Detection of ochratoxin A in cereal samples in different regions of India
2.1 INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by various fungi which affect a wide range of agricultural products meant for human consumption and animal feed. Ochratoxin A (OTA) is one such mycotoxin produced by some species of Aspergillus and Penicillium, particularly A. ochraceus, A. carbonarius, A. niger and P. verrucosum predominantly found in cereal grains, cocoa, spices, oilseeds, coffee beans and legumes resulting in human exposure. However, wheat and its products are the major group of food commodities where this toxin has the greatest impact, due to its usage as staple food for majority of world population. Therefore, concern of food contamination with OTA represents an important source of daily OTA exposure to human beings which may have adverse health implications.

Toxicity of OTA is well documented in many animal species, including human beings (CAST, 2003). OTA has been shown to possess carcinogenic, immunosuppressive, hepatotoxic, and teratogenic potential in several animal species (Kuiper-Goodman and Scott, 1989; Krogh, 1992), and has been classified as a possible carcinogen to humans (Group 2B) by the International Agency for Research on Cancer (IARC) (IARC, 1993). In addition, it is suspected to be involved in Balkan endemic nephropathy (BEN), a chronic tubulointerstitial renal disease, occurring in certain regions of the Balkan Peninsula and is associated with urinary tract tumors (Fink-Gremmels, 2005) which having similarity with OTA induced porcine nephropathy (WHO, 1965; Fuchs and Peraica, 2005; Krogh, 1972). Furthermore, various survey studies in the endemic areas like Yugoslavia and Bulgaria demonstrated that the food commodities like wheat, maize, barley, beans, etc. and animal feed were contaminated by OTA when compared to the control nonendemic areas (Castegnaro, et al., 2006; Vrabcheva et al., 2004). However, ochratoxin A intake was not assessed during late 60’s and early 70’s BEN (WHO, 1965; Krogh, 1972). Furthermore, OTA has been reported to be quite stable during processing like bread making, etc. (Duarte, Pena, & Lino, 2010). OTA is found
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to be absorbed through gastrointestinal tract and lungs of rat with high efficiency (Kumagai and Aibara, 1982; Breitholtz-Emanuelsson, Fuchs, & Hult, 1995) and shows strong binding to albumin of blood thereby ensuring the prolonged biological half-life (Scott, 2005). It is established that OTA is continuously and unavoidably being ingested with food as its detection in human plasma, serum, blood, breast milk and urine of people from many countries have been reported (Coronel, Sanchis, Ramos, & Marin, 2010; Biasucci et al., 2011; Coronel et al., 2011). Consequently, the European Union Scientific Committee for Human Feeding deemed necessary to take prudential measures in order to set a maximum permissible level of 5 μg kg\(^{-1}\) (5 ppb) in raw wheat, barley and rye (European Commission, 2005). Based on risk assessments and the toxicological data on adverse effects of OTA, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has suggested a Provisional Tolerable Weekly Intake (PTWI) of 100 ng kg\(^{-1}\) bw (JECFA, 2001).

Appreciable levels of OTA contamination in wheat, corn, grapes and their byproducts like beer, wine have been reported from various parts of world (JECFA, 2001; Bayman and Baker, 2006). However, OTA in wheat or other cereals from the Indian food produce have not been studied. It is a matter of concern as wheat is a staple food for majority of north Indian population and the consumers are unaware of the exposure risk to OTA because of inadequate data (PFA, 2008). Moreover, due to the widespread nature of fungi in the environment, mycotoxins are considered unavoidable contaminants in foods and feeds; therefore one of the most effective measures to protect the public health is to establish reasonable regulatory levels of these toxins on the basis of valid toxicological data. Therefore, the objective of this chapter is to analyze OTA contamination in wheat, and assesses the likely intake of OTA in Indian population.
2.2 MATERIALS AND METHODS

2.2.1 Chemicals
Standard OTA (>96% purity) was purchased from Sigma Chemical Company (St. Louis, MO). HPLC grade acetonitrile, chloroform, ortho-phosphoric acid, acetic acid was procured from E. Merck (Mumbai, India).

