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FW14007 Trichothecene Mycotoxins in Cereal Products

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Scientific Interpretive Summary

This SIS is prepared by MPI risk assessors to provide context to the following report for MPI risk managers and external readers

The New Zealand Mycotoxin Surveillance Program 06-14 Report Series

FW14007 Trichothecene Mycotoxins in Cereal Products

These reports are the outputs of MPIs ongoing mycotoxin surveillance programme. The nine reports form a series detailing the research undertaken over the last eight years to characterise and quantify the risk to the New Zealand public through the presence of mycotoxins in the food supply.

The nine reports are:

- Risk Profile: Mycotoxin in Foods 2006
- Aflatoxins in Maize Products 2008
- Aflatoxins and Ochratoxin A in Dried Fruits and Spices 2009
- Aflatoxins in Nuts and Nut Products 2010
- Dietary Exposure to Aflatoxins 2011
- Ochratoxin A in Cereal Products, Wine, Beer and Coffee 2011
- Trichothecene Mycotoxins in Cereal Products 2014
- Dietary Exposure to Ochratoxin A and Trichothecene Mycotoxins 2014
- Risk Profile: Mycotoxin in Foods 2014

<u>Trichothecene Mycotoxins in Cereal Products 2014</u>

Trichothecenes were listed as the 3rd highest priority in the 2006 mycotoxin risk profile. They were surveyed across 200 cereal based foods to determine if occurrence represents a risk to public health. Trichothecenes compromises two classes in foods, A and B. Class A Trichothecenes (such as T-2 and HT-2) are considerably more toxic but were considered to be rare in New Zealand foods. Class B trichothecenes (such as Deoxynivalenol (DON) and Nivalenol(NIV)) are more widespread but generally are lower toxicity, although they may be significant contributors to general food poisoning incidents.

Analytical methodology for the survey is well detailed and the results can be viewed with strong confidence as being accurate for current occurrence levels.

DON and NIV were both commonly detected, although levels were low and the highest results occurred in foods containing, or suspected to contain, imported grains. Other Trichothecenes were infrequently detected; diacetoxyscirpenol in three samples, and 15-acetyl DON and T-2 in one sample each.

New Zealand grains appear low in Trichothecene mycotoxins by international standards.

MYCOTOXIN SURVEILLANCE PROGRAMME 2012-2013: TRICHOTHECENE MYCOTOXINS IN CEREAL PRODUCTS

Report Number FW14007

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MYCOTOXIN SURVEILLANCE PROGRAMME 2012-2013: TRICHOTHECENE MYCOTOXINS IN CEREAL PRODUCTS

Prepared for Ministry for Primary Industries
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Client Report FW14007

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May 2014

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SUMMARY

The Mycotoxin Surveillance Programme (MSP) involves investigation of food safety issues associated with mycotoxins in the New Zealand food supply, as identified in a risk profiling exercise carried out in 2005-2006. During 2012-2013, the MSP involved analysis of cereal-based foods for the presence of trichothecene mycotoxins, including deoxynivalenol (DON), 3-acetyl-DON (3ADON), 15-acetyl-DON (15ADON), nivalenol (NIV), fusarenon X (FX), T-2 and HT-2 toxins (T2, HT2), diacetoxyscirpenol (DAS) and neosolaniol (NEO). Analyses were carried out on retained frozen samples from the 2009 New Zealand Total Diet Survey (*n* = 176) and additional fresh samples purchased in March 2014 (*n* = 24).

DON was the most commonly detected toxin (123 of 200 samples), followed by NIV (59 of 200 samples). DAS was detected at low concentrations in 3 samples, while 15ADON and T2 were detected in 1 sample each. However, these infrequently detected toxins were only detected in foods either definitely or potentially of non-domestic origins. Similarly, the highest concentrations of DON were found in foods that were either imported or the major ingredients were potentially imported. It appears that foods produced from New Zealand-produced ingredients contain low levels of DON, by international standards.

It has previously been suggested that fungi capable of producing T2 (and HT2) may not be present in New Zealand. The results of the current study support this proposition, as the only food containing T2 was produced from potentially imported ingredients. Oats are commonly contaminated with T2 in Europe. Rolled oats analysed in the current study were not found to contain detectable T2.

While the Australia New Zealand Food Standards Code does not contain regulatory limits for trichothecene mycotoxins, the concentrations of toxins found in the current study would have been compliant with regulatory limits promulgated in other countries.

Dietary exposure estimates for trichothecene mycotoxins, considering all potential food sources, would assist in placing the results of the current survey in context with respect to human health risks.

1 INTRODUCTION

The Mycotoxin Surveillance Programme (MSP) involves investigation of food safety issues associated with mycotoxins in the New Zealand food supply.

As with other activities of the Ministry for Primary Industries (MPI), activities in this area are directed on the basis of risk. The risk profile of mycotoxins in the New Zealand food supply (Cressey and Thomson, 2006) is viewed as a starting point for this process. The risk profile identified a number of issues to be investigated or clarified.

Effort in previous years have focussed on determination of aflatoxins in a range of foods (Cressey and Jones, 2008; 2009; 2010), culminating in a dietary exposure assessment (Cressey, 2011), and analysis of ochratoxin A (OTA) in dried fruits and spices (Cressey and Jones, 2009) and cereal products, coffee, wine and beer (Cressey and Jones, 2011). During 2012-2013, the MSP involved analyses of cereal products for trichothecene mycotoxins. This is the last of the three priority issues identified in the 2006 risk profile.

1.1 Trichothecene Mycotoxins

1.1.1 Hazard identification

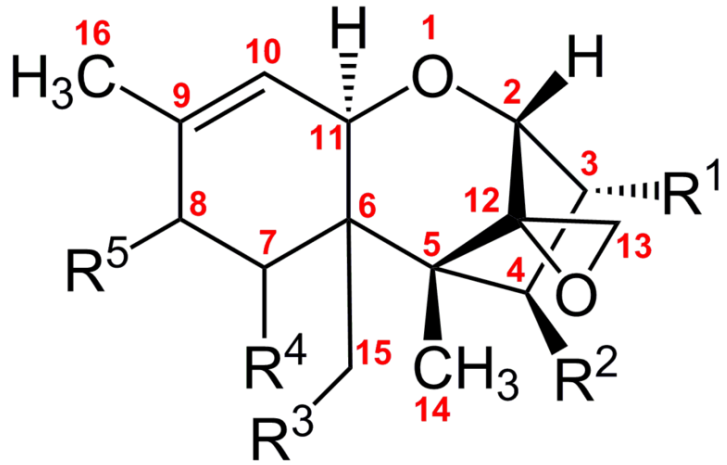
The trichothecenes are a family of approximately 150 structurally related compounds produced by fungi of the genera *Fusarium, Cephalosporium, Myrothecium, Stachybotrys, Trichoderma* and others. Trichothecenes of significance in food are produced by *Fusarium* species, including *F. poae, F. sporotrichioides, F. acuminatum, F. graminearum, F. culmorum, F. crookwellense, F. avenaceum* and *F. equiseti* (Council for Agriculture and Technology, 2003). The toxins in this group that have received the most attention are deoxynivalenol (DON), nivalenol (NIV), T-2 toxin (T2) and HT-2 toxin (HT2), with lesser attention paid to diacetoxyscirpenol (DAS) and other trichothecene toxins. Focus on these toxins has been due to the fact that they are the major toxins formed in foods and/or there is evidence for their involvement in human disease. Trichothecenes have been reported in cereal grain crops worldwide (Schothorst and Van Egmond, 2004).

1.1.2 Structure and nomenclature

The trichothecenes are sesquiterpenoids possessing a tetracyclic 12,13-epoxytrichothecene skeleton. They can be conveniently divided into 4 categories according to similarity of functional groups. The first class is characterised by a functional group other than a ketone at C-8 (type A) and include T2 and HT2, DAS and neosolaniol (NEO). The second category of trichothecenes usually has a carbonyl function at C-8 (type B), typified by DON and NIV. The third category is characterised by a second epoxide group at C-7,8 or C-9,10 (type C), and the fourth contains a macrocyclic ring system between C-4 and C-15 with 2 ester linkages (type D). Type C and type D trichothecenes are not normally associated with food, but may be associated with fungal contamination in the built environment.

Structural summaries are shown in Figure 1.

Figure 1: Structure of type A and B trichothecenes



Type A: T2 (R_1 = OH, R_2 = OAc, R_3 = OAc, R_4 = H, R_5 = $OCOCH_2CH(CH_3)_2$), HT2 (R_1 = OH, R_2 = OH, R_3 = OAc, R_4 = H, R_5 = $OCOCH_2CH(CH_3)_2$), DAS (R_1 = OH, R_2 = OAc, R_3 = Ac, R_4 = H, R_5 = H), NEO (R_1 = OH, R_2 = OAc, R_3 = OAc, R_4 = H, R_5 = OH)

Type B: DON (R_1 = OH, R_2 = H, R_3 = OH, R_4 = OH, R_5 = O), NIV (R_1 = OH, R_2 = OH, R_3 = OH, R_4 = OH, R_5 = O), 3-acetylDON (R_1 = OAc, R_2 = H, R_3 = OH, R_4 = OH, R_5 = O), 15-acetylDON (R_1 = OH, R_2 = H, R_3 = OAc, R_4 = OH, R_5 = O), DON-3-glucoside (R_1 = $OC_6H_{11}O_5$), R_2 = H, R_3 = OH, R_4 = OH, R_5 = O), Fusarenon X (R_1 = OH, R_2 = OAc, R_3 = OH, R_4 = OH, R_5 = O)

4.1.2 Occurrence

Type A trichothecenes (T2, HT2) are frequently associated with *F. tricintum, F. poae, F. sporotrichioides, F. acuminatum, F. equiseti* and *F. semitectum* (WHO, 1990). Trichothecene formation by these fungal species has been reported in Europe and North America and occasionally in Asia, but not in Africa or Australia (Council for Agriculture and Technology, 2003). Type B trichothecenes (DON, NIV) are frequently associated with *F. graminearum* and *F. culmorum* (WHO, 1990). Trichothecene formation by these species, particularly *F. graminearum* appears to be almost universal (Council for Agriculture and Technology, 2003).

Table 1 summarises information on *Fusarium* species occurring and production of trichothecenes in New Zealand crops.

