International Journal of
Medical Toxicology & Forensic Medicine

Summer 2022, Volume 12, Number 3

Research Paper

Survey of the Presence of Nivalenol and Deoxynivalenol in Wheat Flour Factories of Khuzestan Province, Iran

Zahra Nazari Khorasgani[1, 2], Mojtaba Mahdavi[3*], Heibatullah Kalantari[3], Mehdi Goudarzi[4]

1. Deputy of Food & Drug, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
2. Nanotechnology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
3. Department of Toxicology, Pharmacy School, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
4. Medicinal Plant Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Use your device to scan and read the article online

Citation Nazari Khorasgani Z, Mahdavi M, Kalantari H, Goudarzi. Survey of the Presence of Nivalenol and Deoxynivalenol in Wheat Flour Factories of Khuzestan Province, Iran. International Journal of Medical Toxicology and Forensic Medicine. 2022; 12(3):35842. https://doi.org/10.32598/ijmtfm.vi.35842

https://doi.org/10.32598/ijmtfm.vi.35842

Article info:
Received: 18 Aug 2021
First Revision: 02 Feb 2022
Accepted: 16 Mar 2022
Published: 25 May 2022

Keywords:
Nivalenol, Deoxynivalenol, Wheat flour, MycoSep column, HPLC, Iran

ABSTRACT

Background: Wheat is frequently contaminated by Deoxynivalenol (DON) and Nivalenol (NIV), which are type B trichothecenes produced by Fusarium fungi. Most problems related to these contaminants involve prolonged feed intake at low levels of contamination. This study investigated the occurrence of NIV and Deoxynivalenol (DON) in wheat flour in flour factories in Khuzestan Providence, Iran.

Methods: In total, 104 samples were collected in this study. An acetonitrile/water mixture (84:16, v/v) was used to extract the samples. The extracts were filtered and purified using a Whatman No. 4 paper and MycoSepTM 227 column. Then, they were evaporated to dryness at 40˚C under a nitrogen stream. After dissolving the dry residue in the mobile phase, containing a mixture of methanol, acetonitrile, and water (5:5:90, v/v/v), the contents of NIV and DON in the samples were measured in High-Performance Liquid Chromatography (HPLC) system with a column C18 (150 mm×4.6 mm ID, 5 µm) and a UV detector (220 nm).

Results: The results showed that among 104 wheat flour samples, 28 (26.8%) and 54 (51.9%) samples were contaminated with NIV and DON, respectively. The mean and maximum concentrations were 118.75 and 2278 ng/g for NIV, and 593 and 67.88 ng/g for DON, respectively.

Conclusion: Based on the findings, DON and NIV had significantly lower concentrations than the maximum tolerated level (1 µg/g), established by the Institute of Standards and Industrial Research of Iran. Therefore, there were no health risks for consumers at the studied contamination levels.

*** Corresponding Author:**
Mojtaba Mahdavi, PhD.
Address: *Department of Toxicology, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.*
Tel: *+98 (61) 33738378*
E-mail: *mahdavi.mojtaba.tox@gmail.com*

1. Introduction

Wheat is known as an essential product for humans. It is the most consumed agricultural product. Various microorganisms, especially fungi, can contaminate wheat in fields or barns [1]. Trichothecenes are secondary fungal metabolites that can often infect products, such as wheat, corn, barley, sunflower seed, and sorghum during the colonization of Fusarium [2]. Under appropriate conditions, fungus-producing toxic trichothecenes are saprophytes and or plant pathogens that can contaminate plants and agricultural products. Therefore, no doubt that trichothecenes are present in nature, and inevitably, humans and animals are exposed to foods contaminated with these fungal toxins.

The widespread presence of fungus-producing trichothecenes in cereals, animal foods, and vegetables indicates the importance of these metabolites as one of the most important mycotoxin groups or natural toxins [2-4]. Deoxynivalenol (vomitoxin, DON) and Nivalenol (NIV) are classified as type B trichothecenes with similar toxicities [5, 6]. The natural presence of NIV and DON in wheat flour, popcorn, and commercial human food has been recorded in Japan and other countries [7]. Fusarium Head Blight (FHB) is one of the most serious diseases in cereals (wheat) and maize, caused by changing weather patterns and modern agricultural methods, and is responsible for tremendous damage worldwide. This disease is caused by trichothecene mycotoxins, especially DON and NIV, produced by Fusarium graminearum and Fusarium culmorum [6].

