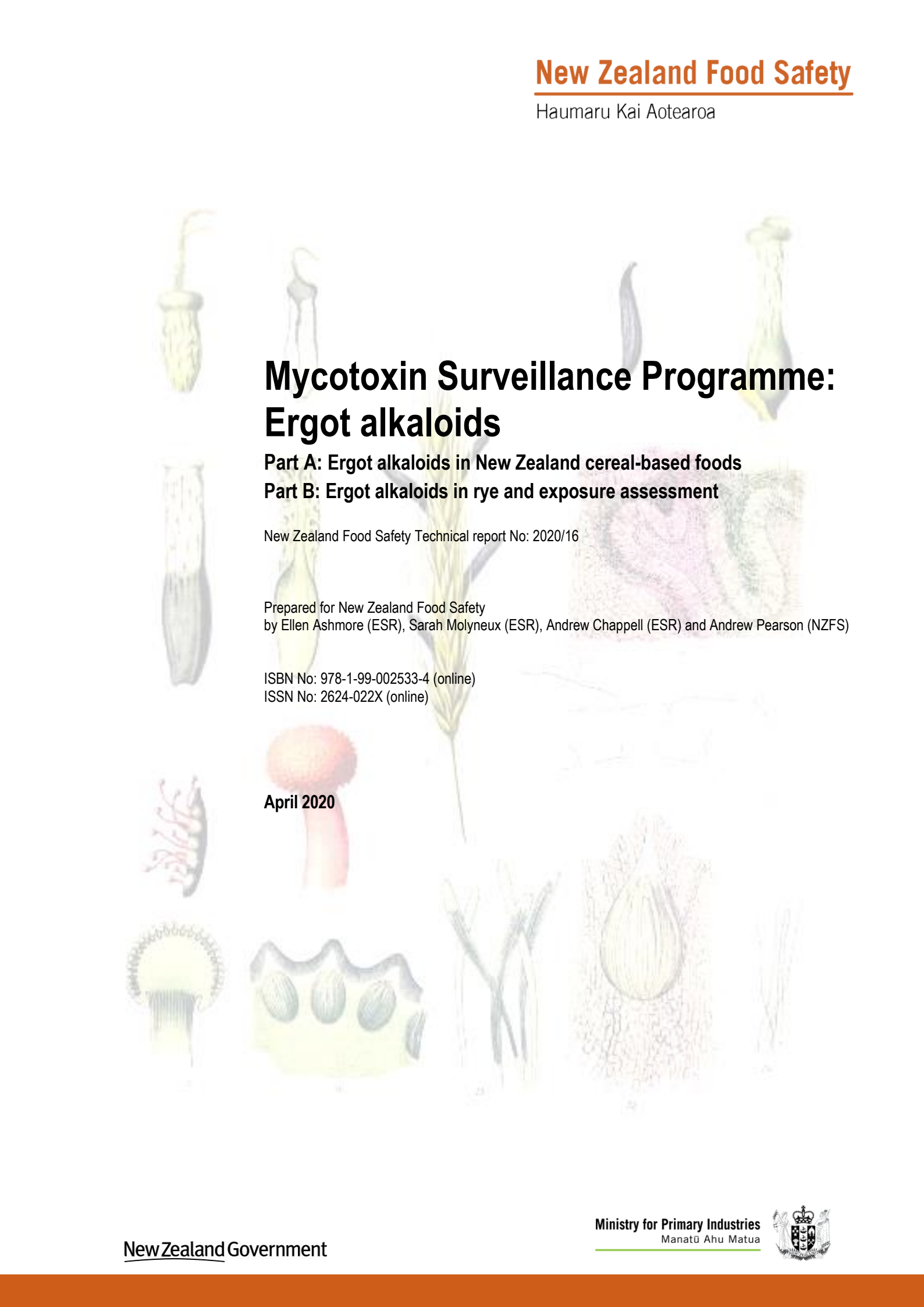
New Zealand Food Safety
Haumaru Kai Aotearoa



Mycotoxin Surveillance Programme: Ergot alkaloids

Part A: Ergot alkaloids in New Zealand cereal-based foods

Part B: Ergot alkaloids in rye and exposure assessment

New Zealand Food Safety Technical report No: 2020/16

Prepared for New Zealand Food Safety
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ISBN No: 978-1-99-002533-4 (online)
ISSN No: 2624-022X (online)

April 2020

New Zealand Government

Ministry for Primary Industries
Manatū Ahu Matua

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Scientific Interpretative Summary

This SIS is prepared by New Zealand Food Safety (NZFS) risk assessors to provide context to the following report for NZFS risk managers and external readers.

Mycotoxin Surveillance Programme: Ergot alkaloids

New Zealand Food Safety runs an ongoing surveillance programme to survey and assess the dietary risk of mycotoxins in the New Zealand food supply.

Ergot alkaloids were listed as a high priority to generate New Zealand occurrence data for in the 2014 Mycotoxin risk profile. The causative fungi for ergot alkaloids, *Claviceps* species, grow in New Zealand conditions and outbreaks of ergot poisoning in livestock are sporadically reported. Two surveys and an exposure assessment were completed to address the potential dietary risks of ergot alkaloids in New Zealand.

The first survey, Part A: Ergot alkaloids in New Zealand cereal-based foods, reports on a survey broadly across different cereal based foods for 12 different ergot alkaloids. The majority of samples had no detectable or minor levels of ergot alkaloids. Only 8% of samples had levels above 10 µg/kg, and overall the concentrations found were on the lower end of international ranges. Rye-containing foods had the highest reported values of ergot alkaloids. A follow-up survey for food produced in a different growing season was identified as useful to quantify the season to season variation that could result from different climatic conditions.

The second survey, Part B: Ergot alkaloids in rye and exposure assessment, reports on the results of the follow-up survey of rye to establish any season to season variation. Forty rye-based foods were tested for 12 different ergot alkaloids. A quarter of samples had levels above 10 µg/kg with the concentrations being consistent with those found in rye-based foods in Part A. These results suggest that the ergot alkaloid content of rye-based foods may be relatively consistent over different growing seasons.
The findings of both surveys were combined to allow an exposure assessment to be completed. The main foods contributing to adult and children dietary exposure were wheat bread, wheat flour, and for adults only, multigrain bread. The New Zealand estimated exposures were far below the health based guidance value set by the European Food Safety Authority.

The findings of the surveys and exposure assessment identify that at present ergot alkaloids are not a significant dietary concern in the New Zealand diet. Consequently, further measures to limit occurrence in the food supply are not required.

ESR
Science for Communities

Mycotoxin Surveillance Programme 2017-2018: Ergot alkaloids in New Zealand cereal-based foods

14 December 2018

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PREPARED FOR:	**Ministry for Primary Industries, project code CFS/17/02, Ergot Alkaloids in New Zealand cereal-based foods, as part of the overall contract for scientific services**
CLIENT REPORT No:	**FW18047**
REVIEWED BY:	**Peter Cressey**

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		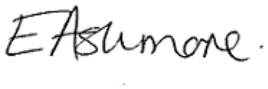
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EXECUTIVE SUMMARY

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

Ergot alkaloids (EAs) are tryptophan derived alkaloids (mycotoxins) produced by fungal *Claviceps* species. There are more than 50 different EAs identified, with the distribution of EAs varying between fungal strains, geographic regions and host plants.

This study analysed cereal foods available on the New Zealand market (*n* = 100) for 12 EAs; ergometrine, ergometrinine, ergotamine, ergotaminine, ergosine, ergosinine, ergocornine, ergocorninine, ergocryptine, ergocryptinine, ergocristine and ergocristinine.

Foods purchased for the study focussed on the major potential dietary sources of EAs for the New Zealand diet, based on an earlier scoping exercise.

Over half of the samples (54%) did not contain quantifiable levels of EAs (limits of quantification were in the range 0.5-1.25 μg/kg for the individual EAs), while over one-third of samples (38%) contained quantifiable concentrations of total EA ≤10 μg/kg. Moderate concentrations of total EA (11-50 μg/kg) were observed in 6% of samples and the remaining 2% of samples had concentrations of total EA >50 μg/kg. The highest concentrations were observed in single samples of rye flour and a rye-based bread.

The concentrations of EAs in foods available in New Zealand were towards the lower end of the range of results reported internationally.

For New Zealand manufactured products, certain food types showed higher concentrations of EAs (rye containing foods) than other food types (oats).

Climatic conditions during the growing season are thought to influence the concentration of EAs. A follow on study, representing a season with different climatic conditions, would provide further monitoring data and could provide information of the impact of climatic variables on EA occurrence. The inclusion of an isotopically-labelled internal standard in the analytical method would be of great benefit, if available.

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 and its update (Cressey, 2014b; Cressey and Thomson, 2006) are viewed as starting points for this process. The risk profile identified a number of issues to be investigated or clarified.

Efforts 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); 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); trichothecene mycotoxins in cereal products (Cressey *et al.*, 2014), culminating in an exposure assessments for ochratoxin A and trichothecene mycotoxins (Cressey, 2014a), completing the initially identified mycotoxin priorities for New Zealand. As a product of revised prioritisation, fumonisins in maize-based products and wine were analysed (Cressey *et al.*, 2017).

Due to the shortage of New Zealand specific prevalence data, ergot alkaloids (EAs) were identified as the topic for the Mycotoxin Surveillance Programme in 2017-2018.

1.1 ERGOT ALKALOIDS

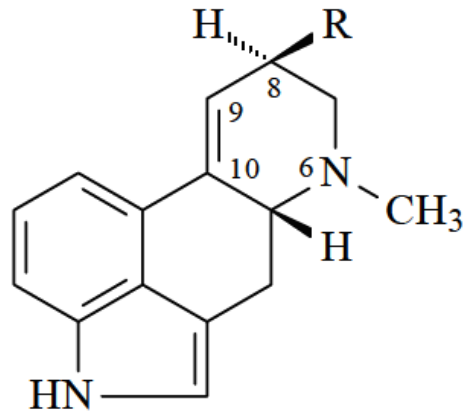
Ergot refers to fungal infestation of cereal plants by fungal *Claviceps* species. During the growth of the plant, ergot replaces grain kernels with large dark-coloured sclerotia (Figure 1). The ergots ripen with the grain and either fall off pre-harvest or are included with harvested grain (Neill, 1941). Once harvested, contamination of cereal-based foods and feeds can occur (Krska and Crews, 2008). Cereals including wheat, rice, rye, corn, sorghum, barley, oats, and millet are all known to be hosts of ergot-producing *Claviceps* species (Lorenz, 1979). Ergot alkaloids (EAs) are tryptophan derived alkaloids (mycotoxins) produced by the *Claviceps* species.

Throughout the Middle Ages, contamination of grains (raw or processed) with EAs caused epidemics of ergotism, exhibiting two major forms: gangrene and convulsions. Gangrene (chronic ergotism) of the hands, feet and limbs occurred due to vasoconstriction properties of ergot, along with an intense burning sensation (De Costa, 2002). Ergotism causing gangrene was previously known as St Anthony's Fire (Flieger *et al.*, 1997). Convulsive ergotism caused hallucinations (De Costa, 2002), delirium, and psychosis (Nemes and Goerig, 2002). Through improvements in agricultural practice, food processing techniques and increased scientific knowledge, ergotism epidemics of the serious nature observed in the Middle Ages are now infrequent. Two outbreaks of ergot poisoning occurred in Ethiopia, in 1977-78 and 2001. The 1977-78 and 2001 outbreaks were attributed to contaminated wild oats which were subsequently mixed with barley for consumption (ergot was not observed on the barley) (Urga *et al.*, 2002). EAs have been shown to have a broad spectrum of pharmacological effects and were used for medicinal purposes. Lysergic acid diethylamide (LSD), a semi-synthetic EA derivative, was discovered in the early 1900s and legally used as a pharmaceutical until its potent psychoactive effects were fully recognised. Today LSD is an illegal drug of abuse (EFSA, 2012).

Figure 1 Ergot sclerotia on rye grass, found in Mid-Canterbury April 2018

There are more than 50 different EAs identified, with the distribution of EAs varying between fungal strains, geographic regions and host plants (Flieger *et al.*, 1997). *C. purpurea* is a common EA producing species and is the *Claviceps* species most commonly associated with infections of commercially-grown cereals in New Zealand. Natural EAs share a common structural feature, an ergoline ring, with a methylated nitrogen (N-6) and various substitutions at C-8 (Figure 2) (Diana Di Mavungu *et al.*, 2011; Flieger *et al.*, 1997; Krska *et al.*, 2008).

Figure 2 The structure of the ergoline ring



Reproduced from Diana Di Mavungu et al. (2011)

Previous studies, as summarised by the European Food Safety Authority (EFSA), have monitored between one and 12 different EAs, with the majority of food surveys reported to EFSA studying 12 EAs (EFSA, 2012). EFSA recommended that studies should monitor six key EAs and their corresponding epimers[1] (12 EAs in total) in order to improve the knowledge of EAs in food (EFSA, 2012). The six major EAs usually analysed are ergometrine, ergotamine, ergosine, ergocristine, ergocryptine (α and β), and ergocornine.

[1] The term epimers refers to molecules that have more than one chiral centre, but only differ by their configuration (conformation) at one of those chiral centres

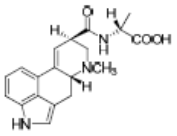
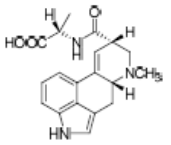
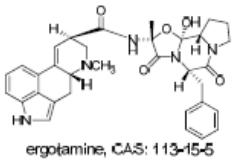
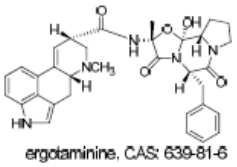
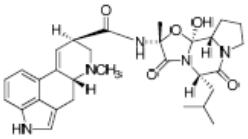
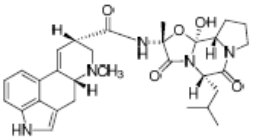
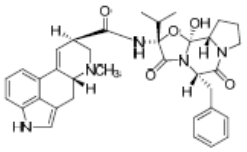
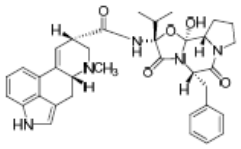
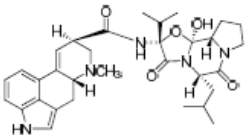
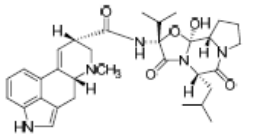
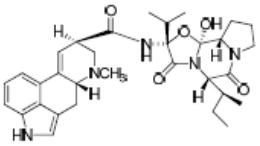
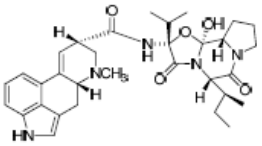
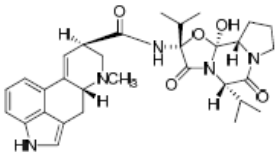
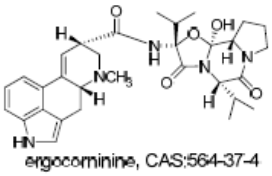
Each corresponding epimer features the suffix –inine. The structures of the major EAs and their epimers are shown in Figure 3.

Ergot alkaloids have recently been assessed by EFSA (EFSA, 2017) and have been identified as a priority by the Codex Committee on Contaminants in Food (CCCF).[2] A recent risk profiling exercise for mycotoxins in the New Zealand food supply identified EAs as priority mycotoxins for further work in the Mycotoxin Surveillance Programme, to address the lack of occurrence data in the New Zealand food supply (Cressey, 2014b).

Previous overseas studies have only detected EAs in grain and grain-based products, although limited analyses have been carried out on non-cereal foods. In particular, rye grain (*Secale cereale*), rye flour and other rye-based products consistently show the highest mean concentrations of EAs of the major cereals (EFSA, 2017). The concentration of EAs in different grains (*n* = 666) as summarised by EFSA (2017) followed the order of rye > spelt > wheat > oats > barley (mean 176, 104, 76, 52, and 47 µg/kg, respectively). A similar concentration order was observed for grain milling products and other grain-based foods. EA profiles in foods and the total amounts are highly variable and may depend on geographic location, climatic conditions during the growing season, fungal genotype and host plant (EFSA, 2012).

[2] http://www.fao.org/fao-who-codexalimentarius/sh-proxy/es/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-735-12%252FCRDs%252Fcf12_CRD02x.pdf Accessed 3 December 2018

Figure 3 Structure of the major ergot alkaloids and their epimeric forms

Lysergic acid derivatives	Isolysergic acid derivatives
 ergometrine (ergonovine, ergobasine) CAS: 60-79-7	 ergometrinine (ergonovinine), CAS: 479-00-5
 ergotamine, CAS: 113-15-5	 ergotaminine, CAS: 639-81-6
 ergosine, CAS: 561-94-4	 ergosinine, CAS: 596-88-3
 ergocristine, CAS: 511-08-0	 ergocristinine, CAS: 511-07-9
 α-ergocryptine (ergocryptine) CAS: 511-09-1	 α-ergocryptinine (ergocryptinine) CAS: 511-10-4
 β-ergocryptine, CAS: 20315-46-2	 β-ergocryptinine, CAS: 19467-61-9
 ergocornine, CAS: 564-36-3	 ergocorninine, CAS:564-37-4

Reproduced from EFSA (2012)

1.2 REGULATORY LIMITS IN NEW ZEALAND

Ergot (in its fungal infestation form) is currently regulated in cereals under the Australia New Zealand Food Standards Code. Schedule 19 – Maximum levels of contaminants and natural toxicants sets out a maximum level (ML) for Ergot in Cereal Grains at 500 mg/kg (section S19-5 Maximum levels of non-metal contaminants).[3] No ML is set for foods for consumption. The Standard applies the concentration of ergot sclerotia in grain, rather than the concentration of the EAs present.

1.3 PROJECT AIMS

This study analysed the six major EAs and their -inine epimers. In addition to the epimeric forms, ergocryptine also occurs as two structure isomers (α- and β-); differing by the position of one methyl group. Only the α- isomers, α-ergocryptine and α-ergocryptinine, were analysed due to the unavailability of analytical standards for the β-isomers. For the remainder of the report, these analytes are referred to as simply ergocryptine and ergocryptinine.

The aim of this project was to provide analytical information on the EA concentrations in cereal-based foods, commercially available in New Zealand. Foods purchased for the study focussed on the major potential dietary sources of EAs for the New Zealand diet, as determined in a preceding scoping exercise.

The analytical information will inform whether further investigation is required. This project included a wide scope of foods, while a follow-up project could be used to focus on foods in which appreciable ergot alkaloid concentrations were found. The information will provide the necessary data to undertake a robust dietary exposure and risk assessment.

[3] https://www.legislation.gov.au/Details/F2017C00333 Accessed 5 November 2018.

2. SAMPLING AND METHODS

2.1 SAMPLING

Cereal-based food samples (*n* = 100) were purchased in Christchurch, New Zealand during February to April 2018. A minimum of 500 g of each food product was purchased from supermarkets and wholefood stores. For certain products sold in packets weighing less than 500 g each, multiple packets with the same batch number and use by/best before date were purchased to make up one analytical sample.

2.2 SAMPLE CHARACTERISTICS

Table 1 provides a summary of the countries of origin for the cereal-based samples (*n* = 100) purchased. The country of origin of a product is not necessarily the country of origin of the raw cereal ingredients. The product packaging provided country of origin details including 'product of', 'grown in', 'made in', 'packed in', and 'from local and imported ingredients'.

Table 1 Country of origin for all samples

Country of origin	Number of samples (*n* = 100)
Australia	7
Belgium	1
Canada	1
Germany	3
Italy	1
New Zealand	58
Turkey	1
UK	2
USA	4
Not provided	22

The food types were designated such that as large as possible a number of samples fell into each category while providing meaningful comparisons between types. The samples were grouped into 14 categories as shown in Table 2. Breakfast cereals – Other contained samples of rye flakes, spelt flakes and an infant-specific oat cereal. Wheat breads comprised of samples where the main ingredient was wheat (*Triticum aestivum*), either as wholemeal or white flour; minor ingredients in some samples included soy flour and/or barley flour (no percentages listed).

Table 2 Food types

Food type	Number of samples (*n* = 100)
Breakfast cereals - Oat	6
Breakfast cereals - Other	4
Breakfast cereals - Wheat	5
Crackers - Oat	3
Crackers - Rye	2
Flours - Oat	5
Flours - Rye	10
Flours - Spelt	5
Flours - Wheat	10
Multigrain bread	15
Rye-based breads	10
Wheat breads	15
Whole grains - Oats	5
Whole grains - Pearled Barley	5

2.3 SAMPLE PREPARATION

The samples were individually homogenised (where required) to a fine powder using a kitchen blender, spice grinder or Thermomix® food processor. The samples were immediately frozen and stored at -18°C after homogenising. All samples were analysed as purchased without further preparation to a ready-to-consume state.

2.4 ANALYTICAL STANDARDS

Analytical standards were purchased from PM Separations and supplied by Chiron, Trondheim, Norway. Ergometrine, ergotamine, ergosine, ergocristine, ergocornine were supplied as 100 µg/mL in dried down 5 mL (i.e. each EA was supplied as 500 µg of dried EA in a vial intended to be reconstituted to 5mL EA standard). Ergometrinine, ergotaminine, ergosinine, α-ergocryptinine, ergocristinine, and ergocorninine were supplied as 25 µg/mL in dried down 5 mL (i.e. each EA was supplied as 125 µg of dried EA in a vial intended to be reconstituted to 5mL EA standard). α-Ergocryptine was supplied as 100 µg/mL in methanol 1 mL.

