

3. Procedure

3.1. The occurrence of mycotoxins in SA grains and grain products

3.1.1. Preamble

From 1986 to 1994, the South African Maize Board commissioned, or itself undertook annual surveys on the mycological infection rates and mycotoxin contamination of commercial maize. These surveys came to a halt when the single channel marketing scheme for domestic maize was discontinued at the start of the 1995/96-marketing season. From 1990 through 1994, the Maize Board also analysed samples of white maize products for a series of mycotoxins, and in 1994, yellow maize feed mill products. In 1992, more than 4 Mt of yellow maize was imported in 83 vessels, all holds of which were sampled upon arrival in a South African port. The samples were analysed for mycotoxin content, before the maize was released for human use or in horse feed and the fungal infection was determined. Much of the data generated by these surveys were published (Viljoen *et al*, 1993; Viljoen *et al*, 1994; Kallmeyer *et al*, 1995; Rheeder *et al*, 1995; Rava, 1995), but only the paper by Rheeder *et al* is generally accessible.

In addition, the Maize Board commissioned the MRC to analyse samples from a shipload of South African yellow maize exported to Taiwan for fungal infection and mycotoxin content (Rheeder *et al*, 1994). This was part of a larger survey of quality changes that take place during the export process (Cronje *et al* 1990).

For purposes of comparison with South Africa, sufficient published data are available to give an understanding of the general levels of FBs and AFLA in maize and maize products in the USA and a few other countries.

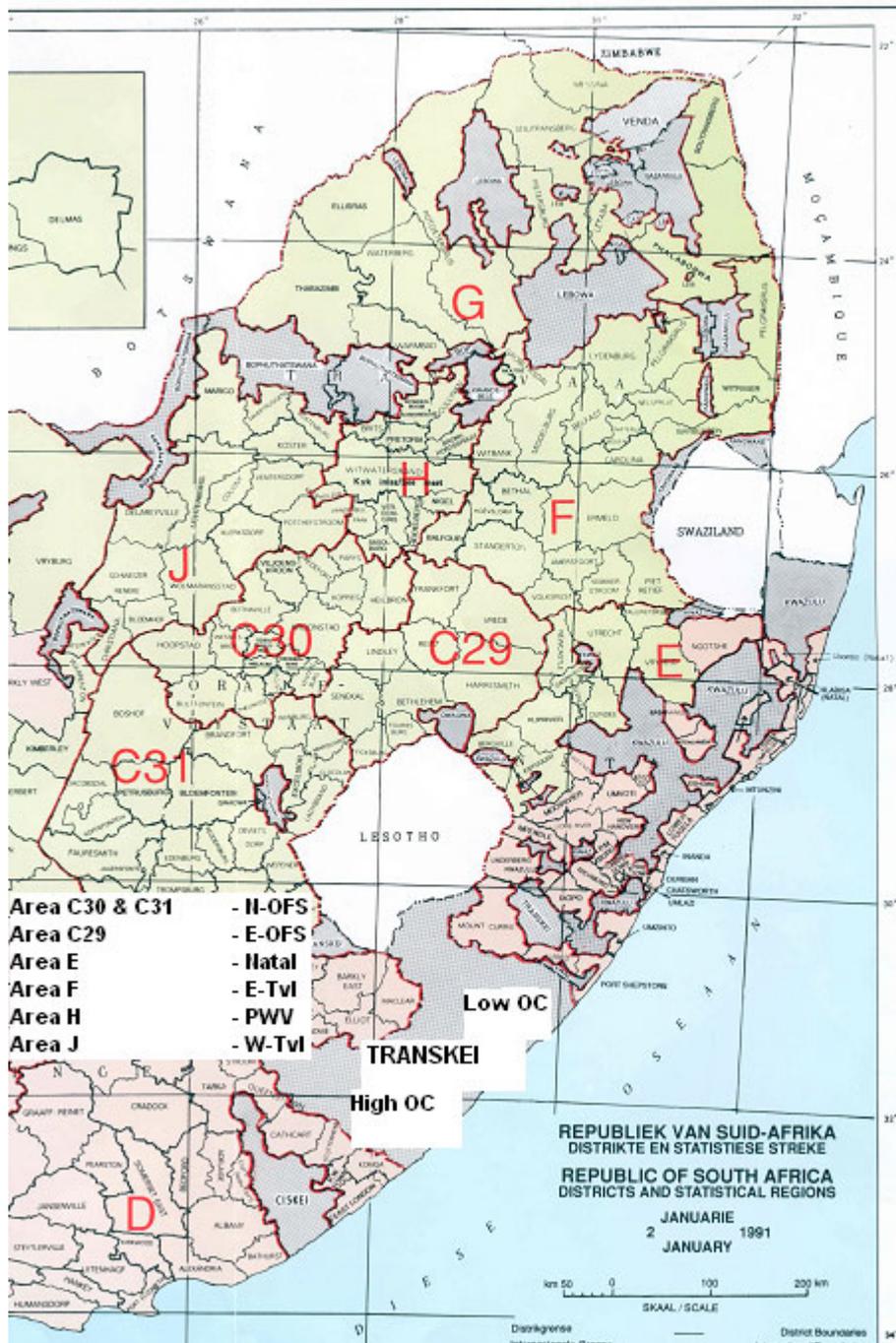


Fig. 1 – Map of the eastern parts of South Africa, showing the maize production areas in 1991 referred to in the text and the ‘high’ and ‘low’ OC incidence areas in Transkei referred to in the literature

3.1.2. Survey procedure

3.1.2.1. Fungi and mycotoxins in South African maize crops

Samples of each year's maize crop were collected from grain silos in the main production areas (Fig. 1), in a way that would ensure the best possible representation of the crop as a whole in the particular area. As farmers delivered their maize to silos, representative samples were taken for grading from each truck or trailer load in the way prescribed in the South African grading regulations for maize (Government Notice No R.2931) i.e. six probes were taken through the depth of the grain at six randomly selected positions in the truck or trailer. Most maize is delivered in 10 to 20t loads. After the load had been graded, the sample was emptied into a bag for that particular grade. Thus, at each silo, a composite sample of each class and grade was made up over the duration of the harvest delivery period from all the grading samples from all consignments delivered to the silo. Compared to this method of sampling, other surveys would be similar to a snapshot of the situation in a specific location at a specific time. A large number of such snapshot surveys would be required to approximate the representation of the crop as a whole of the Maize Board method.