2.2.2 Procurement of cereal samples
A total of 50 wheat samples were collected from different mandis of Lucknow. Out of 50 samples, 7, 16, 10, 10 and 7 wheat samples were from Bihar, Uttar Pradesh, Delhi, Haryana and Punjab, respectively. 15 samples each of maize and barley were collected from different mandis of Lucknow. Out of 15 maize samples, 8 and 7 samples were from Uttar Pradesh and Bihar, respectively. Whereas, out of 15 barley samples, 6, 6 and 3 samples were from Uttar Pradesh, Haryana and Bihar, respectively. All the cereal samples were stored at 4°C and analyzed within a week.

2.2.3 Extraction of OTA from cereal samples
OTA from cereal samples was extracted according to method described in Solfrizzo, Avantaggiato, & Visconti, 1998. Briefly, cereal samples were grinded using a mixer grinder to obtain a 20-mesh sieve size. The ground samples were mixed properly and 10 g of each sample was extracted with 30 ml chloroform and 2.5 ml 0.1 M phosphoric acid by shaking for 30 min on a rotary shaker. The extract was filtered through a fluted filter using Whatman number 1 filter paper. A 10 ml volume of filtrate was collected and evaporated under nitrogen stream at 60°C using Turbovap® LV concentration workstation (Caliper LifeSciences, MA, USA). The residue was reconstituted in 600 µl of acetonitrile–water–acetic acid (41:58:1, v/v) by vortexing for 1 min. The reconstituted extract was defatted with 1 ml n-hexane by vortexing for another 1 min and centrifuged at 6000 rpm for 20 min. Upper phase was discarded and the lower phase was collected and filtered through 0.45 µm syringe driven filter (Sartorius, Germany) for further analysis.
2.2.4 Analysis of OTA by high performance liquid chromatography (HPLC)

The extracted residual solutions were monitored by HPLC (Waters, Vienna, Austria) using a reversed-phase C18-bonded silica symmetry (5 µm) column (4.6 mm x 250mm) (Waters, Vienna, Austria) by injecting into a Rheodyne injector fitted with a 100 µl loop. Elute was monitored using a fluorescence detector (model #474) at an excitation wavelength of 333 nm and emission wavelength of 460 nm, with the mobile phase (acetonitrile: water: acetic acid; 99: 99: 2, v/v/v) delivered from a pump (model #510) under isocratic conditions at a flow-rate of 1.0 ml min⁻¹. For the determination of the minimum detectable limit, different concentrations of OTA reference standard in duplicate were injected on to the HPLC column under similar chromatographic conditions. The chromatograms were recorded and processed by Waters Millennium software. The peaks of OTA in the samples were identified by comparing the retention time with that of reference standard OTA and quantified by comparing the integrated peak area with that of the reference standard.

2.2.5 Recovery Test

Wheat samples showing no OTA contamination were spiked with known quantities of OTA for recovery testing. To analyze the recovery of OTA from wheat grains, the samples in triplicate were spiked with OTA at a concentration of 100, 20, 10, 5, 2.5 and 1.25 µg kg⁻¹, respectively. The spiked samples were extracted and analyzed by the method given in the previous section.

2.2.6 Limit of Detection and Limit of Quantification

In order to determine the linear response, standard OTA in the concentration range of 1.25-320 ng ml⁻¹ were analyzed on HPLC and the lower three values (in triplicates) of the linear range of the calibration curve were used for determination of LOD. For determination of LOQ, three lower spiked concentrations (triplicates) for percentage recovery experiment were used. The LOD and the LOQ were calculated by using the following equations:
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LOD = 3.3 (σ/S) and LOQ = 10 (σ /S), respectively, where σ is the standard deviation of the response and S is the slope of the corresponding calibration curve (Mocak, Bond, Mitchell, & Scollary, 1997).