Animal toxicological studies on DON and NIV show that mycotoxins can prevent the synthesis of proteins, RNA, and DNA and cause neurotoxicity and immunotoxicity in mammals. While acute exposure to DON and NIV can induce vomiting in pigs, chronic exposure may lead to delayed growth and reproductive and immune system disorders. The potential effects of this toxin on humans have been shown, especially in epidemiological studies from China, to cause gastrointestinal symptoms, vomiting, diarrhea, and immune system dysfunction [8, 9]. Although DON and NIV lethality is considered negligible for trichothecenes, reports of contamination with these toxins have continuously risen over the past 10 years and have become an issue for food industries [10].

For the protection of animal and human health, regulatory limits have been established for DON in food and feed by at least 37 countries, while there are no regulatory limits for NIV. However, this mycotoxin is also found with DON in contaminated cereals [5, 6]. Based on a risk assessment by the Scientific Committee of the European Commission, a temporary Tolerable Daily Intake (TDI) has been established for NIV and DON (0.7 and 1 µg/kg BW/day, respectively) [5]. Also, according to the Institute of Standards and Industrial Research of Iran (ISIRI), the maximum tolerated level is 1000 µg/kg for DON in wheat flour [11].

Ahvaz City is located in the Southwest of Iran and is the capital of Khuzestan Province, with a common border with Basra Province in Iraq and the Persian Gulf. Due to particular climatic conditions, such as high temperature and humidity in Khuzestan Province, the production of these toxins is highly probable [12, 13]. Constant monitoring of the level of these natural pollutants in food products for the consumption of humans and animals is a necessary step to reduce the risk of mycotoxins. By keeping the level of toxins within the permissible limit, their consequences for human and animal health can be prevented.

No information is available on the content of NIV and DON in wheat flour produced by factories in this area. In this study, regarding the common consumption of bread among citizens, DON and NIV mycotoxins were measured in wheat flour from flour mills in Ahvaz by the HPLC method. The mean values were compared with the permissible limit specified by ISIRI [11], and the daily intake limit by the European Union [7] for these toxins was determined. This study aimed to evaluate the occurrence of Nivalenol and Deoxynivalenol in wheat flour in flour factories in Khuzestan Providence, Iran

2. Materials and Methods

Chemicals

HPLC grade solvents, including deionized water, methanol, and acetonitrile, were used in the experiments. Sigma (St. Louis, USA) supplied NIV and DON standards. After dissolving the standards in acetonitrile at 0.1 mg/mL as a standard stock solution, they were stored at -20˚C. Romer Labs Inc. (USA) also provided the MycosepTM 227 columns. The paper filter (Whatman No. 4) was obtained from Whatman (Maidstone, UK).

Samples

A total of 104 samples were collected from the region factories (Khuzestan, Ahvaz, Mahzyar, and Jonob

flour mill factories). Overall, 26 composite wheat flour samples (10 grab samples) were collected from each factory and sent to the toxicology laboratory of the Toxicology and Pharmacology Department, Pharmacy School, Ahvaz Jundishapur University of Medical Sciences. Until further analysis, the samples were kept under proper conditions.

Apparatus

In this study, we used a 10ADvp HPLC system (Shimadzu, Japan), consisting of an LC-10ADvp pump (Shimadzu), RF-10AXL UV detector (Shimadzu), DGU-14A Degasser (Shimadzu), SCL-10Avp system controller (Shimadzu), and FCL-10ALvp flow controller (Shimadzu) in the isocratic mode with the LC-Solution software. Capital Co. (England) provided the column (4.6×150 mm), which contained silica particles and was modified by octadecylsilyl groups (diameter, 4 µm).

Extraction and Clean-up

For this purpose, after pouring the mixture of acetonitrile and water (100 mL; 84:16, v/v) into the wheat flour sample (25 g), they were mixed for 3 min. Then, a Whatman No. 4 paper was used to filter the extract, and a MycosepTM 227 column was used to purify the filtrate (8 mL) in line with the manufacturer's instructions. After adding 4 mL of the purified extract to another tube, it was evaporated to dryness at 40˚C under a nitrogen stream. Then, 50 µL of the dry residue mixed in the mobile phase (0.4 mL) was injected into the HPLC system.

