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# Effect of wheat species (*Triticum aestivum* vs *T. spelta*), farming system (organic vs conventional) and flour type (wholegrain vs white) on composition of wheat flour; results of a retail survey in the UK and Germany – 1. Mycotoxin content

Juan Wang[a,b,*], Gultakin Hasanalieva[b,c], Liza Wood.[b], Emilia Markellou.[d], Per Ole Iversen[e,f], Aksel Bernhoft[g], Chris Seal[a], Marcin Baranski.[b,h], Vanessa Vigar[i], Laura Ernst[i], Adam Willson[i], Bronwyn J. Barkla[i,j], Carlo Leifert[e,i,j,*], Leonidas Rempelos[b,*]

[a] Human Nutrition Research Centre, Institute of Cellular Medicine, Newcastle upon Tyne NE2 4HH, UK
[b] School of Agriculture, Food & Rural Development, Nafferton Ecological Farming Group, Newcastle University, Nafferton Farm, Stocksfield, UK
[c] Department of Sustainable Crop and Food Protection, Faculty of Agriculture, Food and Environmental Sciences, Universita Catollica del Sacro Cuore, I-29122 Piacenza, Italy
[d] Department of Phytopathology, Benaki Phytopathological Institute (BPI), Athens, Greece
[e] Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway
[f] Department of Haematology, Oslo University Hospital, Oslo, Norway
[g] Department of Animal Health and Food Safety, Norwegian Veterinary Institute, P.O.Box 750 Sentrum, NO-0106 Oslo, Norway
[h] Department of Functional and Organic Food, Institute of Human Nutrition Sciences, Warsaw University of Life Sciences, Nowoursynowska 159c, 02-776 Warsaw, Poland
[i] Centre for Organics Research, Southern Cross University, Military Rd., Lismore, NSW, Australia
[j] Southern Cross Plant Science, Southern Cross University, Military Rd., Lismore, NSW, Australia

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ABSTRACT

Wheat is one of the main dietary sources for mycotoxins that can cause adverse health effects in humans. Here we report results of a 3-year survey which compared the effects of flour type (whole-grain vs white), wheat species (common vs spelt), and farming system (organic vs conventional) on mycotoxin concentrations in UK and German wheat flour brands.

Wholegrain, conventional and organic flour contained 124, 31 and 9% higher concentrations of T-2/HT-2, DON and ZEA respectively, but concentrations of the three *Fusarium* mycotoxins assessed were ~10 times lower than the EC-maximum contamination levels (MCL). Thirty one percent of flour samples had Ochratoxin A (OTA) concentrations above the MCL (3 μg/kg), but OTA levels were no affected by wheat species, farming system and flour type. Results suggest that both organic and conventional primary production methods and postharvest quality assurance systems are effective for maintaining *Fusarium* mycotoxins, but not OTA concentrations, below the MCL.

## 1. Introduction

Cereal products are the most important source of nutrients and energy in the human diet globally, and common wheat (*Triticum aestivum* L.) is the main cereal species used for human consumption in Europe (McKevith, 2004). Wheat flour is the main ingredient in many staple food products such as breakfast cereals, porridge, pasta, noodles, bread and other bakery products. Currently the majority of wheat products are based on white flour (where the bran and germ are removed from the endosperm during milling) rather than whole-grain

* Corresponding authors at: Human Nutrition Research Centre, Human Nutrition Research Centre, Institute of Cellular Medicine, Newcastle upon Tyne NE2 4HH, UK (J. Wang), School of Agriculture, Food & Rural Development, Nafferton Ecological Farming Group, Newcastle University, Nafferton Farm, Stocksfield, UK (Leonidas Rempelos), Centre for Organics Research, Southern Cross University, Military Rd., Lismore, NSW, Australia (C. Leifert).

*E-mail addresses:* j.wang28@outlook.com (J. Wang), gultekin.hasanaliyeva@gmail.com (G. Hasanalieva), belwood@ucdavis.edu (L. Wood.), e.markellou@bpi.gr (E. Markellou.), p.o.iversen@medisin.uio.no (P.O. Iversen), aksel.bernhoft@vetinst.no (A. Bernhoft), chris.seal@newcastle.ac.uk (C. Seal), marcin_baranski@sggw.pl (M. Baranski.), vanessa.vigar@scu.edu.au (V. Vigar), laura.ernst@scu.edu.au (L. Ernst), adam.willson@scu.edu.au (A. Willson), bronwyn.barkla@scu.edu.au (B.J. Barkla), carlo.leifert@gmail.com (C. Leifert), leonidas.rempelos@newcastle.ac.uk (L. Rempelos).

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flour (where all three components are retained after milling) (Whole Grains Council, 2019). However, the consumption of whole-grain products is recommended by nutritionists, because an increasing number of scientific studies have shown associations between whole-grain consumption and a range of chronic diseases including type 2 diabetes, certain cancers and cardiovascular diseases (Borneo & Len, 2012; Della Pepa, Vetrani, Vitale & Riccardi, 2018; Jones & Engleson, 2010). However, the most recent meta-analysis of randomised clinical trials reported no significant effects of wholemeal consumption on obesity measures (Sadeghi et al., 2020). The health benefits from whole-grain consumption are thought to be associated with the fibre, mineral and (poly)phenol/antioxidant content which is mainly in the bran and germ fraction of the grain (Borneo & Len, 2012; Jones & Engleson, 2010; Thielecke & Nugent, 2018).

However, there is a concern about whole-grain consumption resulting in an increased intake of undesirable compounds that can be found in the bran, especially mycotoxins (Cheli, Pinotti, Rossi, & Dell'Orto, 2013; Edwards et al., 2011; Thielecke & Nugent, 2018). For example, a recent wheat flour survey carried out in China compared 35 whole-grain and 50 "refined" (white) flour samples and found that whole-grain flour contained higher mycotoxin concentrations and was more frequently contaminated with multiple mycotoxin residues than white flour (Zhang et al., 2019). There are very few published studies, which have compared mycotoxin loads in whole-grain and white flour brands in Europe and a study by Edwards et al. (2011) in the UK concluded that relative levels of mycotoxins in the endosperm and bran fraction of wheat grains may differ significantly between years. Surveys at retail level are therefore essential to provide sound advice on the potential impact of mycotoxin intake of European whole-grain consumers.

