5. Conclusions

The conclusions formed through the course of this study are presented in terms of the 12 objectives listed in Section 1.4.

5.1. Existing regulatory, advisory and recommended MTLs for mycotoxins in grain and grain products in various countries

Most of the existing regulations concern AFLA. All 77 countries with mycotoxin regulations have tolerances for AFLA in grains, foods, and/or feeds. Of these, only eight are African countries, leaving about 40 countries in Africa ostensibly without mycotoxin regulation. Except in well-developed countries, it is unlikely that existing MTLs for mycotoxins are routinely enforced.

In the USA, the FDA has set so-called 'action levels' for AFLA in grain, food and feed, and these appear to be enforced through regular monitoring. However, the FDA has no direct jurisdiction over intra-State traded grains and export grains, and at least in Texas contradictory practices are allowed for intra-State traded grains and export grains.

Switzerland has enacted a regulatory MTL of 1 000 ng/g for FBs in maize products and in the USA there are guidance levels of 2 to 4 µg/g (2 000 to 4 000 ng/g) for FBs in foods. Guidance levels in feeds, from 1 µg/g in feed for horses and donkeys to 50 µg/g in feed for poultry raised for slaughter, have also recently been published in the USA. In South Africa, an MTL of 100 to 200 ng/g has been recommended by the MRC for FBs in maize. Throughout this report, this is referred to as the 'recommended level' for RSA maize and maize products. The average FB level in maize products for human consumption was between about 200 and 1 000 ng/g total FBs in different white maize products over several years in the early 1990's in South Africa.

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. 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.

Five countries have also enacted MTLs for ZEA in food, ranging from 30 to 1 000 ng/g. No country has MTLs for ZEA in feeds or feedstuffs. ZEA is rarely found in South African white maize and white maize products for human consumption and if present, it is at insignificant levels.

Russia has an MTL of 100 ng/g for T-2 in cereals for food, and Israel and Canada respectively have MTLs for T-2 and the closely related HT-2 in feeds, ranging from 25 to 100 ng/g. Israel is the only country with an MTL of 1 000 ng/g for DAS in animal feeds. No country has MTLs for NIV, MON, or AME.

Eleven countries have MTLs for OA in cereals, legumes, coffee beans and pig kidneys, and twelve, including South Africa, also for PAT in apples, apple juice and related food products. None of these mycotoxins is found in RSA maize.

It is clear that, for a given substance such as AFLA, or FBs, there is no consistent rationale for setting limits, or for enforcement of control, in different countries. In fact, earlier surveys have indicated that regulatory levels are often set without good scientific evaluation of the need for them, or of the tolerance level at which the regulation is introduced. It is clear that in many countries, particularly developing countries including South Africa, MTLs for mycotoxins in grain and grain products are not enforced on a routine basis and their existence is often little more than an empty gesture. In developed countries like the USA, some routine enforcement appears to take place. However, the federal authorities have limited jurisdiction, and state authorities apply contradictory regulations and actions to intrastate traded grain and export grain. This makes unclear the outcome of problem situations and leaves many gaps for ‘unlawful’ actions.

5.2. The groups of carcinogens of the IARC and mycotoxins considered carcinogens

The IARC of the WHO and the National Toxicology Program of the FDA, classify substances and activities known and suspected to be carcinogenic in humans into four

categories. Group 1 - confirmed human carcinogens; Group 2A – probable human carcinogens; Group 2B – possible human carcinogen and Group 3 – suspected human carcinogen.

However, these classifications do not attempt to portray the risk of causing cancer by any of the substances.

Health authorities worldwide have clearly not considered the fact that any of these substances or activities having become listed as a carcinogen in any of the Groups by itself as sufficient reason to impose regulatory limitations on them. Many listed carcinogens, e.g. alcohol (a Group 1 carcinogen) is consumed without any regulatory health restriction whatsoever. The same applies for a substantial list of substances and activities in the other categories.

Of the mycotoxins, AFLA are listed as a Group 1 carcinogen and 'toxins derived from *Fusarium verticillioides*' (possibly FB_1 and FB_2), Fusarin C, OA and sterigmatocystin are listed as Group 2B carcinogens (possible human carcinogens) (IARC, 1993). Recently, IARC (2002) also evaluated FB_1 as Group 2B.

When tolerance limits for human foods are calculated from toxicological data on experimental animals, JECFA usually applies a safety factor of 100 to 1 000 is for toxins, and 1 000 to 5 000 for carcinogens. The main reason for this difference is that the toxic effects can be more clearly defined by means of toxicological studies on animals, than the carcinogenic effects. Hence, a larger safety factor is applied to carcinogens to compensate for the greater uncertainty. Epidemiological evidence of the risk carcinogens pose to humans is not taken into consideration during the JECFA risk assessment procedure.

