# Risk Assessment Studies Report No. 61

# **Chemical Hazard Evaluation**

# T-2 toxins, HT-2 toxins and 4,15diacetoxyscirpenol in Food

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Centre for Food Safety

Food and Environmental Hygiene Department

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# T-2 toxins, HT-2 toxins and 4,15diacetoxyscirpenol in Food

#### **EXECUTIVE SUMMARY**

Trichothecene mycotoxins are a group of toxic fungal secondary metabolites produced by multiple genera of fungi and can be subcategorized into four major groups, namely type A, B, C, and D. T-2 toxin (T-2), HT-2 toxin (HT-2) and 4,15-diacetoxyscirpenol (DAS) are type A trichothecenes with similar structure. They have been reported to be produced by various *Fusarium* species which grow and invade crops under cool, moist conditions prior to harvest. The main food groups reported to be contaminated with T-2, HT-2 and DAS are cereals and their derived products.

- 2. In animal studies, the immune system was identified to be the target for T-2 toxicity. T-2 and HT-2 induce adverse effects with similar potency but T-2 appears to be more potent than DAS. The International Agency for Research on Cancer (IARC) evaluated "Fusarium sporotrichioides, toxins derived from (T-2 toxin)" in 1993 and concluded that toxins derived from Fusarium sporotrichioides are not classifiable with regard to its carcinogenicity to humans (Group 3 agent).
- 3. In 2022, JECFA re-evaluated the toxicity of T-2, HT-2 and DAS and established a Group tolerable daily intake (TDI) of 25 ng/kg body weight (bw) for T-2, HT-2 and DAS, alone or in combination. The relative potency factor of 0.2 should be applied in comparing the dietary exposure to DAS with the Group TDI.

4. This study serves (i) to examine the levels of T-2, HT-2 and DAS in selected food items available at local markets; (ii) to estimate the dietary exposure to the sum of T-2, HT-2 and DAS of local adult and younger populations; and (iii) to assess the associated potential health risk.

# Methodology

5. A total of 327 samples which have been reported to contain T-2, HT-2 and DAS were collected from the local retailers. These samples were classified into 10 different food groups, including "Cereal Grains", "Flour", "Starch substitute", "Pasta & Noodles", "Breakfast Cereal", "Bakery and Pastry Items", "Savoury Snacks", "Vegetables, Nuts & Seeds", "Vegetable Oils" and "Beverages".

### Results

6. Of the 327 samples analysed, T-2, HT-2 and DAS were not detected in 254 samples (about 78%). 73 samples (about 22%) were reported to contain at least one of these mycotoxins (≥LOD). Among these 73 samples, more than half were from the food group "Cereal grains" (41 samples, 56%), followed by "Breakfast Cereal" (12 samples, 16%) and "Bakery and Pastry Items" (9 samples, 12%). In contrast, all samples in "Starch Substitute" and "Pasta & Noodles" were not detected with T-2, HT-2 and DAS.

- 7. After evaluation, the upper bound a (UB) dietary exposure estimates of local adult population to the sum of T-2, HT-2 and DAS arising from the collected food groups were 0.8455 ng/kg bw/day (3.38% of the Group TDI) and 1.2806 ng/kg bw/day (5.12% of the Group TDI) for average and high (90 percentile) consumers respectively.
- 8. For the younger population, the UB exposure estimates to the sum of T-2, HT-2 and DAS were 1.4703 ng/kg bw/day (5.88% of the Group TDI) and 2.4047 ng/kg bw/day (9.62% of the Group TDI) for average and high consumers respectively.
- 9. None of the food groups was found to be a significant source of dietary exposure to T-2, HT-2 and DAS for the local adult and younger population.

### Conclusion and Recommendations

- 10. The estimated dietary exposures to the sum of T-2, HT-2 and DAS for the average and high consumers of the local adult and younger population were well below the group TDI established by JECFA, suggesting the local adult and younger population are unlikely to experience adverse health effects of T-2, HT-2 and DAS.
- 11. To minimise the risk of trichothecene mycotoxin contamination, members of the general public should purchase cereals and cereal-based products from reliable sources and store them properly in a cool, dry place

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<sup>&</sup>lt;sup>a</sup> An upper bound dietary exposure is estimated by assigning the level of detection to all samples with non-detectable results.

to prevent fungal growth. Additionally, the public should follow the basic dietary advice on healthy eating and maintain a balanced and varied diet to minimise the risk of exposure to contaminants from a limited range of food items. Members of the trade are also advised to store the food commodities properly to avoid susceptible mould growth and increased mycotoxin levels imparted by the mould species.

# T-2 toxins, HT-2 toxins and 4,15-diacetoxyscirpenol in Food

### **OBJECTIVES**

This study aims (i) to examine the levels of T-2 toxin (T-2), HT-2 toxin (HT-2) and 4,15-diacetoxyscirpenol (DAS) in selected food items available at local markets which have been reported to contaminated with T-2, HT-2 and DAS; (ii) to estimate the dietary exposure to the sum of T-2, HT-2 and DAS of local adult and younger populations; and (iii) to assess the associated potential health risk.

## **BACKGROUND**

2. Trichothecene mycotoxins are a group of toxic fungal secondary metabolite produced by multiple genera of fungi<sup>1,2</sup>. The basic structure of all trichothecenes is a tetracyclic ring system with a spiro-epoxide group between C-12 and C-13, and an olefinic double bond between C-9 and C-10<sup>1-4</sup> (Figure 1). Based on the different substituents at various position around this backbone structure, trichothecenes can be subcategorized into four major groups namely type A, B, C, and D<sup>1-5</sup>.

