Mycotoxins in grain and grain products in South Africa and proposals for their regulation

By

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PREFACE

The National Association of Maize Millers (NAMM) and the National Chamber of Milling (NCM) in South Africa commissioned this study in September 2000. It was a sincere effort on their part to discover the realities surrounding the occurrence of mycotoxins in cereal grain staples and their products in South Africa, the threat these may pose to the health of consumers and practical ways to deal with the situation. The driver for their action was the substantial confusion that arose when a lobby of scientists pushed for adoption of maximum tolerable levels (MTLs) for fumonisins previously recommended for consideration by Gelderblom *et al* (1996) and Marasas (1997). These recommendations were based on classical risk assessment methods, including an exposure assessment and a hazard assessment. Based on toxicological data for rats, with a 1000-fold safety factor, these assessments arrived at recommended maximum levels of 100 – 200 ng/g in food. Little epidemiological data were included and socio-economic practicalities were not taken into consideration in these assessments.

Significantly, Prof Marasas and his team of scientists at the Medical Research Council (MRC), including Dr Gelderblom, were not involved in the initiative to push for statutory adoption of these recommendations. Adoption of these levels would have caused a revolution in the grains industry, as is demonstrated within the pages of this thesis. This thesis attempts to consider in a balanced way the relevant scientific information, as well as stakeholder interests, particularly those of consumers from a national health as well as an economic perspective. It offers a pragmatic approach to the setting of MTLs for substances that are potentially harmful to the health of consumers, based on sound scientific evidence. New MTLs for three mycotoxins have been formulated as well as proposals for their practical implementation.

The National Maize Trust has subsequently reimbursed NAMM and NCM for the costs of this study and it stands to its credit that, through this gesture, the maize industry has accepted the outcomes of the study.

Summary

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Degree: PhD

The purpose of the study was to:

- Report on the occurrence of mycotoxins in grain and grain products in South Africa;
- Compare with other countries;
- Weigh the evidence regarding effects on health of test animals, and human and animal consumers;
- Determine the need for statutory measures to regulate mycotoxins in food; and
- Propose practical measures for controlling mycotoxins in grain and grain products in South Africa.

Good mycotoxin data for maize were obtained from the author's surveys. Data on other local grains is lacking. In domestic maize, fumonisins and deoxynivalenol occur regularly, at levels as low or lower than in Argentina and the USA. Other mycotoxins occur rarely, or at very low levels. Deoxynivalenol is likely to occur regularly in domestic wheat. Aflatoxins were virtually absent in domestic maize, but often occur at concerning levels in imported Argentinean and USA maize. The literature show that aflatoxins are acutely and chronically toxic to humans and animals and most countries maintain regulatory Maximum Tolerable Levels (MTLs) for aflatoxins in grain and grain products. Several countries also maintain regulatory MTLs for deoxynivalenol,

based on lesser scientific evidence. The mycotoxin that occurs most frequently in South African maize, is the fumonisin B group of analogues, with fumonisin B_1 the most abundant. Fumonisins are produced by *Fusarium verticillioides* (previously known as *Fusarium moniliforme*) and occur in maize worldwide. Fumonisins cause leukoencephalomalacia in horses, pulmonary oedema in pigs, liver cancer in rats and liver and kidney damage in other animals. A statistical relationship between the occurrence of *F. verticillioides* and fumonisins in maize and oesophageal cancer in humans has been demonstrated in Transkei and in China. The 'toxins derived from *F. moniliforme*' and fumonisin B_1 have been evaluated as Group 2B carcinogens i.e. possibly carcinogenic to humans, by the International Agency for Research on Cancer of the World Health Organisation.

Based on a review of epidemiological and toxicological evidence of the effects of fumonisins on humans and animals, their occurrence in maize and maize products, previously proposed MTLs, and the practical implications of MTLs set for maize and maize products, we propose the following MTLs for total fumonisins in maize and maize products for human consumption:

- 4 µg/g in whole, uncleaned maize;
- 2 µg/g in dry-milled maize products with fat content of $\geq$3.0 %, dry weight basis (e.g., sifted and unsifted maize meal); and
- 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)

These MTLs are too high to address a possible link of fumonisins with neural tube defects in neonates. This potential problem remains to be addressed, possibly by fortification of maize products with folic acid.