5.3. An overview of the relationship between fumonisins and oesophageal cancer

OC became a focus of study in South Africa after a high incidence of the disease was reported in the East London area in the 1950's. An 'epicenter' of high incidence was subsequently found in the Butterworth/Centane area, with comparatively low incidence rates in the Lusikisiki and Bizana area. Investigations on the disease in

South Africa focused almost exclusively on the Transkei and apart from incidence rates, comparatively little attention was given to the occurrence of the disease in other parts of the country.

Many factors have been investigated as possible causes of OC, several of whom showed a relationship with OC incidence. In 1971, a relationship between OC and the brewing of traditional beer from maize was found. At about this time, investigations were being renewed on the relationship between maize infected by *F. verticillioides* and a neurotoxic condition in horses. This lead to the investigation of a possible relationship of the fungal infections of maize produced by subsistence farmers in the Transkei and their associated mycotoxins, with OC incidence.

Several surveys were conducted in the course of this investigation. The procedure applied was to collect maize ears from the storage cribs or huts of subsistence farmers in areas with high and low OC incidences in the Transkei and to examine these for the fungal species infecting the maize. Samples were collected in six seasons (1976, 1977, 1979, 1985, 1986 and 1989) over the period of 1976-1989. Reportedly, farmers store apparently mould-free and visibly mouldy maize ears separately. Maize apparently free of mould is used as food, while visibly mouldy maize is used as animal feed and for brewing beer. As a rule, a single ear each of mouldy and apparently mould-free maize was taken at random from each of a number of households in the high, as well as in the low OC incidence areas. The possibility of unintentional bias in the sampling cannot be excluded.

Fungal infection rates by various fungal species were generally higher in the mouldy maize from the high OC incidence area than in the low incidence area. In the 'good' maize intended for food, the differences were less frequently statistically significant. In the 1985 samples (from 12 households in each of the high, and low OC incidence areas), the levels of various mycotoxins were also tested. Higher levels of DON, NIV, ZEA and MON were found in the low OC incidence area than in the high incidence area. T-2 and DAS were not found.

The most consistent difference in the mycoflora of maize from the high and low OC incidence areas was a significantly higher infection rate of *F. verticillioides* in maize from the high-incidence area. In the 1989 samples for example, the *F. verticillioides*

infection rate of maize kernels in the high and low OC incidence areas was 41.2 and 8.9%, respectively (significant at $P<0.01$), in good (apparently free of mould) maize, and 61.7 and 21.4% respectively, in visibly mouldy maize. The *F. verticillioides* infection rates of commercial maize kernels in South Africa is similar to those in the low OC incidence area of the Transkei (range 1% to 34% over the 5 seasons from 1990 to 1994, and in 1975.

The mycotoxins produced by *F. verticillioides* were chemically characterized in 1988, and the maize samples collected in the Transkei in 1985 and 1989 were analysed for the presence of FB_1 and FB_2. These two are the most abundant of at least 4 FBs naturally produced by *F. verticillioides*. Significantly higher levels of FB_1 and FB_2 were present in the samples of mouldy maize from the high OC incidence areas in both years. In 'good' maize, FB levels were significantly higher in the high OC incidence area in 1985, but not in 1989 samples. It should be noted that the number of samples is small – only 12 households in 1985 and 8 in 1989 in each of the high and low incidence areas were sampled.

Based on these results, a statistical correlation was demonstrated in the Transkei between the *F. verticillioides* infection rates and the FB levels in subsistence maize respectively on the one hand, and OC incidence on the other. This was echoed by similar findings during surveys carried out along similar lines in the LinXian area of China. These findings remain circumstantial since no direct connection between FB intake and the development of OC has yet been demonstrated. Nevertheless, it is concluded that the similarity of the findings in two areas so far apart imply that:

- Relatively high levels of FBs in maize can lead to, or can contribute towards, a high incidence of OC;
- Conversely, the relative absence of FBs in maize products can lead to a low incidence of OC, or helps to prevent development of OC; and
- A similar relationship between FBs in maize products and OC incidence could be expected in the rest of South Africa, where the lifestyle and eating habits of the population are similar to those of Transkeians. (The recommended MTL for FBs in commercial maize products in South Africa (see Section 2.1.3.3) must at least be partly

based on a similar premise, since no other specific health effect in humans caused by FBs appears to be suspected at present.

The relationship between OC incidence and FB levels in maize in parts of South Africa outside the Transkei has, however, not been studied. In the absence of ready data, an effort has been made here to obtain an indication of the existence or not of such a relationship. Based on assumptions considered to be reasonable, this was done using OC incidence rates in black males in different geographical areas of South Africa, and available data on *F. verticillioides* infection rates and FB levels in commercial white maize in the different maize production areas of South Africa.