Mycotoxins are secondary metabolites produced by specific fungi that infect cereal grain in the field and/or during post-harvest storage (Bryla, Waśkiewicz, Ksieniewicz-Woźniak, Szymczyk, & Jędrzejczak, 2018; European Commission, 2006; Magan & Aldred, 2005, 2007; Rachoń, Bobryk-Mamczarz, & Szumiło, 2016; Tangni, Pussemier, Schneider, & Larondelle, 2013). Dietary intake of mycotoxins above certain thresholds can have serious acute and chronic negative health impacts in both humans and livestock, including gastro-intestinal, hormonal, immunosuppressive, mutagenic and carcinogenic effects and cereals are the main source for mycotoxins intake (EFSA, 2006, 2016; European Commission, 2006; Thielecke & Nugent, 2018). The most important mycotoxins found in wheat are Deoxynivalenol (DON), Zearalenone (ZEA) and T-2/HT-2 toxin that are produced by *Fusarium* spp, which infect cereal grains in the field (Bottalico & Perrone, 2002; Bryla et al., 2018; EFSA, 2016; European Commission, 2006) and Ochratoxin A (OTA), which like the aflatoxins, is produced by common mould fungi (*Penicillium* and *Aspergillus* spp) that can infect the cereal grain during storage (Bryla et al., 2018; EFSA, 2006).

The European Commission (EC) has set maximum contamination levels (MCLs) for a range of mycotoxins (aflatoxins, ochratoxin A, patulin, deoxynivalenol and zearalenone) for various food products (European Commission, 2006). The MCLs set by EC for common commercial mycotoxins DON, ZEA and OTA in cereals are 750 μg/kg, 75 μg/kg and 3 μg/kg, respectively. The EC has also made a recommendation for MCLs for the sum of T-2 and HT-2 mycotoxin (50 μg/kg) (European Commission, 2006). The setting of MCLs has resulted in extensive mycotoxin testing regimes being introduced by grain storage and trading companies (often involving the testing of each cereal batch arriving from farms) to avoid grain with mycotoxin loads exceeding MCLs being used for human consumption.

There have been claims that organic crops contain higher levels of mycotoxins because organic standards prohibit the use of fungicides, and that this represent a considerable health risk for consumers (Trewavas, 2001), but these claims have not been substantiated. In contrast, more recent qualitative reviews and studies concluded that there are (a) no significant differences or lower levels of trichothecene mycotoxins and ZEA in organic cereals and (b) no significant difference in OTA concentrations between organic and conventional cereals (Brodal, Hofgaard, Eriksen, Bernhoft, & Sundheim, 2016; Tangni et al., 2013).

However, comprehensive meta-analyses of comparative mycotoxin data are currently not available, since the only meta-analyses that compared mycotoxin levels in organic and conventional foods were based on a very small proportion of the available published evidence (Smith-Spangler et al., 2012). It is therefore important to consider the potential confounding effects of cereal production methods in studies comparing the mycotoxin content of white and whole-grain cereal products.

There are also studies which reported that *Fusarium* head blight infection levels and mycotoxin concentrations in spelt wheat (*Triticum spelta* L.) grain are lower than those found in common wheat (*T. aestivum*) (Mankevičienė, Jablonskyte-Rasce, & Mankevičienė, 2014). Spelt wheat is a hexaploid, hulled wheat species that was used widely in Northern Europe in the past, but is now considered a minor cereal. Spelt is currently increasing its share in the cereal market (Transparency Market Research, 2017) and this is thought to be due to (a) its ability to grow under low input conditions (which make it particularly suitable for organic farming systems) and (b) consumer perceptions that spelt wheat has a higher nutritional value compared with common wheat (Transparency Market Research, 2017). It is therefore also important to consider the potential confounding effect of differences in resistance to mycotoxin producing fungi between wheat species/varieties/genotypes when comparing the mycotoxin content of white and whole-grain cereal products.

The main objectives of this study were therefore to (a) compare mycotoxin loads in white and whole-grain common and spelt wheat flour brands/products available in the UK and Germany (b) study the effect of contrasting primary production methods (organic vs conventional) on mycotoxin loads in wheat flour and (c) identify potential interactions between primary production methods (organic vs conventional), wheat species and post-harvest processing (white vs whole-grain flour) on mycotoxin loads.

The study tested four main hypothesis, which were: (1) whole-grain wheat flour has higher mycotoxin concentrations than white flour, (2) organic wheat flour has similar mycotoxin concentrations to conventional wheat flour, (3) spelt wheat flour has lower mycotoxin concentrations than common wheat flour and (4) farming system, wheat species, but also the year and country in which flour samples are collected, all have confounding effects on differences in mycotoxin concentrations found between whole meal and white flour.

## 2. Material and methods

### 2.1. Retail survey design

The retail survey of wheat flour was conducted over three successive years in the UK (2015 to 2017) and two successive years in Germany (2016 and 2017). The experimental design included 4 factors/variables: country (UK vs Germany), wheat species (*T. aestivum* vs *T. spelta*), farming system (organic vs conventional), and flour type (white vs whole-grain).

Cereal brands were considered as replicates, with only one sample per brand (supermarket own or manufacturers' brands) being used for each combination of country, wheat species, farming system, and flour type per year. This was primarily done to avoid pseudo-replication, since the use of more than one sample per brand could have resulted in both flour samples originating from the same batch of grain used by the millers; different brands were assumed to have been made by different mills or at least from different grain batches.

Due to the small number of spelt wheat brands/samples that could be found in the UK in 2015, the sample collection area for both spelt and common wheat was extended to Germany in 2016. As a result, an

additional factor (country) could be included in the statistical analyses of data from 2016 and 2017 samples. In total, 352 samples were purchased from supermarkets in the UK (Tesco, Waitrose, Sainsbury, Marks &Spencer, Holland& Barrett, Fenwick Food Hall, Morrisons) and Germany (Aldi, Budnikowsky, Combi, Demeter, Denn's Biomarket, Dm, Edeka, Kaufland, LIDL, Nahkauf, Netto, NP-Discount, Reformhaus, Rewe, REAL, Vitalia) and websites in the UK (Allinson, Amazon, Bacheldre Watermill, Buywholefoodonline, Gilchester online, Matthews Gotswold, Sharpham Park, Shipton Mill online, Wessex Mill) in the same period in each year (see Table S1 for the number of samples/flour brands assessed for different wheat flour types in the 2 countries).

Samples were transferred from original packages to vacuum food bags then stored in a −80 °C freezer in sealed containers with silica gel until analysis.

