4. Results and Discussion

4.1. Mycotoxins in grain and grain products consumed in South Africa

4.1.1. Unprocessed commercial South African maize

In only one of 456 samples of 1986 RSA maize examined by the University of Natal, were AFLA detected at more than 5 ng/g. In parallel tests, the Maize Board found no AFLA in this sample. No other mycotoxin was detected in any other sample. The main fungi present were *Stenocarpella* spp., *Fusarium* spp., *Aspergillus* spp. and *Mucor* spp.

Of the 496 samples of 1987 RSA maize analysed, the University of Natal found AFB_1 in 22 samples at levels over 5 ng/g - the statutory limit in South Africa for AFB_1 in food for human consumption. Twelve more samples contained smaller amounts of AFLA. However, the Maize Board's parallel analyses detected no AFLA in any sample. ZEA was found in three samples, in one at a high and in the other two at low levels. No other mycotoxins were detected.

Of the 1988 crop, the University of Natal analysed 277 samples. In addition to the multi-mycotoxin test, thin layer chromatography (TLC) was carried out for AFLA, trichothecenes and ZEA. ZEA was found in one sample at a very low level. No other mycotoxins were found. The major fungi present were *F. verticillioides*, *F. subglutinans, Stenocarpella spp.* and *Alternaria* spp.

It was concluded that a low rate of contamination of RSA maize with mycotoxins was indicated. This was encouraging, but it was felt that the tests were not sufficiently sensitive or specific to give a clear presentation of the situation. It was therefore decided to conduct specific analyses by GC and HPLC in subsequent maize crops for mycotoxins common in maize worldwide.

Dutton & Kinsey (1996a) later published their results on these and other samples. During the period 1984-1993 they examined just over 1600 samples of agricultural commodities, comprising maize, compound animal feeds, oil seeds, soyabean,

fishmeal and forage for fungi and over 20 mycotoxins using a multiscreen augmented with individual assay. AFLA had the highest incidence in over 14% of all samples examined followed by trichothecenes at 10% and then ZEA at 4%. Since 1989 these authors also examined 20 selected maize samples with high levels of *Fusarium* spp. for FB_1. Of these, 90% were positive in 1993. In their tests, incidence of *Fusarium* spp. in maize and maize containing feeds was 32%, which was higher than either *Aspergillus* spp. (27%) or *Penicillium* spp. (12%).

In analyses carried out by the MRC on RSA maize of the 1989 commercial crop (Table 22), *F. subglutinans* and *F. verticillioides* were the most prevalent fungi, followed by *S. maydis* and *F. graminearum*. In maize from the N-OFS and the W-Tvl *F. verticillioides* dominated, while *F. subglutinans* was dominant in maize from the E-OFS, and to a lesser extent in maize from Natal and E-Tvl. There were no differences in infection rates between the three grades of white and yellow maize respectively. On the other hand, infection by *S. maydis* differed significantly between the three grades, illustrating the visibility of *S. maydis* infection and the role it plays in grading, in contrast to *F. verticillioides* and *F. subglutinans*. This implies that grading can be employed to further discriminate against *S. maydis*, but not against *F. verticillioides* and *F. subglutinans*. *S. maydis* was also prevalent in the N-OFS and the W-Tvl. *S. macrospora* was found much less frequently than *S. maydis*. *A. flavus* was rarely found. In Natal, *Penicillium* spp. were found comparatively frequently. In most cases, the infection levels between white and yellow maize were similar, except in the case of *F. subglutinans* and total fungi, where white maize was significantly less infected than yellow maize.

Table 22 - Mean incidence of fungi (% infected kernels) and fumonisin levels (ng/g) in yellow (Y) and white (W) RSA maize of the 1989 crop from different production areas[1]

Fungus	Maize type	N-OFS[1]	E-OFS[1]	Natal[1]	W-Tvl[1]	E-Tvl[1]
		% infected kernels[2]				
F. verticillioides	W	18.4a[3]	2.6c	9.2ab	12.5ab	7.2b
	Y	22.4a	2.9b	5.5b	20.2a	8.6b
F. subglutinans	W	7.7a	17.4a	13.6a	12.6a	9.8a
	Y	15.1a	21.3a	20.0a	14.5a	14.9a
F. graminearum	W	1.3b	4.0a	4.0a	3.4ab	2.0b
	Y	1.8a	2.7a	3.0a	2.5a	2.2a
S. maydis	W	13.2a	3.1b	2.8b	12.2a	5.4b
	Y	14.2a	3.8b	9.4ab	12.2a	5.1ab
Other fungi	W	13.2 abc	16. 6ab	21.1a	10.6c	11.5bc
	Y	14.6a	14.0a	19.3a	16.5a	14.4a
Total fungi	W	53.9a	43.7ab	50.8a	51.3a	36.0b
	Y	68.1a	44.7b	57.5ab	65.9a	45.2b
Mycotoxin	**Maize type**	**ng/g**				
FB_1	W	1 392a	21c	114b	208ab	734ab
	Y	258a	25a	127a	250a	252a

FB_2	W	420a	12b	60ab	81ab	252a
	Y	67a	0a	14a	113a	66a
Total FBs	W	1 812a	33c	174b	289b	986ab
	Y	325a	25a	141a	363a	318a

Based on a total of 68 white and 53 yellow maize samples

Detection limits of mycotoxins were as follows:

FB_1, FB_2, – 20 ng/g; and

AFLA = AFB_1, AFB_2, AFG_1 and AFG_2, - 2 ng/g

AFLA were not found in any samples, or the calculated means for any production area were <0.5 ng/g

[1] See Fig. 1 for location of production areas. N-OFS and E-OFS = northern and eastern Orange Free state respectively; W-Tvl and E-Tvl = western and Eastern Transvaal respectively

[2] Four surface sterilized kernels per petri dish on malted agar and 25 petri dishes per sample

[3] Means in a row followed by the same letter do not differ statistically significantly (P<0.05)

The mycotoxin most frequently detected was FB_1, particularly in white maize from the N-OFS. The highest levels found were 7.02 and 5.23 µg/g of FB_1 and FB_2 together in two first grade samples of white maize from this area. Samples containing 2 to 3 mg FB_1/kg were common from this area. FB_2 commonly occurred together with FB_1. These mycotoxins are produced by *F. verticillioides*, which dominated in this area, and in the W-Tvl. FB levels in the W-Tvl were significantly lower. The levels of infection of yellow maize by *F. verticillioides* were (with the exception of Natal and

E-Tvl) notably, though not significantly higher than that of white maize (Fig. 2), but the levels of FBs in yellow maize were significantly lower. While in 1990 and 1991, the differences in FB levels between white and yellow maize were not significant, the FB level was still notably higher in white maize in spite of a lower infection rate by *F. verticillioides*. No explanation for this anomaly is evident in the data.

The other mycotoxins included in this investigation were found infrequently in 1989, and at insignificant levels. No AFLA were found. The most prevalent of the other mycotoxins was DON, which occurred at levels highly unlikely to be harmful to consumers. It should be noted that the mycotoxin(s) produced by *S. maydis* have as yet not been chemically characterised, therefore these were not included in this study.

The results on 1990, 1991, 1992, 1993 and 1994 RSA maize (Tables 23 through 26) were in general agreement with those of 1989, but significant year-to-year variation in FB content was noticeable. This is not surprising, considering the large year-to-year climatic variation, with the 1991/92 growing season exceptionally dry, and particularly good rainfall in the 1993/94 growing season in all areas. Similar year-to-year climatic variation was evident in single production areas as well.

These surveys confirmed that mycotoxins occur at low levels in commercial maize and, with the exception of FBs, are found infrequently. In most years of the early 1990's, the FBs were particularly prominent in white maize. In 1990, the highest mean levels of FBs were in samples from Natal, the W-Tvl and the N-OFS, and the lowest in maize from the E-Tvl and the E-OFS. In 1990, FB levels in Natal increased, and in 1991, it increased in the E-OFS, compared with each previous year. From year to year FB levels varied considerably and in the 1994 crop particularly high levels of FBs were recorded in white maize of the W-Tvl and to a lesser extent also the E-Tvl. In 1990, 3.1% of all samples contained more than 2 mg/kg, compared to 6.6% in 1989. The highest level found in 1990 was 4.37 mg FB_1 and FB_2/kg compared to 7.02 and 5.23 mg/kg the previous year.

F. subglutinans (and MON in 1990) occurred most frequently in samples from the E-Tvl and the E-OFS. MON was found in only one sample from Natal. *F. graminearum* occurred most frequently in samples from Natal and the E-Tvl, except in 1992, a particularly dry year, when *F. graminearum* levels in all production areas were very

similar and low. In 1990, the highest levels of DON and NIV were found in Natal, and in 1991 in W-Tvl. *F. graminearum* produces DON and NIV as well as ZEA. However, ZEA was not found in a single sample in 1990, and only in two samples in 1991. In 1989, *S. maydis* was most prevalent in the W-Tvl and N-OFS and least prevalent in the E-Tvl and the E-OFS. This changed through the following seasons and in 1992, it was most prevalent in the E-Tvl, E-OFS and Natal, and least so in the W-Tvl and N-OFS. In 1990 and 1991, no AFLA were detected - not even in the samples on which *A. flavus* was found. In 1992, there was a marked increase in the incidence of samples infected *by A. flavus* - 59 out of 118. This is consistent with the drought conditions that occurred during the growing season. However, only 5 of the samples contained AFLA. The highest level detected was about 20 ng/g. Because of drought stress, the maize plants were more susceptible to infection by the fungus. The 1991/92 growing season was one of the driest in the history of RSA maize production. The low incidence of AFLA can probably be ascribed to unsuitable climatic conditions for the production of this mycotoxin.

Fungal infection rates and mycotoxin contamination rates of yellow and white maize differed widely with much year-to-year variation. However, the difference was statistically significant only for DON and only so in 1990. There were no differences between grades as far as *Fusarium* infections were concerned, because most *Fusarium* infected kernels show no signs of infection and appear completely healthy. On the other hand, S. *maydis* infection rates were clearly reflected in the grades, because infected kernels have an obviously mouldy appearance.

FB_3 occurred in 37% of the 1990 crop samples and the levels varied between 20 and 1 670 ng/g. For comparison, FB_1 and FB_2 were found on 83% of the 1990 samples, and on 68% of the 1989 samples. White maize contained more FB_3 than yellow maize, but this was not significant.

The MRC carried out parallel analyses on samples of the 1990 maize crop and published the results, together with their results on the 1989 crop (Rheeder *et al*, 1995). There was excellent agreement between their results and those of the Maize Board.

The toxicology of FBs to humans is still unclear, therefore the significance of the FB levels found cannot be fully judged. However, on the basis of available knowledge, it can be concluded that maximum contamination levels of the magnitude quoted above, gives reason for caution, even though they only occurred in a small number of samples. The mean levels of total FBs in white and yellow maize were far lower than the mean level of approximately 8 000 to 10 000 ng/g in feed known to cause problems in horses (Anonymous, 2001c).

Table 23 - Mean incidence of fungal infected kernels and mycotoxin levels (ng/g) in commercial white (W) and yellow (Y) RSA maize of the 1990 crop from different production areas

Fungus	Maize type	N-OFS[1]	E-OFS[1]	Natal[1]	W-Tvl[1]	E-Tvl[1]
		% infected kernels[2]				
F. verticillioides	W	13.5 a[3]	3.5 b	19.5 a	11.3 a	5.2 b
	Y	14.7 a	6.6 b	12.3 a	17.9 a	9.0 b
F. subglutinans	W	11.1 ab	14.5 a	12.1 ab	7 .7 b	16.0 a
	Y	18.3 a	20.4 a	12.6 b	13.1 b	22 .4 a
F. graminearum	W	0.6 c	1.2 be	2.5 b	1.2 be	4 .5 a
	Y	1.2 b	0.9 b	2.3 b	1.3 b	4.1 a
S. maydis	W	8.4 a	6.0 a	6.5 a	9.4 a	4.2 a
	Y	8.9 b	9.6 b	9.2 b	14.9 a	11.2 ab
S. macrospora	W	0.0	0.0	0.25	0.0	0.0
	Y	0.0	0.0	0.0	0.0	0.07

A. flavus	W	0.03	0.07	0.0	0.04	0.0
	Y	0.05	0.0	0.04	0.21	0.0
Other fungi	W	20.7 ab	15 .3 b	23. 5 a	14.6 b	19.8 ab
	Y	20.3 b	16.9 b	29.0 a	17.0	27.4 a
Total fungi	W	54.4 b	40.6 c	64.4 a	44.2 c	49.7 be
	Y	63.5 b	54.3 c	65.3 b	64.5 b	74.1 a
Mycotoxin	**Maize type**			**ng/g**		
FB_1	W	372 a	224 a	633 a	510 a	209 a
	Y	81 a	87 a	96 a	312 a	104 a
FB_2	W	161 a	91 a	268 a	158 a	69 a
	Y	29 a	42 a	50 a	89 a	44 a
FB_3	W	35 a	23 a	79 a	48 a	28 a
	Y	7 a	9 a	9 a	39 a	11 a
Total FBs	W	567 a	318 a	979 a	716 a	306 a
	Y	117 a	138 a	155 a	440 a	159 a
MON	W	83 a	95 a	0.0 a	0.0 a	498 a
	Y	316 a	89 a	0.0 a	56 a	442 a
DON	W	276 a	0.0 a	624 a	423 a	358 a
	Y	389 a	390 a	600 a	449 a	240 a

NIV	W	86 a	0.0 a	91 a	71 a	7'7 a
	Y	76 a	145 a	67 a	90 a	57 a

Mycological data based on a total of 155 white and 164 yellow maize samples; fumonisin analyses on a total of 66 white and 62 yellow maize samples; other mycotoxins on a total of 30 white and 25 yellow maize samples

Detection limits of mycotoxins were as follows:

DON - 100 ng/g;

NIV, MON and ZEA - 50 ng/g;

FB_1, FB_2, FB_3 – 20 ng/g; and

AFLA = AFB_1, AFB_2, AFG_1 and AFG_2, - 2 ng/g

AFLA were not found in any samples, or the calculated means for any production area were <0.5 ng/g

[1] See Fig. 1 for location of production areas. N-OFS and E-OFS = northern and eastern Orange Free State respectively; W-Tvl and E-Tvl = western and Eastern Transvaal respectively

[2] Four surface sterilized kernels per petri dish on malted agar and 25 petri dishes per sample

[3] Means in a row followed by the same letter are not significantly different ($P<0.05$)

Table 24 - Mean incidence of fungi (% infected kernels) and mycotoxin levels (ng/g) in white (W) and yellow (Y) RSA maize of the 1991 crop from different production areas[1]

Fungus	Maize type	N-OFS[1]	E-OFS[1]	Natal[1]	W-Tvl[1]	E-Tvl[1]
		% infected kernels [2]				
F. verticillioides	W	6.0b[3]	1.4a	9.0b	6.7b	6.3b
	Y	6.0b	2.4a	8.2b	7.1b	6.9b
F. subglutinans	W	6.7a	7.0a	4.5a	6.1a	8.7a
	Y	10.5a	12.6b	8.7a	9.0a	14.3b
F. graminearum	W	1.9a	1.8a	4.5b	2.5a	4.3b
	Y	2.3a	2.2a	4.0a	2.6a	2.9a
S. maydis	W	3.8a	2.7a	3.1a	4.0a	2.7a
	Y	5.6b	3.4a	2.4a	6.5b	7.0b
S. macrospora	W	1.5b	0.0a	0.0a	0.1a	0.0a
	Y	0.14a	0.12a	0.39a	0.28a	0.39a
A. flavus	W	0.05a	0.00a	0.25b	0.02a	0.26b
	Y	0.09a	0.20a	0.17a	0.06a	0.06d
Penicillium spp	W	2.6b	0.7a	3.7b	1.0a	3.1b
	Y	1.5a	1.9a	5.3b	1.1a	3.9b
Other fungi	W	7.3a	7.6a	12.7b	8.2a	16.1c
	Y	13.9a	12.2a	18.7b	10.6a	18.7b

Total fungi	W	29.9b	21.2a	37.8c	28.9b	41.5c
	Y	40.0ab	37.0a	47.8bc	37.1a	54.2c
Mycotoxin	**Maize type**			**ng/g**		
FB_1	W	86a	309a	299a	315a	227a
	Y	23a	64a	124a	299a	483a
FB_2	W	0a	0a	54a	22a	54a
	Y	0a	0a	22a	31a	142a
FB_3	W	0a	15a	0a	17a	9a
	Y	0a	14a	0a	0a	0a
Total FBs	W	86a	324a	353a	344a	290a
	Y	23a	78a	146a	330a	625a
DON	W	446a	324a	200a	762a	50a
	Y	37a	310a	218a	430a	0a
NIV	W	40a	18a	0a	96a	0a
	Y	0a	60a	72a	100a	0a

Mycological data based on a total of 170 white and 182 yellow maize samples and mycotoxin analyses on a total of 84 white and 82 yellow maize samples

Detection limits of mycotoxins were as follows:

DON - 100 ng/g;

NIV - 50 ng/g;

FB_1, FB_2, FB_3 – 20 ng/g; and

AFLA = AFB_1, AFB_2, AFG_1 and AFG_2, - 2 ng/g

AFLA were not found in any samples, or the calculated means for any production area were <0.5 ng/g

[1] See Fig. 1 for location of production areas. N-OFS and E-OFS = northern and eastern Orange Free state respectively; W-Tvl and E-Tvl = western and Eastern Transvaal respectively

[2] Four surface sterilized kernels per petri dish on malted agar and 25 petri dishes per sample

[3] Means in a row followed by the same letter are not significantly different (P<0.05)

Table 25 - Mean incidence of fungi (% kernels infected) in white (W) and yellow (Y) RSA maize of the 1992 crop from different production areas[1]

Fungus	Maize type	N-OFS[1]	E-OFS[1]	Natal[1]	W-Tvl[1]	E-Tvl[1]	PWV[1]
		% kernels infected [2]					
F. verticillioides	W	8.8bc	4.3a	10.9c	15.2d	6.4ab	
	Y	14.6b	6.0a	9.0 a	20.3c	10.2ab	
F. subglutinans	W	3.9a	8.6b	5.0a	3.2a	7.9b	
	Y	7.2ab	14.9c	5.6a	5.0a	8.5b	
F. graminearum	W	0.6a	0.5a	0.6a	0.5a	0.6a	
	Y	0.8a	0.6a	0.5a	0.2a	0.5a	
S. maydis	W	1.3a	4.2ab	6.7be	2.1a	9.6c	
	Y	1.8a	7.7b	8.6b	2.5a	15.8c	
S. macrospora	W	0a	0a	0.09a	0a	0.08a	
	Y	2.8a	0.08a	0a	0.05a	0.12a	

A. flavus	W	17.8b	4.0a	0.4a	15.3b	1.7a	
	Y	7.2c	3.4b	0.4a	11.0d	0. 4a	
Penicillium spp.	W	3.3a	4.4a	4.7a	2.3a	3.3a	
	Y	3.2a	4.6a	4.1a	2.9a	3.0a	
Other fungi	W	42.1b	25.5a	18.7a	41.8b	17.3a	
	Y	33.4c	24.2b	11.1a	34.5c	15.6a	
Total fungi	W	77.8b	51.5a	47.3 a	80.5b	46.9a	
	Y	71.0cd	61.6be	39.3 a	76.5d	54.3b	
Mycotoxin	**Maize type**			**ng/g**			
FB_1	W	183	312	279	459	329	274
	Y	199	70	343	218	202	192
FB_2	W	10	15	17	89	40	49
	Y	25	0	15	35	44	15
FB_3	W	1	1	5	18	16	10
	Y	0	0	7	26	22	3
Total FBs	W	194	328	301	566	385	333
	Y	124	70	365	279	268	211
DON	W	173	0	608	397	332	176
	Y	590	438	179	933	276	217

NIV	W	75	0	114	64	45	0
	Y	78	117	50	208	22	0
ZEA	W	0	8	24	0	0	0
	Y	0	0	0	13	0	7

Based on analyses of a total of 120 white and 118 yellow maize samples

Detection limits of mycotoxins were as follows:

DON - 100 ng/g;

OA, NIV, MON, and ZEA - 50 ng/g;

DAS, T-2 – 250 ng/g

FB_1, FB_2, FB_3 – 20 ng/g; and

AFLA = AFB_1, AFB_2, AFG_1 and AFG_2, - 2 ng/g

AFLA, T-2, DAS, and OA were not found in any samples, or the calculated means for any production area were <0.5 ng/g

[1] See Fig. 1 for location of production areas. N-OFS and E-OFS = northern and eastern Orange Free state respectively; W-Tvl and E-Tvl = western and Eastern Transvaal respectively; PWV = the Pretoria-Witwatersrand-Vereeniging production area

[2] Four surface sterilized kernels per petri dish on malted agar and 25 petri dishes per sample

[3] Means in a row followed by the same letter are not significantly different ($P<0.05$). Means of mycotoxin levels were not compared statistically

Table 26 - Mean incidence of fungi (% kernels infected) in white (W) and yellow (Y) RSA maize of the 1993 and 1994 crops from different production areas