A significant correlation was found between kernel infection rates with *F. verticillioides* and the FB content of the maize. No significant correlation was found between OC incidence and the estimated kernel infection rates of commercial maize consumed in the various areas, nor between OC incidence and the estimated FB content of commercial white maize consumed in the various areas. The trend between OC rates and the estimated long-term average FB content of commercial maize was negative. It was therefore concluded that in the data analysed:

- Fungal infection rates with *F. verticillioides* gave an indication of the levels of FBs in commercial white maize produced in South Africa; and

- There exists no 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.

This is in direct contrast with the findings in the Transkei and it is therefore considered essential that further studies on the possible health effects on humans of FBs in commercial maize be conducted before potentially disruptive MTLs could possibly be considered. So far, the MRC has not taken up the lead of the statistical relationship to conduct a fully-fledged epidemiological study of the role of FBs in the aetiology of OC.

OC incidence rates are available for 174 countries and regions of the world. It appears that:

- There is a higher rate of OC in less developed regions;
- The highest rates of OC occur in remote, isolated areas;
- In Africa, very low rates occur in northern and western Africa, and very high rates in eastern and southern Africa;
- High OC incidence rates occur in widely different regions with reference to lifestyle and staple foods;
- There are large differences in OC incidence rates between countries where maize is a staple;
- There is large variation in the M/F ratio of OC incidence, but in most countries OC in males predominates.
- There appears to be an ethnic predisposition in widely different countries.

The correlation of the peculiar distribution of OC in Africa with supply of the staple foods maize, sorghum and millet (as a rough estimation of consumption) was calculated using data for 23 African countries. A highly significant correlation between OC incidence in males and females, and maize supply was found, but no correlation was found with the other two grains. Thus, there appears to be a statistical relationship between maize consumption and OC incidence in Africa.

5.4. Overview of factors other than fumonisins implicated in oesophageal cancer

In addition to mycotoxins and fungi, many other factors are implicated in the aetiology of cancer in general and OC in particular. Of the many factors that have been investigated, nitrosamines (of which various can occur in some alcoholic beverages, tobacco, and in certain plants and foods) stand out as the only direct causative agent of several cancers, including OC. However, not in the Transkei, nor

in other high OC incidence areas, has a clear epidemiological link between the occurrence of nitrosamines in the environment and the geographic distribution of OC cases been demonstrated. Even in the case of potent OC carcinogens such as certain nitrosamines, it is clear that a whole array of other factors is also involved. Many of these interact with one another in intricate ways. These include folic acid deficiency, vitamin A, smoking and chewing of tobacco, alcohol use, gastro-oesophageal reflux, and deficiency of vitamins and minerals such as zinc, magnesium and selenium. As a simplified example of some of the possible interactions, folic acid deficiency can be caused by low dietary intake, it can be decreased in the body by alcohol use and smoking and possibly by intervention of FB_1 in the folate uptake. Alcohol use prolongs the presence and promotes the entry of carcinogenic substances in the oesophagus and excessive alcohol use promotes gastro-oesophageal reflux, causing acid burns in the oesophagus, which renders the oesophagus vulnerable to tumor development, particularly if certain nitrosamines are also present. To explain the peculiar distribution of OC, it seems likely that at least one other key factor is required, together with exposure to nitrosamines.

There is a decided ethnicity in the predisposition to many cancers, including OC. The results of work in China suggested a major locus underlying susceptibility to OC with sex-specific penetrance, which could explain the observed geographic, sexual, and ethnic distribution patterns of OC. Several genetic links with the development of cancer in general, and OC in particular, have been found so far, including cytochromatic factors and tumor repressor genes.

It is concluded that human OC aetiology has an intricately complex structure in which genetic predisposition and exposure to nitrosamines are probably the key factors. Other factors, including a possible role of mycotoxins, are secondary. Therefore, a simple solution, such as an MTL for FBs in maize products has little chance of being effective. Such a measure would be aimed at only one of several possible secondary aetiological factors. The side effects of such a measure on other sectors of the society and the economy must therefore be carefully considered before it is introduced to solve the OC problem amongst certain groups of the population. The issue of other possible health effects caused by FBs and other mycotoxins in humans must be considered separately from the issue of OC.

5.5. Overview of the toxicology of the mycotoxins covered in this study

Worldwide, the limits on mouldy kernels in the grading systems applicable to commercial grain restrict to a considerable extent the levels of mycotoxins that can be present in commercial grain. Consequently, the high levels of mycotoxins found in maize produced on subsistence farms are highly unlikely to ever occur in commercial grain. Wherever humans or animals have been poisoned by mycotoxins, it has never been by commercial grain as such.

From a South African perspective, and from what has been learnt during the course of this study, only three mycotoxins - AFLA, FBs and DON - need to be singled out as possible mycotoxin contaminants of any real significance in locally produced or imported commercial grains.

Mycotoxins are concentrated in screenings and other milling by-products derived from commercial grain. These are used in feed milling. At times, these materials can contain damaging levels of certain mycotoxins.

Several epidemiological case studies have shown that AFLA are acutely toxic to humans and cause serious liver damage within a short while at a dietary level of about 1.7 µg/g.