### 2.2. Mycotoxin analysis

Mycotoxin analyses were carried out in the quality assurance laboratory of Coastal Grains Ltd., Belford, UK (Northumberland's largest grain co-operative). All samples were analysed using the Rapid One Step Assay (ROSA) test kits (Andover Street, Lawrence, MA, USA; www.charm.com), standard ELISA-based tests used in commercial grain storage facilities and flour mills. The following strips were used to test for levels of specific mycotoxins: ROSA FAST5 DON Quantitative Test; ROSA ZEARQ-FAST5 ZEA Quantitative Test; ROSA OTA Quantitative Test for Feed and Grain, and ROSA T-2/HT-2 Quantitative Test. The ROSA Charm Science Test kit is a quantitative lateral flow test, read in the ROSA-M Reader. All kits were stored at 4 °C and controls provided with the kits were used to ensure that the reader was working correctly. During the analysis progress, all steps followed the procedures prescribed by the supplier of the test kits, Charm Science. Details of the flour extraction, sample preparation protocols and use of the ROSA-M reader are provided in the Supplementary materials.

The standard calibration was used for each test kit and standards and selected samples were tested 5 times to determine a coefficient of variation (CV) for each assay. The CVs for standards and selected samples were 15 and 18% for DON, 20 and 23% for ZEA and 19 and 31% for T-2/HT-2 and 15 and 29% for OTA.

### 2.3. Statistical analysis

Analysis of variance (ANOVA) derived from linear mixed-effects (lme) models (Pinheiro & Bates, 2000) was used to assess the effects and interactions between factors on measured parameters by using the *'nlme'* package in the R (R Core Team, 2018). Flour samples were collected over three years (2015, 2016, 2017) however common wheat flour samples were not collected from Germany in 2015 while white spelt flour was not collected in 2016. Therefore it was not possible to include all five experimental factors (year, country, species, farming system and flour type) in a single analysis and two separate 3-factor ANOVAs were carried out for percentage of samples testing positive and one 4-factor ANOVA was carried out for mean concentration. For data on the percentage of samples testing positive for mycotoxin contamination in different years we therefore used years as replicates and carried out two separate 3-factor ANOVAs with (a) wheat species, farming system and flour type (ANOVA 1) or (b) country, wheat species and farming system (ANOVA 2) as factors (results presented in Table 1). For data on the mean concentration of mycotoxins in flour samples a 4-factor ANOVA with country, wheat species, farming system and flour type as factors was carried out (ANOVA 3; Table 2). The hierarchical nature of the experimental design was designated in the random error structures of the model as: replicate (commercial brand)/ year/ country/ wheat species/ farming system (ANOVA1); replicate (commercial brand)/year/country/wheat species/flour type (ANOVA 2); and replicate (commercial brand)/year/country/farming system (ANOVA 3). For all parameters, it was also checked that the residuals were normally distributed by using the *'qqnorm'* function in R.

**Table 1**
Main effect means ± SE and p-values for the effects of, and interactions between, country (Germany vs UK), wheat species (spelt vs common wheat), farming system (organic vs conventional) and/or flour type (white vs wholegrain) on the % of wheat flour samples testing positive for specific mycotoxins.

| | % of wheat flour samples testing positive for: | | | |
|---|---|---|---|---|
| Factors | DON | T-2/HT-2 | ZEA* | OTA |
| *Country* | | | | |
| Germany (n = 20) | 41 ± 7 | 62 ± 9 | 88 ± 4 | 99 ± 1 |
| UK (n = 22) | 57 ± 8 | 60 ± 9 | 87 ± 5 | 98 ± 1 |
| *Species* | | | | |
| Spelt wheat (n = 22) | 51 ± 8 | 64 ± 9 | 86 ± 4 | 98 ± 1 |
| Common wheat (n = 20) | 48 ± 7 | 58 ± 8 | 89 ± 4 | 99 ± 1 |
| *Farming system* | | | | |
| Conventional (n = 20) | 57 ± 8 | 59 ± 9 | 86 ± 4 | 99 ± 1 |
| Organic (n = 22) | 43 ± 7 | 63 ± 8 | 89 ± 4 | 98 ± 1 |
| *Flour type* | | | | |
| White (n = 20) | 46 ± 7 | 51 ± 8 | 86 ± 5 | 98 ± 1 |
| Wholegrain (n = 22) | 53 ± 8 | 70 ± 9 | 89 ± 3 | 99 ± 1 |
| *ANOVA 1 (p-values)* | | | | |
| *Main Effects* | | | | |
| Species (SP) | NS | NS | NS | |
| Farming System (FS) | NS | NS | NS | |
| Flour Type (FT) | NS | 0.0014 | NS | |
| *Interactions* | | | | |
| SP × FS | NS | 0.0185 | NS | |
| SP × FT | NS | NS | NS | |
| FS × FT | NS | NS | NS | |
| SP × FS × FT | NS | NS | NS | |
| ANOVA 2 (*p*-values) | | | | |
| *Main Effects* | | | | |
| Country (CT) | NS | NS | NS | |
| Species (SP) | NS | NS | NS | |
| Farming System (FS) | 0.0467 | NS | NS | |
| *Interactions* | | | | |
| CT × SP | NS | NS | NS | |
| CT × FS | 0.0393[1] | NS | NS | |
| SP × FS | NS | 0.0058[2] | NS | |
| CT × SP × FS | NS | NS | NS | |

NS, not significant ($p > 0.1$); * ANOVAs were carried out using cube transformed data, means and SE presented were calculated using non-transformed data.
[1] see Table 2 for interaction means ± SE.
[2] see Table S3 for interaction means ± SE.

In order to investigate effect of year on mean concentrations of mycotoxins, year was included as a factor in additional 4-factor ANOVAs for common wheat (ANOVA 4) and spelt wheat (ANOVA 5) with year (2016, 2017 for common wheat and 2015, 2017 for spelt wheat), country, farming system and flour type as factors. The hierarchical nature was designated in the random error structures of the model as: replicate (commercial brand)/year/country/farming system.

In order to further investigate significant ($p < 0.05$) interactions between factors, general linear hypothesis tests (Tukey contrasts) were performed using the *'glht'* function of the *'multcomp'* package in R (Bretz, Hothorn, & Westfall, 2011). The experimental design was reflected in the same random error structures used for the lme models. This method is allowing multiple comparisons in unbalanced models with arbitrary error distribution and hence arbitrary data distribution and variance structure. For all samples, where the mycotoxin concentrations were below the limit of quantification (LOQ), half the LOQ was used as the value in the statistical analyses. The LOQ was 50 μg/kg for DON and 1 μg/kg for OTA, ZEA and T-2/HT-2.