At the completion of harvesting, the composite samples of each class and grade of maize were collected by Maize Board inspectors, thoroughly mixed, and divided into sub-samples through an appropriate divider. The sub-samples thus obtained for each silo were analysed for the fungal infection rate of surface sterilised kernels and for the mycotoxin content of the grain using high performance liquid chromatography (HPLC) for FBs and MON, based on the method described by Shephard *et al* (1990). Gas chromatography (GC) was used for all other mycotoxins. The results of analyses from all the silos within a particular production area were then used to calculate the average levels and the standard deviation for that area. The areas concerned were the production areas as they used to be delimited by the Maize Board before the South African domestic maize trade was deregulated in 1994 (Fig 1). These were the western and eastern Transvaal (W and E-Tvl - area J and F respectively on Fig 1), the northern and eastern Orange Free State (N and E-OFS - area C30 and C29 respectively on Fig 1), the PWV (area H on Fig 1) and Natal (area E on Fig 1). Other production areas were not included in these surveys, as relatively little maize was

produced there. Samples of the 1986, 1987 and 1988 crops were analysed for mycotoxins by the University of Natal, using a multi-mycotoxin test (Dutton, *et al*, undated). The mycotoxins tested for were AFLA, trichothecenes, particularly DON, NIV, DAS, fusarenon X, HT2, T2 and T2-tetraol, and various other mycotoxins, such as CIT, ochratoxin, PAT, penicillic acid, tenuazonic acid and ZEA.

However, multi-mycotoxin methods lack the sensitivity and specificity of methods dedicated to the detection of one, or a group of related toxins. A multi-mycotoxin method can therefore fail to detect a significant level of a specific toxin, or can register false positives for certain toxins. This is less likely to occur with a dedicated technique. Samples of the 1989 and 1990 maize crops were therefore analysed by the MRC by GC and HPLC for FB₁, FB₂, DON, NIV, ZEA, MON, and AFLA in both years, and additionally in 1990, for FB₃. The MRC also determined the percentage of kernels infected by the major fungi. Samples of the 1991, 1992, 1993 and 1994 crops were analysed for fungal infection, AFLA, FB₁, FB₂, FB₃, DON, NIV, T-2, DAS, ZEA, PAT, CIT, OA and AME in the Maize Board's laboratory. The fungal infection rates of maize of the 1989 through 1992 crops from the various production areas were statistically compared using analysis of variance for groups with unequal numbers and the Statpack software package. The average mycotoxin levels in maize of the 1989 through 1991 crops from the various production areas were similarly statistically compared.

3.1.2.2. Mycotoxins in white maize products in South Africa

Samples of various white maize products manufactured from maize of the 1990, the 1991 and the 1994 domestic white maize crops were collected from mills across South Africa and analysed in the Maize Board laboratory for the same series of mycotoxins determined in whole maize. From 1991 onwards, T-2 and DAS were added to the list of mycotoxins analysed. In 1992, the maize crop failed because of drought and the available local white maize supplies were blended with imported yellow maize for the manufacture of maize products for human consumption. No surveys of mycotoxins in maize products were carried out in 1992 and 1993. Some of the results are reported in the literature (Viljoen *et al*, 1993, 1994; Rava 1995) but these papers are not readily available. The results are reported in detail here, and their impact and significance are comprehensively assessed for the first time.

The samplings for these surveys were inevitably of the ‘snapshot’ type, because it was not possible to sample continuously throughout the year at each of the various mills.

The maize products involved in the surveys were unsifted, sifted, special and super maize meal, samp, maize rice and maize flour (see the grading regulations for maize products No R 792 of 27 April 1984, amended by No R 1739 of 17 September 1993). Two by-products of the white maize milling industry were also analysed: maize bran and maize screenings. Maize screenings consist of broken and damaged (i.e. mostly mouldy) grains removed during the cleaning process before conditioning, and maize bran is mainly removed from the kernel during degerming, the first milling step. Both of these by-products are used in animal feeds. In the 1991/92-survey (i.e. maize from the 1991 crop), defatted germ meal, another by-product originating from dry maize milling, was included.

Samples were collected from late in the marketing year, to early the next year. It is therefore reasonable to assume that the products concerned were respectively manufactured from maize of the preceding harvests rather than from the harvest of the year before that, and the results can validly be compared with those on whole white maize of the relevant crops.

Where appropriate, the levels of the different mycotoxins in the various maize products were statistically compared by analyses of variance, using the Statpak computer package, for groups with unequal numbers of samples. Products with less than 10 samples in the group were not included in the statistical analyses. Also, products of the 1994/95-survey were not statistically compared with one another.

3.1.2.3. Mycotoxins in maize feed mill products

In the 1994/95 marketing year, the following yellow maize products and milling by-products were collected from feed mills for mycotoxin analyses (see the grading regulations for maize products No R 792 of 27 April 1984, amended by No R 1739 of 17 September 1993):

- No 1 and no 2 straightrun yellow maize meal;
- Unsifted crushed yellow maize;

- Sifted crushed yellow maize;
- Maize germ meal originating from dry white maize milling;
- Maize bran originating from dry white maize milling; and
- Screenings originating from dry white maize milling.

These samples were analysed for the same series of mycotoxins analysed in white maize products.

3.1.2.4. Fungi and mycotoxins in imported yellow maize

Samples were taken at 27 points (3 points across x 3 points along x 3 depths) of each cargo hold of all 83 shipments of USA maize and ARG maize arriving in South Africa between April 1992 and January 1993. Holds loaded slack, were sampled at 9 to 18 points, depending on the depth of maize in the hold. The samples were analysed in the Maize Board's laboratory for AFLA, FB₁, FB₂ and FB₃ and for infection by the major fungi. The ARG maize was assumed mainly to be of the 1992 crop. USA maize arriving in South Africa between April and the middle of October 1992 was assumed mainly to be of the 1991 crop. USA maize arriving here since the middle of October 1992 was assumed mainly to be of the 1992 crop. Mean levels of FBs and AFLA in the imported maize were compared statistically with those in RSA 1991 and 1992 maize.

