

# Final Project Report

RRF 031 25: Survey of mycotoxin  
contamination in oat foods to inform risk  
analysis and support industry

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## Executive summary

The European Commission recently introduced maximum levels (ML) for the mycotoxins T-2/HT-2 in cereals and occurrence data are now needed to inform risk assessments on UK and European level. This study surveyed the occurrence of T-2 and HT-2, and co-occurrence with other mycotoxins, in oat-based food products.

From UK purchase data, the most-purchased individual food items for food categories oat biscuits (n=11), granola & muesli (n=10) and oatcakes (n=11) were identified, purchased and analysed for 20 mycotoxins using two different LC-MS/MS approaches.

T-2 and HT-2 were the most prevalent mycotoxins, found in 5/11 and 6/11 oat biscuit samples, 6/10 and 6/10 granola & muesli samples and 10/11 and 10/11 oatcake samples. The modified mycotoxin HT-2-glucoside commonly co-occurred in 10/32 oat food samples, while T-2-glucoside was not detected.

Overall, 3/32 oat food samples were contaminated at levels potentially exceeding the EC maximum levels for T-2+HT-2 and further confirmation by an accredited mycotoxin testing facility would be prudent to inform conclusions.

Future work needs to target farm-level interventions to support growers and reduce mycotoxin contamination in the cereal supply chain.

## Background

Chemical food contaminants including fungal mycotoxins are a persistent problem facing the cereal industry. Mycotoxins are frequently found in cereals and their toxicity is well established. Co-contamination of cereals with several mycotoxins as well as mycotoxin metabolites (so called modified mycotoxins) is also frequently reported.

The European Food Safety Authority (EFSA) have recognised the potential risk of mycotoxins for consumers and have recently updated their mycotoxin regulations and introduced maximum levels (ML) for T-2+HT-2 permitted in cereals and cereal foods (EC Reg 2023/915, see table 1). Modified mycotoxins are not presently included in these regulations as robust occurrence data are missing. These regulations directly impact exports of Scottish oat products into the European market.

Table 1. EC Maximum levels of T-2+HT-2 in oats and oat foods.

Commodity	Maximum level T-2 + HT-2 (µg/kg)
Unprocessed oat grains with inedible husk	1250

Oats placed on the market for the final consumer; Milling products of oats (including oat bran); Oat flakes; Bakery wares containing at least 75% milling products of oats	100
Breakfast cereals consisting at least 40 % of milling products of oat grains and whole oat grains	75
Breakfast cereals consisting of less than 40 % of milling products of oat grains and whole oat grains	50
Bakery wares containing at least 75 % milling products of oats	100
Bakery wares except products listed above (containing less than 75% oats)	20

[EC Reg 2023/915](#); amended by [EC Reg 2024/1038](#).

Following the introduction of these limits, EFSA calls for evidence of occurrence of these mycotoxins in relevant food categories to inform risk assessment. Simultaneously, Food Standard Agency (FSA) and Food Standards Scotland (FSS) are conducting a risk assessment for the UK on T-2/HT-2 which will inform future risk management decisions regarding regulatory alignment/divergence with European regulations. This process requires robust evidence on mycotoxin contamination in cereal foods and especially in oats and oat products, where these maximum levels will pose a challenge for UK food producers.

This project, co-constructed with FSS, aims to address this evidence gap and provide levels of mycotoxin contamination in relevant oat food categories. Data will be shared with FSS/FSA as well as EFSA and submitted to relevant future Calls for Evidence by the Committee on Toxicity (COT) and EFSA to inform the risk analysis on national and international levels.

## Methodology

### Activity 1: Sampling

A sampling plan was co-developed with FSS and input from the Food and Drink Federation (to represent industry priorities) to look at oat food products with lower percentage of oats than previously sampled (<75% oat content, based on new mycotoxin regulation introduced in the EU). The sampling plan was informed by purchasing data supplied from Worldpanel Numerator. Up-to-date purchase data (for the period up to September 2025) were obtained for the most-purchased individual food items in the three most relevant food categories: 1) oat biscuits, 2) granola & muesli, and 3) oatcakes. Due to limited data availability for Scottish purchase behaviour, whole GB purchase data were obtained for the total retail market and the 'Free from' market segment, representing the growing segment of gluten-free oat food products. Data were reviewed with FSS and

used to compile the final list of oat foods included in this study, containing 11 oat biscuit, and 10 granola & muesli, and 11 oatcake samples (see table 2).