We propose MTLs for deoxynivalenol of 2 µg/g in cereal grains for food use, and 1 µg/g in cereal grain food products. Finally, we propose that the current regulatory MTLs for aflatoxins be raised from 10 ng/g (total aflatoxins in unprocessed maize) to 20 ng/g.

Ekserp

Mikotoksiene in graan en graanprodukte in Suid-Afrika en voorstelle vir die regulering daarvan

Deur

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Promotor: Prof WFO Marasas; Co-Promotor: Prof MJ Wingfield

Graad: PhD

Die doel met die studie was om:

- Verslag te lewer van die voorkoms van mikotoksiene in graan en graanprodukte in Suid-Afrika;
- Met ander lande te vergelyk;
- Beskikbare data oor die effek op die gesondheid van toetsdiere en menslike en dierlike verbruikers te bestudeer;
- Te bepaal of daar behoefte na statutêre maatreëls is om mikotoksiene in voedsel te reguleer; en
- Praktiese maatreëls aan die hand te doen om mikotoksiene in graan en graanprodukte in Suid-Afrika te reguleer.

Vir mielies is goeie mikotoksiendata beskikbaar vanuit die skrywer se eie opnames. Daar is egter ‘n tekort aan data tov ander grane. Fumonisiene en deoksinivalenol kom dikwels voor in plaaslike mielies teen vlakke soortgelyk of laer as in Argentinië en die VSA. Ander mikotoksiene kom selde voor, of teen baie lae vlakke. Deoksinivalenol kom waarskynlik ook dikwels in plaaslike koring voor. Plaaslike mielies is feitlik totaal vry van aflatoksiene, maar aflatoksiene kom dikwels teen besorgenswaardige vlakke voor in ingevoerde VSA en Argentynse mielies. Uit die literatuur is dit duidelik

dat aflatoksiene akuut sowel as chronies giftig is vir mens en dier en die meeste lande handhaaf regulatoriese Maksimum Aanvaarbare Vlakke (MAVe) vir aflatoksiene in graan en graanprodukte. In verskeie lande is regulatoriese MAVe vir deoksinivalenol ook van krag, maar minder wetenskaplike data is beskikbaar as die basis daarvan. Die mees algemene mikotoksien in Suid-Afrikaanse mielies is die fumonisien B-groep van analoë, waarvan fumonisien B_1 die meeste voorkom. Fumonisiene word deur *Fusarium verticillioides* (voorheen bekend as *Fusarium moniliforme*) geproduseer en word wêreldwyd in mielies aangetref. Fumonisiene veroorsaak leukoencephalomalasia in perde, pulmonêre edeem in varke en nier- en lewerskade in ander diere. 'n Statistiese verwantskap tussen die voorkoms van *F. verticillioides* en fumonisiene in mielies en slukdermkanker by mense is in Transkei en China aangetoon. Die Internasionale Agentskap vir Kankernavorsing van die Wêreld Gesondheidsorganisasie het die 'toxins derived from *F. moniliforme*' en fumonisien B_1 as Groep 2 B karsinogene geëvalueer - d.i. moontlik karsinogenies vir mense.

