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**Occurrence of fusarium mycotoxins in maize imported into the United Kingdom, 2004-7**

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1 **Occurrence of fusarium mycotoxins in maize imported into the**  
2 **United Kingdom from 2004-2007**

3  
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5  
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4 12 This study examined a total of 82 consignments of French and Argentinean raw maize  
5  
6 13 as received at maize mills in the UK during the period 2004-2007. Samples were  
7  
8 14 analysed for deoxynivalenol (DON), nivalenol (NIV), other trichothecenes,  
9  
10 15 zearalenone (ZON) and fumonisins B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub> (FB<sub>1</sub>, FB<sub>2</sub>, and FB<sub>3</sub>) using fully  
11  
12 16 validated analytical methods with limits of quantification of 10 µg kg<sup>-1</sup> for DON, NIV  
13  
14 17 and each fumonisin mycotoxin and 3 µg kg<sup>-1</sup> for ZON. All samples except two  
15  
16 18 containing fumonisins met the EC statutory maximum permissible levels for DON,  
17  
18 19 ZON and FB<sub>1</sub> + FB<sub>2</sub> as operating in 2007. The maximum concentrations of DON,  
19  
20 20 NIV, ZON and FB<sub>1</sub> + FB<sub>2</sub>, found were 444, 496, 165 and 5002 µg kg<sup>-1</sup> respectively.  
21  
22 21 Fumonisin were detected in almost every sample with 65% of Argentinean maize  
23  
24 22 containing more than 1000 µg kg<sup>-1</sup> of FB<sub>1</sub> + FB<sub>2</sub>. In contrast ZON was not detectable  
25  
26 23 in almost 50% of consignments. During this period there was a distinct difference in  
27  
28 24 mycotoxin concentrations between harvests and geographic origin. Flint maize from  
29  
30 25 Argentina usually contained lower concentrations of DON and related trichothecenes  
31  
32 26 and higher levels of fumonisins than maize from France, although concentrations of  
33  
34 27 fumonisins up to 2000 µg kg<sup>-1</sup> or greater occurred in samples from both regions. The  
35  
36 28 incidence and concentrations of fumonisins were similar to those in a similar previous  
37  
38 29 survey while zearalenone concentrations were lower. The distribution of mycotoxins  
39  
40 30 in multi-hold ships was also investigated showing that fumonisins were much more  
41  
42 31 evenly distributed than DON thus indicating their general level in the ship as a whole.  
43  
44 32 The effect of cleaning regimes was found to be very variable, especially for DON,  
45  
46 33 ranging from no removal of mycotoxins to >50% in some instances but was not  
47  
48 34 related to concentration. Evidence here suggests that while cleaning is essential for  
49  
50 35 removing foreign bodies prior to milling it cannot be used as a reliable tool for  
51  
52 36 reducing mycotoxins.  
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3 374  
5  
6 38 **Keywords:** deoxynivalenol, zearalenone, fumonisins, nivalenol, cleaning, maize7  
8 399  
10 40 **Introduction**

11  
12  
13 41 The risk to human health posed by mycotoxins has been assessed by international  
14  
15 42 organisations including WHO/FAO and the European Commission and as a result  
16  
17 43 maximum permissible limits for certain mycotoxins have been introduced. Limits  
18  
19 44 have been set within the EU for DON and ZON in cereals including wheat, maize,  
20  
21 45 oats and for fumonisins in maize (EC 2006a, 2007) and are under consideration for  
22  
23 46 T2-toxin and HT2-toxin. A number of mycotoxins occur in maize often in  
24  
25 47 combination and include aflatoxins (Andersen et al. 1975), ochratoxin A (Shotwell et  
26  
27 48 al. 1969) and a number of fusarium mycotoxins, DON (Gilbert et al. 1983), NIV and  
28  
29 49 other trichothecenes (Vesonder et al. 1973), ZON (Shotwell et al. 1971), fumonisins  
30  
31 50 (Gelderblom et al. 1988), moniliformin (Sharman et al. 1991, Scudamore et al. 1997,  
32  
33 51 1998) and beauvaricin, fusaproliferin and fumaric acid (Ritieni et al. 1997). EU  
34  
35 52 legislation relevant to mycotoxins in raw maize and derived products now applies to  
36  
37 53 aflatoxin, ochratoxin A, DON, ZON and the sum of FB<sub>1</sub> + FB<sub>2</sub>.

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41  
42  
43 55 The total whole maize imported to the UK during 2007 was about 1.2 million tonnes,  
44  
45 56 of which about 0.6 million tonnes was used for human and industrial use and 0.5  
46  
47 57 million tonnes for animal feed. Maize grown in the UK is currently either 'corn on  
48  
49 58 the cob' or is almost entirely used for forage but this could change as new varieties are  
50  
51 59 developed and if the climate becomes more suitable. Maize for potential food use has  
52  
53 60 been grown experimentally for a number of years.