Fungus	Maize type	N-OFS[1]		E-OFS[1]		Natal[1]		W-Tvl[1]		E-Tvl[1]		PWV[1]	
		1993	1994	1993	1994	1993	1994	1993	1994	1993	1994	1993	1994
		% infected kernels[2]											
F. verticillioides	W	28[3]	19	8	6	18	16	34	24	15	12	25	16
	Y	38	26	12	9	19	14	41	27	17	13	24	18
F. subglutinans	W	4	9	14	17	5	9	5	8	16	12	7	9
	Y	8	11	21	19	8	8	7	9	20	16	11	12
F. graminearum	W	0	1	1	3	4	5	0	0	4	4	0	3
	Y	0	1	1	2	3	5	0	1	3	3	2	1
Penicillium spp.	W	5	4	6	2	8	7	7	4	8	4	9	4
	Y	5	1	6	6	6	12	7	4	8	6	7	7
S. maydis	W	3	5	1	2	6	4	3	3	5	5	3	2
	Y	2	8	3	4	11	4	3	4	13	7	10	6
S. macrospora	W	0	0	0	0	0	0	0	0	0	0	0	0
	Y	0	0	0	0	0	0	0	0	0	0	0	0
A. flavus	W	1	0	0	0	0	0	2	0	0	0	0	0
	Y	0	1	0	0	0	0	1	0	0	0	0	0
Other fungi	W	16	19	23	26	21	35	16	17	20	32	19	26
	Y	15	23	22	25	18	35	15	18	17	27	18	24

Total fungi	W	56	56	53	55	62	75	67	56	68	68	63	61
	Y	69	72	63	75	66	82	73	66	78	71	72	68
Mycotoxin	**Maize type**	**ng/g**											
FB_1	W	433	327	118	344	336	496	363	1210	266	742	303	394
	Y	455	627	1027	444	702	275	740	815	437	725	727	776
FB_2	W	109	30	15	9	97	62	98	300	42	91	86	84
	Y	81	202	406	56	157	20	247	210	147	115	226	202
FB_3	W	26	4	3	6	36	29	38	217	16	62	34	92
	Y	30	50	168	8	98	7	128	111	56	32	140	78
Total FBs	W	568	362	136	357	469	587	499	1728	324	895	423	569
	Y	566	879	1601	514	957	303	1115	1136	640	872	1093	1056
DON	W	136	80	110	124	43	148	6	121	20	160	17	397
	Y	135	99	61	157	98	220	0	173	125	213	93	157
NIV	W	0	16	14	15	0	19	0	22	2	20	0	63
	Y	13	29	11	41	15	21	0	25	3	69	16	35
DAS	W	0	0	0	0	0	0	0	0	0	0	0	0
	Y	0	0	0	0	0	0	0	0	0	0	0	0
ZEA	W	0	6	0	8	0	34	0	3	0	18	0	13
	Y	0	3	0	4	0	9	0	14	0	7	0	2
AFLA	W	0	0	0	0	0	0	0	0	0	0	0	0

Y 0 0 0 0 0 1 0 0 0 1 0 1

Based on a total of 178 white and 183 yellow maize samples of the 1993 crop and a total of 164 white and 175 yellow maize samples of the 1994 crop

Detection limits of mycotoxins were as follows:

DON - 100 ng/g;

AME, PAT, CIT, OA, NIV, MON, and ZEA - 50 ng/g;

DAS, T-2 – 250 ng/g

FB_1, FB_2, FB_3 – 20 ng/g; and

AFLA = AFB_1, AFB_2, AFG_1 and AFG_2, - 2 ng/g

PAT, AME, CIT, OA, T-2 were tested for, but not found

[1] See Fig. 1 for location of production areas. N-OFS and E-OFS = northern and eastern Orange Free state respectively; W-Tvl and E-Tvl = western and Eastern Transvaal respectively; PWV = the Pretoria-Witwatersrand-Vereeniging production area

[2] Four surface sterilized kernels per petri dish on malted agar and 25 petri dishes per sample

[3] Means were not compared statistically

During 1994, Dutton & Kinsey (1996b) examined 417 samples of agricultural commodities, comprising: maize, compound animal feeds, oil seeds, soya bean, fish meal and forage for fungi and over 20 mycotoxins using a multi-screen augmented with individual assays. Trichothecenes had the highest incidence of over 19% in all samples received, followed by AFLA at 6% and then ZEA at 3%. Selected samples (73) were analysed for FB_1 and of these, 69 (94%) were found to be positive. They also found that over 70% of the maize and maize containing feed samples was

infected with *Fusarium* spp., which was higher than either *Aspergillus* spp. (19%) or *Penicillium* spp. (33%).

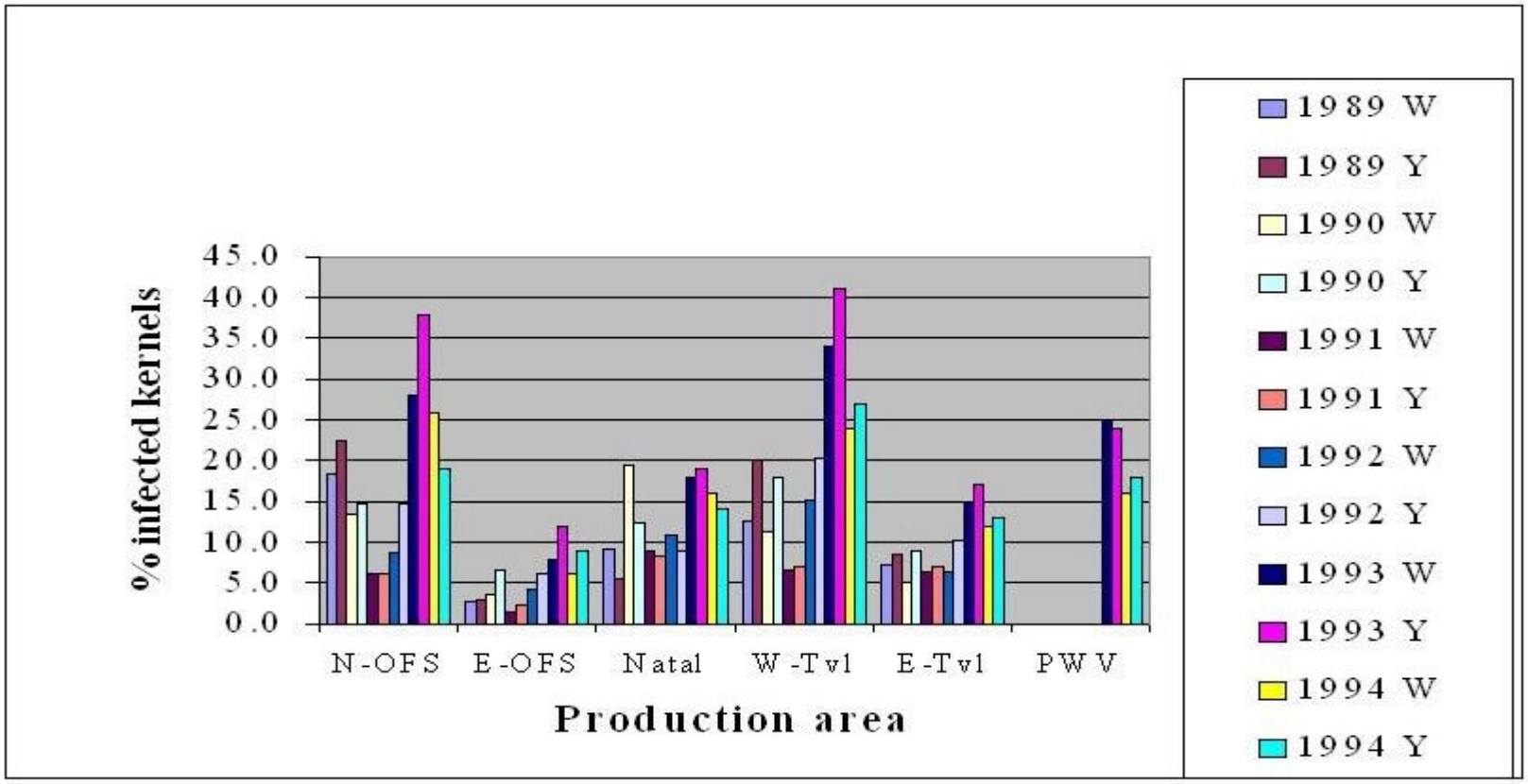

Fig. 2 – Mean percentage white and yellow maize kernels infected by *F. verticillioides* in representative samples of each of six crop years in the main maize production areas of South Africa

Table 27 - Summary of mean mycotoxin content (ng/g) of white maize of the 1989 to 1994 crops in different production areas

	1989	1990	1991	1992	1993	1994	Mean
Total FBs				**ng/g**			
N-OFS[1]	1 812	567	86	207	568	362	600.3
E-OFS[1]	33	318	324	361	136	357	254.8
Natal[1]	174	979	353	350	469	587	485.3
W-Tvl[1]	289	716	354	596	499	1 728	697.0
E-Tvl[1]	986	306	290	405	324	895	534.3
PWV[1]				333	423	569	441.7
MON				**ng/g**			
N-OFS		83					
E-OFS		95					
Natal		0					
W-Tvl		0	344				
E-Tvl		498					
PWV		0					
DON				**ng/g**			
N-OFS	0	276	446	173	136	80	222.2
E-OFS	0	0	324	0	110	124	111.6
Natal	0	624	200	608	43	148	324.6

W-Tvl	0	423	762	397	6	121	341.8
E-Tvl	0	358	50	332	20	160	184.0
PWV	0	0	0	176	17	397	196.7
NIV				**ng/g**			
N-OFS		86	40	75	0	16	43.4
E-OFS		0	18	0	14	15	9.4
Natal		91	0	114	0	19	44.8
W-Tvl		71	96	64	0	22	50.6
E-Tvl		77	0	45	2	20	28.8
PWV		0	0	0	0	63	21.0
ZEA				**ng/g**			
N-OFS		0	0	0	0	6	2.0
E-OFS		0	0	8	0	8	5.3
Natal		0	0	24	0	34	19.3
W-Tvl		0	0	0	0	3	1.0
E-Tvl		0	0	0	0	18	6.0
PWV		0	0	0	0	13	4.3

[1] See Fig. 1 for location of production areas. N-OFS and E-OFS = northern and eastern Orange Free state respectively; W-Tvl and E-Tvl = western and Eastern Transvaal respectively; PWV = the Pretoria-Witwatersrand-Vereeniging production area

Mean values of mycotoxins tested for, but not shown in the table were 0

4.1.2. Mycotoxins in white maize products

The results of the three surveys are summarised in Tables 28, 29 and 30. It appears that maize screenings and maize bran most often contained significantly higher levels of mycotoxins than in any of the milled products. This particularly applies to maize screenings, in which broken and damaged kernels, which are most often mouldy, are concentrated. While the maximum levels of mycotoxins in screenings are similar to those in whole maize, the incidence of samples with high mycotoxin levels is much higher, hence the mean levels in screenings was much higher than in whole maize. For example, over 3 000 µg FB_1 and FB_2/kg in screenings (Table 28), compared with 559 µg total FBs/kg, including FB_3 in 1990 whole white maize.

There was a tendency for the mean FB_1 level in the various maize products to decrease with an increase in refinement from unsifted, to sifted, to special and super maize meal and germless products. The FB_1 content of each product varied considerably, hence the differences were not significant. In places, the tendency was somewhat poorly defined. Bran contained significantly ($P<0.001$) more FB_1 than any of the meals, and in the 1990/91 survey, screenings contained significantly ($P<0.0001$) more FB_1 than bran. The mean FB_1 content of screenings was about 3.6 to 5.5 times higher than that of white maize of the corresponding crop and that of bran about 1.6 to 2.4 times higher. Maximum levels in bran tended to be higher than in screenings. This shows that, during milling, a significant amount of FB_1 is removed with the screenings and bran. With the exception of sifted maize meal in the 1991/92 survey, maize products contained on average less than about half as much FB_1 as whole maize.

The FB_2 content showed a similar - but less clear - pattern to FB_1. Bran contained significantly more FB_2 than any of the meals, and in the 1990/91 survey, screenings contained significantly more FB_2 than bran ($P<0.001$). Again, this shows that a significant part of the FB_2 content of maize is removed with the screenings and the bran.

The levels of FB_1 and FB_2 found here are similar to those found in other studies on commercial South African grain (Sydenham, 1991; Schlechter *et al*, 1998; Thiel *et al*, 1991b; Thiel *et al*, 1992). This confirms that the levels of these two mycotoxins in

South African white maize products are considerably lower than in other countries included in those studies.

The average total FB content in sifted and special maize meal in 1990/91 was about 330 ng/g and about 270 ng/g in 1991/92. These two grades form the bulk of white maize products. Persons consuming 460 g of maize meal per day would have a total FB intake at these contamination levels of between 125 and 152 µg per person per day.

According to the 1991/92-survey, the ZEA content of defatted germ meal was significantly higher than in any other by-product or milled product. In the 1990/91-survey, ZEA levels in bran and screenings were significantly higher than in any milled product. This seems to indicate that some ZEA is concentrated in screenings and bran, but most of it seems to be concentrated in the germ, ending up in the defatted germ meal. This is in agreement with previous studies (Kuiper-Goodman *et al*, 1987). The mean levels found in maize meal etc. can generally be considered as very low and highly unlikely to harm consumers. Interestingly, ZEA was almost completely absent from unprocessed maize.

Table 28 - Mycotoxin content (ng/g) of white maize products in South Africa (1990/91 marketing season)

White maize products	Mycotoxin content (ng/g)						
	FB1	**FB2**	**MON**	**ZEA**	**DON**	**NIV**	**AFLA**
Maize screenings							
Mean	2 096c[1]	968c	-	111b	536	66	0
Maximum	4 335	2 600	-	279	1 400	600	3
Minimum	472	98	-	0	0	0	0
n [2]	15	15	0	15	16	16	16
Maize bran							
Mean	903b	263b	-	94b	76	0	0
Maximum	4 477	1 785	-	521	560	0	0
Minimum	0	0		94	76	0	0
n	23	23	0	25	25	25	25
Unsifted maize meal							
Mean	221a	61a	158b	19a	0	0	0
Maximum	786	308	900	151	0	0	0
Minimum	0	0	0	0	0	0	0
n	22	22	24	25	26	26	26
Sifted maize meal							
Mean	214a	65a	52a	4a	0	0	0
Maximum	1 200	740	632	86	0	0	0

Minimum	0	0	0	0	0	0	0
n	66	66	62	70	72	72	72
Special maize meal							
Mean	200a	69a	53a	3a	0	0	0
Maximum	850	240	380	81	0	0	12
Minimum	0	0	0	0	0	0	0
n	25	25	25	25	27	27	27
Super maize meal							
Mean	134a	24a	28a	0a	0	0	0
Maximum	499	183	300	0	0	0	0
Minimum	0	0	0	0	0	0	0
n	14	14	15	15	16	16	16
Germless products							
Mean	101	0	0	0	0	0	0
Maximum	131	0	0	0	0	0	0
Minimum	66	0	0	0	0	0	0
n	4	4	5	5	9	9	9

Detection limits of mycotoxins were as follows:

DON - 100 ng/g;

NIV, MON and ZEA - 50 ng/g;

FB_1, FB_2, FB_3 – 20 ng/g; and

AFB_1, AFB_2, AFG_1 and AFG_2, - 2 ng/g

[1] Means in a column, followed by the same letter are not significantly different ($P<0.05$)

[2] n = number of samples

Table 29 - Mycotoxin content (ng/g) of white maize products in South Africa (1991/92 marketing season)

White maize products	Mycotoxin content (ng/g)					
	FB1	**FB2**	**ZEA**	**DON**	**NIV**	**T-2**
Maize screenings						
Mean	1 215b[1]	160b	66a	419b	15ab	0a
Maximum [2]	2 130	448	290	1340	200	0
n [3]	18	18	18	18	15	15
Maize bran						
Mean	543a	68ab	65a	423b	60bc	0a
Maximum	5 460	1 342	230	800	420	0
n	27	27	27	25	25	25
DFG meal						
Mean	366a	25a	307b	1120c	100c	0a
Maximum	1 298	202	320	280	200	0
n	22	22	23	22	22	22

Unsifted maize meal						
Mean	79a	0a	12a	12a	0a	0a
Maximum	219	0	100	150	0	0
n	25	25	24	25	26	26
Sifted maize meal						
Mean	371a	29a	19a	11a	0a	0a
Maximum	3 899	757	90	180	0	0
n	52	52	51	51	51	52
Special maize meal						
Mean	125a	3a	13a	15a	0a	0a
Maximum	877	82	180	160	0	0
n	31	31	30	30	31	31
Super maize meal						
Mean	150a	9a	0a	17a	0a	0a
Maximum	806	130	0	200	0	0
n	25	25	25	25	24	24
Germless products						
Mean	119a	6a	20a	0a	0a	0a
Maximum	744	66	80	0	0	0
n	11	11	11	11	11	11

Detection limits of mycotoxins were as follows:

DAS – 250 ng/g – none detected

DON - 100 ng/g;

NIV, MON and ZEA - 50 ng/g;

FB_1, FB_2, FB_3 – 20 ng/g; and

AFB_1, AFB_2, AFG_1 and AFG_2, - 2 ng/g

[1] Means in a column, followed by the same letter are not significantly different ($P<0.05$)

[2] The maximum values observed are as indicated. The minimum values found were 0 in all cases

[3] n = number of samples

Similarly, DON and NIV were particularly highly concentrated in defatted germ meal and to a lesser extent in screenings. This indicates that practically all DON and NIV is removed during cleaning and degerming and very little remains in the product offered for human consumption.

In only one sample of special maize meal, and one sample of screenings, AFLA were found at low levels.

Table 30 - Mycotoxin content (ng/g) of white maize products in South Africa (1994/95 marketing season)

White maize products	Mycotoxin content (ng/g)							
	FB1	FB2	FB3	FBs Total	AFLA Total	DON	NIV	ZEA
Unsifted maize meal								
Mean	827	148	64	1 039	0	179	0	0
Max	3 929	1 100	522	5 551	0	430	0	0
Min	0	0	0	0	0	0	0	0
n [1]	19	19	19	19	19	19	19	19
Sifted maize meal								
Mean	562	87	23	673	0	221	0	2
Max	4 482	1 223	603	6 155	0	850	0	110
Min	0	0	0	0	0	0	0	0
n	47	47	47	47	47	47	47	47
Special maize meal								
Mean	378	32	4	415	0	10	0	4
Max	1 400	507	100	1 773	0	200	0	100
Min	0	0	0	0	0	0	0	0
n	36	36	36	36	36	36	36	36
Super maize meal								
Mean	134	0	0	134	0	22	0	4

Max	871	0	0	871	0	400	0	100
Min	0	0	0	0	0	0	0	0
n	25	25	25	25	24	24	24	24
Maize flour								
Mean	532	0	0	532	0	0	0	0
Max	549	0	0	549	0	0	0	0
Min	514	0	0	514	0	0	0	0
n	2	2	2	2	1	1	1	1
Maize grits								
Mean	554	13	0	567	0	0	0	0
Max	1 800	63	0	1 800	0	0	0	0
Min	0	0	0	0	0	0	0	0
n	5	5	5	5	5	5	5	5
Maize Rice								
Mean	295	0	0	295	0	27	0	0
Max	991	0	0	991	0	300	0	0
Min	0	0	0	0	0	0	0	0
n	11	11	11	11	11	11	11	11
Samp								
Mean	461	3	0	464	0	237	38	0
Max	1 994	41	0	1 994	0	630	300	0

Min	0	0	0	0	0	0	0	0
n	13	13	13	13	13	13	13	13

Detection limits of mycotoxins were as follows:

DAS and T-2 – 250 ng/g – none detected

OA and AME – 50 ng/g – none detected

DON - 100 ng/g;

NIV, MON and ZEA - 50 ng/g;

FB_1, FB_2, FB_3 – 20 ng/g; and

AFB_1, AFB_2, AFG_1 and AFG_2, - 2 ng/g

[1] n = number of samples

In the 1994/95 marketing year, mean levels of FBs and DON in white maize products were considerably higher than in the previous two surveys. This is probably a reflection of the comparatively high FB levels in the 1994 white maize crop from the W-Tvl (mean total FBs 1 728 ng/g). In most years, the W-Tvl is the largest producer of white maize in SA. To a lesser extent, higher FB levels were also evident in white maize grown in the E-Tvl (mean of total FBs, 895 ng/g). An MTL for total FBs of 200 ng/g in maize products for human consumption would have left more than two thirds of all white maize products manufactured in that year legally unsuitable for human consumption.

Persons consuming 460 g of maize meal per day would have a total FB intake at these contamination levels (an average of about 550 ng/g in sifted and special maize meal) of about 253 µg per person per day.

4.1.3. Mycotoxins in maize feed mill products

In the 1994/95 marketing year, a small number of samples of feed mill products (yellow maize) were analysed for their mycotoxin content. Maize germ meal, maize bran and screenings originating from dry milling of white maize and used in the feed milling industry were also analysed. The results are shown in Table 31.

In all products except maize screenings, all mycotoxins that were found were at relatively low mean levels. The mean level of total FBs in screenings was high enough to seriously affect horses, the most sensitive animal species to FBs known. These products were manufactured from maize of the 1994 crop, when abnormally high FB levels were encountered in white maize from the W-Tvl. The W-Tvl usually produces more than 50% of the country's white maize requirements. That year, relatively high FB levels also occurred in maize from the E-Tvl. In some of the bran samples, high FB levels were also found. This confirms that much of the mycotoxin content of unprocessed maize is concentrated in the bran and screenings during the milling process, with only a portion remaining in the white maize products.