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

A shipment of yellow RSA maize of the 1998 crop exported to Taiwan was sampled during outloading from the silos into railway trucks at the points of origin in South Africa prior to shipment, and again at the end-point distributors in Taiwan (Cronje *et al*, 1990; Cronje, 1993; Rheeder *et al* 1994). Most of the maize originated from silos in the E-Tvl production area, with 29% originating from the Pan silo alone. About 27% of the total shipment originated from silos in the W-Tvl production area. The samples were analysed for mycotoxins by the MRC, using HPLC. Surface-sterilized kernels were plated onto two different agar media and the fungal colonies identified.

3.1.3. Fumonisin in foreign maize food products

Reports in the literature of FBs levels in maize products intended for human food have been summarised by Marasas *et al* (1993) and Shephard *et al* (1996a).

3.2. An analysis of the 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

3.2.1. Estimated usage of commercial maize

The relationship between OC incidence and FB levels in maize in parts of South Africa other than the Transkei has not been reported on in the public literature. The existence of such a relationship was therefore investigated here to assist in formulating meaningful MTLs. This was done using OC incidence expressed as a percentage of all cancers within each area, of histologically diagnosed cases in black males, in different geographical areas of South Africa for 1990 and 1991 (Cancer Association, 2000; Sitas, 2002 – personal communications) together with estimated *F. verticillioides* infection rates and FB levels in commercial white maize used to manufacture the white maize products consumed in the various areas. For these estimates *F. verticillioides* infection rates and FB levels of white maize produced in the various production areas of South Africa as determined during the Maize Board surveys were used. Black males are the group with the highest OC incidence rates in South Africa.

The analysis is based on the following assumptions, which are considered to be reasonable:

- It was assumed that exposure of black males to FBs in South Africa takes place mainly through the consumption of commercial maize products;

- It was assumed that exposure over a long period is needed if an external factor such as FBs in staple foods was to contribute towards the development of OC. Since *F. verticillioides* infection rates and the natural FB contamination levels of maize vary considerably from year to year, it was considered reasonable to average the fungal infection rates and the total FBs content (FB₁+ FB₂+ FB₃) in each of the production areas over the six seasons.

The fumonisin content and the percentage *F. verticillioides* infected kernels of white maize used to manufacture the white maize products consumed in the various areas for which data on OC incidence are available, was estimated using the results of the surveys over six seasons (Tables 12 and 13) and Maize Board statistics of maize sold to commercial millers and white maize products sold by commercial millers in various regions of South Africa (Maize Board, 1995). First, the annual average white maize supply in each of the geographic areas was calculated using white maize production statistics for the 10-year period 1985/86 to 1994/95. To obtain a good estimate, the average for a relatively long production period was used because production varies considerably from year to year. Next, the annual average net quantities of white maize products sold by commercial millers in the various geographic areas were calculated per area for the period 1993/94 and 1994/95. It is believed that a good average estimate could be obtained by using statistics for only two years, because consumption of white maize varies little from year to year. Included in the list of maize products were super, special, sifted and unsifted maize meal, maize grits, samp and maize rice. The results of these calculations are given in Table 14.

Not all white maize produced in South Africa is used domestically, some being exported to neighbouring countries, such as Botswana, Swaziland, Namibia and Lesotho. The 'maize equivalent' of the white maize products manufactured in each of the geographic areas was estimated. First an 'extraction rate' was calculated from the total quantity of white maize the Maize Board sold to local millers and the total quantity of maize products sold by millers. This arrived at a figure of 86% i.e. from 100 kg of maize, 86 kg of maize product was manufactured. This is somewhat higher than the 75 – 80% extraction that maize millers in South Africa generally manage to achieve in white maize milling. Using this extraction rate, the total quantity of maize

consumed in each area was calculated and compared to the quantity of maize available from producers in the area. The results of these calculations are also presented in Table 14. Surpluses and shortfalls were made good on an arbitrary basis by assuming the most likely ‘imports’ and ‘exports’ to or from adjacent areas, based on the knowledge that the Maize Board operated a railage system that would ensure the lowest railage costs for the industry as a whole, but not necessarily for individual millers. This meant that not all the maize produced within areas where there was a shortfall was milled and consumed in that area and instead a substantial proportion could flow to shortfall areas further east – see Table 15. Thus the percentage kernels infected by *F. verticillioides* (Table 16) and the fumonisin content (Table 17) of the maize used to manufacture the white maize products consumed in each area was estimated from the proportions sourced from the various production areas and the mean total FB content observed in maize from the various production areas (Tables 12 and 13). For the Eastern Cape, where subsistence maize forms a significant part of the diet, three scenarios were calculated – see Table 17.

In these calculations white maize produced in all areas were taken into consideration, but since not all production areas were included in the surveys on fungi and mycotoxins, these data were not available for maize produced in the Western Cape (W-C), Eastern Cape (E-C), Northern Cape (N-C) and Northern Transvaal (N-Tvl) production areas. (Note that the ‘production areas’ existed long before new provinces were demarcated in 1994). To overcome this lack of data for the calculation of *F. verticillioides* infected kernels and fumonisin content of the maize consumed in relevant areas the averages of these figures for all areas were used. Since the quantities of maize involved in this way were comparatively very small, any possible discrepancies caused by this approach are likely to be small.

Table 12 - Percentage *F. verticillioides* infected kernels in commercial white maize in different maize production areas of South Africa during each of six crop years (two crop years for the PWV area)

Production area	1989	1990	1991	1992	1993	1994	Mean
N-OFS	18.4	13.5	6.0	9.0	28.0	19.0	15.7
E-OFS	2.6	3.5	1.4	4.0	8.0	6.0	4.3
Natal	9.2	19.5	9.0	11.0	18.0	16.0	13.8
W-Tvl	12.5	11.3	6.7	15.0	34.0	24.0	17.3
E-Tvl	7.2	5.2	6.3	6.0	15.0	12.0	8.6
PWV					25.0	16.0	20.5

Data from Kallmeyer *et al*, 1995; see also Section 4.1

Table 13 - Total fumonisin content (FB₁+FB₂+FB₃) (ng/g) of commercial white maize in different maize production areas of South Africa during each of six crop years (three crop years in the PWV area) (Extracted from Table 27)

Production area	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

Table 14 - Mean annual quantities of white maize products sold by millers in various geographic areas of South Africa, the estimated quantities of maize used for manufacturing the products and the estimated surplus or shortfall of white maize produced in the area