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3 62 Maize for human food entering the UK comes principally from France and Argentina  
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6 63 and is purchased on contract requiring consignments to meet specified standards  
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8  
9 64 (including those for mycotoxin concentrations) laid down by the buyer, usually equal  
10  
11 65 to or lower than regulatory limits. Mycotoxin levels may thus not be entirely  
12  
13 66 representative of the mycotoxin concentrations occurring in the countries where the  
14  
15 67 maize is grown but does represent that imported and used by UK Industry. In the  
16  
17  
18 68 earlier study of maize entering the UK, consignments were examined for aflatoxins,  
19  
20 69 ochratoxin A, fumonisins and ZON but did not include DON.  
21

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23 70

24  
25 71 Fumonisin first reported in 1988 have been shown to occur widely, but mainly only  
26  
27 72 in maize (Shephard et al. 1996). Fumonisin, ZON, DON, NIV and other  
28  
29  
30 73 trichothecene mycotoxins are produced by different species of *Fusarium* so that  
31  
32 74 occurrence of a mixture of mycotoxins usually indicates that the maize has been  
33  
34 75 infected by more than one fusarium species. The human consumer is not exposed  
35  
36  
37 76 directly to the quantities of mycotoxins in the raw maize but to the mycotoxins  
38  
39  
40 77 remaining in the food products manufactured after milling and processing of the raw  
41  
42 78 cereal. As maize processing is a complex technology (e.g. Kent and Evers 1994) the  
43  
44 79 levels of mycotoxins in the finished product may not reflect the concentrations in raw  
45  
46  
47 80 maize as the amounts surviving through the food processing chain will depend on the  
48  
49  
50 81 properties of the mycotoxin and on the nature of the process(es).

51  
52 82

53  
54 83 Reliable information about the frequency and concentrations of mycotoxins occurring  
55  
56 84 in maize are required, including trends over the longer term and this survey thus  
57  
58  
59 85 aimed to examine the concentrations of fusarium mycotoxins in raw maize entering  
60  
86 the UK from the harvests in the period 2004 to 2007 and to compare the results where

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3 87 possible with those found previously in the earlier survey targeting the period June  
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6 88 1998 to April 1999 as cited above.  
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## 10 90 **Methods**

### 11 91 *Sampling and analysis*

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14  
15 92 Maize for human consumption in the UK is imported in bulk consignments by boat  
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17  
18 93 and then either transferred directly via conveyer belt to dockside mills or transported  
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20  
21 94 by truck. Maize from France arrives in vessels usually holding 2-6000 tonnes and that  
22  
23 95 from Argentina in larger consignments of 20-30,000 tonnes that may be contained in  
24  
25 96 up to 10 or more holds although not all holds will contain maize and a range of other  
26  
27  
28 97 imports are likely to be carried simultaneously. Samples for this study were supplied  
29  
30 98 by three large UK millers.  
31

32 99  
33

### 34 100 *Official sampling procedures*

35  
36  
37 101 Introduction of statutory limits for fusarium mycotoxins were accompanied by  
38  
39  
40 102 procedures for sampling and for analysis when inspecting for enforcement purposes  
41  
42 103 (EC 2006b). It is however accepted that it may be appropriate to use other sampling  
43  
44  
45 104 methods and procedures for purposes other than for official control. Operation of  
46  
47 105 those sampling procedures here would be both onerous and costly for industry and as  
48  
49  
50 106 sampling was not intended for enforcement purposes, 'alternative' procedures were  
51  
52 107 devised based as far as possible on existing in-house practice in order to disrupt  
53  
54 108 everyday production as little as possible. Samples collected were kept cool and dry out  
55  
56  
57 109 of direct sunlight before sending to the analytical laboratory.  
58

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60

### 111 *Sampling at maize mills*

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2  
3 112 All three millers have in-house sampling protocols at in-take to their mills although  
4  
5 113 each procedure is different. Each company supplied samples based on the internal  
6  
7  
8 114 quality control system in place, or adaptations thereof.  
9

10  
11 115

12  
13 116 Company 1 supplied 30 consignments of raw maize over a 3-year period. Maize was  
14  
15 117 transported from ship to mill in vehicles each containing 28 tonnes. In previous  
16  
17  
18 118 studies from this mill, 20 consecutive lorries were sampled (Scudamore and Patel,  
19  
20 119 2000). In this study one vehicle was isolated on each occasion and 30 incremental  
21  
22  
23 120 samples were taken and bulked to give a 10kg sample for analysis. In further studies a  
24  
25 121 28 tonne load was cleaned and the maize milled to obtain representative samples of  
26  
27  
28 122 the product streams, which included grits, flour and other ingredients.  
29