Table 1: Trichothecene production by *Fusarium* species in New Zealand crops

Crop	Fungal species ('>' indicates order of detection frequency)	Trichothecenes detected	Study reference
Maize (Manawatu)	*F. graminearum* *F. culmorum* *F. subglutinans* *F. acuminatum*	T2, DON, DAS detected, but no details of which species produced which mycotoxins	(Hussein *et al.*, 1987)
Maize (Waikato)	*F. graminearum* >*F. semitectum* > *F. crookwellense**	No analyses carried out for mycotoxins	(Sayer, 1991)
Wheat (Waikato) Wheat (East Coast) Wheat (Manawatu) Wheat (South Island) Barley (Waikato) Barley (East Coast) Barley (Manawatu) Barley (South Island) Oats (East Coast) Oats (South Island) Maize (North Island)	*F. graminearum* >*F. avenaceum, F. crookwellense, F. poae* *F. culmorum* > *F. poae* *F. graminearum, F. culmorum* > *F. avenaceum, F. crookwellense, F. poae* *F. avenaceum* > *F. poae, F. culmorum* *F. graminearum* >*F. avenaceum, F. crookwellense, F. poae* *F. avenaceum, F. culmorum, F, equiseti,*> *F. poae* *F. poae* > *F. avenaceum, F. crookwellense, F. culmorum, F. graminearum* *F. avenaceum, F. poae* > *F. culmorum, F. graminearum* *F. poae* > *F. avenaceum* *F. avenaceum* > *F. culmorum* > *F. poae* *F. graminearum* > *F. crookwellense* > *F. semitectum*	No analyses carried out for mycotoxins	(Sayer and Lauren, 1991)
Maize, wheat, barley, oats (all New Zealand)	*F. graminearum* *F. culmorum* *F. avenaceum* *F. crookwellense* *F. poae* *F. semitectum* *F. equiseti* *F. tricinctum*	NIV, DON NIV, (DAS)# NIV, (DAS)# DAS	(Lauren *et al.*, 1992)
Wheat, barley (North Island)	*F. graminearum* > *F. avenaceum* > *F. poae* > *F. crookwellense* > *F. culmorum*	No analyses carried out for mycotoxins	(Cromey *et al.*, 2001)
Wheat, barley (South Island	*F. avenaceum* > *F. culmorum* > *F. graminearum* > *F crookwellense*	No analyses carried out for mycotoxins	(Cromey *et al.*, 2001)
Maize (Manawatu)	*F. graminearum* > *F. culmorum* > *F. acuminatum, F. subglutinans*	No analyses carried out for mycotoxins	(Hussein *et al.*, 2003)

* Order given here is for field maize. In stored maize the proportion of *F. semitectum* was greater than *F. graminearum.*

Detections of DAS were infrequent in comparison to detections of NIV

While there are differences between different studies, *F. graminearum* appears to be the *Fusarium* species that most commonly infects grain crops in the North Island. *F. graminearum* is associated with production of Type B trichothecenes (NIV, DON). Monds *et al.* (2005) examined the mycotoxin-producing potential of a number of *F. graminearum* isolates from New Zealand grains and found that the isolates either produced NIV or DON or neither, but rarely both NIV and DON in significant amounts.

South Island crops are more likely to be infected with *F. avenaceum*. Bosch *et al*. (1989) demonstrated significant rodent toxicity in extracts from *F. avenaceum* and found high levels of moniliformin in these extracts and a haemorrhagic factor (wortmannin) in 1 extract. There have been no reports of significant trichothecene production by *F. avenaceum* isolates in New Zealand. *F. avenaceum* is the most common *Fusarium* species infecting crops in Northern Europe and has been reported to produce the mycotoxins moniliformin, beauvericin and enniatins (Morrison *et al.*, 2002).

Other species common in New Zealand grain are known trichothecene producers, including *F. culmorum* (DON, NIV), *F. poae* (HT2, T2, DAS, NIV), and *F. crookwellense* (NIV, DAS).

The study of Hussein *et al*. (1987) is unique in reporting detection of T2. Although T2 has been looked for, it has not been detected in subsequent studies.

1.2 Previous Trichothecene Mycotoxin Surveillance in New Zealand

A number of studies have been carried out on trichothecenes (mainly DON and NIV) in New Zealand arable crops (mainly maize and wheat). These are summarised in Table 2.

Table 2: Trichothecenes in New Zealand cereal grains

Crop	Year	Location	Mycotoxin	Proportion positive	Maximum Concentration (µg/kg)		Reference
					DON[1]	NIV	
Maize	1984	Manawatu	DON DAS T2	11/20 6/20[2] 13/20[3]	300		(Hussein *et al.*, 1989)
Maize	1987-1989	Waikato Manawatu Gisborne	NIV/DON NIV/DON NIV/DON	38/38 19/19 16/34	3500 1700 970	1900 1950 3600	(Lauren *et al.*, 1991)
Maize	1992-1994	Waikato, Bay of Plenty, Manawatu, Gisborne	NIV/DON	605/616	8500	7000	(Lauren *et al.*, 1996)
Wheat	1986-1989	Waikato Manawatu Gisborne	NIV/DON NIV/DON NIV/DON	48/48 30/36 3/6	11,950 2310	1270 780 90	(Lauren *et al.*, 1991)
Wheat	1998-2000	Wairarapa, Manawatu, Rangatikei	NIV DON	12/15 14/15	 3250	570	(Cromey *et al.*, 2002)
Wheat	1998-2000	Wairarapa, Manawatu, Rangatikei	NIV[4] DON[4]	12/54 12/54	 1820	930	(Cromey *et al.*, 2002)

Crop	Year	Location	Mycotoxin	Proportion positive	Maximum Concentration (µg/kg)		Reference
					DON[1]	NIV	
Barley	1987-1989	Waikato Manawatu Gisborne	NIV/DON NIV/DON NIV/DON	17/18 14/23 2/3	1000 90 80	530 220 30	(Lauren *et al.*, 1991)
Oats	1989	Gisborne	NIV/DON	2/3	80	610	(Lauren *et al.*, 1991)

NIV = nivalenol DON = deoxynivalenol T2 = T-2 toxin DAS = diacetoxyscirpenol

[1] While there are no Australasian regulatory maximum limits for DON, the European Union has maximum limits of 1750 µg/kg for unprocessed maize, durum wheat and oats and 1250 µg/kg for all other unprocessed cereals. NIV levels are not regulated in any country

[2] DAS concentrations in the range 0.01-0.9 mg/kg were reported

[3] T2 Concentrations in the range 0.005-0.2 mg/kg were reported

[4] Results were only reported for samples with combined NIV + DON levels greater than 0.5 mg/kg

Lauren *et al.* (1991) also examined 102 samples of wheat, 41 samples of barley and 26 samples of oats from South Island sites (Canterbury, South Otago and Southland). While results for these sites were not given in detail, the authors noted that:

- The incidence of trichothecenes in South Island grain crops was low (20%).
- Only DON and NIV-type trichothecenes were detected.
- The level of contamination never exceeded 0.1 mg/kg.

While a number of studies (see Table 2) have determined trichothecene mycotoxins in cereal grains, only 1 study has been carried out on the presence of these toxins in consumer foods (Lauren and Veitch, 1996). The survey determined only DON and NIV. Results are summarised in Table 3.

Table 3: Trichothecenes in New Zealand foods

Food	Number of samples	Number of samples positive (range, mg/kg)	
		NIV*	DON*
Breakfast cereals	20	3 (0.07-0.08)	2 (0.10-0.11)
Extruded snack foods	20	1 (0.05)	ND
Maize meal products (flours, grits)	17	6 (0.06-0.65)	8 (0.05-0.41)
Breads	16	2 (0.06-0.20)	2 (0.04-0.05)
Masa flour products (corn chips, taco shells)	24	2 (0.03-0.05)	1 (0.09)
Snack bars	13	3 (0.03-0.06)	ND
Maize oil	8	ND	ND
Miscellaneous products (corn syrup, brewing sugar)	6	ND	ND
Total	**124**	**17 (13.7%)**	**13 (10.5%)**

NIV = nivalenol DON = deoxynivalenol ND = not detected at detection limit of 0.05 mg/kg

* Where samples were analysed in duplicate, with 1 result being positive and the other not detected, results have been reported here as the mean, with not detected results assigned a concentration of zero. Hence, some results reported here are below the limit of detection for single results.

1.3 Foods Contributing to Trichothecene Mycotoxin Exposure Overseas

A European scientific co-operation (SCOOP) consolidated national European data on levels of trichothecenes in foods and estimates of dietary exposure (SCOOP, 2003). In most cases, the mean estimates of dietary exposure were within the tolerable daily intake (TDI) for DON and NIV. Exposure to T2 and HT2 exceeded the TDI in several countries. In most cases bread, or wheat or flour, was the main contributor to exposure to DON and NIV, although in the UK estimates breakfast cereals often made a greater contribution than bread. For T2 and HT2 toxins, rye, durum (pasta) wheat, oats and beer (barley) were also major contributors in some countries.

The French Total Diet Study found similar results with approximately two-thirds of adult exposure to DON due to bread consumption (Leblanc *et al.*, 2005). Bread was also the major contributor to NIV exposure, followed by pasta, cakes and alcoholic beverages. A Lebanese dietary exposure study found the major contributors to DON exposure were bread, mannakeesh (a local product similar to pizza), cakes and pizza (Soubra *et al.*, 2009). These foods accounted for more than 70% of dietary DON exposure.

2 MATERIALS AND METHODS

2.1 Foods Sampled

2.1.1 Primary sample set

An initial scoping exercise identified cereals and cereal products as foods commonly contaminated with trichothecene mycotoxins.

After consideration of options, it was decided to use stored samples from the 2009 New Zealand Total Diet Study (NZTDS)[1]. These samples constitute a national representative 'snap-shot' of foods consumed in New Zealand at a particular point in time. Foods selected for trichothecene mycotoxin analysis are given in Table 3.

Table 4: New Zealand Total Diet Survey (NZTDS) sample types and sample numbers relevant to trichothecene mycotoxin analyses

Food Group	Food	Number of samples available
Bread	Bread, mixed grain	8
	Bread, wheatmeal	8
	Bread, white	8
Biscuits	Biscuits, chocolate	8
	Biscuits, cracker	8
	Biscuits, sweet plain	8
Breakfast cereals	Cornflakes	8
	Bran flake cereal	8
	Wheat biscuit cereal	8
	Muesli	8
	Oats, rolled (cooked)	8
Bakery products	Cake, plain	8
	Muffin	8
Pasta and noodles	Noodles, instant (cooked)	8
	Pasta, dried (cooked)	8
	Spaghetti in sauce, canned	8
Other cereal-based foods	Rice, white (cooked)	8
	Snack bars	8
	Snacks, flavoured	8
	Pizza	8
	Infant weaning food, cereal-based	8
	Beer	8
	Total	**176**

Foods were classified as either:

- National (N) foods, which were not expected to exhibit any regional variability and included processed foods, such as biscuits, breakfast cereals and beer, which are uniformly available throughout New Zealand. National Foods were sampled in a single location (Christchurch) over 5 weeks on 2 separate occasions

[1] http://www.foodsafety.govt.nz/science/research-projects/total-diet-survey/

(January/February 2009 and July/August 2009). Multiple purchases (up to 10) of 4 leading brands, selected on the basis of market share, were collected on each sampling occasion. Foods were analysed on the basis of composites of individual brands per season to give a total of 4 analyses for each food for each of the 2 seasons.

- Regional (R) foods that may be expected to demonstrate variation in contaminant level depending on the location in which the food was produced. Regional foods include bread and muffins. For the 2009 NZTDS wheat biscuit cereals were classified as regional foods, as it was recognised that North and South Island manufacturers tended to use different grain sources. Regional foods were sampled in each of 4 locations (Auckland, Napier, Christchurch and Dunedin) over 6 weeks on 2 separate occasions (April/May 2009 and October/November/December 2009). Multiple purchases of each food (up to 20) were made in each region, from at least 2 different outlets, such as supermarkets and specialty shops (for example, supermarket, bakery). Foods were prepared and analysed on the basis of composites of individual regions/season to give a total of 4 analyses for each food for each of the 2 seasons.