To test for potential confounding effects of year we carried out two additional 4-factor ANOVAs with year, country, farming system and flour type as factors (results presented in supplementary Tables S1 and

**Table 2**
Main effect means ± SE and p-values for the effects of, and interactions between, country (Germany vs UK), wheat species (spelt vs common wheat), farming system (organic vs conventional) and flour type (white vs wholegrain) on mycotoxin concentrations (μg $kg^{-1}$ DW) in wheat flour samples.

| | Mycotoxin concentration (μg $kg^{-1}$ DW) | | | |
|---|---|---|---|---|
| Factor | DON* | T-2/HT-2* | ZEA* | OTA* |
| *Country* | | | | |
| Germany (n = 158) | 41 ± 6 | 2.3 ± 0.6 | 4.1 ± 0.3 | 2.3 ± 0.1 |
| UK (n = 194) | 65 ± 9 | 2.7 ± 0.5 | 3.4 ± 0.2 | 3.6 ± 0.1 |
| *Wheat species* | | | | |
| Spelt wheat (n = 106) | 33 ± 5 | 1.2 ± 0.2 | 3.5 ± 0.4 | 2.6 ± 0.1 |
| Common wheat (n = 246) | 63 ± 8 | 1.4 ± 0.2 | 3.8 ± 0.2 | 3.2 ± 0.1 |
| *Farming System* | | | | |
| Conventional (n = 181) | 60 ± 6 | 2.4 ± 0.5 | 3.7 ± 0.3 | 2.9 ± 0.1 |
| Organic (n = 171) | 49 ± 9 | 2.6 ± 0.6 | 3.8 ± 0.3 | 3.1 ± 0.1 |
| *Flour Type* | | | | |
| White (n = 214) | 48 ± 7 | 1.7 ± 0.4 | 3.9 ± 0.2 | 2.9 ± 0.1 |
| Wholegrain (n = 138) | 63 ± 9 | 3.8 ± 0.7 | 4.2 ± 0.3 | 3.1 ± 0.1 |
| Maximum contamination level (MCL) μg/kg | 750[#] | 50[##] | 75[#] | 3[#] |
| ANOVA results (*p*-values) | | | | |
| *Main Effects* | | | | |
| Country (CT) | 0.0111 | NS | 0.0483 | < 0.0001 |
| Species (SP) | NS | NS | 0.0286 | NS |
| Farming System (FS) | 0.0060 | NS | NS | NS |
| Flour Type (FT) | NS | < 0.0001 | NS | NS |
| *Interactions* | | | | |
| CT × SP | NS | NS | NS | 0.0002 |
| CT × FS | 0.0481 | NS | NS | NS |
| SP × FS | NS | NS | NS | 0.0051 |
| CT × FT | NS | 0.0006[2] | NS | NS |
| SP × FT | NS | 0.0163[3] | NS | NS |
| FS × FT | NS | 0.0307[4] | NS | NS |
| CT × SP × FS | NS | NS | NS | 0.0392 |
| CT × SP × FT | NS | NS | NS | NS |
| CT × FS × FT | 0.0257 | NS | NS | NS |
| SP × FS × FT | NS | NS | NS | NS |
| CT × SP × FS × FT | 0.0007[1] | NS | NS | 0.0085[1] |

The LOQ was 50 μg/kg for DON and 1 μg/kg for OTA, ZEA and T-2/HT-2.
NS, not significant ($p > 0.1$).
* ANOVAs were carried out on log + 1 transformed data, means and SE presented were calculated with non-log + 1 transformed data.
[#] MCL (EC2006).
[##] Recommended MCL (EC 2006 & 2013).
[1] See Table 3 for interaction means ± SE.
[2] See Table 4 for interaction means ± SE;
[3] See Table 5 for interaction means ± SE.
[4] See Table 6 for interaction means ± SE

S2). For common wheat we used data from 2016 and 2017 only, since no common wheat samples were collected in Germany in 2015 (Table S1). For spelt wheat we included data from 2015 and 2017 only since no white flour spelt wheat samples could be collected in Germany in 2016 (Table S1).

## 3. Results

In the results section the term "significant" is used to describe results that are statistically significant ($p < 0.05$).

### 3.1. Proportion of flour samples testing positive for mycotoxins

When the proportions of samples testing positive for different mycotoxins were compared, nearly all (99%) of samples tested positive for at least one mycotoxin (OTA) (Table 1). There were no significant main effects of country, species, farming system and flour types for the prevalence of DON, ZEA and OTA (Table 1). However, for T-2/HT-2 toxins a significant main effect of flour type ($p = 0.001$) was detected with a higher proportion of mycotoxin positive samples found in wholegrain flour (70%) than in white flour (51%) (Table 1).

There were also significant interactions ($p = 0.019$) between (a) country and farming systems for DON and (b) between wheat species and farming system for both T-2/HT-2 toxins and OTA (Table 1). DON was detected in a significantly higher number of conventional (73%) than organic (44%) flour samples from the UK, but not Germany (41% for conventional vs 42% for organic) (Table S2). T-2/HT-2 was detected in a significantly higher proportion of organic (72%) than conventional spelt (55%), but a significantly lower number of organic (52%) than conventional (64%) common wheat samples (Table S3).

### 3.2. Concentrations of mycotoxins in wheat flour

When the mean concentrations of *Fusarium* mycotoxins were compared, they were found to be more than 10 times lower than the MCLs set by the EC for DON and ZEA or recommended by the EC for T-2/HT-2 (Table 2). In contrast, for OTA (a mycotoxin produced by the common moulds *Penicillium* and *Aspergillus* spp), mean concentrations in the flour sources from the UK (but not Germany) exceeded the MCL (3 μg/kg) set by the EC (Table 2). Overall, a substantial proportion (46% in the UK and 11% in Germany) of flour brands exceeded the MCL.

Significant main effects of country were detected for DON ($p = 0.011$), ZEA ($p = 0.048$) and OTA ($p < 0.001$); samples from Germany had approximately 30% lower DON and 20% lower OTA concentrations than those from the UK, while samples from the UK had approximately 10% lower ZEA concentrations than those from Germany (Table 2). Significant main effects of wheat species ($p = 0.029$), farming system ($p = 0.006$) and flour type ($p < 0.001$) were only detected for ZEA, DON and T-2/HT-2 toxins respectively, with common wheat flour (3.8 μg/kg) having higher ZEA than spelt wheat flour (3.5 μg/kg), conventional flour (60 μg/kg) having higher DON than organic flour (49 μg/kg) and whole-grain flour (3.8 μg/kg) having higher T2/HT-2 concentrations than white flour (1.7 μg/kg) (Table 2).

A wide range of interactions involving all four experimental factors was also detected (Tables 2).

When the 4-way interactions detected for the common mould mycotoxin OTA ($p = 0.009$) and the *Fusarium* mycotoxin DON ($p < 0.001$) were investigated further, different trends were detected for OTA and DON (Table 3).