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31 123

32  
33 124 Company 2 supplied samples from 26 consignments of raw maize over a 3-year  
34  
35 125 period. Sampling was by autosampler from trucks of approximately 20 tonne capacity  
36  
37 126 resulting in 60 incremental samples each of approximately 100 g giving a bulk sample  
38  
39 127 of 6 kg for analysis. Each spearing represented a combination of samples from top,  
40  
41  
42 128 middle and bottom of the truck.  
43

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46  
47 130 Company 3 supplied samples from 26 ship deliveries of maize over a 3-year period.  
48  
49 131 Samples of maize from multiple holds of these ships were in addition taken on six  
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51  
52 132 occasions. Representative intake maize samples were collected from the ship to mill  
53  
54 133 conveyer belt approximately hourly during a continuous eight-hour period  
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56  
57 134 representing a working day run. Eight samples of about 0.5 kg were scooped from the  
58  
59 135 flowing maize streams to provide a composite of about 4 kg of material. Because of  
60



1  
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3 136 the large size of the consignments unloading takes several days and is done hold by  
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5  
6 137 hold. The 4 kg bulk sample was thoroughly mixed and sent for analysis.  
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11 139 *Analytical methods*  
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13 140 In total 82 different ship consignments of whole in-take maize (together with some  
14  
15 141 cleaned samples and milled samples) were examined over an approximate 3-year period  
16  
17 142 spanning four different harvest years. The quantity of sample taken as described earlier  
18  
19 143 was 4 to 10 kg depending on the agreed method of sampling used and a sub sample was  
20  
21 144 sent for analysis. On receipt, the whole sample was ground to pass through a 0.8 mm  
22  
23 145 screen and mixed for 30 minutes in a Gardner horizontal mixer to ensure homogeneity.  
24  
25 146 Samples were stored at -20°C if not analysed immediately.  
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32 148 The analytical methods used were UKAS (ISO 17025) accredited. All reagents were  
33  
34 149 analytical grade and all solvents were of HPLC grade. Mycotoxin standards of DON,  
35  
36 150 NIV, other trichothecenes, ZON and fumonisin B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub>, were purchased from  
37  
38 151 commercial sources. Trichothecene and ZON standards were quantified prior to use by  
39  
40 152 UV spectrometry.  
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47 154 All analyses were conducted with a spiked sample, i.e. a known amount of toxin was  
48  
49 155 added to a sample of ground maize each day prior to extraction, clean-up and HPLC  
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51 156 determination for each batch of 1-5 samples. These results were used to assess  
52  
53 157 recovery and all reported results were corrected using the values obtained. Recoveries  
54  
55 158 in the range 70-110% were considered acceptable.  
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4 160 The spike level for DON, fumonisins and for ZON were 400, 400 and 50  $\mu\text{g kg}^{-1}$   
5  
6 161 respectively. When results were significantly above these levels, they were repeated and  
7  
8 162 spiked at a level appropriate for each toxin.  
9

10  
11 163

12  
13 164 The limit of detection and limit of quantification respectively for each mycotoxin were  
14  
15 165 5  $\mu\text{g kg}^{-1}$  and 10  $\mu\text{g kg}^{-1}$  for DON, the other trichothecenes and each fumonisin  
16  
17 166 mycotoxin and 1.5  $\mu\text{g kg}^{-1}$  and 3  $\mu\text{g kg}^{-1}$  respectively for ZON. The limit of detection  
18  
19 167 is defined as 3 times the electronic baseline noise and the limit of quantification as 6  
20  
21 168 times baseline noise. Calibration curves for each mycotoxin were plotted with the  
22  
23 169 lowest calibration points respectively being equivalent to 10, 3 and 10  $\mu\text{g kg}^{-1}$  for  
24  
25 170 DON, ZON and fumonisins. After analysis samples were retained and stored at -  
26  
27 171 20°C.  
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### 34 35 173 *Analysis*

36  
37 174 DON together with the other trichothecene mycotoxins was determined following the  
38  
39 175 method of Patel *et al.* (1996). ZON was determined using HPLC with fluorescence  
40  
41 176 detection (Scudamore and Patel 2000). The method for fumonisins was a modification  
42  
43 177 of the method originally published by Shephard *et al.* (1990) and modified (Scudamore  
44  
45 178 and Patel, 2000).  
46  
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### 52 53 180 *Method validation and quality control*

54  
55 181 UKAS accreditation ensures that methods are valid for the tasks they are performing,  
56  
57 182 but do not provide an absolute measure of accuracy. Under the Laboratory Quality  
58  
59 183 System a protocol for internal and external proficiency test schemes participation is  
60  
184 defined and the laboratory demonstrates on-going precision and accuracy. This