Use of NZTDS food samples has a number of advantages. They are:

- sampled to be nationally representative. This avoids duplication of sampling and sampling costs.
- homogenised. This avoids further sample preparation costs.
- processed foods and so the inhomogeneous nature of mycotoxin contamination in commodities is less likely to be a problem.

All foods in the 2009 NZTDS had 8 different composite samples analysed. Foods were also prepared 'as consumed'. For the majority of foods included in the current survey, foods are consumed as they are purchased (for example, bread). For foods such as dry pasta and rice, NZTDS samples were subjected to a preparation step prior to analysis. For example, dry pasta was cooked in boiling water. These food preparation steps will often decrease the apparent concentration of trichothecene mycotoxins in the foods, sometimes to undetectable levels. This loss of detectability must be balanced against the greater relevance of prepared foods to human dietary exposure.

The use of composite samples for analysis may 'dilute' occasional individual samples containing elevated concentrations of trichothecene mycotoxins. However, this is consistent with the chronic nature of health concerns relating to trichothecene mycotoxins; risk is related to the long-term average level of exposure, rather than occasional peak levels of exposure.

The NZTDS food samples have been in frozen storage for up to 4 years. Trichothecenes are stable at 120ºC, moderately stable at 180ºC and decompose within 30-40 minutes at 210ºC (JECFA, 2001). While specific information on stability under frozen storage was not identified, scientific literature on trichothecene analysis often describes frozen storage of food samples (-18 to -20ºC) when there is a time period between sampling and analysis (Montes *et al.*, 2012; Ok *et al.*, 2011). While the period of frozen storage is usually not specified in these references, storage periods of up to 2 years could be inferred in some cases.

2.1.2 Additional sample set

Following completion of analysis of the primary sample set, an additional 24 samples were taken to bring the total number of samples to 200. Sample matrices were chosen due to:

- their potential demonstrate to possible year-to-year variation in the trichothecene mycotoxin content of the food supply; and
- being likely major contributors to trichothecene mycotoxin exposure.

Four samples of each of 6 matrices were sampled during March 2014 from supermarkets in Christchurch. The selected matrices were:

- Bread, mixed grain
- Bread, wheatmeal
- Bread, white
- Bran flake cereal
- Muesli
- Snacks, flavoured

2.2 Analytical Methodology

2.2.1 Trichothecene mycotoxins

Trichothecene mycotoxins were analysed for by liquid chromatography-tandem mass spectrometry (LC-MS/MS), using a method based on that of Gottschalk *et al*. (2009).

2.2.1.1 Chemical and reagents

Standards of the type A trichothecenes, T2, HT2, DAS and NEO, and the type B trichothecenes, DON, 3ADON, 15ADON, NIV and FX, were purchased from Sigma-Aldrich. DON-3-G and ^{13}C analogues of T2, HT2, DON and NIV were purchased from Romer Laboratories.

Multisep 227 Trich+ clean-up columns were purchased from Romer Laboratories (Union, MO, USA).

2.2.1.2 Sample preparation

Finely ground and homogenised samples (5 g) were extracted with 20 ml acetonitrile-water (84:16 v/v) by shaking (10 minutes, Chilton Flask Shaker). Extracts were clarified by filtration (0.45 µm PTFE syringe filter). A 6 ml aliquot of clarified extract was purified on a Mycosep 227 Trich+ column. Eluant (4 ml) was collected and evaporated to dryness in a stream of nitrogen at approximately 80ºC. Residues were dissolved in 400 µl of acetonitrile before injection into the LC-MS/MS system.

2.2.1.3 LC-MS/MS instrumentation and parameters

Analyses were performed on an Agilent Technologies 1200 series UPLC coupled to an Agilent Technologies 6410 Triple Quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA).

Analyses were performed in 2 separate runs to accommodate mycotoxins that form negative ions (DON, NIV) and those that form positive ions (all others). All analyses were performed on a Phenomonex Synergi 2.5 μm Polar-RP100A column (100 x 2 mm). Chromatographic conditions were:

- Negative ions. Initial mobile phase; 25% A (5% aqueous methanol, containing 14.2 mM NH_3), 75% B (100% methanol, containing 14.2 mM NH_3) for 1 minute, linear gradient to 95% B at 10 minutes, isocratic to 15 minutes, linear gradient to 75% B at 17 minutes, isocratic to 20 minutes. Flow rate was 0.2 ml per minute, with a column temperature of 35ºC.
- Positive ions. Initial mobile phase; 95% A (5% aqueous methanol, containing 5 mM ammonium formate), 5% B (100% methanol, containing 5 mM ammonium formate) for 1 minute, linear gradient to 95% B at 10 minutes, isocratic to 15 minutes, linear gradient to 5% B at 17 minutes, isocratic to 20 minutes. Flow rate was 0.2 ml per minute, with a column temperature of 35ºC.

The mass spectrometer was operated with gas temperature of 300ºC, gas flow rate 5 L/minute and vapouriser temperature of 220ºC. The nebuliser pressure was 60 psi. Capillary voltage was -2500V for negative ions and +2500V for positive ions. Each toxin was identified by 2 specific fragment ions of 1 precursor ion. Multiple reaction monitoring transitions and conditions are given in Table 5. A dwell time of 400 ms was used for negative ions and 100 ms for positive ions.

Table 5: Multiple reaction monitoring transitions and conditions

Compound	Parent ion (*m/z*)	Product ion 1	Product ion 2	Fragmenter voltage (V)	Collision energy (V)
Negative ion mode					
DON	295	265	138	100	12
^{13}C-DON	310	279	145	100	13
NIV	311	281	187	90	9
^{13}C-NIV	326	295	238	90	11
Positive ion mode					
T2	484	305	215	100	12
^{13}C-T2	508	322	229	100	11
HT2	442	263	215	90	10
^{13}C-HT2	464	278	229	100	10
NEO	400	305	245	80	10
DAS	384	307	247	90	12
FX	372	355	247	90	6
3ADON	356	231	203	100	13
15ADON	356	321	137	100	8
DON-3-G	476	297	249	100	14

DON = deoxynivalenol; NIV = nivalenol; T2 = T-2 toxin; HT2 = HT-2 toxin; NEO = neosolaniol; DAS = diacetoxyscirpenol ; FX = Fusarenon X; 3ADON = 3-acetyldeoxynivalenol; 15ADON = 15-acetyldeoxynivalenol; DON-3-G = deoxynivalenol-3-glucoside

DON-3-G was not included in the list of trichothecene mycotoxins analysed in the method reference (Gottschalk *et al.*, 2009) and it was found that it could not be detected with satisfactory sensitivity in the current study. While DON-3-G was maintained in the mycotoxin screen, the low sensitivity meant it was not detected in any sample and it was not possible to calculate method performance statistics for this mycotoxin.

2.2.1.4 Mycotoxin concentration quantification

Quantification of toxins was done by external calibration at concentrations of 1, 5, 10, 50, 100, 250 and 500 µg/L. Stable ^{13}C-analogues of DON, NIV, T2 and HT2 were spiked into every sample and responses were used to compensate for sample matrix effects. ^{13}C-T2 was used to correct for matrix effects for trichothecene mycotoxins for which no ^{13}C-analogue was available.

2.2.2 Analytical quality control

2.2.2.1 Matrix effects

Matrices can have differential effects on the response of an analyte through mechanisms such as ionisation suppression. Mean matrix effects were quantified by comparison of the response of the ^{13}C-analogues in analytical samples to the response in matrix-free standards. Matrix effects are expressed as a percentage, with a figure close to 100 indicating little impact of the matrix on analyte response, while a figure substantially lower than 100 indicates a marked impact of the matrix on analyte response. Mean matrix effect estimates for matrices included in the current study are given in Table 6.

Table 6: Matrix effects for DON, NIV, T2 and HT2

Food group	Food	Mean matrix effect (%)			
		DON	***NIV***	***T2***	***HT2***
Bread	Bread, mixed grain	47	49	80	92
	Bread, wheatmeal	46	49	79	94
	Bread, white	55	59	89	101
Biscuits	Biscuits, chocolate	75	25	89	92
	Biscuits, cracker	71	39	88	89
	Biscuits, plain sweet	84	23	90	85
Breakfast cereals	Cornflakes	87	34	101	106
	Bran Flake Cereal	55	18	80	84
	Wheat biscuit cereal	66	23	87	83
	Muesli	73	27	83	88
	Oats, rolled (cooked)	63	52	47	60
Bakery products	Cake, plain	55	29	108	112
	Muffin	76	42	86	95
Pasta and noodles	Noodles, instant (cooked)	75	110	61	81
	Pasta, dried (cooked)	79	176	63	76
	Spaghetti in sauce, canned	59	93	53	69
Other cereal-based products	Rice, white (cooked)	70	56	57	72
	Snack Bars	74	35	87	88
	Snacks, flavoured	77	28	92	94
	Pizza	54	68	77	81
	Infant weaning food, cereal based	26	29	37	46
	Beer	41	60	45	63

DON = deoxynivalenol NIV = nivalenol T2 = T-2 toxin HT2 = HT-2 toxin

Matrix effects are most marked for NIV, as an analyte across all matrices, and for cereal-based infant weaning food, as a matrix across all analytes. However, the most profound effects were seen for NIV in bran flake cereal, with approximately 80% suppression of the response for the ^{13}C internal standard.

2.2.2.2 Sensitivity

Limits of detection (LOD) and quantitation (LOQ) were calculated as the concentrations equivalent to a signal 3 and 10 times the average background noise (S/N ratios) (United States Food and Drug Administration, 1996), taking into account the volume of sample used for analysis. LODs and LOQs are summarised in Table 7. LODs and LOQs were adjusted for each matrix by multiplying by the ratio of the response of ^{13}C-analogues in matrix-free standards to the response in the individual matrices (Table 6). For analytes for which no ^{13}C-analogue was available matrix effect figures for T2 were used to adjust the LOD/LOQ.