2.5 ANALYTICAL METHODOLOGY

A summary of the method development details are presented in Appendix A.1. The method was based on Kokkonen and Jestoi (2010).

Each of the 100 samples were extracted by solid phase extraction (SPE) and analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). A fresh calibration curve of 0.4-50 µg/kg was prepared and analysed with each extracted batch of samples.

Briefly, 20 g of sample was extracted with 100 mL acetonitrile/ammonium carbonate in H_2O solution (84:16) and shaken for 1 hour. The extract was filtered through No. 4 Whatman filter paper. The supernatant (4 mL) was passed through a MycoSep© column and 2 mL of the extract evaporated to dryness under nitrogen. The extract was reconstituted with 500 µL acetonitrile and centrifuged at 13,500 rpm for 2 minutes. The supernatant was transferred to a vial for analysis.

Using an Agilent 1200 series liquid chromatograph, mobile phase of (A) 5 mM ammonium formate in water and (B) 5 mM ammonium formate in acetonitrile, 5 µL of sample extract

was loaded onto an Agilent Poroshell HPH-C18 column 1.9 µm, 2.1x100, with a flow rate of 0.18 mL/min. The mobile phase gradient programme is shown in Table 3.

Table 3 Mobile phase gradient programme for analysis of EAs

Time (min)	Mobile phase A (%)[1]	Mobile phase B (%)[2]
0	95	5
10	70	30
11	60	40
25	35	65
25.5	10	90
28	10	90
28.5	95	5
35	95	5

[1] Mobile phase A: 5 mM ammonium formate in water
[2] Mobile phase B: 5 mM ammonium formate in acetonitrile

The analytes were detected by a multimode ion (MMI) source in electrospray ionisation (ESI) and positive ion mode on an Agilent 6410 QQQ. The analysis was conducted with time segments, to increase the dwell time for each transition. The mass spectrometer source parameters were a capillary voltage of 2000 V, corona current of 0 µA and charging voltage of 2000 V. The gas flow rate was 5 L/minute, with a gas temperature of 300°C, nebuliser pressure of 60 psi and a vapouriser temperature of 200°C. Specific mass spectrometric parameters for the EAs are summarised in Table 4, where each compound has two transitions: the first listed is the Quantifier transition, used to determine the concentration of the target species, and the second listed is the Qualifier transition, for increased confidence with the identification of the target species.

Table 4 Mass spectrometer parameters for EAs

Compound	Quantifier/ Qualifier transition (m/z)	Resolution	Dwell time (ms)	Fragmentor Voltage (V)	Collision Energy (V)	Cell Acceleration (V)
Time segment 0 to 14 minutes						
Ergometrine	326.2-223.1	Unit/Wide	150	120	22	2
	326.2-208	Unit/Wide	150	120	28	2
Ergometrinine	326.2-208	Unit/Unit	150	140	28	6
	326.2-265.1	Unit/Unit	150	140	16	7
Time segment 14 to 19.5 minutes						
Ergotamine	582.3-223.1	Wide/Wide	120	130	34	5
	582.3-208.1	Wide/Wide	120	130	46	6
Ergotaminine	582.3-233	Unit/Unit	120	100	34	5
	582.3-297	Unit/Unit	120	100	26	7
Ergosine	548.3-223	Unit/Wide	120	120	32	5
	548.3-208	Unit/Widest	120	120	46	6
Ergosinine	548.3-223.1	Unit/Widest	120	100	34	5
	548.3-263	Unit/Widest	120	100	28	7
Ergocornine	562.4-223.1	Unit/Unit	120	140	36	6
	562.4-268.1	Unit/Unit	120	140	22	7
Ergocryptine	576.4-223.1	Widest/Unit	120	120	40	5
	576.4-305.1	Widest/Unit	120	120	26	2
Ergocristine	610.4-223.1	Unit/Unit	120	100	38	6
	610.4-268	Unit/Unit	120	100	24	7
Time segment 19.5 to 35 minutes						
Ergocorninine	562.4-223.1	Unit/Wide	150	140	36	5
	562.4-277.1	Unit/Wide	150	140	26	7
Ergocryptinine	576.4-223.1	Widest/Unit	150	120	38	5
	576.4-305	Widest/Unit	150	120	26	7
Ergocristinine	610.4-223.1	Widest/Unit	150	180	36	5
	610.4-305.1	Widest/Unit	150	180	26	7

2.5.1 Precision – coefficient of variation

Coefficients of variation (CVs), based on a method standard deviation derived from duplicate analyses of naturally contaminated samples were determined for each of the 12 EAs (IANZ, 2004). Two samples (sample 6 and 26) were naturally contaminated with each of the 12 EAs. The CVs determine the closeness of agreement (precision) between the results of repeated measurements of the same sample, and are reported in Table 5.

The European Commission has specified acceptable CV or relative standard deviation (RSD_r) ranges for regulatory analysis of some mycotoxins depending on their concentration range (European Commission, 2006). Ergot alkaloids are not specified in the regulation. As a guide a comparison with ochratoxin A and patulin can be used, where similar low levels of the mycotoxin are reported. For ochratoxin A concentrations of <1 µg/kg and between 1-10 µg/kg the acceptable CVs are ≤40% and ≤20%, respectively. For patulin concentrations of <20 µg/kg and between 20-50 µg/kg the acceptable CVs are ≤30% and ≤20%, respectively.

CVs determined in the current study are within the acceptable ranges for ochratoxin A and patulin, providing guidance on acceptable reported concentrations.

Table 5 Precision of naturally EA contaminated samples

Ergot Alkaloid	Sample 6 Mean concentration (µg/kg)	Sample 26 Mean concentration (µg/kg)	Mean CV (%)
Ergometrine	9.9	1.3	16.8
Ergometrinine	1.2	0.6	18.1
Ergotamine	19.0	2.6	5.4
Ergotaminine	11.6	4.4	14.6
Ergosine	10.9	1.1	6.8
Ergosinine	5.7	1.5	10.6
Ergocornine	8.0	1.3	6.1
Ergocorninine	5.6	2.6	12.2
Ergocryptine	13.8	4.5	7.7
Ergocryptinine	2.0	1.9	15.8
Ergocristine	20.9	4.3	7.4
Ergocristinine	6.9	7.8	11.3

2.6 METHOD VALIDATION RESULTS

2.6.1 Method sensitivity – Limits of detection and quantification

The limit of detection (LOD); the lowest concentration that is significantly different to the response baseline, was calculated as a signal-to-noise ratio of three (United States Food and Drug Administration, 1996). LODs 0.4 µg/kg for ergometrine and 0.1 µg/kg for all other 11 EAs were assigned. The limit of quantification (LOQ) was calculated as at three times the LOD, consistently reproducible throughout the study and taking into consideration the method dilution factor, resulting an LOQ of 1.25 µg/kg for ergometrine and LOQs of 0.5 µg/kg for all other 11 EAs.

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 concentrations falling between the LOD and LOQ were highlighted as ‘TR’ in Appendix A.4.

2.6.2 Accuracy – spike recovery

Full details of spike recovery methods and results are included in Appendix A3.1.

The European Commission has specified acceptable recovery ranges for regulatory analysis of some mycotoxins depending on their concentration range (European Commission, 2006). Ergot alkaloids are not specified in the regulation. As a guide a comparison with ochratoxin A and patulin can be used, where similar low levels of the mycotoxin are reported. When the samples were spiked in the current study, an array of final concentrations were gained (natural concentration plus spike concentration) therefore the spike recoveries can be compared to a number of the regulations listed by the European Commission (2006). In some situations, for ochratoxin A and patulin, acceptance ranges of 50-120% were specified. The narrowest applicable range specified, depending on the concentration of mycotoxin in the spiked sample, was 70-105%.

In the current study, overall mean recoveries for each EA ranged 57-126%, therefore generally agreeing with the wider acceptable range of 50-120% for the mycotoxin regulations. Seven of the twelve EAs had mean recoveries within the narrowest regulatory range of 70-105%.

For each sample matrix, the minimum and maximum recoveries were more variable (ranging from 5 to 208%), and generally lower for the –ine epimers and higher for the –inine epimers. Interconversion could explain why some recoveries were lower and some higher than desired, when comparing the two epimers of an EA.

In other instances, the sum of the two epimers of an EA were considerably <200% suggesting losses were occurring.

Upon analysis of the recovery results several options for managing the analytical results were considered and not deemed suitable:

- Applying recovery correction for samples. The recoveries were too variable and losses could not be confidently quantified to apply correction factors. Interconversion would affect concentrations of individual EAs but not total EAs.
- To report sum of epimers of the 6 EAs. Whilst some interconversion appears likely, the conversion is not uniform across all matrices nor within matrices.

It was concluded that the current study should continue as planned. The detection of EAs in samples were valid and the subsequent reported concentrations were good estimations regardless of loss or interconversion. The recoveries were within the same order of magnitude as expected and therefore unlikely to affect future exposure assessments.

2.7 FAPAS PROFICIENCY RESULTS

2.7.1 Proficiency testing

Proficiency testing is an essential part of laboratory quality procedure. Proficiency testing provides an independent assessment of the performance of analytical testing compared with other laboratories internationally. Fapas is a respected proficiency testing programme run by Fera in the United Kingdom.[4]

2.7.2 Fapas report

A Fapas proficiency rye flour sample (22149) was procured. The Fapas report[5] produced after the participating external laboratories submitted results stated *inter alia*:

[4] https://fapas.com/ Accessed 28 November 2018
[5] Fapas® - Food Chemistry Proficiency Test Report 22149, Ergot Alkaloids in Rye Flour, March-April 2018.

- For ergocornine, ergocorninine, ergocristine, ergosine and ergotamine the derivation of the Assigned Value was straightforward, and the robust mean was chosen as the Assigned Value.
- For ergocristinine, ergosinine and ergotaminine there were a low number of data points, and the median was used to calculate the Assigned Value.
- For ergocryptine and ergocryptinine the results were multimodal, depending on whether α- and/or β- ergocryptine/ergocryptinine was reported. It was not possible to set an Assigned Value or issue z-scores.
- For ergometrine and ergometrinine there was a lack of consensus between the reported results. It was not possible to set an Assigned Value or issue z-scores.

2.7.3 Analysis of Fapas sample

During the current study, the Fapas proficiency sample was analysed six times (designated A, B, C, D, E, F) and in three different batches (A and B; C, D, and E; F). The results from this study were not submitted to Fapas as the analyses were conducted outside of the required deadline, therefore the results were not included in the z-score and Assigned Value calculations in the Fapas report. The sample was therefore treated as a Quality Control check and used to determine agreement with the Fapas results.

The accuracy of the results (i.e. ESR results compared to the Fapas Assigned Value) was variable; with mean accuracies ranged from 47 to 193%, depending on the EA. Overall, the range of concentrations documented in the Fapas report was considerably wider than the range of results achieved in the current study. In the ESR study, there was some evidence that interconversion of the epimers took place; ergotamine/ergotaminine, ergosine/ergosinine, ergocornine/ergocorninine exhibited alternating low and high accuracies compared to Fapas Assigned Values. All stages of the method were examined to reduce interconversion and it was decided the method was as robust as possible, in the absence of a suitable isotopically-labelled internal standard. The complexity and difficulty of the analysis of the EAs in food samples is reflected in the data generated.

Full results and a comparison assessment are presented in Appendix A.3

3. RESULTS AND DISCUSSION

3.1 SUMMARY OF RESULTS

3.1.1 Percentage of samples with quantified concentrations of ergot alkaloids in the current study

Individual sample results are provided in Appendix A.4. Table 6 summarises the percentage of samples in the current study with quantifiable (positive) EA results, for each food type. A positive sample was identified as containing one or more EAs ≥LOQ, and therefore a robustly quantified result was determined.

Overall, 46% of the samples contained quantifiable concentrations of EAs.

It should be noted that while 54% of samples were reported as less than the LOQ, a small proportion of the samples were noted during the review to contain EAs at levels just below the LOQ and above the LOD. Trace amounts of an individual EA are identified in Appendix A.4.

Wheat breads were the food type most likely to contain EAs, with 80% of samples positive. Multigrain breads were the second most likely to contain EAs, with 67% of samples positive. Low prevalence of EAs was found in oat-based food, with 17, 33, 20, and 20% of breakfast cereal, cracker, flour and whole grain samples positive, respectively. EAs were not quantified in any samples of breakfast cereals – wheat, breakfast cereal – other, and whole grains – pearled barley.

Table 6 Percentage of samples with quantified concentrations of ergot alkaloids (positive samples) for each food type

Food Type	Total samples analysed	Total positive samples	Percentage positive samples
Breakfast cereals - Oat	6	1	17
Breakfast cereals - Other	4	0	0
Breakfast cereals - Wheat	5	0	0
Crackers - Oat	3	1	33
Crackers - Rye	2	1	50
Flours - Oat	5	1	20
Flours - Rye	10	5	50
Flours - Spelt	5	3	60
Flours - Wheat	10	6	60
Multigrain bread	15	10	67
Rye-based bread	10	5	50
Wheat bread	15	12	80
Whole grains - Oats	5	1	20
Whole grains - Pearled Barley	5	0	0
All food types	100	46	46

3.1.2 Percentage of samples with quantified concentrations of ergot alkaloids in overseas studies

In a Polish study, EAs were detected in 94% of rye flour samples (*n*=34) (Bryła *et al.*, 2015).

In a German study, rye breads were noted to show a high percentage of samples with measurable EAs. EAs were detected in 7% of rye crispbread samples (*n*=14) (Bürk *et al.*, 2006).

In a second German study, EAs were detected in 100% of rye flour samples (*n*=9) (Koppen *et al.*, 2013). While in a further German study, EAs were detected in 100% of rye flakes samples (*n*=3) (Müller *et al.*, 2009).

In a British study, EAs were detected in 100% of samples of rye bread (n=8), rye crispbread (n=11), bread mix (rye-based) (n=3), rye-wheat bread (n=1) and rye flour (n=1) (Crews *et al.*, 2009). EAs were detected in 50% of rye crackers (*n*=2) and not detected in all samples of rye flakes (*n*=2).

In a Chinese study, EAs were detected 22% of rye flours (*n*=9) and 6% of wheat flours (*n*=52) samples (Guo *et al.*, 2016).

In a Canadian study, EAs were detected in 33% samples of oat-based cereals (but not specifically designated a breakfast food) (*n*=6) (Lombaert *et al.*, 2003).

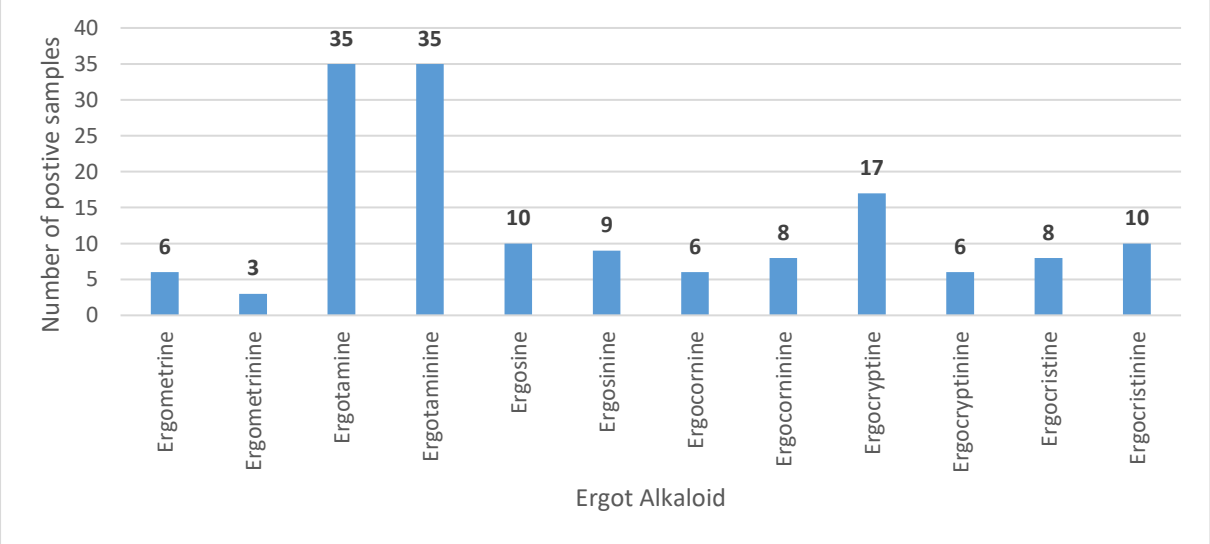
Finally, in a Danish study, EAs were detected in 94% of rye flour (*n*=34) (Storm *et al.*, 2008).

Given the majority of overseas studies focussed predominantly on rye and rye-based products, it is difficult to determine whether the high prevalence of EAs in wheat breads in the current study was to be expected.

3.1.3 Prevalence of the individual ergot alkaloids in the current study

Figure 4 shows the prevalence of the individual EAs across all food types in the current study. The most prevalent EAs were ergotamine and its epimer ergotaminine, each quantified in 35 out of 100 samples. The other 10 EAs were far less prevalent, quantified in ≤17 out of 100 samples. The least prevalent was found to be ergometrinine, quantified in only 3 out of 100 samples.

Figure 4 Prevalence of the individual EAs in cereal based-foods



3.1.4 Prevalence of the individual ergot alkaloids in overseas studies

Ergotamine and ergotaminine were the most frequently occurring EAs reported by Arroyo-Manzanares *et al.* (2018). Ergotamine and ergotaminine prevalence is comparable with the current study.

EFSA (2012) concluded ergotamine, ergocristine, ergosine and ergocornine were generally more abundant than α-/β-ergocryptine and ergometrine.

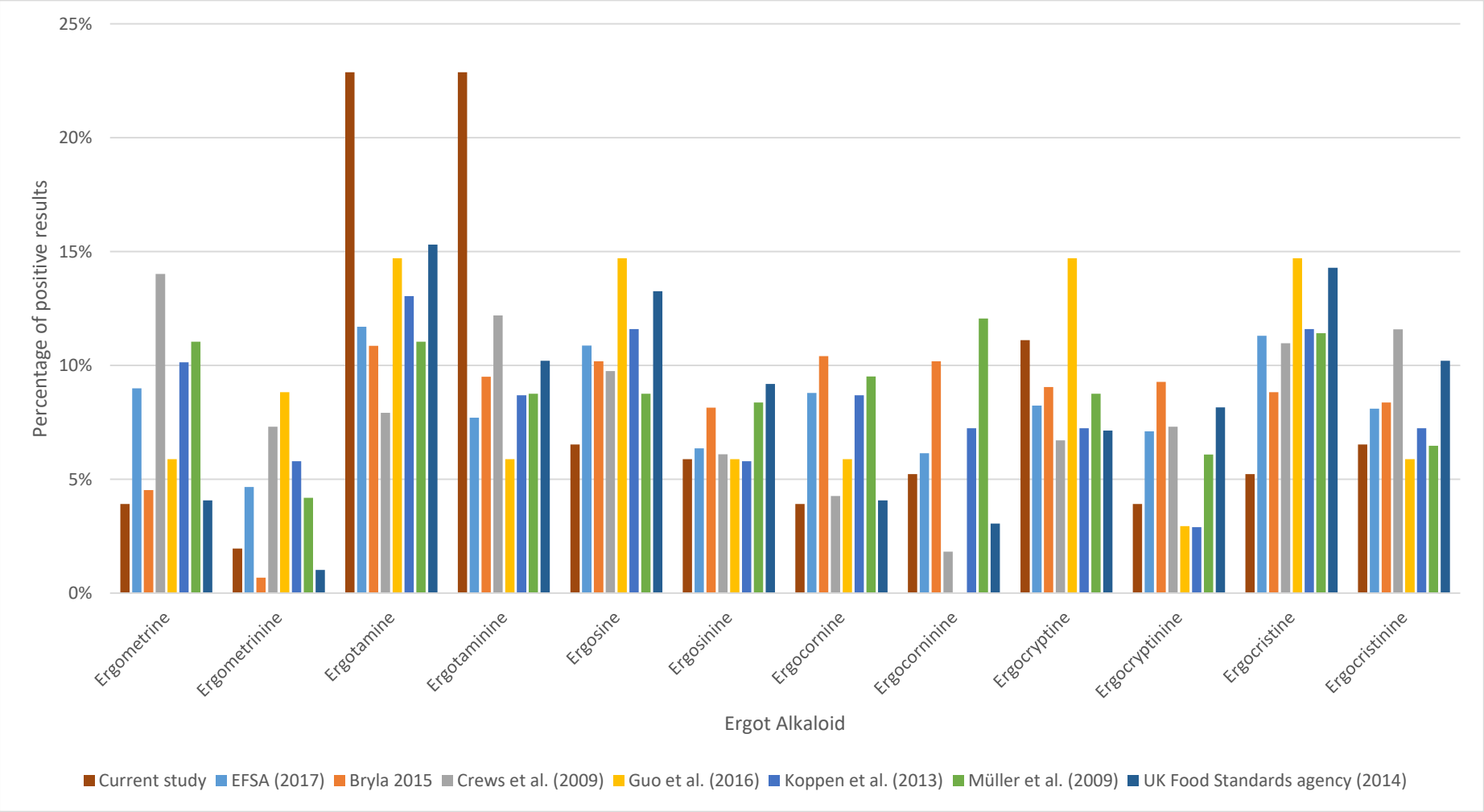
Ergosine was the most frequently occurring EA reported in a Belgian study (Diana Di Mavungu *et al.*, 2012).