Figure 1. Backbone structure of trichothecenes<sup>2</sup>

3. T-2 toxin (T-2), HT-2 toxin (HT-2) and 4,15-diacetoxyscirpenol (DAS) are type A trichothecenes with similar structure<sup>3,5-6</sup>. HT-2 is a primary metabolite of T-2<sup>3,6-8</sup>. Both T-2 and HT-2 have an ester function at the C-8 position, whereas HT-2 also has a hydroxyl group at the C-4 position<sup>2,3,5-6</sup> (Figure 2). DAS differs from T-2 by missing the isovaleryl group at C-8 position<sup>2,9</sup>. They have been reported to be produced by various *Fusarium* species such as *F. sporotrichioides*, *F. poae* and *F. langsethiae* etc. which grow and invade crops under cool, moist conditions prior to harvest<sup>5-7,9-10</sup>.

$$H_3C$$
 $CH_2$ 
 $CH_2$ 
 $CH_2$ 
 $CH_3$ 
 $CH_3$ 

Figure 2. Chemical structure of T-2, HT-2 and DAS<sup>5</sup>

### **Occurrence in Food**

4. The main food groups reported to be contaminated with T-2, HT-2 and DAS are cereals such as wheat, oat, barley, rice, rye, maize and sorghum<sup>2,5-7,9-10</sup>. Their derived products including flour, bread, breakfast cereals, noodles and beer are also found to contain these trichothecenes in some studies<sup>5-7,9-10</sup>. In addition to cereals and cereal-based products, DAS is also detected in coffee beans<sup>5,9-10</sup>.

- 5. In general, a number of process commonly employed in the food industry can lower T-2 and HT-2 levels in food<sup>6</sup>. For example, cleaning, sorting and sieving, and de-hulling of grains lead to a marked overall reduction of up to 98% in the concentration of *Fusarium* toxins<sup>7</sup>. T-2 and HT-2 are more abundant in the outer layers of cereal grains, and are recovered in higher concentrations in husk, bran and germ relative to other milling fractions<sup>6,9</sup>.
- 6. T-2 and HT-2 concentrations in food decrease during cooking at about 150°C<sup>6</sup>. According to some studies, higher temperatures increase the extent of degradation of the toxins<sup>6</sup>. Nevertheless, some studies found that baking and cooking have little effect on T-2 and HT-2 concentrations in food<sup>7</sup>.

# **Toxicity**

7. The immune system was identified to be the target for T-2 toxicity following short-term exposure<sup>6,9</sup>. Feed refusal, reduced weight gain and changes in organ weights are also sensitive toxicological endpoints that observed in various animal species<sup>6,9</sup>. Evidence from animal studies suggested that T-2 and DAS caused similar immunotoxic and haematotoxic effects following oral exposure<sup>3-6</sup>. T-2 and HT-2 induce adverse effects with similar potency<sup>10</sup> but T-2 appears to be more potent than DAS in vitro and in vivo<sup>5-6</sup>.

8. The common mechanisms of toxicity for T-2, HT-2 and DAS involve inhibition effect on protein synthesis by binding to ribosomes, inhibition effect on RNA and DNA synthesis, disruption of cell membranes, and induction of apoptosis particularly in lymphatic and haematopoietic tissue<sup>1,4-5,7-9</sup>.

# Kinetics and Metabolism

- 9. The toxicokinetics of T-2 or HT-2 in humans is not clear at present<sup>7-8,11</sup>. In experimental animals, T-2 is rapidly absorbed after oral exposure and distributed to liver, kidneys and other organs without any particular accumulation<sup>4,7-8</sup>. T-2 is readily transformed into a range of metabolites in the gastrointestinal tract, with HT-2 being the predominant metabolite<sup>7</sup>. Most of the T-2 and its metabolites are then excreted via urine and faeces within 72 hours<sup>4,7</sup>.
- 10. DAS is rapidly absorbed and metabolised with plasma concentration peaking within 30-60 minutes after oral administration<sup>5</sup>. It was found in the gastrointestinal tract, liver, kidney and tissues of lymphohaematopoietic system<sup>5</sup>. Tissue concentrations decreased rapidly with no apparent accumulation in any tissue. Most of the DAS was excreted in urine and faeces within 24 hours<sup>5,9</sup>.

# **Acute Toxicity**

11. The acute oral toxicity (i.e.  $LD_{50}$ ) of T-2 in various animal species ranged from 4 - 10.5 mg/kg body weight (bw)<sup>11</sup>. No oral  $LD_{50}$ 

has been established for HT-2<sup>7</sup>. Following acute oral exposure, T-2 induces oxidative stress, decreased feed intake, emetic, immunotoxic, haematotoxic, hepatic, renal and neurotoxic effects were observed in a variety of experimental animals<sup>4,6,8</sup>.

12. For DAS, the acute oral  $LD_{50}$  in experimental animals are in the range of 2-15 mg/kg bw<sup>5,9</sup>. Acute oral exposure to DAS has effects on feed intake in mice and emetic effects in mink, via a similar mode of action to T-2 and HT-2<sup>6</sup>.

# **Chronic Toxicity**

- 13. Limited long-term studies of toxicity and carcinogenicity for T-2 were identified<sup>6</sup>. JECFA concluded that the mode of action of T-2 induced toxicity is unlikely to include direct interaction with DNA<sup>6</sup>.
- 14. For DAS, tests for genotoxicity in bacterial or eukaryotic in vitro systems gave uniformly negative results<sup>5</sup>. Long-term studies of toxicity or carcinogenicity, and reproductive or developmental toxicity studies conducted by the oral route of exposure in mammalian species were not available<sup>5</sup>.
- 15. The International Agency for Research on Cancer (IARC) evaluated "Fusarium sporotrichioides, toxins derived from (T-2 toxin)" in 1993 and concluded that toxins derived from Fusarium sporotrichioides are not classifiable with regard to its carcinogenicity to humans (Group 3)<sup>11</sup>.

## **Safety Reference Value**

16. In 2022, JECFA re-evaluated the toxicity of T-2, HT-2 and DAS and concluded that the most sensitive, reliable and reproducible effects observed following repeated dietary exposure was reported in the 3-week toxicity study in juvenile pigs<sup>6</sup>. A BMDL<sub>10</sub> of 1.8 μg/kg bw per day based in reduced daily body weight gain was selected as the most appropriate point of departure and a group tolerable daily intake (TDI) of 25 ng/kg bw for T-2, HT-2 and DAS, alone or in combination, was established<sup>6</sup>. The relative potency factor of 0.2 should be applied in comparing the dietary exposure to DAS with the group TDI<sup>6</sup>.

