 34.89	4.20	205.02	30	5
	OTA	69	1.37 ± 5.72	0.24	46.79	5	3
	DON	16	102.22 ± 20.63	96.66	153.02	1000	0
	ZEN	39	<LOQ	–	–	200	0
	FB$_1$	62	85.0 ± 99.92	44.79	608.21	–	0

[a] Positive mean samples > LOD.

Table 5
Deterministic dietary exposures (ng/kg b.w./day) through the consumption of rice contaminated by multiple mycotoxins via people in Tehran. Values exceeding the PMTDI (provisional maximum tolerable daily intake) are shown in bold.

Type of rice	Mycotoxins	Mean Deterministic dietary exposures (ng/kg b.w./day)[a]		Median Deterministic dietary exposures (ng/kg b.w./day)[b]		Maximum Deterministic dietary exposures (ng/kg b.w./day)[c]	
		Adult	Children	Adult	Children	Adult	Children
Domesticsamples	AFB$_1$	**23.78**	**55.48**	**5.99**	**13.97**	**290.35**	**677.49**
	AFT	**29.13**	**67.98**	**9.51**	**22.18**	**322.17**	**751.74**
	OTA	2.47	5.76	0.41	0.95	73.53	171.56
	DON	148.72	347.01	145.62	339.79	220.80	515.20
	ZEN	10.98	25.63	–	–	–	–
	FB$_1$	138.98	324.28	70.70	164.96	955.76	**2230.10**
Imported samples	AFB$_1$	**2.59**	**6.05**	**1.35**	**3.15**	**11.64**	**27.17**
	AFT	**2.28**	**5.32**	**1.52**	**3.56**	**8.34**	**19.47**
	OTA	0.52	1.21	0.33	0.77	1.68	3.92
	DON	184.45	430.39	180.75	421.74	240.46	561.07
	ZEN	10.29	24.02	–	–	–	–
	FB$_1$	115.09	268.55	66.86	156.02	489.88	1143.05
Total samples	AFB$_1$	**18.24**	**42.57**	**3.76**	**8.76**	**290.35**	**677.49**
	AFT	**23.79**	**55.51**	**6.60**	**15.40**	**322.17**	**751.74**
	OTA	2.15	5.02	0.38	0.88	73.53	171.56
	DON	160.63	374.81	151.89	354.42	240.46	561.07
	ZEN	10.86	25.34	–	–	–	–
	FB$_1$	133.57	311.67	70.38	164.23	955.76	**2230.10**

PMTDI values (ng/kg b.w./ day): AFB$_1$ = 1, AFT = 1, FB$_1$ = 2000, OTA = 17, DON = 1000, ZEN = 250.
[a] Calculation based on mean concentration.
[b] Calculation based on median concentration.
[c] Calculation based on maximum concentration.

Table 6
Estimation of the Margin of Exposure of AFB$_1$ in children and adults in Tehran.

Type of rice	Mycotoxins	Mean MoE[a]		Median MoE[b]		Maximum MoE[c]	
		Adult	Children	Adult	Children	Adult	**Children**
Domestic rice (No. = 80)	AFB$_1$	7.15	3.06	28.39	12.17	0.59	0.25
Imported rice (No. = 20)	AFB$_1$	65.56	28.10	125.79	53.91	14.60	6.26
Total rice	AFB$_1$	9.32	3.99	45.26	19.40	0.59	0.25

[a] Calculation based on mean exposure.
[b] Calculation based on median exposure.
[c] Calculation based on maximum exposure.

associated with rice consumption in Tehran was 4.36 and 10.16 HCC cases/year/100,000 individuals in adults and children, respectively.
Table 8 presents the results concerning the risk characterization for FB$_1$, OTA, DON and ZEN using mean, median and maximum HQ (individual mycotoxins) and HI (combined mycotoxins). The results showed that mean HI for adults and median HI for adults and children were

Table 7

Estimation of cancer risk of AFB_1 in children and adults of Tehran.

Type of rice		Mean cancer risk[a]		Median cancer risk[b]		Maximum cancer risk[c]	
		Adult	Children	Adult	Children	Adult	Children
Domestic rice (No = 80)	AFB_1	0.354971	0.83215	0.089807	0.20955	4.355293	10.16235
Imported rice (No = 20)	AFB_1	0.038711	0.09075	0.020271	0.0473	0.174664	0.40755
Total rice	AFB_1	0.272387	0.63855	0.056336	0.13145	4.355293	10.16235

[a] Calculation based on mean exposure.

[b] Calculation based on median exposure.

[c] Calculation based on maximum exposure.

Table 8

Characterization of risk associated with the exposure to 4 mycotoxins (FB1, OTA, DON and ZEN) through consumption of the domestic, imported and all rice samples.