Quantitative Analysis

In the chromatograms, NIV and DON peaks were determined by comparing their retention time with the corresponding standards, quantified by the HPLC system with a UV detector (220 nm) and a column C18 (150 mm×4.6 mm ID, 5 µm). During the process, the column temperature was kept at 40˚C. Also, at a flow rate of 1 mL/min, a mobile phase of acetonitrile, methanol, and water (5: 5: 90, v/v/v) was used.

NIV and DON content was quantified by measuring the peak areas corresponding to NIV and DON in comparison with the combined NIV/DON working standard solutions in the calibration curve (4, 2, 1, 0.5, 0.25, and 0.125 µg/mL with correlations (r) of 0.9999 and 0.9999 for NIV and DON, respectively). In volumetric flasks to perform recovery, appropriate volumes of standard solutions of NIV and DON were added to spiking blank wheat flour at 0.25, 1, and 2 µg/g (six repeats for each level). The clean-up procedure was followed as discussed above.

Estimation of Dietary Intake of NIV and DON

The following formula was calculated to measure the estimated daily intake (EDI; ng/kg BW/day) [14]:

EDI=(essential daily food intake, g/d)×(mean contamination, ng/g)/kg body weight.

According to a survey from Iran, the average wheat flour consumption is 416 g per person each day [15].

Data analysis

Data are presented as Mean±SD and examined in SPSS software v. 20. Differences in the mean concentrations of NIV and DON and the maximum permitted limit of 1 µg/g were calculated by a 1-sample test. The Kolmogorov-Smirnov normality test was done if variables are normally distributed.

Because the distribution of data was not normal, the nonparametric tests of Kruskal-Wallis and Mann-Whitney were used to compare the mean concentrations of NIV and DON between different factories of wheat flour. $P<0.05$ was considered statistically significant.

3. Results

According to the chromatograms, the Retention Time (RT) values of NIV and DON are respectively 6.08 and 11.73 min (Figure 1). The natural concentration of NIV and DON in 104 wheat flour samples is shown in Table 1. NIV was detected in 26.8% (n=28) of the samples at concentrations of 0-2278 µg/kg (mean=118.75 µg/kg), while DON was found in 51.9% (n=54) of the samples at 0-593 µg/kg (mean=67.88 µg/kg). Based on the findings, the limits of detection and quantification (LOD and LOQ, respectively) were 10 and 37.5 µg/kg, respectively.

Table 2 indicates the Mean recovery, as well as the relative SD (RSD %) for the analytical method of NIV and DON content in wheat flour. All recoveries for NIV and DON were above 88.3% and 90.5%, respectively, suggesting the high accuracy of this method. Figure 2 shows the chromatogram of the spiked wheat flour sample (2 µg/mL for NIV and DON). The ISIRI maximum tolerated level (1 µg/g) was significantly higher than the mean concentrations of NIV and DON in the samples ($P<0.001$).

Table 1. Descriptive statistics of Nivalenol (NIV) and Deoxynivalenol (DON) concentration in investigated samples

Factories	Mycotoxin Type	No.	No.(%)	Max, ng/g	Mean±SD (ng/g)
Jonob Flour	DON	26	17(65.4%)	593	78.34±117.45
	NIV	26	3(11.5%)	186	18.61±53.18
Khuzestan Flour	DON	26	10(38.5%)	147	36.34±51.77
	NIV	26	6(23.1%)	2278	199±566.54
Ahvaz Flour	DON	26	16(61.5%)	378	76.96±90.72
	NIV	26	13(50%)	1559	214.28± 409.03
Mahzyar Flour	DON	26	11(42.3%)	536	79.88±127.04
	NIV	26	6(23.1%)	248	42.61±84.17
Factories Total	DON	104	54(51.9%)	593	67.88±101.24
	NIV	104	28(26.8%)	2278	118.75±358.98

International Journal of Medical Toxicology & Forensic Medicine

Table 2. Recoveries and RSD for NIV and DON from spiking into one of the wheat flour samples (n=6)

Sample	Mycotoxin Type	Level (µg/mL)	Mean±SD (%)	RSD (%)
Wheat flour	DON	0.25	90.5±3.5	3.95
		1	92.9±2.9	3.19
		2	95.8±3.6	3.83
	NIV	0.25	88.3±2.9	3.36
		1	93.1±4.45	4.88
		2	94.24±4.34	4.7

International Journal of Medical Toxicology & Forensic Medicine

Using the Kruskal Wallis test, comparing the mean DON contamination level in industrial samples showed no significant difference among factories (P>0.05), while a significant difference was found for NIV. Based on the Mann-Whitney test, differences were found between Ahvaz, Jonob, and Mahzyar factories, while other factories showed no differences. The EDI of NIV and DON in wheat flour was 0.705 and 0.403 µg/kg BW/day, respectively (Table 3).