Table 31 - Mycotoxin content (ng/g) of yellow maize and other maize products used in feed milling in South Africa (1994/95 marketing season)

Feed mill product	Mycotoxin content (ng/g)							
	FB_1	FB_2	FB_3	FBs Total	AFLA Total	DON	NIV	ZEA
No 1 Straightrun yellow maize meal								
Mean	1 200	229	49	1 477	0	56	0	6
Min	0	0	0	0	0	0	0	0
Max	2 437	610	170	3 217	0	300	0	50
n [1]	8	8	8	8	8	8	8	8

No 2 Straightrun yellow maize meal

Mean	506	251	140	897	0	135	0	25
Min	0	0	0	0	0	0	0	0
Max	1 011	502	280	1 793	0	270	0	50
n	2	2	2	2	2	2	2	2

Unsifted crushed yellow maize

Mean	857	250	39	1 146	0	160	0	0
Min	402	0	0	402	0	120	0	0
Max	1 311	500	78	1 889	0	200	0	0
n	2	2	2	2	2	2	2	2

Sifted crushed yellow maize

Mean	581	0	0	581	0	55	0	0
Min	0	0	0	0	0	0	0	0
Max	1 237	0	0	1 237	0	220	0	0
n	4	4	4	4	4	4	4	4

Defatted maize germ meal (from white maize milling)

Mean	437	25	6	468	0	38	0	0
Min	41	0	0	41	0	0	0	0
Max	1 288	200	48	1 288	0	150	0	0
n	8	8	8	8	8	8	8	8

Maize bran (from white maize milling)

Mean	1 324	338	126	1 788	0	658	89	7
Min	0	0	0	0	0	0	0	0
Max	8 180	2 368	2 008	10 948	0	5 350	820	120
n	32	32	32	32	31	31	31	31

Screenings (from white maize milling)

Mean	6 651	1 628	599	8 878	0	1 114	50	16
Min	840	0	0	840	0	0	0	0
Max	15 716	3 718	1 604	20 354	0	4 820	200	60
n	7	7	7	7	7	7	7	7

Detection limits of mycotoxins were as follows:

DAS and T-2 – 250 ng/g – none detected

OA and AME – 50 ng/g – none detected

DON - 100 ng/g;

NIV, MON and ZEA - 50 ng/g;

FB_1, FB_2, FB_3 – 20 ng/g; and

AFB_1, AFB_2, AFG_1 and AFG_2, - 2 ng/g

[1] n = number of samples

4.1.4. Fungi and mycotoxins in imported yellow maize

During the 1992/93 maize imports from the USA and Argentina, no maize from USA Gulf states such as Texas was purchased, because it was known that AFLA levels in maize from these states are often very high. Import contracts stipulated that in no sample should the AFLA content exceed 15 ng/g and the moisture content should not exceed 14.5%. This was in spite of the fact that maize is received for storage in the USA at 15% moisture content, using the AACC 44-15A moisture reference test which itself underestimates the moisture content of maize by about 1.9 percentage points (Paulsen, 1990). The blending of maize to achieve these stipulations was not allowed. The spraying of water on maize during shipping for dust control was not allowed either. It is therefore likely that the imported maize was generally less contaminated by mycotoxins than the bulk of the maize crop in the two countries. The results of mycotoxin analyses on the imported maize are summarized in Table 32.

Table 32 - Mean fumonisin and aflatoxin levels in South African (SA) and imported USA (1991 and 1992 crops), and Argentinean (ARG) maize (1992 crop)

Mycotoxin	USA maize[1]		SA maize		ARG maize[2]
	1991 crop	1992 crop	1991 crop	1992 crop[3]	1991 crop
			ng/g		
AFLA	3.96 bc [4]	2.81 b	0 a	0.85 a	5.00 c
FB_1	952 b	863 b	278 a	239 a	293 a
FB_2	123 b	143 b	35 a	8 a	23 a
FB_3	61 b	45 b	13 a	6 a	13 a
Total FBs	1 136 b	1 051 b	328 a	253 a	329 a

Detection limits of mycotoxins were as follows:

FB_1, FB_2, FB_3 – 20 ng/g; AFLA = AFB_1, AFB_2, AFG_1 and AFG_2, - 2 ng/g

[1] Based on 9 - 27 samples from every hold of all shipments of imported USA maize that arrived in South Africa between April 1992 and January 1993. The total number of shipments involved in this calculation was not recorded, however a total of 70 shipments of USA maize, each >30 kt were received between April 1992 and June 1994

[2] Based on 9 - 27 samples (see text) from every hold of 13 shipments of imported ARG maize, each >30 kt that arrived in South Africa between April and July 1992

[3] Based on the first 42 white maize samples of the 1992 crop that were analysed in that year. A further 78 white maize samples of the 1992 crop were analysed later that year, which reduced the mean for the 1992 crop to <0.5 ng/g

[4] Means in a row, followed by the same letter are not significantly different (P<0.05)

The mean AFLA levels in the imported maize were comparatively low. RSA maize contained significantly less AFLA than USA and ARG maize. The maximum values detected were 136 ng/g in one sample each of 1991 USA, and 1992 ARG maize, and 20 ng/g in one sample of 1992 RSA maize. The mean levels of FBs in USA maize were significantly higher (P<0.05) than in RSA and ARG maize. The maximum levels of total FBs detected were 10 425 ng/g in 1991 USA, 10 486 ng/g in 1992 USA, 4 133 ng/g in 1991 RSA, 1 130 ng/g in 1992 RSA, and 6 387 ng/g in 1992 ARG maize. Low levels of FBs were found in ARG maize shipped from ports on the Parana River (predominantly flint types), while maize shipped from Atlantic ports (predominantly dent types) always contained considerably higher levels of FBs. Of 1991 USA samples, 3.62% contained FBs at levels exceeding 5 000 ng/g, and so did 3.68% of 1992 USA samples and 0.2% of 1992 ARG samples. No samples of 1991 or 1992 RSA maize contained FBs at this level. Particularly disturbing was that in one shipment of USA maize, the entire bottom half of one hold (more than 4 000 metric tons) had a total FB content exceeding 10 000 ng/g. This is high enough to cause mortality in horses, the most sensitive animal species to FBs. It is known that in some years, for example 1989, FBs have occurred at generally much higher levels in USA

maize than the maximum in single samples ever recorded in South Africa. In addition, AFLA also often occur at high levels in USA maize. ARG maize generally contains AFLA at levels considerably higher than USA maize.

The pattern of fungal infection in USA maize varied considerably with each consignment (Table 33). In most shipments, *F. verticillioides* predominated, but in some others *A. flavus* was the major fungus. The infection level of *Penicillium* spp. sometimes exceeded that of *A. flavus*. In contrast with RSA maize, *S. maydis* was very rarely found on USA maize. In ARG maize, *A. flavus* predominated, followed by *Penicillium* spp. *F. verticillioides* occurred at low levels, except in dent maize shipped from Atlantic ports. Again, *S. maydis* was rarely detected.

Table 33 - Mean incidence of fungi in twelve bulk shipments of imported USA maize after arrival in South Africa

Vessel no	*F. verticillioides*	*F. subglutinans*	*F. graminearum*	*A. flavus*	*Penicillium spp*	*S. maydis*	*S. macrospora*	Other fungi	Total fungi
				Mean percentage kernels infected [1]					
1	19.3	2.3	0.4	16.1	8.3	0.1	-	23.8	70.4
2	16.7	2.2	0.4	15.3	7.7	-	0.1	31.1	73.4
3	19.1	3.2	0.6	8.6	11.8	-	0.4	34.1	77.6
4	13.7	3.7	1.8	8. 4	23.6	-	0.2	31.0	82. 4
5	10.9	2.3	0.6	15.7	16.2	0.1	-	32.4	78.2
6	7.6	1.2	0.1	18.4	3.1	-	0.1	18.8	49.3
7	9.3	0.8	0.1	17.5	2.3	-	0.2	21.6	51.7
8	6.0	0.5	0.0	11.3	4.1	0.0	-	18.9	40.8

9	4.5	0.5	0.2	6.4	1.8	0.0	-	8.5	21.9
10	8.4	0.1	0.0	4.7	3.0	0.0	-	18.7	34.9
11	8.1	0.6	0.2	1.6	2.0	0.4	-	18.3	31.1
12	4.0	0.8	0.2	3.7	3.7	0.2	-	19.9	32.5

[1] Four kernels per petri dish on nutrient agar; 25 petri dishes per sample; 9 – 27 sample per cargo hold

4.1.5. Fungi and mycotoxins in a vessel of exported yellow maize

The shipment of RSA maize exported to Taiwan, was analysed for various ear-rot fungi and *Fusarium* mycotoxins (Cronje, 1993; Rheeder *et al*, 1994). The predominant ear-rot fungi, in decreasing order of isolation frequency, were *F. subglutinans*, *F. moniliforme*, *S. maydis* and *F. graminearum*. *A. flavus* and A. *parasiticus* were not isolated from samples prior to export, but a small number of *A. flavus* isolates were found after shipment. The predominant mycotoxins were FB_1 (0-865 ng/g) and FB_2 (0-250 ng/g). Low levels of MON (< or = 390 ng/g) were detected in some samples before shipment. ZEA (25 ng/g), and NIV (120 ng/g) were detected in two out of 32 samples taken in Taiwan. The samples contained no detectable levels of either AFLA (>0.5 ng/g) or DON (>100 ng/g) before or after shipment.

The Maize Board, in parallel analyses on the same series of samples (Cronje *et al*, 1990; Cronje, 1993), found no ZEA at a detection limit of 20 ng/g, nor DON and NIV at a detection limit of 100 ng/g. MON was found in two samples taken during outloading from storage silos, but not in any of the samples taken at the end-users in Taiwan. FBs were detected at a detection limit of 50 ng/g in 27.8% of the samples taken at the storage silos (range 60 – 880 ng/g) and in 43.7% of the samples taken in Taiwan (range 50 – 985 ng/g).

4.1.6. Fumonisins in foreign maize food products

Marasas *et al* (1993) and Shephard *et al* (1996a) summarized the results of FB analyses on South African, Swiss and USA commercial maize-based human foodstuffs (Tables 34 and 35). From these data, and from data of maize imported into South Africa, it is clear that RSA maize contains relatively low levels of mycotoxins, including FBs. If tolerance levels are instituted in South Africa, which a large proportion (up to two thirds in some years) of RSA maize products cannot comply with, alternative sources of similar products are highly unlikely to be found. That would mean severe shortages of maize products, and consumers will have to switch to other grain-based foods, such as rice, pasta and bread. Since about 2 million tons of maize products will have to be replaced by these foods, great upheaval in food markets would be unavoidable. A glut in export maize and feed maize will result as

product labelled unsuitable for human consumption floods the feed markets and the export market to countries with higher, or no MTLs.

4.1.7. Mycotoxins in other grain staples in South Africa

Data for other grain staples in South Africa, similar to the maize data above, are not available, as similar surveys have never been done on other grains in South Africa. Extensive surveys over a period of 14 years from 1982/83 to 1996/97 have been done on the fungi infecting wheat in the 17 production areas in South Africa (Rabie-unpublished). However, it is not known how sampling was done and how representative of commercial wheat the data are. There is no reference to infection rates in different grades. It is unfortunate that the actual mycotoxins occurring in the wheat samples have apparently not been surveyed. It would be misleading to deduce the hazards posed by mycotoxins in wheat from the type of fungus, and the fungal infection rates found. This is very clear from the maize data. The value of the existing data on wheat is therefore limited to demonstrating the major fungal species in wheat and the large year-to-year variation. At best, data from a few 'snapshot' types of mycotoxin surveys have been published, but it is highly unlikely that these would be representative of the situation in South African wheat and sorghum as a whole. It would be risky to base conclusions on these few results, and until supplemental surveys have been carried out they are best ignored.

Table 35 - Fumonisin B_2 levels in commercial maize-based human foodstuffs (from Marasas *et al,* 1993)

Maize Product	Incidence	FB levels (ng/g)		
		South Africa[1]	Switzerland[2]	USA[1]
Meal	Pos/Tot	11/52	0/7	13/16
	Range	0-131	0	0-920
	Mean/Pos	83	0	298
Grits	Pos/Tot	4/18	13/55	5/10
	Range	0-120	0-160	0-1 065
	Mean/Pos	85	100	375
Flakes	Pos/Tot	0/3	0/12	0/2
	Range	0	0	0
	Mean/Pos	0	0	0
Tortillas	Pos/Tot	NT[3]	0/4	0/3
	Range	NT	0	0
	Mean/Pos	NT	0	0

[1] Data from Sydenham *et al* (1991)

[2] Data from Pittet *et al* (1992)

[3] NT = None tested

4.2. Correlation of the geographic distribution of oesophageal cancer in black males and *F. verticillioides* infection rates and fumonisin contamination levels in commercial white maize in South Africa

The estimated kernel infection rates by *F. verticillioides*, the estimated average FB content, and OC incidence in black males in various geographical areas of South Africa are shown in Table 36. The correlations between OC incidence on the one hand, and estimated *F. verticillioides* kernel infection rate or FB level in each of the areas on the other, are also shown.

No significant correlation was found between OC incidence and the estimated kernel infection rates of maize consumed in the various areas, nor between OC incidence and the estimated PDI in the various areas. A significant positive correlation was found between kernel infection rates with *F. verticillioides* and the FB content of the maize. It is therefore concluded that:

- Over the longer term, fungal infection rates with *F. verticillioides* do give an indication of the levels of FBs that can be expected in commercial white maize produced in South Africa; and

There exists no positive correlation between the geographic distribution of OC in South Africa and either the *F. verticillioides* infection rate, or the natural FB levels in commercial white maize produced in South Africa and consumed in the various geographic areas.

Table 34 - Fumonisin B_1 levels in commercial maize-based human foodstuffs in the USA, South Africa and Switzerland (from Marasas *et al,* 1993)

Maize Product	Incidence	FB levels (ng/g)		
		South Africa[1]	Switzerland[2]	USA[1]
Meal	Pos/Tot	46/52	2/7	15/16
	Range	0-475	0-110	0-2 790
	Mean/Pos	138	85	1048
Grits	Pos/Tot	10/18	34/55	10/10
	Range	0-190	0-790	105-2 545
	Mean/Pos	125	260	601
Flakes	Pos/Tot	0/3	1/12	0/2
	Range	0	0-55	0
	Mean/Pos	0	55	0
Tortillas	Pos/Tot	NT[3]	0/4	1/3
	Range	NT	0	0-55
	Mean/Pos	NT	0	55

[1] Data from Sydenham *et al* (1991)

[2] Data from Pittet *et al* (1992)

[3] NT = None tested

Pos/Tot = number of positive samples per total samples tested.

Mean/Pos = mean for all the positive samples

Table 36 - The OC incidence rates in black males in 1990 and 1991[1], the estimated total FB (FB_1+FB_2+FB_3) content (ng/g) of commercial white maize and subsistence maize consumed[2], the estimated average percentage of *F. verticillioides* infected kernels of commercial white maize[3], the estimated per capita maize consumption[4] and the estimated PDI of total FBs[5] in areas of South Africa

					PDI[5]	
Area	**OC1**	**FBs2**	**%[3]**	**g/day[4]**	**ng/g bw/day**	**µg/70-kg person/day**
Eastern Cape[6]	25.6	1 699			7.67	537
		991			4.47	313
		450	11.0	316	2.03	142
Free State	17.4	553	13.6	276	2.18	153
Gauteng Province	15.9	579	16.9	290	2.40	168
KwaZulu Natal	16.5	531	13.1	244	1.85	129
Mpumalanga	6.3	591	11.6	316	2.66	186
Northern Cape	11.1	526	13.9	251	1.89	132
Northern Province	9.6	633	14.9	283	2.56	179
North West Province	5.2	697	17.3	205	2.04	143
Western Cape	18.0	597	15.6	164	1.40	98
			EC-1[7]	**EC-2[7]**	**EC-3[7]**	
Correlation: OC rate/PDI FBs			**0.5806[8]**	**0.4358[8]**	**-0.4222[8]**	
			NS	**NS**	**NS**	

Correlation: OC rate/*F. verticillioides* infection	**-0.3640**
	NS
Correlation: *F. verticillioides*/FBs content	**0.7359**
	***P* < 0.05**

[1] Expressed as a percentage of all cancers in black males within the geographic area - Cancer Association of South Africa, Cancer Information Service, 2000; Sitas 2002 - personal communication

[2, 3,] See Tables 16 and 17

[4] See Table 18 and Sections 3.2.1. and 3.2.2.

[5] Estimated probable daily intake of fumonisins (ng/g body weight/day, or µg/70 kg person/day) through maize. The figure has not been corrected for mycotoxin losses during commercial milling, hence this is an overestimation

[6] Together with other areas, three scenarios were calculated for the Eastern Cape, with different proportions of subsistence maize incorporated – see Section 3.2.2

[7] EC-1,2,3 = Eastern Cape Scenario 1, 2 or 3 – see Section 3.2.2

[8] The value of r, the correlation coefficient

These findings are in contrast with the findings on subsistence maize in Transkei. This indicates that FBs are either not involved in the aetiology of OC, or that there may be a threshold value for FBs in maize below which there is no influence on the development of OC. Ostensibly, this threshold value, if it exists, is above the FB intake levels of consumers of commercial white maize products in South Africa. The FB levels that normally occur in commercial white maize and maize products are often much higher than the recommended MTL of 100 to 200 ng/g. Therefore, a better understanding through epidemiological studies, of the NOAEL in humans is urgently needed. The actual FB intake levels in plate food, the absorption of FBs in

the human gut and the physiological effects on various biomarkers in humans in high and low OC incidence areas all need to be elucidated. Before this has been done, a meaningful decision cannot be taken about the need for MTLs for FBs in food and the level at which they should be introduced. Potentially disruptive MTLs for FBs in commercial maize, based for a large part on the indirect statistical relationship in Transkei, which may prove co-incidental or of secondary importance, should not be introduced without regard to the epidemiology and aetiology of OC and FBs in the rest of South Africa.

These findings, made from an epidemiological viewpoint, support the arguments by Gelderblom *et al* (1996) from a toxicological viewpoint. They argue as follows:

> "Most mathematical models treat all carcinogens as mutagens (genotoxins). They assume that even at low doses, DNA reactive molecules could escape the cell's detoxifying mechanisms and induce mutation in a critical site on the DNA. As a result, many regulatory policies of various countries rely upon the outcome of these models. However, oversimplified speculations on mechanisms of carcinogenesis induced by non-genotoxic carcinogens, such as FBs, should therefore not serve as the basis for risk assessment procedures. Compounds, specifically cancer promoters that act through specific receptors, tend to be active at low doses and it is unclear whether a no-effect threshold exists. On the other hand, compounds that act through a cytotoxic mechanism would be expected to have a no-effect threshold (Cohen & Ellwein, 1990). Below the threshold, cytotoxicity and increased cell proliferation would not occur and thus not increase the tumor risk. Recent studies concerning two compounds, uracil and melamine, that are carcinogenic in the urinary bladder, indicated that urothelial proliferation is a prerequisite for the formation of calculi and tumors (Cohen & Ellwein, 1991). Although these two compounds are carcinogenic in animals, dose-related considerations suggest that they are obviously not carcinogenic since humans are only exposed to doses that are unable to induce urothelial proliferation."

More recently, Chelule *et al*, (2001) surveyed households in rural and urban areas of KwaZulu Natal in South Africa, to assess the exposure of the inhabitants to FB_1. They assessed exposure of the population to FB_1 at three levels, namely, by analysing stored maize, plate-food, and faeces. They examined 50 samples of rural maize (assumedly produced on subsistence farms), 32% of which had levels of FB_1 ranging from 0.1-22.2 mg/kg, whereas 29% of the 28 cooked maize (phutu) samples contained FB_1 ranging from 0.1-0.4 mg/kg – incidence similar, but contamination levels much lower than in the maize samples. The incidence and levels of FB_1 in faeces were 33% and 0.5-39.0 mg/kg, respectively. Again the incidence is similar to that in the maize and

the phutu samples, but while the FB_1 contamination level is similar to that in the maize, it is much higher than in phutu samples. Of the 49 urban maize samples analysed (assumedly commercial maize) 6.1% had a range of 0.2-0.5 mg/kg FB_1, whereas 3 of 44 faecal samples (6%) ranged between 0.6 and 16.2 mg/kg. The FB_1 incidence rate in the urban samples is markedly lower than in the rural samples. No FB_1 was detected in urban phutu samples. Because these levels are lower than those published from regions in South Africa with high incidence of OC, the authors conclude that the risk of OC from FB_1 exposure may be lower in the KwaZulu Natal region.

Shephard *et al* (2002), investigating the effects of cooking on FB levels in maize porridge, found a mean reduction in FB_1 of 23% in cooked compared to uncooked maize meal. The levels in cooked porridge correlated highly significantly with levels in the uncooked meal ($P<0.01$).

4.3. Correlation of oesophageal cancer rates and maize supply in some African countries

The results of the correlation between grain supply and OC incidence in males and females in 23 African countries are presented in Table 37.

A statistically highly significant correlation ($P<0.01$) for both males and females was found between OC rates and maize supply, but not between OC rates and sorghum supply, or between OC rates and millet supply. This indicates a statistical relationship between OC incidence and maize consumption, which could possibly be related to contamination of maize with a mycotoxin such as FB. To confirm such a relationship, actual FB intake figures are essential, but are at present completely lacking.

Contrasting with the significant correlation, the large differences in OC rates between Zimbabwe, Zambia and Malawi are interesting, considering that all three countries almost exclusively rely on maize as a staple.