Table 7: Limits of detection (LOD) and limits of quantitation (LOQ) for trichothecene mycotoxin analyses

Food	LOD/LOQ (µg/kg or µg/L)								
	DON	3ADON	15ADON	NIV	FX	T2	HT2	DAS	NEO
Bread, mixed grain	0.3/1.0	14/46	7/23	0.2/0.7	5/17	0.05/0.17	0.1/0.3	0.2/0.7	0.7/2.3
Bread, wheatmeal	0.3/1.0	14/46	7/23	0.2/0.7	5/17	0.05/0.17	0.1/0.3	0.2/0.7	0.7/2.3
Bread, white	0.3/1.0	13/43	6/20	0.2/0.7	4/13	0.04/0.13	0.1/0.3	0.2/0.7	0.6/2.0
Biscuits, chocolate	0.2/0.7	13/43	6/20	0.4/1.3	5/17	0.05/0.17	0.1/0.3	0.2/0.7	0.6/2.0
Biscuits, cracker	0.2/0.7	13/43	6/20	0.2/0.7	5/17	0.05/0.17	0.1/0.3	0.2/0.7	0.6/2.0
Biscuits, sweet plain	0.2/0.7	12/40	6/20	0.4/1.3	4/13	0.04/0.13	0.1/0.3	0.2/0.7	0.6/2.0
Cornflakes	0.2/0.7	11/36	6/20	0.3/1.0	4/13	0.04/0.13	0.1/0.3	0.1/0.3	0.5/1.7
Bran flake cereal	0.3/1.0	14/46	7/23	0.5/1.7	5/17	0.05/0.17	0.1/0.3	0.2/0.7	0.6/2.0
Wheat biscuit cereal	0.2/0.7	13/43	6/20	0.4/1.3	5/17	0.05/0.17	0.1/0.3	0.2/0.7	0.6/2.0
Muesli	0.2/0.7	13/43	7/23	0.4/1.3	5/17	0.05/0.17	0.1/0.3	0.2/0.7	0.6/2.0
Oats, rolled (cooked)	0.3/1.0	24/79	12/40	0.2/0.7	9/30	0.09/0.30	0.2/0.7	0.3/1.0	1.1/3.6
Cake, plain	0.3/1.0	10/33	5/17	0.3/1.0	4/13	0.04/0.13	0.1/0.3	0.1/0.3	0.5/1.7
Muffin	0.2/0.7	13/43	7/23	0.2/0.7	5/17	0.05/0.17	0.1/0.3	0.2/0.7	0.6/2.0
Noodles, instant (cooked)	0.2/0.7	18/59	9/30	0.1/0.3	7/23	0.07/0.23	0.1/0.3	0.2/0.7	0.9/3.0
Pasta, dried (cooked)	0.2/0.7	18/59	9/30	0.1/0.3	6/20	0.06/0.20	0.2/0.7	0.2/0.7	0.8/2.6
Spaghetti in sauce, canned	0.3/1.0	21/69	11/36	0.1/0.3	8/26	0.08/0.26	0.2/0.7	0.3/1.0	1/3.3
Rice, white (cooked)	0.2/0.7	20/66	10/33	0.2/0.7	7/23	0.07/0.23	0.2/0.7	0.3/1.0	0.9/3.0
Snack bars	0.2/0.7	13/43	6/20	0.3/1.0	5/17	0.05/0.17	0.1/0.3	0.2/0.7	0.6/2.0
Snacks, flavoured	0.2/0.7	12/40	6/20	0.3/1.0	4/13	0.04/0.13	0.1/0.3	0.2/0.7	0.6/2.0
Pizza	0.3/1.0	15/50	7/23	0.1/0.3	5/17	0.05/0.17	0.1/0.3	0.2/0.7	0.7/2.3
Infant weaning food, cereal-based	0.6/2.0	31/102	15/50	0.3/1.0	11/36	0.11/0.36	0.3/1.0	0.4/1.3	1.4/4.6
Beer	0.4/1.3	25/83	12/40	0.2/0.7	9/30	0.09/0.30	0.2/0.7	0.3/1	1.2/4.0

DON = deoxynivalenol NIV = nivalenol T2 = T-2 toxin HT2 = HT-2 toxin
NEO = neosolaniol DAS = diacetoxyscirpenol FX = Fusarenon X
3ADON = 3-acetyldeoxynivalenol 15ADON = 15-acetyldeoxynivalenol

Results falling between the LOD and the LOQ are often referred to as 'trace' amounts, with no quantitative results assigned. In the current study, the concentration corresponding to the analytical response is reported as an 'indicative' value. These appear in this report as quantitative values in brackets.

2.2.2.3 Accuracy

Three quality control materials (FAPAS – Food Analysis Performance Assessment Scheme, operated by the Food and Environment Research Agency, Sand Hutton, York, United Kingdom) were analysed for trichothecene mycotoxins. The materials were:

- T2280 Oat flour
- T2283 Maize flour

- T2285 Breakfast cereal

The laboratory also participated in an inter-laboratory collaborative programme (colab) for multi-mycotoxin methods, organised by CODA-CERVA in Belgium.[2] Results of the analyses of FAPAS and colab samples are shown in Table 8.

Table 8: Analysis of quality control materials

QC Material/Mycotoxin	Mycotoxin concentration (µg/kg)		
	Assigned value	**Satisfactory range**	**ESR analytical results**
T2280 Oat flour			
- T-2 toxin	220	131-308	239
- HT-2 toxin	89.1	49.9-128.3	86
T2283 Maize flour			
- DON	658	434-883	672
T2285 Breakfast cereal			
- DON	344	214-473	311
CODA-CERVA Oat flour			
- DON	2262	1622-2902	2500, 2660
- T-2 toxin	270	165-435	272
- HT-2 toxin	80.6	45.2-116	90.4

DON = deoxynivalenol

Trichothecene mycotoxins (Sigma-Aldrich) were prepared as 1000 µg/L solutions in acetonitrile and spiked into samples of each of the matrices examined at a reporting concentration of 20 µg/kg. Samples were analysed with and without the addition of the mycotoxin spike, with recovery determined as the difference between these results, expressed as a percentage of added mycotoxin. Mean recoveries are reported in Table 9.

Table 9: Spike recoveries for trichothecene mycotoxins in cereals and cereal products

Food	Mycotoxin mean spike recovery (%)								
	DON	**3ADON**	**15ADON**	**NIV**	**FX**	**T2**	**HT2**	**DAS**	**NEO**
Bread, mixed grain	105	NR	74	98	99	102	97	102	95
Bread, wheatmeal	101	119	56	85	96	100	119	109	100
Bread, white	122	118	74	110	111	106	92	111	97
Biscuits, chocolate	79	NR	74	103	120	99	102	124	106
Biscuits, cracker	90	NR	54	76	131	107	100	123	98
Biscuits, plain sweet	110	109	69	102	160	108	95	118	88
Cornflakes	80	NR	61	76	144	107	108	120	80
Bran flake cereal	92	NR	NR	NR	118	94	91	89	74
Wheat biscuit cereal	61	NR	94	78	73	98	106	101	61
Muesli	110	NR	NR	NR	185	99	102	99	77
Oats, rolled (cooked)	94	NR	122	87	181	109	106	139	92

[2] http://www.coda-cerva.be/index.php?option=com_content&view=frontpage&Itemid=263&lang=en accessed 25 February 2014

Food	Mycotoxin mean spike recovery (%)								
	DON	**3ADON**	**15ADON**	**NIV**	**FX**	**T2**	**HT2**	**DAS**	**NEO**
Cake	112	92	95	114	77	104	106	100	96
Muffin	68	NR	58	69	138	101	109	109	88
Noodles, instant (cooked)	122	NR	47	98	167	98	119	128	92
Pasta, dried (cooked)	112	NR	131	21	277	107	95	136	169
Spaghetti in sauce, canned	70	NR	82	68	170	102	108	128	82
Rice, white (cooked)	126	184	75	111	96	114	89	115	95
Snack bars	73	NR	45	76	122	97	104	116	77
Snacks, flavoured	93	102	84	86	116	96	104	117	135
Pizza	90	NR	56	85	126	97	97	112	69
Infant weaning food, cereal	65	NR	108	26	219	94	98	133	112
Beer	84	179	112	82	153	101	106	134	136

NR = not able to be reported due to matrix effects. For 3ADON this was further complicated by high limits of detection

DON = deoxynivalenol NIV = nivalenol T2 = T-2 toxin HT2 = HT-2 toxin
NEO = neosolaniol DAS = diacetoxyscirpenol FX = Fusarenon X
3ADON = 3-acetyldeoxynivalenol 15ADON = 15-acetyldeoxynivalenol

The spike recoveries were generally within the range considered to be acceptable for trace analysis (70-120%). FX and DAS demonstrated consistent over-recovery, most likely due to the lack of appropriate internal standards for these compounds. It was not possible to determine recoveries for 3ADON in most matrices, due to the proximity of the spike level to the limit of detection for this matrix.

The European Commission has specified acceptable recovery ranges for regulatory analysis of some mycotoxins, including T2 and HT2 (60-130%) and DON (60-120%) (European Commission, 2006b). The recoveries shown in Table 3 for these 3 mycotoxins are within the specified recovery ranges, although recovery of DON from white bread, white rice and instant noodles were at the limit of the acceptable recovery range.

2.2.2.4 Precision

The coefficient of variation (CV), based on duplicate analyses of naturally contaminated samples were only able to be determined for NIV and DON. The respective CVs were 9.6 and 5.6%. CVs for other toxins were determined from duplicate analyses of standards and spikes and were in the range 3.8% (HT2) to 13.3% (3ADON). CVs for NIV and DON, based on standards and spikes, were 7.7 and 5.0%, respectively.

Other multi-mycotoxin methods have reported similar CVs. De Boevre *et al.* (2012b) reported CVs in the range 7-26% for a method covering a range of *Fusarium* toxins and their metabolites. Gottschalk *et al.* (2009) reported CVs in the range 1.1-11% for a method very similar to that used in the current study. A further method covering a similar range of trichothecene mycotoxins to the current study reported CVs in the range 4-23% (Rasmussen *et al.*, 2012)

3 RESULTS AND DISCUSSION

3.1 Summary of Results

3.1.1 NZTDS samples

Results for the NZTDS samples analysed in the current study are summarised in Table 10. Full details of results are given in Appendix 1, Table A1.1-3. As the majority of detections were for DON and NIV, only these toxins have been included in this table. In addition to the frequent detection of DON and NIV, 3 other toxins were detected. DAS was detected in 3 samples (cornflakes, rice and pizza), while 15ADON and T2 were detected in the same flavoured snack sample.

Table 10: Trichothecene mycotoxin content of cereal products available on the New Zealand market, sampled for the 2009 New Zealand Total Diet Study

Food type	Food	Number of samples	DON		NIV	
			Number positive (%)	Concentrations in positive samples (µg/kg)	Number positive (%)	Concentrations in positive samples (µg/kg)
Bread	Bread, mixed grain	8	8 (100)	2.0 - 10.4	5 (63)	2.7 - 3.8
	Bread, wheatmeal	8	7 (88)	2.6 - 7.4	4 (50)	3.5 - 6.6
	Bread, white	8	6 (75)	1.3 - 5.4	3 (38)	2.5 - 3.4
Biscuits	Biscuits, chocolate	8	0 (0)	-	0 (0)	-
	Biscuits, cracker	8	8 (100)	4.8 - 22.3	8 (100)	5.8 - 13.5
	Biscuits, sweet plain	8	7 (88)	2.4 - 13.0	6 (75)	6.9 - 16.4
Breakfast cereals	Cornflakes	8	8 (100)	2.4 - 9.5	0 (0)	-
	Bran flake cereal	8	8 (100)	8.9 - 22.3	0 (0)	-
	Wheat biscuit cereal	8	8 (100)	2.4 - 5.0	0 (0)	-
	Muesli	8	8 (100)	1.3 - 9.5	0 (0)	-
	Oats, rolled (cooked)	8	0 (0)	-	0 (0)	-
Bakery products	Cake, plain	8	2 (25)	2.0 - 7.8	3 (38)	2.3 - 7.0
	Muffin	8	0 (0)	-	0 (0)	-
Pasta and noodles	Noodles, instant (cooked)	8	7 (88)	2.6 - 25.1	4 (50)	1.2 - 1.8
	Pasta, dried (cooked)	8	8 (100)	3.0 - 38.0	1 (13)	1.7
	Spaghetti in sauce, canned	8	6 (75)	3.8 - 22.2	3 (38)	1.1 - 1.3
Other cereal-based products	Rice, white (cooked)	8	0 (0)	-	1 (13)	3.9
	Snack bars	8	1 (13)	2.5	1 (13)	2.5
	Snacks, flavoured	8	7 (88)	3.4 - 410	5 (63)	5.0 - 8.6
	Pizza	8	0 (0)	-	0 (0)	-
	Infant weaning food, cereal based	8	0 (0)	-	1 (13)	4.6
	Beer	8	1 (13)	10.9	0 (0)	-

DON = deoxynivalenol NIV = nivalenol

3.1.2 Additional samples

Table 11 gives summary results for the additional foods (*n* = 24) sampled during March 2014. Full details of results are given in Appendix 1, Table A1.4. Only NIV and DON were detected in these additional samples.

