

## Survey of Fumonisin B1 Contamination of Corn in Northern Iran During 2000

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### Abstract

Fumonisin B1 (FB1) is the most abundant of the fumonisin mycotoxins, mainly produced in corn by fungi of the genus *Fusarium*. FB1 has been shown to be hepatocarcinogenic and nephrocarcinogenic in animals. Contamination of corn with FB1 was assayed in samples collected from Mazandaran Province, situated on the Caspian littoral of Iran, in September 2000. In this survey, 38 corn samples were analyzed using HPLC, and all except one showed high levels of FB1 contamination ranged between 1.19 and 12.95 mg/kg. These results confirm the relatively high levels of FB1 contamination in corn of Mazandaran Province.

**Keywords:** Fumonisin; Iran; Corn.

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### Introduction

The fumonisin mycotoxins are produced in corn by a small number of related *Fusarium* species, of which *F. verticillioides* and *F. proliferatum* are the most important as they are common contaminants of corn in many areas of the world (1). There are at least 28 fumonisin analogues that have been identified and these have been classified into series A, B, F and P based on their chemical structures (2, 3), of which fumonisin B1 (FB1) is the most abundant of the group (4). FB1 has been shown to be hepatocarcinogenic and nephrocarcinogenic in the male rat and hepatocarcinogenic in the female mouse (5, 6) and on the basis of

available evidence, the International Agency for Research on Cancer (IARC) has declared the 'toxins produced by *Fusarium moniliforme*' to be possibly carcinogenic to humans (Class 2B carcinogens).

Determining the natural occurrence of fumonisin in human foods and animal feeds is important in establishing the health risk associated with the toxin because global surveys indicated that fumonisins occur naturally at biologically significant levels in corn and a variety of corn-based human foodstuffs and animal feed worldwide (7). There are considerable differences in the extent of human exposure between different maize growing regions. The estimated mean intake of FB1 ranged from 0.2 µg/kg body weight/day in the European type diet to 2.4 µg/kg body weight/day in the African type diet (8). A PMTDI

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(provisional maximum tolerable daily intake) of 2 µg/kg body weight/day for FB1, FB2 and FB3, alone or in combination has been suggested by JECFA (9).

So far, two studies have been performed concerning natural occurrence of fumonisins in corn of Mazandaran Province, I.R. Iran during 1998 and 1999. The results of these studies, which were done in PROMEC (South Africa) and Shaheed Beheshti School of Pharmacy (I. R. Iran) showed relatively high levels of fumonisin in this region (10, 11).

As it was already mentioned, determining the natural occurrence of fumonisin in human foods and animal feeds in different years is important in establishing the health risk associated with the toxin. This study was performed to assess annual variation in FB1 concentration of corn of this region, which was regarded as one of the world's 'hot spots' for esophageal cancer (12).

## Materials and methods

### Sampling

In September 2000, 38 preharvested healthy maize samples from farms of Dasht-e-Naz region in Mazandaran Province, north of Iran, were randomly collected. The sample size was determined based on previous studies which were done concerning FB1 contamination in corn samples of this region. All corn samples were belonged to variety of single cross 704 (sc.704). Each sample consisted of 5 kg of maize ears collected randomly from 10 different points of a farm (0.5 kg, approximately 3 ears per point). Corn ears of each sample, after shelling, were placed in the open air for 2 days. This is an acceptable procedure for drying of samples in field studies. Then samples were milled, mixed and kept at 4°C until analysis.

### Determination of FB1

Each sample was analyzed for the presence of FB1 using the method described in the VICAM Fumonitest Instruction Manual (VICAM 1998) with some modification (13).

Samples (20 g) were extracted in a blender jar by adding 2 g NaCl, 100 ml methanol- water (75:25, v/v) and blending at high speed for 9 min. Extracts were filtered through a fluted

filter paper and 10 ml aliquots were diluted with 40 ml phosphate buffered saline (PBS) and mixed well. Diluted extracts were then filtered through a glass microfiber filter. Ten ml aliquots were passed through Fumonitest affinity column (VICAM, Watertown, USA) at a rate of about 1-2 drops/second until air came through the column. The columns were then washed with 10 ml PBS at the same rate until air came through columns. Fumonisin were eluted from the column with 1.5 ml HPLC grade methanol under gravity. Eluates were dried down under nitrogen gas.

Residues were redissolved in 200 µl acetonitrile-water (50:50, v/v), then 25 µl of this solution was mixed with 225 µl *o*-phthalaldehyde (OPA) reagent, and injected into HPLC exactly 2 min after adding OPA reagent. The detection limit of the method was 0.014 mg/kg.

The accuracy and precision of the method were verified using recovery experiments. First, some samples were ground, thoroughly homogenized and analyzed for FB1. After finding blank corn samples, the ground samples were spiked with different levels of FB1. Mean recoveries from corn spiked at 0.5, 1 and 2 mg/kg were 99.2% (two samples, RSD 4.6%), 82.4% (seven samples, RSD 20.7%) and 86.8% (seven samples, RSD 12.4%), respectively. All of the recoveries were in the acceptable range. Analytical values were not corrected for recovery. Regarding the precision, the RSD obtained were lower than RSD calculated from the Horwitz equation (14). In addition, we (Toxicology lab, Shaheed Beheshti School of Pharmacy) participated in an international proficiency testing (FAPAS® series 22 round 08, November 2002) as an external quality control. The acceptable range is  $+2 > z\text{-score} > -2$ . In our experiment, the obtained *z*-score was 0.4 which is very satisfactory (FAPAS Report No. 2208) (15).