For DON the only flour types for which significant differences between countries could be detected were (a) conventional, whole-grain, common wheat flour (higher concentration in samples from the UK with 116 μg/kg than in samples from Germany with 28 μg/kg) and (b) organic, whole-grain, common wheat flour (higher concentrations in samples from Germany with 110 μg/kg than in the UK samples with 46 μg/kg) (Table 3). Also, DON concentrations in whole-grain, common wheat from Germany were significantly higher in organic (110 μg/kg) compared with conventional samples (28 μg/kg), while in the UK samples concentrations were significantly higher in conventional (116 μg/kg) than organic samples (46 μg/kg). For all other flour types, no significant difference in DON concentrations could be detected between farming systems, and mean concentrations for all flour types were approximately 7 times lower than the MCL of 750 μg/kg set for DON by the EC (Table 3).

For OTA all types of common wheat flour (organic and conventional, white and whole-grain) from the UK had significantly higher OTA concentrations than the same flour types collected in Germany. Also, mean OTA concentration of all types of common wheat flour collected in the UK (but not those collected in Germany) exceeded the MCL for OTA of 3 μg/kg set by the EC (Table 3). However, when spelt flour samples from Germany and the UK were compared, significantly higher OTA levels were only detected in organic whole-grain flour

**Table 3**
Interactions means ± SE for the effects of country (Germany vs UK), species (spelt vs common wheat), farming system (organic vs conventional) and flour type (white vs wholegrain) on DON and OTA concentrations (μg $kg^{-1}$ DW).

| | | | Factor 3 Farming system | | | |
|---|---|---|---|---|---|---|
| | | | Conventional | | Organic | |
| Mycotoxin | Factor 1 | Factor 2 | Factor 4 Flour Type | | | |
| Parameter | Country | Species | White | Wholegrain | White | wholegrain |
| DON | Germany | Spelt wheat | 40 ± 15 A a | 65 ± 26 A ab | 25 ± 11 A a | 26 ± 10 A a |
| | | Common wheat | 31 ± 9 AB a | 28 ± 28 B b | 31 ± 13 B a | 110 ± 41 A a |
| | UK | Spelt wheat | 67 ± 17 A a | 19 ± 9 AB b | 15 ± 8 B a | 35 ± 10 AB b |
| | | Common wheat | 65 ± 11 B a | 116 ± 22 A a | 79 ± 33 BC a | 46 ± 24 C b |
| OTA | Germany | Spelt wheat | 2.8 ± 0.3 AB b | 3.0 ± 0.3 A ab | 2.0 ± 0.2 B b | 2.1 ± 0.2 AB bb |
| | | Common wheat | 1.9 ± 0.2 B c | 1.9 ± 0.4 BC c | 2.3 ± 0.2 AB b | 2.7 ± 0.3 A ab |
| | UK | Spelt wheat | 3.3 ± 0.7 AB ab | 2.4 ± 0.5 AB bc | 2.2 ± 0.4 B b | 3.5 ± 0.5 A a |
| | | Common wheat | 3.5 ± 0.2 B a | 3.7 ± 0.3 AB a | 4.5 ± 0.4 A a | 3.7 ± 0.3 AB a |

For each parameter assessed means labelled with capital letter within the same row or the same lower case letter within the same column are not significant different (Tukey's honestly significant difference test $p < 0.05$).
Pairwise comparisons of means were carried out on log + 1 transformed data, means and SE presented were calculated with non-log + 1 transformed data.

samples from the UK (3.5 μg/kg) compared to Germany (2.1 μg/kg). Mean concentrations which exceeded the MCL for OTA, were only found in conventional white flour and organic whole-grain spelt flour from the UK (Table 3). However, due to the low number of samples collected for each individual flour type in each country the comparisons of 4-way interaction means needs to be interpreted with caution.

When the 2-way interaction between country and flour type for T-2/HT-2 mycotoxins ($p < 0.001$) was examined further, concentrations in white flour were found to be similar in both countries, while concentrations in whole-grain flour were significantly (approximately 90%) higher in flour samples from the UK (2.43 μg/kg) compared with those from Germany (1.27 μg/kg) (Table 4). When flour types within the same country were compared, concentrations of T-2/HT-2 in whole-grain flour were significantly higher than in white flour in the UK (0.83 μg/kg for white vs 2.43 μg/kg for whole-grain), but not in samples from Germany (0.9 μg/kg for white vs 1.27 μg/kg for whole-grain) (Table 4).

When the 2-way interaction between wheat species and flour type for T-2/HT-2 mycotoxins ($p < 0.016$) was examined further, concentrations of T-2/HT-2 in white flour were found to be similar in both spelt and common samples, while concentrations in whole-grain flour were significantly (approximately 100%) lower in spelt (1.25 μg/kg) than in common wheat samples (2.49 μg/kg) (Table 5). Concentrations of T-2/HT-2 in whole-grain flour were significantly higher than in white flour in common but not spelt wheat samples (Table 5).

When the 2-way interaction between farming system and flour type for T-2/HT-2 mycotoxins ($p = 0.031$) was examined further, concentrations in white flour were found to be similar in both conventional and organic samples, while concentrations in whole-grain flour were significantly (approximately 80%) higher in conventional (2.74 μg/kg) than organic flour samples (1.46 μg/kg) (Table 6). Concentrations of T-2/HT-2 in whole-grain flour were significantly higher in conventional but not organic flour samples (Table 6).

When year was included as a factor in separate 4-factor ANOVAs for common and spelt wheat, significant differences in (a) T-2/HT-2 ($p < 0.001$), ZEA ($p < 0.001$) and OTA ($p = 0.002$), but not DON concentrations were detected between common wheat samples collected in 2016 and 2017 (Table S4), while (b) no significant differences in mycotoxin concentrations could be detected between spelt samples taken in 2015 and 2017 (Table S5).

**Table 4**
Interactions means ± SE for the effects of countries (Germany vs UK) and flour types (white vs wholegrain) on T-2/HT-2 concentrations (μg $kg^{-1}$ DW).

| | Factor 1 | Factor 2 Flour Type | |
|---|---|---|---|
| Parameter | Country | White | Wholegrain |
| T-2/HT-2 | Germany | 0.90 ± 0.13 Aa | 1.27 ± 0.25 Ab |
| | UK | 0.83 ± 0.14 Ba | 2.43 ± 0.36 Aa |

For each parameter assessed means labelled with capital letter within the same row or the same lower case letter within the same column are not significant different (Tukey's honestly significant difference test $p < 0.05$).
Pairwise comparisons of means were carried out on log + 1 transformed data, means and SE presented were calculated with non-log + 1 transformed data.