Table 11: Trichothecene mycotoxin content of cereal products available on the New Zealand market, sampled during March 2014

Food	Number of samples	DON		NIV	
		Number positive (%)	Concentrations in positive samples (µg/kg)	Number positive (%)	Concentrations in positive samples (µg/kg)
Bread, mixed grain	4	4 (100)	7.2 – 14.5	0 (0)	-
Bread, wheatmeal	4	4 (100)	3.6 – 10.7	3 (75)	2.5 - 4.8
Bread, white	4	4 (100)	1.4 – 2.2	0 (0)	-
Bran flake cereal	4	4 (100)	5.2 – 10.6	0 (0)	-
Muesli	4	4 (100)	1.0 – 1.8	0 (0)	-
Snacks, flavoured	4	7 (88)	1.1 – 2.6	2 (50)	7.4 - 10.1

DON = deoxynivalenol NIV = nivalenol

Three points should be noted in relation to these additional samples; they are freshly purchased rather than stored frozen, single samples rather than composites, and they were all sampled in Christchurch. While the latter point is not likely to be relevant for nationally distributed foods (bran flake cereal, muesli and flavoured snacks), it has the potential to impact on the distribution of toxin concentrations in bread. This will be discussed in the following section.

3.2 Trichothecene Mycotoxins in Individual Food Types

3.2.1 Bread

Three types of bread were analysed for trichothecene mycotoxins in the current survey (mixed grain, wheatmeal, white). The prevalence and maximum concentration of DON was greatest in mixed grain bread and least in white bread. The prevalence of NIV was also greatest in mixed grain bread for NZTDS samples, but not for additional samples. While sample numbers are small these results are consistent with expectations that white bread should be the least contaminated, as toxin concentrations have been shown to be highest in the bran (outer) layer of wheat, which is largely excluded from white flour (Kostelanska *et al.*, 2011; Ríos *et al.*, 2009; Simsek *et al.*, 2012).

While it is not possible to statistically compare the results for NZTDS samples and the additional samples taken during March 2014, the results do not suggest any marked season-to-season differences. For example, the mean DON content for mixed grain bread samples from Christchurch in the NZTDS was 7.5 µg/kg, while the mean DON content for the additional mixed grain bread samples from Christchurch was 10.1 µg/kg. For the other bread

types the comparable mean DON contents were 5.2 and 8.3 µg/kg for wheatmeal bread and 3.3 and 1.7 µg/kg for white bread, for NZTDS and additional samples, respectively.

NIV was detected in the additional wheatmeal bread samples, but not the mixed grain or white breads. The mean NIV concentration in additional wheatmeal bread samples was 3.8 µg/kg, compared to 5.8 µg/kg in the NZTDS wheatmeal bread samples from Christchurch.

Lauren and Veitch (1996) detected DON and NIV in 2 of 16 New Zealand bread samples, but at substantially higher concentrations than those detected in the current study. The maximum DON and NIV concentrations were 50 and 200 µg/kg, respectively. It should be noted that Lauren and Veitch analysed single samples, whereas the NZTDS samples used in the current study are composites of several individual loaves (3-4 per analytical sample). Compositing has the potential to 'dilute' occasional samples with high concentrations. A student project carried out at ESR did not detect DON in 3 bread samples (LOD = 27 µg/kg), but detected NIV in 1 bread sample at a concentration of 100 µg/kg (Eva Kosanic, 2009, unpublished).

Overseas studies have also reported high prevalence of DON contamination in bread, with at least 50% of bread samples in any survey found to contain DON (Appendix 2, Table A2.1). However, DON concentrations reported in overseas studies are generally higher than those reported in the current study, with mean concentrations commonly in the range 20-120 µg/kg. NIV has been less frequently reported in bread. The French Total Diet Study reported mean NIV concentrations in the range 2.6-6.4 µg/kg for bread (Sirot *et al.*, 2013).

3.2.2 Biscuits

Three types of biscuits were analysed for trichothecene mycotoxins in the current survey (plain, chocolate-coated, cracker). DON and NIV were detected in most samples of cracker and plain sweet biscuit, but not chocolate biscuits. The non-detection of trichothecene mycotoxins in chocolate biscuits is probably due to the higher proportion of non-cereal components in this biscuit type. Chocolate accounts for 38-45% of ingredient content of chocolate biscuits, according to label claims. DON and NIV were detected in all samples of cracker biscuits. This is presumably due to the high cereal content and low moisture content of these products, rather than any differences in the trichothecene mycotoxin content of the raw ingredients.

No previous information on the trichothecene mycotoxin content of New Zealand biscuits was found.

Other studies that have reported analysis of biscuits have reported similar (Ok *et al.*, 2009a; Pacin *et al.*, 2011) or higher (Sirot *et al.*, 2013; Soubra *et al.*, 2009) concentrations of DON, but lower concentrations of NIV (Sirot *et al.*, 2013).

3.2.3 Breakfast cereals

Five types of breakfast cereal were analysed for trichothecene mycotoxins in the current study (cornflakes, bran flake cereal, wheat biscuit cereal, muesli and rolled oats). DON was

detected in all NZTDS breakfast cereal samples, except for rolled oats (porridge). DON was detected in all additional bran flake cereal and muesli samples.

Muesli usually contains oats as the sole or major cereal component. The non-detection of DON in rolled oats and the low concentrations detected in muesli (all but 1 sample less than 5 μg/kg) suggests that New Zealand commercial oats contain low levels of DON contamination. De-hulling of oats has been shown to decrease the DON content by more than 80% (Scudamore *et al.*, 2007).

Examination of raw laboratory information suggests that NIV may be present in a number of breakfast cereal samples. However, the considerable matrix effects observed in these samples meant that results did not meet the criteria for reporting.

DAS was detected in 1 sample of cornflakes, but at a concentration just above the analytical limit of detection (0.2 μg/kg).

The highest DON concentrations were found in bran flake cereal. This is not surprising, as the bran is the exterior layer of the grain and the first point where fungal infection occurs. The mean concentration in NZTDS bran cereal samples (13.7 μg/kg) was markedly higher than in wheat biscuit cereal samples, made from whole wheat flour (3.5 μg/kg). This is consistent with studies on the distribution of DON during wheat milling that showed concentrations of DON in bran to be as much as 3-times the concentration in the whole wheat (Kostelanska *et al.*, 2011; Ríos *et al.*, 2009; Simsek *et al.*, 2012).

Additional samples of bran flake cereal and muesli were analysed, with DON results lower than, but comparable to those detected in the NZTDS samples.

An earlier New Zealand study detected DON in only 2 of 20 breakfast cereal samples, but at considerably higher concentrations (100-110 μg/kg) than found in the current study (Lauren and Veitch, 1996). This earlier study also detected NIV in 3 of 20 breakfast cereals, at concentrations in the range 70-80 μg/kg, in contrast to the current study.

A number of overseas studies have examined the trichothecene mycotoxin content of breakfast cereals (see Appendix 2 for a summary). In most cases the DON content of breakfast cereals reported in overseas studies are markedly higher than those reported in the current study. As an example, cornflake cereals are similar in composition in different countries. In the current New Zealand study, the mean DON concentration in cornflakes was 4.7 μg/kg. A Belgian study reported 5 of 6 samples with DON contents of less than 12 μg/kg, but 1 containing 207 μg/kg of DON (De Boevre *et al.*, 2012b), while a further study by the same group reported a mean DON concentration of 44 μg/kg in 61 samples of cornflakes (De Boevre *et al.*, 2012a). A Lebanese study detected DON in 14 of 20 cornflake samples, with concentrations in the range 60-100 μg/kg (Soubra *et al.*, 2009), while a Spanish study detected DON in 49 of 65 cornflake composites, with a mean concentration of 109 μg/kg (Cano-Sancho *et al.*, 2011).

NIV was infrequently reported in breakfast cereals in overseas studies.

3.2.4 Bakery products

Two types of bakery product were included in the current survey (muffins and cake). No trichothecene mycotoxins were detected in muffins. DON (2 samples) and NIV (3 samples) were detected in cake samples. The lower prevalence of trichothecene mycotoxin detection in these products than in bread is probably due, at least in part, to the use of white flour for cake and muffin production. Also, flour makes up a lower proportion of the total ingredients used for cake and muffin production than bread production.

No comparative New Zealand information was found for similar products.

DON has been reported in cakes in 2 overseas studies (see Appendix 2), with mean concentrations greater than 50 μg/kg (Sirot *et al.*, 2013; Soubra *et al.*, 2009).

3.2.5 Pasta and noodles

Three types of pasta or noodles were included in the current survey (instant noodles, pasta cooked from dried and canned spaghetti in sauce). DON was detected in 21 of 24 samples of pasta or noodles. NIV was detected in 8 of 24 samples, but only at very low concentrations (<3 μg/kg). The highest mean (16.7 μg/kg) and individual (38 μg/kg) concentrations of DON were found in pasta (cooked from dry pasta). Pasta samples differ from the other products in this group in being manufactured exclusively from durum wheat flour. The sample set also includes a high proportion of imported products and the pasta with the highest DON concentration originated from Italy.

Two of the 8 canned spaghetti in sauce samples contained DON concentrations greater than 10 μg/kg (13.8 and 22 μg/kg). One of these samples was manufactured in Italy, while the other was manufactured in New Zealand from "local and/or imported ingredients".

Two of the 8 noodle samples contained DON concentrations greater than 10 μg/kg (17.4 and 25 μg/kg). It should be noted that all of these products were manufactured in Asia (Malaysia, Thailand, Indonesia or China).

No comparative New Zealand information was found for similar products.

Overseas studies have mostly examined dry noodles or pasta, so results of different studies are not strictly comparable.

A Malaysian study reported DON concentrations in dry instant noodles to be consistently less than 1 μg/kg (Moazami and Jinap, 2009), compared to a mean concentration of 9.2 μg/kg in the current study for cooked noodles. An Argentinean study estimated the mean concentration of DON in noodles to be in the range 7-9 μg/kg (Pacin *et al.*, 2011).

A number of studies in European countries have included analysis of pasta for trichothecene mycotoxins (see Appendix 2). Mean DON concentrations in pasta have been reported in the range 50-100 μg/kg (González-Osnaya *et al.*, 2011; Raiola *et al.*, 2012; Reinhold and

Reinhardt, 2011; Sirot *et al.*, 2013), although 1 study reported a higher mean concentration of 226 μg/kg (Cano-Sancho *et al.*, 2011). The highest DON concentration found in pasta in the current study (38 μg/kg) was in an Italian product. Assuming 60% water absorption during cooking, this would equate to a concentration of approximately 60 μg/kg in the dry product.