# **Regulatory Control**

- 17. The Codex Alimentarius Committee (Codex) and other jurisdictions such as Australia, Canada, Mainland China, New Zealand and the United States have not established any standards for T-2, HT-2 and DAS in food.
- 18. In the European Union, as set in Commission Regulation (EU) 2024/1038 of 9 April 2024 amending Commission Regulation (EU) 2023/915 on maximum levels for certain contaminants in food, the maximum levels for the sum of T-2 and HT-2 toxins in certain types of food (Appendix I) have come into force in July 2024<sup>12</sup>.
- 19. In Hong Kong, although there is no specific standard of T-2, HT-2 and DAS in foods, it is stipulated in the Public Health and Municipal

Services Ordinance (Cap 132) that all food available for sale in Hong Kong must be fit for human consumption.

#### SCOPE OF STUDY

20. This study focused on analysis of foods which have been reported to contain T-2, HT-2 and DAS, mainly cereal and cereal-based products. They were chosen based on (i) the occurrence data reported in the literature, (ii) the popularity among the local population, and (iii) the availability in the local market during the sampling period.

#### METHODOLOGY AND LABORATORY ANALYSIS

## Methodology

- 21. A total of 327 food samples were collected from local retailers such as supermarkets, online shops and grocery stores from October to December 2023. The samples were classified into 10 different food groups, namely "Cereal Grains", "Flour", "Starch substitute", "Pasta & Noodles", "Breakfast Cereal", "Bakery and Pastry Items", "Savoury Snacks", "Vegetables, Nuts & Seeds", "Vegetable Oils" and "Beverages". A total of 61 food items were included in the sampling list (Appendix II). All items, except flours, starch substitutes and vegetable oils, were tested in "as consumed" form, i.e. raw food were cooked prior to analysis of T-2, HT-2 and DAS.
- 22. A potency factor of 0.2 was applied to the analytical results of DAS in accordance with JECFA's evaluation and then summed up with the results of T-2 and HT-2. The combined results of DAS, T-2 and HT-2 were then combined with food consumption information captured from the Second Hong Kong Population-based Food Consumption Survey (2018-

- 2020) and Food Consumption Survey in the Younger Population (2021-2022) to obtain the dietary exposures of the local adult and younger population respectively.
- 23. The estimation of dietary exposures was performed using an inhouse developed web-based computer system, Exposure Assessment System (EASY). The exposure to the sum of T-2, HT-2 and DAS from the selected food items for consumers was obtained by summation of exposures from relevant food items. The mean and 90<sup>th</sup> percentile exposure levels were used to represent the dietary exposure levels of average and high consumers of the local adult and younger population respectively. The estimated exposure levels were compared with the group TDI of 25 ng/kg bw for T-2, HT-2 and DAS and were expressed as a percentage of this benchmark.

# **Laboratory Analysis**

- 24. Laboratory analysis of T-2, HT-2 and DAS was conducted by the Food Research Laboratory (FRL) of the Centre for Food Safety (CFS). All samples were tested for the presence of T-2, HT-2 and DAS.
- 25. Levels of T-2, HT-2 and DAS in food samples were analysed by ultra-performance liquid chromatography tandem mass spectroscopy (UPLC-MS/MS). Stable isotopes labelled T-2, HT-2 and DAS were spiked quantitatively into a measured amount of sample. The sample was then extracted by shaking with a mixture containing acetonitrile, water and

acetic acid. The sample extract was further purified by clean-up column containing a combination of specific adsorbents for T-2, HT-2 and DAS analysis. After sample purification, the sample solution was concentrated and subjected to instrumental analysis. Limits of detection (LODs) of T-2, HT-2 and DAS were  $0.05~\mu g/kg$  in samples.

# **Treatment of Analytical Values Below the LOD**

26. In this study, data were treated by both the lower bound (LB) and upper bound (UB) approaches <sup>b</sup>. The approaches compare the two extreme scenarios, based on the consideration that the true value for results smaller than LOD may actually be any value between zero and the LOD. The LB scenario assumes that the chemical is absent; therefore, to results reported as <LOD a value of zero is assigned. The UB scenario assumes that the chemical is present at the level of the LOD; thus, to results reported as <LOD a value of the corresponding LOD is assigned.

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<sup>&</sup>lt;sup>b</sup> The protocol for assigning concentration values to non-detectable results is critical to the dietary exposure estimate. Concentrations should err on the side of nutritional or toxicological caution, while remaining scientifically defensible. For chemicals likely to be present in food (e.g. naturally occurring contaminants and mycotoxins), both the lower and upper bounds should be calculated for the mean food concentration. The lower bound is obtained by assigning a zero value to those samples in which the chemical is not detected (ND) and using these values to estimate the dietary exposure. An upper bound dietary exposure is estimated by assigning the LOD to all samples with ND.

#### **RESULTS AND DISCUSSION**

# Occurrence of T-2, HT-2 and DAS

- 27. Of the 327 samples analysed, T-2, HT-2 and DAS were not detected in 254 samples (about 78%). 73 samples (about 22%) were reported to contain at least one of these mycotoxins (≥LOD).
- Among these 73 samples, more than half were from the food group "Cereal grains" (41 samples, 56%), followed by "Breakfast Cereal" (12 samples, 16%) and "Bakery and Pastry Items" (9 samples, 12%). All samples in "Starch Substitute" and "Pasta & Noodles" were not detected with T-2, HT-2 and DAS.
- 29. 10 out of 73 samples were detected with all 3 mycotoxins being analysed. 6 of them were from the food group "Breakfast Cereal" while 4 samples were from "Cereal Grains".
- 30. The mean levels of the sum of T-2, HT-2 and DAS of different food groups were summarised in Table 1. The food group "Breakfast Cereal" had the highest mean level of the sum of T-2, HT-2 and DAS (2.687-2.726  $\mu$ g/kg, LB-UB), followed by "Vegetable Oils" (1.075-1.222  $\mu$ g/kg, LB-UB) and then "Cereal Grains" (0.304-0.381  $\mu$ g/kg, LB-UB). The lowest mean level of the sum of T-2, HT-2 and DAS was found in the food group "Savoury Snacks" (0.005-0.113  $\mu$ g/kg, LB-UB). The levels of the sum of T-2, HT-2 and DAS in different food items were summarised in the Appendix 1.