Table 2. List of food items included

Rowett ID	Food Category	Product description	Organic/gluten free	% oat content
2025-1550	Oat biscuits	Milk Chocolate Oaties		34% oat flakes
2025-1553	Oat biscuits	Milk chocolate biscuits		30% wholegrain rolled oats
2025-1556	Oat biscuits	Wholemeal wheat flour Oaties		46% oat flakes
2025-1559	Oat biscuits	Dark Chocolate Chips Wholegrain Oats~Wheat Free biscuits		59% wholegrain oats
2025-1562	Oat biscuits	Chocolate Chips Oaties	Gluten Free	60% wholegrain oats
2025-1564	Oat biscuits	Oaties	Gluten Free	68% wholegrain oats
2025-1567	Oat biscuits	Biscuit Breaks Oats & Stem Ginger	Gluten Free	66% wholegrain oats
2025-1571	Oat biscuits	Rolled Oats Biscuits	Gluten Free	42% wholegrain rolled oats, 17% wholegrain oat flour
2025-1573	Oat biscuits	Oaties		42% oats
2025-1576	Oat biscuits	Oaty Flapjack slices	Gluten free	36% oats
2025-1578	Oat biscuits	Oaty Flapjacks	Gluten Free	43% wholegrain rolled oats
2025-1552	Granola, Muesli	Raisins & Almond Crunchy Oat Granola		70% wholegrain oat flakes
2025-1555	Granola, Muesli	No Added Sugar Swiss Style Muesli		oat flakes, % not reported
2025-1558	Granola, Muesli	Raisin & Almond Granola		68% wholegrain oat flakes
2025-1561	Granola, Muesli	Fruit & Nut Muesli		27% wholegrain oat flakes
2025-1563	Granola, Muesli	Tropical Granola		64% wholegrain oat flakes
2025-1566	Granola, Muesli	Mixed nuts granola	Gluten Free	59% oats
2025-1568	Granola, Muesli	Wheat free, fruit and seed muesli	Gluten Free	60% wholegrain oats
2025-1570	Granola, Muesli	Blueberries, Raspberries Strawberries & Yoghurt Chunks granola	Organic, Gluten free	60% wholegrain rolled oats
2025-1575	Granola, Muesli	Wheat and milk free fruity muesli	Gluten Free	53% oats
2025-1580	Granola, Muesli	Almond, Apple & Raisin Granola with Coconut chips & cinnamon	Gluten Free	66% rolled oats
2025-1551	Oatcakes	Rough oatcakes		90% wholegrain oats
2025-1554	Oatcakes	Cheese oatcakes		71% wholegrain oats
2025-1557	Oatcakes	Wheat Free Fruit & Seed Oatcakes		68% wholegrain oats
2025-1560	Oatcakes	Super seeded oatcakes	Organic	76% wholegrain oats
2025-1565	Oatcakes	Cheese oatcakes	Gluten Free	72% wholegrain oats
2025-1569	Oatcakes	Thin oatcakes		76% wholegrain oats
2025-1572	Oatcakes	Cheese oatcakes		70% wholegrain oats
2025-1574	Oatcakes	Black pepper oatcakes bites		58% oatmeal
2025-1577	Oatcakes	Thin Oatcakes		74% oats

2025-1579	Oatcakes	Original Oatcakes	Gluten Free	90% wholegrain oats
2025-1581	Oatcakes	Super seeded oatcakes	Organic, Gluten free	77% wholegrain oats

## Activity 2.: Method validation (2a) & sample analysis (2b)

### *Mycotoxin standards*

All mycotoxins used, T-2 toxin (T-2), [<sup>13</sup>C<sub>24</sub>] T-2, HT-2 toxin (HT-2), [<sup>13</sup>C<sub>22</sub>] HT-2, diacetoxyscirpenol (DAS), [<sup>13</sup>C<sub>19</sub>] DAS, neosolaniol (NEO), deoxynivalenol (DON), [<sup>13</sup>C<sub>15</sub>] DON, nivalenol (NIV), [<sup>13</sup>C<sub>15</sub>] NIV, zearalenone (ZEN), [<sup>13</sup>C<sub>18</sub>] ZEN, α-zearalenone (α-ZEL), β-zearalenone (β-ZEL), aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), [<sup>13</sup>C<sub>17</sub>] AFB<sub>1</sub>, aflatoxin G<sub>1</sub> (AFG<sub>1</sub>), ochratoxin (OTA), fumonisin B<sub>1</sub> (FB<sub>1</sub>) and fumonisin B<sub>2</sub> (FB<sub>2</sub>) were purchased from Romer Labs Ltd., Tulln, Austria. DAS-3-α, D-glucoside (DAS-Glc), T-2-3-α, D-glucoside (T-2-Glc) and HT-2-3-β, D-glucoside (HT-2-Glc) were obtained from Dr. Mark Busman and Dr Susan McCormick, Mycotoxin Prevention and Applied Microbiology Unit, USDA-ARS-NCAUR in the USA. NIV-3-β, D-glucoside (NIV-Glc) was obtained from Dr. Tomoya Yoshinari, National Institute of Health Sciences, Japan. ZEN-14-β, D-glucoside (ZEN-Glc), α-ZEL-Glc and β-ZEL-Glc standard used in this study were previously synthesised as part of FSA-funded project FS102101. Working solutions for all mycotoxins were prepared in acetonitrile (ACN) and stored at 4 °C.