Area of consumption ¹	Production 10-year mean ²	Products sold 2-year mean ³	Maize equivalent of products sold ⁴	Maize surplus or shortfall ⁵
	(kt/year)			
W-Cape	1.5	42.4	49.5	-48.0
N-Cape	19.2	21.7	25.4	-6.2
E-Cape	22.3	201.0	234.5	-212.0
E-OFS	152.2	167.2	194.4	-42.2
N-OFS	1 493.3	188.8	220.3	1 210.0
Natal	119.0	527.0	614.9	-496.0
North-West	1 686.0	192.4	224.5	1 462.0
Limpopo	74.9	415.7	485.0	-410.0
Mpumalanga	392.8	245.8	286.8	106.0
Gauteng	152.4	464.1	541.5	-389.0
Total	4 113.6	2 298.9	2 682.3	1 174.0

¹ The areas of consumption are equivalent to the provinces that were delimited in 1994, except for E-OFS and N-OFS, which are both in the Free State Province

² The mean production is the annual mean calculated for the 10-year period 1984/85 – 1994/95

³ The figures represent the annual mean calculated for the 2-year period 1993/94 – 1994/95 for all white maize products manufactured by dry roller milling for human consumption, and sold in each of the geographic areas

⁴ The average quantities of white maize milled for domestic human consumption were calculated as the mean for each consumption area and are about 14% more than the quantity of maize product derived from the maize. This translates to an extraction rate of about 86%, which is 6 – 9 percentage points higher than the extraction rate actually achieved by large commercial mills. The reason for the discrepancy is not clear, but the estimates appear sufficiently accurate

⁵ Maize equivalent of products sold minus production

Table 15 - Estimated quantities of white maize sourced from the various production areas to manufacture the white maize products sold for human consumption in various geographic areas of South Africa

Area of consumption	Subsistence maize (kt)	Quantity of commercial maize sourced from various production areas for supply of white maize products (kt)										
		N-OFS	E-OFS	W-Tvl	E-Tvl	Natal	PWV	W-C	N-C	E-C	N-Tvl	Total
W-Cape		48.0						1.5				49.5
N-Cape		6.2							19.2			25.4
E-Cape ¹	0	50.4	63.0			99.0				22.3		234.7
	189.3 ²	50.4	63.0			99.0				22.3		424.0
	390.2 ³	50.4	63.0			99.0				22.3		624.9
E-OFS		100.0	62.0	32.0								194.0
N-OFS		25.9										25.9
Natal		435.0	106.0		53.0	20.0						614.0

N-West			224.5								224.5
Limpopo			310.0	100.0						75.1	485.1
Mpumalanga			100.0	187.0							287.0
Gauteng	168.0		168.0	53.0		152.4					541.4
Total	833.5	231.0	834.5	393.0	119.0	152.4	1.5	19.2	22.3	75.1	2 681.5

¹ See section 3.2.2

²The total quantity of white subsistence maize produced in 2000/2001, an above average crop year

³The quantity of subsistence maize required in addition to commercial maize to increase per capita consumption in the Eastern Cape to 316 g/70-kg person/day, if it is assumed that maize consumption in Transkei equals that in Mpumalanga, the highest in the rest of South Africa

Table 16 - Estimated percentage *F. verticillioides* infected kernels in commercial white maize used to manufacture the white maize products sold by millers in various geographic areas of South Africa

Area of consumption	Estimated contribution to % <i>F. verticillioides</i> infected kernels in maize sourced from each production area for manufacturing of white maize products										
	N-OFS	E-OFS	W-Tvl	E-Tvl	Natal	PWV	W-Cape	N-Cape	E-Cape	N-Tvl	Total
W-Cape	15.22						0.41				15.63
N-Cape	3.82							10.12			13.93
E-Cape	3.37	0.51			5.82				1.27		10.98
E-OFS	8.09	0.59	2.85								11.54
N-OFS	15.70										15.70
Natal	11.12	0.76		0.74	0.45						13.07
N-West			17.30								17.30

University of Pretoria etd – Viljoen, J H (2003)

Limpopo		11.06	1.77		2.07	14.90
Mpumalanga		6.03	5.60			11.63
Gauteng	4.87	5.37	0.84	5.77		16.85

Table 17 - Estimated total fumonisin content of commercial white maize used to manufacture the white maize products sold by millers in various geographic areas of South Africa, as well as in subsistence maize used in the Eastern Cape

Area of consumption	FBs contribution in subsistence maize (kt)	Contribution to total fumonisin content of commercial maize sourced from various production areas for manufacturing white maize products sold in different geographic areas (ng/g)										
		N-OFS	E-OFS	W-Tvl	E-Tvl	Natal	PWV	W-C	N-C	E-C	N-Tvl	Total
W-Cape		582						15				597
N-Cape		146							380			526
E-Cape ¹		129	68			205				48		450 ²
	541	129	68			205				48		991 ³
	1 211	129	68			205				48		1 661 ⁴
E-OFS		309	81	115								506
N-OFS		600										600

Natal	425	44	46	16	531
N-West		697			697
Limpopo		445	110		78 633
Mpumalanga		243	348		591
Gauteng	186	216	52	124	579

¹ See Section 3.2.2

²Total FBs in 234.7 kt of commercial maize (Tables 13 and 27)

³ Total FBs in 234.7 kt of commercial maize (Tables 13 and 27) and 189.3 kt subsistence maize (based on analyses of 18 samples of ‘healthy’ maize over 2 crop years – Rheeder *et al*, 1992)

⁴ Total FBs in 234.7 kt of commercial maize (Tables 13 and 27) and 390.2 kt subsistence maize (based on FB analyses of 18 samples of ‘healthy’ maize over 2 crop years – Rheeder *et al*, 1992) Incorporating subsistence maize in the Eastern Cape

Maize grown by the developing sector in South Africa is mainly for own use – referred to here as subsistence maize. The South African Department of Agriculture (2001 – URL <http://www.nda.agric.za/docs/Trends2001/trends.htm#Maize>) estimated production of subsistence maize in 2000/2001 at 258.124 kt: 189.299 kt of white maize and 68.825 kt of yellow maize. Estimated yield was approximately 0.5 t/ha. In comparison, the commercial maize crop for the 2000/01-production season was estimated at 7.193 Mt, with an estimated yield of 2.66 t/ha – substantially more than the average yield of just over 2.0 t/ha for the 10-year period 1986/87 – 1995/96. Annually, the South African population consumes a total of about 2.68 Mt of commercial white maize (Table 15). If the 189.299 kt white subsistence maize crop of 2000/2001 is taken as an average crop, subsistence maize forms about 6.5% of the total average quantity of white maize consumed by the South African population. However, the bulk of subsistence maize is produced in remote parts of the country, particularly the Transkei region of the Eastern Cape Province, and it forms an important part of the diet in this area. Accurate, detailed production data for subsistence maize per geographic area are not readily available, therefore an effort was made here to estimate the proportion that subsistence maize might form of total maize intake, and hence the fumonisin intake.