4. Discussion

Several methods and approaches have been designed to measure NIV and DON content in different agricultural commodities [10]. However, there is no available

Table 3. EDI of NIV and DON from wheat flour to body weight (Assuming 70 kg)

Sample Food	Mycotoxin Type	Amount (g/d)	Flour (ng/g)	Estimated Daily Intake (µg/kg bw/d)
Wheat flour	NIV	416	118.75	0.705
	DON	416	67.88	0.403

International Journal of Medical Toxicology & Forensic Medicine

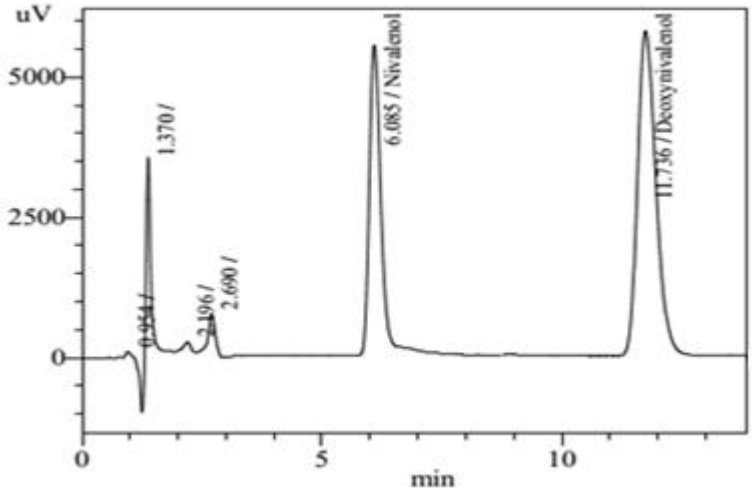

Figure 1. High-performance liquid chromatography-UV Chromatogram of 2 µg/mL nivalenol and deoxynivalenol standard solution

technique for determining these mycotoxins in wheat flour, using HPLC with MycoSep column clean-up in Iran. This study confirms that using MycoSep column clean-up before HPLC-UV is a suitable method for quantification of NIV and DON in wheat flour. The results indicated that the concentration of DON in all the investigated samples was below the maximum tolerated level by ISIRI and is safe for human consumption [11].

Our findings showed that the mean and maximum level of DON contamination in wheat flour samples was lower than the numbers reported in several studies [1, 16, 17].

Tunisia, Bensassi et al. reported DON concentrations of 7200 to 54000 µg/kg in wheat flour (n=65; 83% positivity) [16]. In addition, Pinto et al. found 300-70000 µg/kg of DON in wheat samples from Argentina (n=19; 78.9% positivity) [1]. Li Cui et al. also reported DON levels of 259-4972 µg/kg in China (n=50; 89.3% positivity) [17].

According to LOD, the analytical performance in our study was lower than or similar to other studies [17-19].

Exposure assessment is an essential component in the risk evaluation of chemical compounds. Long-term exposure to low concentrations of mycotoxins through diet can cause health problems [20]. Considering the results of this study for NIV and DON contamination in the samples, the estimated daily exposure to wheat flour was 0.705 and 0.405 µg/kg bw/d in average consumers,