Gebaseer op 'n oorsig van epidemiologiese en toksikologiese gegewens met betrekking tot die effek van fumonisiene op mens en dier, die voorkoms van fumonisiene in mielies en mielieprodukte, MAVe wat voorheen aan die hand gedoen is, en die praktiese implikasies wat MAVe vir die mieliebedryf inhou, word die volgende nuwe MAVe vir fumonisiene (totaal) in mielies en mielieprodukte vir menslike verbruik aan die hand gedoen:

- 4 µg/g in heel, onskoongemaakte mielies;
- 2 µg/g in mielieprodukte van die droëmaalbedryf, met 'n vetinhoud $\geq$3.0 %, droëmassabasis (bv. gesifte en ongesifte mieliemeel); en
- 1 µg/g in mielieprodukte van die droëmaalbedryf, met 'n vetinhoud <3.0 %, droëmassabasis (bv. mieliegruis, brouersgruis, stampmielies, mielierys, super and spesiale mieliemeel)

Hierdie vlakke is egter onvoldoende om 'n moontlike verband tussen fumonisiene en neuraalbuisdefekte by pasgeborenes aan te spreek. 'n Oplossing vir dié probleem moet elders gevind word, moontlik deur fortifisering van mielieprodukte met foliensuur.

Ten opsigte van deoksinivalenol word ‘n MAV van 2 µg/g vir graan bestem as voedsel aan die hand gedoen, en 1 µg/g vir graanprodukte. Laastens word aan die hand gedoen dat die huidige regulatoriese MAV vir aflatoksiene van 10 ng/g (totale aflatoksiene in onverwerkte mielies) na 20 ng/g verhoog word.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY AND ABBREVIATIONS USED

AFMA – Animal Feed Manufacturers Association in South Africa

AFB_1, AFB_2, AFG_1, AFG_2 AFM_1, AFM_2 - Aflatoxin B_1, B_2, G_1, G_2, M_1 & M_2 respectively

AFLA - aflatoxins

AME - Alternariol monomethyl ether

ARG maize – yellow maize imported from Argentina

ASIR – age standardised incidence rate

BGYF - bright green yellow fluorescence

Carcinogen – a substance that causes cancer in animals and/or humans

CFSAN – Center for Food Safety and Nutrition of the FDA

CIT - citrinin

CVM – Center for Veterinary Medicine of the FDA

DAS - Diacetoxyscirpenol

DON - Deoxynivalenol

E-OFS – Eastern Orange Free State

E-Tvl – Eastern Transvaal

ENSO – El Nino Southern Oscillation

FAO – Food and Agriculture Organization of the United Nations

FBs – Two or more of fumonisin B_1, B_2, B_3, B_4

FB_1, FB_2, FB_3, B_4 – fumonisin B_1, B_2, B_3 and B_4 respectively

FDA – Food and Drug Administration in the USA

Feed – products intended for animal consumption

Feed components – products intended for mixing with other products in predetermined ratios to produce a balanced ration for animal use

FGIS - Federal Grain Inspection Service in the USA

Food – products intended for human consumption

Fungi – a diverse group of plants that lack chlorophyll and which obtain their food as saprophytes from dead organic matter, and/or as parasites from other living organisms

GLC – Gas liquid chromatography

HBV – hepatitis B virus

HCV – hepatitis C virus

HFB – hydrolysed fumonisins through alkali treatment

HPLC – High Pressure Liquid Chromatography

HT-2 – HT-2 toxin

IACs - Immunoaffinity columns; ELISA or antibody test kits

kt – kiloton, or thousand metric tons

LEM - leucoencephalomalacia, a condition caused by FBs in horses, where cavities develop in the white matter of the brain

MBN - methylbenzylnitrosamine

Mixed feed – a balanced ration consisting of a mixture of feed components, intended for animal consumption

MON - Moniliformin

MRC – The Medical Research Council in Tygerberg, South Africa

Mt – Megaton, or million metric tons

MTL – maximum tolerable level

Mycotoxicoses - diseases in animals and humans resulting from the consumption of mycotoxins

Mycotoxins – secondary metabolites produced by fungi, some of which are toxic to plants animals and humans, and some are toxic and carcinogenic to animals and humans

N-OFS – northern Orange Free State

***N*-MBN** – *N*- methylbenzylnitrosamine

NIV - Nivalenol

NOAEL – no observed adverse effect level

NS – statistically not significant

OA – ochratoxin A

OC – oesophageal cancer

PAT - patulin

PDI – probable daily intake

ppb – parts per billion, or ng/g, or µg/kg, or mg/metric tonne

ppm – parts per million, or µg/g, or mg/kg, or g/metric tonne.