**Table 5**
Interactions means ± SE for the effects of species (spelt vs common wheat) and flour types (white vs wholegrain) on T-2/HT-2 concentrations (μg $kg^{-1}$ DW).

| | Factor 1 | Factor 2 Flour Type | |
|---|---|---|---|
| Parameter | Species | White | Wholegrain |
| T-2/HT-2 | Spelt wheat | 1.04 ± 0.20 A a | 1.25 ± 0.22 A b |
| | Common wheat | 0.81 ± 0.10 B a | 2.49 ± 0.38 A a |

For each parameter assessed means labelled with capital letter within the same row or the same lower case letter within the same column are not significant different (Tukey's honestly significant difference test $p < 0.05$).
Pairwise comparisons of means were carried out on log + 1 transformed data, means and SE presented were calculated with non-log + 1 transformed data.

**Table 6**
Interactions means ± SE for the effects of farming system (organic vs conventional) and flour types (white vs wholegrain) on T-2/HT-2 concentrations (μg $kg^{-1}$ DW).

| | Factor 1 | Factor 2 Flour type | |
|---|---|---|---|
| Parameter | Farming system | White | Wholegrain |
| T-2/HT-2 | Conventional | 0.80 ± 0.11 B a | 2.74 ± 0.50 A a |
| | Organic | 0.95 ± 0.15 A a | 1.46 ± 0.20 A b |

For each parameter assessed means labelled with capital letter within the same row or the same lower case letter within the same column are not significant different (Tukey's honestly significant difference test $p < 0.05$).
Pairwise comparisons of means were carried out on log + 1 transformed data, means and SE presented were calculated with non-log + 1 transformed data.

## 4. Discussion

Although the health benefits of increasing wholegrain consumption are well documented (Borneo & Len, 2012; Della Pepa et al., 2018; Jones & Engleson, 2010) concerns have been raised about the safety of whole-grain consumption, because it may increase exposure to mycotoxins, toxic metals and acrylamide, since especially mycotoxin contamination of cereals continues to be a serious threat to public health globally (Thielecke & Nugent, 2018).

In the study reported here, the mean concentrations of the *Fusarium* mycotoxins DON, T-2/HT-2 and ZEA in whole-grain and white flour brands from both Germany and the UK were all more than 10 times lower than the maximum contamination levels (MCL) set by the EC. The very low *Fusarium* mycotoxin and similar OTA concentrations found in wholegrain and white flour, suggest that there is no increased health risk from mycotoxin exposure for consumers that follow current nutritional recommendations to increase wholegrain consumption.

However, although OTA concentrations were overall very similar in whole-grain and white flour, mean concentrations of OTA were 20% higher than the MCL in samples from the UK and only around 20% lower than the MCL in samples from Germany. These results should be viewed with some concern, since OTA has been shown to have nephrotoxic and immunosuppressive effects in all experimental animal systems used in tests, and is suspected to also have carcinogenic and teratogenic effects at low concentrations (EFSA, 2016; Ostry, Malir, Toman, & Grosse, 2017). The IARC currently classifies OTA as "*possibly carcinogenic*" (group 2B) to humans, but it is expected to be re-classified as "*probably carcinogenic*" (group 2A) in the near future (Ostry et al., 2017).

Retail surveys, such as the one presented here, aim to quantify and estimate variation in mycotoxin levels in the currently available flour brands available to consumers. However, for flour samples collected in supermarkets it is virtually impossible to obtain information on specific farming system parameters, that are known to affect mycotoxin levels in grain such as (a) location and pedoclimatic conditions, (b) agronomic practices (e.g. fertilisation, crop protection, tillage, rotation design) used on cereal producing farms, (c) harvest parameters (e.g. climatic conditions, grain dry matter content), (d) post-harvest treatments used (e.g. grain drying and cleaning) and (e) quality assurance systems used to minimise mycotoxin contamination in grain used to produce the flour brands available in the UK and Germany (Bernhoft, Clasen, Kristoffersen, & Torp, 2010; Bernhoft, Torp, Clasen, Løes, & Kristoffersen, 2012; Magan & Aldred, 2005, 2007; Paulsen & Weißmann, 2002; Wu, 2019). It is also likely, that flour was made from cereal batches produced in a wide range of different regions and imported grains in both countries. Thus, it is impossible to provide definitive explanations for the differences in mycotoxin concentrations reported here. In the discussion of the differences between whole-grain and white flour produced (a) in different countries (UK vs Germany) and years/production seasons, (b) from contrasting wheat species (spelt vs common wheat) and (c) with different agronomic protocols (organic vs conventional) we have therefore related the results of the survey to previous experimental and farm survey based studies that focused on identifying climatic, crop genetic, agronomic, grain processing and quality assurance related parameters affecting mycotoxin levels.

### 4.1. Effect of year/production season and country

The finding that mycotoxin concentrations and profiles differed between sampling years and countries was expected since environmental conditions during the growing seasons and harvesting are known to have a substantial effect on fungal infection levels, and mycotoxin loads and composition in cereal grains (Bernhoft et al., 2012; Magan & Aldred, 2005, 2007; Wu, 2019).

The finding of overall higher concentrations of the *Fusarium* mycotoxins DON and ZEA in samples from the UK than Germany is therefore likely to reflect, at least partially contrasting climatic conditions in the different years and countries where flour samples were collected.

However, difference in agronomic practices (e.g. contrasting rotation designs) and post-harvest processing and quality assurance protocols used in Germany and the UK may also have contributed (see also Sections 4.2 and 4.3 below).

### 4.2. Effect of flour type

Milling practices that remove the outer layers of the grain are described as one way to minimise dietary exposure to mycotoxins (Cheli et al., 2013; Zhang et al., 2019). In the survey reported here, significantly higher mycotoxin loads in whole-grain than white flour were only detected for one *Fusarium* mycotoxin (T-2/HT-2), but T-2/HT-2 concentrations in whole-grain flour were still more than 12 times lower than the MCL.

The very low levels of contamination with *Fusarium* mycotoxins and very similar OTA levels detected in whole-grain and white flour suggest that there is no substantial difference in health risk associated with consumption of white and whole-grain flours in the UK and Germany. Based on the results of this study there is no justification for changing the current dietary recommendations to increase whole-grain consumption (Borneo & Len, 2012; Tangni et al., 2013; Thielecke & Nugent, 2018). However, the finding that mean OTA concentrations were above the MCL for 6 out of 8 flour types in the UK and only slightly lower than the MCL for the two other UK and all German sourced flour types should be viewed with some concern. While cereal and cereal products are the main dietary source for *Fusarium*-mycotoxins (e.g. DON, ZEA, T-2/HT-2), a wider range of foods, including pulses, coffee, cacao, grape juice, dry vine fruits, wine, nuts and spices can significantly contribute to dietary OTA intake (EFSA, 2006).