Eight-point calibration curves (DON 1.95–375 ng/mL; T-2, HT-2, T2-Glc, HT-2-Glc, 0.21–40 ng/mL; ZEN, αZEL, β-ZEL 0.39–75 ng/mL; FB<sub>1</sub> 1.79–343.4 ng/mL; FB<sub>2</sub> 0.92–176.2 ng/mL; OTA 0.04–8 ng/mL; AFB<sub>1</sub>, AFG<sub>1</sub> 0.01–1.5 ng/mL;) were used to quantify all analytes.

### *Modified AOAC method*

#### Sample processing

On receipt, samples were logged and unique identifying numbers (IDs) assigned. All samples were ball milled (Retsch model MM400) for one minute (×3), with cleaning between samples to avoid cross contamination. All samples were stored at room temperature until further analysis.

Samples were processed in accordance with the recently published AOAC method (Leeman et al., 2025) with minor modifications. In brief, 2.5 g of homogenised sample were extracted with 10 mL of extraction solvent (50% acetonitrile) for 30 min at 300 rpm using an orbital shaker, IKA® VXR basic (Thomson Scientific, England, UK). Samples were centrifuged at room temperature (3000 × g, 10 minutes using a PK 121R centrifuge, Thomson Scientific, EC) and 4 mL of supernatant diluted in 46 mL phosphate buffered saline (PBS) (Sigma Aldrich) and filtered through glass microfiber filter paper (GF/A

diameter 110 mm, Whatman). The filtered extracts (50 mL) were then loaded onto immunoaffinity columns (IAC, R- Biopharm Rhone Ltd, UK) at a flow rate of approximately 2 mL/min, washed with 20 mL ammonium acetate (20 mM) at a flow rate of 5 mL/min and air was passed through the column before eluting with 1.5 mL concentrated methanol (with 3-fold backflushing). An additional 1.5 mL of water was passed through the column, giving a total eluent volume of 3 mL (in 50% methanol).

500  $\mu$ L aliquots of eluents (including samples, spikes, and blank extracts) were evaporated to dryness (on a Reacti-Therm™ Model 18790 heating module and Reacti-Vap™ Model 18780 evaporating unit) under a stream of Nitrogen at 50°C, reconstituted in 100  $\mu$ L 50% methanol and then transferred into LC vials with glass inserts. The contents of the vials were mixed and 20  $\mu$ L thereof injected into the LC-MS/MS system.

Mycotoxins were analysed using a Shimadzu Nexera X2 LC system coupled to a Shimadzu 8060 mass spectrometer with an ESI source as described by (Leeman et al., 2025). Chromatographic separation was achieved on a Phenomenex Luna Omega C18 column (3  $\mu$ m Polar, 100  $\times$  3 mm) using 1mM Ammonium Formate, 0.1% Formic, 5% Methanol (A) and 1mM Ammonium Formate, 0.1% Formic, 98% Methanol (B). After 0.5 min at 60% A and 40% B, solvent B was increased to 100% over 4 min, held for 2 min, and followed by a 4 min re-equilibration. The flow rate was 600  $\mu$ L/min with a 20  $\mu$ L injection. The MS operated in positive ion modes under with similar ESI conditions. Quantification was performed using MRM as described by (Leeman et al., 2025).

#### Method validation

For recovery experiments, selected matrix samples (1 oat biscuit, 1 oat muesli, 1 oatcake) were spiked in triplicate (spike levels described in table 3). Blanks for the same samples were also analysed in triplicate. For each spike, 12.5  $\mu$ L of the spike solution was added onto the surface of 2.5 g of sample and left uncovered in the fume hood overnight to allow the solvent to evaporate. Spiked samples were then processed as described above.

To determine matrix effects, eight-point matrix-matched calibration curves were prepared in triplicate for selected matrix samples (1 oat biscuit, 1 oat muesli, 1 oatcake) in blank extracts at the same concentrations as the standard multi-mycotoxin calibration curves. The 20  $\mu$ L thereof injected into the LC-MS/MS system.

Matrix effects were further analysed by assessing signal suppression/enhancement (SSE%) by comparing slopes of solvent standard curves for each mycotoxin with slopes of matrix-matched standard curves. Limit of quantification (LOQ) was determined as a signal-to-noise ratio of 1/10 for each mycotoxin in each matrix. LOQ values for both methods are given in results tables 4 and 6.

### *Stable-isotope dilution assay (SIDA) method*

To facilitate testing the oat products for T-2-Glc, HT-2-Glc and a range of other modified mycotoxins, an established SIDA method was used (Daud et al. 2023).