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2  
3 185 laboratory analysis uses in-house reference material, participation in sample testing  
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5  
6 186 schemes such as FAPAS (www.fapas.co.uk/fapas.cfm) and Certified Reference  
7  
8 187 Materials (CRM's) intermittently. Acceptable results have been achieved in the  
9  
10 188 FAPAS Scheme for DON, ZON and FB<sub>1</sub> and FB<sub>2</sub> in cereals during rounds during the  
11  
12 189 past 8 years .  
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15  
16 19017  
18 191 **Results and discussion**19  
20 192 Recoveries obtained for each analytical method fell within the acceptable range  
21  
22 193 throughout the study (70-110%) and all results presented are corrected for recovery.  
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2425  
26 19427  
28 195 From data for maize consumption it would seem that the companies participating in  
29  
30 196 this work process between 50 % and 100 % of the raw maize used for human food in  
31  
32 197 the UK. In total 82 consignments of raw maize received at the ports or on entry to  
33  
34 198 maize mills during 2004-2007 were examined comprising 56 from France and 26  
35  
36 199 from Argentina.  
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3839  
40 20041  
42 201 **INSERT: TABLE 1**43  
44 20245 202 Table 1 gives the concentration of mycotoxins found in samples taken from the 26  
46  
47 203 ships from Argentina. Twenty of the samples represented the concentrations from an  
48  
49 204 individual hold of 2 - 3000 tonnes in a single ship while 6 samples were the mean  
50  
51 205 concentrations of samples taken from either 6 or 7 individual holds in that ship. Every  
52  
53 206 consignment contained fumonisins, the lowest concentration of FB<sub>1</sub> + FB<sub>2</sub> being 446  
54  
55 207 µg/kg and the maximum being 5002 µg kg<sup>-1</sup>. Concentrations in 2 consignments  
56  
57 208 exceeded the current maximum allowed concentrations but these occurred in the first  
58  
59 209 year of the study before regulations for fumonisins came into force. 65 % of samples  
60

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3 210 contained between  $1000 \mu\text{g kg}^{-1}$  and  $3999 \mu\text{g kg}^{-1}$  although this was much less than the  
4  
5  
6 211 97 % of total fumonisins found in this range in the survey of 2000 although that study  
7  
8 212 only covered a 10-month period. Levels of DON and ZON in the current study were  
9  
10 213 much lower rarely occurring at more than 10 % of the statutory limits while a level of  
11  
12 214  $496 \mu\text{g/kg}$  of NIV was found in one consignment. However, while DON was detected  
13  
14 215 in every sample ZON was undetected in 50 % of the consignments. NIV occurred in  
15  
16 216 65 % of samples, although the mean value of  $57 \mu\text{g kg}^{-1}$  resulted from a heavily  
17  
18 217 skewed distribution where a few consignments contained relatively high  
19  
20 218 concentrations so that the median value was only  $13 \mu\text{g kg}^{-1}$ . In the earlier study ZON  
21  
22 219 was greater than  $20 \mu\text{g kg}^{-1}$  in every consignment with a maximum value of  $584 \mu\text{g}$   
23  
24 220  $\text{kg}^{-1}$  (cf.  $42 \mu\text{g kg}^{-1}$  here).

25  
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29  
30 **INSERT: TABLE 2**  
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33 222  
34  
35 223 Results for French consignments of maize are given in Table 2 and are presented  
36  
37 224 separately for the 2 mills and combined to give the means for all French consignments  
38  
39 225 to enable comparison with the Argentinean maize. The maize used by the 2 French  
40  
41 226 mills originates from different growing areas. The concentrations of mycotoxins in  
42  
43 227 maize imported by Mill 2 were somewhat higher than those from Mill 1 (except for  
44  
45 228 NIV) although this could merely reflect different purchasing quality specifications set  
46  
47 229 by the two Companies rather than climate or other factors. Concentrations of DON  
48  
49 230 and ZON were higher on average in French maize than in the Argentine maize while  
50  
51 231 fumonisin concentrations were lower. Mean concentrations of NIV were quite similar  
52  
53 232 from all areas.  
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3 234 The reduction of ZON (and probably DON) concentrations since 2000 may in part be  
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5  
6 235 due to the introduction of limits for these mycotoxins that were introduced in 2006  
7  
8 236 while limits for fumonisins were not introduced until 2007.  
9

10 237

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12  
13 238 Previous studies of French maize indicated that amounts of fumonisins (and  
14  
15 239 aflatoxins) decreased with higher latitudes, probably reflecting the cooler climate  
16  
17 240 (Scudamore and Patel 2000). Fumonisin levels (and aflatoxin concentrations) were  
18  
19 241 higher in Argentinean maize. In contrast, there was no clear trend in the results for  
20  
21 242 ZON. This was suggested as reflecting the relative climate in the regions and is  
22  
23 243 consistent with the conditions necessary for the growth of the fungi responsible for the  
24  
25 244 formation of these mycotoxins.  
26  
27  
28  
29