Table 37 - The average supply of sorghum, millet and maize in kg per capita per year[1] (calculated over the 4 years 1987 to 1990) in each of 23 African countries[2], and the OC rate (ASIR world population per 100 000) in males and females in each of the countries[3]

Country	OC Rate		Grain supply (ave kg/capita/year)		
	Females	Males	Maize	Sorghum	Millet
Algeria	0.9	0.5	1.0	0.1	0
Angola	0.9	7.9	29.0	0.0	5.65
Belize	1.4	3.4	23.8	0.0	0
Benin	1.2	2.1	58.9	18.0	3.02
Botswana	11.9	27.7	57.2	39.6	1.12
Burkina Faso	1.2	2.1	22.6	88.3	69.60
Burundi	4.9	11.6	29.4	1.7	0.55
Gambia	0.6	0.7	10.0	8.1	42.30
Ghana	1.2	2.1	34.1	8.0	7.40
Malawi	25.7	45.5	151.0	1.0	1.10
Mali	0.6	1.64	20.8	54.4	81.90
Morocco	0	4.09	16.4	0.9	0.17
Mozambique	4.96	11.6	40.0	10.8	0.30
Namibia	2.29	8.33	42.6	4.3	36.20
Niger	0.63	2.48	1.5	43.8	155.50
Nigeria	1.55	2.32	30.7	43.1	35.90

Rwanda	0	0.99	13.9	18.2	0.10
South Africa	12.36	33.7	97.9	3.6	0.15
Swaziland	4.52	31.47	32.6	1.0	0
Tanzania	8.43	9.5	82.5	8.7	4.50
Uganda	8.35	16.97	18.0	6.3	22.82
Zambia	2.99	7.77	153.7	3.0	1.40
Zimbabwe	6.08	23.6	116.4	6.5	10.25
Correlation: OC Rate (Females)/Grain supply			**0.6629[4]**	**-0.2003[4]**	**-0.2851[4]**
			***P*<0.01**	**NS**	**NS**
Correlation: OC Rate (Males)/Grain supply			**0.6157[4]**	**-0.276[4]**	**-0.3322[4]**
			***P*<0.01**	**NS**	**NS**

[1]Per capita supplies in terms of product weight are derived from the total supplies available for human consumption (i.e. food) by dividing the quantities of food by the total population actually partaking of the food supplies during the reference period, i.e. the present in-area (de facto) population. The per capita supply figures shown therefore represent the average supply available for the population as a whole and are taken as an approximation to per capita consumption.

[2]FAO, 2000

[3]Ferlay *et al*, 1999

[4]The value of r, the correlation coefficient

4.4. Aetiology of liver, kidney and brain cancer in South Africa and in Africa in relation to maize and maize products

4.4.1. Correlation of the geographic distribution of liver, kidney and brain cancer in black males and *F. verticillioides* infection rates and fumonisin contamination levels in commercial white maize in South Africa

The estimated kernel infection rates by *F. verticillioides*, the estimated average FB content, and the incidence of liver, kidney and brain cancer in black males as a percentage of all cancers in each area in various geographical areas of South Africa are shown in Table 38. The correlations between OC incidence on the one hand, and estimated *F. verticillioides* kernel infection rate or FB level in each of the areas on the other, are also shown.

A significant correlation was found between kernel infection rates with *F. verticillioides* and the FB content of the maize. No correlation was found between liver, kidney and brain cancer incidence in black males and the estimated kernel infection rates of commercial maize used for manufacturing white maize products consumed in the various areas, nor between liver, kidney and brain cancer incidence and the estimated FB content of commercial white maize used for manufacturing white maize products consumed in the various areas. It is therefore concluded that:

- Over the longer term, fungal infection rates with *F. verticillioides* do give an indication of the levels of FBs that can be expected in commercial white maize produced in South Africa; and
- There exists no correlation between the geographic distribution of liver, kidney and brain cancer in South Africa and either the *F. verticillioides* infection rate, or the natural FB levels in commercial white maize produced in South Africa and consumed in the various geographic areas.

These results differ from those of Ueno *et al* (1997). Maize samples, collected in 1993, 1994 and 1995 from agricultural stocks for human consumption in Haimen (Jiangsu County) and Penlai (Shandong Province), high- and low-risk areas for primary liver cancer in China, respectively, were analysed for FBs, AFLA and trichothecenes. In 1993, levels and positive rates of FBs and DON were significantly higher in Haimen than in Penlai. In 1994, FB contamination levels and rates in the two areas were comparable to those observed in 1993 in Haimen. AFB_1 occurred widely in 1993 and 1994, but the positive rates as well as levels were not significantly different between the areas. In 1995, FB contamination in Haimen was significantly higher than in Penlai. The contamination level, as well as positive rate in 1993 and 1995, were 10-50-fold higher in Haimen than in Penlai, and the authors therefore suggest that FBs may be a risk factor for promotion of primary liver cancer in endemic areas, along with the trichothecene DON. They assumed that co-contamination with AFLA, potent hepatocarcinogens, played an important role in the initiation of hepatocarcinogenesis.

4.4.2. Correlation of liver, kidney and brain cancer rates and grain supply in some African countries

Table 39 presents the correlation coefficients between per capita supply of sorghum, millet and maize (calculated over the 4 years 1987 to 1990) and liver, kidney and brain cancer rates in males and females in 23 African countries.

No statistically significant correlation for either males or females was found between any of the cancer incidence rates and grain supply, for any of the grains.

Table 38 - Incidence of liver, kidney and brain cancer incidence in black males in 1990 and 1991 in different geographic areas of South Africa[1], the estimated total FB (FB_1+FB_2+FB_3) content (ng/g)[2] of commercial white maize and of subsistence maize in the Eastern Cape, the estimated average percentage of *F. verticillioides* infected kernels[3], the estimated per capita maize consumption[4] and the estimated PDI of total FBs[5] in areas of South Africa

Area	Kidney[1]	Brain[1]	Liver[1]	FBs[2]	%[3]	g/day[4]	PDI[5]
Eastern Cape[6]	0.61	0.220	4.30	1699	-	316	7.67
				991	-	316	4.47
				450	11.0	316	2.03
Free State	0.83	0.100	2.35	553	13.6	276	2.18
Gauteng Province	1.12	0.710	3.95	579	16.9	290	2.40
KwaZulu Natal	1.06	0.770	5.92	531	13.1	244	1.85
Mpumalanga	0.00	0.000	6.25	591	11.6	316	2.66
Northern Cape	0.38	0.000	3.45	526	13.9	251	1.89
Northern Province	0.78	0.000	8.53	633	14.9	283	2.56
North West Province	0.00	0.000	6.90	697	17.3	205	2.04
Western Cape	0.89	2.410	2.79	597	15.6	164	1.40

Correlation: Cancer rate/estimated *F. verticillioides* kernel infection rate of maize consumed in the area	**Kidney**	**0.1330[7] NS**
	Brain	**0.2648[7] NS**
	Liver	**0.0659[7] NS**
Correlation: Cancer rate/estimated FB content of	**Kidney**	**-0.0680[7] NS**

maize consumed in the area

	Brain	**-0.2614[7] NS**
	Liver	**-0.0067[7] NS**
Correlation: *F. verticillioides* infection/FBs		**0.7360[7] *P*<0.05**

[1]Expressed as a percentage of all cancers of black males in each area (National Cancer Association of South Africa, 2000; Sitas, 2002)

[2,3]See Tables 16 and 17

[4]See Table 18 and Sections 3.2.1. and 3.2

[5]Estimated probable daily intake of fumonisins (ng/g body weight/day) through maize. The figure has not been corrected for mycotoxin losses during commercial milling, hence this is an over-estimation

[6]Together with other areas, three scenarios were calculated for the Eastern Cape, with different proportions of subsistence maize incorporated. Only the first scenario, with maximum inclusion of subsistence maize in the Eastern Cape and highest FBs levels is analysed here

[7] The value of r, the correlation coefficient

Table 39 - The correlation of average per capita supply of sorghum, millet and maize (calculated over the 4 years 1987 to 1990) (FAOSTAT Database), and the liver, kidney and brain cancer rate in males and females in 23 African countries

Type of cancer	Gender	Correlation (r)		
		Maize	Sorghum	Millet
Liver	M	0.0312 NS	0.4431 NS	0.4007 NS
	F	0.1314 NS	0.3671 NS	0.4168 NS
Kidney	M	0.0238 NS	0.0365 NS	0.2632 NS
	F	-0.2150 NS	0.1146 NS	0.3451 NS
Brain	M	0.0008 NS	-0.2042 NS	-0.2700 NS
	F	-0.0633 NS	-0.1552 NS	-0.3003 NS

4.5. Aetiology of NTD in South Africa in relation to the occurrence of fumonisins in maize and maize products

4.5.1. The link between NTD and fumonisins

Hendricks (1999) reports that in most years, between one and five equine leukoencephalomalacia clusters occur in Texas, but in contrast, 40 to 60 clusters involving approximately 100 horses occurred in Texas during the autumn of 1989, indicating high levels of FBs in the maize crop in Texas that year. Maize linked to 45 equine leukoencephalomalacia clusters had FB_1 levels ranging from <1 to 126 µg/g (Ross *et al*, 1991b) and the mean level in 14 clusters was 10.8 µg/g (Thiel *et al*, 1991b). Similarly, FB_1 levels ranging from <1 to 330 µg/g in maize screenings were associated with porcine pulmonary oedema outbreaks over the same time period (Ross *et al*, 1991a). This indicates that a significant proportion of the crop contained FBs at unusually high levels. As has been shown in Section 4.1.3 and Tables 28, 29 and 30, maize screenings that are removed from grain during the milling process, always contain much higher levels of all the mycotoxins present in the maize. Consequently, where such screenings are utilized in animal feeds, toxicity problems often occur. Of all the animal species, horses are particularly sensitive to FBs, showing severe effects at dietary levels around 10 µg/g. Pigs show an effect at dietary levels around 100 µg/g.

In April 1991 three anencephalic infants were delivered at a Brownsville (Cameron County) hospital within 36 hours by Mexican-American women who conceived in the Lower Rio Grande Valley during 1990 (Texas Department of Health, unpublished report, in Hendricks, 1999). Three more were delivered over the next 6 weeks. Cameron County women who conceived during 1990-1991 had a substantially higher NTD rate (27 per 10 000 live births) than those who conceived during 1986-1989 (15 per 10 000 live births). Most of the increase was accounted for by a doubling of the anencephaly rate from 10 to 20 per 10 000 live births. A case-control study showed that a lower hematocrit was a risk factor, but offered no clue about the origin of the cluster (Texas Department of Health, unpublished report, in Hendricks, 1999).

During this time period, U.S. maize-based foodstuffs also had relatively elevated levels of FBs; 16 maizemeal samples collected from May 1990 through April 1991 had an average total FB level (FB_1 and FB_2) of 1.22 µg/g (Sydenham *et al*, 1991). These levels are two to three times higher than those seen in maize-based foodstuffs collected from South Texas from 1995 through 1997 i. e. between 400 and 600 ng/g (Texas Department of Health and United States Food and Drug Administration, unpublished data in Hendricks, 1999). Unlike non-Hispanic whites in North America, Mexican-Americans in Texas consume a great deal of maize, in the form of tortillas. For instance, Canadian adults consume, on average, about 17 g of maize-based foods per day (Kuiper-Goodman *et al*, 1996). In contrast, Mexican-American women on the Texas-Mexico border consume approximately 90 g of maize per day from tortillas alone (Hendricks, 1999). Thus, Hendricks reasons, it is likely that Mexican-American women along the border were exposed to elevated levels of FBs in maize products during the critical time period.

The levels mentioned here, mean that a woman of 70 kg eating say 100 g of maize-based foodstuffs per day, containing 600 ng/g of total FBs, is ingesting about 60 µg of FBs per 70 kg person each day. Under such conditions, NTD incidence rates appear to be on par with world standards, and if FBs are a factor in NTD, this level can be accepted as a NOAEL for NTD in humans. At an FBs content of approximately 1.2 mg/kg as in the case of the suspected critical period for the cluster of NTD reported by Hendricks (1999), women would be ingesting122 µg of FBs/70 kg person/day.

Hendricks argues further that FB exposure as a risk factor for NTD is supported epidemiologically by a few descriptive NTD studies. She reasons that, although blacks typically have lower NTD rates than both Hispanic and non-Hispanic whites, the NTD rate for blacks in the Transkei region of South Africa is about 10 times higher than that for blacks in Cape Town (61 vs. 5.5 per 10 000 live births) (Ncayiyana, 1986; Cornell *et al*, 1983). A similar high NTD rate (57 per 10 000 live births) has been documented for the Hebei Province of China (Moore *et al*, 1997). As previously mentioned, both of these geographic areas have elevated levels of FBs in maize-based foodstuffs. Working from figures reported by Rheeder *et al* (1992) for FB levels in 'good' Transkeian subsistence maize of 1985 and 1989, Transkeians in the high OC area are estimated to have a PDI of FBs through apparently uninfected

maize used for food, of about 959 µg of FBs/70 kg person/day. These figures will be analysed further in Section 4.5.3.

4.5.2. Other studies on NTD incidence in South Africa

Delport *et al* (1995) studied the spectrum of clinical problems and outcomes in infants born at an urban academic hospital in South Africa. The incidence of congenital anomalies and the outcomes of affected infants of live born infants born over a 3-year period, 1 May 1986 to 30 April 1989, at Kalafong Hospital, Pretoria, were recorded. A total of 17 351 live born infants were examined and the total congenital anomalies incidence was 118.7 per 10 000 live births. The central nervous system was the system most frequently involved (23.0 per 10 000 live births), followed by the musculoskeletal system (21.3 per 10 000 live births). The commonest individual congenital anomaly was Down syndrome (13.3 per 10 000 live births), followed by neural tube defects (9.9 per 10 000 live births) and ventricular septal defects (6.9 per 10 000 live births). In 11% (22.5 per 10 000 live births) of neonatal deaths, infant loss was attributable to congenital anomalies. It was concluded that the incidence of congenital anomalies in black South African neonates, born in an urban setting, is of the same order as in other developed and developing countries.

Venter *et al* (1995) studied the incidence and spectrum of congenital anomalies in live born neonates born in Mankweng Hospital, Sovenga, a rural hospital in the Northern Transvaal, over the period 12 June 1989 to 31 December 1992. Of a total of 10 380 neonates born during this period, 7.617 (73.4%) were examined within the first 24 hours of life. Congenital anomalies were found in 149.7 live births per 10 000, which is higher than in the study by Delport *et al* (1995) in an urban environment. The higher incidence is largely as a result of higher incidences of neural tube defects (35.5 per 10 000 live births) and Down syndrome (21.0 per 10 000 live births).

4.5.3. The epidemiological relationship of NTD with fumonisin intake

Urban consumers of white maize products in South Africa, such as in Cape Town and Pretoria, consume an estimated average of 276 g of white maize product per 70 kg person per day (Gelderblom *et al*, 1996). This is about 3 times as much as the 90 g per

day of Mexican-American women on the Texas-Mexico border (Hendricks, 1999). The average total FBs content of the white maize products sifted, special and super maize meal in the 1990/91 and 1991/92 seasons is about 230 ng/g, calculated from the figures given in Tables 28 and 29. In urban areas, maize consumers would therefore have had a PDI of FBs of about 63 µg per 70 kg person per day. Consumers in rural areas, who consume about 460 g of white maize product per person per day (Gelderblom *et al*, 1996), would be ingesting FBs at the rate of about 106 µg per person per day. From these estimates of PDI of FBs, and the NTD incidence rates in the studies above, and those quoted by Hendricks (1999), Table 40 was compiled, and the correlation between estimated FB intake and NTD incidence calculated. The correlation was statistically significant at $P<0.05$, indicating a positive relationship.

It should be taken into account that FB levels in maize can vary considerably from year to year, and also from consignment to consignment within a year, as indicated by the maximum and minimum levels found in samples during these surveys. In both the W-Tvl and N-OFS for instance, average levels in commercial white maize for a year as high as 1.7 to 1.8 µg/g, or about 2.5 times as much as the long term average, have been recorded in some years. The effect, if any, of these high level years on NTD incidence is not evident in the data above, but it should be kept in mind that it is likely that exposure for a relatively short period of only a few weeks during early pregnancy could cause the disorder. Therefore, long-term average FB levels are not entirely satisfactory indicators of PDI of FBs linked to NTD. If the PDI of FBs by pregnant women during the critical first 6 weeks of pregnancy can be more accurately estimated greater clarity on the possible link between FB intake and NTD incidence can be obtained.

Table 40 - NTD incidence rates per 10 000 live births, and estimated PDI of fumonisins in parts of South Africa and the USA

Locality	**NTD rate**	**PDI [1] FBs/day**
Cape Town	5.5	63
Pretoria	9.9	63
N-Tvl	35.5	106
Transkei	61	959
USA (high incidence year)	27	122
USA (normal year)	15	60
Correlation	**0.8731[2]**	***P*<0.05**

[1] µg/70-kg person/day

[2] The value of r, the correlation coefficient

Other complicating factors are probably also involved in the aetiology of NTD. For example, it is unlikely that the higher incidence of NTD in the rural northern Transvaal, compared to Pretoria, can be ascribed only to a higher intake of FBs, as better health care and better general nutrition in urban areas may also play a role. At this very preliminary stage, however, there can be little doubt that an average daily intake of FBs of around 60 µg per person per day is a safe level in terms of NTD. This translates to an MTL of 130 ng/g in maize products for rural consumers in South Africa, and to 217 ng/g for urban consumers, which are within the MTL range recommended by the MRC for FBs in maize in South Africa (See Section 2.1.3.3). However, it is a small and well-defined section of the population who might need

protection. Such protection, if needed, may be achieved through other means much more effectively than a blanket MTL for FBs in maize products, without the disruption of the maize industry that MTLs of this level would bring (See Sections 4.9.1 and 4.9.2.3).

4.5.4. Animal studies on the effect of fumonisins on foetal bone development and NTD

Lebepe-Mazur *et al* (1995) studied the effects of FBs on foetal bone and organ development in rats. Groups of 5-6 pregnant F344/N rats were orally dosed from day 8 to 12 of gestation with 30 or 60 mg purified FB_1/kg body weight, or with a fat-soluble extract of *F. proliferatum*/maize culture derived from an amount of maize culture that would provide approximately 60 mg FB_1/kg. A fat-soluble extract contains no FBs. Control rats were dosed with water or maize oil. Food intake was monitored daily during dosing. Foetal bone development was examined after staining with alizarin red, whereas internal organ development was examined in hematoxylin and eosin-stained tissue sections. Although group differences in maternal body weight were not statistically significant, weight was 6% less in dams dosed with 60 mg FB_1/kg compared with the control group ($P<0.12$). Relative litter weight was significantly suppressed by 60 mg FB_1/kg. Ossification of the sternebrae and vertebral bodies was significantly impaired by FB_1 treatment. Weight of litters from mothers treated with a fat-soluble extract of *F. proliferatum*/maize culture, which contains no FBs, was not suppressed and bone development was not impaired. It was concluded that FB_1 is fetotoxic to rats by suppressing growth and foetal bone development.

Flynn *et al* (1994) evaluated the embryotoxicity of aminopentol, the total hydrolysis product of FB_1, in cultured rat embryos. Gestation day 9.5 embryos were exposed to 0, 3, 10, 30, 100 or 300 µM aminopentol throughout the entire 45-hr culture period. At 100 µM aminopentol, growth and overall development were reduced significantly. There was also a significant increase in the incidence of abnormal embryos. Of the embryos, 29% had NTD, and 36% had other abnormalities. At 300 µM aminopentol, the incidence of NTD was 15%, and 85% of the embryos had other abnormalities. These findings suggest that aminopentol, at concentrations of 100 µM and above, can induce NTD in organogenesis-stage cultured rat embryos. However, these NTDs are

in conjunction with significant overall retardation of growth and development as well as significant increases in the incidence of other defects. These studies also showed, when compared with previous findings, that aminopentol is over 100-fold less toxic than FB_1 to cultured rat embryos.

On the other hand, LaBorde *et al* (1997) investigated the embryotoxic potential of FB_1 in New Zealand White rabbits. Animals were dosed by gavage daily on gestation day 3-19 with purified FB_1 at 0.10, 0.50, or 1.00 mg/kg/day. Maternal lethality occurred at the 0.50 and 1.00 mg/kg/day doses. When examined on gestation day 29, there were no differences in maternal body weight, maternal weight gain, maternal organ weights, number of nonlive implantations, and number of malformations. Foetal weight was decreased at 0.50 and 1.00 mg/kg/day (13 and 16%, respectively); this was true for male and female pups. Foetal liver and kidney weights were also decreased at these doses. Analysis of embryonic sphinganine to sphingosine ratios demonstrated no differences between control and treated embryos on gestation day 20, although these ratios were increased in maternal urine, serum, and kidney when compared to control animals. These data suggest that FB_1 did not cross the placenta and that the observed decreased foetal weight was probably the result of maternal toxicity, rather than any developmental toxicity produced by FB_1.

4.5.5. Epidemiological studies of NTD in Mexico

As in all toxicological tests, the doses given to test animals in these tests are much higher than those implicated in NTD in humans and also much higher than humans are ever likely to be exposed to. However, these findings may nonetheless indicate the first direct effect of FBs on human health. Follow-up epidemiological studies in humans across the world have so far been extremely limited. As part of an effort to determine levels of FBs in human food, Stack (1998) devised a liquid chromatographic method for determining FB_1 and the total hydrolysis product of FB_1 (HFB_1) in tortillas. HFB_1 is formed through hydrolysis of FB_1 during the alkali treatment (nixtamalization) of maize for the preparation of masa. The method gave average recoveries from tortillas spiked with FB_1 and HFB_1 at 250, 500, and 1000 ng/g, of 86.5% for FB_1 and 82.6% for HFB_1. Tortillas (54) and masa (8) from the Texas-Mexico border were analysed for FB_1 and HFB_1. Average amounts of FB_1 and HFB_1 in tortillas were 187 and 82 ng/g, respectively. Average amounts of FB_1 and

HFB_1 in masa were 262 and 64 ng/g, respectively. The author concludes that the results show that FB_1 and its hydrolysis product are present in tortillas consumed by a population experiencing an increased incidence of neural tube defects. Dombrink-Kurtzman & Dvorak (1999) found that the highest level of hydrolyzed FB_1 detected in masa and tortillas was 0.1 µg/g. The amount of FB_1 was significantly higher in Mexican samples (0.21 – 1.80 µg/g, mean = 0.79 µg/g) than in samples purchased in the United States (0.04 – 0.38 µg/g, mean = 0.16 µg/g). However, these FB_1 levels are similar to those for total FBs in South African white maize products, where no increased effect on NTD is evident in urban areas.