2.2.7 LC-MS Confirmation

For additional confirmation, both the standard and wheat extract were subjected to LC-MS analysis which was performed on Thermo Finnigan LCQ advantage max ion trap mass spectrometer having Finnigan surveyor HPLC system connected to it. The column was reversed-phase symmetry column packed with 5 µm C18 bonded silica gel (Waters, Vienna, Austria). The 20 µl sample was introduced into the ESI source through Finnigan surveyor autosampler. The mass spectra were scanned in the range of 150-1000 da and the maximum ion injection time was set at 200 nS. Electrospray ionization in the positive mode was used. The ion spray voltage was set at 5.3 KV and capillary voltage at 40 V. The MS scan run up to 60 min and the spectra’s print outs are averaged of over 4-6 scans at peak top in total ion chromatogram (TIC). The MS data reductions were performed by using Xcalibur 1.4 SR 1.

2.3 RESULTS

2.3.1 Quantitative analysis of OTA in cereal samples

A linear HPLC response to OTA standards was observed for a concentration range of 1.25-320 ng ml\(^{-1}\) injected onto the column, where the lowest point of calibration curve was 1.25 ng ml\(^{-1}\) (Figure 2.1). The correlation coefficient \(r = 0.995\) (n = 3) was obtained for concentration (ng ml\(^{-1}\)) versus peak area with a linear equation as \(y = -2.37 \times 10^3 + 8.19 \times 10^3\) (Table 2.1). The limit of detection (LOD) of OTA was 0.6 ng ml\(^{-1}\). The recovery in spiked triplicate samples ranged from 71% to 92% for the added levels of 100, 20, 10, 5, 2.5 and 1.25 µg kg\(^{-1}\) (Table 2.2) and the limit of quantitation (LOQ) of OTA was found to be 0.71 µg kg\(^{-1}\). The HPLC profile of standard OTA and OTA extracted from wheat sample is shown in Figure 2.2. The retention time (RT) of standard OTA and that of OTA extracted from wheat samples was 12.6 min.
Table 2.3 shows the quantitative analysis of OTA in various wheat samples from different regions of India. Of the 50 wheat samples, 60% were found to be contaminated with OTA within the range of 1.36-21.17 µg kg\(^{-1}\), while the remaining samples had no detectable contamination above the LOD. The highest OTA content (21.17 µg kg\(^{-1}\)) was found in a wheat sample collected from Bihar state. Table 2.4 shows the quantitative analysis of OTA in various maize samples from different regions of India. Only 2 samples from Uttar Pradesh were found to be contaminated with OTA within the range of 3.95-6.48 µg kg\(^{-1}\), while the remaining samples had no detectable contamination above the LOD. Table 2.5 shows the quantitative analysis of OTA in various barley samples from different regions of India. Of the 15 barley samples, 46% were found to be contaminated with OTA within the range of 1.08-5.12 µg kg\(^{-1}\), while the remaining samples had no detectable contamination above the LOD. The highest OTA content (5.12 µg kg\(^{-1}\)) was found in a barley sample collected from Uttar Pradesh.

### 2.3.2 LC-MS confirmation

Since OTA levels were high in a few samples, identification of HPLC peak was considered necessary and the eluted peaks from positive samples were collected for LC-MS analysis. A total ion chromatogram (TIC) of standard OTA and sample extract showed the resolution of OTA in positive ion mode with an RT of 10.85 min (Figure 2.3). The ESI-MS spectra in wheat samples matched the standard, having a major ion peak at 402, thus confirming the presence of OTA in cereal samples (Figure 2.4).

### 2.3.3 Intake pattern of OTA through wheat

Among all the cereals, wheat is the most important and widely consumed cereal in north Indian states. Therefore, north Indian population is at major risk towards exposure to OTA through wheat. The average weekly intake per capita of OTA through wheat for the population of individual states is shown in Table 2.6. The maximum average intake of OTA through wheat was found in Punjab state (249 ng kg\(^{-1}\) bw) as compared with others. However, weekly
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intake based on 95th percentile value of OTA contamination was highest in Uttar Pradesh (466 ng kg\(^{-1}\) bw).