30 245

31  
32 246 *Differences between seasons*  
33

34  
35 247 Table 3 shows the results separated into the relative harvest years 2004 to 2007.  
36  
37 248 Although the relative number of samples is small and is not the same for each season,  
38  
39 249 the quantity of maize represented by the samples is high and should give a good  
40  
41 250 indication of the relative levels of mycotoxins in these years. Hence fumonisins  
42  
43 251 occurred at their highest levels by far in 2004 in maize from Argentina that suffered a  
44  
45 252 prolonged drought prior to harvest, and in maize from France in 2006, a year in which  
46  
47 253 fumonisins reached very high levels in parts of Europe during a very hot, dry summer.  
48  
49 254 DON, ZON and NIV were all at their lowest levels in the 2005 harvests in maize from  
50  
51 255 both Argentina and France.  
52  
53  
54  
55

56 256

57 **INSERT: TABLE 3**

58  
59 257 Although this survey indicates the concentrations of DON, ZON and fumonisins in  
60  
258 imported raw maize over the years 2004-2007, care should be exercised in comparing

1  
2  
3 259 maize from different sources and seasons as this will be affected by physical treatment  
4  
5  
6 260 of these grain consignments before shipping selection before export to ensure it will  
7  
8  
9 261 meet the specifications of the buyer. Constant monitoring of maize is required at  
10  
11 262 source before export to avoid the arrival of a ship containing maize with unacceptable  
12  
13 263 levels of mycotoxins (e.g. in excess of regulatory limits) as this would represent a  
14  
15  
16 264 huge problem and a potential financial disaster.  
17

18 265

19  
20 266 ***Relationship between fumonisin mycotoxins***

21  
22  
23 267 Legislation for fumonisins is for the combined concentration of FB<sub>1</sub> + FB<sub>2</sub>. Melcion  
24  
25 268 et al. (1997), studied the biosynthesis of fumonisins in culture by various strains of  
26  
27 269 *Fusarium moniliforme*. Some strains only produced FB<sub>1</sub> while others varied widely in  
28  
29  
30 270 the amounts and proportions of the various analogues obtained. Rheeder et al. (2002)  
31  
32 271 reported that FB<sub>1</sub> typically accounts for 70 to 80 % of the total fumonisins produced,  
33  
34  
35 272 while FB<sub>2</sub> usually makes up 15 to 25 % and FB<sub>3</sub> usually makes up from 3 to 8 % when  
36  
37 273 cultured on corn or rice or in liquid medium.  
38

39 274

40 **INSERT: TABLE 4**

41  
42 275 The relationship between levels of FB<sub>1</sub> FB<sub>2</sub> and FB<sub>3</sub> for all samples of intake maize is  
43  
44 276 given in Table 4 as the mean ratios of FB<sub>1</sub> to FB<sub>2</sub> and FB<sub>1</sub> to FB<sub>3</sub> in the three areas  
45  
46  
47 277 from which the maize originates and is similar irrespective of origin. Combining all  
48  
49 278 results gives a ratio of 3.6 for FB<sub>1</sub> to FB<sub>2</sub> with a SD of 23 % On the basis of these  
50  
51  
52 279 results it is suggested that results found for FB<sub>1</sub> allows extrapolation to a working  
53  
54 280 value for FB<sub>1</sub> + FB<sub>2</sub>.  
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56 281

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59 282 ***Variation in mycotoxin concentrations in different holds of multi-hold ships***

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3 283 During this work the opportunity arose to obtain samples from different holds in a  
4  
5  
6 284 single ship as there appears little or no information about how the distribution of  
7  
8 285 mycotoxins vary in such a situation. This was carried out in 5 ships in which the  
9  
10 286 maize from either 6 or 7 different holds each containing several thousand tonnes of  
11  
12 287 maize could be examined. Each ship was loaded with maize from different suppliers  
13  
14  
15 288 from Argentina, but all originating from the same general area.

17  
18 289 **INSERT: TABLE 5**

19  
20 290 The distribution of mycotoxins between holds in 5 different vessels is given in table 5.  
21  
22 291 The variability between concentrations from the different holds indicated by % SD  
23  
24  
25 292 between samples showed that the % SD for fumonisin values were usually much  
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27 293 lower than those for DON, ZON and NIV confirming the visual picture shown in  
28  
29 294 Figure 1. Despite the variability observed, the maximum value for DON, ZON and  
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31 295 NIV from any individual hold was still never more than 3 times that of the mean  
32  
33 296 concentration of maize in the ship. The maximum value for FB<sub>1</sub>+ FB<sub>2</sub> was only 1.7  
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35 297 times the mean in vessel E and this value was lower in the other vessels. No sample in  
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37 298 any hold exceeded the EC statutory limits set for fumonisins despite their relatively  
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39 299 high mean concentration, for example, in ship B being more than 50 % of the statutory  
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41 300 limit.