Where EAs prevalence was reported as the sum of the epimers, the distribution of the EA epimers followed the order ergocryptine > ergocristine > ergosine > ergocornine > ergometrine > ergotamine (Mulder *et al.*, 2015); and ergocryptine > ergocornine > ergocristine > ergotamine (ergometrine not studied) (Storm *et al.*, 2008). Both of the studies reported ergotamine epimer as the lowest prevalence in their studies, the opposite to the current study.

Overseas results are shown in Figure 5. This shows that overseas studies show great variability in the distribution of individual EAs (Bryła *et al.*, 2015; Crews *et al.*, 2009; EFSA, 2017; Guo *et al.*, 2016; Koppen *et al.*, 2013; Müller *et al.*, 2009; UK Food Standards Agency, 2014).

There was little agreement between the prevalence of the individual EAs in the overseas studies and the current study. EA profiles may depend on climatic conditions, location, fungal genotype and host plant (EFSA, 2012).

Figure 5 Prevalence of the individual EAs compared with overseas studies



3.1.5 Summary of ergot alkaloids results by food type

Table 7 to Table 9 summarise the results for the 12 EAs in the cereal-based foods analysed in the current study. The mean concentrations and the range are of the positive samples only. Three samples contained quantifiable concentrations of all 12 EAs, they were samples of flour – rye (sample 6), flour – oat (sample 26) and rye-based bread (sample 44). The individual sample results are provided in Appendix A.4.

Table 7 Ergometrine, ergometrinine, ergotamine, and ergotaminine content in cereal-based foods in New Zealand

		Ergometrine		Ergometrinine		Ergotamine		Ergotaminine	
Food Type	Total samples analysed	No. positive samples	Mean (Range) µg/kg	No. positive samples	Mean (Range) µg/kg	No. positive samples	Mean (Range) µg/kg	No. positive samples	Mean (Range) µg/kg
Breakfast cereals - Oat	6	1	2.8	0	-	0	-	0	-
Breakfast cereals - Other	4	0	-	0	-	0	-	0	-
Breakfast cereals - Wheat	5	0	-	0	-	0	-	0	-
Crackers - Oat	3	0	-	0	-	0	-	0	-
Crackers - Rye	2	0	-	0	-	0	-	0	-
Flours - Oat	5	1	1.6	1	0.7	1	3.2	1	5.5
Flours - Rye	10	1	21.6	1	2.2	5	6.1 (0.6-23.2)	3	6.4 (0.9-16.3)
Flours - Spelt	5	1	3.8	0	-	2	8.7 (2.1-15.4)	2	3.3 (1.8-4.9)
Flours - Wheat	10	1	2.5	0	-	5	2.4 (1.1-6.6)	4	1.8 (0.8-3.8)
Multigrain bread	15	0	-	0	-	9	0.8 (0.5-1.3)	8	1.0 (0.5-2.0)
Rye-based bread	10	1	1.9	1	0.5	5	2.0 (0.6-4.8)	5	1.8 (0.5-4.9)
Wheat bread	15	0	-	0	-	8	1.3 (0.5-5.1)	12	1.1 (0.6-4.0)
Whole grains - Oats	5	0	-	0	-	0	-	0	-
Whole grains - Pearled Barley	5	0	-	0	-	0	-	0	-
All food types, mean (range)	100	6	5.7 (1.6-21.6)	3	1.2 (0.5-2.2)	35	2.6 (0.5-23.2)	35	2.0 (0.5-16.3)

Table 8 Ergosine, ergosinine, ergocornine, and ergocorninine content in cereal-based foods in New Zealand

		Ergosine		Ergosinine		Ergocornine		Ergocorninine	
Food Type	Total samples analysed	No. positive samples	Mean (Range) µg/kg	No. positive samples	Mean (Range) µg/kg	No. positive samples	Mean (Range) µg/kg	No. positive samples	Mean (Range) µg/kg
Breakfast cereals - Oat	6	0	-	0	-	0	-	0	-
Breakfast cereals - Other	4	0	-	0	-	0	-	0	-
Breakfast cereals - Wheat	5	0	-	0	-	0	-	0	-
Crackers - Oat	3	0	-	0	-	0	-	0	-
Crackers - Rye	2	0	-	0	-	0	-	0	-
Flours - Oat	5	1	1.4	1	1.9	1	1.7	1	3.2
Flours - Rye	10	1	10.4	1	6.0	1	7.8	1	11.7
Flours - Spelt	5	2	1.9 (0.6-3.1)	1	2.3	1	1.5	1	2.3
Flours - Wheat	10	1	0.9	1	0.7	0	-	0	-
Multigrain bread	15	3	1.0 (0.5-1.9)	2	1.2 (0.6-1.8)	1	0.8	2	1.3 (0.8-1.9)
Rye-based bread	10	2	2.1 (0.8-3.5)	3	1.9 (0.6-4.3)	2	3.3 (0.9-5.7)	2	4.8 (0.9-8.6)
Wheat bread	15	0	-	0	-	0	-	0	-
Whole grains - Oats	5	0	-	0	-	0	-	1	0.9
Whole grains - Pearled Barley	5	0	-	0	-	0	-	0	-
All food types	100	10	2.4 (0.5-10.4)	9	2.1 (0.6-6.0)	6	3.1 (0.8-7.8)	8	3.8 (0.8-11.7)

Table 9 Ergocryptine, ergocryptinine, ergocristine, and ergocristinine content in cereal-based foods in New Zealand

		Ergocryptine		Ergocryptinine		Ergocristine		Ergocristinine	
Food Type	Total samples analysed	No. positive samples	Mean (Range) µg/kg	No. positive samples	Mean (Range) µg/kg	No. positive samples	Mean (Range) µg/kg	No. positive samples	Mean (Range) µg/kg
Breakfast cereals - Oat	6	0	-	0	-	0	-	0	-
Breakfast cereals - Other	4	0	-	0	-	0	-	0	-
Breakfast cereals - Wheat	5	0	-	0	-	0	-	0	-
Crackers - Oat	3	1	0.5	0	-	0	-	0	-
Crackers - Rye	2	0	-	0	-	0	-	1	0.5
Flours - Oat	5	1	5.6	1	2.4	1	5.4	1	9.7
Flours - Rye	10	3	3.2 (0.6-16.7)	1	4.5	1	26.6	1	22.1
Flours - Spelt	5	3	4.1 (0.6-6.0)	2	1.2 (1.1-1.3)	2	2.1 (0.6-3.6)	1	2.6
Flours - Wheat	10	2	1.2 (0.6-1.7)	0	-	1	1.2	1	0.9
Multigrain bread	15	2	1.1 (0.6-1.7)	1	0.9	1	1.2	3	1.5 (0.7-2.7)
Rye-based bread	10	3	3.3 (0.8-7.5)	1	2.4	2	4.3 (1.1-7.5)	2	4.2 (0.8-7.5)
Wheat bread	15	1	1.4	0	-	0	-	0	-
Whole grains - Oats	5	1	0.8	0	-	0	-	0	-
Whole grains - Pearled Barley	5	0	-	0	-	0	-	0	-
All food types	100	17	3.2 (0.5-16.7)	6	2.1 (0.9-4.5)	8	5.9 (0.6-26.6)	10	4.9 (0.5-22.1)

From the results presented in Table 7 to Table 9, a single sample of rye flour reported the four highest individual EA concentration levels of 26.6, 23.2, 22.1 and 21.6, µg/kg, for ergocristine, ergotamine, ergocristinine and ergometrine, respectively. The total EA content of the sample was found to be 169.0 µg/kg.

Of the positive samples, the mean concentration of individual EAs ranged from 1.2 µg/kg (ergometrinine) to 5.9 µg/kg (ergocristine).

3.1.6 Summary of total EA concentrations

Total EA concentrations were calculated as the summation of the 12 individual EA results. Left-censored data (<LOQ) were assigned the value of zero. Table 10 summarises the total EA concentrations determined in the study.

Table 10 Summary total EA concentrations

Total EA concentration	Not quantified	Range 0.5-10 µg/kg	Range 11-50 µg/kg	Range 50-100 µg/kg	Range >100 µg/kg
No. of samples	54	38	6	1	1

3.1.7 Summary of mean total EA concentrations

Lower-bound

Mean total EA concentrations were calculated as the summation of the 12 individual EA results for each food type, divided by the number of samples for the food type. Left-censored data (<LOQ) were assigned the value of zero, creating lower-bound (LB) mean total EA concentrations.

Table 11 shows the LB mean total EA concentration of positive samples and all samples, by sample type.

In the current study, the LB mean total EA concentration of positive samples in the different flour samples followed the order oat > rye > spelt > wheat. Note that although oat flour had the highest mean EA concentration, only 1 out of 5 oat flour samples were positive.

For LB mean total EA concentration of all samples, the flour samples followed the order rye > spelt > oat > wheat. The order of this data set is comparable to the results reported by EFSA (2017).

The LB mean total concentration of EAs of positive samples and all samples in the different bread samples followed the order rye > multigrain > wheat.

The concentration of EAs in different grains (*n* = 666) as summarised by EFSA (2017) followed the order of rye > spelt > wheat > oats > barley (mean 176, 104, 76, 52, and 47 µg/kg, respectively). A similar concentration order was observed for grain milling products and other grain-based foods.

The current study suggests rye products available in New Zealand are potentially more likely to contain higher concentrations of EAs than foods based on other cereal types and this would be consistent with international data. Further analytical data from future crop growing seasons would provide a firmer conclusion.

Table 11 LB mean total EA concentration (positive samples and all samples)

Food Type	Mean total EA concentration positive samples (µg/kg)	Mean total EA concentration all samples (µg/kg)
Breakfast cereals - Oat	2.8	0.5
Breakfast cereals - Other	-	-
Breakfast cereals - Wheat	-	-
Crackers - Oat	0.5	0.2
Crackers - Rye	0.5	0.3
Flours - Oat	42.5	8.5
Flours - Rye	36.2	18.1
Flours - Spelt	19.8	11.9
Flours - Wheat	4.6	2.8
Multigrain bread	3.3	2.2
Rye-based bread	15.3	7.7
Wheat bread	2.1	1.7
Whole grains - Oats	1.9	0.3
Whole grains - Pearled Barley	-	-
All food types	11.8	4.9

Upper-bound

Mean total EA concentrations were calculated as the summation of the 12 individual EA results for each food type, divided by the number of samples for the food type. Left-censored data of <LOQ were assigned the value of LOQ (0.5 or 1.25), and <LOD were assigned the value of LOD (0.1 or 0.4), creating upper-bound (UB) mean total EA concentrations.

Table 12 shows the UB mean total EA concentration of all samples.

Table 12 UB mean total EA concentration (positive samples and all samples)

Food Type	Mean total EA concentration all samples (µg/kg)
Breakfast cereals - Oat	2.0
Breakfast cereals - Other	1.5
Breakfast cereals - Wheat	1.5
Crackers - Oat	1.9
Crackers - Rye	3.3
Flours - Oat	9.7
Flours - Rye	19.8
Flours - Spelt	14.2
Flours - Wheat	4.6
Multigrain bread	4.7
Rye-based bread	9.5
Wheat bread	3.7
Whole grains - Oats	1.9
Whole grains - Pearled Barley	1.5
All food types	6.4

3.1.8 EAs by sample country of origin

Half of samples labelled with New Zealand as country of origin of the product were positive for EAs (29 out of 58 samples), although the descriptions included ‘product of’, ‘grown in’, ‘made in’, ‘packed in’, and ‘from local and imported ingredients’. Therefore, in some instances it is unknown whether all or any of the ingredients were grown in New Zealand. Half of samples with an unknown country of origin were also positive for EAs (11 out of 22 samples). With the ambiguous country of origin labelling it is difficult to conclude further outcomes from the study. While it is difficult to draw general conclusions, these results suggest that cereal-based products manufactured in New Zealand are as likely to be

contaminated with EAs as cereal-based products manufactured elsewhere and available in New Zealand.

Table 13 EAs by sample country of origin

Country of origin	Number of samples (*n* = 100)	No. positive samples
Australia	7	0
Belgium	1	0
Canada	1	1
Germany	3	1
Italy	1	1
New Zealand	58	29
Turkey	1	0
UK	2	1
USA	4	2
Not provided	22	11

3.2 ERGOT ALKALOIDS IN FOOD TYPES AND COMPARISON TO INTERNATIONAL DATA

The following sections compare EAs in the designated food types with international data, where available. Each section opens with a statement of results from the current study. The international data relates to food for human consumption at retail level, rather than animal feeds, harvested grains-prior to processing, field-samples, or the fate of EAs during food production.

In addition to the overseas comparator studies discussed in the following sections, several other studies have analysed retail foods for EAs, but did not provide suitable data for comparison with the results of the current study (Diana Di Mavungu *et al.*, 2011; Liao *et al.*, 2013; Lopez *et al.*, 2016; Malysheva *et al.*, 2014; Mohamed *et al.*, 2006; Mulder *et al.*, 2015; Piñeiro *et al.*, 1996; Reinhard *et al.*, 2008).

3.2.1 Breakfast cereal – Oat

Six samples of breakfast cereals, where the most predominant component was oats, were analysed. One positive sample was found to contain just one EA (ergometrine) at a concentration of 2.8 µg/kg.

In a Canadian study, samples of oat-based cereals (but not specifically designated a breakfast food) were analysed for five EAs (ergosine, ergotamine, ergocornine, α-ergocryptine and ergocristine) (Lombaert *et al.*, 2003). Two samples out of six (33%) contained EAs at detectable levels, with both samples reporting a total EA concentration of 5 µg/kg.

3.2.2 Breakfast cereal – Other

Four samples of breakfast cereals – other were analysed. This category included samples of rye flakes and spelt flakes. No EAs were quantified in these samples.

In a British study, samples of rye flakes were analysed for 12 EAs (Crews *et al.*, 2009). In the two samples analysed, none of the 12 EAs were detected, with LOQs of 0.5-2.8 µg/kg.

In a German study, samples of rye flakes were analysed for 12 EAs (Müller *et al.*, 2009). EAs were detected in all three samples analysed, with mean individual EA concentrations ranging from not detected to 5.5 µg/kg, for ergometrinine and ergocornine, respectively. The maximum individual EA concentration ranged from not detected to 16.6 µg/kg (ergocornine). The mean and maximum total EA concentrations were 26.4 and 66.2 µg/kg, respectively.

3.2.3 Breakfast cereal – Wheat

Five samples of wheat-based breakfast cereals were analysed. No EAs were quantified in any sample in this sample category. No comparative data were found for wheat-based breakfast cereals.

3.2.4 Crackers – Oat

Three samples of crackers, where the main ingredient was oats, were analysed. EAs were quantified in one sample, which contained just one EA (ergocryptine) at a concentration of 0.5 µg/kg.

3.2.5 Crackers – Rye

Two samples of crackers, where the main ingredient was rye, were analysed. EAs were only quantified in one sample which was found to contain just one EA (ergocristinine) at a concentration of 0.5 µg/kg.

In a German study, samples of rye crispbread were analysed for six EAs (ergometrine, ergocornine, ergotamine, α-ergocryptine and ergocristine) and reported as total EA content (Bürk *et al.*, 2006). One sample out of 14 (7%) contained EAs at detected levels, with a total concentration of 28 µg/kg.

In a British study, samples of rye crispbread were analysed for 12 EAs (Crews *et al.*, 2009). Eleven samples were analysed and all contained EAs at detectable levels, with a mean total EA concentration of 76 µg/kg (range 2-340 µg/kg). All 12 EAs were detected in one sample (9%). The same study also considered rye crackers, as a separate food type. One out of two samples (50%) contained EAs at detectable levels, with a total EA concentration of 9 µg/kg.

3.2.6 Flours – Oat

Five samples of oat flour were analysed for EAs in the current study. EAs were only quantified in one sample, however, that sample gave positive results for all 12 EAs. The mean concentration of individual EAs in the positive sample was 3.5 µg/kg, with a range of 0.7 to 9.7 µg/kg, and a total EA content of 42.5 µg/kg. The total EA content was the third highest reported in the current study and the sample was a pre-packaged product purchased in a wholefoods store (country of origin Canada).

3.2.7 Flours – Rye

Ten samples of rye flour were analysed and 50% contained quantifiable levels of EAs. In the positive samples, the mean concentration of individual EAs was 11.0 µg/kg, with a mean range of 0.6 (ergotamine and ergocryptine) to 26.6 µg/kg (ergocristine). The LB and UB mean total EA concentration of all samples was 18.1 and 19.8 µg/kg, respectively. The most prevalent EA was ergotamine which was quantified in all five positive samples. One sample gave positive results for all 12 EAs. Its mean individual EA concentration was 14.1 µg/kg, with a range of 2.2 to 26.6 µg/kg, and total EA content of 169.0 µg/kg. The total EA content was the highest reported in the current study and the sample was obtained from a bulk food bin at a wholefoods store (country of origin not specified).

In a British study, one sample of rye flour was analysed for 12 EAs (Crews *et al.*, 2009) and reported a total EA concentration of 9 µg/kg.

In a Chinese study, samples of rye flour were analysed for 13 EAs (α-ergocryptine and β-ergocryptine were reported separately) (Guo *et al.*, 2016). Two samples out of nine (22%) contained EAs at detectable levels. One positive sample had a total EA concentration of 800 µg/kg, and nine individual EAs were detected with the range of contribution being 1.99 (ergosinine) to 593 µg/kg (ergocristine). The other positive sample had a total EA concentration of 30.75 µg/kg, and seven individual EAs were detected with the range of contribution being 2.00 (ergocornine) to 8.04 µg/kg (ergotamine).

In a German study, samples of rye flour were analysed for 12 EAs (Koppen *et al.*, 2013). Nine samples were analysed and 100% contained EAs at detectable levels. The mean total EA concentration was 64.8 µg/kg, with a range of 5.8 to 178 µg/kg. Two samples were positive for all 12 EAs, with total EAs content of 144 and 178 µg/kg. The sample with the highest total EA content also reported the highest individual EA of 39.0 µg/kg (α-ergocryptine).

In a second German study, samples of rye flour were analysed for 12 EAs (α-ergocryptine and α-ergocryptinine) (Müller *et al.*, 2006). A sample of naturally contaminated rye flour had a total EA content of approximately 1600 µg/kg.

In a further German study, samples of rye flour were analysed for 12 EAs (Müller *et al.*, 2009). Twenty-two samples were analysed and 100% contained EAs at detectable levels. The mean individual EA concentration ranged from 1.8 (ergotmetrinine) to 27.0 µg/kg (ergocristine). The maximum individual EA concentrations ranged from 15.8 to 133 µg/kg, for ergometrinine and ergocristine, respectively. The mean and maximum total EA concentrations were 138 and 715 µg/kg, respectively.

In a Danish study, samples of organic and conventional rye flour were analysed for ergocornine, α-ergocryptine, ergocristine, ergometrine, ergotamine, and their corresponding epimers (Storm *et al.*, 2008). Thirty-four samples were analysed and 94% contained EAs at detectable levels. The mean total EA content was 46 µg/kg, with a range of not detected to 234 µg/kg. The most common EAs were ergotamine and α-ergocryptine, and their corresponding epimers. Differences between organic and conventional rye flour samples were not significant.

In a Polish study, rye flour (*n*=34) was analysed for 12 EAs (Bryła *et al.*, 2015). The mean total EA content was 106 µg/kg, the range was 0.9 to 1216 µg/kg, and 32 out of 34 samples were positive (94%).

In a Swiss study, 15 samples of rye flour were analysed for 16 EAs (including α- and β-ergocryptine; α- and β-ergocryptinine; ergostine and ergostinine) (Reinhard *et al.*, 2008). The samples were collected across three different years and reported as total EA content, with a reported range of 18-519 µg/kg.

In a Scientific Opinion piece produced by EFSA (2012), rye milling products (*n*=511) were analysed for ‘at least six EAs’. The mean LB total EA concentration and mean UB total EA concentration was 124 and 155 µg/kg, respectively.

Only the British study of Crews *et al*. (2009) reported a lower mean total EA concentration for rye flour than found in the current study.

3.2.8 Flours – Spelt

Five samples of spelt flour were analysed and 60% contained quantifiable levels of EAs. In the positive samples the mean concentration of individual EAs was 3.1 µg/kg, with a mean range of 1.2 (ergocryptinine) to 8.7 µg/kg (ergotamine). The LB and UB mean total EA concentration of all samples was 11.9 and 14.2 µg/kg, respectively. The most prevalent EA was ergocryptine, quantified in all three positive samples. One sample gave a positive result for 11 out of 12 EAs. Its mean individual EA concentration was 2.5 µg/kg, with a range not detected to 5.8 µg/kg, and a total EA content of 30.3 µg/kg. The total EA content was the fourth highest reported in the current study and the sample was a common brand purchased in the supermarket (country of origin USA).