#### Sample processing

In brief, 2.0 g milled samples were extracted with 8 mL of extraction solvent (79% acetonitrile, 20% H<sub>2</sub>O, 1% acetic acid) for 90 min (on an orbital shaker IKA® VXR basic, Thomson scientific, UK at 1200 rpm). Samples were centrifuged at room temperature (2000 × g, 5 minutes, PK 121R centrifuge, Thomson scientific, EC). 100 µL of extracts were dried under nitrogen, reconstituted in 200 µL of 10% acetonitrile and 1% acetic acid and spiked with the internal standard mix and 15 µL thereof injected into the LC-MS/MS system.

Mycotoxins and their [<sup>13</sup>C]-labelled standards were analysed using a Shimadzu Nexera X2 LC system coupled to a Shimadzu 8060 mass spectrometer with an ESI source as described by (Daud et al., 2023). Chromatographic separation was achieved on a Phenomenex Gemini C18 column (150 × 3 mm, 3 µm) using 0.1 mM ammonium acetate (A) and methanol (B). After 2 min at 100% A, solvent B was increased to 100% over 12 min, held for 3 min, and after 15 min there was a 0.1 min step where B was dropped to 0% then held to a total time of 17 min. The flow rate was 400 µL/min with a 15 µL injection. The MS operated in positive and negative ion modes under with similar ESI conditions and quantification was performed using MRM. Eight-point calibration curves (DON 0.625–500 ng/mL; HT-2, HT-2-Glc, 0.3125–250 ng/mL; NIV, T-2, T-2-Glc, ZEN, 0.1563–125 ng/mL) were used to quantify all analytes. Stable-isotope labelled internal standards were used as follows: DON <sup>13</sup>C<sub>15</sub> (50 ng/mL) was used to quantify DON; NIV <sup>13</sup>C<sub>15</sub> (50 ng/mL) was used to quantify NIV; HT-2 <sup>13</sup>C<sub>22</sub> (50 ng/mL) was used to quantify HT-2 and HT-2-Glc; T-2 <sup>13</sup>C<sub>24</sub> (50 ng/mL) was used to quantify T-2 and T-2-Glc; ZEN <sup>13</sup>C<sub>18</sub> (25 ng/mL) was used to quantify ZEN.

#### Method validation

Both methods are validated in-house, but not fully accredited. Recovery was assessed by spiking 0.5 g of sample in triplicate with a multi-mycotoxin standard mix (spike levels described in table 5). After spiking, the samples were incubated for 30 minutes at 37°C to evaporate the spiking solvent and then extracted as described above.

## **Results**

### Activity 3. Data analysis and reporting

### Results using the modified AOAC method

Three representative matrix samples (matrix 1 oat biscuit, matrix 2 oat muesli, matrix 3 oatcake) were spiked in triplicate to determine recovery and matrix effects in the method.

Table 3. Spiking levels, mean recovery, and mean matrix effect (signal suppression/enhancement SSE) using the modified AOAC method.

	DON	T-2	HT-2	ZEN	$\alpha$ -ZEL	$\beta$ -ZEL	AFB <sub>1</sub>	AFG <sub>1</sub>	OTA	FB <sub>1</sub>	FB <sub>2</sub>
Spike level (ng/g food)	37.5	4	4	7.5	7.5	7.5	0.15	0.15	0.8	34.3	17.6
Matrix 1 recovery %	101.5	48.7 <sup>a</sup>	12.4 <sup>a</sup>	31.8 <sup>a</sup>	31.6 <sup>a</sup>	65.6	42.3 <sup>a</sup>	51.5	80.2	99.8	84.6
Matrix 2 recovery %	102.1	83.8	61.7	81.8	56.6	96.6	66.7	75.8	86.0	93.5	82.5
Matrix 3 recovery %	108.3	88.1	25.0 <sup>a</sup>	74.5	61.9	98.9	56.3	61.8	87.1	96.2	88.9
Matrix 1 SSE %	104.5	59.6	80.2	67.9	81.2	87.1	49.3 <sup>a</sup>	57.4	83.2	94.1	90.4
Matrix 2 SSE %	106.4	67.5	91.5	67.0	81.0	91.7	58.3	60.3	87.9	96.4	94.0
Matrix 3 SSE %	105.1	83.9	94.2	83.7	103.8	110.6	86.5	87.6	96.6	97.9	93.9

<sup>a</sup> recovery outside the acceptable range of 60-140%.

Recoveries were observed to be lower for matrix 1 (oat biscuit) compared to other matrices for the majority of mycotoxins tested. Poor recovery was observed for HT-2 across all matrices, especially matrix 1 (oat biscuit) and matrix 3 (oatcake), hence results in table 4 were not corrected for recovery. T-2-Glc and HT-2-Glc were also included in spiking experiments, but recovery was highly variable and outside the acceptable range (0-41%) in all matrices tested due to low binding to immunoaffinity columns. Results for these modified mycotoxins are not presented.

