Introduction

In Japan there are some pathogens responsible for causing fusarium head blight of wheat. These pathogens belong to Fusarium species (Fusarium graminearum, F. avenaceum, F. culmorum, F. crookwellense, F. sporotrichioides, F. poae, F. tricinctum, F. acuminatum, F. equiseti, F. semitectum, F. kyushuense, Microdochium nivale (formerly F.nivale)). The principal toxins produced by these Fusarium species are trichothecenes, such as deoxynivalenol (DON), 3-acetyledeoxynivarenol (3-ADON), 15-acetyledeoxynivalenol (15-ADON), Diacetoxyscirpenol (DAS), nivalenol (NIV), fusarenon X (FX), T-2 toxin and Zearalenone (ZEN).

Among these mycotoxins, DON, T-2 toxin and HT-2 toxin were evaluated at the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2001. As for DON, the provisional maximum tolerable daily intake (PMTDI) has been established at a level of 1 µg/kg of body weight per day, based on the results of a 2-year feeding study in mice. With the determination of PMTDI for DON, the Ministry of Health, Labor and Welfare of Japan has started the study and surveillance on the risk assessment of DON in cereals to set the standard from 2001 and they have recommended a level of 1.1 mg/kg of DON in wheat as the provisional standard in 2002.

This paper aims to introduce the toxicities of mycotoxins related with fusarium head blight and the ground of setting the provisional standard.

Toxicities of trichothecene mycotoxins and zearalenone

Trichothecene mycotoxins, especially T-2, HT-2, DON and NIV possess common biochemical and cellular toxicities. These are 1) the strong inhibitory effect on the protein synthesis by binding to the ribosomes. 2) the inhibitory effect on RNA and DNA synthesis. 3) toxic effects on cell membranes (it seems to be anti-oxidation reaction). Also, their capacity to inhibit protein synthesis is thought to induce apoptosis in thymus, lymphatic and haematopoietic tissue via mitogen activated
protein kinases (MAP kinases). The different trichothecenes differ the inhibitory activity. For T-2 toxin, HT-2, DON and NIV, general toxicity and immunotoxicity of trichothecenes are considered to be critical effects. The crops contaminated with trichothecenes resulted in serious food poisoning with nausea, vomiting and diarrhea. After the World war II, the critical food poisoning occurred by milletin Russia. This disease caused the decrease of circulating white blood cell number. It is called alimentary toxic aleukia (ATA) and a major causal toxin was thought to be T-2 toxin produced by Fusarium sporotrichioides and F. poae. The immunotoxicity, which is chronic effect of trichothecenes mycotoxins results in the decrease of host resistance. Selective upregulates serum IgA caused by dietary expose to DON or NIV induces the IgA nephropathy. The effect as the cancer promoter is seems to be responsible for the immunotoxicity.

Zearalenone, which is produced by F. graminearum and F. culmorum, interacts with oestrogen receptors. This toxin induces apparent hyperoestrogenism. Even though all of mammalian species are target of zearalenone, female pigs are considered to be most sensitive animal species. Other typical toxicities of zearalenone are not recognizes, but the oral exposure to high dose zearalenone has been reported to cause hepatocellular adeomas in B6C3F1 mice and apoptosis in vitro.

**International regulation of trichothecenes and zearalenone**

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) and European Commission (EC) had evaluated the risk of trichothecens and zearalenone and established provisional maximum tolerable daily intake (PMTDI) of these toxins (Table 1). JECFA has set PMTDI for deoxynivalenol (DON), T-2 roxin, HT-2 toxin and zearalenone. EC has established a full TDI to DON and temporary TDIs to nivalenol, zearalenon and T-2 toxin and HT-2 toxin.

**Setting provisional standard for tolerable level of DON contamination in wheat**

Prior to setting PMTDI by JECFA, some investigators in Japan have pointed out a possibility that DON intake over the PMTDI in Japan if no regulation is set for DON contamination levels in domestic wheat in Japan because high levels of DON were found in Japanese domestic wheat frequently. Therefore the Ministry of Health, Labour and Welfare (MHLW) organized the reserach group to survey current situation of DON contamination levels in domestic wheat in 2001-2002. As

<table>
<thead>
<tr>
<th>mycotoxin</th>
<th>PMTDI (µg/person kg/day)</th>
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<tbody>
<tr>
<td></td>
<td>JECFA</td>
</tr>
<tr>
<td>DON</td>
<td>1</td>
</tr>
<tr>
<td>NIV</td>
<td>-</td>
</tr>
<tr>
<td>T-2 and HT-2</td>
<td>0.06</td>
</tr>
<tr>
<td>ZEA</td>
<td>0.5</td>
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</tbody>
</table>

* temporary TDI
the regulation for DON was urgently needed to prevent adverse health, the Joint Committee of Food Standard and Toxicity Committee under the Food Sanitation Council recommended setting a provisional standard for the tolerable level of DON in wheat. Based on the surveillance data in 2001-2002 and daily average intake of wheat, the research group proposed some simulations about the tolerable level of DON in unpolished wheat (Table 2). On May 21, 2002, the Food Sanitation Council has set the tolerable level of DON in unpolished wheat at 1.1 mg/kg as a provisional standard when the reduction of DON concentration in final products is estimated about 50%.