In a Swiss study, three samples of spelt flour were analysed for 16 EAs (including α- and β-ergocryptine; α- and β-ergocryptinine; ergostine and ergostinine) (Reinhard *et al.*, 2008). The samples were collected across three different years and reported as total EA content, with a reported range of 19-51 µg/kg.

3.2.9 Flours – Wheat

Ten samples of wheat flour were analysed and 60% contained quantifiable levels of EAs. From the positive samples the mean concentration of individual EAs was 1.4 µg/kg, with a mean range of 0.7 (ergocryptine) to 2.5 µg/kg (ergometrine). The mean total EAs concentration of the positive samples was 4.6 µg/kg. The most prevalent EA was ergotamine and it was quantified in five out of the six positive samples.

In a Chinese study, samples of whole wheat flour were analysed for 13 EAs (α-ergocryptine and β-ergocryptine were reported separately) (Guo *et al.*, 2016). Three samples out of 52 (6%) contained detectable levels of EAs. The highest individual EA concentration was ergocristine at 45.0 µg/kg, contributing 47% of the total EA concentration of 96 µg/kg. The other two positive samples had total EA concentrations of 9.5 and 81 µg/kg.

In a Swiss study, 40 samples of wheat flour were analysed for 16 EAs (including α- and β-ergocryptine; α- and β-ergocryptinine; ergostine and ergostinine) (Reinhard *et al.*, 2008). The samples were collected across three different years and reported as total EA content, with a reported range of 1-211 µg/kg.

In a Scientific Opinion piece produced by EFSA (2012), wheat milling products (*n*=191) were analysed for 'at least six EAs'. The mean LB total EA concentration and mean UB total EA concentration was 30 and 39 µg/kg, respectively.

3.2.10 Multigrain bread

Fifteen samples of multigrain bread were analysed and EAs were quantified in 67% of samples in this category. In the positive samples the mean individual EA concentration was 1.1 µg/kg, with a mean range of 0.8 (ergotamine) to 1.5 µg/kg (ergocristinine). The LB and UB mean total EA concentration of all samples was 2.2 and 4.7 µg/kg, respectively. The most prevalent EA was ergotamine and it was quantified in nine out of the ten positive samples.

In a Scientific Opinion piece produced by EFSA (2012), multigrain bread and rolls (*n*=18) were analysed for 'at least six EAs'. The mean LB total EA concentration and mean UB total EA concentration was 17 and 23 µg/kg, respectively.

3.2.11 Rye-based bread

Ten samples of rye-based bread were analysed and EAs were quantified in 50% of samples in this category. In the positive samples the mean individual EA concentration was 2.7 µg/kg, with a mean range of 0.5 (ergometrinine) to 4.8 µg/kg (ergocorninine). The LB and UB mean total EA concentration of all samples was 7.7 and 9.5 µg/kg, respectively. The most prevalent EAs were ergotamine and ergotaminine, and they were quantified in all five positive samples. One sample contained quantifiable levels of all 12 EAs. Its mean individual EA concentration was 4.9 µg/kg, with a range of 0.5 to 8.6 µg/kg, and a total EA content of 59.0 µg/kg. The total EA content was the second highest reported in the study and the sample was a common brand purchased in the supermarket (country of origin 'Made in New Zealand').

In a German study, samples of rye bread were analysed for six EAs (ergometrine, ergocornine, ergotamine, α-ergocryptine and ergocristine) reported as total EA content (Bürk *et al.*, 2006). EA content of >10 µg/kg was detected in 14 out of 23 samples (61%). One mixed-grain bread (grouped in the rye bread category) contained a total EA concentration of 258 µg/kg. Additionally, samples of pumpernickel bread (a bread typically made from coarsely ground wholemeal rye) were analysed as a separate category to the rye breads detailed above (Bürk *et al.*, 2006). Total EA content at the LOQ (0.1 to 1.0 µg/kg) was reported for 17 out of 20 samples (85%). The highest total EA concentration in pumpernickel was 47 µg/kg. Finally, samples of rye-bread rolls were analysed as a separate category to

the rye breads detailed above (Bürk *et al.*, 2006). EAs were detected in three out of nine samples (33%) with total EAs of 11, 31, and 91 µg/kg.

In a British study, samples of rye bread were analysed for 12 EAs (Crews *et al.*, 2009). Eight samples were analysed and 100% contained detectable EAs, with a mean total concentration of 40 µg/kg (range 1-171 µg/kg). Four out of eight samples (50%) contained detectable levels of all 12 EAs. Additionally, one sample of rye-based bread, designated as a separate category to the rye breads detailed above, was analysed for 12 EAs (Crews *et al.*, 2009). The sample had a total EA concentration of 19 µg/kg. Furthermore, samples of rye-based bread mix were analysed for 12 EAs (Crews *et al.*, 2009). Three samples were analysed and 100% contained EAs at detectable levels, with a mean total concentration of 7 µg/kg (range 3-13 µg/kg).

In a Swiss study, samples of bread (with rye) were analysed for 16 EAs (including α- and β-ergocryptine; α- and β-ergocryptinine; ergostine and ergostinine) (Reinhard *et al.*, 2008). The samples were collected across three different years and reported as total EA content, with a reported range of 17-477 µg/kg.

In a Scientific Opinion piece produced by EFSA (2012), rye bread and rolls (*n*=24) were analysed for 'at least six EAs'. The mean LB total EA concentration and mean UB total EA concentration was 30 and 45 µg/kg, respectively.

3.2.12 Wheat bread

Fifteen samples of wheat bread were analysed and EAs were quantified in 80% of samples in this category. In the positive samples the mean individual EA concentration was 1.3 µg/kg, with a mean range of 1.1 (ergotaminine) to 1.4 µg/kg (ergocryptine). The LB and UB mean total EA concentration of all samples was 1.7 and 3.7 µg/kg, respectively. The most prevalent EA was ergotaminine and it was quantified in all twelve positive samples. It is worth noting that, although EAs were quantified more frequently in wheat bread than rye bread, the mean total EAs concentration in wheat bread was only 14% of the mean concentration in rye bread in New Zealand.

In a Scientific Opinion piece produced by EFSA (2012), wheat bread and rolls (*n*=29) were analysed for 'at least six EAs'. The mean LB total EA concentration and mean UB total EA concentration was 3 and 15 µg/kg, respectively.

3.2.13 Whole grain – Oats

Five samples of whole oat grains were analysed for EAs in the current study. EAs were only quantified in one sample. The positive sample contained two EAs (ergocryptine and ergocorninine) at concentrations of 0.8 and 0.9 µg/kg, respectively.

3.2.14 Whole grain – Pearled Barley

Five samples of pearled barley grains were analysed for EAs in the current study. No EAs were quantified in any sample in this food category. This is consistent with the results of a data consolidation carried out by EFSA (2012).

4. CONCLUSIONS

The prevalence and concentrations of EAs in this study were variable across food types, with only occasional high results. Over half of the samples (54%) did not contain quantifiable levels of EAs, followed by over a third of samples (38%) with low concentrations of total EA (≤10 µg/kg). Moderate concentrations of total EA (11-50 µg/kg) were observed in 6% of samples and the remaining 2% of samples had high concentrations of total EA (>50 µg/kg). The highest concentrations were observed in a single sample of rye flour and a rye-based bread.

The concentrations of EAs in foods available in New Zealand were towards the lower end of the range of results reported internationally.

For New Zealand manufactured products, certain food types showed higher concentrations of EAs (rye containing foods) than other food types (oats).

In the foods studied, ergotamine and ergotaminine were by far the most prevalent EAs. Overseas studies showed great variability in the patterns of individual EAs. In general, there was little agreement between the prevalence of the individual EAs in the overseas studies and the current study.

Climatic conditions during the growing season are thought to influence the concentration of EAs. A follow on study, representing a season with different climatic conditions, would provide further monitoring data and could provide information of the impact of climatic variables on EA occurrence. The inclusion of an isotopically-labelled internal standard in the analytical method would be of great benefit, if available.

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APPENDIX A: RESULTS

A.1 Method Development

A standard extraction and detection method for EAs has yet to be evaluated and agreed by the international science community. During scoping of the project, a method by Kokkonen and Jestoi (2010) was identified as a suitable starting point for the extraction and detection of the 12 EAs. It utilised MycoSep© 150 Ergot columns (Romer Labs Inc.) for sample clean-up, liquid chromatography-tandem mass spectrometry (LC-MS/MS) for identification and detection, and a mobile phase of ammonium carbonate/acetonitrile.

After initial method development with analytical standards, contamination of the LC-MS/MS instrument was observed and an alternative mobile phase investigated. The alternative mobile phases were: ammonium hydroxide/acetonitrile; ammonium formate/acetonitrile; ammonium formate/methanol; and ammonium formate/formic acid/methanol. The criteria for accepting a change of mobile phase were no loss of sensitivity compared to the original method and good signal-to-noise ratio. It was determined that a mobile phase of ammonium formate/acetonitrile was a suitable alternative.

The retention and separation of ergosine, ergotamine, ergocornine, ergocryptine, ergocristine, and their epimers on the LC column was good, regardless of the mobile phase gradient programme. Ergometrine and ergometrinine epimers shared common mass-to-charge (m/z) transitions so it was important to fully resolve their peaks. Both compounds were highly sensitive to subtle mobile phase gradient changes with two outcomes unsuitable: co-eluting; fully resolving but with ergometrine eluting over an extended period of time. The final mobile phase gradient was chosen to achieve a balance of restricting the lengthy eluting time of ergometrine and fully resolving its peak before ergometrinine was eluted. A less than optimal peak shape of ergometrine had to be accepted in order to produce suitable, robust calibration curves.

A.2 Method Validation Results

A.2.1 Accuracy – spike recovery

The performance of the developed analytical method was assessed. Firstly, the SPE column was assessed by spiking a blank extraction solution with a known concentration (10 µg/kg) of each of the 12 EAs. An acceptable range of recoveries were observed for all EAs (range 76 to 108%).

Secondly, accuracy of the developed method was assessed by spiking a known concentration (10 µg/kg) of the each of the 12 EAs into food samples from different matrices. The samples were analysed to determine what proportions of the added EAs were recovered by the analytical method. The mean recovery results and range (if more than one spike per matrix) are shown in Table 14 and Table 15. Generally, across all of the sample matrices recoveries were variable, suggesting that losses were occurring or interconversion between –ine and –inine epimers. Interconversion could explain why some recoveries were lower and some higher than 100%, when comparing the two epimers of an EA.

The inclusion of an isotopically-labelled internal standard would have enabled correction for interconversion, losses and matrix effects, including signal/ionisation suppression or enhancement during LC-MS/MS analysis. A suitable internal standard was not available at the time of this study.

Table 14 Accuracy – spike recovery for ergometrine, ergometrinine, ergotamine, ergotaminine, ergosine and ergosinine. Mean recovery (Range) %.

	Ergometrine	Ergometrinine	Ergotamine	Ergotaminine	Ergosine	Ergosinine
Breakfast cereals - Oat	44 (35-54)	48 (45-52)	78 (59-97)	84 (46-122)	67 (38-97)	83 (50-116)
Breakfast cereals - Other	90	87	107	81	70	76
Breakfast cereals - Wheat	82	65	87	104	104	114
Crackers - Oat	81	66	92	131	115	137
Crackers - Rye	66	57	90	125	116	144
Flours - Oat	55	75	75	149	79	136
Flours - Rye	54 (32-69)	65 (48-86)	76 (63-90)	115 (107-134)	79 (47-95)	97 (68-112)
Flours - Spelt	81	81	99	159	118	135
Flours - Wheat	68 (60-76)	72 (61-83)	81 (70-92)	117 (116-119)	91 (74-107)	106 (99-113)
Multigrain bread	41 (34-46)	45 (40-49)	51 (44-60)	93 (72-128)	68 (48-95)	92 (71-110)
Rye-based bread	13 (5-21)	20 (5-35)	63 (40-86)	81 (55-108)	57 (38-76)	75 (51-98)
Wheat bread	44 (28-54)	47 (33-57)	58 (45-69)	119 (106-144)	92 (82-111)	106 (92-127)
Whole grains - Oats	57	75	79	114	79	114
Whole grains - Pearled Barley	56	68	110	195	103	172

Table 15 Accuracy – spike recovery for ergocornine, ergocorninine, ergocryptine, ergocryptinine, ergocristine and ergocristrinine. Mean recovery (Range) %.

	Ergocornine	Ergocorninine	Ergocryptine	Ergocryptinine	Ergocristine	Ergocristinine
Breakfast cereals - Oat	65 (45-85)	124 (82-167)	96 (68-123)	103 (77-129)	69 (40-98)	86 (71-101)
Breakfast cereals - Other	62	129	110	118	70	95
Breakfast cereals - Wheat	102	122	108	119	100	122
Crackers - Oat	102	152	103	113	105	134
Crackers - Rye	109	150	111	120	118	128
Flours - Oat	86	119	97	75	78	95
Flours - Rye	71 (49-93)	129 (104-177)	93 (89-100)	110 (87-153)	79 (66-92)	97 (68-119)
Flours - Spelt	84	136	101	108	84	123
Flours - Wheat	71 (61-82)	150 (148-152)	98 (86-110)	112 (105-118)	73 (60-85)	116 (113-119)
Multigrain bread	52 (49-57)	118 (108-137)	77 (69-88)	105 (97-111)	61 (56-68)	136 (92-208)
Rye-based bread	55 (44-65)	95 (62-128)	87 (68-106)	75 (56-94)	64 (50-79)	81 (56-106)
Wheat bread	58 (46-74)	113 (87-127)	89 (79-94)	102 (91-111)	78 (62-106)	156 (115-208)
Whole grains - Oats	82	111	95	46	87	90
Whole grains - Pearled Barley	99	195	148	148	122	193

A.3 Fapas sample

A.3.2 ESR determined value compared with Fapas report

Table 16 compares the Fapas sample concentration as determined by ESR with the results reported by Fapas, based on data submitted from other laboratories. The accuracy of the results (i.e. ESR results compared to the Fapas Assigned Value) was variable; accuracies ranged from 47 to 193%. Overall, the range of concentrations documented in the Fapas report was considerably wider than the range of results achieved in the current study. Even for the EAs with Fapas Assigned Values, the results in the Fapas report showed little agreement between the laboratories due to the large range in reported concentrations.

The variability could be due to a number of factors: differing sample masses used for the extractions, extraction solvents, sample clean-up materials, mobile phases, and detection methods. The report noted a wide variety of analytical methods. This is not surprising given the lack of published standard methodology for EA determination in foods.

From the accuracy values, it could be suggested that interconversion of the epimers took place; ergotamine/ergotaminine, ergosine/ergosinine, ergocornine/ergocorninine exhibited alternating low and high accuracies. All stages of the method used in this study were examined to reduce interconversion and it was decided the method was as robust as possible, in the absence of a suitable isotopically-labelled internal standard.

ESR individual EA concentrations in the FAPAS sample are shown in Table 17.

Table 16 ESR determined value vs Fapas Assigned Value

Ergot Alkaloid	Number of analyses	ESR determined value, mean µg/kg [1]	Fapas Assigned Value, µg/kg [2]	Accuracy (ESR vs Fapas) %	ESR determined value range, µg/kg	Fapas range, µg/kg [3]	ESR determined mean value within Fapas range?	ESR LOQ, µg/kg	Fapas LOQ range, µg/kg
Ergometrine	6	5.5	Not issued	-	3.5-9.1	2.1-17.3	Yes	1.25	0.13-20
Ergometrinine	6	Detected but below LOQ	Not issued	-	-	0.1-16.6	-	0.5	0.50-38.4
Ergotamine	6	5.1	10.90	47	3.1-8.1	6.3-16.7	No	0.5	0.25-20
Ergotaminine	6	4.2	3.10	135	2.0-7.9	2.01-9.6	Yes	0.5	0.25-20
Ergosine	6	5.3	11.30	47	2.6-8.1	8-16	No	0.5	0.25-20
Ergosinine	6	4.3	4.30	101	1.7-6.1	0.6-11.3	Yes	0.5	0.50-12
Ergocornine	6	7.3	15.30	48	3.4-11.2	11-94	No	0.5	0.50-20
Ergocorninine	6	10.7	8.47	126	8.5-13.0	1-13.6	Yes	0.5	0.25-20
Ergocryptine	6	4.2	Not issued	-	1.7-6.6	6-161.6	No	0.5	0.25-20
Ergocryptinine	6	4.9	Not issued	-	3.5-6.4	4.5-39.9	Yes	0.5	0.25-20
Ergocristine	6	17.7	9.17	193	10.0-24.4	4.75-21.9	Yes	0.5	0.25-20
Ergocristinine	6	5.1	3.90	131	3.2-8.8	0.5-6.2	Yes	0.5	0.25-20

[1] Determined value, mean µg/kg: results were not corrected for recovery
[2] Fapas Assigned Value, µg/kg: results were corrected for recovery
[3] Fapas range, µg/kg:

- Results for Fapas laboratory number 16 in the report were omitted from range in the table above as all results were 10 to 100 x greater than the majority of submitted results (this was considered to be a reporting error).
- The Fapas range does not include results reported as the sum of -ine and –inine.
- The Fapas minimum result does not include reporting of <LOQ, as the LOQ was typically higher than the consensus of the numeric results

Table 17 ESR individual EA concentrations in the FAPAS sample

Sample	Sample type	Ergometrine	Ergometrinine	Ergotamine	Ergotaminine	Ergosine	Ergosinine	Ergocornine	Ergocorninine	Ergocryptine	Ergocryptinine	Ergocristine	Ergocristinine
A	FAPAS	5.1	<0.5	3.4	7.9	2.6	1.7	3.4	12.7	6.2	6.4	10.0	6.5
B	FAPAS	4.2	<0.5	3.1	7.0	2.9	4.0	4.9	13.0	6.6	6.3	11.5	8.8
C	FAPAS	3.5	<0.5	5.9	2.5	6.6	4.8	8.7	8.6	3.3	3.7	17.3	3.5
D	FAPAS	5.8	<0.5	4.4	2.2	7.4	4.3	8.8	8.5	4.0	3.6	20.2	3.2
E	FAPAS	5.6	<0.5	8.1	3.4	8.1	6.1	11.2	9.3	3.3	3.5	24.4	4.1
F	FAPAS	9.1	<0.5	5.6	2.0	4.4	5.0	7.1	12.1	1.7	6.0	22.9	4.5

A.3.3 Precision of Fapas results - coefficient of variation (CV)

Table 18 below shows the precision of Fapas results. Coefficients of variation (CVs) are based on a method standard deviation derived from two or more analytical results. The six samples were analysed in three batches (A and B; C, D, and E; F). Calculation of a CV for fewer than two results is not possible (i.e. sample F).

Briefly, intra-batch results showed closer precision than inter-batch results. On the whole, neither the first batch (A and B) or the second batch of (C, D and E) exhibited greater precision than each other.