PWV – Pretoria, Witwatersrand, Vereeniging area

RSA maize – locally produced South African white or yellow maize

Squamous cells or squamous epithelium – tile-like cells on the surface layers of a body tissue

t – metric ton

T-2 - T-2 toxin

TDI – Tolerable daily intake: the daily intake of a toxin that should be harmless

TLC – Thin layer chromatography

USA maize – yellow maize imported from the United States of America

W-Tvl – western Transvaal

WHO – World Health Organization of the United Nations

ZEA – Zearalenone

1. Introduction

One of the most important food safety aspects in foods and feeds made of cereal grains today is contamination with mycotoxins. Attempts at regulating mycotoxin levels in foods are a long way from being fully effective or are not always the best way to address the problem. Very often the extent of 'the problem' is not very well known, either because the toxicology of the mycotoxin is imperfect, or the level of exposure of consumers is not very clear. This thesis is an attempt to look at some of these issues concerning grain and grain products in South Africa, and the mycotoxins that are of interest.

1.1. What are mycotoxins?

Mycotoxins are chemicals that are sometimes - certainly not always - produced by fungi occurring in food and feed. Particular fungi produce specific mycotoxins. Under a given set of environmental conditions, specific fungi often dominate in particular food crops, either during the growing stage, and/or after harvest. Mycotoxins can be considered as natural toxic substances that can adversely affect human and animal consumers, including causing cancer in some cases. Some mycotoxins also adversely affect plants and/or micro-organisms. One of the best-known mycotoxins is penicillin, used as an antibiotic for treatment against disease organisms.

Mycotoxins have probably been present in food and feed since early in the history of of humankind. Some of their effects have been known for hundreds of years. The technology to detect and chemically characterize them has only really developed in the last 40 years, particularly since 1980. Very small quantities of many of the important mycotoxins can now be detected and accurately measured in foods and feeds. In addition to those already known, many others are known to exist, but have not yet been chemically characterized. Scientists are now identifying toxic compounds in food faster than the information can be processed. However, to maintain perspective, it must be remembered that these substances have always been there, that humans have always been eating the food in which they occur and in the case of many substances, only the dose makes the poison.

1.2. Where do mycotoxins come from in grain?

Fungi that infect growing crop plants, or foodstuffs in storage, produce mycotoxins. However, mycotoxins are not necessarily produced at all times when fungi are actively growing on grain, dead plant material, or in live plants. The range of environmental conditions, especially the humidity and temperature, under which a fungus will produce a mycotoxin, is generally much narrower than the range in which fungal growth can take place. Thus, the presence of a fungus, even at a high infection rate, does not necessarily mean that there will also be mycotoxins present. In addition, there are large differences between different strains of a given fungal species in their ability to produce mycotoxins. On the other hand, mycotoxins that have been produced by a fungus can remain in plant materials long after all signs of fungal infection have disappeared.

Theoretically, preventing fungal infection of the growing plant or the stored commodity can prevent mycotoxin contamination of food. In practice, however, mycotoxins in food are unavoidable, because fungi are ubiquitous and there is no cost-effective way available to prevent fungal infection of crops in the field. The only real prospect of achieving this is to develop plant varieties that are resistant to fungal infection, either through conventional plant breeding or through genetic modification. In storage, fungal growth can be limited by storing grain as dry and as cool as possible. Reliable moisture measurement in stored grain is essential to this end, since changes as small as 0.5% in the moisture content of cereal grains can have a significant effect on fungal growth and the production of mycotoxins.