Matrix effects were further analysed by assessing signal suppression/enhancement (SSE%) by comparing slopes of solvent standard curves for each mycotoxin with slopes of matrix-matched standard curves. Very minor matrix effects were observed for most mycotoxins (table 3) due to the use of immunoaffinity column clean-up. AFB<sub>1</sub> in matrix 1 was the only observation of significant signal suppression (<50%).

In oat food samples, T-2 and HT-2 toxins were most prevalent mycotoxins quantifiable in 5/11 and 6/11 oat biscuit samples, 6/10 and 6/10 granola & muesli samples and 10/11 and 10/11 oatcake samples (table 4). Recovery of HT-2 from spiked oat biscuit and oatcake samples were low (table 3) and results presented in table 4 can only be considered semi-quantitative, leading to a likely underestimation of HT-2 as well as the sum of T-2+HT-2 in oat biscuits and oatcakes. Keeping this in mind, none of the samples analysed would exceed the maximum permitted levels for T-2+HT-2, although HT-2 levels are likely to be higher than reported here.

Table 4. Mycotoxin co-contamination in individual oat food samples (as ng/g food) using the modified AOAC method.

Sample ID	Food category	DON	T-2	HT-2	T-2+HT-2	ZEN	AFB <sub>1</sub>	OTA	FB <sub>1</sub>	FB <sub>2</sub>
	LOQ (ng/g food)	18.75	1.00	1.00		7.50	0.08	0.20	1.07	0.54
2025-1550	Oat biscuits <sup>a</sup>	<LOQ	<LOQ	1.36 <sup>e</sup>	1.86 <sup>f</sup>	nd	nd	<LOQ	nd	nd
2025-1553	Oat biscuits <sup>a</sup>	nd	2.28	<LOQ	2.78 <sup>f</sup>	nd	nd	nd	nd	nd
2025-1556	Oat biscuits <sup>a</sup>	<LOQ	2.16	1.68 <sup>e</sup>	3.84	nd	nd	nd	nd	nd
2025-1559	Oat biscuits <sup>a</sup>	nd	7.58	4.16 <sup>e</sup>	11.74	nd	nd	<LOQ	nd	nd
2025-1562	Oat biscuits <sup>a</sup>	nd	<LOQ	2.22 <sup>e</sup>	2.72 <sup>f</sup>	nd	<LOQ	nd	nd	nd
2025-1564	Oat biscuits <sup>a</sup>	nd	9.45	3.05 <sup>e</sup>	12.50	nd	nd	nd	nd	nd
2025-1567	Oat biscuits <sup>a</sup>	nd	<LOQ	<LOQ	<LOQ	nd	nd	nd	nd	nd
2025-1571	Oat biscuits <sup>a</sup>	nd	<LOQ	<LOQ	<LOQ	nd	nd	nd	nd	nd
2025-1573	Oat biscuits <sup>a</sup>	nd	<LOQ	<LOQ	<LOQ	nd	nd	nd	nd	nd
2025-1576	Oat biscuits <sup>a</sup>	nd	<LOQ	<LOQ	<LOQ	nd	nd	nd	nd	nd
2025-1578	Oat biscuits <sup>a</sup>	nd	8.89	3.89 <sup>e</sup>	12.78	nd	nd	nd	nd	nd
2025-1552	Granola, Muesli <sup>b</sup>	nd	3.70	4.18	7.88	nd	nd	nd	nd	nd
2025-1555	Granola, Muesli <sup>b</sup>	nd	4.54	8.18	12.72	nd	nd	nd	nd	nd
2025-1558	Granola, Muesli <sup>b</sup>	nd	4.66	6.96	11.62	nd	nd	nd	nd	nd
2025-1561	Granola, Muesli <sup>c</sup>	nd	1.01	1.71	2.72	nd	nd	nd	nd	nd
2025-1563	Granola, Muesli <sup>b</sup>	nd	10.32	9.11	19.43	nd	nd	nd	nd	nd
2025-1566	Granola, Muesli <sup>b</sup>	nd	1.34	1.83	3.17	nd	nd	<LOQ	nd	nd
2025-1568	Granola, Muesli <sup>b</sup>	nd	<LOQ	<LOQ	<LOQ	nd	nd	nd	nd	nd
2025-1570	Granola, Muesli <sup>b</sup>	nd	nd	nd	nd	nd	nd	nd	nd	nd
2025-1575	Granola, Muesli <sup>b</sup>	nd	nd	nd	nd	nd	nd	nd	nd	nd
2025-1580	Granola, Muesli <sup>b</sup>	nd	nd	nd	nd	nd	nd	nd	nd	nd
2025-1551	Oatcakes <sup>d</sup>	nd	15.93	14.97 <sup>e</sup>	30.90	nd	nd	0.246	nd	nd
2025-1554	Oatcakes <sup>a</sup>	nd	9.78	7.66 <sup>e</sup>	17.44	nd	nd	0.269	nd	nd
2025-1557	Oatcakes <sup>a</sup>	<LOQ	3.90	5.58 <sup>e</sup>	9.47	nd	nd	<LOQ	nd	nd
2025-1560	Oatcakes <sup>d</sup>	nd	1.43	1.00 <sup>e</sup>	2.43	nd	nd	nd	nd	nd
2025-1565	Oatcakes <sup>a</sup>	nd	<LOQ	<LOQ	<LOQ	nd	nd	nd	nd	nd
2025-1569	Oatcakes <sup>d</sup>	nd	2.49	3.14 <sup>a</sup>	5.63	nd	nd	<LOQ	nd	nd
2025-1572	Oatcakes <sup>a</sup>	nd	3.59	2.93 <sup>a</sup>	6.52	nd	nd	<LOQ	nd	nd
2025-1574	Oatcakes <sup>a</sup>	<LOQ	3.14	1.66 <sup>a</sup>	4.80	nd	nd	<LOQ	nd	nd
2025-1577	Oatcakes <sup>a</sup>	nd	1.40	1.53 <sup>a</sup>	2.93	nd	nd	<LOQ	nd	nd
2025-1579	Oatcakes <sup>d</sup>	nd	2.33	1.29 <sup>a</sup>	3.62	nd	nd	0.248	nd	nd
2025-1581	Oatcakes <sup>d</sup>	nd	2.97	2.73 <sup>a</sup>	5.70	nd	nd	nd	nd	nd