Table 18 Fapas sample precision

Ergot Alkaloid	CV %, samples A-B	CV %, samples C-E	CV %, samples A-F
Ergometrine	10	21	16
Ergometrinine	-	-	-
Ergotamine	5	24	36
Ergotaminine	7	18	57
Ergosine	5	9	43
Ergosinine	39	15	33
Ergocornine	18	12	39
Ergocorninine	1	4	19
Ergocryptine	4	10	34
Ergocryptinine	<1	2	27
Ergocristine	7	14	30
Ergocristinine	15	11	42

A.4 Results

ND = Not Detected

TR = Trace result: >LOD and <LOQ

Table 19 Sample results, individual EA and total EA

Sample	Sample type	Ergometrine	Ergometrinine	Ergotamine	Ergotamininine	Ergosine	Ergosinine	Ergocornine	Ergocorninine	Ergocryptine	Ergocryptinine	Ergocristine	Ergocristinine	TOTAL
1	Flours - Rye	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	Flours - Rye	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	Flours - Rye	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	Flours - Rye	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	Flours - Rye	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	Flours - Rye	21.61	2.23	23.19	16.26	10.36	5.96	7.80	11.74	16.70	4.50	26.63	22.05	169.0
7	Flours - Rye	ND	ND	1.97	0.88	TR	ND	ND	ND	0.61	TR	ND	ND	3.5
8	Flours - Rye	ND	ND	0.65	TR	ND	ND	ND	ND	ND	ND	ND	ND	0.6
9	Flours - Rye	ND	ND	3.96	2.10	TR	TR	TR	TR	1.02	TR	TR	TR	7.1
10	Flours - Rye	ND	ND	0.68	TR	ND	ND	ND	ND	ND	ND	ND	ND	0.7
11	Flours - Spelt	TR	ND	15.38	4.88	0.58	TR	ND	ND	6.01	1.13	TR	TR	28.0
12	Flours - Spelt	TR	ND	TR	ND	ND	ND	TR	ND	0.57	ND	0.60	TR	1.2
13	Flours - Spelt	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
14	Flours - Spelt	3.83	TR	2.09	1.80	3.14	2.26	1.55	2.27	5.82	1.32	3.59	2.60	30.3
15	Flours - Spelt	TR	ND	ND	ND	ND	ND	ND	ND	ND	ND	TR	TR	ND
16	Flours - Wheat	TR	ND	6.57	3.80	TR	TR	ND	ND	1.71	TR	ND	ND	12.1
17	Flours - Wheat	ND	ND	1.21	1.16	ND	ND	ND	ND	ND	ND	ND	ND	2.4

Sample	Sample type	Ergometrine	Ergometrinine	Ergotamine	Ergotamininine	Ergosine	Ergosinine	Ergocornine	Ergocorninine	Ergocryptine	Ergocryptinine	Ergocristine	Ergocristinine	TOTAL
18	Flours - Wheat	ND	ND	1.49	0.77	ND	ND	ND	ND	ND	ND	ND	ND	2.3
19	Flours - Wheat	ND	ND	1.08	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.1
20	Flours - Wheat	ND	ND	1.68	1.37	ND	ND	ND	ND	0.63	TR	ND	ND	3.7
21	Flours - Wheat	ND	ND	TR	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
22	Flours - Wheat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
23	Flours - Wheat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
24	Flours - Wheat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
25	Flours - Wheat	2.51	TR	TR	TR	0.91	0.67	TR	TR	TR	ND	1.20	0.93	6.2
26	Flours - Oat	1.60	0.71	3.22	5.52	1.41	1.93	1.69	3.24	5.63	2.42	5.40	9.72	42.5
27	Flours - Oat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
28	Flours - Oat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
29	Flours - Oat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
30	Flours - Oat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
31	Whole grains - Oats	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
32	Whole grains - Oats	ND	ND	ND	ND	ND	ND	TR	0.95	0.75	ND	ND	ND	1.7
33	Whole grains - Oats	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
34	Whole grains - Oats	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
35	Whole grains - Oats	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
36	Whole grains - Pearled Barley	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Sample	Sample type	Ergometrine	Ergometrinine	Ergotamine	Ergotamininine	Ergosine	Ergosinine	Ergocornine	Ergocorninine	Ergocryptine	Ergocryptinine	Ergocristine	Ergocristinine	TOTAL
37	Whole grains - Pearled Barley	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
38	Whole grains - Pearled Barley	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
39	Whole grains - Pearled Barley	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
40	Whole grains - Pearled Barley	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
41	Rye-based bread	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
42	Rye-based bread	ND	ND	0.58	0.87	TR	0.58	ND	TR	ND	TR	ND	TR	2.0
43	Rye-based bread	ND	ND	2.49	2.01	TR	TR	TR	TR	0.79	TR	TR	TR	5.3
44	Rye-based bread	1.86	0.52	4.79	4.87	3.49	4.30	5.67	8.57	7.51	2.37	7.53	7.53	59.0
45	Rye-based bread	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
46	Rye-based bread	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
47	Rye-based bread	ND	ND	0.79	0.53	0.77	0.80	0.87	0.93	1.72	TR	1.14	0.78	8.3
48	Rye-based bread	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
49	Rye-based bread	ND	ND	1.16	0.93	ND	ND	ND	ND	TR	ND	ND	ND	2.1
50	Rye-based bread	ND	ND	TR	ND	ND	ND	ND	ND	TR	ND	ND	ND	ND
51	Wheat bread	ND	ND	5.11	4.04	TR	TR	ND	ND	1.35	TR	ND	ND	10.5
52	Wheat bread	ND	ND	0.98	1.58	ND	TR	ND	ND	TR	TR	ND	TR	2.6

Sample	Sample type	Ergometrine	Ergometrinine	Ergotamine	Ergotamininine	Ergosine	Ergosinine	Ergocornine	Ergocorninine	Ergocryptine	Ergocryptinine	Ergocristine	Ergocristinine	TOTAL
53	Wheat bread	ND	ND	0.74	0.80	ND	ND	ND	ND	TR	ND	ND	ND	1.5
54	Wheat bread	ND	ND	0.64	0.72	ND	ND	ND	ND	TR	ND	ND	ND	1.4
55	Wheat bread	ND	ND	TR	0.65	ND	ND	ND	ND	ND	ND	ND	ND	0.6
56	Wheat bread	ND	ND	TR	0.61	ND	TR	ND	ND	ND	ND	ND	ND	0.6
57	Wheat bread	ND	ND	TR	0.56	ND	ND	ND	ND	ND	ND	ND	ND	0.6
58	Wheat bread	ND	ND	1.16	1.19	ND	ND	ND	ND	ND	ND	ND	ND	2.3
59	Wheat bread	ND	ND	0.73	1.21	ND	ND	ND	ND	TR	TR	ND	ND	1.9
60	Wheat bread	ND	ND	TR	TR	ND	ND	ND	ND	ND	ND	ND	ND	ND
61	Wheat bread	ND	ND	0.51	0.58	ND	ND	ND	ND	ND	ND	ND	ND	1.1
62	Wheat bread	ND	ND	TR	TR	ND	ND	ND	ND	ND	ND	ND	ND	ND
63	Wheat bread	ND	ND	TR	TR	ND	ND	ND	ND	ND	ND	ND	ND	ND
64	Wheat bread	ND	ND	TR	0.87	ND	ND	ND	ND	ND	ND	ND	ND	1.4
65	Wheat bread	ND	ND	0.83	0.89	ND	ND	ND	ND	ND	ND	ND	ND	1.7
66	Multigrain bread	TR	ND	1.28	2.01	1.94	1.83	0.82	1.90	1.69	0.86	1.19	2.72	16.2
67	Multigrain bread	TR	ND	0.76	0.96	0.59	0.60	TR	0.78	0.60	TR	TR	1.05	5.3
68	Multigrain bread	ND	ND	ND	TR	ND	TR	ND	ND	ND	ND	TR	ND	ND
69	Multigrain bread	ND	ND	0.86	1.14	TR	TR	ND	ND	TR	TR	ND	TR	2.0
70	Multigrain bread	ND	ND	TR	0.83	TR	TR	ND	ND	ND	ND	ND	ND	0.8
71	Multigrain bread	ND	ND	0.76	0.81	TR	TR	ND	ND	TR	ND	ND	TR	1.6
72	Multigrain bread	ND	ND	TR	TR	TR	TR	ND	TR	ND	ND	ND	TR	ND
73	Multigrain bread	ND	ND	TR	TR	ND	ND	ND	ND	ND	ND	ND	ND	ND
74	Multigrain bread	ND	ND	1.03	0.82	TR	TR	ND	ND	TR	TR	ND	TR	1.9

Sample	Sample type	Ergometrine	Ergometrinine	Ergotamine	Ergotamininine	Ergosine	Ergosinine	Ergocornine	Ergocorninine	Ergocryptine	Ergocryptinine	Ergocristine	Ergocristinine	TOTAL
75	Multigrain bread	ND	ND	0.53	TR	ND	ND	ND	ND	ND	ND	ND	ND	0.5
76	Multigrain bread	ND	ND	TR	TR	ND	ND	ND	ND	ND	ND	ND	ND	ND
77	Multigrain bread	TR	ND	0.64	0.54	0.52	TR	ND	TR	TR	TR	TR	0.66	2.4
78	Multigrain bread	ND	ND	0.53	TR	ND	ND	ND	ND	TR	ND	ND	ND	0.5
79	Multigrain bread	ND	ND	0.81	0.55	ND	ND	ND	ND	TR	ND	ND	ND	1.4
80	Multigrain bread	ND	ND	TR	TR	ND	ND	ND	ND	ND	ND	ND	ND	ND
81	Breakfast cereals - Other	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
82	Breakfast cereals - Other	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
84	Crackers - Rye	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
85	Crackers - Rye	ND	ND	TR	TR	TR	TR	ND	TR	TR	TR	TR	0.52	0.5
86	Breakfast cereals - Wheat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
87	Breakfast cereals - Wheat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
88	Breakfast cereals - Wheat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
89	Breakfast cereals - Wheat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
90	Breakfast cereals - Wheat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
91	Breakfast cereals - Oat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Sample	Sample type	Ergometrine	Ergometrinine	Ergotamine	Ergotaminine	Ergosine	Ergosinine	Ergocornine	Ergocorninine	Ergocryptine	Ergocryptinine	Ergocristine	Ergocristinine	TOTAL
92	Breakfast cereals - Oat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
93	Breakfast cereals - Oat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
94	Breakfast cereals - Oat	2.77	TR	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.8
95	Breakfast cereals - Oat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
96	Breakfast cereals - Oat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
106	Breakfast cereals - Other	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
107	Breakfast cereals - Other	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
112	Crackers - Oat	ND	ND	ND	TR	ND	ND	ND	ND	ND	ND	ND	ND	ND
113	Crackers - Oat	ND	ND	ND	TR	ND	ND	ND	ND	0.51	ND	ND	ND	1.0
114	Crackers - Oat	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

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Science for Communities

Mycotoxin Surveillance Programme 2019-2020: Ergot alkaloids in rye and exposure assessment

20 March 2020

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PREPARED FOR: **Ministry for Primary Industries, project code CFS/18/05, Ergot Alkaloids in Rye, as part of the overall contract for scientific services**

CLIENT REPORT No: **FW20008**

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GLOSSARY OF COMMON ACRONYMS

ANS	Adult Nutrition Survey
CNS	National Children's Nutrition Survey
EA	Ergot Alkaloid
EFSA	European Food Safety Authority
ESR	Institute of Environmental Science and Research Limited
FAO	Food and Agriculture Organization of the United Nations
FSANZ	Food Standards Australia New Zealand
LB	Lower bound
MPI	Ministry for Primary Industries
NNS	National Nutrition Survey
TDI	Tolerable Daily Intake
UB	Upper bound
WHO	World Health Organization
µg/kg bw/day	Micrograms per kilogram body weight per day

EXECUTIVE SUMMARY

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

Ergot alkaloids (EAs) are tryptophan-derived alkaloids (mycotoxins) produced by fungal *Claviceps* species. More than 50 different EAs have been identified, with the distribution of EAs varying between fungal strains, geographic regions and host plants.

This study analysed rye-based foods available on the New Zealand market (*n* = 40) for 12 EAs; ergometrine, ergometrinine, ergotamine, ergotaminine, ergosine, ergosinine, ergocornine, ergocorninine, ergocryptine, ergocryptinine, ergocristine and ergocristinine.

Foods purchased for the study were rye containing products – the most commonly contaminated food type based on results of the mycotoxin surveillance programme 2017-2018 - Ergot alkaloids in cereal-based foods.

Over half of the samples (60%) contained quantifiable levels of EAs (limits of quantification were in the range 0.5-1.25 μg/kg for the individual EAs). Just over one-third of samples (35%) contained quantifiable concentrations of total EAs ≤10 μg/kg. Concentrations of total EA in the range 11-50 μg/kg were observed in 20% of samples and the remaining 5% of samples had concentrations of total EAs >50 μg/kg. The highest concentration was observed in a sample of rye flour.

When taken with the results for rye-based products found in the 2018 EA in New Zealand cereal based foods study, these results suggest that the EA content of rye-based foods may be relatively consistent over different growing seasons.

Mean upper bound (UB) estimates of dietary EA exposure were 0.0094 μg/kg bw/day for adults and 0.025 μg/kg bw/day for children. The main contributors to adult dietary EA exposure were wheat bread, wheat flour, and multigrain bread.

Food contributors to dietary exposure in children were similar, except there was very little contribution from multigrain bread.

The New Zealand estimates are much lower than those reported in Europe (0.09 μg/kg bw/day for adults, 0.20 μg/kg bw/day for children). The European Food Safety Authority have calculated a TDI for total EAs of 0.6 μg/kg bw/day.

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), work in this area is directed on the basis of risk. The risk profile of mycotoxins in the New Zealand food supply and its update (Cressey 2014b, Cressey and Thomson 2006) are viewed as starting points for this process. The risk profiles identified a number of issues to be investigated or clarified.

Efforts 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); 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); trichothecene mycotoxins in cereal products (Cressey et al. 2014), culminating in an exposure assessment for ochratoxin A and trichothecene mycotoxins (Cressey 2014a), completing the initially identified mycotoxin priorities for New Zealand. As a product of revised prioritisation, fumonisins in maize-based products and wine were analysed (Cressey et al. 2017) and a dietary exposure assessment performed (Cressey 2018).

Due to the shortage of New Zealand specific prevalence data, ergot alkaloids (EAs) were identified as the topic for the Mycotoxin Surveillance Programme in 2017-2018 (Ashmore and Molyneux 2018). The results of this initial surveillance of many cereal types identified rye and rye products as the food types most frequently contaminated with EAs in the New Zealand food supply. Therefore, a further study of the EA content of rye containing foods was initiated under the Mycotoxin Surveillance Programme in 2019.

1.1 ERGOT ALKALOIDS

Ergot refers to fungal infestation of cereal plants by fungal *Claviceps* species. During the growth of the plant, ergot replaces grain kernels with large dark-coloured sclerotia (Figure 1). The ergots ripen with the grain and either fall off pre-harvest or are included with harvested grain (Neill 1941). Once harvested, contamination of cereal-based foods and feeds can occur (Krska and Crews 2008). Cereals including wheat, rice, rye, corn, sorghum, barley, oats, and millet are all known to be hosts of ergot-producing *Claviceps* species (Lorenz 1979). Ergot alkaloids (EAs) are tryptophan derived alkaloids (mycotoxins) produced by the *Claviceps* species.

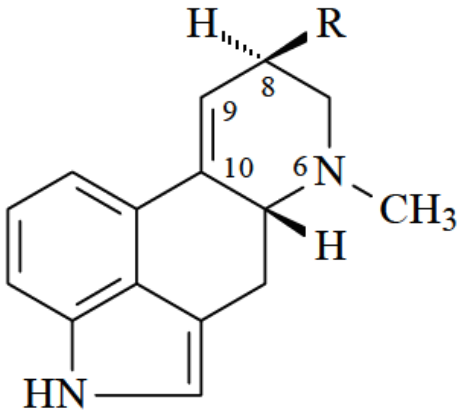
Throughout the Middle Ages, contamination of grains (raw or processed) with EAs caused epidemics of ergotism, exhibiting two major forms: gangrenous and convulsive. Gangrene (chronic ergotism) of the hands, feet and limbs occurred due to vasoconstriction properties of EAs, and was accompanied by an intense burning sensation (De Costa 2002). Ergotism causing gangrene was previously known as St Anthony's Fire (Flieger et al. 1997). Convulsive ergotism caused hallucinations (De Costa 2002), delirium, and psychosis (Nemes and Goerig 2002). Through improvements in agricultural practice, food processing techniques and increased scientific knowledge, ergotism epidemics of the serious nature observed in the Middle Ages are now infrequent. Two outbreaks of ergot poisoning occurred in Ethiopia, in 1977-78 and 2001. The 1977-78 and 2001 outbreaks were attributed to contaminated wild oats which were subsequently mixed with barley for consumption (ergot was not observed on the barley) (Urga et al. 2002).

EAs have been shown to have a broad spectrum of pharmacological effects and are used for medicinal purposes. Lysergic acid diethylamide (LSD), a semi-synthetic EA derivative, was discovered in the early 1900s and legally used as a pharmaceutical until its potent psychoactive effects were fully recognised. Today LSD is an illegal drug of abuse (EFSA 2012).

Figure 1 Ergot sclerotia on rye grass, found in Mid-Canterbury April 2018

More than 50 different EAs have been identified, with the distribution of EAs varying between fungal strains, geographic regions and host plants (Flieger et al. 1997). *C. purpurea* is a common EA-producing species and is the *Claviceps* species most commonly associated with infections of commercially-grown cereals in New Zealand. EAs share a common structural feature, an ergoline ring, with a methylated nitrogen (N-6) and various substitutions at C-8 (Figure 2) (Diana Di Mavungu et al. 2011, Flieger et al. 1997, Krska et al. 2008).

Figure 2 The structure of the ergoline ring



Reproduced from Diana Di Mavungu et al. (2011)

Previous studies, as summarised by the European Food Safety Authority (EFSA), have monitored between one and 12 different EAs, with the majority of food surveys reported to EFSA including 12 EAs (EFSA 2012). EFSA recommended that studies should monitor six

key EAs and their corresponding epimers[1] (12 EAs in total) in order to improve the knowledge of EAs in food (EFSA 2012). The six major EAs usually analysed are ergometrine, ergotamine, ergosine, ergocristine, ergocryptine (α and β), and ergocornine. Each corresponding epimer features the suffix –inine. The structures of the major EAs and their epimers are shown in Figure 3.

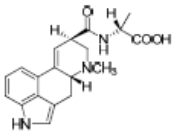
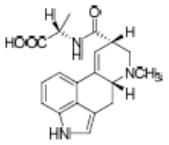
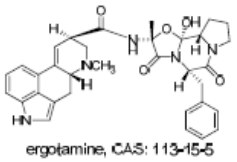
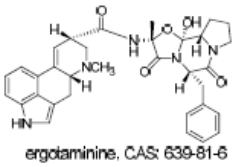
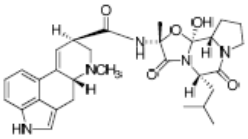
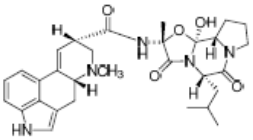
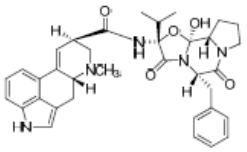
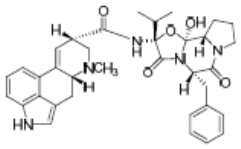
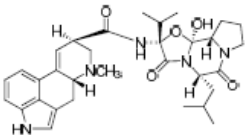
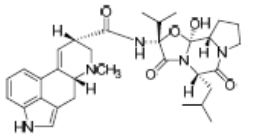
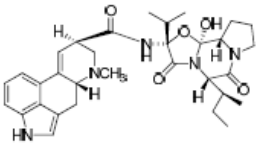
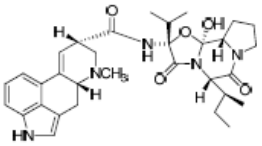
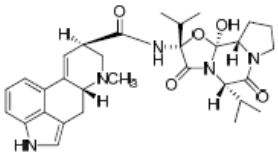
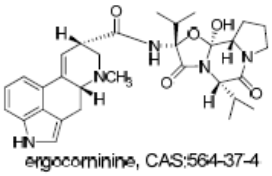
EAs have recently been assessed by EFSA (EFSA 2017) and have been identified as a priority by the Codex Committee on Contaminants in Food (CCCF).[2] A recent risk profiling exercise for mycotoxins in the New Zealand food supply identified EAs as priority mycotoxins for further work in the Mycotoxin Surveillance Programme, to address the lack of occurrence data in the New Zealand food supply (Cressey 2014b).

Previous overseas studies have only detected EAs in grain and grain-based products, although only limited analyses have been carried out on non-cereal foods. In particular, rye grain (*Secale cereale*), rye flour and other rye-based products consistently show the highest mean concentrations of EAs of the major cereals (EFSA 2017). The concentration of EAs in different grains (*n* = 666) as summarised by EFSA (2017) followed the order of rye > spelt > wheat > oats > barley (mean 176, 104, 76, 52, and 47 µg/kg, respectively). A similar concentration order was observed for grain milling products and other grain-based foods. A 2018 New Zealand study looking at the total EA content of New Zealand cereal-based foods found that the concentration of total EAs in different flour products (n=30) followed the order rye > spelt > oat > wheat (Ashmore and Molyneux 2018). EA profiles in foods and the total amounts are highly variable and may depend on geographic location, climatic conditions during the growing season, fungal genotype and host plant (EFSA 2012).

[1] The term epimers refers to molecules that have more than one chiral centre, but only differ by their configuration (conformation) at one of those chiral centres

[2] http://www.fao.org/fao-who-codexalimentarius/sh-proxy/es/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-735-12%252FCRDs%252Fcf12_CRD02x.pdf Accessed 3 December 2018

Figure 3 Structure of the major ergot alkaloids and their epimeric forms

Lysergic acid derivatives	Isolysergic acid derivatives
 ergometrine (ergonovine, ergobasine) CAS: 60-79-7	 ergometrinine (ergonovinine), CAS: 479-00-5
 ergotamine, CAS: 113-15-5	 ergotaminine, CAS: 639-81-6
 ergosine, CAS: 561-94-4	 ergosinine, CAS: 596-88-3
 ergocristine, CAS: 511-08-0	 ergocristinine, CAS: 511-07-9
 α-ergocryptine (ergocryptine) CAS: 511-09-1	 α-ergocryptinine (ergocryptinine) CAS: 511-10-4
 β-ergocryptine, CAS: 20315-46-2	 β-ergocryptinine, CAS: 19467-61-9
 ergocornine, CAS: 564-36-3	 ergocorninine, CAS:564-37-4

Reproduced from EFSA (2012)

1.2 REGULATORY LIMITS IN NEW ZEALAND

Ergot (in its fungal infestation form) is currently regulated in cereals under the Australia New Zealand Food Standards Code. Schedule 19 – Maximum levels of contaminants and natural toxicants sets out a maximum level (ML) for ergot in cereal grains at 500 mg/kg (section S19-5 Maximum levels of non-metal contaminants).[3] No ML is set for foods for consumption. The Standard applies to the concentration of ergot sclerotia in grain, rather than the concentration of the EAs present.

1.3 PROJECT AIMS

The current project analysed the six major EAs and their -inine epimers. In addition to the epimeric forms, ergocryptine also occurs as two structure isomers (α- and β-); differing by the position of one methyl group. Only the α- isomers, α-ergocryptine and α-ergocryptinine, were analysed due to the unavailability of analytical standards for the β-isomers. For the remainder of the report, these analytes are referred to as simply ergocryptine and ergocryptinine.

