Contamination of staple cereals with deoxynivalenol and nivalenol in Japan

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Summary

The provisional regulation level for deoxynivalenol in wheat is 1.1 mg/kg in Japan. However, the regulation level for nivalenol and the occurrence of trichothecenes in rice still remain as matters to be settled. In this paper, we described a risk assessment for daily exposure of trichothecene mycotoxin based on the data obtained for wheat and rice harvested in 2002. By employing the weighted mean of trichothecene contamination levels, the trichothecenes risk in Japan was evaluated. The tolerable daily intake of the trichothecenes in Japanese dietary intake was also described.

Key words: deoxynivalenol, nivalenol, wheat, rice

Introduction

Trichothecenes are one of the major mycotoxins produced by toxigenic Fusarium species and currently, the contamination of deoxynivalenol (DON) in cereals is a worldwide problem to be solved. Based on the results of a 2-year feeding study in mice, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established a level of 1 μg/kg of body weight per day as the provisional maximum tolerable daily intake (PMTDI) for DON in 200110. In line with this, the Ministry of Health, Labor and Welfare of Japan recommended a level of 1.1 mg/kg of DON in wheat as the provisional standard for local governments in 2002, and further investigations are required to establish the administrative guideline based on Article 7 of the Food Sanitation Law of Japan.
Concerning the effects on human health of the exposure to DON and/or nivalenol (NIV), the most important mission is to clarify the actual risk level in the daily consumption of contaminated staple cereals, such as wheat and rice. Surveys for DON in wheat have been carried out in numerous countries\textsuperscript{1,2}. Our previous surveys had demonstrated the co-contamination of DON and NIV in wheat samples harvested from 21 countries\textsuperscript{3}. As for rice, it is the most popular cereal in Japan, and Japanese people consume more than eight million tons of rice per year. However, there is limited information about trichothecene contamination in rice.

In this paper, we described the contamination of DON and NIV in imported and domestic wheat, and domestic rice of the 2002 harvest taking the producing districts into consideration. In addition, the corresponding limit of the tolerable daily intake (TDI) was evaluated based on the Japanese prospective intake of wheat. The TDI of DON and NIV in dietary intake were also considered.

**Materials and Methods**

**Samples** A total of 199 domestic wheat samples of the 2002 crop, and 20 and 178 imported wheat samples of the 2001 and 2002 harvests, respectively, were obtained from the Ministry of Agriculture, Forestry and Fisheries in Japan. A total of 124 rice samples were obtained from farms and retail stores in Japan. The samples of domestic wheat and rice were obtained from 4 regions of Japan: the North (Hokkaido and Tohoku districts; 70 wheat and 37 rice), the Central-east (Kanto and Hokuriku districts; 37 wheat and 43 rice), and the Central-west (Tokai, Kinki and Chugoku districts; 43 wheat and 30 rice), and the South (Kyushu and Shikoku districts; 49 wheat and 14 rice). One kg of each sample collected was blended well, and a 500 g portion was smashed and passed through a 1 mm sieve. All the sample were stored at 4 °C until use.

**Chemicals and reagents** DON and NIV standards were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). The commercial cartridge columns, Autoprep MF-T and MultiSep #227, were purchased from Showa Denko (Tokyo, Japan) and Romer Labs, Inc. (MO, USA), respectively. Organic solvents were analytical reagent grade.

**Preparation of standards solution** DON and NIV standards were separately dissolved in acetonitrile, prepared as 100 μg/ml stock solutions and stored at 4 °C in the dark until use. For working standards of LC and LCMS analysis, each stock solution of DON and NIV was mixed in a vial, and evaporated to dryness at 40 °C under gentle stream of nitrogen. The working standard was dissolved in a mobile phase for LC and LCMS.

**Apparatus and chromatographic conditions** The LC system consisted of a Shimadzu Model LC-10AD pump (Shimadzu, Kyoto, Japan) equipped with a UV detector (SPD-10A, Shimadzu), an auto-injector (SIL-10A, Shimadzu) and a system controller (SCL-10A, Shimadzu). A Shodex Silica C18M 4E reversed-phase column (250 x 4.6 mm I.D.,
5 μm) was used. The separation was maintained at 40 °C. The mobile phase was acetonitrile-methanol-water (5:5:90, v/v/v) at a flow rate of 1.0 ml/min. The detection was measured at 220 nm.