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49 302 ***Effect of cleaning***

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51 303 Maize on intake is subjective to a comprehensive cleaning process that removes  
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53 304 stones, metal objects cobs and other foreign bodies. A succession of sieves, aspirators  
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55 305 and separation systems will then remove broken maize seeds and dust. The  
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57 306 effectiveness of cleaning for removing mycotoxins from maize has been reported  
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59 307 previously as being very variable. Scudamore and Patel (2000) studied the effect of  
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3 308 cleaning on the levels of aflatoxins, zearalenone and fumonisins in maize in a large  
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6 309 commercial mill. Aflatoxin and fumonisin concentrations were reduced by about 30 -  
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8 310 40 % but little reduction was observed in the levels of zearalenone. No significant  
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10 311 reduction of aflatoxin was observed earlier by Brekke *et al.* (1975) while cleaning also  
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12 312 appears to be relatively ineffective for deoxynivalenol (Scott *et al.*, 1984; Abbas *et al.*,  
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14 313 1985; Seitz *et al.*, 1985).

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18 314 **INSERT: TABLE 6**

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22 316 **INSERT: TABLE 7**

23  
24 317 Tables 6 to 8 present results showing the effect of cleaning on mycotoxin  
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26 318 concentrations in the 3 mills. Where analytical results for a matched pair of raw maize  
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28 319 and clean maize are available the apparent reduction or change in mycotoxin  
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30 320 concentration has been calculated for each mycotoxin. The mean change in  
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32 321 concentration achieved for each mycotoxin for all samples was then calculated and  
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34 322 these values are given in the tables. The changes achieved in mills 1, 2 and 3 were +5  
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36 323 %, 28 % and -1 % respectively for DON, + 11, + 23 % but -54% for ZON and + 4 %,  
37  
38 324 + 18 % and - 48 % for FB<sub>1</sub>. Results for FB<sub>2</sub> and for FB<sub>3</sub> were similar to those for FB<sub>1</sub>  
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40 325 within each mill.

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42 326 **INSERT: TABLE 8**

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44 327 The concentrations of mycotoxins in the consignments of maize studied are  
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46 328 also given in the tables but these do not appear to influence the degree of cleaning  
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48 329 achieved.. Despite the difference in scale, the principles of operation were similar.  
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50 330 Other reports of maize cleaning at the industrial scale show similar variability and so  
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52 331 the effectiveness of cleaning is probably more related to the condition of the grain  
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54 332 consignment than to the specific mill involved. The effect varied from being  
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3 333 ineffective on some occasions to substantially reducing mycotoxins in others with up  
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6 334 to 80 % of the mycotoxins being removed at the extreme. The effects on DON  
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9 335 appeared to be extremely variable although this is most likely to be an artefact of  
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11 336 sampling.

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15 338 ***Occurrence of other Fusarium mycotoxins***

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18 339 The only mycotoxins detected in intake maize other than DON, ZON, NIV and  
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20 340 fumonisins were low levels of 3-ac DON, 15-ac DON, fusarenone-X, HT2 and T2  
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22 341 although T2 and HT2 were never detected here in Argentinean maize. When these  
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25 342 trace concentrations were found in intake maize, they could usually be confirmed after  
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27 343 milling by the higher levels in the milling streams such as bran, meal and germ in  
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29 344 which mycotoxins are concentrated (results to be reported elsewhere).

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35 346 **Conclusions**

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37 347 One or more fusarium-derived mycotoxins were present in quantitative amounts in  
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39 348 every sample of raw maize examined. Total fumonisin concentrations were frequently  
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41 349 greater than 1000  $\mu\text{g kg}^{-1}$ , but since legislation for fumonisins was introduced, no  
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43 350 consignment contained  $\text{FB}_1 + \text{FB}_2$  above the statutory limit of 4000  $\mu\text{g kg}^{-1}$  while no  
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45 351 concentrations of DON, ZON and NIV exceeded 500  $\mu\text{g kg}^{-1}$ , 200  $\mu\text{g kg}^{-1}$  and 500  $\mu\text{g}$   
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47 352  $\text{kg}^{-1}$  respectively. Mycotoxins levels varied between source and season. Sampling from  
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49 353 different holds in large ship consignments suggested that variation in the  
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51 354 concentrations of mycotoxins in the vessel tended to be less for fumonisins than for  
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53 355 DON.