Although there is some contradictory evidence, strong evidence also exists of a relationship between AFLA in plate food and the occurrence of primary liver cancer in humans in several countries. Humans are very much more resistant to the hepatocarcinogenic property of AFLA than experimental animals. Indications are that an AFLA intake above about 5.0 ng/kg body weight/day results in a rise in the incidence rate of primary liver cancer from a very low base. If the total intake at this level came from maize meal, it would translate to a dietary level of 0.76 ng/g for consumers eating 460 g of maize meal per person per day.

Contradicting epidemiological data from India and Costa Rica indicate that a dietary level of 15 ng/g has no effect on consumers.

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It is nonetheless also clear that the aetiology of primary liver cancer in humans is multifactorial and in addition to exposure to AFLA, HBV and HCV infection, several other factors play an important role.

In contrast with the AFLA scenario, not a single incident of acute intoxication of humans by FBs has been recorded. This also applies to the Transkei, where FB levels as high as 142 µg/g were found in some samples and where mouldy maize is reportedly used to make traditional beer, of which some Transkeieans consume large quantities.

An overview of toxicological studies on a variety of farm animals by the FDA's CVM is reproduced in totality and was used to indicate possible physiological loci in humans where health problems might occur for the purposes of our study.

The CVM study demonstrated large differences in susceptibility to FBs between different animal species. Horses and rabbits were classified as particularly sensitive for FBs, with damage to brain and liver tissue most evident. A maximum total FBs level in the feed of 1 µg/g was recommended for horses. Swine and catfish were classified as moderately sensitive, with pulmonary oedema and liver and kidney damage the most evident in pigs. A maximum total FBs level in the feed of 10 µg/g was recommended for swine and catfish. Ruminants and mink were classified as moderately tolerant and a maximum total FBs level in the feed of 30 µg/g was recommended. Poultry were found quite tolerant and a maximum total FBs level in the feed of 50 µg/g was recommended. The liver and kidney are the organs where damage is most evident.

No synergistic interaction between a nitrosamine - a known OC initiator - and FB_1 in the rat oesophagus was found when the two compounds were administered together. At exposure levels of more than 50 µg/g, FBs have been shown to initiate and promote liver and kidney cancer in male laboratory rats and liver cancer in female laboratory mice. There is no toxicological or epidemiological evidence that FBs initiate or promote OC in animals. There is no epidemiological evidence that FBs are linked to any kind of cancer in animals.

In a study on the effect of FBs on the sphingosine/sphinganine ratio – a possible biomarker for FB exposure - in vervet monkeys, the animals tolerated for a period of

60 weeks dietary intakes equivalent in humans to about 45 and 121 µg/g. Such levels would be fatal to horses and pigs within weeks and would cause liver cancer in rats and mice. This possibly indicates a very high tolerance to FBs in primates.

Human epidemiological studies currently available demonstrate only inconclusive statistical associations between FBs in maize produced on subsistence farms in the Transkei and in China and human OC in these, but not in other areas. These studies are limited by the lack of controlled conditions, particularly for established confounding risk factors e.g. alcohol consumption and exposure to nitrosamines. The statistical evidence has not been followed up with fully-fledged epidemiological studies, consequently actual FB intake from plate food and beer have not been established. Therefore, the results of these studies do not allow any definitive conclusions to be made about OC causation in humans.

The sphingosine/sphinganine ratios in blood serum and urine of humans living in areas where FB levels in maize are around 600 ng/g, are not significantly different to those in humans living in areas where FBs in maize were virtually absent. In another study, (Qiu & Liu, 2001) urinary sphingosine/sphinganine ratios in urine of humans appeared to be affected only when FB_1 exposure was high.

The toxicology of DON in humans is still poorly understood, but the main overt effect of DON at low dietary concentrations appears to be a reduction in food consumption (anorexia), while higher doses induce vomiting (emesis). DON is known to alter brain neurochemicals and it suppresses normal immune response to pathogens and simultaneously induces autoimmune-like effects, which are similar to human immunoglobulin A nephropathy. This may be of importance in relation to the present AIDS epidemic in South Africa and should be investigated.

5.6. Incidence of liver, kidney and brain cancer in Africa in relation to grain consumption, and in South Africa in relation to the occurrence of fumonisins in maize

During the course of the present study it transpired that three mycotoxins occur regularly at possibly significant levels in domestic and/or imported commercial grain in South Africa: AFLA, FBs and DON. Therefore, only these three warrant attention from the aspect of establishing MTLs.

AFLA are acutely and chronically toxic to humans, causing liver damage. In spite of some contrary evidence, there is strong evidence that AFLA are carcinogenic in humans. The threat from AFLA to human health is therefore sufficiently clear to justify institution of MTLs and to provide a rational basis for estimating meaningful MTLs.