The aim of this project was to provide analytical information on the EA concentrations in rye-based foods commercially available in New Zealand in 2019. Foods purchased for the study focussed on the major potential dietary sources of rye for the New Zealand diet, with rye being the cereal containing the highest concentrations of EAs in the New Zealand diet in a previous study sampled in 2018 (Ashmore and Molyneux 2018).

The analytical information will suggest whether the concentration of EAs in rye is consistent between growing seasons.

The results of this study were combined with results from the previous study, and provided the necessary data to undertake a robust dietary exposure and risk assessment.

[3] https://www.legislation.gov.au/Details/F2017C00333 Accessed 5 November 2018.

2. SAMPLING AND METHODS

2.1 SAMPLING

Rye-based (where rye was the main ingredient, or listed as an ingredient in labelling) food samples (*n* = 40) were purchased in Christchurch, New Zealand during September to November 2019. A minimum of 500 g of each food product was purchased from supermarkets and wholefood stores. For certain products sold in packets weighing less than 500 g each, multiple packets with the same batch number and use by/best before date were purchased to make up one analytical sample.

2.2 BRAND INFORMATION

Brand name, manufacturer or supplier, batch number details, use by or best before date, and country of origin of each sample purchased were recorded from the packaging, where available.

2.3 SAMPLE CHARACTERISTICS

Table 1 provides a summary of the countries of origin for the rye-based samples (*n* = 40) purchased. The country of origin of a product is not necessarily the country of origin of the raw cereal ingredients. The product packaging provided descriptions of the origin of ingredients including 'product of', 'grown in', 'made in', 'packed in', and 'from local and imported ingredients'.

Table 1 Country of origin for all samples

Country of origin	Number of samples (*n* = 40)
Australia	4
Canada	1
Germany	5
New Zealand	18
Sweden	1
Turkey	1
UK	3
Not provided	7

The samples were grouped into four categories as shown in Table 2. Rye-based breads included samples where either the main ingredient was rye (*Secale cereale*), or rye was listed as an ingredient in the labelling. Rye was present either as whole grain, whole meal, kibbled grain or flour. Other ingredients in some samples included wheat flour and/or spelt, oat and barley (no percentages listed).

Table 2 Food types

Food type	Number of samples (*n* = 40)
Flours - Rye	15
Rye-based breads	12
Rye-based crackers/crispbread	10
Kvas[1]	3

[1] Kvas (Kvass) is a traditional fermented Slavic and Baltic beverage commonly made from rye bread.

2.4 SAMPLE PREPARATION

The samples were individually homogenised (where required) to a fine crumb using a kitchen blender. The samples were immediately frozen and stored at -18°C after homogenising. All samples were analysed as purchased without further preparation to a ready-to-consume state.

2.5 ANALYTICAL STANDARDS

Analytical standards were purchased from PM Separations and supplied by Chiron, Trondheim, Norway. Ergometrine, ergotamine, ergosine, ergocristine, ergocornine were supplied as 100 µg/mL in dried down 5 mL (i.e. each EA was supplied as 500 µg of dried EA in a vial intended to be reconstituted to 5mL EA standard). Ergometrinine, ergotaminine, ergosinine, α-ergocryptinine, ergocristinine, and ergocorninine were supplied as 25 µg/mL in dried down 5 mL (i.e. each EA was supplied as 125 µg of dried EA in a vial intended to be reconstituted to 5mL EA standard). α-Ergocryptine was supplied as 100 µg/mL in methanol (1 mL).

2.6 ANALYTICAL METHODOLOGY

The method used was identical to that used for the investigation of EAs in New Zealand cereal-based foods as part of the Mycotoxin Surveillance Programme 2017-2018 (Ashmore and Molyneux 2018) and was based on the method of Kokkonen and Jestoi (2010).

Each of the samples was extracted by solid phase extraction (SPE) and analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). A fresh set of calibration standards in the range of 0.4-50 µg/kg was prepared and analysed with each batch of extracted samples.

Briefly, 20 g of sample was extracted with 100 mL acetonitrile/ammonium carbonate 200 mg/L in H_2O (84:16) and shaken for 1 hour. The extract was filtered through No. 4 Whatman filter paper. An aliquot of the filtrate (4 mL) was passed through a MycoSep© cartridge and 2 mL of the extract evaporated to dryness under nitrogen. The extract was reconstituted with 500 µL acetonitrile and centrifuged at 13,500 rpm for 2 minutes. The supernatant was transferred to a vial for analysis.

Using an Agilent 1200 series liquid chromatograph, mobile phase of (A) 5 mM ammonium formate in water and (B) 5 mM ammonium formate in acetonitrile, 5 µL of sample extract was loaded onto an Agilent Poroshell HPH-C18 column 1.9 µm, 2.1x100, with a flow rate of 0.18 mL/min. The mobile phase gradient programme is shown in Table 3.

Table 3 Mobile phase gradient programme for analysis of ergot alkaloids

Time (min)	Mobile phase A (%)[1]	Mobile phase B (%)[2]
0	95	5
10	70	30
11	60	40
25	35	65
25.5	10	90
28	10	90
28.5	95	5
35	95	5

[1] Mobile phase A: 5 mM ammonium formate in water
[2] Mobile phase B: 5 mM ammonium formate in acetonitrile

The analytes were detected by a multimode ionisation (MMI) source in electrospray ionisation (ESI) and positive ion mode on an Agilent 6410 mass spectrometer. The analysis was conducted with time segments, to increase the sensitivity for each transition. The mass spectrometer source parameters were a capillary voltage of 2000 V, corona current of 0 µA and charging voltage of 2000 V. The gas flow rate was 5 L/minute, with a gas temperature of 300°C, nebuliser pressure of 60 psi and a vapouriser temperature of 200°C. Specific mass spectrometric parameters for the EAs are summarised in Table 4, where each compound has two transitions: the first listed is the Quantifier transition, used to determine the concentration of the target species, and the second listed is the Qualifier transition, for increased confidence of the identification of the target species.

Table 4 Mass spectrometer parameters for ergot alkaloids

Compound	Quantifier/ Qualifier transition (m/z)	Resolution	Dwell time (ms)	Fragmentor Voltage (V)	Collision Energy (V)	Cell Acceleration (V)
Time segment 0 to 14 minutes						
Ergometrine	326.2-223.1	Unit/Wide	150	120	22	2
	326.2-208	Unit/Wide	150	120	28	2
Ergometrinine	326.2-208	Unit/Unit	150	140	28	6
	326.2-265.1	Unit/Unit	150	140	16	7
Time segment 14 to 19.5 minutes						
Ergotamine	582.3-223.1	Wide/Wide	120	130	34	5
	582.3-208.1	Wide/Wide	120	130	46	6
Ergotaminine	582.3-233	Unit/Unit	120	100	34	5
	582.3-297	Unit/Unit	120	100	26	7
Ergosine	548.3-223	Unit/Wide	120	120	32	5
	548.3-208	Unit/Widest	120	120	46	6
Ergosinine	548.3-223.1	Unit/Widest	120	100	34	5
	548.3-263	Unit/Widest	120	100	28	7
Ergocornine	562.4-223.1	Unit/Unit	120	140	36	6
	562.4-268.1	Unit/Unit	120	140	22	7
Ergocryptine	576.4-223.1	Widest/Unit	120	120	40	5
	576.4-305.1	Widest/Unit	120	120	26	2
Ergocristine	610.4-223.1	Unit/Unit	120	100	38	6
	610.4-268	Unit/Unit	120	100	24	7
Time segment 19.5 to 35 minutes						
Ergocorninine	562.4-223.1	Unit/Wide	150	140	36	5
	562.4-277.1	Unit/Wide	150	140	26	7
Ergocryptinine	576.4-223.1	Widest/Unit	150	120	38	5
	576.4-305	Widest/Unit	150	120	26	7
Ergocristinine	610.4-223.1	Widest/Unit	150	180	36	5
	610.4-305.1	Widest/Unit	150	180	26	7

2.6.1 Precision – coefficient of variation

Coefficients of variation (CVs), based on a method standard deviation derived from duplicate analyses of naturally contaminated samples were determined for each of the 12 EAs (IANZ 2004) in the previous study of EAs in New Zealand cereal-based foods as part of the Mycotoxin Surveillance Programme 2017-2018 (Ashmore and Molyneux 2018).

2.7 METHOD PERFORMANCE CHARACTERISTICS

2.7.1 Method sensitivity – Limits of detection and quantification

The limit of detection (LOD) was 0.4 µg/kg for ergometrine and 0.1 µg/kg for all other 11 EAs. The limit of quantification (LOQ) was 1.25 µg/kg for ergometrine and 0.5 µg/kg for all other 11 EAs, as determined in the previous study (Ashmore and Molyneux 2018).

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 concentrations falling between the LOD and LOQ were highlighted as 'TR' in Appendix A:

2.7.2 Accuracy – spike recovery

Full details of spike recovery methods and results are included in Appendix B:

The European Commission has specified acceptable recovery ranges for regulatory analysis of some mycotoxins depending on their concentration range (European Commission 2006). Ergot alkaloids are not specified in the regulation. As a guide a comparison with ochratoxin A and patulin can be used, where similar low levels of the mycotoxin are reported. When the samples were spiked in the current study, an array of final concentrations were gained (natural concentration plus spike concentration) therefore the spike recoveries can be compared to a number of the regulations listed by the European Commission (2006). In some situations, for ochratoxin A and patulin, acceptance ranges of 50-120% were specified. The narrowest applicable range specified, depending on the concentration of mycotoxin in the spiked sample, was 70-105%.

In the current study, overall mean recoveries for each EA were in the range 23-120%. If recoveries for ergometrine and ergometrinine are excluded, recoveries were in the range 61-120%, therefore generally agreeing with the wider acceptable range of 50-120% for the mycotoxin regulations. Six of the twelve EAs had mean recoveries within the narrowest regulatory range of 70-105%.

As was found in the previous study (Ashmore and Molyneux 2018), for each sample matrix the recoveries were generally lower for the –ine epimers and higher for the –inine epimers. Interconversion (Crews 2015) could explain why some recoveries were lower and some higher than desired, when comparing the two epimers of an EA.

In other instances, the sum of the two epimers of an EA was considerably <200% suggesting losses were occurring.

The detection of EAs in samples were considered valid and the subsequent reported concentrations were good estimations regardless of loss or interconversion. The recoveries were within the same order of magnitude as expected and therefore unlikely to affect the dietary exposure assessment.

2.8 FAPAS QUALITY CONTROL RESULTS

2.8.1 Certified quality control sample

Certified quality control sample analysis is an essential part of laboratory quality assurance procedures. Analysis of a certified quality control sample provides an independent assessment of the performance of analytical testing compared with other laboratories

internationally. Fapas is a respected provider of certified quality control material run by Fera Science Limited (Fera) in the United Kingdom.[4] A Fapas certified quality control rye-flour sample was obtained, with assigned values and z-scores for 8 of the EAs analysed in the current study, with the exclusion of ergometrine, ergometrinine, ergocryptine and ergocryptinine.

2.8.2 Analysis of Fapas certified quality control sample

During the current study, the Fapas certified quality control sample was analysed three times (designated A, B and C) and in three different batches.

The accuracy of the results (i.e. ESR results compared to the Fapas Assigned Value) was variable; with mean accuracies ranged from 48 to 154%, depending on the EA. The ESR result for four of the eight EAs was within the acceptable range of results given by Fapas, however the ESR values for the other four EAs were outside of this range. The four EAs that were not within range were less than 1 µg/kg outside of the acceptable range. In the previous ESR study, there was some evidence that interconversion of the epimers took place; when the concentrations of the epimers were added together they all fell within the Fapas acceptable range. This finding is consistent with the original EA study undertaken at ESR (Ashmore and Molyneux 2018) .

Full results and a comparison assessment of analyses of the Fapas material are presented in Appendix B:

[4] https://fapas.com/ Accessed 28 November 2018

3. RESULTS AND DISCUSSION

3.1 SUMMARY OF RESULTS

3.1.1 Percentage of samples with quantified concentrations of ergot alkaloids in the current study

Individual sample results are provided in Appendix A:. Table 5 summarises the percentage of samples in the current study with quantifiable (positive) EA results, for each food type. A positive sample was identified as containing one or more EAs at concentrations ≥LOQ, and therefore a robustly quantified result was determined.

Overall, 60% of the samples contained quantifiable concentrations of EAs.

It should be noted that while 40% of samples were reported as less than the LOQ, a small proportion of the samples were noted during the review to contain EAs at levels just below the LOQ and above the LOD. Samples containing trace amounts of an individual EA are identified in Appendix A:

Rye-based crackers/crispbread were the food type most likely to contain quantifiable concentrations of EAs, with 80% of samples positive. Rye-based breads were the second most likely to contain quantifiable concentrations of EAs, with 67% of samples positive. EAs were not quantified in any Kvas samples.

Table 5 Percentage of samples with quantified concentrations of ergot alkaloids (positive samples) for each food type

Food Type	Total samples analysed	Total positive samples	Percentage positive samples
Flours - Rye	15	8	53%
Rye-based bread	12	8	67%
Crackers/Crispbread - Rye	10	8	80%
Kvas	3	0	0%
All food types	40	24	60%

3.1.2 Percentage of samples with quantified concentrations of ergot alkaloids in overseas studies

The overseas studies mentioned below had limits of quantitation (LOQ) in the range of 0.1 – 4 µg/kg, depending on the study, the sample type, and the individual EA. The ESR method was more sensitive than most of the comparison studies, with an LOQ for the current study of 0.5 – 1.25 µg/kg.

In a Polish study, EAs were detected in 94% of rye flour samples (*n*=34) (Bryła et al. 2015).

In a German study, EAs were detected in 61% of rye breads at a concentration of greater than 10 µg/kg. EAs were detected in 7% of rye crispbread samples (*n*=14) (Bürk et al. 2006).

In a second German study, EAs were detected in 100% of rye flour samples (*n*=9) (Koppen et al. 2013). While in a further German study, EAs were detected in 100% of rye flakes samples (*n*=3) (Müller et al. 2009).

In a British study, EAs were detected in 100% of samples of rye bread (n=8), rye crispbread (n=11), bread mix (rye-based) (n=3), rye-wheat bread (n=1) and rye flour (n=1) (Crews et al. 2009). EAs were detected in 50% of rye crackers (*n*=2) and not detected in all samples of rye flakes (*n*=2).

In a Chinese study, EAs were detected 22% of rye flours (*n*=9) and 6% of wheat flours (*n*=52) samples (Guo et al. 2016).

In a Canadian study, EAs were detected in 33% samples of oat-based cereals (but not specifically designated a breakfast food) (*n*=6) (Lombaert et al. 2003).

Finally, in a Danish study, EAs were detected in 94% of rye flour (*n*=34) (Storm et al. 2008).

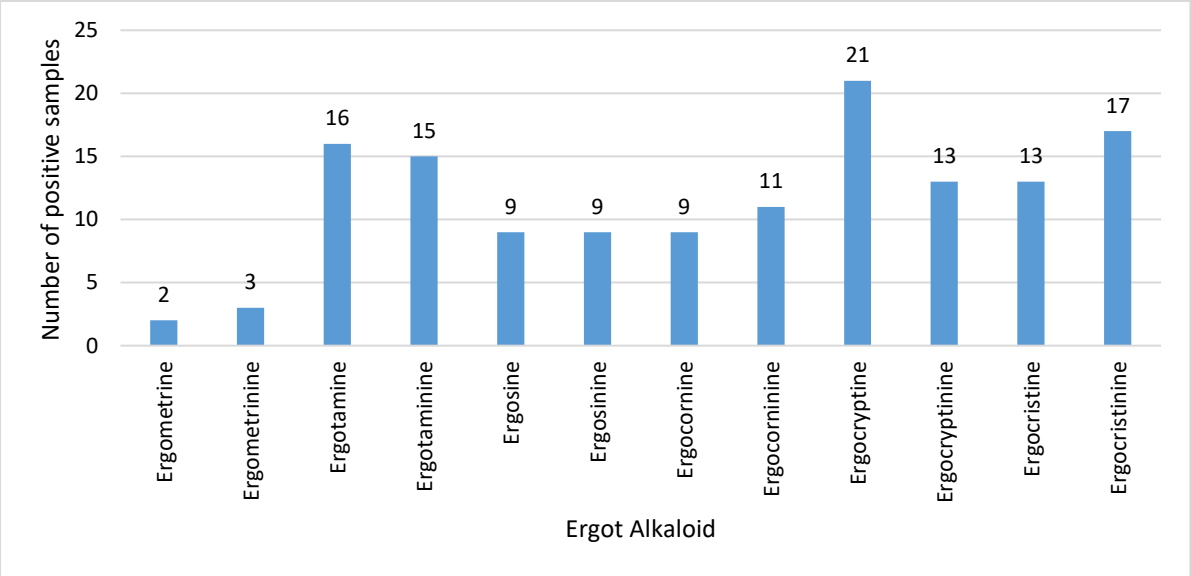
3.1.3 Prevalence of the individual ergot alkaloids in the current study

Figure 4 shows the prevalence of the individual EAs across all food types in the current study. The most prevalent EA was ergocryptine, quantified in 21 out of 40 samples. Ergocristinine, ergotamine and ergotaminine were the next most prevalent EAs, quantified in 17, 16 and 15 samples respectively. The least prevalent were found to be ergometrine and ergometrinine, quantified in only 2 and 3 out of 40 samples respectively.

This is in contrast to the previous New Zealand study on cereal-based foods (Ashmore and Molyneux 2018), where ergotamine and ergotaminine were the most frequently occurring EAs.

Overseas results also showed high variability in the order of occurrence of individual EAs.

Figure 4 Prevalence of the individual ergot alkaloids in rye-based-foods



3.1.4 Summary of ergot alkaloids results by food type

Table 6 summarises the analytical results for the 12 EAs in the rye-based foods analysed in the current study. The mean concentrations and the range are of the positive samples only. No samples contained quantifiable concentrations of all 12 EAs, there were 7 samples that contained 11 of the 12 EAs, which were rye flour (2 samples), rye-based bread (4 samples) and rye crackers/crispbread (1 sample). The individual sample results are provided in Appendix A:.

Table 6 Ergot alkaloid content (µg/kg) of rye-based foods in New Zealand

Ergot Alkaloid	Food Type	Flours - Rye	Rye-based bread	Crackers/crispbread	Kvas	All Food Types
	Total samples analysed	15	12	10	3	40
Ergometrine	No. Positive samples	2	0	0	0	2
	Mean (range)	1.6 (1.4-1.9)	-	-	-	1.6 (1.4-1.9)
Ergometrinine	No. Positive samples	0	2	1	0	3
	Mean (range)	-	0.5 (0.5-0.5)	0.7	-	0.6 (0.5-0.7)
Ergotamine	No. Positive samples	7	6	3	0	16
	Mean (range)	3.4 (1.8-6.1)	2.9 (0.6-6.8)	1.2 (0.7-1.9)	-	2.5 (0.6-6.8)
Ergotaminine	No. Positive samples	7	5	4	0	16
	Mean (range)	2.9 (0.8-6.0)	3.6 (0.9-6.9)	1.9 (0.5-4.6)	-	2.8 (0.5-6.9)
Ergosine	No. Positive samples	4	4	1	0	9
	Mean (range)	1.9 (1.0-3.2)	2.8 (2.2-3.7)	1.34	-	2.0 (1.0-3.7)
Ergosinine	No. Positive samples	4	4	1	0	9
	Mean (range)	1.6 (1.1-2.3)	2.8 (2.2-3.9)	3.0	-	2.5 (1.1-3.9)
Ergocornine	No. Positive samples	4	4	1	0	9
	Mean (range)	3.1 (0.6-6.2)	1.4 (1.0-1.6)	1.0	-	1.8 (0.6-6.2)
Ergocorninine	No. Positive samples	4	4	3	0	11
	Mean (range)	2.8 (0.8-4.6)	2.9 (2.2-3.2)	2.4 (0.7-5.9)	-	2.7 (0.7-5.9)
Ergocryptine	No. Positive samples	7	8	6	0	21
	Mean (range)	8.5 (1.1-24.7)	2.2 (0.6-4.1)	1.3 (0.6-2.8)	-	4.0 (0.6-24.7)
Ergocryptinine	No. Positive samples	5	4	4	0	13
	Mean (range)	1.7 (0.5-3.6)	1.0 (0.9-1.1)	1.5 0.7-3.6)	-	1.4 (0.5-3.6)
Ergocristine	No. Positive samples	5	5	4	0	13
	Mean (range)	3.6 (0.6-6.6)	1.6 (1.3-1.8)	1.9 (0.5-4.6)	-	2.1 (0.5-6.6)
Ergocristinine	No. Positive samples	5	5	7	0	17
	Mean (range)	4.1 0.7-6.5)	2.5 (1.1-3.3)	2.4 (0.9-6.4)	-	3.0 (0.7-6.5)

3.1.5 Summary of total ergot alkaloid concentrations

EAs are usually considered to be of equal toxicity and the EA content of a food sample may conventionally be considered in terms of 'total EAs'; the sum of the concentrations of the individual EAs. Total EA concentrations were calculated as the summation of the 12 individual EA results. Table 7 summarises the LB total EA concentrations determined in the study.