Table 1: Mean level of the sum of T-2, HT-2 and DAS (μg/kg)

Detected in Food Groups

| Food Group         | No. of samples | % of samples <lod< th=""><th>Mea</th><th>n of T-2, HT-2 &amp; 1</th><th>DAS (μg/kg)</th><th>* [range]</th></lod<> | Mea                  | n of T-2, HT-2 & 1 | DAS (μg/kg) | * [range]     |
|--------------------|----------------|---|----------------------|--------------------|-------------|---------------|
|                    |                |   | Lowe                 | r bound            | Upp         | er bound      |
| Breakfast Cereal   | 17             | 29  | 2.687                | [0-23.718]         | 2.726       | [0.11-23.718] |
| Vegetable Oils     | 6              | 33  | 1.075                | [0-3.43]           | 1.222       | [0.11-3.44]   |
| Cereal Grains      | 190            | 78  | 0.304                | [0-7.038]          | 0.381       | [0.11-7.038]  |
| Bakery & Pastry    | 51             | 82  | 0.168                | [0-4.3]            | 0.264       | [0.11-4.31]   |
| Items              |                |   |                      |                    |             |               |
| Beverages          | 6              | 67  | 0.027                | [0-0.1]            | 0.120       | [0.11-0.16]   |
| Flour              | 6              | 67  | 0.019                | [0-0.08]           | 0.119       | [0.11-0.14]   |
| Vegetables, Nuts & | 9              | 78  | 0.008                | [0-0.036]          | 0.115       | [0.11-0.136]  |
| Seeds              |                |   |                      |                    |             |               |
| Savoury Snacks     | 6              | 83  | 0.005                | [0-0.028]          | 0.113       | [0.11-0.128]  |
| Starch Substitute  | 6              |   |                      | < LOD in all san   | nples       |               |
| Pasta & Noodles    | 30             |   | < LOD in all samples |                    |             |               |
| Total              | 327            | 78  |                      |                    |             |               |

<sup>\*</sup> Rounded to 3 decimal places

# Dietary Exposure to the sum of T-2, HT-2 and DAS

# **Adult Population**

31. The dietary exposure estimates to the sum of T-2, HT-2 and DAS of the local adult population arising from the collected food items are shown in Table 2. For dietary exposures to the sum of T-2, HT-2 and DAS of the local adult population, the LB and UB exposure estimates for average consumers are 0.0753 and 0.8455 ng/kg bw/day (0.3% and 3.38% of the Group TDI), while for high consumer (90th percentile), the LB and

UB exposure estimates are 0.1907 and 1.2806 ng/kg bw/day (0.76% and 5.12% of the Group TDI). The results indicated that both average and high consumers were unlikely to experience adverse effects of T-2, HT-2 and DAS.

Table 2: Dietary Exposure to the sum of T-2, HT-2 and DAS of Local
Adult Population and the % Contribution to the Group TDI

|                   | Average Consumers | High Consumers            |  |
|-------------------|-------------------|---------------------------|--|
|                   | (LB-UB)           | (90th Percentile) (LB-UB) |  |
| Dietary Exposure  | 0.0753 - 0.8455   | 0.1907 - 1.2806           |  |
| (ng/kg bw/day)    | 0.0733 – 0.6433   |                           |  |
| % contribution to | 0.3% - 3.38%      | 0.76% - 5.12%             |  |
| Group TDI         | 0.3% - 3.38%      | 0.70% - 3.12%             |  |

32. Further analysis of dietary exposures of individual age-gender groups of the adult population to T-2, HT-2 and DAS is shown in Table 3. The results also indicated that both average and high consumers of all age-gender groups were unlikely to experience adverse effects of T-2, HT-2 and DAS.

<u>Table 3. Dietary Exposure to the sum of T-2, HT-2 and DAS by Age-Gender Groups of Local Adult Population</u>

|             |                | Dietary Exposure (ng/kg bw/day) |                            |  |
|-------------|----------------|---------------------------------|----------------------------|--|
|             |                | Average Consumers               | <b>High Consumers</b>      |  |
|             |                | (LB-UB)                         | (90th Percentile) (LB-UB)  |  |
| Age 18-49   | Male           | 0.0643-0.9035                   | 0.1504-1.3549              |  |
|             | Female         | 0.0701-0.8019                   | 0.1628-1.2723              |  |
| Age 50-64   | Male           | 0.0907-0.9456                   | 0.2659-1.3937              |  |
|             | Female         | 0.0894-0.7925                   | 0.1998-1.2400              |  |
| >Age 65     | Male           | 0.0719-0.8516                   | 0.1840-1.2573              |  |
|             | Female         | 0.0758-0.7685                   | 0.1897-1.1586              |  |
| Age 18- 65+ | Male           | 0.0737-0.9052                   | 0.1943-1.3458              |  |
|             | Female         | 0.0768-0.7923                   | 0.1851-1.2388              |  |
| Age 18- 65+ | Female<br>Male | 0.0758-0.7685<br>0.0737-0.9052  | 0.1897-1.15<br>0.1943-1.34 |  |

# Younger Population

33. The dietary exposure estimates to the sum of T-2, HT-2 and DAS of the local younger population arising from the collected food items are shown in Table 4. For dietary exposures to the sum of T-2, HT-2 and DAS of the younger population, the LB and UB exposure estimates for average consumers are 0.3407 and 1.4703 ng/kg bw/day (1.36% and 5.88% of the Group TDI) respectively, while for high consumer (90<sup>th</sup> percentile), the LB and UB exposure estimates are 0.7078 and 2.4047 ng/kg bw/day (2.83% and 9.62% of the Group TDI) respectively. The results indicated that both average and high consumers were unlikely to experience adverse effects of T-2, HT-2 and DAS.

<u>Table 4: Dietary Exposure to the sum of T-2, HT-2 and DAS of Local</u>
<u>Younger Population and the % Contribution to the Group TDI</u>

|                                    | Average Consumers<br>(LB-UB) | High Consumers<br>(90 <sup>th</sup> Percentile) (LB-UB) |
|------------------------------------|------------------------------|---|
| Dietary Exposure<br>(ng/kg bw/day) | 0.3407 - 1.4703              | 0.7078 - 2.4047   |
| % contribution to Group TDI        | 1.36% - 5.88%                | 2.83% - 9.62%   |

34. Further details on the analysis of the dietary exposures of the younger population subgroups (children and adolescents, i.e. aged 6-11 and aged 12-17) are presented in the following paragraphs.