The toxicology of DON is somewhat obscure, but it is not acutely toxic or carcinogenic in humans. However, at levels that often occur in commercial grain, it causes disease in animals. It occurs worldwide in both wheat and maize, and possibly also in grain sorghum. While the threat from DON to human health is far from clear, its common occurrence warrants action. As toxicological data are insufficient to institute MTLs on a rational basis, MTLs would have to be instituted on an arbitrary basis. This could be done without causing upheaval in the local grain industries.

Based on present knowledge, FBs may possibly have two effects on human health: OC and neural tube defects.

Previous workers found a statistical relationship in Transkei and China, between the incidence of OC and infection rates of subsistence maize by *F. verticillioides*. A weaker statistical relationship of OC with FB contamination of subsistence maize has also been demonstrated. On that basis, Gelderblom *et al* (1996) and Marasas (1997) recommended a very low MTL of 100-200 ng/g for FBs in commercial maize. However, in the present study it was demonstrated that a relationship exists neither between the incidence of OC and estimated FB levels in commercial maize in South

Africa, nor between OC incidence and estimated infection rates of commercial maize by *F. verticillioides*.

In animals, FBs damage mainly brain, liver and kidney tissue. Humans who subsist on a maize-based diet constantly ingest FBs, but there are no reports of damage to these tissues in humans. However, the possibility of cancer in these organs in humans, because of exposure to FBs, needs to be investigated before meaningful MTLs for FBs can be formulated. Therefore, the correlation between cancer of each of these organs in black males in South Africa and estimated *F. verticillioides* infection rates on the one hand, and FB levels on the other in commercial maize in different parts of the country was calculated. The results indicate that there is no relationship between FB levels in commercial maize and the incidence of liver, kidney or brain cancer in black males in South Africa.

Furthermore, the correlation between cancer of each of these organs in males and females and the supply (as a rough estimate of consumption) of maize, grain sorghum and millet in 23 African countries was also calculated. The results indicate that there is no relationship between the consumption of maize, grain sorghum and millet and liver, kidney and brain cancer incidence in Africa.

It is concluded that natural levels of FBs in staples play no role in the occurrence of liver, kidney or brain cancers in humans. This is of importance when MTLs for FBs are considered.

5.7. Neural tube defects and mycotoxins

An NTD is the failure of the spinal canal or the skull to close around the nerve tissue inside during the first 6 weeks of fetal development.

The causes of NTD are multifactorial and include a body fever in the pregnant woman during the first weeks of pregnancy, folic acid deficiency in her diet and several other proven and suspected factors.

In 1995 a possible link between a cluster of NTDs in the south of Texas and exposure to FBs in a diet with a high maize content, was highlighted as a further possible cause of NTD.

Statistical analysis of available data from South Africa and the USA in our study has shown a significant relationship between estimated FB intakes and the incidence rates of NTD.

Animal experiments have demonstrated an effect during gestation on foetal organ development, including bone development and NTD in rats exposed to FBs. However, no similar effect was observed in rabbits.

A possible physiological mechanism, whereby FBs affect availability of folic acid to the foetus and thus the development of an NTD, has been put forward.

It is concluded that the possibility exists that exposure to FBs in the diet during the early weeks of pregnancy may be an additional cause of the development of an NTD in the foetus.

From the available data the NOAEL is a dietary intake of FBs (or HFBs) of about 60 μg/70 kg person/day. This level of intake in early pregnancy does not cause a rise in NTD incidence and can be considered as safe in terms of NTD.

This level translates to an MTL of 130 ng/g in maize products for rural consumers in South Africa, who consume on average 460 g of maize products per day, and to 217 ng/g for urban consumers, who consume on average 276 g of maize product per day.

It is clear that the section of the population that could possibly be at risk from FBs as a cause of NTD is less than 0.47% and their vulnerability is limited to a very specific period. It is therefore concluded that protection against any possible NTD caused by FBs in maize products could probably be more effectively achieved through other means than MTLs of 130 to 217 ng/g. MTLs of this order for FBs would cause serious disruption in the maize industry, which would harm maize consumers economically.

5.8. Overview of the occurrence of mycotoxins in South African grains and grain products and the possible risks of natural mycotoxin levels to consumers

Through surveys carried out by the Maize Board on maize from the main production areas over the 6 crop years 1989 – 1994, extensive data, representative of the situation in commercial maize in South Africa, are available on the fungi and mycotoxins that occur in white and yellow South African maize. The period included years of high, as well as extremely low rainfall, and it is likely that a large part of all possible variation in fungal and mycotoxin levels in commercial RSA maize is represented in the data from these surveys.

AFLA are almost completely absent in South African white and yellow maize even on occasion of severe drought during the maize-growing season. In the USA and Argentina, AFLA occur frequently, often at levels 10 to 20 times as high as the South African MTL. In maize imported from these countries, AFLA were found in some samples at levels 10 to 20 times as high as the South African MTLs for AFLA.