Type of rice	Mycotoxin	Mean HQ[a]			Median HQ[b]			Maximum HQ[c]			HI for mean HQ		HI for median HQ		HI for maximum HQ	
			Adult	Children	Adult	Children	Adult	Children		Adult	Children	Adult	Children	Adult	Children
Domestic rice (No = 80)	OTA	0.15	0.34	0.02	0.06	**4.33**	**10.09**		0.41	0.95	0.21	0.48	**5.03**	**11.73**
	DON	0.15	0.35	0.15	0.34	0.22	0.52							
	ZEN	0.04	0.10	–	–	–	–							
	FB_1	0.07	0.16	0.04	0.08	0.48	**1.12**							
Imported rice (No = 20)	OTA	0.03	0.07	0.02	0.05	0.10	0.23		0.31	0.73	0.23	0.55	0.58	**1.36**
	DON	0.18	0.43	0.18	0.42	0.24	0.56							
	ZEN	0.04	0.10	–	–	–	–							
	FB_1	0.06	0.13	0.03	0.08	0.24	0.57							
Total rice	OTA	0.13	0.30	0.02	0.05	**4.33**	**10.09**		0.4	0.93	0.21	0.48	**5.05**	**11.77**
	DON	0.16	0.37	0.15	0.35	0.24	0.56							
	ZEN	0.04	0.10	–	–	–	–							
	FB_1	0.07	0.16	0.04	0.08	0.48	**1.12**							

Values exceeding one are shown in bold. PMTDI values (ng/kg b.w. day): $AFB_1 = 1$, AFT = 1, $FB_1 = 2000$, OTA = 17, DON = 1000, ZEN = 250.

[a] Calculation based on mean Deterministic dietary exposures.

[b] Calculation based on median Deterministic dietary exposures.

[c] Calculation based on maximum Deterministic dietary exposures.

Table 9

Mycotoxins contamination in rice samples in different provinces of Iran.

Toxin	Region	Type of rice	No. of samples	Level (ng g^{-1})	Quantification Method	Ref.
OTA	Mashhad	Domestic and imported	182	0.20−4.80	HPLC with fluorescence detector	[35]
AFB_1	Mazandaran	Domestic	40	0.29−2.92	ELISA	[34]
AFB_1				1.89−10.0		[36]
AFB_2	Hormozgan, East Azarbayejan	Imported	71	0.14−8.41	HPLC with fluorescence detector	
AFG_1				0.05−0.79		
AFG_2				0.012−0.19		
AFB_1				0.090−3.30	HPLC with fluorescence detector	[57]
AFT	Bushehr	Imported	152	0.15−4.27		
ZEN	ShahreKord	Domestic	20	89.0	ELISA	[58]
AFB_1				3.90−30.83		
AFB_2						
OTA	Tehran	Domestic	65	0.6−1.260.65−11.544.95−215.46	LC-MS/MS	[59]
ZEN						
FB_1				4.95−215.46		
AFB_1			18	0.34- 2.46		
AFB_2						
AFG^1	Tehran	Domestic and imported	62	0.79- 1.09	HPLC	[60]
OTA						
ZEN						
AFB_1	Ardabil, Azarbayjan east, Azarbayjan west, Bushehr, Charmahale Bakhtiary, Esfahan, Fars, Ghazvin, Ghom, Gilan, Golestan, Hamedan, Hormozgan, Ilam, Kerman, Kermanshah, Khoozestan, Khorasan north, Khorasan Razavi, Khorasan south, Kohkilooie va boir ahmad, Kordestan, Lorestan, Markazi, Mazandaran, Semnan, Sistan va balooc, Tehran, Yazd, Zanjan	Domestic	256	0.00−5.80	HPLC with fluorescence detector	[61]
AFT				0.10−6.30		

below one, and mean HI for children was close to one.

The maximum HQ for OTA in adults and children through consumption of domestic and total rice and FB_1 in children through consumption of domestic and total rice were higher than one. Maximum HI for the simultaneous exposure to FB_1, OTA, DON and ZEN through consumption of domestic rice showed the highest values, well higher than one.

4. Discussion

Mycotoxins are natural toxic compounds produced by fungal species; if high levels of contamination are present in food, they cause health hazards and even death in humans and animals. In the present study, results revealed that the different types of mycotoxins naturally occur in rice samples. Several studies conducted in Iran deal with the occurrence of mycotoxins in rice (Table 9).