About 100 000 fungi have been identified, of which over 400 are considered potentially toxic. About 20 of these produce toxic compounds - or families of compounds - which cause problems in one or more parts of the world (De Koe, 1993). A handful predominates in grain crops in South Africa. These, together with the most important mycotoxins that each produces if conditions are suitable, are given below:

In maize

Fungal species	Main mycotoxins produced	Reference
Fusarium verticillioides (Previously known as *F. moniliforme*)	Fumonisins (FBs)	Gelderblom *et al* (1988); Thiel *et al* (1991a); Marasas (2001); JECFA (2002)
Fusarium subglutinans	Moniliformin (MON)	Kriek *et al* (1977); Marasas (2001)
Fusarium graminearum	Deoxynivalenol (DON), or nivalenol (NIV), zearalenone (ZEA)	Marasas *et al* (1984a); Marasas (2001)
Aspergillus flavus	Aflatoxins	IARC (1993); JECFA (1998)
Penicillium spp	OA, Citrinin (CIT), Patulin (PAT)	Scott (1994)
Stenocarpella maydis	Unidentified, causing diplodiosis in cattle and sheep	Rabie *et al* (1985a); Kellerman *et al* (1985)
Stenocarpella macrospora	Diplosporin	Gorst-Allman *et al* (1983)
Alternaria alternata	Alternariol monomethyl ether (AME)	Visconti & Sibilia (1994)

In wheat

Fungal species	Main mycotoxins produced	Reference
Alternaria alternata	AME	Visconti & Sibilia (1994)
Eurotium spp	Sterigmatocystin	Scott (1994)
Fusarium graminearum	DON or NIV, ZEA	Marasas *et al* (1984a); Marasas (2001)
Fusarium crookwellense	NIV, ZEA	Marasas *et al* (1984a); Marasas (2001)
Fusarium culmorum	DON, ZEA	Marasas *et al* (1984a); Marasas (2001)
Fusarium equiseti	Diacetoxyscirpenol (DAS)	Marasas *et al* (1984a)
Penicillium spp	CIT, OA, penicillic acid	Scott (1994)
Aspergillus flavus	Aflatoxins	IARC (1993); JECFA (1998)

In grain sorghum and sorghum malt

Fungal species	Main mycotoxins produced	Reference
Alternaria alternata	AME	Bosman *et al* (1991); Visconti & Sibilia (1994)
Phoma sorghina	Tenuazonic acid?[1]	Rabie & Lübben (1984)
Fusarium verticillioides	FB?	Rabie & Lübben (1984)
Fusarium thapsinum	MON	Marasas *et al* (1984a);

		Marasas (2001); Leslie & Marasas (2001)
Fusarium subglutinans	MON?	Rabie & Lübben (1984)
Fusarium chlamydosporum	Not known	Rabie & Lübben (1984)
Fusarium andiyazi	Not known	Marasas *et al* (2001); Marasas (2001)
Aspergillus flavus	Aflatoxins?	Rabie & Lübben (1984)
Rhizopus spp	Rhizonin A and unknown mycotoxins	Rabie *et al* (1985b)
Epicoccum spp	Not known	Bosman *et al* (1991)
Gonatobotrys spp	Not known	Bosman *et al* (1991)
Cladosporium spp	Not known	Bosman *et al* (1991)

[1]? – It is unclear whether the relevant mycotoxin occurs naturally in the particular crop plant in South Africa.

Some of the mycotoxins mentioned above rarely occur in South Africa, or are generally considered relatively harmless, and were therefore not included in the study.

The fungi listed above are not host specific, but environmental conditions in specific crops in specific countries are often more suitable for fungal growth or mycotoxin production than in other crops or in other countries.

1.3. Purpose of the study

The broad purposes of the study were:

- To report on the occurrence of certain mycotoxins in grain and grain products in South Africa, compared with other countries;
- To weigh the evidence on their effects or suspected effects on the health of test animals, and human and animal consumers;
- To determine where statutory measures might be needed to regulate their presence in food and to propose practical measures that can work in the South African grain storage and trading system;
- To consider means other than legislative regulation to deal with any real problem;
- To consider the practical application of a regulatory system.