According to the most recent EFSA opinion (EFSA, 2006) there is strong evidence for "*site specific renal toxicity as well as DNA damage and genotoxic effects of OTA*" and some epidemiological evidence *"for distinct renal diseases and otherwise rare tumours of the kidneys in certain endemic regions of the Balkan Peninsula"*. EFSA reports the lowest observed adverse effect level as being 8 μg/kg body weight per day and applied a composite uncertainty factor of 450 when setting the tolerable weekly intake (TWI) at 120 ng/kg body weight for OTA (EFSA, 2006). Dietary exposure of adult European consumers to OTA is estimated to range from 15 to 60 ng OTA per kg body weight per week and is therefore well below the TWI set by EFSA (EFSA, 2006). However, there are uncertainties about the accuracy of estimates for (a) the lowest observed adverse effect and (b) total dietary OTA exposure. A more detailed risk assessments should therefore be considered in the future, to clarify whether there concerns about the close to and above MCL concentrations found for OTA are justified.

High OTA levels in cereal grains and flour have often been associated with delayed or inefficient grain drying post-harvest and/or poor storage facilities (Magan & Aldred, 2005, 2007; Wu, 2019). Further reductions in OTA levels in wheat flour may therefore come from a detailed review and improvements of post-harvest critical control points, including the quality assurance protocols (e.g. the OTA concentrations set as thresholds during testing) by grain storage/marketing companies and/or millers.

It is interesting to note that higher T-2/HT-2 concentrations in whole-grain than white flour were only detected in flour of UK origin, which may be explained by the differences in climatic conditions and/or contrasting *Fusarium* species profiles in the UK and Germany especially during the period before grain harvest (Bernhoft et al., 2012). It is well established that the *Fusarium* species and mycotoxin profiles associated with *Fusarium* head blight (FHB) in wheat and other small grain cereals differs considerably between climatic zones in Europe and that mycotoxin production is affected by environmental conditions, especially temperature and rain/humidity (Bernhoft et al., 2012;

Bottalico & Perrone, 2002). For example, wet/humid and warm conditions before harvest were shown to significantly increase both T-2/HT-2 contamination in barley, oats and wheats, and lower temperatures before grain harvest was found to increase DON concentrations (Bernhoft et al., 2012). The types/profiles of mycotoxins produced on cereal grains differs between *Fusarium* species (Bottalico & Perrone, 2002; Kokkonen, Ojala, Parikka, & Jestoi, 2010). For example, the most frequently found mycotoxins associated with FHB (DON and ZEA) are produced primarily by the pathogenic species *F. graminearum* and *F. culmorum*. In contrast, the occurrence of T-2/HT-2 toxin, which is considered to be substantially more toxic than DON, has been linked mainly to sporadic epidemics of *F. sporotrichioides* and *F. langsethiae* (previously classified as *F. poae*) which are thought to be less plant pathogenic (Bottalico & Perrone, 2002). These *Fusarium* species may therefore mainly infect the outer layers of the grain and not the endosperm, which would explain the lower concentrations in white flour in the UK. Contrasting T-2/HT-2 levels may also have been due to differences in *Fusarium* disease pressure and grain infection levels (Edwards et al., 2011).

### 4.3. Effect of wheat species

Spelt wheat (*T. spelta*), unlike most other cereals including common wheat, has husks/glumes and some studies have concluded that the husks act as physical barrier that reduces fungal colonisation and mycotoxin production in wheat grains/kernels (Bryla et al., 2018; Mankevičienė et al., 2014). This is consistent with results presented here which found (a) overall lower ZEA concentrations in spelt compared with common wheat flour, (b) lower T-2/HT-2 concentrations in whole-grain spelt flour than whole-grain common wheat, (c) lower DON concentrations in conventional whole-grain spelt flour than in conventional whole-grain common wheat flour in the UK and (d) lower OTA concentrations in conventional, whole-grain and organic white spelt than common wheat flour in the UK.

There is also a range of other studies, which reported that the relative resistance to mycotoxin producing fungi is higher in spelt than other wheat species (Konvalina et al., 2016; Mankevičienė et al., 2014). For example, a study by Rachoń et al. (2016) compared *Fusarium* mycotoxin (including DON, T-2, ZEA) concentrations in common, durum, spelt, einkorn and spelt winter-wheat genotypes and showed that spelt wheat had the lowest mycotoxin levels. It should be pointed out that most spelt wheat varieties also have longer stems/straws than modern common wheat varieties grown in Europe, which is also thought to reduce the risk of grain infections by *Fusarium* spp (Mesterhazy, 1995).

However, it is important to consider that the mycotoxin concentrations found in flour are determined by three main factors or critical control points (CCPs). These are the (a) climatic and agronomic parameters during the growth and harvest of cereals in the field, (b) post-harvest treatments (e.g. drying) and storage and (c) mycotoxin testing-based quality assurance (QA) protocols used by seed storage/marketing/processing companies (Bryla et al., 2018; Wu, 2019). More detailed farm survey-based approaches which monitor mycotoxin levels and background conditions along the supply chain (from field to supermarket shelf) would therefore be required to explain differences found between spelt and common wheat flour.

### 4.4. Effect of farming system

Apart from variety choice and climatic conditions during the growing season (especially after tillering) and at harvest, the level of mycotoxin contamination in cereals is affected by (a) agronomic management factors including crop protection, tillage, fertilisation, and rotation design/pre-crop (Heier, Jain, Kogel, & Pons-Kühnemann, 2005; Paulsen & Weißmann, 2002) and (b) postharvest management practices (e.g. drying and cleaning of harvested grain and storage conditions) (Bryla et al., 2018; Wu, 2019).

Fungicides are widely used in conventional cereal production to control foliar diseases (including *Fusarium* head blight), but are prohibited under organic farming standards. It has therefore been argued that organic cereal crops are at a higher risk from fungal diseases and mycotoxin contamination (Trewavas, 2001). However, this claim has not been substantiated (Bernhoft et al., 2012; Brodal et al., 2016), and studies into the effect of fungicides application on *Fusarium* head blight severity, *Fusarium* grain infection and mycotoxin levels have often shown variable and/or contradictory results (Bernhoft et al., 2012; Bryla et al., 2018; Heier et al., 2005; Wu, 2019). There is also evidence that the use of growth regulators and some fungicides may increase mycotoxin production, for example, due to stress imposed on the fungus (Ellner, 2005; Mankevičienė, Supronienе, Dabkevičius, & Auskalniene, 2008). In the study reported here organic wheat flour, overall, had 20% lower DON but similar contamination levels of T-2/HT-2, ZEA and OTA. For DON these results are consistent with recent qualitative reviews (Bernhoft et al., 2010; Brodal et al., 2016) and the only previous meta-analysis of comparative DON contamination data (Smith-Spangler et al., 2012) which reported significantly lower levels of DON in organic compared with conventional common wheat samples (SMD, −0.94 [CI, −1.27 to −0.62]; $P < 0.01$; $I^2 = 63$).