Generally, FBs occur in RSA maize at relatively low levels compared to maize from the USA, but most maize contains some FBs. During the first years of the 6 years over which the Maize Board surveys stretched, FBs were found at higher levels in white, than in yellow maize, but the situation was very variable in most production areas.

In white maize, FBs were most prevalent in maize from the N-OFS and the W-Tvl production areas, the main production areas for white maize in South Africa. In some years FBs occurred in white maize in these two areas at mean levels approaching 2 000 ng/g, about 10 to 20 times as high as the recommended MTL for South Africa. There is no direct evidence that the observed levels are a threat to human health.

FBs occurred in imported ARG yellow maize at mean levels similar to the mean levels in South African maize. In imported USA maize, FBs occurred at mean levels considerably higher than in South African maize.

Of the other mycotoxins covered in this study, only DON, NIV and MON were frequently found, but only at low levels. MON was tested for in only 1 year in most areas and in 2 years in one area and no firm conclusions can be made about MON on that basis. ZEA was found very infrequently, and at very low levels. The other mycotoxins covered in this study were not found in maize, nor were any OA, PAT or CIT ever found in maize during these surveys. With the possible exception of DON, which occurred regularly at moderate levels, none of these mycotoxins appears to be a cause for concern in South African maize regarding human or animal health.

Surveys of mycotoxins in white maize products over 3 marketing years within a four-year period showed that mycotoxins generally occur at much lower levels in white maize products than in whole maize. The levels tend to decrease as the degree of refinement of the product increases. This tendency is more pronounced in the case of some mycotoxins than others. Defatted germ meal, maize screenings and maize bran from white maize milling, utilized in the feed milling industry, contained mycotoxins at considerably higher mean levels than whole maize, or milled products and could on occasion threaten animal health.

The mean levels and frequency of occurrence of mycotoxins in South African white maize products are low in general. In years when the FB content of white maize in the main production areas are relatively high, the mean FB content of a large proportion of white maize products is likely to exceed by a large margin the recommended MTL of 100 – 200 ng/g. In 'normal' years, the total FB content of maize products often exceeded 1 000 ng/g and sometimes 4 500 ng/g.

The mean levels and frequency of occurrence of AFLA and FBs in maize products for human food in South Africa are considerably lower than in similar products in the USA and Argentina. In years when relatively high levels of FBs occur in white maize in South Africa, an alternative source that can comply with an MTL of 200 or even 300 ng/g is highly unlikely to be found. An MTL of this level, if enforced, will eventually have a disastrous effect on maize farmers, the maize milling industry and consumers who rely on maize as a staple food in South Africa.

As yet, there is no clear evidence that the FB levels in commercial maize in South Africa pose any threat whatsoever to consumer health. The statistical relationship of

OC incidence with FBs in maize produced on subsistence farms in the Transkei could very well be co-incidental. FB levels in commercial maize in South Africa are on par with those in subsistence maize in the low OC incidence area of the Transkei where OC incidence is moderately low in world terms. Exposure to other mycotoxins in locally produced commercial maize in South Africa clearly poses no threat to consumer health.

Similar data to the maize data are not available for other grain staples in South Africa, and until further surveys are conducted, it would be risky to form conclusions in respect of mycotoxins in these grains. Worldwide, DON is frequently found in wheat and wheat products, often at relatively high levels.

5.9. Estimate of the highest MTLs for mycotoxins that can be adopted in grain and grain products in South Africa, without jeopardizing the safety of consumers

Of the 77 countries with MTLs for mycotoxins, only Canada has so far consistently approached the need for and the setting of limits from a scientific basis. Recently, the USA applied a good scientific approach for setting guidance levels for FBs in feed and food. Apart from MTLs for mycotoxins in food, no other type of measure has so far been introduced as a regulatory measure to limit human exposure to a mycotoxin. Economic and social considerations have not been brought into account when introducing regulatory measures.

Any possible need for regulation should be determined based on a human exposure assessment, while the type of measure, or the level of an MTL needed, should be based on a hazard assessment.

Other considerations when considering MTLs include regulations of trading partners, commercial interests and sufficiency of food supply.

By applying the work procedure outlined above, new MTLs for AFLA, FBs and DON are proposed, independent of existing or previously proposed MTLs. A basis for determination of compliance is also proposed, which was previously lacking. The basis of compliance is the mycotoxin level in one representative sample of any

consignment of grain or grain product. A consignment is defined as any distinguishable unit of grain, from a pallet to a ships hold.

The risk of human exposure to AFLA in South Africa could not clearly be estimated from the data available and there remain several uncertainties. One of these relates to current moisture problems in stored wheat because of the use in South Africa of an unproven, non-standard reference test for calibrating electronic moisture meters. This could create conditions favourable for AFLA production in stored wheat. Another relates to imported maize, which frequently contains AFLA, but where the frequency and scale of imports can vary indefinitely. Apart from these, the general indications are that the risk of exposure is small, mainly because of very low AFLA levels in local commercial maize and maize products.