3.2.6 Rice

DON was not detected in any rice sample analysed in the current study. NIV was detected in 1 sample, at a concentration of 3.9 μg/kg. DAS was also detected in the same sample, at a concentration of 1.2 μg/kg.

A student project carried out at ESR detected NIV, but not DON in 1 of 3 rice samples (Eva Kosanic, 2009, unpublished). The concentration detected (28 μg/kg) was substantially higher than found in the current survey, although analyses were carried out on uncooked rice, while the current study analysed cooked rice. While no studies were found on the impact of cooking of rice on mycotoxin levels, it has been reported that 30% of DON was transferred to the soak water during parboiling of rice (Leite *et al.*, 2012).

Few studies of trichothecene mycotoxins in rice have been reported. Ok *et al*. (2011) detected DON in 10 of 65 and NIV in 23 of 65 rice samples. The mean concentrations were 4 and 10 μg/kg, respectively. A study in Ecuador reported DON occurrence in paddy rice, but not in polished rice (Ortiz *et al.*, 2013). A Spanish study reported detection of DON, but not NIV, in 50% of rice samples, with a mean concentration of 5.5 μg/kg (Rodríguez-Carrasco *et al.*, 2013)

3.2.7 Snack bars

DON was detected in 1 snack bar sample at a concentration of 2.5 μg/kg, while NIV was detected in 2 of 8 snack bar samples, with a maximum concentration of 5.4 μg/kg.

The New Zealand study of Lauren and Veitch (1996) detected NIV in 3 of 13 snack bar samples, but did not detect DON in any sample. The NIV concentrations detected were in the range 30-60 μg/kg. While the small number of samples mean any conclusions should be drawn with caution, it is interesting to note that both New Zealand studies found NIV to be the most commonly detected trichothecene mycotoxin in this sample type.

It was not possible to determine if any overseas studies had analyses matrices equivalent to our definition of a snack bar.

3.2.8 Snacks, flavoured

Flavoured snacks were the food category found to have the highest concentrations of DON (410 μg/kg) amongst NZTDS samples. The high-DON product was also the only sample tested that contained detectable quantities of 15ADON, the acetylated form of DON, and T2. This food category contains 3 sub-categories; corn chips, extruded maize-based products and products made from wheat or a mixture of cereals. The highest DON concentrations (26, 60

and 410 µg/kg) were detected in the 3 corn chip samples. DON was only detected in 1 of 2 extruded maize-based products (9.0 µg/kg) and 2 of 3 products produced from wheat (6.6 and 7.6 µg/kg). Levels of NIV in corn chips were no greater than levels detected in wheat-containing products. NIV was not detected in extruded maize-based products.

For the corn chips analysed in this study, the ingredients were reported to be 'local and imported'. It is not possible to say whether these toxin levels are due to domestically-produced or imported corn/maize.

Additional samples were obtained in March 2014 of corn chips (n = 2) and extruded maize-based products (n = 2). DON concentrations in all products were less than 3 µg/kg. NIV was detected in 1 sample of each product type. The DON concentrations in corn chips were in sharp contrast to those found in NZTDS samples. It is uncertain whether this is a seasonal effect or due to the corn raw material coming from a different source.

Lauren and Veitch (1996) analysed 24 'masa flour products', including corn chips and taco shells. DON was only detected in 1 sample at a concentration of 90 µg/kg. NIV was detected in 2 samples with concentrations in the range 30-50 µg/kg.

While it is uncertain whether the products are exactly analogous, DON was detected in 56 of 71 'corn snacks' in a Spanish study, with a maximum concentration of 304 µg/kg (Cano-Sancho *et al.*, 2011). T2 was detected in about 10% of samples, with a maximum concentration of 70 µg/kg. Another study in Valencia, Spain detected DON in 13 of 57 baked corn-based snacks and 12 of 63 fired corn-based snacks (Castillo *et al.*, 2008). The maximum concentration detected was 132 µg/kg. NIV was only detected in 1 sample, at a concentration of 56 µg/kg.

3.2.9 Pizza

DON and NIV were not detected in any pizza sample analysed in the current study. DAS was detected in 1 sample, at a concentration near the limit of detection (0.3 µg/kg).

No previous New Zealand studies have analysed pizza for the presence of trichothecene mycotoxins.

Three overseas studies were found that included analysis of pizza for trichothecene mycotoxins (Pacin *et al.*, 2011; Sirot *et al.*, 2013; Soubra *et al.*, 2009). In all 3 studies the mean DON concentration was in excess of 20 µg/kg (see Appendix 2).

3.2.10 Infant weaning food, cereal-based

NIV was detected in 1 sample of cereal-based weaning food, at a concentration of 4.6 µg/kg. No other trichothecene mycotoxins were detected in this food type.

No previous New Zealand information on trichothecene mycotoxins in this food type has been reported.

A German study reported detection of a range of trichothecene mycotoxins in 5 samples of infant food (Gottschalk *et al.*, 2009). The most frequently detected toxin was DON (5 samples, mean concentration 24 µg/kg), followed by HT2 (4 samples, mean concentration 1.3 µg/kg) and 15ADON (3 samples, mean concentration 1.5 µg/kg) NIV was detected in 2 samples, with a mean concentration of 8.8 µg/kg.

3.2.11 Beer

DON was detected in 1 of the composite samples of beer analysed in the current study at a concentration of 10.9 µg/kg. No other trichothecene mycotoxins were detected in this sample type.

Previous New Zealand studies on trichothecene mycotoxins in food did not include analysis of beer.

The results of the current study are comparable to a Spanish study, which detected DON in 1 of 71 beer samples, at a concentration of 12 µg/kg (Cano-Sancho *et al.*, 2011). However, most other studies have reported a greater prevalence and higher concentrations of DON in beer (Anselme *et al.*, 2006; Belajová and Rauová, 2008; Cantrell, 2008; Ok *et al.*, 2009a; Varga *et al.*, 2012).

3.3 Regulatory Limits for Trichothecene Mycotoxins

The Joint Australia New Zealand Food Standards Code (the Code) does not include limits for any trichothecene mycotoxin in any food.

In 2003, worldwide regulations for mycotoxins were reviewed (Van Egmond and Jonker, 2004). It should be noted that several countries with separate trichothecene regulations in 2003 were listed as 'EU candidate' countries. Several of these countries (Bulgaria, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Romania, Slovakia, and Slovenia) have since been admitted to the EU and their mycotoxin regulations will now come under the entry for the EU[3]. Worldwide regulations for trichothecene mycotoxins are summarised in Table 12.

Table 12: International regulatory limits for trichothecene mycotoxins in 2003

Country	Commodity description	Toxin	Regulatory limit (µg/kg)
Armenia	All foods	T2	100
	Wheat	DON	700
	Barley	DON	1000
Belarus	Wheat	DON	700
	Barley	DON	1000
	Infant foods	DON	Not allowed

[3] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R1881:20100701:EN:PDF

Country	Commodity description	Toxin	Regulatory limit (µg/kg)
	Grain, flour, groats	T2	Unknown
	Infant food	T2	Not allowed
Bulgaria	Processed cereal products	DON	1000
	Cereals, subject to further treatment	DON	2000
	Maize and maize products	DON	1000
	Processed cereal products	T2	100
Canada	Domestic, uncleaned soft wheat	DON	2000
	Soft wheat flour (adult food)	DON	1200
	Soft wheat flour (infant food)	DON	600
China	Wheat and wheat flour, maize and maize flour	DON	1000
Cuba	Imported cereals	DON	300
Czech Republic	Corn, rice, maize	DON	2000
	Flour	DON	1000
Estonia	Wheat and wheat products	DON	700
	Barley and barley products	DON	1000
	Cereal and cereal products	T2	100
European Union	Cereal products, as consumed	DON	500
	Flour, used as raw material	DON	750
Hungary	Milled cereal products	DON	1000
	Edible bran	DON	1200
	Milled cereal products	T2	300
Iran	Wheat, barley, maize, rice	DON	1000
Japan	Wheat and wheat products	DON	1100
Latvia	Cereals	DON	1000
	Cereals	T2	100
Moldova	Wheat and wheat flour	DON	700
	Barley and barley flour	DON	1000
	Cereals and cereal flour	T2	100
Russian Federation	Wheat	DON	700
	Barley	DON	1000
	Barley	T2	100
Singapore	Cereal and grain products	DON	Not given
	Wheat, maize, rice	T2	20
	Wheat, rice for production of children's food	T2	0.5
	Maize for production of children's food	T2	1
Switzerland	Cereal grains	DON	1000
Ukraine	Wheat of other than strong, hard varieties, flour, bread	DON	500
	Grain-based and fruit-vegetable-dairy mix baby foods	DON	200
	Wheat of strong, hard varieties	DON	1000
	Grains, flour, etc.	T2	100

Country	Commodity description	Toxin	Regulatory limit (μg/kg)
USA	Finished wheat products for human consumption	DON	1000
Uruguay	Wheat flour and by-products	DON	1000

DON = deoxynivalenol T2 = T-2 toxin

As part of an assessment of public health risks associated with T2 and HT2, EFSA reviewed regulatory limits for these toxins in non-EU countries (EFSA, 2011). Limits for cereals and cereal products were most commonly 100 μg/kg, except for Hungary, where the limit was 300 μg/kg. In Armenia, Moldova, the Russian Federation and Ukraine the limit applies to T2 toxin, while in Norway it applies to the sum of T2 and HT2. Norway also has a separate limit of 50 μg/kg for cereals and cereal products for infants and young children.

In 2006, the European Commission enacted regulations setting maximum limits for several mycotoxins, including DON (European Commission, 2006a). Maximum limits for DON were (in μg/kg):

Unprocessed cereals, other than durum wheat, oats and maize	1250
Unprocessed durum wheat and oats	1750
Unprocessed maize	1750
Cereals intended for direct human consumption, cereal flour (including maize flour, maize meal and maize grits), bran as end product marketed for direct human consumption and germ, with the exception of foodstuffs for infants and young children	750
Pasta (dry)	750
Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals	500
Processed cereal-based foods and baby foods for infants and young children	200

Provisions for maximum limits for T2 and HT2 were indicated in these regulations, but were not established, pending further assessment by EFSA.

A further regulation, issued in 2007, amended the limits for DON in unprocessed maize (European Commission, 2007):

Unprocessed maize, with the exception of unprocessed maize intended to be processed by wet milling	1750
Milling fractions of maize with particle size > 500 micron falling within CN code 1103 13 or 1103 20 40 and other maize milling products with particle size > 500 micron not used for direct human consumption falling within CN code 1904 10 10	750
Milling fractions of maize with particle size ≤500 micron falling within CN code 1102 20 and other maize milling products with particle size ≤500 micron not used for direct human consumption falling within CN code 1904 10 10	1250

CN = Combined Nomenclature

The setting of limits for DON was considered to also be protective against potential impacts of 3ADON, 15ADON and NIV, due to co-occurrence with DON.