The study is based on an analysis of the knowledge available in the published scientific literature, and surveys of mycotoxins in maize carried out by the South African Maize Board, which existed between 1939 and 1997 to administer a marketing scheme for maize. The information was used to address a number of specific objectives, listed below. First, the abstracts, or full papers of more than 1 500 published papers, a few selected textbooks, conference proceedings and web pages were obtained that deal with the mycotoxins involved, and related issues. The references, with authors, title, source, keywords and a hyperlink where appropriate, were incorporated in a database to enable quick and easy searches for papers on any given topic. Each objective was then dealt with individually. Lastly, this thesis was compiled from the results of the analyses of data related to each of the various objectives.

1.4. Objectives

Based on the broad purposes of the study, specific objectives were formulated. The objectives were to:

- Gather information on regulatory/advisory/recommended maximum tolerable levels (MTLs) of AFLA, FBs, DON, ZEA, NIV, T-2, MON, DAS and AME in maize, wheat and grain sorghum and their products intended for human and animal consumption in the USA, Europe, Canada, Australia, Japan, Africa, China and other Asian countries. More specifically, the grains and grain products the indicated MTL applies to, whether the MTL indicated is regulatory, advisory, or recommended, the known effects of each mycotoxin on humans and animals, and which mycotoxins are considered to be carcinogens, and which are not, needed to be indicated.

- Overview categories of carcinogens of the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) and of the mycotoxins considered being carcinogens.

- Overview the relationship between the FBs and oesophageal cancer (OC) in SA, China, France, Iran & USA.

- Overview other factors implicated in OC.

- Overview toxicological studies with the mycotoxins listed above in humans and animals.

- Overview the aetiology of liver, kidney and brain cancer in SA in relation to the occurrence of the mycotoxins listed above.

- Overview the aetiology of Neural Tube Defects in SA in relation to the occurrence of the mycotoxins listed above.

- Overview the occurrence of mycotoxins in SA grains, grain products, and the possible risks of natural levels to consumers.

- Estimate the highest MTLs that can be allowed in SA for the mycotoxins listed above, without jeopardizing the safety of consumers.

- Discuss the probable implications of existing and newly proposed MTLs for the local grain milling industry, and for major grain trading partners on international trade in grains and grain products, with reference to naturally occurring levels of the mycotoxins listed above.

- Overview available test methods for the mycotoxins listed above in grains and grain products.

- Recommend test methods, sampling methods and testing procedures to be considered for adoption by the grains industry in South Africa, together with MTLs for the mycotoxins listed above.

2. Literature survey

2.1. Regulatory/advisory/recommended levels of important mycotoxins in maize, wheat and grain sorghum and their products intended for human and animal consumption in various countries

By 1995, data on the MTLs for mycotoxins for 90 countries were available. Some 77 countries have enacted or proposed regulations for control of mycotoxins in food and/or animal feed (Van Egmond, 1993; 1995a; 1995b; Anonymous, 1997). These have primarily been aimed at the aflatoxins (AFLA), but in 15 countries limits also apply to ochratoxin A (OA), PAT, ZEA, DON and a few others. Some 13 countries were known to have no regulations concerning MTLs for mycotoxins in food or feed, and of 40 more, mainly in Africa, no data were available and it is not known whether they have regulations or not (Anonymous, 1997). In this section, regulatory, advisory or recommended limits for mycotoxins are overviewed.

2.1.1. Explanation of terminology as used

Regulatory MTLs are fixed by legislation and state the substances concerned, the MTL in specified commodities, and the intended uses of the commodities. Sampling and testing methods are sometimes specified, as is the interpretation of results. The point between field and final consumption at which the MTL applies can be specified, or implied. Ideally, the steps permissible to allow utilization of commodities in which MTLs are exceeded should also be outlined but are often lacking.

Advisory MTLs, also called 'guidance levels', are officially published by a country's health authorities, but are not binding on the authorities or on industry. The purpose is to invite comment from interested parties, ostensibly with a view of introducing suitable regulatory limits at an appropriate stage in the future.

Recommended MTLs are levels recommended by knowledgeable scientists, but which have not been officially adopted or publicly supported by health authorities.