As a first step, the per capita consumption of commercial white maize by maize consumers was estimated by dividing the estimated quantities of white maize (from Table 15) used to manufacture commercial white maize products in different parts of the country by the maize consuming population in that area (Table 18). The maize consuming population was assumed to consist wholly of the population group ‘African/Black’ (1996 population census – URL: <http://www.statssa.gov.za/default3.asp>). The effect of this assumption is that the per capita maize consumption, and consequently the FBs intake is slightly overestimated. Next, the area with the highest per capita white maize consumption – 316 g/70-kg person/day in Mpumalanga, where it is thought that little subsistence maize is grown – was taken as the benchmark for the maximum per capita maize consumption. The per capita consumption of commercial maize in the Eastern Cape was subtracted from the figure for Mpumalanga on the assumption that in Transkei total consumption was similar to that in Mpumalanga and the difference between total consumption and

consumption of commercial maize was made up by usage of subsistence maize. Thus, in Transkei, on average an estimated 119 g of commercial maize is consumed, plus an estimated 197 g of subsistence maize/person/day, for a total of 316 g/70-kg person/day. This estimate for Mpumalanga and Transkei is considerably below the estimate of 460 g/70-kg person/day for rural consumers by Gelderblom *et al* (1996). However, the estimate involves a total amount of 390.2 kt of subsistence maize in Transkei alone, which outstrips by a considerable margin the 258.124 kt (total for white and yellow subsistence maize) produced in the country as a whole in an average year like 2000/2001. Therefore, as a third scenario, the total available quantity of 189.3 kt of white subsistence maize was taken into account (see Tables 16 and 17).

While our estimates of maize consumption in rural areas are substantially lower than that of Gelderblom *et al* (1996), our estimate of per capita consumption in Gauteng, an urban environment, is 290 g/70-kg person/day, slightly higher than the 276 g/70-kg person/day estimate by Gelderblom *et al* (1996). Corrected for the 86% extraction rate we worked on, our estimate for consumption of maize product in Gauteng is 247 g/70-kg person/day.

A similar procedure was not followed for other parts of the country for incorporating subsistence maize in per capita consumption estimates. It is thought more likely that the bulk of the shortfall compared to maize consumption in Mpumalanga is made up by other starchy foods such as bread, rice and potatoes, rather than by subsistence maize. This is certainly true for metropolitan areas such as Gauteng, where subsistence maize grown around townships is exclusively consumed as a vegetable, similar to sweet corn.

Finally, three scenarios for the FBs levels in maize consumed in EasternCape were calculated (Table 17), firstly, based on commercial maize only, secondly, based on maize consumption of 234.7 kt commercial, as well as 189.3 kt subsistence maize, and thirdly based on maize consumption of 234.7 kt commercial, as well as 390.2 kt subsistence maize to the ratio of 116:197 g/70-kg person/day. A total FBs content of 1.94 mg/kg in 'healthy' subsistence maize determined in 18 samples over two crop years was used – see Section 4.6.3.2.2. These data were used in correlations of

estimated FBs in maize, with incidence of OC, liver, kidney and brain cancer in different areas of South Africa.

Table 18 - Estimated per capita consumption of commercial white maize in various geographical areas of South Africa

Geographic area	Commercial maize used (kt/yr)¹	Maize consumers (millions)²	Maize consumption (g/person/day)³
W-Cape	49.5	0.827	164
N-Cape	25.4	0.277	251
E-Cape	234.7	5.418	119 ⁴
Free state	219.9	2.184	276
KwaZulu-Natal	614.0	6.888	244
N-West	224.5	3.003	205
Limpopo	485.1	4.704	283
Mpumalanga	287.0	2.492	316
Gauteng	541.4	5.110	290
Total	2681.5	30.903	238

¹ From Table 15

² 1996 population census - URL: <http://www.statssa.gov.za/default3.asp>

³ The quantity of maize products manufactured from the maize is 86% of the maize quantity indicated

⁴ This figure does not include home grown subsistence maize, which forms a substantial proportion of maize consumed in the E-Cape in particular

3.3. The correlation of oesophageal cancer rates and maize supply in some African countries

The very large differences between OC rates in African countries (see Section 2.3.3) are particularly interesting and have been analysed further. Few data are available on mycotoxin levels in cereals in any African country besides South Africa. In western Cameroon, Ngoko *et al* (2001) assessed the fungal incidence and mycotoxin contamination of farm-stored maize (assumedly non-commercial subsistence farms) and compared grain samples from three villages each in two agroecological zones over time. Maize samples were collected at 2 and 4 months after stocking from 72 farmers' stores in 1996 and 1997 in the Humid Forest and Western Highlands of Cameroon. Of the fungi found in 1996, *Nigrospora* spp. were the most prevalent in both the Humid Forest (32%) and Western Highlands (30%) area. *F. verticillioides* (22%) and *F. graminearum* (27%) were also isolated from these samples. In 1996, no significant difference in fungal incidence was found among villages in the Western Highlands for samples collected 2 months after harvest, but at 4 months incidence was significantly higher.

However, the annual supply of sorghum, millet and maize per capita per year was obtained (FAO, 2000) over the 4 years 1987 to 1990 for each of 23 African countries, and the annual average calculated as a rough estimate of consumption. The OC rates in males and females (ASIR, world population, per 100 000) in each of the countries were also obtained (Ferlay *et al*, 1999). The correlations between OC rates and the various grain supplies were calculated on the assumption that supply is related to consumption of each of the cereals in each of the countries.