Mycotoxins contamination was also in agreement with previous findings in IR Iran [57,59]. Nazari et al. [59] indicated that FB_1 was observed in 6% of imported rice samples with a mean level of 75.37 mg kg^{-1} [59]. In another survey [62], reported that 9.2 % of rice samples contaminated with FB_1 with a mean level of 110.6 mg kg^{-1} and ranged 54.48–176.58 mg kg^{-1} [62]. Several studies have been published about contamination of cereals and foods with mycotoxins in Malaysia, Nigeria, Korea, UK, Japan, China and Beirut [63–69].

Our finding indicated about 40 % of samples were contaminated with AFT at mean level of 15.13 µg kg^{-1} which was lower than national maximum limit for AFT in rice (30 µg kg^{-1}). AFB_1 was found in 15 out of 80 rice samples in the range of 0.03 to 184.66 µg kg^{-1}. Among 80 evaluated samples 3.75 % were contaminated with OTA above the maximum permitted level (5 µg kg^{-1}).

Tavakoli et al. [60] indicated that 54.8 % samples of imported rice samples were contaminated with AFB_1 [60] In another study, 76.97 % of samples were contaminated by AFT with the mean level of 0.671 ng g^{-1} [60,61]. AFB_1 contents were considerabely higher in Iranian rice samples compared to imported samples measured AFB_1. The maximum and minimum contents of AFB_1 in Iranian rice samples ranged from 0.1-184.77 µg kg^{-1}. The maximum AFT level was obtained in Iranian rice samples (205.29 µg kg^{-1}). Among imported rice samples, the maximum levels of AFB_1 and AFT were in the ranged from 0.1 to 7.41 and 1.0-8.40 µg kg^{-1}.

Our findings indicated that OTA was positive in 69 out of 100 rice samples with a maximum and mean level of 46.79 µg kg^{-1} and 1.5 µg kg^{-1}. Three samples were above the ML for OTA in rice that set in Iran (5 µg kg^{-1}). Contamination of rice samples with ZEN and DON were lower than the Institute of Standard and Industrial Research (ISIRI) limits [51]. The mean level of FB_1 in samples was too lower than the MLs for total FBs set by Institute of Standard and Industrial Research (ISIRI) limits [51]. ZEN, DON and FB_1 were found in 39 %, 16 % and 62 % of the samples with mean levels of 6.1 µg kg^{-1}, 102.2 µg kg^{-1} and 99 µg kg^{-1}, respectively. No detectable level was detected for FB_2. Our finding is in agreement with other research in Iran that indicate high incidence of AFB_1 in rice [57,59,61].

Rashedi et al. [58] found that 2 out of 20 samples were contaminated to ZEN with a mean level of 89 mg/kg [58]. Nazari et al. [59] reported that ZEN was detected in 35 % of imported rice samples in Iran with mean level of 60.38 mg kg^{-1}. ZEN contamination in domestic rice samples is consistent with previous reports from Iran [59] and the contamination levels were lower than LOQ value of ZEN. FB_1 contamination occurred in 62 % of rice samples with range from 1.72 to 14.73 mg kg^{-1} that are in agreement with the findings of Alizadeh et al. [70] who reported FB_1 contamination in 40.9 % of rice samples with mean level of 21.6 mg kg^{-1} [70]. Rice is considered as the main food in diet, thus toxigenic fungi and their metabolites are a significant problem worldwide [71]. In IR Iran, natural contamination of fungi and mycotoxin production is related to the humid subtropical climate during the rice-growing season and storage conditions [57,59,61,62].

The data presented here in suggest a low incidence of DON, ZEN and FBs in rice samples, and are in agreement with those of other studies [59, 61]. Co-occurrence of AFB_1 contamination with FB_1, OTA and DON were observed in 35.6 %, 32.67 % and 27.7 % of rice samples, respectively. Co-occurrence of OTA and AFB_1 was observed in 2% of the samples. This result is of particular importance given the potential effect of synergism and the combined effects of these mycotoxins on human health. Such simultaneous contamination of toxins has also been observed in other studies [59,65,66].

There is little data on daily dietary exposure of mycotoxins in rice in Iran. In a survey of ZEA that conducted by Yazdanpanah et al. [72] on the 72 samples of rice, bread, puffed corn snack and wheat flour the exposure assessment of the Tehran population to ZEA was much lower than the tolerable daily intake estimated by JECFA. The dietary intakes of ZEA for male and female adults were 0.00297 and 0.00478 µg/kg bw/day, respectively, which was lower than the values in this study [72]. In the study on exposure assessment of Tehran population to AFB_1, the mean estimated daily intake of AFB_1 from all analyzed foods was 3.62 ng/kg bw/day, and the mean dietary intake of AFB_1 from rice alone was estimated 3.49 ng/Kg bw/day which is 3.5 times higher than the guidance value of 1 ng AF/kg bw/day [73]. This result is in agreement with the present study.