4.5.6. By what mechanisms could fumonisins induce NTDs?

Hendricks (1999) speculates as follows:

"Folate is needed for biochemical reactions involving one-carbon metabolism, such as the biosynthesis of purines and thymidine, the regeneration of methionine from homocysteine, and histidine metabolism. The folate receptor, one of two systems responsible for folate uptake into cells, is found in membrane domains enriched in cholesterol and sphingolipids, and is a glycosylphosphatidylinositol (GPI)-anchored protein (Lacey *et al*, 1998). This high-affinity receptor is responsible for transport of folate into cells with elevated folate requirements, such as placenta, kidney, and breast. By the time of organogenesis, the fetus is dependent on maternally derived folic acid. This continuous need for folic acid is not usually a problem because the placenta concentrates this water-soluble vitamin 3:1 in favor of the fetus (Henderson *et al*, 1995). It has recently been shown that treatment of Caco-2 cells with FB_1 inhibits folate receptor-mediated transport of 5-methyltetrahydrofolate in both a time- and concentration-dependent fashion (Stevens & Tang, 1997). It is not unreasonable to assume that blocking placental uptake of this water-soluble vitamin for a few critical days might induce an NTD.

"Competitive inhibition of folate uptake is not the only possible mechanism through which FBs could induce NTDs. FBs are sphingosine analogs and inhibit the reactions catalyzed by ceramide synthase, resulting in a paucity of sphingolipids synthesized downstream of the synthase, and a disruption of cellular functions dependent on these sphingolipids (Wang *et al*, 1992; Merrill *et al*, 1993; Merrill *et al*, 1995). Ceramides

and sphingosine derivatives are second messengers that trigger apoptosis in a variety of human cell lines (Tolleson *et al*, 1996). Sphingolipids have important roles in membrane and lipoprotein structure, cell-cell communication, interactions between cells and the extracellular matrix, regulation of growth factor receptors, and as second messengers for a wide range of factors including tumor necrosis factor, interleukin 1, and nerve growth factor (Merrill *et al*, 1993)."

Recently, Sadler *et al* (2002) exposed neurulating mouse embryos to fumonisin or folinic acid in whole embryo culture and assessed them for effects on growth and development. Fumonisin exposure inhibited sphingolipid synthesis, reduced growth, and caused cranial neural tube defects in a dose dependent manner. Supplemental folinic acid ameliorated the effects on growth and development, but not inhibition of sphingolipid synthesis. It is concluded that fumonisin has the potential to inhibit embryonic sphingolipid synthesis and to produce embryotoxicity and neural tube defects. Folic acid can reverse some of these effects, supporting results showing that fumonisin disrupts folate receptor function.

4.6. Estimate of the highest MTLs that can be allowed in South Africa for fumonisins, aflatoxins and deoxynivalenol, without jeopardizing the safety of consumers

4.6.1. The current approach to regulation of human exposure to mycotoxins

To date, about 77 countries have enacted or proposed regulations for mycotoxins in food and feed – see Section 2.1 for details. These are all based on MTLs for mycotoxins in certain food commodities and no use is made of other possible measures to minimize exposure to mycotoxins. To introduce appropriate regulations and to set rational MTLs, various scientific, technological, economic and social factors should ideally be brought into account. These include toxicological data, data on dietary exposure, epidemiological data, the distribution of mycotoxins over commodities, legislation of other countries with which trade relations exist, methods of analysis, commercial interests and sufficiency of food supply (Van Egmond & Dekker, 1995). Most of these factors are addressed in the various sections of this report. However, few countries have formally presented their rationale for the need to regulate, or for the selection of a particular maximum tolerable level. For example, most countries' MTLs for AFLA in food are based on vague statements of the carcinogenic risk for humans (Van Egmond, 1993). The general approach is that exposure to a potential human carcinogen that cannot be totally avoided, should be limited to the lowest practical level. However, the definition of practicality varies, depending on whether the country is an importer or producer of the potentially contaminated commodity and on the actual levels of contamination experienced. Several countries claim to have made a hazard evaluation (Belgium, Canada, India, The Netherlands, Switzerland, South Africa, United Kingdom, United States), but specifics are scarce (Stoloff et *al*, 1991; Van Egmond, 1993). In their surveys on the rationale of countries for setting limits for mycotoxins other than AFLA, Stoloff *et al* (1991) found that no rationales were provided, except for Canada, where risk assessment was done for DON, ZEA and OA. Recently, the USA applied a good

scientific approach for setting guidance levels for FBs in feed and food - see Section 2.5.3.1 for details. In South Africa, recommended levels for FBs are based on toxicological and some epidemiological data (Marasas, 1997), while estimates of acceptable levels of total fumonisins in maize are based on TDI (based on NOEL in rats/1000 and NOEL in rats/100) (Gelderblom *et al*, 1996). However, many other important factors have not been considered. It is apparent that in most countries either the scientific basis for regulation of mycotoxins is non-existent, or the science has not been fully utilized (Stoloff et *al*, 1991; Van Egmond, 1993). Considerations related to trade, economic and social aspects are mostly completely ignored.

4.6.2. Formulating a proposal for MTLs for aflatoxins in grain and grain products

4.6.2.1. Assessment of human exposure to aflatoxins in South Africa

4.6.2.1.1. Estimate of direct aflatoxin intake

Local maize and grain sorghum

AFLA are practically completely absent from locally produced commercial maize and dry milled maize products manufactured thereof, and probably also from grain sorghum and sorghum products (See Section 4.1). A possible exception is sorghum beer, where particularly floor malting practices could possibly create conditions suitable for growth of *A. flavus*, and perhaps also for AFLA production. Unfortunately, very few test data are available on AFLA production during the malting of grain sorghum. AFLA intake from this source is therefore uncertain, but probably very low.

Local wheat

In stored wheat in South Africa in recent years, a non-standard moisture reference test has been used for calibrating electronic moisture meters for moisture testing during harvest intake at storage silos, which clearly underestimates the wheat moisture content. The proof for this is seen in moisture problems, usually ascribed to bin leakage, which have been regularly experienced during 1998 and 1999 in silo bins in which wheat is stored. Far fewer 'bin leakages' are experienced in bins containing

other grains. Clearly, the correct moisture content of wheat in storage in South Africa is not known, and is sufficiently high in places for caking and sprouting to occur. Where wheat pockets contain more than 18% moisture, growth of *A. flavus* could take place, as this fungus does occur in wheat in South Africa. The production of AFLA in wheat is possible, because grain temperatures in wheat generally exceed 25°C, which is a suitable temperature for AFLA production. This is a second possible source of AFLA intake, the importance of which is presently uncertain. However, using a proven standard moisture reference method for calibrating electronic moisture meters could easily eliminate this source.

Nuts and groundnuts

Probably the main source of AFLA in the diet of South Africans is nuts, particularly groundnuts, which are often contaminated with AFLA. A grading system that discriminates against mouldy groundnuts and regulatory MTLs for AFLA in nuts are in force and are apparently strictly applied by the trade. Groundnuts are also sorted for the confectionery market and discoloured or damaged nuts are removed. Only nuts low in AFLA content are used for direct human consumption, but no routine testing by the official health authorities takes place. These circumstances indicate a low AFLA content in nuts used for human consumption. However, AFLA levels and human consumption of nuts have not been investigated as it falls outside the scope of the present study. AFLA intake from this source is therefore also uncertain.

Imported maize

An important sporadic source of AFLA exposure in the South African diet is imported maize. Depending on the stage weather cycles such as the El Nino Southern Oscillation (ENSO) cycle, South Africa from time to time suffers droughts, which can be severe enough to force the importation of most of the maize needed to meet the demand for human consumption. Approximately 2.7 Mt of maize is annually needed for processing into 2.2 – 2.3 Mt of various food products for human consumption. In 1992/93, more than 4 Mt of maize was imported for both human and animal consumption. The average AFLA content of USA maize was between 3 and 4 ng/g, and 5 ng/g in Argentine maize (Section 4.1.4; Table 32). Some individual samples contained as much as 150 ng/g of AFLA, particularly Argentine maize. Moreover, in

the USA, mycotoxin levels in export grain are not under the jurisdiction of the FDA (See Section 2.1.2.1). AFLA exposure of humans through imported maize can be significant. However, it is difficult to estimate potential AFLA intake from this source, because the frequency of imports, the quantities imported, the source countries and the degree of AFLA contamination can all vary unpredictably.

A reasonably accurate estimate of human exposure to AFLA through direct dietary intake is not possible with the present information, and has probably never been done before, except in studies like those by Van Rensburg (1977). However, it is believed that direct AFLA intake in South Africa is probably very low, compared to many other countries.

4.6.2.1.2. Estimate of indirect intake through animal products from animals that were fed aflatoxin contaminated feeds

AFLA exposure through animal products occurs almost exclusively through milk, since dairy cows excrete a large proportion of the AFLA they ingest in their milk. The official health authorities do not monitor AFLA levels in milk, and it is unlikely that dairy companies do. MTLs of 0.05 ng/g for milk and 20 ng/g in feed for dairy cows are in force, but are not routinely monitored by any government authority. It is not known if feed manufacturers monitor AFLA levels in the feed components they use. Many dairy farmers mix their own feeds, using feed components purchased as cheaply as possible. Farmers do not have testing facilities and would want to avoid the cost of using commercial testing services to determine AFLA levels in feed components. The AFLA level in locally produced commercial maize used in mixed feeds is practically zero, but important possible sources of AFLA in feed components are peanut oilcake, peanut meal and imported maize. Peanut oil cake and peanut meal come from the lower grades of groundnuts with higher AFLA levels, used for oil extraction. It is concluded that human AFLA exposure through milk is uncertain and cannot be estimated with current information.

4.6.2.1.3. Estimate of food intake and PDI of aflatoxins

Gelderblom *et al* (1996) estimated the intake of maize products by urban consumers as 276g/70 kg person/day, and for rural consumers as 460g/70 kg person/day. Our own estimates, based on the quantities of white maize milled to produce maize

products sold in various geographic areas are somewhat lower (Table 18). Nonetheless, the estimates by Gelderblom *et al* (1996) are accepted, for the higher maize and FB intakes err on the safe side. Similar estimates for the intake of peanuts and milk in South Africa could not be found. The data available are therefore insufficient for a reasonably accurate estimate of the PDI of AFLA by humans in South Africa, but the intake from commercial food products is probably very low.

4.6.2.1.4. Estimate of absorption of aflatoxins in the human gut

Table 41 shows that AFLA, probably together with other nutrients, are readily absorbed from the human gut. A high concentration – more than 70% of the AFLA concentration of the stomach contents - was found in a victim's liver, with lower concentrations in other organs. Probably because other nutrients are also absorbed, the AFLA concentration of the faeces nonetheless remained almost the same as that of the stomach contents. Unfortunately, the proportion of AFLA that was absorbed from the food is not clear from the available data. Nonetheless, it is clear that significant absorption of AFLA takes place in the human alimentary canal.

Table 41 - AFB_1 concentration in autopsy specimens from Reye's syndrome cases poisoned with AFB_1 (Shank *et al*, 1971)

Specimen	AFB_1 concentrations (ng/g or /ml fluid)
Brain	1-4
Liver	93
Kidney	1-4
Bile	8
Stool	123
Stomach content	127
Intestinal content	81

4.6.2.1.5. Evidence from human tissue of exposure to aflatoxins

Table 41 clearly demonstrates that human exposure to AFLA can be reflected in the AFLA content of various tissues, particularly the liver and excreta, as well as other indicators such as dark urine and signs of jaundice. However, no survey data could be found in South Africa with regard to human tissues for exposure to AFLA.

The risk of human exposure to AFLA in South Africa cannot clearly be estimated from the available data and there remain several uncertainties. In general, the indications are that the risks are small, mainly because of very low AFLA levels in local commercial maize and maize products. The risk will certainly increase if more maize is to be imported.

4.6.2.2. Health hazard assessment

4.6.2.2.1. Assessment of the toxicological effects of aflatoxins on humans, experimental animals and farm animals

AFLA are acutely toxic to humans and animals and many cases of acute poisoning have been recorded – see Section 1.5.2.2.1. In humans, a dietary intake of 1.7 mg/kg AFLA leads to serious liver damage within a short period. AFLA at low dietary levels are chronically toxic to humans (Yadgiri *et al*, 1970, Amla *et al*, 1971, Krishnamachari *et al*, 1975), farm animals and experimental animals – see Section 2.5.2.2.2. Exposure to sub-acute doses over an extended period leads to the development of liver cancer in rats, and liver damage in many other animals.

4.6.2.2.2. An epidemiological assessment of possible effects of aflatoxins on humans

A strong correlation has been demonstrated between the incidence of liver cancer and AFLA intake from food on the plate, spanning several countries (van Rensburg, 1977). The relationship suggests that AFLA intake above 5.0 ng/kg body weight/day results in elevated incidence of primary liver cancer from a very low incidence base rate of 2 cases per 100 000 (Table 11; Section 2.5.2.2.3). If the total intake of 5.0 ng/kg body weight/day came from maize meal, this intake level translates to a dietary

level of 0.76 ng/g for consumers eating 460 g of maize meal per person per day, such as rural blacks in South Africa.

On the other hand, the PDI of AFLA by the Indian population was estimated to be in the range of 4-100 ng/kg body wt/day (Vasanthi & Bhat 1998). This intake of between 280 and 7 000 ng/70 kg person/day, translates to a dietary level of between 0.61 and 15 ng/g in maize meal for persons eating 460 g of maize meal per day. In India, there also is a high infection rate of HBV and HCV, an important co-factor in the aetiology of liver cancer. The liver cancer incidence rate in India is nonetheless very low (2.63 in males and 1.22 in females per 100 000 ASIR – Ferlay *et al*, 1999), compared with the rest of the world.

Similarly, in Costa Rica, AFLA levels in maize are high (average 147 ng/g) (see Section 1.5.2.2.4), but liver cancer incidence is moderate (6.57 in males and 3.85 in females per 100 000 ASIR - Ferlay *et al*, 1999). Unfortunately, the AFLA intake in Costa Rica was not calculated in the study concerned. However, if it is assumed that only 150 g of maize is consumed per person per day and that maize products contain only one third the AFLA levels of unprocessed maize, the average AFLA intake would be approximately 2.4 µg per person per day. This translates to a dietary level of about 16 ng/g for people eating 460 g of maize product per day.

From both a toxicological and an epidemiological viewpoint, there is clear evidence that AFLA are a health hazard to humans. Indications are that a dietary level of about 15 ng/g in high volume staples should not lead to an increase in incidence of liver cancer. This holds true even under conditions of poor nutritional status and high infection rates with HBV and HCV.

4.6.2.3. Other considerations

4.6.2.3.1. Regulations of international trading partners

Traditionally, in times of local shortages, South Africa has imported wheat and maize from the USA and Argentina, and additionally, wheat from Canada and Australia. The USA and Argentina each maintain a regulatory MTL of 20 ng/g for AFLA in maize (Anonymous, 1997), however, this does not apply to export maize. Australia has an MTL of 5 ng/g for AFLA in all foods. When maize was imported from the

USA and Argentina in the 1980's, high AFLA levels were prevalent in imported maize, and this caused an outcry in the local media, which caused considerable harm to the maize industry. During the 1992/93 imports, special contract specifications were needed to meet the existing South African MTL of 10 ng/g for AFLA in maize. This caused considerable difficulty and quality control measures had to be specially implemented in the source countries before the grain was shipped. In a significant number of samples, the South African MTL was nonetheless exceeded, sometimes by a factor of 15 and purchases from Argentina were discontinued after only 13 shipments. In spite of AFLA levels in some samples exceeding 150 ng/g, the average AFLA content in maize from the USA calculated over all shipments was between 3 and 4 ng/g, and 5 ng/g in ARG maize.

4.6.2.3.2. Commercial interests

Millers and feed millers are exposed to substantial claims for damages if their products should harm the health of humans or livestock, especially if they do not comply with regulatory MTLs. In fact, it could be said that a single human death caused by AFLA in maize meal might cause sufficient emotional response that it could close down a multi million Rand corporation. MTLs for hazardous contaminants in food therefore do not only protect consumers, but also commercial interests.

4.6.2.3.3. Sufficiency of food supply

During the 1992/93 maize imports, maize that could not meet the 10 ng/g South African MTL for human consumption, was redirected to animal use. In many other African countries that imported maize through South African ports, however, no such opportunity existed, in fact AFLA levels were probably not even tested by the importing country. It is known that maize imported by other African countries often had much higher AFLA levels than the maize imported by the Maize Board (Viljoen, unpublished data). In some of those countries, people were perishing of hunger, so the choice was simple, even if they were aware of the high AFLA levels in some of their imports. Had South Africa been solely dependent on Argentina for supply, the average AFLA levels in our imports would undoubtedly have been significantly higher. There is a real possibility that it could have culminated in a choice between

complying with our MTL, and not having sufficient maize supplies for human consumption. Such a choice would have forced a reappraisal of the basis for the existing MTLs, which clearly are largely on an arbitrary basis. Against this background new proposals are put forward for consideration in Section 4.6.5 to replace the existing MTLs for AFLA in maize in South Africa while the MTLs in maize products remain unchanged. The data on mycotoxin levels in commercial maize products in Sections 4.1.1 and 4.1.2 indicate that higher MTLs in unprocessed maize is highly unlikely to cause the existing MTLs for maize products to be exceeded because of the losses in mycotoxin levels that occur during commercial milling.

4.6.3. Formulating a proposal for MTLs for fumonisins in grain and grain products

4.6.3.1. Assessment of human exposure to fumonisins in South Africa

4.6.3.1.1. Estimate of direct fumonisin intake

FBs occur mainly in maize and maize products. The FB levels in these products have been thoroughly investigated – see Section 4.1 for details. *F. verticillioides* also infects various other food plants, and FBs are known to occur in grain sorghum and sorghum products, but details are unavailable.

From Tables 28 through 30, can be calculated that the average total FB content in sifted and special maize meal was about 330 ng/g in 1990/91, about 270 ng/g in 1991/92 and about 550 ng/g in 1993/94. Sifted and special maize meals form the bulk of white maize products sold in rural areas, where per capita maize consumption is highest. Consumers in rural areas would be ingesting FBs at an average rate of between 124 and 253 µg/70 kg person/day. There is considerable year-to-year and spatial variation in FB levels, depending on the FB content of white maize in particular production areas (See Table 27). The average levels in maize products are approximately one third of the levels in maize. FB levels in imported (yellow) maize from the USA appear to be three to four times higher than in home grown commercial maize, both white and yellow. Therefore, in years of maize imports, like 1992/93, FB intake probably increases. Details of FB levels in white USA maize are unavailable.

It can be concluded that consumers of maize in South Africa, like elsewhere, are constantly exposed to FBs through direct intake in a maize-based diet. The level of exposure and its variation is well defined.

4.6.3.1.2. Estimate of indirect intake through animal products from animals that were fed fumonisin contaminated feeds

The studies by the CVM (Section 2.5.3.1) show that FBs are poorly absorbed 'orally' in all farm animals tested to date. Oral bio-availability averaged about 4% in swine and 0.7% in laying hens. Most of the ingested FB_1 and FB_2 are excreted in the faeces unchanged. The CVM believe FB residues in meat, milk and eggs are unlikely to be a public health concern.

The CVM believes further testing in cattle livers may need to be considered. Feeding cattle a diet containing about 129 µg/g FB_1 (based on consuming 3% of their body weight in food per day) for 30 days resulted in liver FB_1 levels up to 4.6 µg/g. However, the $FB_1 + FB_2 + FB_3$ level in the total diet of this study was estimated to be about 185 µg/g. This is more than six times higher than the CVM recommendations of <30 µg/g in rations of cattle fed for slaughter.

It is therefore concluded that indirect intake of FBs through contaminated animal products is insignificant.

4.6.3.1.3. Estimate of food intake and PDI of fumonisins

Gelderblom *et al* (1996) estimated the intake of maize products by urban consumers as 276 g/70 kg person/day, and for rural consumers as 460 g/70 kg person/day. At these levels, the calculated direct FB intake from maize products containing between 270 and 550 ng/g (the levels found in sifted and special maize meal) therefore ranges between about 125 and 253 µg per person consuming 460 g of sifted or special maize meal per day. This respectively corresponds to about 1.8 to 3.6 µg/kg body weight/day, for rural consumers eating commercial maize products. This is much lower than the intake of 47 - 355 µg/g body weight/day calculated by Marasas (1997) for consumers eating maize produced on subsistence farms in the Transkei.

Excluding the Eastern Cape, where estimates are more uncertain, our own estimates of commercial maize consumption range between 164 g/70-kg person/day in the Western Cape, and 316 g/70-kg person/day in Mpumalanga (Table 18). These figures represent unprocessed maize and have not been corrected for milling extraction, which in our calculations came to 86%. At these intake levels and with the estimated fumonisin levels in the maize commercially milled in different parts of the country as shown in Tables 17, the PDI varies from 1.40 µg/kg body weight/day in the Western Cape to 2.66 µg/kg body weight/day in Mpumalanga. Again, these figures have not been corrected for disappearance of mycotoxins as a result of commercial milling and are therefore an over estimation.

It is concluded that consumers of commercial maize products in South Africa regularly ingest FBs. The average FB intake of rural consumers is between 125 and 253 µg per person per day, or up to 3.6 µg/kg body weight/day depending on annual contamination levels in commercial maize.