3.4. Incidence of liver, kidney and brain cancers in Africa in relation to grain consumption, and in SA in relation to the occurrence of fumonisins in maize

3.4.1. Preamble

In Section 4 (Results and Discussion), it is shown that only three mycotoxins occur regularly or are likely to occur regularly at levels that are, or could be, significant for human or animal health in locally produced, and/or imported commercial wheat and maize, and possibly in grain sorghum as well. These are AFLA, FBs and DON. AFLA rarely occur in locally produced grain, but are an important contaminant in imported ARG and USA maize. FBs are ubiquitous in imported, as well as locally produced maize, and are possibly significant in grain sorghum. DON occurs in locally produced and probably also in imported maize, and can reach significant levels in ARG, USA and Canadian wheat. There is paucity of public data on its occurrence in Australian wheat, which is often imported to South Africa, but it seems likely to occur in Australian wheat, particularly wheat from areas that receive rain during harvest time, like northern New South Wales and southern Queensland. It is probably also present at significant levels in locally produced wheat and grain sorghum, particularly in years when scab, or head blight is prevalent.

As shown in Section 2.5.2, AFLA are acutely toxic to animals as well as humans and, in spite of some contradictory evidence, there is substantial evidence that it is an important aetiological factor in liver cancer in humans. The role of AFLA in human and animal health is therefore clear and consequently, most countries maintain regulatory MTLs in the low ng/g's range for AFLA in food and feed (see Section 2.1.2 for details).

Relatively little is known about the human health effects of DON, but there is consensus that DON is one of the least acutely toxic trichothecenes to animals (see Section 2.5.4). There is no evidence of chronic intoxication of humans or animals by DON and DON appears not to be carcinogenic. In spite of the gaps in toxicological knowledge about DON, there is relatively little concern from toxicological and

epidemiological points of view about its effects on human health. The main concern about DON springs from the regularity of its occurrence in various grains, at levels that are known to affect animals. In a few countries where DON in staples may regularly reach $\mu\text{g/g}$ levels, regulatory MTLs in the high ng/g 's, or low $\mu\text{g/g}$ range for DON are maintained (Section 2.1.4).

A comparatively large body of knowledge is available on the toxicology of FBs in animals (Section 2.5.3). FBs are acutely toxic to horses at dietary levels around 8 to 10 $\mu\text{g/g}$ fed over some weeks. Many fatal cases of LEM in horses caused by FBs in the field occurred sporadically over the last 100 years. FBs occasionally occur in apparently sound commercial grain at levels that can seriously affect horses. The FDA recently adopted a guidance level of 1 $\mu\text{g/g}$ in horse rations.

FBs are also acutely toxic to pigs at dietary levels around 50 to 90 $\mu\text{g/g}$, causing many outbreaks of porcine pulmonary oedema in the field in the USA. It is highly unlikely that grain would still appear sound and healthy when it contains FBs at these levels. The FDA adopted a guidance level of 10 $\mu\text{g/g}$ in the total ration for pigs (see the FDA's Centre for Veterinary Medicine's 'Background Paper in Support of Fumonisin Levels in Animal Feed' - Section 2.5.3.1).

No cases of acute intoxication by FBs have been reported for other farm animals. In male rats FBs fed over an extended period at a dietary level greater than 50 $\mu\text{g/g}$ cause liver and kidney cancer, and liver cancer in female mice.

In all animals, damage to the liver and the kidneys was evident, and in horses the brain tissue is damaged by FBs. These appear to be the main organ loci damaged by FBs in animals.

There is no direct evidence of acute or chronic intoxication of humans by FBs. FBs are ubiquitous in maize and most maize contains some FBs. In countries where maize is a staple, humans are constantly ingesting FBs at dietary levels ranging from near zero to around 4 or 5 $\mu\text{g/g}$ – see Sections 2.5.3.4, 4.1.1, 4.1.2, 4.1.4, 4.1.5, and 4.1.6. Based on the main loci of damage in animals, the correlation between the estimated FBs content of white maize consumed in various parts of South Africa, and the incidence of liver, kidney and brain cancer in black males in the different areas have been calculated as a further attempt to elucidate the possible chronic effects of FBs in

humans. In addition, the per capita maize, sorghum and millet supply (as a rough estimate of consumption) in 23 African countries have been correlated with the incidence of liver, kidney and brain cancer in males and females in these countries.

3.4.2. 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

On the same basis as has been done in Section 3.2 with regard to OC, the correlation between liver, kidney and brain cancer incidence in black males and estimated FB levels in white maize consumed in different geographic parts of South Africa was calculated. The incidence of histologically diagnosed cases of liver, kidney and brain cancer in black males, in different geographical areas of South Africa for 1990 and 1991 were obtained from the Cancer Association of South Africa (Cancer Information Service, 2000 - Personal communication). These data were then correlated with available data on the *F. verticillioides* infection rates and FB levels in commercial white maize in the different maize production areas of South Africa (Table 38).

3.4.3. Correlation of liver, kidney and brain cancer rates in males and females with grain supplies in other African countries

There are large differences between liver, kidney and brain cancer rates in African countries. Little data are available on mycotoxin levels in cereals in African countries other than South Africa, however, Table 19 gives the average supply of sorghum, millet and maize per capita per year (calculated over the 4 years 1987 to 1990) in each of 23 African countries. The cancer rates for each of the three cancers in males and females (ASIR, world population, per 100 000) in each of the countries were obtained (Ferlay *et al*, 1999). The correlation between cancer rates and grain supplies were calculated on the assumption that supply is related to consumption of each of the cereals in each of the countries, and that intake of FBs is related to maize consumption.