2.4 DISCUSSION

The tropical climate of India is conducive to fungus growth, resulting in contamination of cereal grains during pre- and post-harvest stages. Fungal spores may infect cereal crops in the field and at harvest. Infected grain may later contaminate those products already in storage leading to mycotoxin contamination including OTA. Furthermore, contamination of wheat grains and other cereals with OTA has been reported from various parts of world (Ayalew, Fehrmann, Lepschy, Beck, & Abate, 2006; Hajjaji, et al., 2006; Jørgensen & Jacobsen, 2002; Czerwiecki, Czajkowska, & Witkowska-Gwiazdowska, 2002). Thus, a constant monitoring for OTA in wheat and other cereals is necessary to ensure the safety of Indian food produce.

Wheat (\textit{Triticum aestivum} L.) is one of the most important staple food crops worldwide including India. It is one of the major sources of carbohydrates and protein for majority of Indian population. The average annual consumption per capita for wheat in India was 65 kg/person/year in 2009-10 (FAS, 2011). India is second largest producer and consumer of wheat in the world and this cereal is second to rice in fulfilling the nutritional requirement of Indian population (FAS, 2011; Lawrance, 2011). The present study suggests that 85% wheat samples from Bihar, 12% samples from Uttar Pradesh, 42% samples from Punjab, and 20% samples from Delhi were found to exceed the EU limit of 5ppb. Whereas, none of the wheat samples collected from Haryana exceeded the EU limit. Maize and Barley are also consumed widely in India, and the present study showed that 13% and 46% samples, respectively, were contaminated with OTA. These data confirms the contamination of Indian cereal produce with OTA, which may be due to poor agricultural practices and storage conditions. As yet Indian regulatory standards have not been prescribed for OTA in food products (PFA, 2008).
Studies related to intake of contaminants or additives depend on the consumption of particular food commodity in which those contaminants and additives are present. Since, wheat is one of the staple foods of India, the consumption of this food commodity in the population of different states have already been assessed (NSSO, 2001). Based on the consumption profile of wheat and the presence of average and 95\textsuperscript{th} percentile values of OTA in wheat in the present study, the intake of OTA was found to exceed (2-4 folds) the PTWI of 100 ng kg\textsuperscript{-1} bw (JECFA, 2001). Thus our study indicates the possible prolonged exposure risk of Indian population to OTA through consumption of contaminated wheat, which may further lead to chronic toxicological implications like previously observed in Balkan population during Balkan Endemic Nephropathy (BEN) (Castegnaro, et al., 2006). Furthermore, recent studies indicate that there are ~7 million patients in India having chronic kidney problems and out of these aetiological factor for ~400,000 cases (5.4\%) is unknown (Dash and Agarwal, 2006). Keeping chronic toxicological manifestations of OTA in mind it can be hypothesized that presence of OTA in wheat grains could be one of the unknown factors responsible for kidney problems in Indian population and needs further elucidation. This is perhaps the first study to evaluate the exposure risk of OTA in Indian human population, comprising of almost 1/6th of the global population, which requires immediate legislative and therapeutic attention so as to safe guard the health at large.

In conclusion, this study indicates towards contamination of Indian cereals with higher levels of OTA in some samples, which may be due to less awareness towards good agricultural practices and the climatic conditions of India is more favorable for fungal growth either in field or during storage. Therefore, it is necessary to create awareness in farmers towards mycotoxin producing fungi along with proper handling and storage of cereal grains so that exposure risk to OTA can be minimized at large.


Figure 2.1:
Linearity of concentration dependent detection of standard ochratoxin A (OTA) by High Performance Liquid Chromatography (HPLC)

The values of ochratoxin A injected to HPLC are in the concentration range of 6.25-3200 ng ml\(^{-1}\).
Figure 2.2:
High Performance Liquid Chromatography (HPLC) profile of ochratoxin A

(A) HPLC chromatogram of ochratoxin A standard (50 ng ml\(^{-1}\)).
(B) HPLC chromatogram of ochratoxin A (5 µg kg\(^{-1}\)) extracted from wheat grain.