From both a toxicological and an epidemiological viewpoint, there is clear evidence that AFLA are a health hazard to humans. The maximum tolerable AFLA intake level, unlikely to be hazardous to human health, appears to be about 5 µg/kg body weight/day, translating to a dietary level of about 15 ng/g under South African conditions.

In the USA and Argentina - main sources of imported maize for South Africa - MTLs of 20 ng/g for AFLA in maize apply to grain used locally. Special measures are required to assure that maize imported from these countries meets this specification. The present South African MTL of 10 ng/g can only be met if grain is purchased on an identity preserved basis, at increased cost. An unrealistically low MTL for AFLA could create difficulty in sourcing import supplies. Existence of a regulatory MTL for AFLA, which millers comply with, can safeguard millers against claims for damages from consumers.

An MTL of 20 ng/g in uncleaned, unprocessed cereal grains intended for food use, and 10 ng/g in grain products for food, with not more than 5 ng/g AFB_1, is proposed for AFLA.

FBs are ubiquitous in maize and humans in South Africa who rely on commercial maize products as a staple, are constantly exposed to FBs. Consumers in rural areas are ingesting FBs in commercial maize products at an estimated average rate of between 124 and 253 µg/70 kg person/day or between 1.8 and 3.6 µg/kg body

weight/day. Depending on the hazard this exposure poses, there may exist a need for measures to reduce exposure.

With the possible exception of neural tube disorders in newborn infants, the hazard posed by these levels of FBs to human health appears to be insignificant. The only remaining possible threat to human health demonstrated so far consists of a statistical relationship between OC incidence and FBs in subsistence maize in parts of the Transkei. No such relationship could be found in the commercial maize areas of South Africa. Estimated ingestion rates of between 1.6 and 49.3 µg/kg body weight/day in the Lusikisiki/Bizana area of Transkei do not result in an elevated incidence of OC. OC incidence in this area is moderately low.

In Argentina, FB intake of 11.3 ng/g of body weight/day was estimated for child maize consumers (1-5 years old). No adverse effects were evident.

In animal tests, FBs have not been shown to cause OC. In animals, FBs cause damage to liver, kidney and brain tissue, but there is no evidence of similar damage in humans constantly ingesting FBs. In rats and mice, FBs at high dietary levels over an extended period induced and/or promoted kidney and/or liver cancer, but in human maize consumers there is no statistical relationship between exposure to FBs in commercial maize and cancer of the brain, liver and kidneys.

Exposure to FBs in maize at up to 580 ng/g had no effect on the serum and urine Sa/So ratios in humans. It is therefore highly unlikely that any evidence of human exposure to FBs will be found in Sa/So ratios in the commercial maize areas of South Africa.

Based on these results, it was concluded that a safety factor of 1 000 for extrapolating from animal toxicology data was unnecessarily cautious. A safety factor of 50 should be sufficient, considering that FBs are non-genotoxic and that clear evidence of a threshold limit exists for their cancer initiating action in rats.

The USA has set guidance levels of between 2 and 4 µg/g for FBs in maize-based foods. An MTL for maize, much lower than these values would create severe difficulties for South Africa in sourcing import maize and could result in artificial food shortages.

Impractical, difficult to comply with MTLs for FBs can expose millers to non-compliance claims and could cause huge trade losses.

Based on these considerations, the following MTLs for FBs are proposed:

- 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).

Insufficient data are available to estimate with reasonable accuracy the exposure of humans to DON in South Africa. However, DON occurs widely in local maize, and probably in wheat, barley and grain sorghum too. DON is the most common mycotoxin in USA, ARG and Canadian wheat. Human exposure in South Africa is therefore probably significant, and regulation could be necessary.

Because of insufficient toxicological and epidemiological data, the health hazard DON poses to humans is not clear. However, the immuno-suppressive properties of DON in humans could be of particular importance in relation with the current AIDS epidemic in South Africa.

With so much information unavailable, it is impossible to rationally formulate a proposal for MTLs for DON. It could therefore be acceptable to institute arbitrary MTLs for DON in South Africa, based on the MTLs in use in other countries.

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, including Canada and the USA. No difficulties in food supply are envisaged at such MTLs.

Thus, an MTL for DON of 2 µg/g in unprocessed grains, and 1 µg/g in finished foods is proposed.

5.10. Implications for the international grain trade and for millers in South Africa of MTLs for mycotoxins in grains and grain products

From the broad perspective, the advantages for a country to maintain MTLs for undesirable contaminants in grain and other food products outweigh the difficulties and disadvantages it may create. However, higher standards only come with increased costs in the purchase price, as well as in testing, supervision and control to ensure that grain shipped actually complies with MTL specifications.