<sup>a</sup> indicates products with where EC ML for T-2+HT-2 of 20 ng/g would apply

<sup>b</sup> indicates products with where EC ML for T-2+HT-2 of 75 ng/g would apply

<sup>c</sup> indicates products with where EC ML for T-2+HT-2 of 50 ng/g would apply

<sup>d</sup> indicates products with where EC ML for T-2+HT-2 of 100 ng/g would apply

<sup>e</sup> values for HT-2 in oat biscuits and oatcakes were not corrected for recovery (as <30%)

<sup>f</sup> for sum T-2+HT-2 calculations if one toxin is <LOQ it is replaced by 1/2 LOQ

ZEN, FB<sub>1</sub> and FB<sub>2</sub> were not detected in any samples. One sample was contaminated with AFB<sub>1</sub> below LOQ. Ochratoxin a was quantifiable in three oatcake samples and detectable below LOQ in eight further samples, but no samples exceeded the maximum permitted levels for OTA in bakery wares (2-4 ng/g, EC reg 2023/915).  $\alpha$ -ZEL,  $\beta$ -ZEL and AFG<sub>1</sub> were measured but not detected in any sample (data not shown).

### Results using the SIDA method

A stable isotope dilution (SIDA) method previously developed for unprocessed cereals (Daud et al. 2023) was used to confirm the results obtained with the modified AOAC method. Spiking experiments show acceptable recoveries for all mycotoxins assessed except DON-Glc and NIV-Glc, although some recoveries were high (> 140% for T-2 and ZEN in oatcakes and T-2-Glc in oat biscuits (table 5).

Table 5. Spiking levels and mean recovery using the SIDA method (Daud et al. 2023).

	<b>DON</b>	<b>DON-Glc</b>	<b>NIV</b>	<b>NIV-Glc</b>	<b>T-2</b>	<b>HT-2</b>	<b>T2-Glc</b>	<b>HT2-Glc</b>	<b>ZEN</b>	<b>ZEN-Glc</b>
Spiking level (ng/g food)	300.0	75.0	75.0	75.0	75.0	150.0	75.0	150	75.0	75.0
Matrix 1 recovery %	107.7	13.6 <sup>a</sup>	nd <sup>a</sup>	nd <sup>a</sup>	139.4	131.0	147.5	134.4	127.2	125.8
Matrix 2 recovery %	96.4	29.4 <sup>a</sup>	85.2	19.2 <sup>a</sup>	119.2	115.9	107.2	109.3	94.7	147.4
Matrix 3 recovery %	126.0	20.6 <sup>a</sup>	109.9	30.0 <sup>a</sup>	145.2	131.5	108.8	130.4	145.4	104.4

<sup>a</sup>recovery outside the acceptable range

Using the SIDA method (table 6), T-2 and HT-2 toxins were confirmed as the most commonly quantifiable mycotoxins in oat biscuits (3/11 and 4/11), granola & muesli (4/10 and 4/10), and oatcakes (5/11 and 7/11). This prevalence is lower compared to results using the modified AOAC method due to higher LOQ for the SIDA method. Samples contaminated with high levels of T-2 and HT-2 were consistently identified by both methods (e.g. samples 2025-1551, 2025-1554, 2025-1563). However, concentrations of HT-2 measured with the SIDA method were higher compared to the modified AOAC method. A recent FSS report (MacDonald et al. Fera 2022) also reports slightly higher levels of T-2 and HT-2 in oat food products using similar SIDA and AOAC methods, although the discrepancy is not as pronounced as in the current study. The discrepancy is partly explained by the low recovery of HT-2 from two matrices using the modified AOAC method and not correcting results for recovery.