Chromatographic conditions: column: reversed phase Symmetry\(^{®}\) (5 µm); mobile phase: water/acetonitrile/acetic acid (99:99:2, v/v/v); flow-rate: 1.0 ml min\(^{-1}\); tunable fluorescence detector at excitation and emission wavelength of 333 nm and 460 nm, respectively, injection volume: 100 µl.
Figure 2.3:
The total ion chromatogram (TIC) of
(A) standard ochratoxin A and
(B) ochratoxin A extracted from wheat grain.
Figure 2.4:
Electrospray Ionization Mass Spectrometry (ESI-MS) spectra of ochratoxin A showing peak at m/z 402.
(A) ESI-MS spectra of standard ochratoxin A.
(B) ESI-MS spectra of ochratoxin A extracted from wheat grain.
Table 2.1:
Linear regression equation, linearity range, regression coefficient, LOD and LOQ for Ochratoxin A

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Equation $Y=b+mx^a$</th>
<th>Linearity Range, (ng ml$^{-1}$)</th>
<th>Regression coefficient</th>
<th>LOD (ng ml$^{-1}$)</th>
<th>% RSD (n=9)</th>
<th>LOQ (µg kg$^{-1}$)</th>
<th>% RSD (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTA</td>
<td>$-2.37 \times 10^3 + 8.19 \times 10^3$</td>
<td>1.25-320</td>
<td>0.995</td>
<td>0.6</td>
<td>4.38-14.98</td>
<td>0.71</td>
<td>1.79-4.19</td>
</tr>
</tbody>
</table>

LOD (Limit of Detection) and LOQ (Limit of Quantitation) was calculated by injecting different concentration of standard solutions and spiked sample extract respectively using the below mentioned formula.

\[
\text{LOD} = 3.3 \times \sigma/S \\
\text{LOQ} = 10.0 \times \sigma/S
\]

where $\sigma$ is the standard deviation of the response  
$S$ is the slope of the corresponding calibration curve
Table 2.2:

Recovery and Relative Standard Deviation obtained for Ochratoxin A

<table>
<thead>
<tr>
<th>Amount added (ng/gm)</th>
<th>Average Peak Area (n=3)</th>
<th>Amount recovered (ng/gm)</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>735070</td>
<td>92.13</td>
<td>92</td>
<td>8.369834</td>
</tr>
<tr>
<td>20</td>
<td>135352</td>
<td>16.93</td>
<td>84</td>
<td>3.134387</td>
</tr>
<tr>
<td>10</td>
<td>72809</td>
<td>8.58</td>
<td>85</td>
<td>3.12432</td>
</tr>
<tr>
<td>5</td>
<td>37405</td>
<td>3.76</td>
<td>75</td>
<td>1.79221</td>
</tr>
<tr>
<td>2.5</td>
<td>19687</td>
<td>2.17</td>
<td>86</td>
<td>4.197663</td>
</tr>
<tr>
<td>1.25</td>
<td>9650</td>
<td>0.89</td>
<td>71</td>
<td>3.792505</td>
</tr>
</tbody>
</table>

The recovery in spiked triplicate samples ranged from 71% to 92% for the added levels of 100, 20, 10, 5, 2.5 and 1.25 µg kg⁻¹.
Table 2.3:

Ochratoxin A in wheat samples from different states of India.

<table>
<thead>
<tr>
<th>Location</th>
<th>Positive over total samples&lt;sup&gt;a&lt;/sup&gt;</th>
<th>OTA contamination (µg kg&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>95&lt;sup&gt;th&lt;/sup&gt; Percentile (µg kg&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average ± SE&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Median</td>
</tr>
<tr>
<td>Bihar</td>
<td>6 (7)</td>
<td>9.4±2.3</td>
<td>7.26</td>
</tr>
<tr>
<td>Uttar Pradesh</td>
<td>9 (16)</td>
<td>5.0±1.8</td>
<td>2.93</td>
</tr>
<tr>
<td>Delhi</td>
<td>8 (10)</td>
<td>3.8±0.7</td>
<td>3.07</td>
</tr>
<tr>
<td>Haryana</td>
<td>2 (10)</td>
<td>3.0±1.13</td>
<td>3.0</td>
</tr>
<tr>
<td>Punjab</td>
<td>4 (7)</td>
<td>7.33±2.14</td>
<td>6.47</td>
</tr>
<tr>
<td>Total</td>
<td>29 (50)</td>
<td>5.76±0.9</td>
<td>4.13</td>
</tr>
</tbody>
</table>

The average, median and 95<sup>th</sup> percentile values have been tabulated by Microsoft excel.