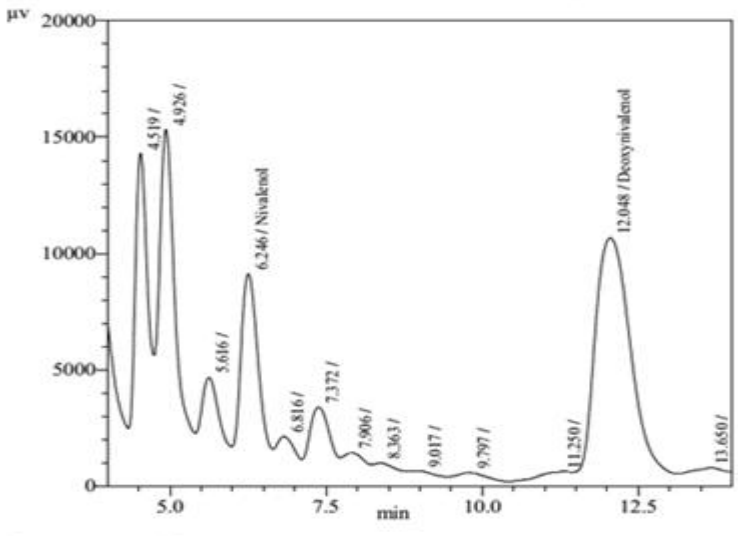

Figure 2. High-performance liquid chromatography-UV chromatogram of spiked wheat flour sample with 2 µg/mL for nivalenol and deoxynivalenol

respectively. These contamination levels are below the standards for wheat flour consumption (0.7 and 1 µg/kg bw/d). At this level of contamination, there were no health risks for consumers [21].

Climate majorly influences wheat contamination with Fusarium graminearum and Fusarium culmorum [22]. Considering the tropical conditions of Khuzestan, the wheat harvest season begins mainly from late April and early May (rainy season). Fusarium graminearum and Fusarium culmorum attack before harvest, and farms are consequently exposed to FHB disease and the natural occurrence of these toxins. Therefore, there is a need to monitor different climatic conditions; this might be one of the reasons for low to average concentrations of these toxins in the present study.

5. Conclusion

Based on the findings, in wheat flour samples, the mean DON and NIV concentrations were significantly lower than the ISIRI maximum threshold (1 µg/g). Therefore, at these contamination levels, consumers were not exposed to any health risks.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This study was part of a Master's thesis submitted by Mojtaba Mahdavi, granted by the Deputy of Research of Ahvaz Jundishapur University of Medical Sciences (No.: FDRC-5).

Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

References

[1] Pinto VE, Terminiello LA, Basilico JC, Ritieni A. Natural occurrence of nivalenol and mycotoxigenic potential of Fusarium graminearum strains in wheat affected by head blight in Argentina. Brazilian Journal of Microbiology. 2008; 39(1):157-62. [DOI:10.1590/S1517-83822008000100031] [PMID] [PMCID]

[2] Moosavi M, Rezaei M, Kalantari H, Behfar A, Varnaseri G. l-carnitine protects rat hepatocytes from oxidative stress induced by T-2 toxin. Drug and Chemical Toxicology. 2016; 39(4):445-50. [DOI:10.3109/01480545.2016.1141423] [PMID]

[3] Sudakin DL. Trichothecenes in the environment: Relevance to human health. Toxicology letters. 2003; 143(2):97-107. [DOI:10.1016/S0378-4274(03)00116-4] [PMID]

[4] World Health Organization (WHo). Joint FAO/WHO Expert Committee on Food Additives. Meeting (71st: 2009: Geneva, Switzerland) & International Programme on Chemical Safety. Safety evaluation of certain food additives: prepared by the Seventy-first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). World Health Organization; 2010. https://apps.who.int/iris/handle/10665/44269

[5] EFSA Panel on Contaminants in the Food Chain (CONTAM). Scientific opinion on risks for animal and public health related to the presence of nivalenol in food and feed. EFSA Journal. 2013; 11(6):3262. [DOI:10.2903/j.efsa.2013.3262]

[6] Sugita-Konishi Y, Nakajima T. Nivalenol: The mycology, occurrence, toxicology, analysis, and regulation. In: Rai M, Varma A editors. Mycotoxins in food, feed and bioweapons. Berlin: Springer; 2010. [DOI:10.1007/978-3-642-00725-5_15]

[7] Schothorst RC, van Egmond HP. Report from SCOOP task 3.2.10 collection of occurrence data of fusarium toxins in food and assessment of dietary intake by the population of EU member states. Subtask: Trichothecenes. Toxicology Letters. 2004; 153(1):133-43. [DOI:10.1016/j.toxlet.2004.04.045] [PMID]