# Children (6-11 years old)

35. The dietary exposure estimates to the sum of T-2, HT-2 and DAS of children arising from the collected food items are shown in Table 5. For dietary exposures to the sum of T-2, HT-2 and DAS of children, the LB and UB exposure estimates for average consumers are 0.4694 and 1.8328 ng/kg bw/day (1.88% and 7.33% of the Group TDI) respectively, while for high consumers (90<sup>th</sup> percentile), the LB and UB exposure estimates are 1.2601 and 2.9497 ng/kg bw/day (5.04% and 11.80% of the Group TDI) respectively. The results indicated that both average and high consumers were unlikely to experience adverse effects of T-2, HT-2 and DAS.

Table 5: Dietary Exposure to the sum of T-2, HT-2 and DAS of Children and the % Contribution to the Group TDI

|                                    | Average Consumers<br>(LB-UB) | High Consumers<br>(90 <sup>th</sup> Percentile) (LB-UB) |
|------------------------------------|------------------------------|---|
| Dietary Exposure<br>(ng/kg bw/day) | 0.4694 - 1.8328              | 1.2601 – 2.9497   |
| % contribution to<br>Group TDI     | 1.88% - 7.33%                | 5.04% - 11.80%  |

# Adolescents (12-17 years old)

of adolescents arising from the collected food items are shown in Table 6. For dietary exposures to the sum of T-2, HT-2 and DAS of adolescents, the LB and UB exposure estimates for average consumers are 0.2046 and 1.0870 ng/kg bw/day (0.82% and 4.35% of the Group TDI) respectively, while for high consumers (90<sup>th</sup> percentile), the LB and UB exposure estimates are 0.3077 and 1.7155 ng/kg bw/day (1.23% and 6.86% of the Group TDI) respectively. The results indicated that both average and high consumers were unlikely to experience adverse effects of T-2, HT-2 and DAS.

Table 6: Dietary Exposure to the sum of T-2, HT-2 and DAS of Adolescents and the % Contribution to the Group TDI

|                   | Average Consumers | High Consumers            |  |
|-------------------|-------------------|---------------------------|--|
|                   | (LB-UB)           | (90th Percentile) (LB-UB) |  |
| Dietary Exposure  | 0.2046 – 1.0870   | 0.3077 – 1.7155           |  |
| (ng/kg bw/day)    | 0.2010 1.0070     |                           |  |
| % contribution to | 0.82% - 4.35%     | 1.23% - 6.86%             |  |
| <b>Group TDI</b>  | 0.0270 - 4.3370   | 1.23% - 0.80%             |  |

37. The dietary exposure estimates for adolescents are further broken down by gender, having considered the gender differences in diet among adolescents (Table 7). The results indicated that both average and high consumers were unlikely to experience adverse effects of T-2, HT-2 and DAS.

Table 7. Dietary Exposure to the sum of T-2, HT-2 and DAS by Gender

Groups of Adolescents

|        | Average Consumers | High Consumers            |
|--------|-------------------|---------------------------|
|        | (LB-UB)           | (90th Percentile) (LB-UB) |
| Male   | 0.2164-1.1894     | 0.2170-1.7656             |
| Female | 0.1922-0.9787     | 0.3683-1.5745             |

# **Major Food Contributor**

- 38. Contributions of food groups to overall LB dietary exposure to the sum of T-2, HT-2 and DAS for an average consumer of adult and younger populations are shown in Tables 8 and 9, respectively.
- 39. The LB was considered to be better reflecting the actual food group contribution to the overall exposure to T-2, HT-2 and DAS since it was not influenced by the high numbers of samples with results below the LOD in some food groups. The percentages of Group TDI of T-2, HT-2 and DAS were also calculated using the LB estimation.

# **Adult Population**

40. Compared with other food groups, "Cereal Grains" has the highest contribution to the overall exposure to T-2, HT-2 and DAS, followed by "Beverages" and "Bakery & Pastry Item". Nonetheless, the results revealed that none of the food groups was identified as a significant source of dietary exposure to T-2, HT-2 and DAS for the local adult population as the percentage contributions were all less than 5% of the Group TDI of T-2, HT-2 and DAS.

Table 8. Average Dietary Exposure to T-2, HT-2 and DAS of Local Adult

<u>Population</u>

| Food Groups              | Dietary Exposure | % Contribution to       | % contribution to |
|--------------------------|------------------|-------------------------|-------------------|
|                          | (ng/kg bw/day)   | <b>Dietary Exposure</b> | <b>Group TDI</b>  |
| Cereal Grains            | 0.02560          | 33.98%                  | 0.1024%           |
| Beverages                | 0.01621          | 21.52%                  | 0.0648%           |
| Bakery & Pastry Items    | 0.01477          | 19.60%                  | 0.0591%           |
| Breakfast Cereal         | 0.01306          | 17.34%                  | 0.0522%           |
| Vegetable Oils           | 0.00368          | 4.89%                   | 0.0147%           |
| Flour                    | 0.00186          | 2.47%                   | 0.0074%           |
| Savoury Snacks           | 0.00012          | 0.15%                   | 0.0005%           |
| Vegetables, nuts & seeds | 0.00004          | 0.05%                   | 0.0002%           |
| Total                    | 0.07534          | 100%                    | 0.3%              |

# Younger Population

41. Compared with other food groups, "Breakfast Cereal" has the highest contribution to the overall exposure to T-2, HT-2 and DAS, followed by "Bakery & Pastry Item" and "Cereal Grains". Nonetheless, the results revealed that none of the food groups was identified as a significant source of dietary exposure to T-2, HT-2 and DAS for the younger population as the percentage contributions were all less than 5% of the Group TDI of T-2, HT-2 and DAS.