Table 19 - The average supply of sorghum, millet and maize in kg per capita per year¹ (calculated over the 4 years 1987 to 1990) in each of 23 African countries², and the cancer rates (ASIR world population per 100 000 per year) in males and females³ in each of the countries

Country	Brain		Kidney		Liver		Maize	Sorghum	Millet
	F	M	F	M	F	M			
Algeria	2.25	4.86	0.95	1.09	0.98	1.54	1.00	0.1	0.00
Angola	0.12	0.14	0.55	0.27	4.3	6.66	29.0	0.0	5.65
Belize	4.56	5.78	2.76	3.94	3.98	5.36	23.8	0.0	0.00
Benin	0.67	1.59	0.96	1.57	6.67	22.15	58.9	18.0	3.03
Botswana	1.08	1.35	0.73	0.65	6.54	18.07	57.2	39.6	1.13
Burkina Faso	0.67	1.59	0.96	1.57	6.67	22.15	22.6	88.3	69.60
Burundi	0.46	0.84	0.76	1.5	5.27	17.25	29.4	1.7	0.55
Gambia	0	0	0.37	0.32	9.57	30.4	10.0	8.1	42.30
Ghana	0.67	1.59	0.96	1.57	6.67	22.15	34.1	8.0	7.40
Malawi	0	0.62	0.11	0.67	4.62	13.23	151.0	1.0	1.10
Mali	0.18	0.47	1.7	1.47	17.0	47.98	20.8	54.4	81.93
Morocco	2.36	2.93	0.33	2.49	2.11	5.99	16.4	0.9	0.18
Mozambique	0.46	0.84	0.76	1.5	5.27	17.25	40.0	10.8	0.30
Namibia	0.18	0.08	1.31	1.95	2.53	7.66	42.6	4.3	36.20
Niger	0.11	0.07	1.91	3.19	10.4	27.22	1.5	43.8	155.5
Nigeria	0.59	1.86	0.85	1.46	3.96	16.79	30.7	43.1	35.9

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Rwanda	0.28	0.22	0.28	0.62	10.6	35.9	13.9	18.2	0.10
South Africa	1.73	2.51	1.8	2.89	6.74	20.53	97.9	3.6	0.15
Swaziland	0	0.45	0.91	0.23	6.35	26.09	32.6	1.0	0.00
Tanzania	0.18	1.03	1.18	1.07	4.62	15.89	82.5	8.7	4.50
Uganda	0.32	0.42	1.69	0.87	3.43	9.18	18.0	6.3	22.83
Zambia	0.12	0.16	0.32	1.91	8.35	23.02	153.7	3.0	1.40
Zimbabwe	3.4	3.35	1.32	1.98	14.9	28.87	116.4	6.5	10.25

¹Per 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. 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

3.5. The epidemiology of neural tube defects (NTD) in relation to the occurrence of fumonisins in maize and maize products

3.5.1. What is an NTD and what causes it?

– after <http://orpheus.ucsd.edu/otis/Hyperthermia.html#h4> accessed October 2000.

The neural tube is the spine and the skull, surrounding and protecting the spinal cord and brain. Neural tube defects occur when the spine or skull does not close properly around the nerve tissue during early foetal development. This closure is normally completed by the beginning of the 6th week of pregnancy. Once closed, the neural tube does not reopen. This implies that there is only a limited period that any cause of NTD can have an effect.

An opening in the spinal column is called spina bifida, while an open skull defect is called anencephaly. The majority of infants with spina bifida grow to adulthood, but infants with anencephaly have a severely underdeveloped brain and usually die at, or shortly after birth. Normally, about 10 to 20 out of every 10 000 births has a neural tube defect, but the figure can vary considerably with time and place. The severity of the defect can also vary considerably.

Increased body temperature of pregnant women, such as fever caused by illness, exceeding 101°F for an extended period of time during the first 6 weeks of pregnancy, is one of several risk factors for NTD. Another known risk factor is folic acid deficiency in the diet of pregnant women and in many countries pregnant women receive supplemental folic acid as part of their health care during pregnancy.

Hardness of drinking water and consumption of potato affected by blight have been put forward as possible aetiological factors for spina bifida, but these have not been proven. High fluoride content in the diet has also been linked to increased incidence of NTD. A genetic predisposition, based on the strong ethnic predisposition is an additional factor being investigated. The aetiology of NTD is clearly multifactorial and as an additional possible causative factor, a possible link between high FB levels

in maize and a cluster of NTD in neonates delivered by Mexican-American women who conceived in the Lower Rio Grande Valley, has been put forward (Hendricks, 1999).

3.5.2. An epidemiological interpretation of the possible relationship of NTD in South Africa and elsewhere with fumonisin intake

Whereas the possible cancer initiating and cancer promoting effects of FBs in humans are likely to be the result of long term exposure, any possible effect with regard to NTD is likely to be caused by short term exposure during the critical stage of pregnancy with regard to NTD. Therefore, if FB contamination of food is a cause, it is likely that there should be a direct and immediate link between cause and effect. To investigate a possible relationship between FB intake and the incidence of NTD, the PDI of FBs in various areas were estimated and correlated with NTD incidence at the time, in those areas. First, the average FB content of white maize products in the 1990/91 and 1991/92 marketing years were calculated from the data in Tables 28 and 29. Next, published data from studies at four localities in South Africa (Delpont *et al*, 1995; Venter *et al*, 1995) and at two different times in the southern USA (Hendricks, 1996) were used to compile a data set on which the correlation analysis was performed.

3.6. Estimated DON content of white maize consumed in SA

The same procedure described in Section 3.2 was applied to estimate the DON content of white maize used to manufacture white maize products for domestic consumption in South Africa, and the PDI of DON through white maize (Tables 20 and 21).

Table 20 - Estimated DON content of commercial white maize used to manufacture the white maize products sold by millers in various geographic areas of South Africa, as well as in subsistence maize used in the Eastern Cape

Area of consumption	Contribution to total DON content of commercial maize sourced from various production areas for manufacturing white maize products sold in different geographic areas (ng/g)										
	N-OFS	E-OFS	W-Tvl	E-Tvl	Natal	PWV	W-Cape	N-Cape	E-Cape	N-Tvl	Total
W-Cape	215.5						6.6				222.0
N-Cape	54.0							164.3			218.3
E-Cape	47.7	30.0			136.9			20.6			235.2
E-OFS	114.5	35.7	56.4								206.6
N-OFS	222.2										222.2
KwaZulu-Natal	157.4	19.3		15.9	10.6						203.1
N-West			341.8								341.8
Limpopo			218.4	37.9						33.6	290.0

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Mpumalanga		119.1	119.9			239.0
Gauteng	69.0	106.1	18.0	33.2		226.2

Table 21 - Estimated PDI of DON through commercial white maize used to manufacture white maize products for domestic consumption in SA

Area	DON ¹	Consumption ²	PDI ³	PDI ⁴
E-Cape	235	316	1.06	74.2
E-OFS	207	276	0.82	57.4
N-OFS	222	276	0.87	60.9
Gauteng	226	290	0.87	60.9
KwaZulu-Natal	203	244	0.71	49.7
Mpumalanga	239	316	1.08	75.6
N-Cape	218	251	0.78	54.6
Limpopo	290	283	1.17	81.9
N-West	342	205	1.00	70.0
W-Cape	222	164	0.52	36.4

¹ DON content of white maize (ng/g) – calculated from Tables 15 and 20

² maize consumption in g/person/day - See Table 18 and Sections 3.2.1. and 3.2.2.