However, contrasting effects of farming systems on DON contamination were detected between countries and/or flour types found in the study reported here. For example, substantial differences in DON concentrations were detected for (a) whole-grain common wheat flour in Germany (approximately 4 times higher in organic flour), (b) white spelt flour in the UK (approximately 4 times higher in conventional flour) and (c) whole-grain common wheat flour in the UK (approximately 2 times higher in conventional flour). This variation could be due to differences in (a) agronomic protocols used for spelt and common wheat and/or (b) both agronomic protocols used and preharvest climatic conditions between the UK and Germany, since levels of contamination with *Fusarium* mycotoxins is determined primarily by agronomic parameters (e.g. rotation design, tillage, fertilisation and crop protection regimes) and environmental conditions before harvest. For example, Paulsen and Weißmann (2002) identified 13 agronomic/farm management factors that may affect mycotoxin formation and contamination in food and feed crops, and may also explain differences in *Fusarium* mycotoxin contamination between organic and/or conventional crop farming systems.

For OTA, the results reported here contradict a range of studies carried out in the early 2000s which reported significantly higher OTA levels in organic cereals or cereal products and described limited access to grain drying facilities and poor on-farm storage conditions as the main reasons for the higher OTA levels in organic wheat/cereal products at that time (Magan & Aldred, 2007). OTA contamination levels are mainly determined by climatic conditions during harvest and post-harvest treatment (especially drying to reduce grain moisture to acceptable levels) and storage conditions which prevent infection growth and mycotoxin production by common mould fungi, such as *Aspergillus* and *Penicillium* spp (Magan & Aldred, 2007). However, our results are consistent with the findings of a more recent meta-analysis of studies which compared mycotoxin contamination in organic and conventional cereals, which reported that OTA levels in organic cereals/cereal products have decreased over time and are now similar to those found in conventional cereals (Wang, 2019). These changes in OTA contamination in organic wheat grain and products over time are thought to be mainly due to improved access to state-of-the-art grain drying and storage facilities and better post-harvest quality assurance (Wang, 2019).

### 4.5. Potential limitations of the ELISA based analysis method

It should be pointed out that standard commercial ELISA-based test kits were used for mycotoxin analysis in the study reported here. These test kits are used widely for quality assurance by grain storage and

processing companies but have higher detection limits and are considered less accurate at estimating mycotoxin concentrations than other mycotoxin assessment methods (e.g. HPLC or GC based assays). However, due to the high cost of HPLC, HPLC-MS or GC–MS based analyses and the large number of samples that needed to be examined to identify potentially confounding effects of country, flour type and wheat species we used the commercial ELISA-based test kits in this study. Results show that the method used was sufficiently sensitive to identify both main effects and interaction between factors. However, more sensitive analytical protocols should be considered in future experiments into the effects of pedoclimatic conditions, wheat species/genotype, flour types and farming systems, to quantify mycotoxin levels in cereals more accurately (Nuryono, Noviandi, Böhm, & Razzazi-Fazeli, 2005).

## 5. Conclusion

Overall, the results of the retail survey study show that whole-grain flour and white flour has similar concentration of DON, ZEA and OTA, while the T-2/HT-2 mycotoxin which was found at significantly higher concentrations in wholemeal flour. These results therefore contradict hypothesis 1. However, since T-2/HT-2 concentrations in both whole-grain and white flour were more than 12 times lower than the MCL set by the EC, this difference is highly unlikely to have any health implications. These results clearly suggest that in the UK and Germany (a) there is no difference in mycotoxin-exposure from white and whole-grain wheat consumption and (b) concerns about mycotoxin loads should not restrict nutritional recommendations to switch from white to whole-grain consumption, given the strong evidence for health benefits of whole-grain (Borneo & Len, 2012; Jones & Engleson, 2010; Thielecke & Nugent, 2018).

Results also show that overall, there is no increased risk of dietary mycotoxin exposure from organic wheat flour as suggested in the past (Trewavas, 2001), which confirms hypothesis 2. Only DON concentrations were on average, over all flour types, slightly (22%) but significantly higher in conventional wheat flours and in the UK nearly twice as many conventional than organic flour samples tested positive for DON. However, given that DON concentrations were more than 12 times lower in the conventional and organic samples than the MCL this is also highly unlikely to be of nutritional relevance.

However, the finding that OTA concentrations in both organic and conventional wheat flour were close to, and for a substantial number of samples above the current MCL set by the EU should be of concern and lead to a re-examination of the critical control points pre- and post-harvest (e.g. drying, storage, mycotoxin testing) where OTA contamination can be minimised.

The finding of similar mycotoxin concentrations in spelt and common wheat flour also contradicts hypothesis 3. However, this study demonstrated significant effects of the country and years in which samples were taken on mycotoxin loads and profiles and a wide range of interactions with agronomic protocols and wheat species. This supports hypothesis 4 and is consistent with the well documented effects of climatic conditions, wheat genetics, agronomic practices and post-harvest storage, processing and quality assurance protocols on mycotoxin loads discussed above.

## CRediT authorship contribution statement

**Juan Wang:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Gultakin Hasanalieva:** Investigation, Methodology. **Liza Wood.:** Investigation. **Emilia Markellou.:** Conceptualization, Writing - review & editing. **Per Ole Iversen:** Validation, Writing - review & editing. **Aksel Bernhoft:** Conceptualization, Validation, Writing - review & editing. **Chris Seal:** Methodology, Project administration, Resources, Supervision, Validation, Writing - review & editing. **Marcin Baranski.:** Data curation, Formal analysis, Validation. **Vanessa Vigar:** Conceptualization, Writing - review & editing. **Laura Ernst:** Writing - review & editing. **Adam Willson:** Writing - review & editing. **Bronwyn J. Barkla:** Validation, Writing - review & editing. **Carlo Leifert:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing - original draft, Writing - review & editing. **Leonidas Rempelos:** Data curation, Formal analysis, Investigation, Supervision, Visualization, Writing - original draft, Writing - review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.127011.

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