The existing regulatory MTL of 10 ng/g AFLA (of which 5 ng/g may be AFB_1) in food grains holds little implications for millers as far as locally produced grains are concerned, because natural AFLA levels in local grains, with the possible exception of wheat, are low. The existing regulation does not specify the basis for compliance.

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

The new MTL of 20 ng/g proposed for AFLA in unprocessed grains is in line with those in the major supplier countries, which will make it easier to source import grain. The proposed MTL of 10 ng/g for AFLA in finished products is the same as the existing MTL and can easily be complied with. Thus consumer interests are not jeopardized by the higher MTL proposed for unprocessed grains.

The recommended MTL of 100 to 200 ng/g for FBs in (unprocessed) maize will seriously affect millers, maize producers, and consumers. Maize-based foods everywhere contain FBs, often at considerably higher levels than in South Africa; alternative sources are therefore not easily available. An MTL of 200 ng/g in maize or maize products is impossible to comply with and would culminate in severe shortages of maize and maize products considered suitable for human use. The shortfall will raise prices for maize products. Shortages will have to be made up by other starchy foods such as wheat, rice and potatoes, which will cause havoc in these industries at the volumes required. Maize unsuitable for human consumption will find

its way to the export or animal feeds markets with a severe impact on these markets. The health benefits to consumers are obscure.

On the other hand, most commercial maize in most crop years in South Africa can comply with an MTL of 4 µg/g for FBs. This MTL will therefore have a minimal negative effect on the domestic maize industry. There is no reason to believe that FBs at these levels in commercial maize have been detrimental to consumer health anywhere in the world. An MTL of 4 µg/g in maize will prevent the importation of maize that could be harmful to sensitive animals such as horses and pigs, without rendering impossible the sourcing of maize for importation.

The newly proposed MTL of 2 µg/g DON in grains intended for food 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.

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

Tests for mycotoxins fall in several categories, some of which require sophisticated laboratory facilities, while others can be done with relatively basic facilities.

Tests requiring only basic laboratory facilities include TLC tests and those based on immunoaffinity. The immunoaffinity tests come in kit form, of which a disposable affinity column or 'well' is the main component. These tests are accurate and lend themselves to a variety of applications, including testing at grain silos, mills, and feed mills. Immunoaffinity testing have therefore become widely accepted.

Briefly, the mycotoxin is extracted from the sample using solvents, the affinity column is used to extract the mycotoxin from the solvents for cleanup, and the mycotoxin is then converted to a fluorescing derivative (e.g. Vicam). The fluorescence is measured to quantify the mycotoxin. Either HPLC (in a sophisticated laboratory), or a fluorometer (in a basic laboratory) can be used for quantification.

Alternatively, some immunoaffinity systems (e.g. Neogen) convert the mycotoxin to a coloured substance, which can be quantified by spectrophotometry.

An estimate of the capital cost to set up a basic laboratory to facilitate one technician for immunoaffinity testing for mycotoxins by fluorometry would be between R250 000 and R300 000. Included in the estimate are two fluorometers for quantification, three or four high-speed blenders, two laboratory mills, glassware and other basic apparatus. The cost of a building and furniture are excluded.

The cost of consumables for immunoaffinity testing, such as test columns, developers, filter papers etc is from about R120.00 per test for the AFLA test to about R172.00 for the FB test. Testing for three mycotoxins can therefore cost more than R500.00 per sample. If each sample represents a 10-ton grain parcel, consumables for mycotoxin testing can add R50.00 per ton to the cost of grain handling and storage.

If a skilled technician could manage to complete 2.5 immunoaffinity tests per hour, labour costs would come to about R8.00 per sample, for all three mycotoxins.

While the immunoaffinity methods require relatively unsophisticated testing facilities, the total capital cost, as well as the running costs, remain high and this limits the scale on which the tests can be applied.

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

Mycotoxins are not evenly distributed in grain, grain products or mixed feeds. Therefore, taking a representative sample for mycotoxin needs special care. Sampling procedures are given for sampling grain and grain products in bulk in vehicles, silo bins and ships holds, as well as for grain and grain products in stacked bags or packages.

If mycotoxins are to be tested for on a routine basis, two options are available:

- To test each load at storage silos during harvest intake;
- To test the grain after harvest intake, either in the silo bin before any grain is outloaded, or in the truck during dispatch.

The advantages and disadvantages of the various options and sub-options are briefly listed and the cost bracket of each is roughly estimated. Sampling and testing at harvest intake would give maximum sensitivity for the detection and management of mycotoxins, but could cost more than R60.00/ton to execute. Sampling and testing during dispatch from storage silos can reduce the cost to less than R12.50/ton but it puts the onus for managing the mycotoxin situation and for losses on the buyer. Sampling and testing the grain in silo bins before outloading reduces the costs to an insignificant amount and it leaves the onus for losses on grain suppliers. However, it also reduces sensitivity for detection and management of contaminated grain stocks, which could lead to unexpected grain shortages, or finished product unsuitable for human consumption. Millers need to consider these options.