<u>Table 9: Average Dietary Exposure to T-2, HT-2 and DAS of Younger Population</u>

| Food Groups              | Dietary Exposure | % Contribution to       | % contribution to |
|--------------------------|------------------|-------------------------|-------------------|
|                          | (ng/kg bw/day)   | <b>Dietary Exposure</b> | <b>Group TDI</b>  |
| Breakfast Cereal         | 0.28063          | 82.37%                  | 1.1225%           |
| Bakery & Pastry Items    | 0.02388          | 7.01%                   | 0.0955%           |
| Cereal Grains            | 0.02388          | 7.01%                   | 0.0955%           |
| Vegetable Oils           | 0.01008          | 2.96%                   | 0.0403%           |
| Flour                    | 0.00159          | 0.47%                   | 0.0064%           |
| Savoury Snacks           | 0.00049          | 0.14%                   | 0.0020%           |
| Vegetables, nuts & seeds | 0.00014          | 0.04%                   | 0.0006%           |
| Total                    | 0.3407           | 100%                    | 1.36%             |

# **Comparison with Other Places**

- 42. Dietary exposures of T-2, HT-2 and DAS or their sum have been reported in several studies, which were conducted by China National Center for Food Safety Risk Assessment (CFSA), the European Food Safety Authority (EFSA), French Agency for Food and Environmental and Occupational Health & Safety (ANSES).
- 43. JECFA also reviewed studies from different countries and provided a summary of the range of exposure estimates to T-2 and HT-2 derived from the scientific literature. The committee re-evaluated the combined dietary exposure to T-2, HT-2 and DAS in 2022 and considered the risks associated with combined dietary exposure to only the sum of T-2 and HT-2 was adopted because the dietary exposure estimates to DAS were much lower than the estimates of combined dietary exposure to T-2 and HT-2<sup>1</sup>. Table 10 summaries the reported level of average dietary exposure to T-2, HT-2 and DAS or their sum in overseas studies and this

study. It is noted that the average dietary exposures to T-2, HT-2 and DAS for local adult and younger populations are in the low range.

44. Caution should be taken when comparing the results from different studies. Apart from the test methods adopted, other factors such as research methodology, sampling strategies, approaches of capturing and handling consumption data, limit of detection, would affect the outcome of the studies.

Table 10: Reported level of average dietary exposure to T-2, HT-2 and DAS or their sum in overseas studies and this study

|                                  |                   | Ave                      | day)       |           |           |
|----------------------------------|-------------------|--------------------------|------------|-----------|-----------|
| Report/Study                     | Mycotoxin         | Mycotoxin Children/ Your |            | Adult     |           |
|                                  |                   | LB                       | UB         | LB        | UB        |
| ANSES (2011) <sup>13</sup>       | T-2               | 4.0                      | 38.0       | 1.78      | 19.6      |
|                                  | HT-2              | 10.5                     | 53.1       | 7.16      | 32.2      |
| EFSA (2017) <sup>3</sup>         | Sum of T-2 & HT-2 | 4.37-18.0                | 15.4-62.1  | 1.82-10.2 | 13.4-26.4 |
| $(2018)^9$                       | DAS               | 0.2-27.9                 | 23.5-174.3 | 0.2-8.9   | 12.2-66.2 |
| CFSA (2018) <sup>14</sup>        | Sum of T-2 & HT-2 | NA                       |            | 52        |           |
| <b>JECFA</b> (2020) <sup>5</sup> | Sum of T-2 & HT-2 | 0.8-53                   | 8.2-169    | 0.3-27    | 2.7-60    |
| <b>Current study</b>             | Sum of T-2, HT-2  | 0.2046                   | 1.8328     | 0.0753    | 0.8455    |
| (2024)                           | and DAS           |                          |            |           |           |

# **Uncertainties and Limitations of the Study**

45. In this study, only selected food items that were commonly consumed and reported to be more likely to contain T-2, HT-2 and DAS were sampled. As there are limited published studies on T-2, HT-2 and DAS in local foods, the samples collected in this study may not cover all

foods in the local diet containing high levels of T-2, HT-2 or DAS, in particular those consumed in small amount.

While higher accuracy and precision in exposure estimation could be achieved with analysis of more samples, compromises had to be made about the use of finite resources. Batch-to-batch variation is likely to be present in T-2, HT-2 and DAS levels even for the same product, the results of this study represented only a snapshot of the levels of these mycotoxins in the selected locally available foods.

#### CONCLUSIONS AND RECOMMENDATIONS

- 47. In this study, 78% of samples (254 out of 327 samples) were not detected with T-2, HT-2 and DAS, and only 73 samples (22%) were found to contain at least one of these mycotoxins. The food group "Breakfast Cereal" was found to contain the highest mean levels of the sum of T-2, HT-2 and DAS, followed by "Vegetable Oils".
- 48. The estimated dietary exposures to the sum of T-2, HT-2 and DAS for the local average and high consumers of the adult and younger population were well below the group TDI established by JECFA. All in all, the study findings revealed that dietary exposure to the sum of T-2, HT-2 and DAS would be unlikely to pose health risks to the average and high consumers of the local adult and younger populations.
- 49. To minimise the risk of trichothecene contamination, members of the general public should purchase cereals and cereal-based products from reliable sources and store them properly in a cool, dry place to prevent fungal growth. Additionally, the public should follow the basic dietary advice on healthy eating and maintain a balanced and varied diet to minimise the risk of exposure to contaminants from a limited range of food items.
- 50. Members of the trade are advised to store the food commodities properly. Safe moisture levels should be maintained with minimum temperature changes to avoid susceptible mould growth and increased mycotoxin levels imparted by the mould species. For details, please refer

to Codex's "Code of Practice for the Prevention and Reduction of Mycotoxin Contamination in Cereals".