The sum of T-2+HT-2 could potentially be close to or above the new EC ML for some samples. Overall, the potential exceedances (3/32 samples, 9.4% using the SIDA method) are higher than in the FSS report on a smaller set of similar oat food products (0/10 samples MacDonald et al. Fera 2022). Further confirmation of potential ML exceedances by an accredited mycotoxin testing facility would be prudent to inform conclusions.

Modified mycotoxins T-2-Glc and HT-2-Glc were also analysed using the SIDA method. T-2-Glc was not quantifiable in any samples, confirming the earlier FSS report (MacDonald et al. FERA 2022). HT-2-Glc was found in 10/32 samples, always co-occurring with HT-2 at ratios of 64-299% of HT-2. No commercial reference standard is available for HT-2-Glc, but results from this survey clearly indicate significant co-contamination with this modified mycotoxin in oat foods.

Table 6. Mycotoxin co-contamination in individual oat food samples (as ng/g food) using the SIDA method.

Sample ID	Food category	DON	NIV	T-2	HT-2	T-2+HT-2	T2-Glc	HT2-Glc	ZEN
	LOQ (ng/g food)	25	12.5	3.1	6.3		12.5	12.5	6.3
2025-1550	Oat biscuits <sup>a</sup>	nd	nd	nd	<LOQ		nd	20.55	<LOQ
2025-1553	Oat biscuits <sup>a</sup>	nd	nd	nd	<LOQ		nd	nd	nd
2025-1556	Oat biscuits <sup>a</sup>	nd	nd	nd	<LOQ		nd	nd	nd
2025-1559	Oat biscuits <sup>a</sup>	nd	nd	5.59	16.73	<b>22.33</b>	nd	nd	nd
2025-1562	Oat biscuits <sup>a</sup>	nd	nd	nd	10.75	10.75	nd	nd	nd
2025-1564	Oat biscuits <sup>a</sup>	nd	nd	6.41	10.07	16.49	nd	17.57	nd
2025-1567	Oat biscuits <sup>a</sup>	nd	nd	nd	nd		nd	nd	nd
2025-1571	Oat biscuits <sup>a</sup>	nd	nd	nd	<LOQ		nd	nd	nd
2025-1573	Oat biscuits <sup>a</sup>	nd	nd	nd	<LOQ		nd	nd	nd
2025-1576	Oat biscuits <sup>a</sup>	nd	nd	nd	nd		nd	nd	nd
2025-1578	Oat biscuits <sup>a</sup>	nd	nd	6.32	12.15	18.47	nd	11.42	nd
2025-1552	Granola, Muesli <sup>b</sup>	nd	nd	7.07	11.73	18.80	nd	30.70	nd
2025-1555	Granola, Muesli <sup>b</sup>	nd	nd	7.54	13.59	21.13	nd	nd	nd
2025-1558	Granola, Muesli <sup>b</sup>	nd	nd	7.69	15.66	23.35	<LOQ	42.57	nd
2025-1561	Granola, Muesli <sup>c</sup>	nd	nd	nd	<LOQ		nd	24.77	nd
2025-1563	Granola, Muesli <sup>b</sup>	nd	nd	12.65	20.21	32.86	nd	60.48	nd
2025-1566	Granola, Muesli <sup>b</sup>	nd	nd	nd	<LOQ		nd	nd	nd
2025-1568	Granola, Muesli <sup>b</sup>	nd	nd	nd	nd		nd	nd	nd
2025-1570	Granola, Muesli <sup>b</sup>	nd	nd	nd	nd		nd	nd	nd
2025-1575	Granola, Muesli <sup>b</sup>	nd	nd	nd	nd		nd	nd	nd
2025-1580	Granola, Muesli <sup>b</sup>	nd	nd	nd	nd		nd	nd	nd
2025-1551	Oatcakes <sup>d</sup>	nd	nd	22.78	45.99	68.77	nd	29.39	nd
2025-1554	Oatcakes <sup>a</sup>	nd	nd	16.48	31.91	<b>48.39</b>	nd	30.89	nd