Table 7 Summary LB total ergot alkaloid concentrations

	LB Total EA concentration			
	Not quantified	Range 0.5-10 µg/kg	Range 11-50 µg/kg	Range >50 µg/kg
No. of samples	16	14	8	2

3.1.6 Summary of mean total ergot alkaloid concentrations

Lower-bound

Mean total EA concentrations were calculated as the summation of the 12 individual EA results for each food type, divided by the number of samples for the food type. Left-censored data (<LOQ) were assigned the value of zero, creating lower-bound (LB) mean total EA concentrations.

In the current study, the LB mean total EA concentration of positive samples was slightly higher for the flour samples than the bread samples, and then the cracker samples. No EAs were detected in the Kvas.

The current study confirms the results of the 2018 study that rye products available in New Zealand are likely to contain EAs. This has been consistent over two years and likely different growing seasons.

Upper-bound

Mean total EA concentrations were calculated as the summation of the 12 individual EA results for each food type, divided by the number of samples for the food type. Left-censored data of >LOD but <LOQ were assigned the value of LOQ (0.5 or 1.25 µg/kg), and <LOD were assigned the value of LOD (0.1 or 0.4 µg/kg), creating upper-bound (UB) mean total EA concentrations.

Table 8 shows the LB mean total EA concentration of positive samples and all samples, and the UB mean total of all samples by sample type.

Table 8 LB and UB mean total ergot alkaloid concentration

Food Type	Mean total EA concentration positive samples (µg/kg)	LB Mean total EA concentration all samples (µg/kg)	UB Mean total EA concentration all samples (µg/kg)
Flours - Rye	19.4	12.9	14.7
Rye-based bread	15.2	10.1	11.8
Crackers/Crispbread - Rye	7.4	5.9	8.5
Kvas	0.0	0.0	1.5
All food types	15.4	9.2	11.3

3.1.7 Ergot alkaloids by sample country of origin

Just over half of samples labelled with New Zealand as country of origin of the product were positive for EAs (11 out of 18 samples) (Table 10), although the descriptions included 'product of', 'grown in', 'made in', 'packed in', and 'from local and imported ingredients'. Therefore, in some instances it is unknown whether all or any of the ingredients were grown in New Zealand. Just over half of samples with an unknown country of origin were also positive for EAs (4 out of 7 samples). With the ambiguous country of origin labelling it is difficult to conclude further outcomes from the study. While it is difficult to draw general conclusions, these results suggest that rye-based products manufactured in New Zealand are as likely to be contaminated with EAs as rye-based products manufactured elsewhere and available in New Zealand.

Table 9 Ergot alkaloids by sample country of origin

Country of origin	Number of samples (*n* = 40)	No. positive samples (%)
Australia	4	1 (25)
Canada	1	1 (100)
Germany	5	3 (60)
New Zealand	18	11 (61)
Sweden	1	1 (100)
Turkey	1	0 (0)
UK	3	3 (100)
Not provided	7	4 (57)

3.2 ERGOT ALKALOIDS IN FOOD TYPES

The following sections describe the concentrations of EAs in the designated food types from the current study and compares them to previous New Zealand and overseas studies.

3.2.1 Crackers – Rye

Ten samples of crackers/crispbread, where the main ingredient was rye, were analysed. EAs were quantified in eight samples, which contained between one and 11 EAs. Crackers/crispbreads were most likely to contain ergocristinine (found in 7 of the 8 positive samples) and ergocryptine (found in 6 of the 8 positive samples). In the positive samples, the mean concentration of individual EAs was 1.7 µg/kg, with a mean range of 0.7 µg/kg (ergometrinine) to 3.0 µg/kg (ergosinine). The mean total EA concentration of positive cracker/crispbread samples was 7.3 µg/kg (range 0.6 - 33.0 µg/kg).

In the previous study (Ashmore and Molyneux 2018), two samples of crackers, where the main ingredient was rye, were analysed. EAs were only quantified in one sample which was found to contain just one EA (ergocristinine) at a concentration of 0.5 µg/kg.

In a German study, samples of rye crispbread were analysed for six EAs (ergometrine, ergocornine, ergotamine, α-ergocryptine and ergocristine) and reported as total EA content (Bürk et al. 2006). One sample out of 14 (7%) contained EAs at detectable levels, with a total concentration of 28 µg/kg.

In a British study, samples of rye crispbread were analysed for 12 EAs (Crews et al. 2009). Eleven samples were analysed and all contained EAs at detectable levels, with a mean total EA concentration of 76 µg/kg (range 2-340 µg/kg). All 12 EAs were detected in one sample (9%). The same study also considered rye crackers, as a separate food type. One out of two samples (50%) contained EAs at detectable levels, with a total EA concentration of 9 µg/kg.

The total EA concentrations in the current study are lower than those reported overseas, and higher than those reported previously in a small number of New Zealand samples (Ashmore and Molyneux 2018). The percent of positive samples was consistent with that observed in overseas studies.

3.2.2 Flours – Rye

Fifteen samples of rye flour were analysed and 53% contained quantifiable levels of EAs. In the positive samples, the mean concentration of individual EAs was 3.2 µg/kg, with a mean range of 1.6 (ergometrine and ergosinine) to 8.5 µg/kg (ergocryptine). The LB mean total EA concentration of positive samples was 19.2 µg/kg. The most prevalent EAs were ergotamine, ergocryptine and ergotaminine which were all quantified in seven out of eight positive samples. Two samples gave positive results for all EAs except ergometrinine. These two samples had the highest total EA concentration of the rye flour samples, at 55.2 and 66.5 µg/kg.

In the previous New Zealand study, ten samples of rye flour were analysed and 50% contained quantifiable levels of EAs. In the positive samples, the mean concentration of individual EAs was 11.0 µg/kg, with a mean range of 0.6 (ergotamine and ergocryptine) to 26.6 µg/kg (ergocristine). The LB and UB mean total EA concentration of all samples was 18.1 and 19.8 µg/kg, respectively. The most prevalent EA was ergotamine which was quantified in all five positive samples. One sample gave positive results for all 12 EAs. For this sample the mean individual EA concentration was 14.1 µg/kg, with a range of 2.2 to 26.6 µg/kg, and total EA content of 169.0 µg/kg.

In a British study, one sample of rye flour was analysed for 12 EAs (Crews et al. 2009) and reported a total EA concentration of 9 µg/kg.

In a Chinese study, samples of rye flour were analysed for 13 EAs (α-ergocryptine and β-ergocryptine were reported separately) (Guo et al. 2016). Two samples out of nine (22%) contained EAs at detectable levels. One positive sample had a total EA concentration of 800 µg/kg, and nine individual EAs were detected with the range of contribution being 1.99 (ergosinine) to 593 µg/kg (ergocristine). The other positive sample had a total EA concentration of 30.75 µg/kg, and seven individual EAs were detected with the range of contribution being 2.00 (ergocornine) to 8.04 µg/kg (ergotamine).

In a German study, samples of rye flour were analysed for 12 EAs (Koppen et al. 2013). Nine samples were analysed and 100% contained EAs at detectable levels. The mean total EA concentration was 64.8 µg/kg, with a range of 5.8 to 178 µg/kg. Two samples were positive for all 12 EAs, with total EAs content of 144 and 178 µg/kg. The sample with the highest total EA content also reported the highest individual EA of 39.0 µg/kg (α-ergocryptine).

In a second German study, samples of rye flour were analysed for 12 EAs (Müller et al. 2006). A sample of naturally contaminated rye flour had a total EA content of approximately 1600 µg/kg.

In a further German study, samples of rye flour were analysed for 12 EAs (Müller et al. 2009). Twenty-two samples were analysed and 100% contained EAs at detectable levels. The mean individual EA concentration ranged from 1.8 (ergometrinine) to 27.0 µg/kg (ergocristine). The maximum individual EA concentrations ranged from 15.8 to 133 µg/kg, for ergometrinine and ergocristine, respectively. The mean and maximum total EA concentrations were 138 and 715 µg/kg, respectively.

In a Danish study, samples of organic and conventional rye flour were analysed for ergocornine, α-ergocryptine, ergocristine, ergometrine, ergotamine, and their corresponding epimers (Storm et al. 2008). Thirty-four samples were analysed and 94% contained EAs at detectable levels. The mean total EA content was 46 µg/kg, with a range of not detected to

234 µg/kg. The most common EAs were ergotamine and α-ergocryptine, and their corresponding epimers. Differences between organic and conventional rye flour samples were not significant.

In a Polish study, rye flour (*n*=34) was analysed for 12 EAs (Bryła et al. 2015). The mean total EA content was 106 µg/kg, the range was 0.9 to 1216 µg/kg, and 32 out of 34 samples were positive (94%).

In a Swiss study, 15 samples of rye flour were analysed for 16 EAs (including α- and β-ergocryptine; α- and β-ergocryptinine; ergostine and ergostinine) (Reinhard et al. 2008). The samples were collected across three different years and reported as total EA content, with a reported range of 18-519 µg/kg.

In a Scientific Opinion piece produced by EFSA (2012), rye milling products (*n*=511) were analysed for 'at least six EAs'. The mean LB total EA concentration and mean UB total EA concentration was 124 and 155 µg/kg, respectively.

The total concentrations of EAs in the current study were at the lower end of those reported overseas. There was also a lower percentage of positive samples.

3.2.3 Rye-based bread

Twelve samples of rye-based bread were analysed and EAs were quantified in 67% of samples. In the positive samples the mean individual EA concentration was 2.2 µg/kg, with a mean range of 0.5 (ergometrinine) to 3.6 µg/kg (ergotaminine). The LB mean total EA concentration of positive samples was 14.8 µg/kg. The most prevalent EA was ergocryptine, which was quantified in all eight positive samples. Two samples contained quantifiable levels of 11 EAs. These two bread samples contained total EA content of 25.3 and 29.9 µg/kg and were country of origin 'Made in New Zealand'.

In the previous New Zealand study, ten samples of rye-based bread were analysed and EAs were quantified in 50% of samples in this category. In the positive samples the mean individual EA concentration was 2.7 µg/kg, with a mean range of 0.5 (ergometrinine) to 4.8 µg/kg (ergocorninine). The LB and UB mean total EA concentration of all samples was 7.7 and 9.5 µg/kg, respectively. The most prevalent EAs were ergotamine and ergotaminine, and they were quantified in all five positive samples. One sample contained quantifiable levels of all 12 EAs. Its mean individual EA concentration was 4.9 µg/kg, with a range of 0.5 to 8.6 µg/kg, and a total EA content of 59.0 µg/kg.

In a German study, samples of rye bread were analysed for six EAs (ergometrine, ergocornine, ergotamine, α-ergocryptine and ergocristine) reported as total EA content (Bürk et al. 2006). EA content of >10 µg/kg was detected in 14 out of 23 samples (61%). One mixed-grain bread (grouped in the rye bread category) contained a total EA concentration of 258 µg/kg. Additionally, samples of pumpernickel bread (a bread typically made from coarsely ground wholemeal rye) were analysed as a separate category to the rye breads detailed above (Bürk et al. 2006). Total EA content above the LOQ (0.1 to 1.0 µg/kg) was reported for 17 out of 20 samples (85%). The highest total EA concentration in pumpernickel was 47 µg/kg. Finally, samples of rye-bread rolls were analysed as a separate category to the rye breads detailed above (Bürk et al. 2006). EAs were detected in three out of nine samples (33%) with total EAs of 11, 31, and 91 µg/kg.

In a British study, samples of rye bread were analysed for 12 EAs (Crews et al. 2009). Eight samples were analysed and 100% contained detectable EAs, with a mean total concentration of 40 µg/kg (range 1-171 µg/kg). Four out of eight samples (50%) contained detectable levels of all 12 EAs. Additionally, one sample of rye-based bread, designated as a separate category to the rye breads detailed above, was analysed for 12 EAs (Crews et al. 2009). The sample had a total EA concentration of 19 µg/kg. Furthermore, samples of rye-based bread mix were analysed for 12 EAs (Crews et al. 2009). Three samples were

analysed and 100% contained EAs at detectable levels, with a mean total concentration of 7 µg/kg (range 3-13 µg/kg).

In a Swiss study, samples of bread (with rye) were analysed for 16 EAs (including α- and β-ergocryptine; α- and β-ergocryptinine; ergostine and ergostinine) (Reinhard et al. 2008). The samples were collected across three different years and reported as total EA content, with a reported range of 17-477 µg/kg.

In a Scientific Opinion piece produced by EFSA (2012), rye bread and rolls (*n*=24) were analysed for 'at least six EAs'. The mean LB total EA concentration and mean UB total EA concentration was 30 and 45 µg/kg, respectively.

The concentrations of total EAs in the current study are largely consistent with results reported from overseas studies, and with similar percentage of positive samples. The concentration and percentage of positive samples was also similar to the previous study of New Zealand samples (Ashmore and Molyneux 2018).

3.2.4 Kvas

No detectable levels of any EAs were found in Kvas. The spike recoveries for Kvas were lower than those for the other food types, due to the liquid sample diluting the final solution. Even with the lower recovery taken into account the Kvas samples were not found to contain quantifiable levels of EAs.

No other studies investigating EA content of rye-based beverages were observed.

4. DIETARY EXPOSURE ASSESSMENT

4.1 ERGOT ALKALOID DIETARY EXPOSURE ASSESSMENT

For dietary exposure to chemicals, exposure can be defined as:

$$E_i = \frac{\sum_k Q_{ik} \times C_{ik}}{bw_i}$$

Where Ei is the exposure of individual i to some chemical at some specified point in time, $Q_{i,k}$ is the amount of food k consumed by individual i, $C_{i\,k}$ is the concentration of the chemical of interest in food k consumed by individual i and bw_i is the body weight of individual i. For deterministic (point) estimates of exposure, these parameters (concentration, food consumption and body weight) are represented by population averages or selected percentiles. For dietary modelling, food consumption and body weight will be represented by actual reported values for an individual on one particular day or on several days, depending on the structure of the dietary survey.

In the current study, chronic dietary ergot alkaloid exposure was determined by a dietary modelling method using 24-hour dietary recall records from the 2009 Adult Nutrition Survey (University of Otago and Ministry of Health 2011) for adults 15 and over and 24-hour dietary recall records from the 2002 National Children's Nutrition Survey (Ministry of Health 2003) for children 5-15 years.

4.1.1 Ergot Alkaloid concentration data

Exposure to EAs are of concern due to their acute and chronic toxicity, which is well characterised from various outbreaks of human poisoning (WHO 1990). The current assessment only considers chronic dietary exposure. For chronic dietary exposure assessment the most appropriate concentration metric for each food type is the mean concentration, including both samples with quantifiable and non-quantifiable analytical results, as over time this will be the expected concentration value that individuals will be exposed to.

The dataset for EAs in New Zealand foods contains a reasonably low proportion of left censored data (<50%), due to the selection of foods expected to contain appreciable concentrations of EAs. Left censoring refers to the situation where the distribution of observed results is truncated at the left hand end due to the limitations of measurement technologies (not detected). However, it was common to find detectable concentrations of one or more, but not all EAs. The exposure assessment was performed using the concentration of total EAs in the sample. The total EA content is simply the sum of the concentrations of each of the 12 individual EAs that were assayed in each sample. In the case of samples with some individual EAs not detected, two figures were calculated. The upper bound assigned a value of the LOQ to any EA detected at >LOD and <LOQ and a value of the LOD to any EA detected at <LOD, while the lower bound total assigned a concentration value of zero to any EAs below the LOQ.

These two concentrations were carried through all calculations and into the exposure assessment, giving an upper and lower bound estimate of dietary exposure. This is analogous to the EFSA exposure assessment studies (EFSA 2017).

Analytical concentration results for the dietary exposure assessment were taken from both this current rye-based EA study, and the 2018 cereal based study (Ashmore and Molyneux 2018).

4.1.2 Food consumption information

National Nutrition Survey (NNS) records

Periodic national nutrition surveys are carried out in New Zealand. The most recent are the 2009 Adult Nutrition Survey (09ANS) covering adult New Zealanders, aged 15 years and over (University of Otago and Ministry of Health 2011) and the 2002 National Children's Nutrition Survey (02CNS) covering New Zealand children aged 5-15 years (Ministry of Health 2003).

These two surveys included 24-hour dietary recall records (24HDR). These include a complete listing of all foods consumed by an individual during one 24-hour period and the amount of the food consumed. Days of the week and time of year are randomised across the survey to avoid bias due to these factors. The 09ANS contains 24HDR records for 4,721 respondents and the 02CNS contains 24HDR records for 3,098.

24HDR records were used to provide individual estimates of dietary EA exposure for the surveyed day.

4.1.3 Mapping of NNS foods to ergot alkaloid-containing foods

The NNSs contain over 10,000 unique food descriptors. In order to estimate the EA concentration of each of these foods it is necessary to map the foods for which EA concentrations are available to the list of unique NNS food descriptors. Three situations arise:

- The food description for the food analysed for EAs is sufficiently similar to the NNS food descriptor to allow direct application of the determined EA concentration;
- The NNS food is unrelated to any food analysed for EAs and no EA contribution is attributed to it; or
- The NNS food is similar to or contains (as part of a recipe) one of the foods analysed for EAs.

Appendix C: identifies the foods for which EA concentration values were available, the wider group of foods that they were chosen to represent and the mean EA concentration used. Where foods were not related to samples analysed in this survey (for example, shellfish) no mapping was carried out. These foods were assigned to an 'Other' category, with a zero EA concentration.

It should be noted that the mapping may exaggerate EA exposure in some instances. Samples were selected on the assumption they would be a contributor to EA exposure, and those results were mapped to other foods that were a close match, for example a crumpet was mapped as wheat bread.

4.1.4 Body weights

The dietary modelling approach generates an estimate of EA exposure for each respondent in the 09ANS or 02CNS and the corresponding actual body weights are used in this approach to convert the individual dietary exposure estimates to a 'per kg body weight' basis.

4.2 EXPOSURE ASSESSMENT RESULTS

4.2.1 Dietary total ergot alkaloid exposure assessment

Estimates of dietary EA exposure derived from dietary modelling are summarised in Table 10.

Table 10: Estimates of dietary ergot alkaloid exposure for the New Zealand population, including sub-groups

Population sub-group	Mean estimated dietary EA exposure (95th percentile) (µg/kg body weight/day)	
	Lower bound[5]	Upper bound
All children (5-15 years)	0.012 (0.028)	0.025 (0.059)
All adults (15+ years)	0.0046 (0.012)	0.0094 (0.022)
Child (5-6 years)	0.016 (0.033)	0.033 (0.069)
Female (11-14 years)	0.0082 (0.019)	0.017 (0.039)
Male (11-14 years)	0.010 (0.023)	0.021 (0.046)
Male (19-24 years)	0.0064 (0.015)	0.013 (0.030)
Female (25+ years)	0.0039 (0.0096)	0.0080 (0.019)
Male (25+ years)	0.0047 (0.011)	0.0097 (0.022)

4.2.2 Major food contributors to dietary ergot alkaloid exposure

For adult New Zealanders, 49% of dietary EA exposure was from consumption of wheat breads, 27% from other wheat flour-containing foods, and 15% from multigrain breads (Figure 5). These foods were included in the earlier 2017-2018 survey.

For New Zealand children, 60% of dietary EA exposure was from consumption of wheat breads, 30% from other wheat flour-containing foods, and 10% from other foods (Figure 6).

Many food descriptors contained in the 09ANS and 02CNS were not sufficiently similar to any analysed food in the 2018 or 2020 EA surveys to allow assignment of an EA concentration. These foods were not mapped, and given a zero EA concentration figure for the exposure estimates. Such foods included chocolate, fruits and vegetables, meats, and dairy products; foods in which EA contamination would not be expected.

[5] Upper and lower bound refers to treatment of left censored analytical data. Lower bound exposure estimates are derived by assuming that analytical values below the limit of quantification (LOQ) are true zero values, while upper bound exposure estimates are derived by assigning a value of the LOQ to any EA detected at >LOD and <LOQ and a value of the LOD to any EA detected at <LOD.