The Codex Committee on Contaminants in Food are currently developing a proposal for maximum levels of DON in cereals and cereal-based foods (Codex Committee on Contaminants in Food, 2013). The proposed limits are (μg/kg):

Raw cereal grains (wheat, maize and barley)	2000
Flour, semolina, meal, flakes (and possibly grits and starch) derived from wheat, maize or barley	1000
Cereal-based foods for infants and young children	500

The 7th Session of CCCF (CCCF7) agreed to the limits for raw and processed cereals and agreed to establish the maximum limit for foods for infants and young children at 200 μg/kg (Codex Alimentarius Commission, 2013). A maximum limit for bran products was not proposed, but member countries were encouraged to collect and submit data on DON in bran products. An electronic working group (EWG), led by Japan and Canada, was formed to prepare a working paper on the extension of the maximum limits for DON to its acetylated derivatives. While the levels were agreed at CCCF7, they were not progressed by the Codex Commission, and the discussions are ongoing and the issue will be revisited in March 2015

Results from the current study of foods available for consumption in New Zealand comply with all of the maximum limits outlined above.

4 CONCLUSIONS

Cereal-based food samples (n = 200) were analysed for a range of trichothecene mycotoxins, including those for which maximum limits exist in other countries. DON was the most commonly detected toxin, followed by NIV. DAS was detected at low concentrations in 3 samples, while 15ADON and T2 were detected in 1 sample each. However, these infrequently detected toxins were only detected in foods either definitely or potentially of non-domestic origins. Similarly, the highest concentrations of DON were found in foods that were either imported or the major ingredients were potentially imported. It appears that foods produced from New Zealand-produced ingredient contain low levels of DON, by international standards.

It has previously been suggested that fungi capable of producing T2 (and HT2) may not be present in New Zealand (Cressey and Thomson, 2006). The results of the current study support this proposition, as the only food containing T2 was produced from potentially imported ingredients. Oats are commonly contaminated with T2 in Europe. Rolled oats analysed in the current study were not found to contain detectable T2.

While the Australia New Zealand Food Standards Code does not contain regulatory limits for trichothecene mycotoxins, the concentrations of toxins found in the current study would have been compliant with regulatory limits promulgated in other countries.

Dietary exposure estimates for trichothecene mycotoxins, considering all potential food sources, would assist in placing the results of the current survey in context with respect to human health risks.

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APPENDIX 1 DETAILS OF SAMPLES ANALYSED IN THE CURRENT SURVEY

Table A1. 1: Deoxynivalenol (DON) concentrations in New Zealand Total Diet Study (NZTDS) samples of cereal products (including beer)

Food type	Food	DON (µg/kg or µg/L)							
		First Sampling[1]				Second Sampling[1]			
		1	2	3	4	1	2	3	4
Bread	Bread, mixed grain (R)	3.6	10.4	6.7	2.0	4.2	4.6	8.1	2.9
	Bread, wheatmeal (R)	ND	4.8	7.4	2.6	3.5	5.6	5.5	2.7
	Bread, white (R)	ND	3.1	5.4	ND	3.0	3.4	3.4	1.3
Biscuits	Biscuits, chocolate (N)	ND	ND	ND	ND	ND	ND	ND	ND
	Biscuits, cracker (N)	4.9	5.2	7.8	5.4	22	4.8	9.0	5.4
	Biscuits, sweet plain (N)	ND	2.4	4.5	3.8	4.2	13.0	4.3	4.7
Breakfast cereals	Cornflakes (N)	3.4	5.8	3.2	9.5	4.6	2.4	3.8	4.8
	Bran flake cereal (N)	14.3	9.2	14.6	12.3	16.2	8.9	22	11.9
	Wheat biscuit cereal (R)	2.9	3.3	3.7	2.4	4.2	2.7	5.0	3.7
	Muesli (N)	1.8	3.5	1.3	4.6	1.8	3.4	3.7	9.5
	Oats, rolled (cooked) (N)	ND	ND	ND	ND	ND	ND	ND	ND
Bakery products	Cake, plain (R)	ND	7.8	ND	ND	ND	2.0	ND	ND
	Muffin (R)	ND	ND	ND	ND	ND	ND	ND	ND
Pasta and noodles	Noodles, instant (cooked) (N)	ND	4.5	17.4	5.7	2.6	3.8	25	5.4
	Pasta, dried (cooked) (N)	3.0	11.0	38	11.7	24	15.0	28	3.3
	Spaghetti in sauce, canned (N)	13.8	3.8	ND	ND	5.6	8.5	22	8.7
Other cereal-based foods	Rice, white (cooked) (N)	ND	ND	ND	ND	ND	ND	ND	ND
	Snack bars (N)	2.5	ND	ND	ND	ND	ND	ND	ND
	Snacks, flavoured (N)	9.0	7.6	6.6	60	26	410	3.4	ND
	Pizza (R)	ND	ND	ND	ND	ND	ND	ND	ND
	Infant weaning food, cereal based (N)	ND	ND	ND	ND	ND	ND	ND	ND
	Beer (N)	ND	10.9	ND	ND	ND	ND	ND	ND

[1] For nationally distributed foods the sample numbers represent different brands or composites of different brands. For regionally produced foods numbers represent the sampling location (1 = Auckland, 2 = Christchurch, 3 = Dunedin, 4 = Napier). See section 2.1.1 for details of sampling dates for R and N foods

R = regional food N = nationally distributed food
ND = Not detected, See Table 7 for matrix specific limits of detection

Table A1. 2: Nivalenol (NIV) concentrations in New Zealand Total Diet Study (NZTDS) samples of cereal products (including beer)

Food type	Food	NIV (µg/kg or µg/L)							
		First Sampling[1]				Second Sampling[1]			
		1	2	3	4	1	2	3	4
Bread	Bread, mixed grain (R)	ND	3.5	2.7	ND	3.2	3.2	ND	3.8
	Bread, wheatmeal (R)	ND	5.0	4.2	ND	ND	3.5	6.6	ND
	Bread, white (R)	ND	2.5	ND	ND	ND	2.6	3.4	ND
Biscuits	Biscuits, chocolate (N)	ND	ND	ND	ND	ND	ND	ND	ND
	Biscuits, cracker (N)	8.2	9.3	13.5	8.0	6.5	6.2	12.0	5.8
	Biscuits, sweet plain (N)	ND	ND	6.9	10.3	16.4	9.8	8.6	11.4
Breakfast cereals	Cornflakes (N)	ND	ND	ND	ND	ND	ND	ND	ND
	Bran flake cereal (N)	ND	ND	ND	ND	ND	ND	ND	ND
	Wheat biscuit cereal (R)	ND	ND	ND	ND	ND	ND	ND	ND
	Muesli (N)	ND	ND	ND	ND	ND	ND	ND	ND
	Oats, rolled (cooked) (N)	ND	ND	ND	ND	ND	ND	ND	ND
Bakery products	Cake, plain (R)	ND	7.0	3.4	2.3	ND	ND	ND	ND
	Muffin (R)	ND	ND	ND	ND	ND	ND	ND	ND
Pasta and noodles	Noodles, instant (cooked) (N)	ND	1.8	ND	1.2	ND	1.5	1.4	ND
	Pasta, dried (cooked) (N)	ND	ND	1.7	ND	ND	ND	ND	ND
	Spaghetti in sauce, canned (N)	ND	ND	ND	ND	1.3	1.3	ND	1.1
Other cereal-based products	Rice, white (cooked) (N)	ND	ND	ND	ND	ND	ND	3.9	ND
	Snack bars (N)	2.5	ND	ND	ND	ND	ND	ND	ND
	Snacks, flavoured (N)	ND	5.0	6.1	5.0	8.6	ND	5.4	ND
	Pizza (R)	ND	ND	ND	ND	ND	ND	ND	ND
	Infant weaning food, cereal based (N)	4.6	ND	ND	ND	ND	ND	ND	ND
	Beer (N)	ND	ND	ND	ND	ND	ND	ND	ND

[1] For nationally distributed foods the sample numbers represent different brands or composites of different brands. For regionally produced foods number represent the sampling location (1 = Auckland, 2 = Christchurch, 3 = Dunedin, 4 = Napier). See section 2.1.1 for details of sampling dates for R and N foods

R = regional food N = nationally distributed food
ND = Not detected, see Table 7 for matrix specific limits of detection

Table A1. 3: Other trichothecene mycotoxin concentrations in New Zealand Total Diet Study (NZTDS) samples of cereal products (including beer)

Food type	Mycotoxin concentration (µg/kg or µg/L)[1]							
	First Sampling[2]				Second Sampling[2]			
	1	2	3	4	1	2	3	4
Diacetoxyscirpenol (DAS)								
Cornflakes (N)	ND	ND	ND	ND	ND	ND	(0.2)	ND
Rice, white (N)	ND	ND	ND	ND	ND	ND	1.2	ND
Pizza (R)	ND	ND	ND	ND	ND	ND	(0.3)	ND
15-acetyldeoxynivalenol (15ADON)								
Snacks, flavoured (N)	ND	ND	ND	ND	ND	41	ND	ND
T-2 toxin (T2)								
Snacks, flavoured (N)	ND	ND	ND	ND	ND	0.4	ND	ND

[1] Concentration figures in brackets are indicative only and relate to analytical results which lie between the limit of detection and the limit of quantitation of the analytical method.

[2] For nationally distributed foods the sample numbers represent different brands or composites of different brands. For regionally produced foods number represent the sampling location (1 = Auckland, 2 = Christchurch, 3 = Dunedin, 4 = Napier). See section 2.1.1 for details of sampling dates for R and N foods

R = regional food N = nationally distributed food

ND = Not detected, See Table 7 for matrix specific limits of detection

Table A1. 4: Deoxynivalenol (DON) and nivalenol (NIV) concentrations in samples of cereal products taken during March 2014

Food type	Sample[1]			
	1	2	3	4
DON concentration (µg/kg)				
Bread, mixed grain	14.5	11.4	7.2	7.4
Bread, wheatmeal	10.7	9.9	3.6	9.0
Bread, white	2.2	1.8	1.5	1.4
Bran flake cereal	7.7	8.8	10.6	5.2
Muesli	1.8	1.0	1.6	1.1
Snacks, flavoured	1.7	1.1	2.6	2.4
NIV concentration (µg/kg)				
Bread, mixed grain	ND	ND	ND	ND
Bread, wheatmeal	4.8	ND	2.5	4.2
Bread, white	ND	ND	ND	ND
Bran flake cereal	ND	ND	ND	ND
Muesli	ND	ND	ND	ND
Snacks, flavoured	7.4	ND	ND	10.1

[1] All samples were obtained from supermarkets in Christchurch during March 2014

ND = Not detected, see Table 7 for matrix specific limits of detection

APPENDIX 2 INTERNATIONAL CONTEXT

Results of some overseas studies of trichothecenes in foods comparable to those analysed in the current study are summarised in Table A2.1.