The LCMS system consisted of a LC Model Agilent 1100 (Agilent Technologies, CA) fitted with a Zorbax eclipse XDB C18 (150 x 2 mm I.D., 5 μm) (Agilent Technologies), an automated sampler, and a mass spectrometric detector equipped with orthogonal spray-atmospheric pressure photo-ionization (APPI) (Model Agilent 1100MSD SL). The column temperature was kept at 40 °C. The mobile phase was programmed as a linear gradient from methanol-water containing 10 mM ammonium acetate (10:90, v/v) to (60:40, v/v) in 20 min. The flow rate was 0.2 ml/min. The APPI-negative ionization at 100 V for fragmentor voltage and the selected ion monitoring (SIM) mode were used for the determination of DON and NIV.

**Preparation of samples** DON and NIV were extracted from 50 g of finely ground sample with 200 ml acetonitrile-water (85:15, v/v). The solvent was constantly shaken for 30 min, and centrifuged at 1,650 x g for 5 min. The supernatant was filtered through a glass microfiber filter GF/B (Whatman Ind. Ltd., Maidstone, UK). A 10 ml portion of the filtrate was applied to the cartridge column. After passing through at a flow rate of 1 ml/min, the first 3 ml eluate was discarded. The following 5 ml eluate was collected in a tube. A 2 ml portion of the eluate was transferred to another tube and evaporated to dryness at 40 °C under gentle stream of nitrogen.

**LCMS and reversed phase LC analyses** The purified test samples were dissolved in 1 ml of the mobile phase solution, and vortex-mixed. Ten μl aliquots of the standard and sample solutions were applied to the LCMS/APPI. The selected ions (m/z) were monitored at 355 and 371 for DON and NIV, respectively. For LC analysis, 20 μl each of the standard and sample solutions were injected.

**Results and Discussion**

As shown in Table 1, 81 out of 199 (41 %) domestic wheat samples analyzed were positive for DON, and the mean concentration and range were 160 μg/kg and 0-2,100 μg/kg, respectively. Among these samples, 10 samples were found to be exceeding the allowable limits (1.1 mg/kg). Yields of wheat grains and DON concentrations detected varied in the 4 regions examined. The South region showed the highest mean concentration of DON. However, the amount of wheat grains harvested in the South region is only 19 % of the whole domestic wheat production. Therefore, the weighted mean analysis was introduced to estimate local variation of DON concentrations based on the ratio of wheat production. As a result, the DON concentration in the South region was reduced by 80 % when compared with the un-weighted mean concentration, and it appeared that both North and South regions are mostly responsible for the DON
contamination of wheat grains cultivated in Japan. In imported wheat, the range and mean concentrations were 0-680 µg/kg and 60 µg/kg, respectively, and DON levels were below the provisional regulation level in Japan. Regarding the total estimation of DON exposure through intake of wheat grains, the data revealed that the Japanese exposure to DON through daily consumption of wheat grains harvested in 2002 did not exceed the PMTDI of DON (Table 1).

Table 1. Natural occurrence of deoxynivalenol and nivalenol in wheat grains.

<table>
<thead>
<tr>
<th>Source and region</th>
<th>Yield or imported amounts (million ton)</th>
<th>No. sample tested</th>
<th>Positive (Incidence %)</th>
<th>Concentration (µg/kg)</th>
<th>Range</th>
<th>Mean</th>
<th>Weighted mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DON  NIV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Domestic wheat (2002)</td>
<td>0.83</td>
<td>199</td>
<td>81 (41) 68 (34)</td>
<td>0-2100 0-640</td>
<td>160</td>
<td>59</td>
<td>169 43</td>
</tr>
<tr>
<td>North</td>
<td>0.51</td>
<td>70</td>
<td>30 (43) 4 (6)</td>
<td>0-2100 0-90</td>
<td>120</td>
<td>4</td>
<td>74 2</td>
</tr>
<tr>
<td>Central-east</td>
<td>0.12</td>
<td>37</td>
<td>3 (8) 4 (11)</td>
<td>0-290 0-260</td>
<td>13</td>
<td>20</td>
<td>2 3</td>
</tr>
<tr>
<td>Central-west</td>
<td>0.04</td>
<td>43</td>
<td>5 (12) 14 (33)</td>
<td>0-110 0-300</td>
<td>10</td>
<td>41</td>
<td>0.5 2</td>
</tr>
<tr>
<td>South</td>
<td>0.16</td>
<td>49</td>
<td>43 (88) 46 (94)</td>
<td>0-1800 0-640</td>
<td>480</td>
<td>183</td>
<td>93 35</td>
</tr>
<tr>
<td>Imported wheat (2002)</td>
<td>5.85</td>
<td>178</td>
<td>45 (25)</td>
<td>0-680</td>
<td>60</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Imported wheat (2001)</td>
<td>20</td>
<td>9</td>
<td>45 (45)</td>
<td>0-5</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>6.68</td>
<td>377</td>
<td>126 (33) 77 (35)</td>
<td>0-2100 0-640</td>
<td>113</td>
<td>54</td>
<td>73 6</td>
</tr>
</tbody>
</table>