## Results and discussion

The results of the occurrence of FB1 in healthy corn from Mazandaran Province during 2000 are given in Table 1. As observed, all samples except one are positive for FB1 at

levels varying from 1.19 to 12.95 mg/kg with a mean level of 5.67 mg/kg. Figure 1 shows the level of contamination of corn with FB1 over the 3-year period (1998-2000) in this region. A comparison of these results indicates higher mean and maximum levels during 2000 than those found during 1999 (mean=3.18, max=7.66 mg/kg) and 1998 (mean=2.27, max=3.98 mg/kg) (10, 11). The mean concentration of FB1 over 3 consecutive years (1998-2000) was 4.4 mg/kg, which is higher than the recommended Iranian level of 1000 µg/kg for FB1 and FB2 combined (16). The Iranian maximum tolerated limits for mycotoxins in foods and feeds have been set by Institute of Standard and Industrial Research of I.R. Iran in 2002 (16).

Worldwide survey of fumonisin contamination of corn and corn-based products demonstrated the general distribution of fumonisin around the world, which reflects the ubiquitous nature of the main producing fungi (17), but limited data were available on annual variation in fumonisin concentration in maize harvested in consecutive years.

Surveys conducted annually from 1988 to 1991 in Iowa in the mid Western USA showed high levels of FB1 contamination with annual means between 2.5 and 3.3 mg/kg (18), whereas the mean value for corn samples analyzed from Iowa in 1992 yielded contrasting results and showed a dramatic decrease in FB1 contamination with a mean of 0.05

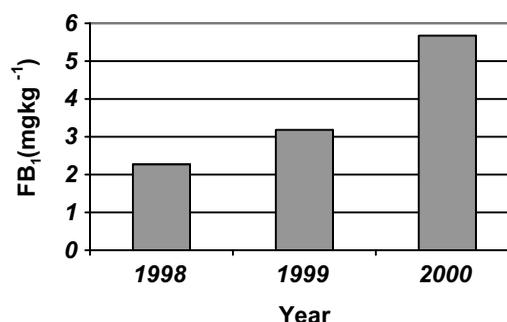


Figure 1. FB1 levels in Mazandaran corn samples during 1998-2000.

mg/kg (19). Similar data were available for South Africa between 1989 and 1993, which show the variation from 0.17 up to 0.68 mg/kg in yellow corn but relatively low variation in white corn (17). The average concentration of FB1 in Linxian county, China, has been varied from 0.68 in corn in 1989 (20) to 30.4 mg/kg in fine corn in 1991 (21). The FB1 mean concentrations over 3 consecutive years (1998-2000) in corn of Dasht-e-Naz region in Mazandaran Province, northern Iran showed considerable variation, too. On the other hand, FB1 surveys in Isfahan Province, I. R. Iran over 2 consecutive years (1998-1999) showed that the mean concentration of FB<sub>1</sub> is 0.2 mg/kg (10, 11), which is quite below than the recommended Iranian maximum level of 1 mg/kg for fumonisins (16). The mean FB1 level (0.2 mg/kg) is comparable to the mean reported for fumonisins from South Africa during 1989-1993 and Pennsylvania, USA in 1992 (17, 19). Although the corn samples of Dasht-e-Naz

Table 1. FB1 levels in maize from Mazandaran Province in Iran during 2000

Sample	FB <sub>1</sub> (mg/kg)	Sample	FB <sub>1</sub> (mg/kg)	40 Sample41	FB <sub>1</sub> (mg/kg)
1	5.56	14	8.61	27	5.56
2	5.38	15	2.87	28	7.80
3	9.47	16	5.73	29	7.35
4	2.58	17	9.30	30	5.30
5	8.64	18	3.67	31	6.83
6	6.67	19	3.51	32	6.38
7	8.91	20	9.43	33	6.43
8	1.45	21	7.67	34	1.82
9	1.53	22	8.03	35	5.85
10	1.85	23	8.52	36	1.19
11	12.95	24	2.22	37	Nd <sup>a</sup>
12	2.24	25	7.59	38	5.19
13	6.97	26	7.16		

Mean ± SD= 5.67 ± 2.99 mg/kg (mean of all samples).

Median = 5.79 mg/kg (median of all samples).

region in Mazandaran Province were highly contaminated with FB1, only 7.5% of these samples were contaminated with zearalenone and the contamination levels were lower than maximum tolerated limits for this mycotoxin in Iran (22). In fact, the corn of this region due to the favorable environmental conditions (humidity and temperature) is susceptible to contamination with fumonisins. In this study, we analyzed fumonisins in corn and other parameters including water activity were not determined. Therefore, from agricultural point of view, future efforts should be made to insect control and hybrid evaluation to determine which hybrid has the greatest resistance to *Fusarium* fungi. On the other hand, the announcement by the FDA of draft guidance levels for industry, the moves within various European countries to set tolerance limits on fumonisin contamination of corn and recent decision by JECFA to assign a PMTDI of 2 mg/kg body weight/Day, will lead to further surveillance of the natural occurrence of fumonisins. This will improve our knowledge of both actual levels as well as the annual variations that may occur in many areas of the world.

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