4.6.3.1.4. Estimate of absorption of fumonisins in the human gut

No data could be found indicating absorption of FBs in the human alimentary canal. Chelule *et al* (2001) assessed exposure of a rural population of KwaZulu Natal, South Africa to FB_1 by analysing stored maize, plate-ready food, and faeces. Of the 50 rural maize samples examined 32% had levels of FB_1 ranging from 0.1-22.2 mg/kg, whereas 29% of the 28 cooked maize (phutu) samples contained FB_1 at levels ranging from 0.1-0.4 mg/kg. Of the faeces samples, 33% contained FB_1 at 0.5-39.0 mg/kg – higher than in the maize and the plate ready phutu. Of the 49 urban maize samples analysed 6.1% contained 0.2-0.5 mg/kg FB_1, whereas 3 of 44 faecal samples (6%) contained between 0.6 and 16.2 mg/kg FB_1. No FB_1 was detected in urban phutu samples. Again, FB_1 levels in the faecal samples appear to be much higher than in the maize and phutu samples.

Absorption by animals is only between 4% (in swine) and 0.75% (in laying hens) (Section 2.5.3.1). In vervet monkeys, dietary levels equivalent to 121 µg/g for 60 weeks apparently caused little more than elevated serum sphinganine:sphingosine ratios (Shephard *et al*, 1996b), indicating that some FBs were absorbed. In the report,

no mention was made of mortality, while dietary levels of this magnitude are acutely toxic to pigs at 4% absorption.

4.6.3.1.5. Evidence from human tissue of exposure to fumonisins

No data are available on the physiological effects (bio-marker effects) in humans of FBs in commercial maize products. However, van der Westhuizen *et al* (1999) conducted a study on human volunteers in the Transkei and KwaZulu-Natal in South Africa and in the Bomet district, western Kenya to determine the Sa/So ratios in the plasma and urine of males and females consuming a staple diet of maize grown on subsistence farms (referred to as home-grown maize, as opposed to commercial maize). Maize samples were randomly collected from the same region where the volunteers lived. Mean total FB level was 580 ng/g (n = 40) in Transkeian maize. This level is similar to the long-term averages in commercial maize in South Africa (see Table 27). It is also almost identical to the estimated average level of 550 ng/g in sifted and special maize meal in1994/95, a year when FB levels in commercial maize was comparatively high in South Africa. It is believed that maize grown on subsistence farms is crushed and the whole grain meal used for preparing food. Therefore, no contaminants are removed, unlike in commercial milling, where the maize is cleaned, (which removes broken and mouldy kernels) and degermed (removing the germ and bran) before milling. The FB concentration in the Transkei maize reported on by van der Westhuizen *et al* (1999) was similar to that in commercial maize meal in 1994/95. In the 1994 maize crop, FB levels in white maize in the W-Tvl were about three times the normal levels. Therefore, the results of this study are relevant to commercial maize and maize products in South Africa. In the KwaZulu-Natal province, no FB (n = 17) was detected (<10 ng/g) in the maize. In Kenya, only one of seven samples was contaminated with 60 ng/g FBs.

At these levels of contamination, no significant differences were found in the sphinganine/sphingosine ratios between males and females within the regions, nor between the different regions ($P<0.05$). It can be concluded that exposure to FBs in maize at up to 580 ng/g has no observable effect on the serum and urine sphinganine/sphingosine ratios in humans. It is therefore highly unlikely that any evidence of human exposure to FBs will be found in sphinganine/sphingosine ratios in the commercial maize areas of South Africa.

4.6.3.2. Health hazard assessment of fumonisins

4.6.3.2.1. Assessment of the toxicological effects of fumonisins on humans, experimental animals and farm animals

Unlike AFLA, no incidents of acute intoxication of humans by FBs have been reported. FBs are acutely toxic to horses and rabbits (see Section 2.5.3.1) at dietary levels >5 µg/g under field conditions, causing damage to the brain tissue, the liver and kidneys. FBs are also acutely toxic to pigs and channel catfish at dietary levels > 40 µg/g, causing pulmonary oedema and damage to the liver and kidneys in pigs. More than 23 µg/g (10 µg/g in another study) FBs in the diet of pigs caused elevated sphinganine/sphingosine ratios in various tissues. The lowest estimated NOAEL of various biomarkers in pigs was 18 µg/g. Chronic toxicity of FBs to farm animals has not been very well documented, but there are no reports of cancer development in farm animals caused by FBs. In the rat oesophagus, no synergistic interaction between a nitrosamine - a known OC initiator - and FB_1 was found when the two compounds were administered together. In 2-year feeding studies (Anonymous, 1999) of laboratory rats and mice on diets containing 0, 5, 15, 80 or 150 µg/g (males), or 0, 5, 15, 50, or 80 µg/g (females) FB_1, survival was significantly less in animals receiving feed containing 80 µg/g FB_1 than in control groups. These dietary levels are equivalent to intake levels of about 0.25, 0.8, 2.5 and 7.5 mg/kg body weight/day in male rats. At 2 years, there was a significant increase in the incidences of renal tubule adenoma in male rats dosed at 150 µg/g and of renal tubule carcinoma in 50 and 150 µg/g males. Apoptosis of renal tubule epithelium was significantly increased in males exposed to 15 µg/g or more for 26 weeks. Hyperplasia of renal tubule epithelium was significantly increased in 50 and 150 µg/g males at 2 years. According to these studies, FB intake of 0.8 mg/kg body weight/day (dietary level 15 µg/g), can be taken as a conservative estimate of the NOAEL in rats. This is similar to the NOAEL of 18 µg/g in pigs. A NOAEL of 0.8 mg/kg body weight/day was used by Marasas (1997) as the basis for calculating his recommended MTL of 100 – 200 ng/g for FBs in maize, incorporating a safety factor of 1 000.

It is also clear from these studies that in all animals where damage to tissue occurs, the liver and kidneys are important target organs.

4.6.3.2.2. An epidemiological assessment of possible effects of fumonisins on humans

Acute toxicity, oesophageal cancer, and liver or kidney damage

In the Transkei, total FB levels >140 µg/g were found in some maize samples grown on subsistence farms (Rheeder *et al*, 1992). Mouldy maize is reportedly used to brew traditional opaque beer, of which some Transkeians consume large quantities. No incidents of acute toxicity have been recorded.

Marasas (1997) estimated the FB levels in mouldy and 'healthy' maize in an area of the Transkei with high OC incidence at respectively 54 and 7.1 µg/g. These estimates are based on FB levels found in a total of about 18 samples analysed during two surveys in maize grown on subsistence farms – see Section 2.3.2 for details. (The basis of his calculation is unknown and our own calculation gave a result of 43.0 and 1.94 µg/g in mouldy and 'healthy' maize respectively. Our calculation is based on the FBs levels in 18 samples each of 'healthy' and mouldy maize collected in the high OC incidence area of Transkei during two seasons - Rheeder *et al*, 1992). Based on these data, Marasas estimated FB intake in the Butterworth/Centane area of Transkei, where there is a high OC incidence, at between 46.6 and 354.9 µg/kg body weight/day. Such intake levels would be acutely toxic to horses and pigs respectively. There are no reports of human fatalities, or of liver and kidney damage in humans caused by FBs and the concern about these levels of intake was linked solely to the high incidence of OC in the area. No data are available that directly link the actual exposure of humans in the area to FBs, as reflected by FB levels in plate food, bio-marker effects and FBs in human excreta, with OC. It is clear that humans are far less sensitive to fumonisins than horses and rabbits; growing colts and rabbits could be poisoned with 2.24 µg/g of fumonisins in a complete feed, while mature horses could be adversely affected by about 4.25 µg/g of fumonisins in a complete feed (See Section 2.5.3.1). It is therefore appropriate for MTLs for these sensitive animals to be lower than those for humans.

Recently, the values of 54 and 7.1 µg/g given by Gelderblom *et al* (1996) were confirmed as being incorrect (Marasas – pers. comm., 2002). They have recalculated the values for total FBs from data on the individual mouldy and healthy maize

samples in the high OC area, given in the PhD Thesis by Sydenham (1994) as follows:

- Mouldy samples (18 samples): 43.4 µg/g;
- Healthy samples (18 samples): 2.0 µg/g.

In the Lusikisiki/Bizana area of Transkei, OC incidence is moderately low in terms of world standards. The average FB levels in mouldy and healthy maize grown on subsistence farms in this area were about 0.239 and 7.5 µg/g respectively, calculated from figures published by Rheeder *et al* (1992). Based on these figures, FB intake in the low incidence area is between 1.6 and 49.3 µg/kg body weight/day. These intake levels are similar, to considerably higher, than the estimated PDIs of between 1.8 and 3.6 µg/kg body weight/day in the commercial maize areas of South Africa. No correlation was found between OC incidence in black males (the section of the population at highest risk for OC) and estimated FB levels in commercial maize used to manufacture the maize products consumed in the nine South African provinces – see Tables 13 through 17 and Table 36. From an epidemiological viewpoint, these intake levels can therefore be considered as NOAELs for OC in humans.

Although maize is not the staple food in Argentina, maize consumption is very important among children. In one study (Solovey *et al*, 1999), maize meal contained an average of about 891 ng/g FBs. A daily FB intake of 11.3 ng/g of body weight was estimated for child maize consumers (1-5 years old) based on an average consumption of 200 g of maize meal/day. According to Kuiper-Goodman (1999), young children are more vulnerable to exposure than the average population because of their lower body weight. Nonetheless, no incidents of liver and kidney damage to children in Argentina have been reported and the incidence of OC in Argentina is moderate (11.04 in males, per 100 000 ASIR – Ferlay *et al*, 1999). This is further epidemiological evidence of a NOAEL in humans. This intake level is equal to a NOAEL in rats, extrapolated to humans, with a safety factor of about 70.
Since the intake levels in the Lusikisiki/Bizana areas do not result in elevated OC incidence, it can be speculated that the FB intake levels in this area can be accepted as NOAELs for OC in humans. Urinary and/or blood Sa/So ratios in humans only become elevated at high dietary FB_1 levels comparable to those in the

Butterworth/Centane area of Transkei (van der Westhuizen *et al,* 1999; Qiu & Liu, 2001). In addition, primates appear to be much more tolerant of at least the acute toxicity of FBs (Shephard *et al*, 1996b) than rats and probably also of chronic toxicity and carcinogenicity. It can further be reasoned that clear epidemiological evidence of a NOAEL for development of human OC and absence of liver and kidney damage in young children eliminates the need for a safety factor as high as 1 000 when setting MTLs from rat data. A safety factor as low as 50 could be considered sufficient when extrapolating from rat data, considering that FBs are non-genotoxic and clear evidence of a threshold limit exists for their cancer initiating action in rats (Gelderblom *et al*, 1994). FBs are either not cancer initiators in humans or the levels that normally occur in commercial maize or maize products are well below the threshold limits for initiating cancer development. Based on a 50-fold safety factor applied to rat data, 2 µg/g of FBs in food should be safe for humans. This would allow up to 8 µg/g FBs in maize, since less than one quarter to about one half of the level in maize is found in milled products of various grades of refinement.

Neural tube defects

A possible link exists between exposure to FBs during early pregnancy and neural tube defects (NTD) in newborn infants - see Section 4.5. Similar effects have been demonstrated in experimental animals.

Epidemiological evidence suggests that an average daily intake of FBs of around 60 µg/70 kg person/day (about 0.86 µg/kg body weight/day) is probably a safe level in terms of NTD (see Section 4.5.1). This translates to an MTL of 130 ng/g in food for rural consumers in South Africa. This level is often exceeded by a considerable margin in commercial maize products. However, only a small (about 0.47% of the population at 3% population growth rate) and well-defined section (pregnant women in their first 6 weeks of pregnancy) of the population might be at risk. Therefore, if protection is needed, this could probably be achieved more effectively through other means than MTLs. The physiological mechanism involved could be an effect on the availability of folic acid to the foetus (Hendricks, 1999). Possible measures include abstaining from maize products during the critical period, supplemental folic acid at higher levels than normal to maize consumers during early pregnancy and fortification of maize products with folic acid.

4.6.3.3. Other considerations

4.6.3.3.1. Regulations of international trading partners related to fumonisins

So far, only Switzerland has enacted a regulatory MTL of 1 µg/g for FBs in food – see Section 2.1.3.1. This is an arbitrary level and is not based on scientific consideration (Zoller *et al*, 1994). Therefore, the Swiss MTL cannot form part of the basis for debating realistic MTLs for South Africa. Switzerland is not a source country for South African grain imports, and from this aspect their MTL is of little consequence to the South African maize industry. The Swiss may have trouble to find maize for import that can comply with their MTL for FBs in food and may offer an attractive market for products that can comply.

The USA has set a wide range of guidance levels for FBs in feeds for different animal species – see Section 2.1.3.2. In food, the following guidance levels have been set:

Product	**Total fumonisins (FB1+FB2+FB3) µg/g**
Degermed dry milled maize products (e.g., flaking grits, maize grits, maize meal, maize flour with fat content of < 2.25 %, dry weight basis)	2 µg/g
Whole or partially degermed dry milled maize products (e.g. flaking grits, maize grits, maize meal, maize flour with fat content > 2.25 %, dry weight basis)	4 µg/g
Dry milled maize bran	4 µg/g
Cleaned maize intended for masa production	4 µg/g
Cleaned maize intended for popcorn	3 µg/g

It is often argued that maize products form only a minor part of the diet of Europeans or North Americans and MTLs for FBs can therefore be set considerably higher than would for instance be required in many African countries, where maize is a staple

(e.g. Marasas *et al*, 2000). However, it should be remembered that these limits have actually been set bearing in mind the interests of people in the USA who, for a large part of their starch needs, are dependent on maize products. These include gluten intolerant people and certain ethnic groups.

The USA guidance levels for food have only been set for finished products and maize one step from a ready to eat product. No guidance levels for maize being normally traded has been set, which means that these guidance levels do not directly affect maize producers in the USA.

4.6.3.3.2. Commercial interests

Millers and feed millers are exposed to claims for damages if their products should harm the health of humans or livestock, especially if they do not comply with regulatory MTLs. Currently, there is no evidence suggesting that millers and feed millers run any risks in this regard relevant to FBs in maize. Feed millers are exposed to some risk with regard to horses and pigs, if maize screenings and maize bran, which in some years may contain high FB levels, are used as feed components in balanced feeds for horses and swine. Apart from that, there is no direct danger of damages caused by FBs. However, impractical, difficult to comply with MTLs can expose millers to non-compliance claims and can create a situation where substantial trading losses could be suffered. This will be dealt with in more detail in Section 4.9. An MTL of 1 to 2 µg/g for maize products, and 4 µg/g for maize in South Africa would be in line with the guidance levels in the USA. It would limit trading losses and it would not lend itself to be used as a trade barrier.

4.6.3.3.3. Sufficiency of food supply

FBs are ubiquitous in maize and probably also occur in grain sorghum. In Sections 4.1.1 and 4.1.4 it has been shown that FB levels in RSA maize are probably some of the lowest amongst major world maize suppliers to international markets. In contrast with USA and ARG maize, RSA maize is also practically completely free of AFLA. Setting MTLs for FBs in South Africa that are difficult to comply with, will cause a real problem of finding maize for sufficient food supply in South Africa. This will have serious knock-on effects on other food grains and the feed grains market. The entire grain chain, from maize producers through to consumers will be seriously

affected and the poorest sections of the consumer community will be hit the hardest. See Section 4.9 for details.

4.6.4. Formulating a proposal for MTLs for deoxynivalenol in grain and grain products

4.6.4.1. Assessment of human exposure to deoxynivalenol in South Africa

4.6.4.1.1. Estimate of direct deoxynivalenol intake

DON contamination of maize and maize products in South Africa has been thoroughly investigated, but little is known about DON levels in South African grain sorghum, wheat, barley and their products, including beer. Worldwide, DON is the main mycotoxin in wheat, barley and their products (Trucksess *et al*, 1993; Zakharova *et al*, 1994; Furlong *et al*, 1995; Ruprich & Ostry, 1995; Zakharova *et al*, 1995; Pacin *et al*, 1997; Scott, 1997; Gonzalez *et al*, 1998). It could therefore be be speculated that the situation is no different in South Africa. However, without detailed, specific information it is not possible to estimate direct intake of DON in South Africa.

4.6.4.1.2. Estimate of indirect intake of deoxynivalenol through animal products from animals that were fed deoxynivalenol contaminated feeds

Similarly, without information available about DON levels in important feedstuffs and in animal food products in South Africa, indirect intake of DON via these products cannot be estimated.

4.6.4.1.3. Estimate of food intake and PDI of deoxynivalenol

Our estimates for DON intake through white maize vary between 1.17 ng/g body weight per day for Limpopo and 0.52 ng/g body weight per day for the Western Cape (Table 21). No estimates of the average DON intake through wheat and barley products in South Africa are available. Without this information and without data on contamination levels of grain sorghum, wheat, barley and their products, the PDI cannot be accurately estimated. However, the indications are that it could be

substantial, particularly in years when rains damage the wheat and barley crops when ready for harvest.

4.6.4.1.4. Estimate of absorption of deoxynivalenol in the human gut

No estimate of the uptake of DON in the human alimentary canal could be found.

4.6.4.1.5. Evidence from human tissue of exposure to deoxynivalenol

No research seems to have been focused on this aspect in South Africa and no data could be found in the international literature.

4.6.4.2. Health hazard assessment of deoxynivalenol

4.6.4.2.1. Assessment of the toxicological effects of deoxynivalenol on humans, experimental animals and farm animals

From the available data (Section 2.5.4) it appears that DON is one of the least toxic tricothecenes, however, the immuno-suppressive properties of DON in humans could be of particular importance in relation to the current AIDS epidemic, particularly in Africa. However, no data are available.

4.6.4.2.2. An epidemiological assessment of possible effects of deoxynivalenol on humans

DON appears likely to be ingested in significant quantities by all grain consumers in South Africa and mainly by wheat and barley consumers in many other countries, like Argentina, Canada, the USA, Eastern Europe and Russia (Trucksess *et al*, 1993; Zakharova *et al*, 1994; Furlong *et al*, 1995; Ruprich & Ostry, 1995; Zakharova *et al*, 1995; Pacin *et al*, 1997; Scott, 1997; Gonzalez *et al*, 1998: Solovey et *al*, 1999). For example, DON levels in bakery products in Argentina in one study ranged from 200 ng/g to 2800 ng/g with an average of 464 ng/g. 92% of samples contained DON (Pacin *et al*, 1997). In maize products in South Africa, the average DON levels ranged between <10 to >200 ng/g. There is little doubt that DON will also be found in other grain products, particularly wheat and barley in South Africa. No immediate effect on consumers is evident, but the question remains to be answered about the immuno-suppressive role of DON in AIDS.

With so much information unavailable, it is impossible to rationally formulate a proposal for MTLs for DON. Moreover, where there is reason for concern, but insufficient information, the normal procedure is to increase the safety factor when extrapolating MTLs for humans from animal data (Kuiper-Goodman, 1999). However, wheat and barley are the main crops contaminated by DON and their products are consumed as staples over large parts of the world. It could therefore be acceptable to institute arbitrary MTLs for DON in South Africa, based on the MTLs in use in other countries. Thus, an MTL of 2 µg/g in unprocessed grains, and 1 µg/g in finished foods is proposed.

4.6.4.3. Other considerations

4.6.4.3.1. Regulations of international trading partners related to deoxynivalenol

Five countries have enacted MTLs for DON, ranging from 500 to 1 000 ng/g in foods and from 1 000 to 10 000 ng/g in feeds (Section 2.1.4). Amongst these are important source countries for imported wheat, like Canada and the USA. In South African white maize, DON has been found at average levels up to about 760 ng/g in different crop years and in white maize products for human consumption at average levels up to about 220 ng/g. No country has a regulatory MTL for DON in unprocessed maize.

4.6.4.3.2. Commercial interests

To avoid possible claims for damages, it would be in the interest of millers and feed millers for a regulatory MTL for DON to be introduced in South Africa. MTLs similar to those in other countries should not lead to trading difficulties.

4.6.4.3.3. Sufficiency of food supply

MTLs for DON of 2 µg/g in unprocessed grains and 1 µg/g in finished foods should not disqualify large stocks for food use and should not lead to artificial food shortages.

4.6.5. Summary of proposed MTLs for certain mycotoxins in grain and grain products intended for human consumption

The following new MTLs are proposed for AFLA, FBs and DON in maize, wheat, barley, grain sorghum and their products to replace existing MTLs or as completely new MTLs where none exist at present:

4.6.5.1. Aflatoxins

- 20 ng/g in unprocessed, uncleaned cereal grains intended for food use;
- 10 ng/g in grain products for food, with not more than 5 ng/g AFB_1.

4.6.5.2. Fumonisins

- 4 µg/g in whole, uncleaned grain intended for human consumption;
- 2 µg/g in dry milled grain products with fat content of ≥3.0 %, dry weight basis (e.g., sifted and unsifted maize meal);
- 1 µg/g in dry-milled maize products with fat content of <3.0 %, dry weight basis (e.g., flaking grits, brewers grits, samp, maize rice, super and special maize meal).

4.6.5.3. Deoxynivalenol

- 2 µg/g in uncleaned cereal grains intended for food use;
- 1 µg/g in cereal grain products intended for food use.

4.6.6. The basis for determination of compliance of grain with MTLs

It is proposed that the basis for compliance to any MTL should be the level of the mycotoxin concerned in one representative sample of a consignment – see Section 4.8 for detailed proposals on sampling procedures.

In the case of unprocessed grain in bags, a consignment will be a rail, or road truck containing bagged grain, a bag stack, or a pallet with bagged grain. In the case of grain in bulk, a consignment will be a bulk rail or road truck, a silo bin, or any other bulk container containing grain, irrespective of its size and to what capacity it has been filled.

In the case of packaged cereal products, a consignment will be a pallet, a stack or a truck containing packaged product.

In the case of cereal products stored or transported in bulk, a consignment will be a bulk bin or bulk rail or road truck containing product, irrespective to what capacity it has been filled.