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| 1.9     | T-2 and HT-2 toxins  | Maximum level (µg/kg)      | Remarks  |  |  |
|---------|--|----------------------------|--|--|--|
|         |  | Sum of T-2 and HT-2 toxins | For the sum of T-2 and HT-2 toxins, maximum levels refer to lower bound concentrations, which are calculated on the assumption that all the values below the limit of quantification are zero.   |  |  |
| 1.9.1   | Unprocessed cereal grains except products listed in 1.9.1.1, 1.9.1.2, 1.9.1.3 and 1.9.1.4  | 50                         | Except unprocessed maize grains intended to be processed by wet milling and except rice.  The maximum level applies to unprocessed cereal grains placed on the market for first-stage processing (°).  |  |  |
| 1.9.1.1 | Unprocessed malting barley grains  | 200                        | The maximum level applies to unprocessed malting barley grains placed on the market for first-stage processing (*).  |  |  |
| 1.9.1.2 | Unprocessed barley grains other than malting barley grains   | 150                        | The maximum level applies to unprocessed barley grains placed on the market for first-stage processing (°).  |  |  |
| 1.9.1.3 | Unprocessed maize grains and unprocessed durum wheat grains  | 100                        | Except unprocessed maize grains for which it is evident, e.g. through labelling or destination, that they are intended for use in a wet milling process only (starch production).  The maximum level applies to unprocessed maize grains and unprocessed durum wheat grains placed on the market for first-stage processing (*). |  |  |
| 1.9.1.4 | Unprocessed oat grains with inedible husk  | 1 250                      | The maximum level applies to unprocessed oat grains with husk placed on the market for first-stage processing (*). The maximum level applies to the oat grains with the inedible husk included.  |  |  |
| 1.9.2   | Cereals placed on the market for the final consumer except products listed in 1.9.2.1 and 1.9.2.2  | 20                         | Except rice.   |  |  |
| 1.9.2.1 | Oats placed on the market for the final consumer   | 100                        |  |  |  |
| 1.9.2.2 | Barley, maize and durum wheat placed on the market for the final consumer  | 50                         |  |  |  |
|         |  | I                          |  |  |  |
| 1.9.3   | Milling products of cereals except products listed in 1.9.3.1 and 1.9.3.2  | 20                         | Except milling products of rice.   |  |  |
| 1.9.3.1 | Milling products of oats (including oat bran)  | 100                        |  |  |  |
| 1.9.3.2 | Bran from cereals other than oats and milling products of maize  | 50                         |  |  |  |
| 1.9.4   | Bakery wares except products listed in 1.9.5, pasta, cereal snacks and breakfast cereals except products listed in 1.9.6, 1.9.7 and 1.9.8  | 20                         | Except rice products.<br>Including small bakery wares.<br>Pasta means pasta (dry) with a water content of approximately 12 %.  |  |  |
| 1.9.5   | Bakery wares containing at least 90 % milling products of oats   | 100                        | Except rice products. Including small bakery wares.  |  |  |
| 1.9.6   | Oat flakes   | 100                        |  |  |  |
| 1.9.7   | Breakfast cereals consisting of at least 50 % of cereal bran, milling products of oat grains, milling products of maize grains, whole oat grains, barley grains, maize grains or durum wheat grains, and consisting of less than 40 % of milling products of oat grains and whole oat grains | 50                         |  |  |  |
| 1.9.8   | Breakfast cereals consisting of at least 50 % of cereal bran, milling products of oat grains, milling products of maize grains, whole oat grains, barley grains, maize grains or durum wheat grains, and of at least 40 % of milling products of oat grains and whole oat grains             | 75                         |  |  |  |
| 1.9.9   | Baby food and processed cereal-based food for infants and young children (*)   | 10                         | Except rice products.  The maximum level applies to the dry matter (3) of the product as placed on the market.   |  |  |
| 1.9.10  | Food for special medical purposes intended for infants and young children (*)  | 10                         | Except rice products.  The maximum level applies to the dry matter (3) of the product as placed on the market.   |  |  |

Appendix II

# Mean level of the Sum of T-2, HT-2 and DAS (μg/kg) Detected in <u>Different Food Items</u>

| Food Item                | No. of     | % of  | Mean of Sum of T-2, HT-2 & DAS (μg/kg) [range] |                  |              |              |  |
|--------------------------|------------|---|--|------------------|--------------|--------------|--|
|                          | samples    | samples   |  |                  |              |              |  |
|                          |            | <lod< th=""><th></th><th></th><th></th><th></th></lod<> |  |                  |              |              |  |
|                          |            |   | Lower bound                                    |                  | Upper bound  |              |  |
| <b>Cereal Grains</b>     | <u>190</u> | <u>78</u>   | <u>0.304</u>                                   | [0-7.038]        | <u>0.381</u> | [0.11-7.038] |  |
| White Rice               | 13         |   | < LOD in all samples                           |                  |              |              |  |
| Red Rice                 | 13         | 77  | 0.005  | [0-0.038]        | 0.113        | [0.11-0.138] |  |
| Brown Rice               | 13         | 85  | 0.157  | [0-0.19]         | 0.124        | [0.11-0.29]  |  |
| Ten Rice                 | 12         | 67  | 0.059  | [0-0.46]         | 0.162        | [0.11-0.52]  |  |
| Glutinous Rice           | 13         | 92  | 0.001  | [0-0.016]        | 0.111        | [0.11-0.116] |  |
| Oat Rice                 | 12         | 83  | 0.394  | [0-3.78]         | 0.488        | [0.11-3.79]  |  |
| Risotto                  | 12         |   | < LOD in all samples                           |                  |              |              |  |
| Millet                   | 13         | 85  | 0.613  | [0-7.038]        | 0.7056       | [0.11-7.038] |  |
| Quinoa                   | 13         |   | < LOD in all samples                           |                  |              |              |  |
| Barley                   | 12         | 83  | 0.214  | [0-1.4]          | 0.311        | [0.11-1.46]  |  |
| Buckwheat                | 12         |   | < LOD in all samples                           |                  |              |              |  |
| Dried Corn               | 12         | 92  | 0.064  | [0-0.77]         | 0.166        | [0.11-0.78]  |  |
| Pearl Barley             | 13         | 38  | 0.572  | [0-2.2]          | 0.673        | [0.11-2.3]   |  |
| Wheat                    | 12         | 58  | 0.215  | [0-0.84]         | 0.296        | [0.11-0.85]  |  |
| Wheat Bran               | 3          | 33  | 2.804  | [0-4.402]        | 2.844        | [0.11-4.402] |  |
| Wheat Germ               | 12         | 25  | 1.534  | [0-6.27]         | 1.573        | [0.11-6.28]  |  |
| Flour                    | <u>6</u>   | <u>67</u>   | <u>0.019</u>                                   | [0-0.08]         | <u>0.119</u> | [0.11-0.14]  |  |
| Wheat Flour              | 3          |   | < LOD in all samples                           |                  |              |              |  |
| Rice Flour               | 3          | 33  | 0.037  | [0-0.08]         | 0.127        | [0.11-0.14]  |  |
| <b>Starch Substitute</b> | <u>6</u>   |   | < LOD in all samples                           |                  |              |              |  |
| Cornstarch               | 3          |   |  | < LOD in all sar | nples        |              |  |
| Wheat Starch             | 3          | -   |  |                  |              |              |  |
| Pasta & Noodles          | <u>30</u>  |   | < LOD in all samples                           |                  |              |              |  |
| Instant Noodles          | 3          |   | < LOD in all samples                           |                  |              |              |  |
| Egg Noodles              | 3          |   |  |                  |              |              |  |
| Dan Dan Noodles          | 3          |   |  |                  |              |              |  |
| Ramen                    | 3          |   |  |                  |              |              |  |