The overriding consideration when recommending an MTL is usually to recommend a level that will be safe for humans, with little consideration for practical aspects affected by the MTL.

On the one hand, recommendations are based on an exposure assessment, where the probable daily intake (PDI) of the population is estimated on the grounds of the levels of the substance occurring in foodstuffs and consumption of the contaminated foodstuffs. On the other hand, it is based on a hazard assessment, where the hazard to humans is estimated from toxicological studies in experimental animals, extrapolated to humans, with a safety factor of 100 to 1 000 for toxins, and 1 000 to 5 000 for carcinogens (Stoloff *et al*, 1991; Van Egmond, 1993; 1995a; 1995b, Anonymous, 1997; Marasas, 1997). Where available, observations of suspected effects on specific communities, such as known cases of human intoxication together with the levels of occurrence of the substance(s) in foods at the time, are also used for the hazard assessment.

MTLs for animal feeds are established much more easily through direct toxicological studies on the animal species affected.

2.1.2. Existing limits for aflatoxin

AFLA are toxic to animals, particularly poultry, and are also carcinogenic in many test animals. It is the most potent carcinogen in rats, causing liver cancer. Mice are much less susceptible to the carcinogenic effects of AFLA, and other substances are more potent carcinogens than AFLA in mice. In humans, AFLA are listed by the International Agency for Research on Cancer (IARC) of the World Health Organisation (WHO) of the United Nations (UN) as a Group 1 substance (confirmed human carcinogen) (see section 2.2.1). It is believed that AFLA, linked with hepatitis B and hepatitis C virus (HBV and HCV) infection, are the main cause of liver cancer in humans in many parts of the world (e.g. IARC, 1993; JECFA, 1998). There are, however, also confounding factors and some contradictory evidence concerning the importance of AFLA in liver cancer in humans (e.g. Dhir & Mohandas, 1998) and some scientists remain unconvinced – see section 2.5.2.2.4. Worldwide, AFLA are the most regulated of all the mycotoxins, more than 77 countries having adopted

regulatory AFLA levels in unprocessed grain, nuts, feed and food. A few examples are presented below to demonstrate the general trend.

2.1.2.1. USA

The Food and Drug Administration (FDA) regulates the interstate shipment of corn (maize) and action levels for AFLA in maize, various nuts, oilcake and animal feeds. AFLA is just one of many listed substances of which contamination of food and feed is considered 'unavoidable'. The following is a quote from a publication on the Internet at http://vm.cfsan.fda.gov/~lrd/fdaact.html (Anonymous, 2000a):

> "Action levels for poisonous or deleterious substances are established by the FDA to control levels of contaminants in human food and animal feed.
>
> Action levels and tolerances are established based on the unavoidability of the poisonous or deleterious substances and do not represent permissible levels of contamination where it is avoidable. The blending of a food or feed containing a substance in excess of an action level or tolerance with another food or feed is not permitted, and the final product resulting from blending is unlawful, regardless of the level of the contaminant.
>
> Action levels and tolerances represent limits at or above which FDA will take legal action to remove products from the market. Where no established action level or tolerance exists, FDA may take legal action against the product at the minimal detectable level of the contaminant.
>
> The action levels are established and revised according to criteria specified in Title 21, Code of Federal Regulations, Parts 109 and 509 and are revoked when a regulation establishing a tolerance for the same substance and use becomes effective."

For AFLA in food and feed, the FDA has set the action levels in the USA (Anonymous, 2000a) presented in Table 1.