4.7. Overview of available test methods for the mycotoxins included in this study in grains and grain products

4.7.1. Categories of analytical tests (After Duncan & Hagler, Undated; Woloshuk, 2000)

4.7.1.1. Ultraviolet light

Ultraviolet light or the so-called black light method is used by grain buying stations in the USA as a screening test for AFLA contamination. An ultraviolet light with wavelength of 365 nm is normally used to detect kernels or portions of kernels that glow with a bright green yellow fluorescence (BGYF). This is strictly a presumptive test and indicates only that the causal fungus, *A. flavus*, was growing on the living kernel and does not indicate the presence of AFLA or other mycotoxins. BGYF is best seen in cracked maize rather than whole kernels. When examining maize for BGYF, there should be a colour standard or an authentic BGYF for comparison. The presence of the fungus does not necessarily mean that AFLA is present. The compound that produces the fluorescence is kojic acid, not AFLA. Other fungi may also produce kojic acid. Therefore, a follow-up chemical test is necessary for the actual detection of AFLA. Ultraviolet light is a useful presumptive screening method, to indicate which grain lots require an analytical test.

4.7.1.2. Minicolumn method

The minicolumn method was used until recent years as a rapid test for AFLA. A minicolumn is a small column containing silica gel and Florisil (or other adsorbents) to which sample extracts are applied for detection of AFLA. If properly used, the minicolumn test is capable of giving good results for AFLA. Buying stations in the USA often used it to test for AFLA as a follow up on black light positive samples, particularly during years when AFLA problems were common. The method can detect AFB_1 as low as 5 ng/g in cottonseed products, but cannot be used analytically because it lacks resolution, and more importantly, because it does not definitely identify AFB_1. Normally, a sample is called positive for AFB_1 if an AFLA-like fluorescing material is found absorbed to the florisil layer of the column. Generally, the test sample is compared to known AFLA positive samples (usually at 20 and 100 ng/g). Like the black light method, the minicolumn has often been mishandled and misused and is no longer recommended, it has been replaced by antibody-based test kits which have become widely available over the last few years.

4.7.1.3. Fluorometric-iodine method (Genter *et al*, 2000)

This method was originally developed for detecting AFLA. Iodine is used to convert AFB_1 into a more intensely fluorescent derivative, which is then quantified, using a simple photo-fluorometer and filter combination. The instrument is adjusted to read directly in ng/g of AFLA. This method also has the advantage of using fewer solvents, which makes it much safer for the operator. More recently, the fluorometer has been used in combination with antibody test kits, to analyse AFLA, FBs and ZEA. The antibody test kits are used to extract and clean up the mycotoxin from the sample and the fluorometer is then used for quantification after addition of a ‘developer’ to increase fluorescence. The fluorometer is easy to use at grain silos or mills. As an example, the test procedure for AFLA is briefly as follows (Anonymous, 2001a):

Sample preparation:

- Put sample through divider.
- Clean mill and mill sub-sample through coarse screen.
- Thoroughly mix milled sample.
- Mill subsample through fine screen.

Extraction:

- Weigh sample into blender.
- Add 5 g NaCl (salt).
- Add appropriate Methanol:Water extraction solvent.
- Cover and blend for 1 minute.
- Pour extract into fluted filter paper setup.
- Extract Dilution
- Pipette specified amount of filtered extract into a clean container.
- Dilute with specified amount of de-ionized water.
- Filter through microfibre filter.

Affinity Chromatography:

- Set up affinity column.
- Pass filtered extract though column.
- Wash twice with deionised water.
- Elute AFLA from column with HPLC grade methanol.
- Collect in a glass cuvette.

- Add diluted Aflatest Developer to eluent in cuvette.
- Place cuvette in calibrated fluorometer and measure fluorescence.

4.7.1.4. Thin layer chromatography (TLC)

The Association of Official Analytical Chemists approved various TLC methods for mycotoxins. The mycotoxins are extracted from the grain sample using solvents. The extract is concentrated and spotted on chromatograms. The presence of spots on thin layer chromatograms with RF values similar to or identical with those of the particular mycotoxin is a tentative identification. To confirm the presence of the mycotoxin, the suspect spot is reacted with other reagents in a new solvent system and by comparing with known standards. Relatively simple laboratory facilities are needed and some TLC tests for mycotoxins are available as commercial test kits. This method is mostly used by analytical laboratories, but can easily be set up at grain silos and mills.

Romer Labs Inc., 1301 Stylemaster Drive, Union, Missouri 63084, Tel (314) 583-8600 offer the Mycotest test kits for AFLA, vomitoxin (DON), and ZEA. The price in the USA is $379 for 25 tests or about R172.00 per test (March 2002 exchange rate of about R11.50/US$), excluding overheads and labour. All three mycotoxins may be detected with one kit. Romer's "MYCOTEST" uses TLC technology. Maize samples are ground and extracted. The extracts are then spotted onto a TLC plate. One TLC plate can be spotted with extracts from several samples. The TLC plate is developed, dipped into an aluminium chloride solution and heated. The mycotoxins are then visualised by viewing the plate under long-wave ultraviolet irradiation (black light). Mycotoxin standards are also available making it possible to visually estimate the quantity of the mycotoxins present by comparing the fluorescence against that of the standard.

TLC analysis probably takes as long as fluorometry and the result is only approximately quantitative, therefore it does not lend itself to regulatory purposes.

4.7.1.5. High performance liquid chromatography (HPLC)

This method requires sophisticated and expensive equipment and an expert technologist. It is very reliable. It is used by analytical laboratories, but not by grain silos or mills. Antibody test kits are now often used for mycotoxin extraction and cleanup from the sample, followed by HPLC for quantification. The cost usually runs to hundreds of Rands per sample, depending on the throughput. During the 1992 maize imports, the Maize Board laboratory in Pretoria managed to test up to about 180 samples from ships holds within 24 hours after docking by HPLC. Thus, a central testing facility could be more cost-effective than testing on-site at mills or silos, using ELISA test kits and fluorometric detection.

4.7.1.6. Mass Spectrometry

There is no more definitive confirmation of the identity of any mycotoxin than mass spectroscopy because this method is a direct characterization of the molecule. Very expensive, sophisticated equipment is used, requiring a highly skilled technologist to operate. Therefore, only a few research laboratories use this method. The cost runs to hundreds of Rands per sample.

4.7.1.7. Immunoaffinity columns (ELISA, or antibody test kits) (Scott & Trucksess, 1997)

Immunoaffinity columns (IACs) are widely used for cleanup and isolation of mycotoxins extracted from foods and biological fluids, particularly AFLA, OA, and FBs. The columns are prepared by binding antibodies specific for a given mycotoxin to a specially activated solid-phase support and packing the support suspended in aqueous buffer solution into a cartridge. The mycotoxin in the extract or fluid binds to the antibody, impurities are removed with water or aqueous solution, and then the mycotoxin is desorbed with a miscible solvent such as methanol. Further separation can be performed with IAC, followed by HPLC quantification, either off-line or on-line in an automated system, or by fluorometry.

Laboratories that developed the antibodies have used IACs but they are now also available commercially. Among commercial IACs, Aflatest P is used as the cleanup

step in an LC method and in a solution fluorometry method for maize, peanuts, and peanut butter. This method was adopted as an AOAC INTERNATIONAL Official Method after evaluation through an international collaborative study. As part of a fluorometer-based test kit, Aflatest P was further certified by the AOAC Research Institute to measure total AFLA in 10 grain types and grain products. IACs can concentrate the analyte from a large amount of sample, allowing detection limits at low parts-per-trillion levels in some cases (e.g., for AFM_1 and OA in liquid food matrixes). Regeneration of IACs for reuse in AFLA, OA, FB, and ZEA analyses has been investigated.

Commercial antibody test kits for screening or quantification are currently available for AFLA, ZEA, DON, T-2 toxin, OA, and FBs. These antibody methods, while they are still being improved, are good if used properly. The mycotoxin test kits in Table 42 have been tested and found to perform in a variety of laboratories (Anonymous 2000e).

Table 42 - Some of the commercially available antibody test kits (Anonymous 2000e)

Manufacturer	**Mycotoxins detected**	**Test kit name**
Editek	AFLA	EZ-Screen
P O Box 908, 1238 Anthony Rd.	Ochratoxin	
Burlington, NC 27215	T-2	
Phone: (910) 226-6311	ZEA	
Fax: (910) 229-4471		
International Diagnostic System Corp.	AFLA (4 Kits)	1. Afla 20 Cup
2620 S. Cleveland Ave.		2. Afla 10 Cup
Suite 100, St. Joseph,		3. Afla 5 Cup
MI 49085		4. Afla B1

Phone: (616) 428-8400 Fax: (616) 428-0093	ZEA (2 Kits)	1. One Step ELISA, Quantitative Test 2. I. D. Block, ELISA Antibody
Neogen Corporation 620 Lesher Place Lansing, MI 48912 Phone:(517)372-9200 (800) 234-5333 Fax:(517) 372-2006	AFLA T-2 DON ZEA FB AFM_1 Ochratoxin	AgriScreen Veratox
VICAM, 313 Pleasant St, Watertown, MA 02172 Phone: (800) 338-4381 (617) 926-7045 Fax: (617) 923-8055 Or 29 Mystic Avenue, Sommerville, Massachusetts 02145	AFLA FB Ochratoxin ZEA	Aflatest-P Fumonitest Ochratest Zearalatest

4.7.1.7.1. The Vicam Test Kits

November 2000 costs of columns were from about R80.00 each for the AFLA test columns to about R112.00 each for the FB test columns. Each mycotoxin and each grain sample requires a separate test. The cost of columns alone when testing three mycotoxins could be around R300.00 per sample. Other materials required, such as solvents, chemicals, developers, filter papers etc, are sold separately and will add about R40.00 per test. Vicam's columns use immunoaffinity chromatography technology. The columns contain beads chemically fused to antibodies specific for the mycotoxins. A maize sample is ground and extracted with a methanol/water solution. The extract is then run through the affinity column and the mycotoxin binds to the antibody on the beads. Other materials in the extract do not bind and are washed off the column. The mycotoxin is then removed from the column, using methanol. To visualise and measure the level of mycotoxin, a derivative of the mycotoxin must be made using a 'developer' and measured with a fluorometer.

4.7.1.7.2. FumoniTest™ from Vicam

FumoniTest™ from VICAM (URL:http://www.vicam.com/vicamy2k/fumonitest.html) produces precise numerical results. It can be performed in less than 15 minutes (excluding sample preparation and extraction), requires no special skills, and is sensitive, simple and quick for parts per million levels. FumoniTest™ is also ideal as the cleanup step for any HPLC analysis for precise results in parts per billion. FumoniTest™ has a long shelf life. The limit of detection is 250 ng/g when quantifying with a fluorometer and 160 ng/g when using HPLC for quantification. The testing procedure is as follows:
Extract Sample

- Grind and weigh sample
- Blend sample with salt and methanol/water mixture
- Filter
- Dilute and Filter

- Dilute a portion of filtered extract
- Filter
- Absorb and Elute
- Pass a portion of filtrate over FumoniTest™ affinity column
- Wash column with buffers
- Elute FBs from the column with methanol and collect in a cuvette.

Measure

- Add developers and place cuvette into a calibrated fluorometer and read results in µg/g, or
- Inject Eluate into HPLC
- Determine FB concentration by HPLC.

Ordering Information

Cat. No. Description

G8008 / G8009 FumoniTest™ Series-4 Fluorometer Basic Equipment Package
G8008, 110 V for U.S.A. / G8009, 220 V for international
Includes Series-4 Fluorometer, Series-4 printer paper, Mycotoxin Instructional Video, waste collection beaker, filter funnels (65mm), glass syringe (10mL), disposable cuvettes, FumoniTest™ calibration standards, Kim-Wipes tissues, microfibre filters (1µm), VICAM fluted filter paper (24cm), single position pump stand, cuvette rack, wash bottle (500mL), bottle dispenser for methanol (500mL), 2 glass blender jars (500mL), graduated cylinder (250mL), commercial blender with stainless steel container, digital scale and adapter, graduated cylinder (50mL), disposable plastic beakers, micro-pipet tips (50µL), micro-pipettor (20 µl), micro-pipettor (1 ml), micro-pipet tips (1 ml) and FumoniTest™ instruction manual.

Each item available individually.

Cat. No. Description

G1008 FumoniTest™ Columns, Fluorometer & HPLC, 25/box

33060 FumoniTest™ calibration standards

34000 Cuvettes, 250/pack

35016 Methanol, HPLC Grade, 4 x 4 L bottles

G5005 FumoniTest™ Developer A Fluorometer, 15 ml (for 15 tests)

G5003 FumoniTest™ Developer A-HPLC, 5 ml (for 22 tests)

G5004 FumoniTest™ Developer B-HPLC / Fluorometer, 500 ml (for ± 200 tests)

The cost per test including columns, developers, calibration standards, solvents, filter paper and other materials, but excluding overheads and labour, is about R257.00 in March 2002.

4.7.1.7.3. The Neogen Test Kit

Test kits are available for AFLA, vomitoxin (DON), ZEA, FB, T-2 and ochratoxin. Price: US$80-130 for 24 test wells; each mycotoxin requires a separate kit or between about R38.00 and R62.00 per test well (March 2002), excluding other materials, overheads and labour. A different test well is needed to test each mycotoxin.

Neogen's "AGRI-SCREEN" and "VERATOX" use ELISA technology. Antibodies specific for a mycotoxin are adhered to the wall of a microwell. A solution of mycotoxin chemically conjugated to an enzyme is provided with the kit. A maize sample to be tested for mycotoxin is ground and extracted. The extract is then mixed with a fixed amount of the mycotoxin-enzyme solution and placed into the microwell. The mycotoxin from the extracted maize sample and mycotoxin-enzyme conjugate then compete for binding to the antibodies in the microwell. As the mycotoxin in the maize sample increases, it competes with the mycotoxin-enzyme conjugate.

The assay procedure measures how much of the conjugate actually binds to the antibodies by first thoroughly washing the microwell and adding a colourless substrate to it. The enzyme present in the microwell converts the substrate to a blue

coloured product; the more mycotoxin-enzyme-conjugate in the microwell, the more intense the blue colour. Because maize samples with mycotoxin will result in less binding of the mycotoxin-enzyme conjugate, positive samples will be lighter blue. Determination of the mycotoxin is done by visual comparison of the maize sample with positive and negative controls. Quantitative measurements can be obtained if a spectrophotometer is available.

4.7.2. Infrastructure and labour for on-site immuno-affinity testing

The laboratory apparatus required to facilitate a single technician for maximum throughput would consist of at least two fluorometers, four high-speed blenders, two laboratory mills, a laboratory scale, sufficient beakers, pipettes, funnels etc and other basic laboratory ware. The total cost (November 2000) would be between R250 000 and R300 000.

The entire immuno-affinity test procedure can take more than 60 minutes to complete, since the two filtering steps are slow. A skilled technician, running tests for all three mycotoxins on at least three samples simultaneously, could probably test no more than 30 samples in a 12-hour day, or about 2.5 tests per hour, including three mycotoxins. At a remuneration of R20.00 per hour, the labour cost per test is R8.00.

To perform at this level, each technician would require at least 10 square meters of laboratory space, with sufficient electricity, water and sewage.

4.8. Recommendations of test methods, sampling methods and testing procedures to be adopted together with MTLs for fumonisins, aflatoxins and deoxynivalenol

4.8.1. Preamble

In Section 4.6.5, newly proposed MTLs for AFLA, FBs and DON were summarised. A basis for determining compliance was also proposed (Section 4.6.6), being the level of the relevant mycotoxin in a representative sample of a grain or product lot or consignment. A lot or consignment could be any distinguishable unit, from a pallet stacked with packaged product, up to a complete silo bin or ship's hold containing bulk grain, flour etc. In the next sections, sampling procedures and different options for the practical execution of the testing of grain or grain products for compliance to any MTLs that may be adopted, are discussed.

Divergent procedures are evident in the literature consulted on the sampling and testing of grain for compliance to MTLs for mycotoxins. Therefore, what follows is based on my own experience in mycotoxin sampling and analysis on a large scale under practical South African grain storage and handling conditions.

4.8.2. Sampling grain for mycotoxin analysis

4.8.2.1. General principles

Mycotoxins are not evenly distributed in grain, grain products or mixed feeds. Therefore, taking a feed or grain sample, which will give a result in mycotoxin analyses representative of the lot from which it was taken as a whole, is difficult. Nearly 90 percent of the error associated with mycotoxin assays can be attributed to how the sample was collected. This is because only 1 to 3 percent of the kernels in a contaminated lot actually contains mycotoxin, and these contaminated kernels are rarely evenly distributed within the grain bulk. Over- or under-representation of contaminated kernels in the sample gives a skewed result for the lot as a whole.

Various types of sampling procedure can be employed, each of which is best suited to a particular situation. The following are distinguished:

- **Uniform sampling**. In this method, a composite sample is taken in a planned way from *all* parts of the whole lot. The average of the lot is represented in the sample. The samples are combined into one sample and thoroughly mixed. If the lot is large, the sample needs to be large as well if all the variation in the large lot is to be realistically represented in the sample. Usually, the sample is too large for the entire sample to be analysed; therefore, thorough mixing and dividing into smaller, uniform portions is necessary. Special grain dividers are used to split the sample into equal portions, one or more of which is then analysed.

- **Selective sampling**. In this method, a composite sample is made up by selecting small samples from sections of the lot that are likely to contain the lowest quality. If the sample passes the criterion, the chances are that the entire lot complies with the criterion.

- **Random sampling**. In this method, a sample is taken from a section of a lot in a haphazard, unplanned way on the assumption that the lot is uniform in terms of the property being analysed, and that the sampler is unbiased. Random sampling is probably the most used and the most misused method of sampling in the grain industry. True random sampling avoids even the subtlest bias, by selecting samples blindly, or by means of a lottery system. It is a useful method where a lot is uniform and not all parts of the lot can be sampled with ease.

- **Combined random and uniform sampling**. In this method, a composite sample is taken by randomly selecting several sections of the lot, from which the sample is composed. This is the method mostly used for sampling bulk grain for grading purposes. The lots sampled in this way are rarely larger than 50 tons and the number of points sampled rarely exceeds 10. For larger grain lots, the number of sampling points needs to be increased and consequently the size of the

composite sample increases. After thorough mixing, a grain divider is used to split the composite sample into manageable sub-samples for analysis.

4.8.2.2. Specific sampling procedures

4.8.2.2.1. Sampling from bulk rail or road trucks

The grading regulations stipulate that a composite sample should be made up by using a grain probe to sample each truckload through its entire depth in at least six randomly selected sampling positions. Sampling for grading purposes is sufficient also for mycotoxin analysis, as long as the lot being sampled is relatively homogenous and does not exceed 100 tons.

4.8.2.2.2. Sampling bulk grain in silo bins and ships holds

It is well known that most grain silos are funnel flow silos, where grain flows from the top out of the silo bin. Therefore, if a silo bin was completely empty at the beginning of harvest intake, it is possible to obtain a representative sample by running the centre core from the grain outlet. However, this can be done only once, because thereafter the centre core consists of grain from the top surface that has flowed down the centre and it no longer represents the grain in the bin from top to bottom. The composite sample is composed by frequently taking grain from the grain stream at the grain outlet until the very first sign of an indentation appears on the grain surface at the apex in the top of the bin.

Once some grain has been let out from a newly filled silo bin, future sampling has to be done from the grain surface. A composite sample is taken with a pneumatic grain sampler (Probe-A-Vac) from at least three randomly selected points on the grain surface, through the entire depth of the grain in the bin. The points should be at least 2 m away from one another and at least 1.5 m away from the centre of the bin. At each point, the entire sample is collected as the sampler probe moves deeper through the grain.

The same method is followed when sampling bulk grain in a ships hold, but here the grain must be sampled from at least 16 points in a 4 x 4-grid pattern on the grain surface within the open hatch.

Sample size from large grain bulks in silos and ship holds should be at least 2 kg for each 100 t of grain in the bin or hold.

4.8.2.2.3. Sampling from a grain conveyor

Bulk grain can be sampled by scooping from a belt conveyor at regular intervals. However, grain sampled in this way is representative only of the grain that has been outloaded and not of the grain remaining in the bin. Grain should be scooped alternately from the top and bottom surfaces of the grain on the conveyor. The bottom surface can be accessed at a point where the grain is thrown off the belt and travels through the air for a short distance. It is important to sample from the bottom surface because fines sift through to the bottom very soon after the grain from the bin outlet has landed on the belt. Sampling only from the top surface underestimates many quality properties, including insect infestation and mycotoxin contamination. Sample size should be at least 2 kg for each 100 t or less of grain moved.

4.8.2.2.4. Sampling bagged grain

Grain in bags stacked on a warehouse floor, a pallet or a vehicle should be sampled by probing all the bags around the surface areas of the stack. A sample is composed from all probes. Sample size should be at least 2 kg for each 100 tons in the stack, or a minimum of 2 kg for all smaller stacks.

4.8.2.2.5. Sampling packaged products in stacks

Packaged products wrapped in polythene and stacked on pallets are best sampled from the conveyor before they are wrapped and stacked. Depending on the size of the packages, as many whole packages as needed to compose a sample of at least 2 kg for every 100 tons or less of product, should be removed at random to make up a sample.

4.8.2.3. Sample preparation

After a composite grain sample has been collected, it is thoroughly mixed and the whole sample coarsely milled through a 14-mesh screen. The milled sample is thoroughly mixed and a 1 to 2 kg sub-sample is then milled through a 20-mesh screen. A sufficiently sized portion of the finely milled material is then used for analysis.

If the original composite sample is larger than 10 kg, splitting through a divider a number of times, until at least 5 kg remains, can reduce it to a more manageable size. This sub-sample is coarsely milled and further treated as described above.