2025-1557	Oatcakes <sup>a</sup>	nd	nd	7.26	23.33	<b>30.60</b>	nd	63.36	nd
2025-1560	Oatcakes <sup>d</sup>	nd	nd	nd	<LOQ		nd	nd	nd
2025-1565	Oatcakes <sup>a</sup>	nd	nd	nd	nd		nd	nd	nd
2025-1569	Oatcakes <sup>d</sup>	nd	nd	nd	10.34	10.34	nd	nd	nd
2025-1572	Oatcakes <sup>a</sup>	nd	nd	4.98	9.81	14.79	nd	nd	nd
2025-1574	Oatcakes <sup>a</sup>	nd	nd	nd	9.18	9.18	nd	nd	nd
2025-1577	Oatcakes <sup>a</sup>	nd	nd	nd	<LOQ		nd	nd	nd
2025-1579	Oatcakes <sup>d</sup>	nd	nd	3.25	<LOQ	6.40 <sup>e</sup>	nd	nd	nd
2025-1581	Oatcakes <sup>d</sup>	nd	nd	nd	6.56	6.56	nd	nd	nd

<sup>a</sup> indicates products with where EC ML for T-2+HT-2 of 20 ng/g would apply

<sup>b</sup> indicates products with where EC ML for T-2+HT-2 of 75 ng/g would apply

<sup>c</sup> indicates products with where EC ML for T-2+HT-2 of 50 ng/g would apply

<sup>d</sup> indicates products with where EC ML for T-2+HT-2 of 100 ng/g would apply

<sup>e</sup> for sum T-2+HT-2 calculations if one toxin is <LOQ it is replaced by 1/2 LOQ

Levels exceeding the EC ML are highlighted in **bold**.

DON, NIV, and ZEN-Glc were not detected in any sample, ZEN was detectable but <LOQ in one sample (table 6). NIV-Glc was detected in one sample, but data are not shown due to unacceptable recovery (table 5). DON-Glc, DAS, DAS-Glc,  $\alpha$ -ZEL and  $\beta$ -ZEL, ZEN-Glc,  $\alpha$ -ZEL-Glc and  $\beta$ -ZEL-Glc were measured but not detected in any sample (data not shown).

## Summary

This study assessed the multi-mycotoxin contamination in oat food products which form an important part of the UK retail market. The study used two methods for mycotoxin extraction and quantification, and both datasets confirm high prevalence of T-2 and HT-2 in oat food products. Some oat food products could potentially exceed the ML for T-2+HT-2 when determined with the SIDA method, while the modified AOAC method is likely to underestimate HT-2 levels due to low recovery from some oat food matrices. Further confirmation by an accredited testing facility is required to ascertain if ML exceedances have occurred in this sample set.

Co-contamination with HT-2-Glc was frequently observed (10/32 samples or 31%) further increasing the potential risk of human exposure.

OTA was detected at low but quantifiable levels in three oatcake samples and traces of OTA <LOQ were detected in five further oatcake samples, one granola and two biscuit samples.

Other *Fusarium* mycotoxins (DON, NIV, DAS, NEO, ZEN), their modified forms and other mycotoxins (AFB<sub>1</sub>, AFG<sub>1</sub>, FB<sub>1</sub>, FB<sub>2</sub>) were not detected in any oat food products.

The high prevalence of T-2/HT-2 toxins in final food products highlights the issues of contamination and carry-over across the entire cereal supply chain. Cereal processing steps of de-husking remove part of the mycotoxin contamination, but a considerable proportion still enters the food chain. Data gaps remain regarding the levels of mycotoxins in cereal ingredients used in cereal food manufacture and further work is needed to quantify mycotoxin carry-over into final food products.

A recent FSA/FSS risk assessment for T-2/HT-2 (COT/2026/02) highlights a potential concern for consumer health, especially in infants and toddlers, and for some oat-based foods in adults and vegetarians/vegans (mainly oat porridge). The risk assessment uses data on T-2/HT-2 levels in ready-to-eat foods (including oat porridge, biscuits, muesli and infant cereals) and considers total dietary exposure, but concludes that the evidence base is very limited and more data are required. Data from this project will be shared with FSA/FSS and future work is required to further strengthen the evidence base.

### **Recommendations**

Further work is needed to quantify the levels of mycotoxins in cereal ingredients and their carry-over into final food products, and potential exceedances of MLs need to be confirmed by an accredited testing lab.

Tackling the mycotoxin problem in cereal production needs to focus on the primary producer and offer support to minimise the contamination risk for cereal commodities. Climate is an important driver of fungal disease and mycotoxin risk, but agronomy practices also influence outcomes. Identifying potential low-risk varieties of oats can support growers in their decision making. Changes to regenerative agronomy practices, designed to minimize negative impact on soil health, biodiversity and sustainability, need to be assessed for their potential impact of mycotoxin risks in cereal commodities.

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