The further study on surveillance of DON levels in wheat and rice in 2002-2003 and reduction of DON concentration in processing and cooking

After the setting of provisional standard for unpolished wheat, MHLW organized further study on surveillance of the current situation and reduction of DON concentration in processing and cooking to assess the exposure level of DON in detail. Further surveillance results of DON levels in unpolished wheat in 2002-2003 revealed that the averages of DON concentration in imported wheat (n=178) and domestic wheat (n=199) were 60 ng/g and 160 ng/g, respectively. Taking into consideration of the weighted average of supply flow, the average of DON contamination was calculated as 71 ng/kg. In domestic rice (n=124), the average was 2.6 ng/g. As the DON level in rice was too low, the contribution to the amount of exposure to DON might be neglected.

The reduction study using the natural contaminant wheat showed that milling process reduced DON level by 55.4%. The cooking study using the natural contaminant flour found that DON level reduced by 71.1% in cooked noodle but did not in bread. These studies concluded that the retention level of DON from wheat to final product could be conservatively assessed at 44.2% in bread and at 12.9% in noodle.

Based on the results of the study performed in 2002-2003, we recalculated level of DON contamination in wheat and flour. When the reduction of DON in final product was taken into consideration, the concentration of DON in unpolished was allowed until 1,913 µg/kg for all age and 858 µg/kg for 1-6 years old (Table 3). The research group assessed that the mean intake of DON in Japan were 1.94 µg/day/person for all age and 1.31 µg/day/person for 1-6 years old children. The intakes were ranked 3.7% of PMTDI for all age and 8.3% of PMTDI for 1-6 years old children (Table 4).
As the exposure level of DON was very low, MHWL decided to continue the regulation of unpolished wheat regarding DON contamination by using the provisional standard. In order to establish the standard of DON, more data on DON contamination are needed.

**Further approach**

Recently, many cases of co-contamination occurrence with trichothecenes have been reported in wheat, barley and other cereals[9-14]. Regarding the establishment of DON standard, the effects of combined exposure to several trichothecenes and ZEA should be taken into account in risk assessment.

**References**

4 ) Hinoshita, F., Suzuki, Y., Yokoyama, K., Hara, S., Yamada, A., Ogura, Y., Hashimoto, H., Tomura, S.,...

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### Table 3. Tolerable level of DON contamination in wheat and flour satisfying PMTDI (Based on the research on 2002-2003)

<table>
<thead>
<tr>
<th>Age (weight (kg))</th>
<th>Intake of rice (g)</th>
<th>Intake of wheat (g)</th>
<th>not taking into consideration of reduction in noodle and bread (µg/kg)</th>
<th>taking into of consideration of reduction in noodle and bread (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>94.3</td>
<td>1,251</td>
<td>1,913</td>
<td>853</td>
</tr>
<tr>
<td>1-6 years (15.9)</td>
<td>64.1</td>
<td>556</td>
<td>850</td>
<td>380</td>
</tr>
<tr>
<td>Yes</td>
<td>160.4</td>
<td>1,247</td>
<td>1,908</td>
<td>851</td>
</tr>
<tr>
<td>1-6 years (15.9)</td>
<td>64.1</td>
<td>554</td>
<td>846</td>
<td>378</td>
</tr>
</tbody>
</table>

DON level in unpolished wheat (µg/kg) = PMTDI/Intake of wheat x reduction in flour x \{(the rate for which noodle consumption x the retention in noodle) + (the rate for which bread consumption x the retention in bread) + (the rate for which others product consumption x the retention in others)\}

Retention in flour: 44.6 % The rate for which noodle consumption: 46.5 % The retention in noodle: 28.9 % The rate for which bread consumption: 45.6 % The retention in bread: 97.1 % The rate for which others product consumption: 7.6 % the retention in others: 100 %

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### Table 4. The percentage to PMTDI of the intake amount (Based on the research on 2002-2003)

<table>
<thead>
<tr>
<th>Age</th>
<th>the intake of wheat (g)</th>
<th>body weight (kg)</th>
<th>the intake of DON (µg/day/person)</th>
<th>the percentage to PMTDI(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All age</td>
<td>94.3</td>
<td>52.6</td>
<td>1.94</td>
<td>3.7</td>
</tr>
<tr>
<td>1-6 years old</td>
<td>64.3</td>
<td>15.9</td>
<td>1.31</td>
<td>8.3</td>
</tr>
</tbody>
</table>

The average of domestic wheat; 0.16 mg/kg, The amount of product; 540.000 ton
The average of imported wheat; 0.06 mg/kg, The amount of product; 4,560,000 ton
The weighted average; 0.071 mg/kg
赤カビ病に関わるマイコトキシンの毒性と小麦におけるDON汚染レベルの暫定基準について

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赤カビ病に起因する主なカビ毒は、デオキシニバレノール、ニバレノール、T-2トキシンなどのトリコテセン系マイコトキシンとセサラレンである。トリコテセン系マイコトキシンの共通の毒性には、消化管障害、免疫毒性などがあり、ガンへのプロモーター作用を示唆する報告もある。我が国の赤カビ病において最も頻繁に検出されるカビ毒は、デオキシニバレノール、ニバレノールであるが、デオキシニバレノールに関しては2002年に暫定基準値が設定された。その根拠を2つの厚生科学研究研究（平成13年度および平成14年度）の結果をもとに示した。

キーワード：マイコトキシン、赤カビ病、デオキシニバレノール、暫定基準値