Table A2. 1: Recent overseas studies on the trichothecene mycotoxin content of cereal products, including beer

Country	Year	Food(s)	Toxin	Number of samples, positive/total (%)	Range of positive results (µg/kg)	Reference
Argentina	NS				Mean	(Pacin *et al.*, 2011)
		French bread	DON	50/64	43	
		Vienna bread		34/46	30	
		Crackers		10/26	15-17	
		Noodles		1/12	7-9	
		Pizza		6/8	29	
Austria, Hungary, Croatia, Serbia	2011-2012	Beer				(Varga *et al.*, 2012)
		- Pale	DON	118/217	5.4-89	
			DON-3-G	142/217	3.6-81	
		- Wheat	DON	36/46	5.2-50	
			DON-3-G	32/46	3.5-28	
		- Dark	DON	14/47	11-45	
			DON-3-G	28/47	4.2-26	
		- Bock	DON	18/20	7.2-27	
			DON-3-G	20/20	2.4-33	
		- Non-alcoholic	DON	5/19	3.2-26	
			DON-3-G	9/19	2.0-6.6	
		- Shandy	DON	13/25	4.2-13	
			DON-3-G	20/25	1.8-7.9	
Belgium	2003-2004	Beer	DON			(Anselme *et al.*, 2006)
		- Conventional		27/40	6-22	
		- Organic		32/40	6-14	
Belgium	NS	Bread	DON	6/6	<12-102	(De Boevre *et al.*, 2012b)
			3ADON	6/6	29-51	
			15ADON	4/6	<12-18	
			DON3G	5/6	26-29	
			T2	3/6	<18	
			HT2	3/6	<10	
		Cornflakes	DON	5/6	<12-207	
			3ADON	5/6	29-52	
			15ADON	5/6	<12-17	
			DON3G	3/6	24-28	
			T2	3/6	<18	
			HT2	5/6	<10	
Belgium	2010-2011			*n*	Mean (maximum)	(De Boevre *et al.*, 2012a)
		Fibre-enriched bread	DON	52	34 (138)	
			3ADON		14 (74)	
			15ADON		9 (45)	
			DON-3-G		34 (425)	
		Bran-enriched bread	DON	36	25 (127)	
			3ADON		16 (59)	
			15ADON		7 (45)	
			DON-3-G		21 (103)	

Country	Year	Food(s)	Toxin	Number of samples, positive/total (%)	Range of positive results (µg/kg)	Reference
		Cornflakes	DON	61	44 (718)	
			3ADON		31 (431)	
			15ADON		10 (194)	
			DON-3-G		13 (63)	
		Oatmeal	DON	13	18 (91)	
			3ADON		45 (116)	
			15ADON		7 (27)	
			DON-3-G		28 (97)	
Canada	1999-2001				Maximum	(Roscoe *et al.*, 2008)
		Breakfast cereals	DON	72/156	940	
			NIV	1/156	60	
			HT2	1/156	20	
Czech Republic	2010	White flour products	DON	16/17	13-350	(Malachova *et al.*, 2011)
			DON-3-G	14/17	5-30	
			NIV	0/17	-	
		Mixed flour products	DON	32/36	13-431	
			DON-3-G	28/36	7-41	
			NIV	1/36	-	
		Breakfast cereals	DON	2/7	31-347	
			DON-3-G	6/7	19-66	
			NIV	1/7	31	
		Snacks	DON	21/34	13-320	
			DON-3-G	28/34	11-94	
			NIV	1/34	-	
Europe	2006-2007	Beer	T2	*n* = 393	Max = 2.67	(Cantrell, 2008)
			HT2		Max = 2.26	
			DON		Max = 76	
			NIV		Max = <LOQ	
France	NS			*n*	Mean	(Sirot *et al.*, 2013)
		Bread and dried bread products	DON	14	132	
			3ADON		0.2-3.5	
			15ADON		0-3.0	
			NIV		2.6-6.4	
			T2		0.4-4	
			HT2		2.1-8	
		Breakfast cereals	DON	6	8.5-10.8	
			3ADON		0-3.0	
			15ADON		0.5-4.2	
			NIV		1.5-6.5	
			T2		1.0-5.3	
			HT2		1.0-5.3	
		Pasta (cooked)	DON	4	56	
			3ADON		0-3.0	
			15ADON		0-3.0	
			NIV		6.5-10	
			T2		0.8-4.8	
			HT2		3.0-10	
		Sweet or savoury biscuits and bars	DON	8	58-62	
			3ADON		0-3.0	
			15ADON		1.9-4.5	

Country	Year	Food(s)	Toxin	Number of samples, positive/total (%)	Range of positive results (µg/kg)	Reference
			NIV		1.9-4.5	
			T2		1.1-5.6	
			HT2		1.5-6.5	
		Pastries and cakes	DON	18	54-55	
			3ADON		0-3.0	
			15ADON		0-3.0	
			NIV		0-3.0	
			T2		0-3.0	
			HT2		0.5-4.2	
		Pizzas, quiches and savoury pastries	DON	4	101	
			3ADON		0-3.0	
			15ADON		0-3.0	
			NIV		0-3.0	
			T2		0.8-4.8	
			HT2		0-3.0	
Germany	2006-2007	Breakfast cereals	T2+HT2	73/97	0.2-87.3	(Usleber, 2008)
		Wheat bran	T2+HT2	13/13	1.3-26.1	
		Oat bran	T2+HT2	22/24	0.2-29.2	
		Oat flakes	T2+HT2	133/135	0.2-79.5	
		Barley grits	T2+HT2	7/7	0.8-1.7	
		Spelt flakes	T2+HT2	3/8	0.2-1.9	
		Multi-grain flakes	T2+HT2	7/10	0.2-5.4	
		Muesli-type cereals	T2+HT2	169/180	0.2-48.6	
		Bread and rolls	T2+HT2	178/238	Max = 10.3	
		Fine bakery products	T2+HT2	247/294	Max = 66.2	
		Pasta	T2+HT2	304/355	Max = 16.9	
		Infant foods	T2+HT2	231/297	Max = 30.6	
		Beer, malt drinks	T2+HT2	26/33	Max = 1.5	
		Confectionery	T2+HT2	23/24	Max = 5.6	
		Soy products	T2+HT2	18/32	Max = 4.4	
					Max	(Gottschalk *et al.*, 2009)
Germany	2005-2006	Wheat flour	T2	33/39	1.2	
			HT2	38/39	11	
			DAS	1/39	0.25	
			NEO	1/39	0.17	
			DON	39/39	613	
			3ADON	15/39	5.2	
			15ADON	33/39	8.8	
			NIV	22/39	77	
			FX	0/39	-	
		Whole wheat flour	T2	11/11	0.25	
			HT2	11/11	2.2	
			DAS	0/11	-	
			NEO	1/11	0.04	
			DON	11/11	131	
			3ADON	7/11	1.8	
			15ADON	7/11	2.1	
			NIV	10/11	62	
			FX	0/11	-	
		Semolina	T2	5/13	0.08	

Country	Year	Food(s)	Toxin	Number of samples, positive/total (%)	Range of positive results (µg/kg)	Reference
			HT2	7/13	0.67	
			DAS	0/13	-	
			NEO	0/13	-	
			DON	13/13	172	
			3ADON	2/13	0.47	
			15ADON	6/13	0.70	
			NIV	4/13	35	
			FX	1/13	0.18	
		Wheat bran	T2	10/10	1.9	
			HT2	10/10	22	
			DAS	2/10	0.25	
			NEO	9/10	0.40	
			DON	10/10	1163	
			3ADON	7/10	15	
			15ADON	8/10	26	
			NIV	9/10	96	
			FX	0/10	-	
		Infant food	T2	2/5	0.15	
			HT2	4/5	1.3	
			DAS	0/5	-	
			NEO	0/5	-	
			DON	5/5	24	
			3ADON	1/5	1.5	
			15ADON	3/5	3.2	
			NIV	2/5	8.8	
			FX	0/5	-	
Germany	2009				Mean (maximum)	(Reinhold and Reinhardt, 2011)
		Bread	DON	7/13	53 (54)	
		Pre-baked rolls		9/33	78 (168)	
		Ciabatta		15/24	115 (338)	
		Pasta		13/28	87 (126)	
Italy	NS	Pasta, dry	DON	22/27	35-450	(Raiola *et al.*, 2012)
Lebanon	2005	Biscuits	DON	10/20	60-70	(Soubra *et al.*, 2009)
		Bread		22/40	80-700	
		Cakes		15/20	60-100	
		Cornflakes		14/20	60-100	
		Pizza		10/20	100-200	
Malaysia	NS	Instant noodles	DON	17/30	0.7-1.0	(Moazami and Jinap, 2009)
Slovakia	NS	Beer	DON	8/14	6-33.2	(Belajová and Rauová, 2008)
South Korea	2007-2008	Breakfast cereals	DON	6/18	9-37	(Ok *et al.*, 2009b)
		Beer		3/26	8-29	
		Bread		3/8	38-78	
		Biscuits		3/8	23-35	
		Rice		13/149	4-128	
South Korea	2009				Mean (maximum)	(Ok *et al.*, 2011)
		Breakfast cereals	DON	13/18	23 (58)	

Country	Year	Food(s)	Toxin	Number of samples, positive/total (%)	Range of positive results (µg/kg)	Reference
			NIV	11/18	122 (1097)	
			FX	7/18	3 (10)	
			3ADON	2/18	0.6 (6)	
			15ADON	5/18	0.7 (4)	
		Rice	DON	10/65	4 (32)	
			NIV	23/65	10 (45)	
			FX	10/65	1.4 (15)	
			3ADON	8/65	0.6 (10)	
			15ADON	30/65	2.0 (24)	
Spain (Catalonia)	2008	Wheat flakes	T2	6/27	<14-75	(Cano-Sancho *et al.*, 2011; Cano-Sancho *et al.*, 2012)
			HT2	14/27	<12-183	
			DON	20/27[1]	Max = 437	
		Cornflakes	T2	7/65	<19	
			HT2	35/65	<10-65	
			DON	49/65[1]	Max = 580	
		Beer	T2	0/71	-	
			HT2	0/71	-	
			DON	1/71[1]	12	
		Bread	T2	1/103	<36	
			HT2	16/103	<10-75	
			DON	43/103[1]	Max = 739	
		Sweet corn	T2	6/72	<45-256	
			HT2	8/72	<8-84	
			DON	2/72[1]	Max = 139	
		Corn snacks	T2	8/71	<18-70	
			HT2	24/71	<20-895	
			DON	56/71[1]	Max = 304	
		Pasta	T2	14/70	<21	
			HT2	32/70	<10-80	
			DON	52/70[1]	Max = 946	
Spain	2009	Breakfast cereals	DON	38/148	32-468	(Montes *et al.*, 2012)
			3ADON	0/148	-	
			15ADON	0/148	-	
			NIV	4/148	<19-42	
			FX	2/148	<15-57	
Spain	NS	Bread	DON	21/75	12-147	(González-Osnaya *et al.*, 2011)
			T2	2/75	39,68	
		Pasta	DON	47/75	11-623	
			T2	7/75	29-260	
Spain (Valencia)	2005	Corned based				(Castillo *et al.*, 2008)
		- Breakfast cereals	DON	22/55	30-121	
			NIV	6/55	51-107	
		- Baked snacks	DON	13/57	36-132	
			NIV	1/57	56	
		- Fried snacks	DON	12/63	26-80	
			NIV	0/63	-	

DON = deoxynivalenol
NIV = nivalenol
T2 = T-2 toxin
HT2 = HT-2 toxin
NEO = neosolaniol
DAS = diacetoxyscirpenol
FX = Fusarenon X
3ADON = 3-acetyldeoxynivalenol

15ADON = 15-acetyldeoxynivalenol DON-3-G = deoxynivalenol-3-glucoside

[1] For DON, the positive samples are those with a concentration greater than the LOQ
max = maximum reported concentration
NS = not stated
LOD = limit of detection LOQ = limit of quantification