[8] Pestka J. Toxicological mechanisms and potential health effects of deoxynivalenol and nivalenol. World Mycotoxin Journal. 2010; 3(4):323-47. [DOI:10.3920/WMJ2010.1247]

[9] Pestka JJ. Deoxynivalenol: Toxicity, mechanisms and animal health risks. Animal Feed Science and Technology. 2007; 137(3):283-98. [DOI:10.1016/j.anifeedsci.2007.06.006]

[10] Ran R, Wang C, Han Z, Wu A, Zhang D, Shi J. Determination of deoxynivalenol (DON) and its derivatives: Current status of analytical methods. Food Control. 2013; 34(1):138-48. [DOI:10.1016/j.foodcont.2013.04.026]

[11] Annola K, Karttunen V, Keski-Rahkonen P, Myllynen P, Segerback D, Heinonen S, et al. Transplacental transfer of acrylamide and glycidamide are comparable to that of antipyrine in perfused human placenta. Toxicology Letters. 2008; 182(1-3):50-6. [DOI:10.1016/j.toxlet.2008.08.006] [PMID]

[12] Behfar A, Nazari Khorasgani Z, Alemzadeh Z, Goudarzi M, Ebrahimi R, Tarhani N. Determination of aflatoxin m1 levels in produced pasteurized milk in ahvaz city by using HPLC. Jundishapur Journal of Natural Pharmaceutical Products. 2012; 7(2):80-4. [DOI:10.5812/jjnpp.4707] [PMID] [PMCID]

[13] Jalali A, Khorasgani Z, Goudarzi M, Khoshlesan N. HPLC determination of patulin in apple juice: A single-center study of Southwest area of Iran. Journal of Pharmacology and Toxicology. 2010; 5(5):208-14. [DOI:10.3923/jpt.2010.208.214]

[14] dos Santos JS, Souza TM, Ono EYS, Hashimoto EH, Bassoi MC, de Miranda MZ, et al. Natural occurrence of deoxynivalenol in wheat from Paraná State, Brazil and estimated daily intake by wheat products. Food chemistry. 2013; 138(1):90-5. [DOI:10.1016/j.foodchem.2012.09.100] [PMID]

[15] Yazdanpanah H, Zarghi A, Shafaati AR, Foroutan SM, Aboul-Fathi F, Khoddam A, et al. Exposure assessment of the Tehran population (Iran) to zearalenone mycotoxin. Iranian Journal of Pharmaceutical Research. 2012; 11(1):251. [PMID] [PMCID]

[16] Bensassi F, Zaied C, Abid S, Hajlaoui MR, Bacha H. Occurrence of deoxynivalenol in durum wheat in Tunisia. Food Control. 2010; 21(3):281-5. [DOI:10.1016/j.foodcont.2009.06.005]

[17] Cui L, Selvaraj JN, Xing F, Zhao Y, Zhou L, Liu Y. A minor survey of deoxynivalenol in fusarium infected wheat from the Yangtze-Huaihe river basin region in China. Food Control. 2013; 30(2):469-73. [DOI:10.1016/j.foodcont.2012.08.011]

[18] Setyabudi F, Nuryono N, Wedhastri S, Mayer H, Razzazi-Fazeli E. Limited survey of deoxynivalenol occurrence in maize kernels and maize-products collected from Indonesian retail market. Food Control. 2012; 24(1):123-7. [DOI:10.1016/j.foodcont.2011.09.013]

[19] Barros G, García D, Oviedo S, Ramirez M, Torres A, Chulze S. Deoxynivalenol and nivalenol analysis in soybean and soy flour. World Mycotoxin Journal. 2008; 1(3):263-6. [DOI:10.3920/WMJ2008.1052]

[20] Ames BN. Dietary carcinogens and anticarcinogens oxygen radicals and degenerative diseases. Science. 1983; 221(4617):1256-64. [DOI:10.1126/science.6351251] [PMID]

[21] Leblanc J-C, Tard A, Volatier J-L, Verger P. Estimated dietary exposure to principal food mycotoxins from the first French total diet study. Food Additives & Contaminants. 2005; 22(7):652-72. [DOI:10.1080/02652030500159938] [PMID]

[22] Canady RA, Coker RD, Egan SK, Krska R, Kuiper-Goodman T, Olsen M, et al. Deoxynivalenol. Geneva: World Health Organisation. [Link]

This Page Intentionally Left Blank