4.8.3. Practical application of MTLs for aflatoxins, fumonisins and deoxynivalenol in grain and grain products

4.8.3.1. Options for consideration

The enforcement of MTLs for mycotoxins in grain and grain products can create a need for substantial infrastructure and significant additional costs to handling and storing grain. Included in these costs are the direct, visible costs of sampling and testing, and the indirect, often invisible, costs of redirecting grain not suitable for human use to other uses. Also included, are the costs of finding grain that can comply with the set standards and of switching to alternative foods. In the end, the consumer pays for all these costs. Therefore, the costs and benefits of instituting MTLs should be carefully considered, as well as the choice of an enforcement program. The options to choose from are as follows:

- Not to institute an MTL;
- To institute an MTL, but not to enforce it;
- To institute an MTL, but to only test where an apparent problem emerges;
- To institute an MTL and to routinely test for compliance raw grains only, either on samples collected randomly or according to a set sampling procedure;

- To institute an MTL and to routinely test for compliance only consumer ready products, either according to a set sampling procedure or on samples collected randomly;

- A combination of 4 and 5 i.e. to institute an MTL and to routinely test raw grains as well as grain products, either according to a set sampling procedure or on samples collected randomly.

The ideal is to sample and test for compliance as early as possible in the grain chain. The fourth option is therefore considered the most suitable. The various sub-options for this option, (i.e. at harvest intake, or during dispatch to buyers, or in the depot storage bin, or upon receival at mills), using a set sampling procedure, will therefore be discussed further.

4.8.3.2. Routine testing at harvest intake

This option entails the testing of each load for compliance to the MTL when the farmer delivers it to a storage silo, mainly during harvest time. From the point of view of millers, the advantages of testing grain during harvest intake are that:

- The producer is penalized if he delivers grain not complying with a regulatory MTL. This creates an incentive for producers to press for the development of varieties less susceptible to fungal infection and the possibility of a more lasting solution to the problem of mycotoxins in grain;

- No additional sampling is required, since samples are taken for grading anyway, which can also be analysed for mycotoxins.

- Relatively small grain packages are tested, so there is less likelihood of the discovery of grain lots or finished product that do not comply with the MTL later in the handling chain.

- Where a large proportion of the crop in the service area of a grain silo exceeds the MTL, there exists an opportunity to blend incoming loads so that the maximum quantity of grain possible can still comply with

the MTL. A system has been developed for calculating running averages of various quality properties during grain intake, which could be made available for the purpose.

The disadvantages are that:

- Testing facilities, capable of keeping up with a grain intake rate of approximately 300 loads per silo per day during peak harvest, need to be established at storage silos. The capital cost of this could be between R2.5 million and R3.0 million per grain silo, to facilitate about 10 technicians;

- In addition, the cost of consumables to test for three mycotoxins will add more than R50.00/t, as farmers' loads are only about 10 t each and each load and each mycotoxin requires a separate test costing between R120 and R172;

- The cost of labour would add about R0.80 per ton. The total costs, including electricity, water, and building rent could therefore be more than R60 per ton;

- The testing infrastructure is mainly used during the grain intake season only;

- Segregation facilities at storage silos are already under strain, and to separately store additional categories of grain imposed by compliance and non-compliance to MTLs for mycotoxins, will add to the difficulties. This constraint can be partly alleviated by using the system for calculating running averages during grain intake.

- Additional testing would be required to detect spoilage during storage.

Overall, the capital requirements and running costs of this option are prohibitive and the benefits to consumers may prove cost-ineffective.

4.8.3.3. Routine testing after harvest intake

In this case, the grain is taken in as usual, without testing for MTL compliance of each load at delivery to the storage silo. From here, three sub-options can be considered:

- To sample and test each rail or road truck when dispatched to a mill;
- To sample and test the grain in each individual silo bin before grain is outloaded from the bin.
- To sample and test each rail or road truck upon arrival at a mill.

The sub-option of testing grain lots upon receival at mills is fraught with a multitude of practical problems for both large and small mills. This option will therefore not be analysed further.

4.8.3.4. Sampling and testing of truckloads on dispatch to mills

This option entails the testing of each load for compliance with the MTLs either at the storage silo when it is dispatched to a mill, or at the mill upon receival.

The advantages of this option are as follows:

- The capital costs of setting up testing facilities can be reduced to less than one tenth of that required for testing at crop intake, because the outloading rate is much slower than the harvest intake rate. The cost of setting up a basic laboratory at each silo should therefore be between R250 000 and R300 000 to facilitate on average one technician per silo;
- Testing facilities are utilized throughout the year, which will make it easier to recruit suitable staff;
- The running costs of testing are reduced to less than a quarter of that of testing at crop intake, because loads dispatched to mills are generally more than 4 times as large as the loads farmers deliver to silos.

Running costs could therefore come to about R12.50 per ton to test for three mycotoxins;

- The size of the grain parcels tested is still relatively small, which reduces the likelihood of the discovery of finished product that does not comply with the MTLs.

The disadvantages are that:

- Millers will have no redress of grain suppliers for supplying non-compliant grain;
- There may be a strain on rail siding facilities at grain silos whilst the sampling and testing is in progress;
- Therefore, most non-compliant truckloads will have to be dispatched to the mill in any case; and
- Millers will have to decide how best to deal with non-compliant truckloads, either by blending the grain in with compliant grain or by selling it off to another miller or as animal feed.

Although the testing costs involved in this option could be acceptable, there are many difficulties, which could make it unattractive to millers.

4.8.3.5. Sampling and testing of individual silo bins before grain is outloaded

This option entails the testing of each silo bin at each storage silo for compliance with the MTLs before any new season grain is outloaded from it. Each bin is treated as a grain pool, with all farmers who have grain in that particular bin partaking in the pool. The grain in the bin is sampled and tested as a unit. The advantages of this option are that:

- The onus is on producers to supply MTL compliant grain;

- If the grain in a bin does not comply, an opportunity may exist for millers to blend non-compliant grain with compliant grain to render more grain compliant to the relevant MTL;

- Testing facilities need not necessarily be set up at all storage silos and testing could be done by a central laboratory; and

- The running cost of sampling and testing is reduced to an absolute minimum, and could be as little as a few cents per ton.

The disadvantages are that:

- Relatively large grain parcels are tested and non-compliant grain pockets of several truckloads could escape detection until later in the grain chain;

- For the same reason, more grain may be found non-compliant and in some years millers may experience difficulty to find sufficient supplies of compliant grain;

- Silo-owners and grain producers might be unwilling to support this option.

4.9. Possible implications of MTLs for mycotoxins in South Africa and major grain trading partners on international trade in grains and grain products

4.9.1. General considerations

From a broad perspective, the existence or absence of MTLs, or differences between the MTLs for mycotoxins in grain importing and exporting countries carries certain advantages and disadvantages. Some of these are listed in Table 43.

Table 43 - Some advantages and disadvantages of having, or not having MTLs from a country's broad perspective

Advantages	Disadvantages
A country with an MTL, importing grain	
Consumer safety warranted	Difficulty to source MTL compliant grain
Fewer losses of imported grain found to be unsuitable for use	Higher grain purchase price
	Added costs for regulation and monitoring
A country without an MTL, importing grain	
Low purchase price, or grain donated	Consumer safety is compromised
Ease of finding grain suppliers	Susceptible to dumping of contaminated, or high moisture grain
No added costs for regulation and monitoring	Larger losses of grain found to be unsuitable for certain uses

A country without an MTL, exporting grain

No added costs for regulation and monitoring	Lower selling prices
	Difficulty to find markets
	Difficulty to meet clients' import requirements
	Own consumers' health compromised

A country with an MTL, exporting grain

Safety of own, and overseas consumers warranted	Added costs for regulation and monitoring
Better selling prices realised	
A wide selection of markets are available	

4.9.1.1. Difficulty of harmonization between countries

One of the main problems to surface where countries maintain MTLs, is caused by differences in the MTLs of different countries. Clearly, these differences are the result of different national needs caused by differences in the kinds of mycotoxins that contaminate grain in different parts of the world, and in eating habits and mycotoxin intake among countries. Sometimes the practicalities around an MTL also play a role in the setting of an MTL. In South Africa, AFLA are rarely found in commercial grain, hence one of the lowest MTLs in the world is in use here, and can be complied with easily. In specific states in the USA, on the other hand, MTLs for AFLA in maize intended for intra-state animal feed uses, are much higher than the FDA action

levels set for maize crossing state borders. Countries need to be autonomous and serve their own interests. The interests of countries differ widely and it is therefore difficult to use a harmonized approach. Differences between the MTLs of countries could be used as trade barriers, unless all parties agree on the approach for deriving safe levels and can see that their own interests have been addressed. Other impeding factors relate to procedures adopted for data collection, data interpretation and analysis.

4.9.1.2. Effects of MTLs on desirability of grain from specific sources and on price

Low mycotoxin levels in grains meeting an MTL specification, could popularise a country's export grain and grain products and effect a price premium. Conversely, real or potentially high mycotoxin levels in grains from a country where no MTL specification applies, or where the grain cannot comply to the importer's MTL specification, can lose export markets or result in price discounts. For example, Thai maize was formerly popular for its bright yellow colour and high protein content. Today, however, these qualities are ignored as a result of high levels of AFLA. This has seriously affected the demand for Thai maize, which now trades at a US$10-20 per tonne discount on the world market (Tangthirasunan, 1998). ARG maize also trades at a discount, at least partly because control over AFLA levels and moisture content was lacking in the past. RSA maize, on the other hand, traditionally trades at a price premium of between $15 and $25 per tonne on international markets, because of absence of AFLA, low moisture content and other desirable quality characteristics. On the debit side, the cost of testing should be considered. This cost depends on the test program used and the number of mycotoxins included in the testing. For example, routine testing at harvest intake of 10-ton grain parcels for three mycotoxins could add about R100.00 per ton to the cost of grain handling and storage.

4.9.1.3. Need for, and cost of testing, supervision and control

In 1992, South Africa imported more than 4 Mt of maize from Argentina and the USA. Import contracts stipulated that the average total AFLA content of any consignment should not exceed 15 ng/g. In no individual sample should the total AFLA content exceed 20 ng/g. Various procedures were put in place to ensure that these stipulations would be met, including the appointment of supervisory companies

in the source countries. In addition, in the USA, the Maize Board was able to establish a working relationship with the Federal Grain Inspection Service (FGIS) to help ensure that all quality specifications would be met in grain shipped to South Africa. No similar governmental body existed in Argentina and the Board had to rely solely on their appointed supervisor to ensure that only maize that meet the quality specifications would be shipped. However, from the very first shipment from Argentina, AFLA levels in a number of samples exceeded as much as 100 ng/g upon arrival in South Africa. Each sample represented roughly 300 tons of maize therefore the Board believed that this posed a real threat to consumer health and a possible outcry in the press, similar to that in the 1980's. The Board therefore discontinued maize purchases from Argentina after only 13 shipments (about 15% of the total requirement) were received, in spite of the better price at which ARG maize was available.

Where traders or millers import relatively small quantities of grain, it is anticipated they would have much greater difficulty to meet stringent MTLs such as 200 ng/g for FBs.

4.9.1.3.1. Elevated cost of imported grain that can meet local MTLs

The existing MTL for AFLA in food maize in South Africa is 10 ng/g, of which no more than 5 ng/g AFB_1 is allowed. In the USA, the MTL for AFLA in food grain is 20 ng/g however the FDA action levels do not apply to export grain. During the 1992 maize imports, the best that could be agreed upon was 15 ng/g. Any lower limit would require identity preserved handling of grain parcels, with hugely elevated costs. The average AFLA levels in USA maize nonetheless turned out considerably lower than the South African MTL for human use and little USA maize had to be redirected to animal use. These controls nonetheless elevated the grain purchase price and handling costs.

4.9.2. Specific considerations

It has been shown that in grain in South Africa three mycotoxins are at present of concern. These are AFLA, FBs and DON. New MTLs for these three mycotoxins in grain and grain products were proposed. Apart from these proposals, a regulatory MTL for AFLA has been in existence for years, and recently, an MTL for FBs in grain has been recommended by Marasas (1997). The implications for millers of existing, recommended and newly proposed MTLs will now be discussed.

4.9.2.1. Summary of existing/recommended and proposed MTLs

Aflatoxins:

- An existing regulatory MTL of 10 ng/g (of which 5 ng/g may be AFLA B1) in food grains;
- New MTLs (as summarised in Section 4.5.6) to replace the existing MTL above, of 20 ng/g in uncleaned whole maize intended for food use, and 10 ng/g (of which 5 ng/g may be AFB_1) for cereal products. The term 'uncleaned grain' refers to grain not yet cleaned for the purposes of milling and not to 'grain cleaning' as done at storage silos).

Fumonisins:

- An MTL of 200 – 300 ng/g in maize and maize products, as recommended by the MRC;
- Newly proposed MTLs of 4 µg/g in uncleaned, whole maize intended for human consumption, 2 µg/g in dry milled grain products for human consumption with a fat content < 2.0% wet weight basis, and 1 µg/g in dry milled products for human consumption with fat content of $\geq$ 2.0 %, wet weight basis).

DON:

- A newly proposed MTL of 2 µg/g in uncleaned cereal grains intended for food use;
- A newly proposed MTL of 1 µg/g in dry milled cereal grain products intended for food use.

4.9.2.2. Aflatoxins

4.9.2.2.1. Implications for millers of the existing MTL

The existing MTL of 10 ng/g for AFLA in food grains and grain products holds little implications for millers as far as locally produced grains are concerned, because natural AFLA levels in maize are low. Local commercial maize easily complies with the MTL and no routine testing is required.

A possible exception is stored wheat. The present use of an unproved, non-standard moisture reference test has resulted in moisture problems in stored wheat, possibly creating conditions suitable for the production of AFLA in wheat.

Damage to the health of consumers caused by AFLA exceeding the existing MTL can expose millers to large claims for compensation.

Imported maize cannot easily comply with the existing MTL and millers may have difficulty to find maize for import at a reasonable price. AFLA are not normally found in imported wheat.

4.9.2.2.2. Implications for millers of the newly proposed MTLs for aflatoxins

The newly proposed MTLs for AFLA should make life easier for millers, without compromising consumer interests. The proposed MTL in unprocessed grains are in line with those in the major supplier countries, which will make it easier to source import grain. On the other hand, the proposed MTL in finished products is the same as the existing MTL. Because grain cleaning before milling removes more than half of the mycotoxins in grain, no extra input will be needed to comply with the MTL for grain products when grain containing 20 ng/g of AFLA is used for milling. The

higher MTL for unprocessed local grain does not create an opportunity for 'upward blending', simply because locally produced grains with high AFLA levels is not available in SA, provided moisture control in stored grains is of a high standard.

4.9.2.3. Fumonisins

4.9.2.3.1. Implications for millers of the MTL for fumonisins recommended by the MRC

The MTL of 100 to 200 ng/g recommended by the MRC for FBs in (unprocessed) maize holds serious implications, not only for millers, but also for the rest of the maize industry, including consumers. The aspects that would be affected are the availability of maize and maize products that comply with the MTL for FBs and the supply and utilization of maize and maize products that do not comply.

Availability of maize and maize products

The average FB levels in white RSA maize from various production areas, calculated over six years, all exceeded 200 ng/g (Table 27). This means that the major portion of the crop would be labelled unsuitable for human consumption. This would have obvious and serious implications for the entire maize industry and particularly for consumers. However, if the recommendation was ambiguous and the intention of the MRC was for an MTL of 100 to 200 ng/g for finished maize products, a large proportion of maize product would still be found unsuitable for human consumption. Particularly, in years like 1989 and 1994 when FB levels in white maize in respectively the N-OFS and the W-Tvl were at relatively high levels, a very large proportion of finished product would be labelled unsuitable for humans.

The bulk of the white maize crop by far (about 70%) comes from the N-OFS and the W-Tvl and blending with maize from areas with lower FB levels is neither a cost-effective, nor a practical option. Even if maize from only these two areas could be blended in each of the two years, it would not bring the FB content down to below the MTL. The average level would still be about 1 000 ng/g in maize and between approximately 300 and 500 ng/g in different finished products.

Table 44 - Total FBs (ng/g) in white maize from different areas and different crops in South Africa

	1989	1990	1991	1992	1993	1994	Mean
N-OFS	1 812	567	86	207	568	362	600.3
E-OFS	33	318	324	361	136	357	254.8
Natal	174	979	353	350	469	587	485.3
W-Tvl	289	716	354	596	499	1 728	697.0
E-Tvl	986	306	290	405	324	895	534.3
PWV				333	423	569	441.7

It would also be impossible to obtain sufficient maize or maize products from alternative sources that could comply with an MTL of 100 – 200 ng/g in maize or in finished products. Some estimates state that about one third of maize products in the Netherlands would not comply with the Swiss MTL of 1 000 ng/g (de Nijs *et al*, 1998a). In 349 samples of maize from 18 countries worldwide, FB_1 was present in 93% of the samples. The median FB_1 content of all samples was 420 ng/g, and the average contamination level was 1 359 ng/g of FB_1. Total FBs (FB_1, FB_2 and FB_3) would be considerably higher.

In another survey (De Nijs *et al*, 1998b), 78 maize-containing foods obtained from retail stores in the Netherlands were analysed for FB_1 contamination. Thirty-six per cent of the samples contained FB_1 in the range of 8 ng/g (limit of detection) to 1 430 ng/g. Forty-six per cent of samples like maize for bread production or popcorn, maize flour and polenta, contained FB_1 in the range of 8 - 380 ng/g. Twenty-six per cent of the processed foods (tostados, canned maize, maize starch, maize bread, popped maize, flour mixes, maize chips and cornflakes) contained FB_1 in the range of 8 – 1 430 ng/g.

These surveys show that maize-based foods everywhere contain FBs, often at considerably higher levels than in South Africa. An MTL of 200 ng/g in maize or maize product is therefore impossible to comply with and would culminate in severe shortages of maize and maize products considered suitable for human use. The shortfall will have to be made up by other starchy foods such as wheat, rice and potatoes. If only 25% of maize-based foods need to be replaced with these products, a quantity of around 500 to 600 kt of finished products is involved. An extra burden of this magnitude on the wheat and other staple food industries in the country would cause havoc and the cost of these products to consumers would rocket, which is likely to force poor sections of the consumer population to use grain meant for animal feed, thereby nullifying the intended protection of the MTL for FBs.

Utilization of maize and maize products that do not comply with an MTL of 200 ng/g.

Worldwide, the markets for white and yellow maize are distinctly different markets, but white maize or maize products that cannot be used as food will be offered on the feeds market, or for export. These markets will be destabilized and prices are likely to plummet if 500 to 600 kt of white maize product suddenly became available on the feeds market. The quantity annually coming available will vary depending on FB levels, which are totally unpredictable. The effects on maize producers would be disastrous and many of them would go out of business. The resulting white maize shortfall in following years will have to be made up by imports of maize or other staple grains, if import maize that can comply with the MTL cannot be found. As has been shown, imported maize is generally of a lower mycotoxicological quality than RSA maize.

In the USA, grain that is unsuitable for human or animal use because of non-compliance with an MTL for mycotoxins can be used for producing fuel alcohol. That option is not available in South Africa, since the market for fuel alcohol is fully serviced by the oil-from-coal process. A small amount of maize is used in South Africa for producing distilled alcoholic beverages. The irony of this option, if it could become viable, is that alcohol is a Group 1 carcinogen. So, to avoid exposure of consumers to a suspected human carcinogen, the contaminated maize would be turned into a confirmed human carcinogen!

Clearly then, an MTL of 100 – 200 ng/g or even 300 ng/g in maize or in finished maize products would cause havoc in the grain industry. On top of that, the health benefits to consumers are obscure, because no definitive detrimental effect of FBs on human health has been demonstrated yet. Nonetheless, a detrimental effect on human health is possible, because FBs are acutely toxic to horses at levels commonly found in foods. Horses are obviously much more sensitive to FBs than humans and most other animals. FBs are also acutely toxic to pigs at very high levels not found in commercial grain or in foods. In addition, FBs are carcinogenic to rats and mice following chronic exposure to high levels not found in commercial grain or in foods. FBs are present at low levels in many maize–based foods and humans are constantly exposed to these. Therefore, for the sake of safety, some maximum limit of human exposure is desirable. This limit should be determined in a rational way, making use of all the available information. To this end and on this basis, the MTLs formulated in the present study are being proposed. The implications for millers of the proposed MTLs are discussed next.

4.9.2.3.2. Implications for millers of the proposed MTLs for fumonisins

Most maize in most crop years can comply with an MTL of 4 µg/g and this MTL will have a minimal effect on the maize industry in general, and millers in particular. Unfortunately, with the data presently available, it is not possible to estimate more precisely the proportion of the crop that could be labelled unsuitable for human consumption in 'good' and 'bad' FB years. However, the quantities are likely to be small enough for it to be practical and cost-effective to blend in any maize containing FBs at levels exceeding the MTL, with low FB-content maize. Blending before milling would preclude the occurrence of some lots of finished product exceeding the relevant MTLs. For example, the maximum levels of 5.5 and 6.1 µg/g found in 1994/95 in samples of sifted and unsifted maize meal respectively (Table 30) could probably be avoided in this way.

An MTL of 4 µg/g is also realistic in terms of finding maize for import. It is of the same order as the guidance levels for FBs in the USA, and could therefore not be used as a trade barrier. At the same time, it could ensure that apparently healthy maize containing FBs at levels as high as 10 µg/g is not imported.

4.9.2.4. Deoxynivalenol

Implications for millers of the MTLs for DON proposed in Chapter 10

The proposed MTL of 2 µg/g DON in grains intended for foods use will not create difficulties in grain supply, either from local sources or from overseas, and it would ensure that only healthy grain is imported and milled.