<sup>a</sup> Values in parentheses indicate total number of samples analyzed

<sup>b</sup> SE = Standard Error
Table 2.4:

Ochratoxin A in maize samples from different states of India.

<table>
<thead>
<tr>
<th>Location</th>
<th>Positive over total samples&lt;sup&gt;a&lt;/sup&gt;</th>
<th>OTA contamination (µg kg&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>95&lt;sup&gt;th&lt;/sup&gt; Percentile (µg kg&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average ± SE&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Median</td>
</tr>
<tr>
<td>Bihar</td>
<td>0 (7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Uttar Pradesh</td>
<td>2 (8)</td>
<td>5.22±1.24</td>
<td>5.22</td>
</tr>
<tr>
<td>Total</td>
<td>2 (15)</td>
<td>5.22±1.24</td>
<td>5.22</td>
</tr>
</tbody>
</table>

The average, median and 95<sup>th</sup> percentile values have been tabulated by Microsoft excel.

<sup>a</sup> Values in parentheses indicate total number of samples analyzed
<sup>b</sup> SE = Standard Error
Table 2.5:

Ochratoxin A in barley samples from different states of India.

<table>
<thead>
<tr>
<th>Location</th>
<th>Positive over total samplesᵃ</th>
<th>OTA contamination (µg kg⁻¹)</th>
<th>95ᵗʰ Percentile (µg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average ± SEᵇ</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>Bihar</td>
<td>2 (3)</td>
<td>2.60±1.49</td>
<td>2.60</td>
</tr>
<tr>
<td>Uttar Pradesh</td>
<td>4 (6)</td>
<td>3.01±0.85</td>
<td>2.83</td>
</tr>
<tr>
<td>Haryana</td>
<td>1 (6)</td>
<td>2.20</td>
<td>2.20</td>
</tr>
<tr>
<td>Total</td>
<td>7 (15)</td>
<td>2.78±0.57</td>
<td>2.20</td>
</tr>
</tbody>
</table>

The average, median and 95ᵗʰ percentile values have been tabulated by Microsoft excel.

ᵃ Values in parentheses indicate total number of samples analyzed

ᵇ SE = Standard Error
Table 2.6:

State wise consumption pattern of wheat and assessment of intake pattern of OTA through wheat consumption.

<table>
<thead>
<tr>
<th>State</th>
<th>Average Consumption&lt;sup&gt;a&lt;/sup&gt; (Kg/Capita/Week)</th>
<th>Weekly intake of OTA (ng kg&lt;sup&gt;-1&lt;/sup&gt; bw) Mean</th>
<th>95&lt;sup&gt;th&lt;/sup&gt; percentile</th>
<th>Saturation of PTWI&lt;sup&gt;b&lt;/sup&gt; (Folds) Mean</th>
<th>95&lt;sup&gt;th&lt;/sup&gt; percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bihar</td>
<td>1.3125</td>
<td>205</td>
<td>393</td>
<td>2.05</td>
<td>3.93</td>
</tr>
<tr>
<td>Uttar Pradesh</td>
<td>1.9565</td>
<td>163</td>
<td>466</td>
<td>1.63</td>
<td>4.66</td>
</tr>
<tr>
<td>Haryana</td>
<td>2.1</td>
<td>105</td>
<td>140</td>
<td>1.05</td>
<td>1.40</td>
</tr>
<tr>
<td>Punjab</td>
<td>2.044</td>
<td>249</td>
<td>419</td>
<td>2.49</td>
<td>4.19</td>
</tr>
<tr>
<td>Delhi</td>
<td>1.54</td>
<td>97</td>
<td>179</td>
<td>0.97</td>
<td>1.79</td>
</tr>
</tbody>
</table>

<sup>a</sup> Source: Based on data provided in National Sample Survey Organization, Department of Statistics, Government of India (2001).

<sup>b</sup> PTWI of Ochratoxin A is 100 ng kg<sup>-1</sup> bw (JECFA, 2001).