| Knife-cut Noodles                  | 3         |           |              |                  |              |                |
|------------------------------------|-----------|-----------|--------------|------------------|--------------|----------------|
| Udon                               | 3         | _         |              |                  |              |                |
| Rice Noodles                       | 3         | _         |              |                  |              |                |
| Flat Noodles                       | 3         | _         |              |                  |              |                |
| Vietnamese Rice                    | 3         | _         |              |                  |              |                |
| Noodles                            |           |           |              |                  |              |                |
| Soba                               | 3         |           |              |                  |              |                |
| <b>Breakfast Cereal</b>            | <u>17</u> | <u>29</u> | <u>2.687</u> | [0-23.718]       | <u>2.726</u> | <u>[0.11-</u>  |
|                                    |           |           |              |                  |              | <u>23.718]</u> |
| Oatmeal                            | 3         | 67        | 0.103        | [0-0.31]         | 0.180        | [0.11-0.32]    |
| Corn Flakes                        | 3         | 33        | 0.405        | [0-0.884]        | 0.441        | [0.11-0.884]   |
| Breakfast Cereal                   | 5         | 20        | 7.823        | [0-23.718]       | 7.859        | [0.11-         |
| (Ready-to-eat)                     |           |           |              |                  |              | 23.718]        |
| Cereal Bar                         | 3         | 33        | 0.847        | [0-1.56]         | 0.890        | [0.11-1.57]    |
| Granola                            | 3         | 0         | 0.835        | [0.36-1.588]     | 0.839        | [0.37-1.588]   |
| Bakery & Pastry                    | <u>51</u> | <u>82</u> | <u>0.168</u> | [0-4.3]          | <u>0.264</u> | [0.11-4.31]    |
| <u>Items</u>                       |           |           |              |                  |              |                |
| White bread                        | 3         | _         |              | < LOD in all sam | ples         |                |
| Wheat bread                        | 3         |           |              |                  |              |                |
| Pizza crust                        | 3         | 67        | 0.767        | [0-0.23]         | 0.170        | [0.11-0.29]    |
| Bagel                              | 3         | 0         | 0.607        | [0.21-1.15]      | 0.633        | [0.27-1.16]    |
| Paratha                            | 3         | _         |              | < LOD in all sam | ples         |                |
| Multigrain Bread                   | 3         |           |              |                  |              |                |
| Bread Crumbs                       | 3         | 67        | 0.023        | [0-0.07]         | 0.117        | [0.11-0.13]    |
| Saltine Crackers                   | 3         | _         |              | < LOD in all sam | ples         |                |
| Sandwich Crackers                  | 3         |           |              |                  |              |                |
| Digestive Biscuit                  | 3         | 33        | 1.587        | [0-4.3]          | 1.630        | [0.11-4.31]    |
| Maltose Biscuit                    | 3         | 67        | 0.183        | [0-0.55]         | 0.277        | [0.11-0.61]    |
| Chocolate flavored                 | 3         | 67        | 0.377        | [0-1.13]         | 0.453        | [0.11-1.14]    |
| Cracker                            |           |           |              |                  |              |                |
| Sponge Cake 3 < LOD in all samples |           | ples      |              |                  |              |                |
| Swiss Roll                         | 3         | _         |              |                  |              |                |
| Cake with Whipped                  | 3         |           |              |                  |              |                |
| Cream                              |           | _         |              |                  |              |                |
| Cake                               | 3         | _         |              |                  |              |                |
| Cheesecake                         | 3         |           |              |                  |              |                |
| Savoury Snacks                     | <u>6</u>  | <u>83</u> | <u>0.005</u> | [0-0.028]        | <u>0.113</u> | [0.11-0.128]   |

| Potato Chips       | 3        | 67        | 0.009                | [0-0.028]   | 0.116        | [0.11-0.128] |  |
|--------------------|----------|-----------|----------------------|-------------|--------------|--------------|--|
| Popcorn            | 3        |           | < LOD in all samples |             |              |              |  |
| Vegetables, Nuts & | 9        | <u>78</u> | <u>0.008</u>         | [0-0.036]   | <u>0.115</u> | [0.11-0.136] |  |
| Seeds              |          |           |                      |             |              |              |  |
| Soybean            | 3        |           | < LOD in all samples |             |              |              |  |
| Sunflower Seed     | 3        | 67        | 0.012                | [0-0.036]   | 0.119        | [0.11-0.136] |  |
| Chestnut           | 3        | 67        | 0.011                | [0-0.032]   | 0.117        | [0.11-0.132] |  |
| Vegetable Oils     | <u>6</u> | <u>33</u> | <u>1.075</u>         | [0-3.43]    | 1.222        | [0.11-3.44]  |  |
| Olive Oil          | 3        | 67        | 0.093                | [0-0.28]    | 0.187        | [0.11-0.34]  |  |
| Sunflower Oil      | 3        | 0         | 2.057                | [0.92-3.43] | 2.257        | [0.98-3.44]  |  |
| Beverages          | <u>6</u> | <u>67</u> | <u>0.027</u>         | [0-0.1]     | <u>0.120</u> | [0.11-0.16]  |  |
| Coffee             | 3        |           | < LOD in all samples |             |              |              |  |
| Beer               | 3        | 33        | 0.053                | [0-0.1]     | 0.130        | [0.11-0.16]  |  |