³ Estimated probable daily intake of DON (ng/g body weight/day) through maize. The figure has not been corrected for mycotoxin losses during commercial milling, hence this is an overestimation

⁴ Estimated probable daily intake of DON (µg/70-kg person/day) through maize, not corrected for mycotoxin losses during commercial milling

3.7. Estimating the highest MTLs that can be allowed in SA for selected mycotoxins, without jeopardizing the safety of consumers

3.7.1. The rationale for estimating realistic MTLs for mycotoxins

The need for regulatory control measures and the actual limits set for mycotoxins in food were estimated by applying the following procedure, which was based on Kuiper-Goodman (1994; 1995; 1999) and Miller Jones (1992):

3.7.1.1. Determining the need for a control measure on the basis of a human exposure assessment

This consists of the following:

- An estimate of the direct intake of mycotoxins;
- An estimate of indirect intake through animal products from animals that were fed mycotoxin contaminated feeds;
- An estimate of food intake and the PDI of the mycotoxin under consideration;
- An estimate of absorption of mycotoxins in the human gut;
- Evidence of the mycotoxin in human tissue (blood, urine etc) or other physiological evidence of exposure (biomarkers).

Once a need to reduce human exposure has been recognized, the next step is to determine what measures are needed to achieve this. This strongly depends on the hazard the exposure poses to human health; hence a hazard assessment to human health was carried out next.

3.7.1.2. Assessment of the hazards to human health that a mycotoxin poses

This consists of:

- An assessment of the toxicological effects on humans, experimental and farm animals;
- An epidemiological assessment of possible effects on humans including the effects, as well as the absence of effects where humans have been exposed.
- Other considerations concerning social aspects, trade and industry, including:
 - Existing regulations of international trading partners;
 - The effect of an MTL on commercial interests; and
 - The effect of an MTL on sufficiency of food supply.

Based on this rationale, the background information overviewed in Section 2 of this thesis and the results of our own analyses presented in Section 4 are applied to formulate proposals for MTLs for AFLA, FBs and DON in cereal grains in South Africa.

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

A basis for compliance to MTLs for mycotoxins in cereal grains is proposed, based on practical considerations with regard to where and when samples can be obtained during normal handling procedures for grain and grain products.

3.8. Estimation of the possible implications of MTLs for mycotoxins in SA and major grain trading partners on international trade in grains and grain products

Possible implications of the existence of MTLs for mycotoxins in grain and grain products in SA with regard to international trade were considered in the following general contexts:

- The advantages and disadvantages to trading partners of having MTLs for mycotoxins in grain;
- The difficulty of harmonization between trading partners;
- The effects of MTLs on desirability of grain from specific sources and on price;
- The need for, and cost of testing, supervision and control with specific reference to the elevated cost of imported grain able to meet local MTLs.

Implications of the existence of specific MTLs for AFLA, FBs and DON in grain and grain products in SA with regard to international trade were also considered in the following contexts:

- Implications for South African millers of the currently existing MTLs or recommended MTLs;
- Implications for millers of the MTLs for AFLA, FBs and DON newly proposed in the current study with regard to:
- Availability of grain supplies capable of meeting the proposed MTLs;
- Utilisation of grain that does not meet MTLs.

3.9. Formulating a proposal for the practical application of MTLs for mycotoxins in cereal grains

3.9.1. Overview of analytical tests for mycotoxins in grain

The various qualitative and quantitative tests available for testing for mycotoxins in cereal grains were briefly reviewed, from the point of view of their suitability for use during normal grain handling for storage, trading and milling, as well as their relative cost. Several commercially available tests considered suitable for use under practical industrial conditions were reviewed in more detail with regard to the basis of the test, available packaging, facilities and equipment required and the cost of test kits. The infrastructure and labour required for on-site immunoaffinity testing of grain for mycotoxins were also considered against the background of normal practical conditions in the grain industry.

3.9.2. Formulating proposals for sampling methods and sample preparation to be adopted together with MTLs for aflatoxins, fumonisins and deoxynivalenol

Sampling of grain and grain products is overviewed in general, followed by considering sampling for mycotoxins in specific situations in the grains and milling industries in South Africa. The following specific sampling situations are covered:

- Sampling from bulk rail or road trucks;
- Sampling bulk grain in silo bins and ships holds;
- Sampling from a grain conveyor;
- Sampling bagged grain;
- Sampling packaged products in stacks.

This is followed by considering the procedure for sample preparation.

3.9.3. Practical execution of a sampling and testing program on grain and grain products for compliance to MTLs for aflatoxins, fumonisins and deoxynivalenol

The factors that play a role, and the advantages and disadvantages of various options that could be considered for executing routine testing of grain and grain products for compliance to the proposed MTLs for AFLA, FBs and DON are put forward, and the relative costs are discussed. The options considered are:

- Routine testing at harvest intake;
- Routine testing after harvest intake;
- Sampling and testing of truckloads of grain on dispatch to mills; and
- Sampling and testing of individual silo bins before grain is outloaded.

3.10. Possible implications of MTLs for mycotoxins in SA and major grain trading partners on international trade in grains and grain products

The implications of MTLs for mycotoxins in SA and major grain trading partners on international trade in grains and grain products are considered in the context of general and specific considerations. General implications discussed include:

- The advantages and disadvantages for grain importers and exporters of having official MTLs for mycotoxins in grain;
- Difficulties of harmonizing MTLs between countries;
- Effects of MTLs on desirability of grain from specific sources and on price;
- The need for, and cost of testing, supervision and control.

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Specific implications for millers in South Africa are discussed with regard to AFLA, FBs and DON in respect of existing or recommended MTLs and the MTLs proposed in this study and the occurrence of these mycotoxins in domestic and imported cereal grains in South Africa.