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3 357 Information about the fate of mycotoxins during processing is required to assist with  
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6 358 the estimation of daily intake of mycotoxins from maize products and relate these with  
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9 359 the levels in raw maize. The acquisition of these data should be encouraged to ensure  
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11 360 that they are available for any discussion relating to further EU legislation for  
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13 361 mycotoxins in raw cereals and cereal-based foods.  
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18 363 Sampling according to the methods proscribed by the EC Regulations for enforcement  
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20 364 purposes is difficult, time consuming and not always practically possible but  
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22 365 alternative samples for quality control may be suitable. The UK Food Standards  
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25 366 Agency has recommended guidelines to help clarify sampling requirements for use by  
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27 367 enforcers and trade.  
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32 369 Despite severe problems of very high concentrations of fumonisins reported from  
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34 370 Europe in maize from the 2006 harvest, UK maize millers have been able to import  
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36 371 maize that in most instances has mycotoxin levels significantly below the current  
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38 372 statutory limits. Should it become agronomically and economically feasible to grow  
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40 373 maize with the appropriate characteristics needed for products for human consumption  
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42 374 in the UK, it could be anticipated that concentrations of fumonisins would be low  
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44 375 compared to those in maize from hotter parts of Europe although frequency and levels  
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46 376 of DON and ZON might be higher.  
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54 378 Cleaning tends to reduce mycotoxin concentrations somewhat, but the results obtained  
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56 379 are very variable and unpredictable. However it is concluded here that the cleaning  
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58 380 operations are essential for removing foreign bodies prior to milling rather than as a  
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60 381 tool for reducing mycotoxins.

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7 383 Results obtained from this survey provide important information on the occurrence  
8 384 and distribution of DON, ZON, NIV and fumonisins in maize. This information is  
9 385 relevant both to the UK and to the EC and these data give a valuable indication of the  
10 386 effectiveness of recently introduced EC limits for these mycotoxins.  
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### 17 388 **Acknowledgements**

18 389 These studies were funded by the UK DEFRA, Food Quality and Safety LINK  
19 390 Programme and the UK Food Standards Agency and would not have been possible  
20 391 without the enthusiastic cooperation and assistance from the staff of the three UK  
21 392 maize mills.  
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461 Table 1: Occurrence of mycotoxins in raw maize from Argentina, 2004-2007 harvests

No. of Consignments	Parameter	Mycotoxin, $\mu\text{g kg}^{-1}$ (EC maximum)						
		DON (1750)	ZON (350)	FB <sub>1</sub>	FB <sub>2</sub>	FB <sub>3</sub>	FB <sub>1</sub> + FB <sub>2</sub> (4000)	NIV
26	Mean all results	89	15	1426	476	245	1902	57
	Median all	74	10	1210	393	229	1653	13
	Range all	16-220	<3-42	345-3813	101-1230	74-711	446-5002	<10-496
	<10	0	13	0	0	0	0	9
	10-99	18	13	0	0	3	0	13
	100-349	8	0	1	11	19	0	3
	350-999	0	0	8	13	4	7	1
	1000-3999	0	0	17	2	0	17	0
>4000	0	0	0	0	0	2	0	

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Table 2 : Mycotoxins in raw maize from France ( UK intake mills 1 and 2), 2004-2007

No. of Consignments	Parameter	Mycotoxin, $\mu\text{g kg}^{-1}$ (EC maximum)						
		DON (1750)	ZON (350)	FB <sub>1</sub>	FB <sub>2</sub>	FB <sub>3</sub>	FB <sub>1</sub> + FB <sub>2</sub> (4000)	NIV
30	Mean, mill 1	139	16	320	83	49	403	39
	Median, mill 1	41	<3	289	59	31	362	27
	Range, mill 1	<10-444	<3-86	<10-1010	<10-269	<10-180	<10-1279	<10-134
26	Mean, mill 2	270	37	541	155	89	696	31
	Median, mill 2	254	23	439	120	67	543	22
	Range, mill 2	19-932	<3-165	14-2590	<10-811	<10-501	19-3401	<10-170
56	Mean, all	200	26	423	115	68	538	35
	Median, all	131	11	293	61	47	365	25
	<10	1	23	2	7	8	2	15
	10-99	24	31	9	25	38	7	37
	100-349	18	2	20	20	9	19	4
	350-999	13	0	18	4	1	21	0
	1000-3999	0	0	7	0	0	7	0
	>4000	0	0	0	0	0	0	0

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Table 3: Mean fusarium mycotoxin concentrations in maize from different harvest years as received in the UK

Country (consignments )	Harvest year	Mycotoxin, $\mu\text{g kg}^{-1}$				
		DON	ZON	FB <sub>1</sub>	FB <sub>1</sub> + FB <sub>2</sub>	NIV
Argentina						
( 4 )	2004	117	27	2714	3648	14
( 8 )	2005	45	6	1095	1439	16
( 9 )	2006	82	6	1455	1952	109
( 5 )	2007	147	26	693	906	64
France						
(12)	2004	273	41	316	398	54
(21)	2005	52	4	319	408	17
(19)	2006	338	39	697	893	46
(4)	2007	232	51	469	600	34