Data for NIV in Table 1 shows that the domestic wheat harvested in 2002 and imported wheat in the 2001 harvest were contaminated with low levels of NIV. The highest incidence of NIV in Japan was found in the South region. In the imported wheat grains, NIV concentrations were very low. These data indicate that the contamination of wheat with NIV is an important problem only for the domestic grains.

For a risk assessment of DON and NIV exposure due to wheat consumption, the data were arranged to give the ratio of exposure levels to TDI values, which were proposed by the JECFA and European Science Committee as 1 µg and 0.7 µg for DON and NIV, respectively (Table 2). Exposure levels to DON through daily consumption of wheat products were 7 and 15 % for adults and children (1-6 years), respectively, and those of NIV were 0.8 and 1.7 % for adults and children, respectively, of the corresponding TDI values. The summarized data suggest that the present contamination of wheat products with trichothecenes is within acceptable levels, however, continuous monitoring systems for the contamination levels will be required to avoid unexpected
risk of exposure to high concentrations of trichothecenes.

Rice is the staple food of the Japanese, and most people take boiled and/or cooked rice daily. Out of the 124 domestic rice samples analyzed for DON and NIV, the number of positive sample was 4 and 15, respectively. Mean concentrations in all samples tested were 0.7 and 0.6 µg/kg for DON and NIV, respectively. The exposure levels estimated from these concentrations were very low, that is 0.1 % of TDI for adults and 0.2 % of TDI for 1-6 year old children. However, data for the occurrence of trichothecenes in domestic rice was limited, and further surveys will be required.

In conclusion, the data obtained in this study suggest that the problem of trichothecenes in Japan is related to geographical distribution of wheat and rice fields. In particular, wheat samples from the North and South regions were found to be highly contaminated with trichothecenes. The evaluation of human risk from the exposure to trichothecenes needs to be carefully conducted from the standpoint of PMTDI. Therefore, it should be emphasized that reducing the risk of exposure is attendant to reducing the contamination levels in commodities.

Table 2. Risk estimation of trichothecenes in wheat grains in Japan.

<table>
<thead>
<tr>
<th>Item</th>
<th>Weighted mean</th>
<th>Mean (µg/kg)</th>
<th>Ratio of toxins per TDI¹ (%) exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Deoxynivalenol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td>73</td>
<td>7 *²</td>
<td></td>
</tr>
<tr>
<td>Children (1-6 years old)</td>
<td>73</td>
<td>15 *³</td>
<td></td>
</tr>
<tr>
<td><strong>Nivalenol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td>6</td>
<td>0.8 *⁴</td>
<td></td>
</tr>
<tr>
<td>Children (1-6 years old)</td>
<td>6</td>
<td>1.7 *⁵</td>
<td></td>
</tr>
</tbody>
</table>

¹ TDI : 1 µg/kg body weight per day as provisional maximum TDI for DON established by the JECFA in 2001 and 0.7 µg/kg body weight per day as temporary TDI for NIV assigned by the European Science Committee in 2002
² Mean concentrations (µg) x 0.5(reduction) x 94.3 g (prospective intake of wheat per day)/50 µg (TDI : 50 kg body weight per day) x 100
³ Mean concentrations (µg) x 0.5(reduction) x 64.1 g (prospective intake of wheat per day)/16 µg (TDI : 16 kg body weight per day) x 100
⁴ Mean concentrations (µg) x 0.5(reduction) x 94.3 g (prospective intake of wheat per day)/35 µg (TDI : 50 kg body weight per day) x 100
⁵ Mean concentrations (µg) x 0.5(reduction) x 64.1g (prospective intake of wheat per day)/11 µg (TDI : 16 kg body weight per day) x 100
References