Figure 5: Ergot alkaloid exposure from major contributing food groups in adults

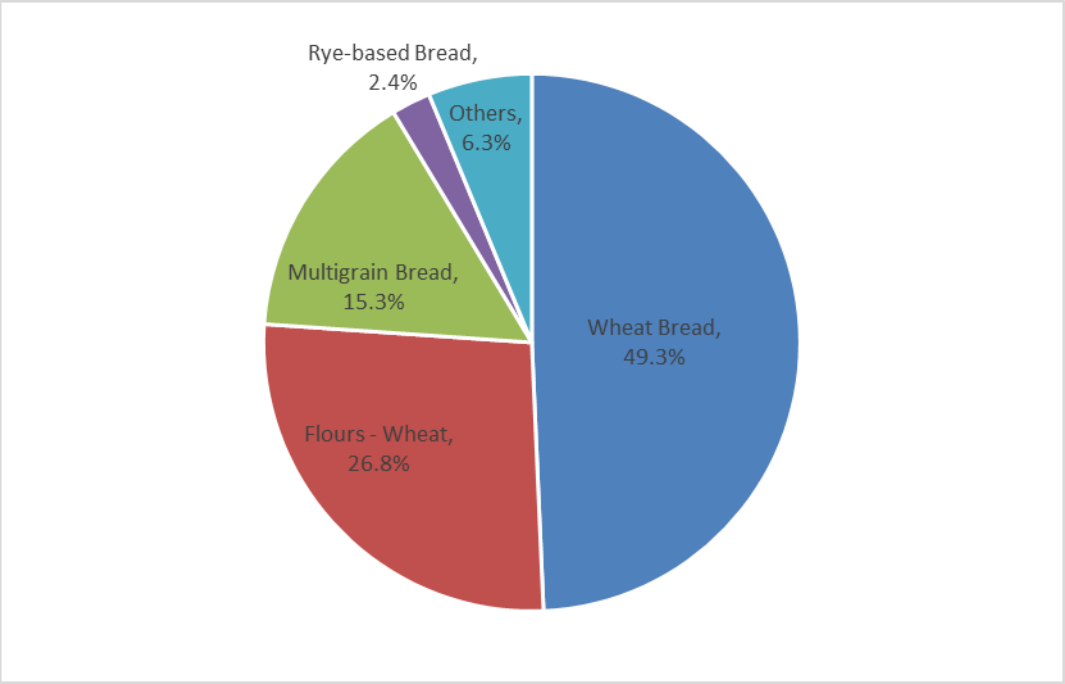
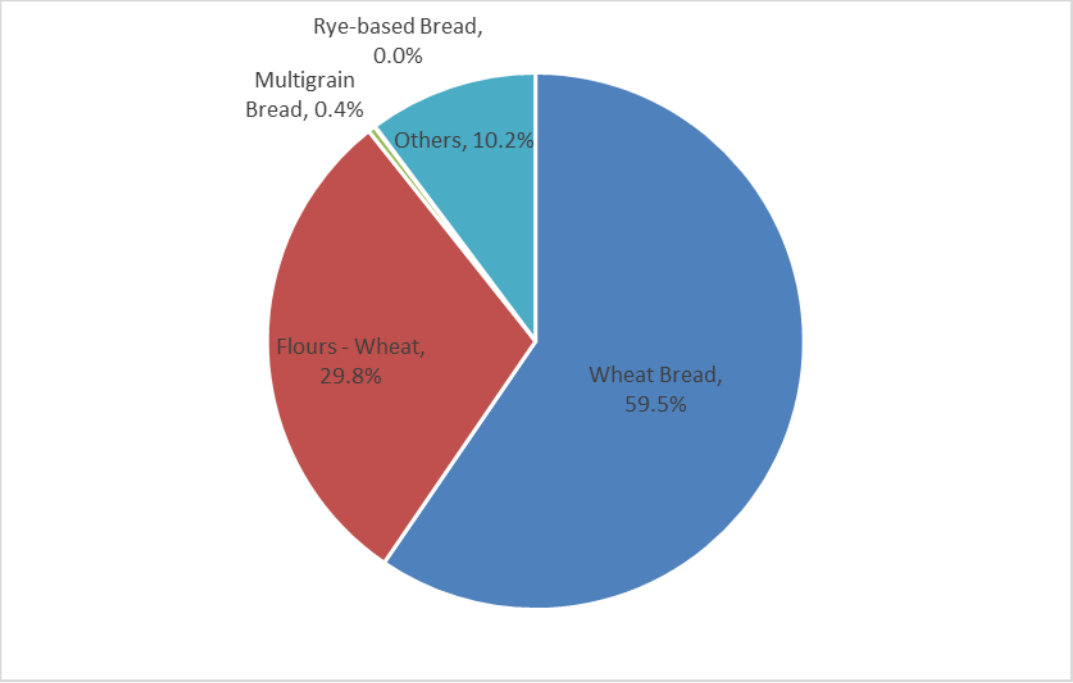


Figure 6: Ergot alkaloid exposure from major contributing food groups in children



4.2.3 Comparison with international exposure estimates

Dietary EA exposure assessments have been performed and collated using data from 35 dietary surveys from 19 different European countries (EFSA 2017). A summary of the European studies, and comparison with this New Zealand exposure assessment are shown in Table 11. It should be noted that the EFSA assessment derived estimates of chronic dietary exposure for each age group from food consumption studies in a number of European countries. The figures in Table 12 are the medians of these individual study

estimates. Age ranges for the New Zealand dietary exposure estimates have been selected to align as closely as possible with the age ranges used for the EFSA estimates.

Dietary EA exposure in New Zealand is considerably lower than in European studies, mainly due to the higher concentration of EAs in food analysed in Europe. For example, the mean upper bound concentration of EAs in rye bread used in exposure estimates in Europe was 66.8 µg/kg, but only 10.8 µg/kg in this New Zealand study.

Table 11: Dietary exposure to ergot alkaloids – comparison between this study, and the median of the mean exposure level across different surveys (EFSA, 2017)

Age class	Mean estimated dietary EA exposure (µg/kg body weight/day)			
	New Zealand (current study)		EFSA summary (median)	
	Upper bound	Lower bound	Upper bound	Lower bound
3 – 10 years	-	-	0.20	0.05
5 – 15 years	0.025	0.012	-	-
10 – 17 years	-	-	0.12	0.03
10 – 14 years	0.020	0.0095	-	-
15 – 17 years	0.012	0.0062	-	-
18 – 64 years	0.0094	0.0047	0.09	0.02
65 – 74 years	0.0081	0.0038	0.09	0.02
75 years and over	0.0083	0.0039	0.09	0.02

4.2.4 Risk Characterisation

The EFSA Panel on Contaminants in the Food Chain (CONTAM) in 2012 (EFSA 2012) performed a risk assessment considering both chronic and acute exposure to EAs. They concluded a group tolerable daily intake (TDI), based on the sum of all measured EAs, should be 0.6 µg/kg bw/day. This determination assumed equal relative potency of each EA measured. The Panel noted that dietary exposure estimates relate to a limited number of food groups, and additional contribution from other food groups cannot be discounted.

Dietary exposure to EAs in New Zealand is approximately 2.5 times higher in children than adults. The mean dietary exposure (0.025 µg/kg bw/day) and P95 dietary exposure (0.053 µg/kg bw/day) in children are both much lower than the EFSA TDI value of 0.6 µg/kg bw/day.

5. CONCLUSIONS

Ergot alkaloid (EA) concentrations were measured in selected New Zealand foods by ESR in 2018 and 2020. Foods were selected based on their composition/ingredients, and the expectation of EAs being present, based on international literature.

The results of these analyses have been used to generate chronic dietary EA exposure estimates, based on food consumption reported in the 2009 Adult Nutrition Survey and 2002 National Children's Nutrition Survey.

There was no major variation in prevalence or concentrations of EAs in this study between samples in a food type. Just under half of the samples (40%) did not contain quantifiable levels of EAs, followed by over a third of samples (35%) with low concentrations of total EA (≤10 µg/kg). Concentrations of total EA in the range 11-50 µg/kg were observed in 20% of samples and the remaining 5% of samples had high concentrations of total EA (>50 µg/kg). The highest concentrations were observed in samples of rye flour.

In the foods studied, ergocryptine and ergocristinine were the most prevalent EAs.

The total EA concentration in rye flour and rye breads were similar between the current and 2018 New Zealand studies. However, the profiles of individual EAs were variable between the studies.

The concentrations of EAs in foods available in New Zealand were towards the lower end of the range of results reported internationally, and this was reflected in the exposure estimates. Mean estimates of adult chronic dietary exposure to EAs (0.0094 µg/kg bw/day) were markedly lower than in Europe (0.18 µg/kg bw/day). This appears to be primarily a reflection of lower EA concentrations in foods available in New Zealand, although differences in food consumption patterns will also contribute.

Major contributing food groups to dietary EA exposure were wheat bread, wheat flour, and multigrain bread for adults. Given that the wheat flour analysed contained a reasonably low concentration of EAs compared with overseas surveys, this exposure assessment may underestimate the exposure from consumption of imported foods containing wheat flour.

Foods containing higher concentrations of EAs, such as rye flour and rye bread, did not contribute appreciably to dietary exposure in the New Zealand population since these foods were not consumed in high quantity or frequency.

The dietary EA exposure assessment calculated that consumption in New Zealand is approximately 10 fold lower than similar studies in Europe. This is primarily due to the lower EA concentrations measured in cereal and rye-based foods.

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APPENDIX A: RESULTS

Table 12 Sample results, individual ergot alkaloids and total ergot alkaloids

Sample	Sample type	Ergometrine	Ergometrinine	Ergotamine	Ergotaminine	Ergosine	Ergosinine	Ergocornine	Ergocorninine	Ergocryptine	Ergocryptinine	Ergocristine	Ergocristinine	TOTAL[1]
1	Rye Flour	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	Rye Flour	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	Rye Flour	ND	ND	TR	TR	TR	ND	ND	ND	ND	ND	ND	ND	0.0
4	Rye Flour	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	Rye Flour	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	Rye Flour	ND	ND	1.7	0.8	ND	ND	ND	ND	1.1	ND	ND	ND	3.7
7	Rye Flour	1.4	TR	5.3	3.5	3.2	2.3	6.2	4.6	24.7	3.6	6.6	5.2	66.5
8	Rye Flour	TR	TR	2.6	2.2	1.8	1.3	1.5	1.6	5.1	1.0	4.3	5.4	26.8
9	Rye Flour	ND	ND	2.3	2.0	TR	TR	TR	TR	1.7	TR	TR	0.7	6.7
10	Rye Flour	0.0	0.0	1.8	2.1	1.0	1.1	0.6	0.7	2.7	0.5	1.3	2.8	14.8
11	Rye Flour	TR	ND	6.1	6.0	TR	TR	ND	TR	5.1	0.6	TR	ND	17.9
12	Rye Flour	1.9	TR	3.6	3.7	1.6	1.8	4.2	4.5	19.4	2.8	5.3	6.5	55.2
13	Rye Flour	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
14	Rye Flour	TR	ND	TR	TR	TR	TR	ND	ND	ND	ND	0.6	TR	0.6
15	Rye Flour	ND	ND	0.5	0.5	ND	ND	ND	ND	ND	ND	ND	ND	0.9
16	Rye-based Bread	TR	0.5	2.7	2.7	2.7	2.7	1.6	3.0	3.4	0.9	1.8	3.3	25.3
17	Rye-based Bread	ND	ND	TR	0.8	TR	TR	0.6	TR	0.9	TR	TR	TR	2.3
18	Rye-based Bread	TR	TR	6.8	6.9	2.2	2.2	1.0	2.2	3.0	1.0	1.3	2.1	28.5
19	Rye-based Bread	TR	TR	3.4	3.4	2.6	2.5	1.6	3.2	3.4	1.1	1.8	3.1	26.0

Sample	Sample type	Ergometrine	Ergometrinine	Ergotamine	Ergotaminine	Ergosine	Ergosinine	Ergocornine	Ergocorninine	Ergocryptine	Ergocryptinine	Ergocristine	Ergocristinine	TOTAL[1]
20	Rye-based Bread	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
21	Rye-based Bread	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
22	Rye-based Bread	ND	ND	TR	ND	TR	0.0	TR	TR	1.8	TR	TR	TR	1.8
23	Rye-based Bread	ND	ND	ND	ND	ND	ND	ND	ND	0.8	ND	ND	TR	0.8
24	Rye-based Bread	TR	0.5	3.3	4.1	3.7	3.9	1.5	3.1	4.1	0.9	1.8	3.0	29.9
25	Rye-based Bread	ND	ND	0.5	TR	ND	ND	ND	ND	0.9	ND	1.5	1.0	4.0
26	Rye-based Bread	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
27	Rye-based Bread	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
28	Rye Cracker/Crispbread	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
29	Rye Cracker/Crispbread	TR	0.7	1.9	4.5	1.3	3.0	1.0	5.8	2.8	3.6	1.9	6.3	33.0
30	Rye Cracker/Crispbread	ND	ND	TR	0.5	ND	TR	TR	0.7	1.0	0.9	0.6	2.2	6.0
31	Rye Cracker/Crispbread	ND	ND	0.7	1.4	ND	TR	TR	0.8	1.5	0.7	0.5	3.4	8.8
32	Rye Cracker/Crispbread	ND	ND	TR	TR	TR	TR	ND	TR	TR	TR	TR	1.1	1.1
33	Rye Cracker/Crispbread	ND	ND	TR	TR	TR	TR	ND	TR	0.6	TR	ND	TR	0.6
34	Rye Cracker/Crispbread	ND	ND	0.8	1.4	ND	TR	ND	TR	0.9	TR	TR	2.1	5.2
35	Rye Cracker/Crispbread	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
36	Rye Cracker/Crispbread	ND	ND	TR	TR	TR	TR	ND	TR	TR	ND	TR	0.9	0.9
37	Rye Cracker/Crispbread	ND	ND	TR	TR	TR	TR	ND	TR	0.8	0.7	TR	1.0	2.5
38	Kvas	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
39	Kvas	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
40	Kvas	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[1] Total ergot alkaloid concentrations have been calculated as a sum of the individual ergot alkaloid concentrations, assuming that all ND and TR values are equal to zero

ND = Not Detected

TR = Trace result: >LOD and <LOQ

APPENDIX B: QUALITY CONTROL

B.1 Accuracy – spike recovery

The performance of the analytical method was assessed. Accuracy of the developed method was assessed by spiking a known concentration (10 µg/kg) of the each of the 12 EAs into food samples from different matrices. The samples were analysed to determine what proportions of the added EAs were measurable (recovered) by the analytical method. The mean recovery results and range (if more than one spike per matrix) are shown in Table 13 and Table 14. Generally, across all of the sample matrices recoveries were variable, suggesting that losses were occurring or interconversion between –ine and –inine epimers. Interconversion could explain why some recoveries were lower and some higher than 100%, when comparing the two epimers of an EA. Recoveries for Kvas were consistently lower than that for the other food types, most likely because this liquid sample dilutes the extract.

The inclusion of an isotopically-labelled internal standard would have enabled correction for interconversion, losses and matrix effects, including signal/ionisation suppression or enhancement during LC-MS/MS analysis. A suitable internal standard was not available at the time of this study.

Table 13 Accuracy – spike recovery for ergometrine, ergometrinine, ergotamine, ergotaminine, ergosine and ergosinine. Mean recovery (Range) %.

	Ergometrine	Ergometrinine	Ergotamine	Ergotaminine	Ergosine	Ergosinine
Flours - Rye	29 (23-36)	30 (25-33)	81 (64-108)	101 (96-105)	73 (68-81)	90 (82-97)
Rye-based bread	21 (13-28)	27 (20-35)	58 (46-69)	67 (47-87)	65 (63-66)	74 (67-82)
Crackers/Crispbread - Rye	23 (20-26)	22 (20-24)	87 (83-90)	119 (117-122)	82 (79-84)	108 (102-114)
Kvas	6	6	36	35	31	32

Table 14 Accuracy – spike recovery for ergocornine, ergocorninine, ergocryptine, ergocryptinine, ergocristine and ergocristrinine. Mean recovery (Range) %.

	Ergocornine	Ergocorninine	Ergocryptine	Ergocryptinine	Ergocristine	Ergocristinine
Flours - Rye	64 (58-71)	120 (106-144)	93 (87-100)	115 (107-124)	74 (68-84)	141 (116-182)
Rye-based bread	50 (45-55)	107 (100-113)	99 (82-116)	66 (62-70)	54 (53-55)	85 (77-93)
Crackers/Crispbread - Rye	82 (79-85)	111 (110-113)	114 (110-118)	215 (163-267)	69 (81-110)	114 (100-188)
Kvas	33	49	59	47	42	59

B.2 Fapas sample

B.2.1 ESR determined value compared with Fapas assigned value

Table 15 compares the Fapas sample concentration as determined by ESR with the results assigned by Fapas. The accuracy of the results (i.e. ESR results compared to the Fapas Assigned Value) was variable; accuracies ranged from 48 to 154%.

The variability could be due to a number of factors: differing sample masses used for the extractions, extraction solvents, sample clean-up materials, mobile phases, and detection methods.

From the accuracy values, it could be suggested that interconversion of the epimers took place; ergotamine/ergotaminine, ergosine/ergosinine, ergocornine/ergocorninine exhibited alternating low and high accuracies. All stages of the method used in this study were examined to reduce interconversion and it was decided the method was as robust as possible, in the absence of a suitable isotopically-labelled internal standard.

ESR individual EA concentrations in the FAPAS sample are shown in Table 16.

Table 15 ESR determined value vs Fapas assigned value

Ergot Alkaloid	Number of analyses	ESR determined value, mean µg/kg [1]	Fapas Assigned Value, µg/kg [2]	Accuracy (ESR vs Fapas) %	ESR determined value range, µg/kg	Fapas range, µg/kg	ESR determined mean value within Fapas range?	ESR LOQ, µg/kg
Ergometrine	3	2.0	Not assigned	-	0.9-2.6	-	-	1.25
Ergometrinine	3	-	Not assigned	-	-	-	-	0.5
Ergotamine	3	7.2	10.90	66	5.2-8.5	6.1-15.7	Yes	0.5
Ergotaminine	3	4.8	3.10	154	4.4-5.2	1.74-4.46	No	0.5
Ergosine	3	5.5	11.30	48	3.4-6.5	6.3-16.2	No	0.5
Ergosinine	3	3.7	4.30	85	3.5-3.8	2.41-6.19	Yes	0.5
Ergocornine	3	13.0	15.30	85	11.8-14.0	8.5-22	Yes	0.5
Ergocorninine	3	12.5	8.47	148	8.8-17.6	4.74-12.2	No	0.5
Ergocryptine	3	45.2	Not assigned	-	38.8-50.5	-	-	0.5
Ergocryptinine	3	4.8	Not assigned	-	2.9-6.5	-	-	0.5
Ergocristine	3	6.7	9.17	74	5.4-7.7	5.13-13.2	Yes	0.5
Ergocristinine	3	5.3	4.0	133	2.8-7.0	2-5	No	0.5

[1] Determined value, mean µg/kg: results were not corrected for recovery
[2] Fapas assigned value, µg/kg: results were corrected for recovery

Table 16 ESR individual ergot alkaloid concentrations in the FAPAS sample

Sample	Sample type	Ergometrine	Ergometrinine	Ergotamine	Ergotaminine	Ergosine	Ergosinine	Ergocornine	Ergocorninine	Ergocryptine	Ergocryptinine	Ergocristine	Ergocristinine
A	FAPAS	2.5	<0.5	8.5	4.7	6.5	3.5	13.1	1.8	38.8	2.9	7.1	2.8
B	FAPAS	0.9	<0.5	8.0	4.4	6.5	3.8	14.0	4.0	46.4	5.0	5.4	6.2
C	FAPAS	2.6	<0.5	5.2	5.2	3.4	3.6	11.8	4.8	50.5	6.5	7.7	7.0

B.2.2 Precision of Fapas results - coefficient of variation (CV)

Table 17 below shows the precision of Fapas results between batches. Coefficients of variation (CVs) are based on a method standard deviation derived from two or more analytical results. The three samples were analysed in three batches.

Table 17 Fapas sample inter-batch precision

Ergot Alkaloid	CV %, samples A-C
Ergometrine	40
Ergometrinine	-
Ergotamine	20
Ergotaminine	7
Ergosine	27
Ergosinine	14
Ergocornine	7
Ergocorninine	30
Ergocryptine	3
Ergocryptinine	30
Ergocristine	11
Ergocristinine	34

APPENDIX C: MAPPING OF FOODS

Mapping of foods for which EA information was available to National Nutrition Survey foods

Table 18: Food group mapping for dietary exposure assessment

Food group description	National nutrition survey descriptors included	LB Mean EA concentration (µg/kg)	UB Mean EA concentration (µg/kg)
Beverage - Rye Based	Any beverage based on rye	0.0	1.5
Breakfast Cereal - Oat	Any oat based breakfast cereals, “Muesli” and “Muesli” bars	0.5	2.0
Breakfast Cereal - Other	Any breakfast cereal not based on oats or wheat, such as rye or spelt flakes	0.0	1.5
Breakfast Cereal - Wheat	Any breakfast cereal based on wheat	0.0	1.5
Crackers - Oat	Any crackers or crispbread made predominantly from oat	0.2	1.9
Crackers/Crispbread - Rye	Any crackers or crispbread made predominantly from rye	4.9	7.7
Flours - Oat	Any foodstuff specified as containing oat flour	8.5	9.7
Flours - Rye	Any foodstuff specified as containing rye flour	14.9	16.7
Flours - Spelt	Any foodstuff specified as containing spelt flour	11.9	14.2
Flours - Wheat	Any foodstuff containing flour, eg. biscuit, wheat cracker, dumpling, pasta, pastry, wafer, sausage. Where flour type was not specified, wheat flour was assumed.	2.8	4.6
Multigrain Bread	Any bread or bread product specified to be multigrain	2.2	4.7
Rye-based Bread	Any bread or bread product specified to be based on rye	8.9	10.8
Wheat Bread	All wheat breads plus croissant, crumpet, donut, eclair, wraps, wheat tortilla, muffin split, pita bread, pizza base	1.7	3.7
Wholegrain - Oats	Any foodstuff containing whole oats	0.3	1.9
Wholegrain - Pearled Barley	Any foodstuff containing barley	0.0	1.5

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