The exposure of consumers to aflatoxins in Tehran (adults and children) was above the toxicological reference values of AFT at all consumption levels. Also, the maximum exposure of adults and children of domestic rice consumer to OTA in Tehran was above the toxicological reference values of OTA. Combined intake of different mycotoxins at different concentration levels may lead to a higher risk than their single intake [74]. Nonetheless, we can conclude that the dietary exposures associated with the consumption of rice are considered a risk for public health.

There have been many reports about dietary exposure to toxigenic mycotoxins from different countries, which have concentrated on staple foods such as rice [69,75–77]. Epidemiological studies indicate that contamination of food with AFB_1 is the major risk factor for human liver cancer. Areas with a high exposure to AFB_1 coincide with areas with a high prevalence of HCC [78]. According to the results, the population in Tehran is at a high risk due to AFB_1 exposure through rice consumption.

According to finding of estimated exposure and risk assessment for AFB_1 in rice in Japan, cancer risk and MOE were 0.04 and 107, respectively. Worldwide occurrence of aflatoxins showed that cancer risk and MOE of cereal including rice was $0.057-0.467$ and $56-10$, respectively. Further reports from Africa, Brazil, China and Japan for aflatoxins indicated that cancer risk and MOE from different cereal including rice were estimated $0.1-70.1$ and $121.4-0.2$; $0.0731-0.0753$ and $25.8-25.0$; $0.003-0.2$ and $24.7-0.5$; 0.0021 and 209 respectively [79].

The study of Alizadeh et al., showed fumonisin contamination of foods, especially in rice as the most commonly used cereal. They concluded that rice may be considered as a risk factor for esophageal cancer (EC) in Golestan Province in IR Iran [70]. Rheeder et al. [80] and Marasas [81] proposed positive correlation between fumonisin contamination of cereals and the risk of EC [80,81].

Although our results showed that mean and median HI were below one, in real life, consumers are exposed to mycotoxins through consumptions of other foods too. As far as we know, this study reports, for the first time, the risk assessment of multiple-mycotoxins through domestic and imported rice consumption in adults and children in Tehran.

This study also provides the first insight on the cancer risk and MOE of AFB_1 associated with rice intake in Tehran. Human health risk valuation from aflatoxins contact via rice by children and adults showed a significant adverse health risk to humans since all calculated values for MOE was below 10,000. Since mycotoxins are natural food contaminants and considering toxicity of AFT as carcinogenic and mutagenic toxins, special attention should be dedicated to the AFT contamination of foods. Therefore, this investigation will allow health authorities to

assess mycotoxin contamination in foods in domestic markets and use the results as a reference for food management.

5. Conclusions

In the present study the results indicated that the levels of AFT, AFB_1, DON, ZEN and FUMs in Iranian rice samples were higher than imported samples. In total domestic and imported samples date showed that, sixteen rice samples exceeded the maximum limit for AFB_1 in rice that set in Iran (5 µg kg^{-1}). The estimated AFB_1 intake for the adults and children of Tehran through rice consumption was higher than established for both carriers and non-carriers of hepatitis B virus. This study provides the first insight on the cancer risk and MOE of AFB_1 associated with rice intake in Tehran. Cancer risk in adult and child populations due to consumption of domestic rice was higher than cancer risk due to consumption of imported rice.

Totally, the population in Tehran could be at risk of cancer due to AFB_1 exposure through rice consumption and considering the real-life exposure scenario in which an individual is exposed to mycotoxins from other foods even if mycotoxins are found at very low concentrations, long-term exposure of combinations of other compounds might have the potential to induce adverse health effects.

Therefore, further studies are necessary for the monitoring mycotoxins in different food products as well as estimating average dietary exposure and health risk assessment of mycotoxins for main foods in IR Iran. For management strategies, it is needed to focus more on the reduction of mycotoxins contamination. There is a variety of risk management options regard to mycotoxins that help to ensure a safe food supply. These range from prevention of mould growth and setting of regulatory limits, to diversion into alternate uses.

CRediT authorship contribution statement

Samira Eslamizad: Risk assessment methodology, Writing-Reviewing and Editing. **Hassan Yazdanpanah:** Conceptualization, Consultant, Writing- Reviewing and Editing. **Zahra Hadian:** Conceptualization, Management, Writing- Reviewing and Editing. **Christina Tsitsimpikou:** Reviewing and Editing. **Marina Goumenou:** Reviewing and Editing. **Mohammad Hossein Shojaee AliAbadi:** Methodology, Validation. **Mahdie Kamalabadi:** Writing. **Aristides Tsatsakis:** Reviewing and Editing.

Declaration of Competing Interest

There is no conflict of interests.

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