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5 4856 486 Table 4: Mean ratios values for the concentrations of FB<sub>1</sub> to FB<sub>2</sub> and FB<sub>1</sub> to FB<sub>3</sub> in  
7 487 raw maize

	Mill 1 (French)		Mill 2 (French)		Mill 3 (Argentine)		ALL	
	FB <sub>1</sub> : FB <sub>2</sub>	FB <sub>1</sub> : FB <sub>3</sub>	FB <sub>1</sub> : FB <sub>2</sub>	FB <sub>1</sub> : FB <sub>3</sub>	FB <sub>1</sub> : FB <sub>2</sub>	FB <sub>1</sub> : FB <sub>3</sub>	FB <sub>1</sub> : FB <sub>2</sub>	FB <sub>1</sub> : FB <sub>3</sub>
Mean	3.9	7.0	3.7	6.5	3.1	6.1	3.6	6.7
Median	4.0	6.7	3.7	6.0	3.2	5.4	3.7	6.0
Range	2.5-6.1	4.5-16.3	2.2-5.8	3.4-14.7	1.9-4.0	3.6-15.3	1.9-6.1	3.4-16.3
SD, %	20	38	21	40	17	48	23	42

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489 Table 5: Variation in mycotoxin concentrations in maize taken from different holds of  
 490 ships from the Argentine

Ship Ref. (holds sampled)	parameter	Mycotoxin, $\mu\text{g kg}^{-1}$			
		DON	ZON	FB <sub>1</sub> + FB <sub>2</sub>	NIV
A (6)	Mean	38	4	1789	10
	Median	30	<3	1856	7
	Range	12-86	<3-15	1231-2459	<10-23
	SD, %	69	123	27	70
B (6)	Mean	42	18	2058	108
	Median	48	16	2170	91
	Range	<10-69	6-40	1238-2488	<10-243
	SD, %	58	65	21	89
C (7)	Mean	65	6	1653	91
	Median	65	4	1528	97
	Range	11-118	<3-17	1230-2506	<10-153
	SD, %	61	95	31	69
D (7)	Mean	148	15	446	<10
	Median	137	13	414	<10
	Range	63-284	11-21	158-653	-
	SD, %	49	29	39	-
E (6)	Mean	218	37	1383	46
	Median	134	17	1312	28
	Range	43-630	9-100	456-2365	<10-153
	SD, %	99	104	55	126
SD, % mean of all		67	83	35	88

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Table 6: Effect of cleaning on mycotoxin concentrations, mean of all consignments, mill 1

	mycotoxin				
	DON	ZON	FB <sub>1</sub>	FB <sub>2</sub>	FB <sub>3</sub>
Number of calculations†	12	4	14	12	12
Mean change, %	+5	+11	+4	+4	+3
Median change, %	+3	-24	-10	-16	-9
Range mean change, %	-58 to +143	-38 to +233	-47 to +102	-65 to +152	-42 to +58
Range of concentrations*	10-519	3-86	10-1010	10-269	19-180
% SD	49	74	47	65	34

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† only pairs of raw and cleaned maize are included in which both values are equal to or above the quantitative limit. These are the numbers of values used for the calculations.

\*  $\mu\text{g kg}^{-1}$  in raw maize

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Table 7: Effect of cleaning on mycotoxin concentrations, mean of all consignments, mill 2

	Mycotoxin				
	DON	ZON	FB <sub>1</sub>	FB <sub>2</sub>	FB <sub>3</sub>
Number of calculations†	9	6	8	7	7
Mean change, %	+28	+23	+18	+43	+5
Median change, %	+32	+27	+6	+3	+6
Range mean change, %	-51 to +276	-64 to +231	-30 to +247	-14 to +384	-25 to +137
Range of concentrations*	42-743	14-130	14-1250	23-440	14-191
% SD	55	63	47	75	23

504 † only pairs of raw and cleaned maize are included in which both values are equal to or above  
505 the quantitative limit. These are the numbers of values used for the calculations.

506 \*  $\mu\text{g kg}^{-1}$  in raw maize  
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Table 8: Effect of cleaning on mycotoxin concentrations, mean of all consignments, mill 3

	Mycotoxin				
	DON	ZON	FB <sub>1</sub>	FB <sub>2</sub>	FB <sub>3</sub>
Number of calculations†	10	8	10	10	10
Mean change, %	-1	-54	-48	-52	-51
Median change, %	-11	-67	-57	-61	-66
Range mean change, %	-75 to +143	-74 to -12	-77 to 0	-84 to +3	-83 to +4
Range of concentrations*	44-191	5-42	377-2740	128-1230	74-711
% SD	5419	62	52	51	59

512 † only pairs of raw and cleaned maize are included in which both values are equal to or above  
513 the quantitative limit. These are the numbers of values used for the calculations.

514 \*  $\mu\text{g kg}^{-1}$  in raw maize  
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