Table 1 - FDA action levels for aflatoxins in food and feed in the USA

Commodity	Action Level (ng/g)	Reference
Animal Feeds		
Corn and peanut products intended for finishing (i.e., feedlot) beef cattle	300	CPG 683.100
Cottonseed meal intended for beef, cattle, swine, or poultry (regardless of age or breeding status)	300	CPG 683.100
Corn and peanut products intended for finishing swine of 100 pounds or greater	200	CPG 683.100
Corn and peanut products intended for breeding beef cattle, breeding swine, or mature poultry	100	CPG 683.100
Corn, peanut products, and other animal feeds and feed ingredients but excluding cottonseed meal, intended for immature animals	20	CPG 683.100
Corn, peanut products, cottonseed meal, and other animal feed ingredients intended for dairy animals, for animal species or uses not specified above, or when the intended use is not known	20	CPG 683.100
Brazil nuts	20	CPG 570.200

Foods	20	CPG 555.400
Milk	0.5 (AFM_1)	CPG 527.400
Peanuts and Peanut products	20	CPG 570.375
Pistachio nuts	20	CPG 570.500

It is important to note, however, that the FDA does not have direct authority over maize for export or maize that remains solely and exclusively in intrastate commercial channels. AFLA occurs regularly and sometimes at very high levels in maize in all southeastern Corn Belt states, particularly when droughts occur during the growing season. AFLA is most prevalent in Texas and Georgia. Texas, and probably also other states, has its own prescriptions of how maize should be handled in which FDA action levels for AFLA are exceeded. This also allows blending (Krausz, 1998, accessed September 2000). (Unfortunately, subsequent efforts to access the URL where this information was published were unsuccessful and gave the following message: "HTTP Error 403 – Forbidden. Internet Explorer").

In Texas,

> "Aflatoxin-contaminated corn may legally be blended with less contaminated corn if the concentration of aflatoxin is not greater than 500 parts per billion (ppb) prior to blending. The contaminated corn cannot be blended with corn containing greater than 20 ppb of aflatoxins. The blending process must reduce the aflatoxin concentration to 200 ppb or less, and then the blended corn can ONLY be used for feeder lot cattle. The blended grain can only be used in Texas and cannot enter interstate transport. Any attempts at blending must be preceded by a permit and verification by the Office of the Texas State Chemist"

(Krausz, 1998).

And further on:

> "Aflatoxin -contaminated corn may be legally ammoniated in Texas if the initial aflatoxin level does not exceed 1 000 ppb. The ammoniation process must reduce the aflatoxin level to 200 ppb or less, and the ammoniated corn must be used only for feeder lot cattle. If it is reduced to 50 ppb or less, it can be used for deer corn. The ammoniated corn must be used in Texas and cannot enter interstate transport.

> Any attempts at ammoniation must be preceded by a permit and verification by the Office of the Texas State Chemist"

(Krausz, 1998).

2.1.2.2. Europe

The European Union has regulations setting MTLs for aflatoxin B_1 (AFB_1) in feedstuffs, ranging from 5 ng/g AFB_1 in 'complementary feedstuffs', to 200 ng/g in raw feedstuff materials, such as groundnuts and groundnut products, various other oilseeds and their products, and maize and maize products (Anonymous, 1997). In addition, all European countries have regulatory MTLs for AFLA in foods or in many cases for AFB_1 only. For example, an MTL of 5 ng/g AFB_1 in the edible parts of pistachio nuts applies in the Netherlands (Scholten & Spanjer, 1996). In all foods in Germany a maximum of 4 ng/g of AFB_1, aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2) is allowed, of which not more than 2 ng/g may be AFB_1 (Anonymous, 1997).

2.1.2.3. Canada

In Canada, regulatory MTLs of 15 ng/g of AFB_1, AFB_2, AFG_1 and AFG_2 applies to nuts and nut products for human consumption, and of 20 ng/g of all AFLA to animal feeding stuffs. A zero tolerance of all mycotoxins applies to feedstuffs for reproducing animals (Anonymous, 1997).

2.1.2.4. Australia

An MTL of 5 ng/g AFB_1, AFB_2, AFG_1 and AFG_2 applies to all foods, and an MTL of 15 ng/g AFB_1, AFB_2, AFG_1 and AFG_2 applies to peanut butter, nuts and the nut proportion of products (Anonymous, 1997).

2.1.2.5. Japan

An MTL of 10 ng/g AFB_1 applies to all foods, and an MTL of 1 000 ng/g AFB_1 applies to imported peanut meal for use in animal feeds (Anonymous, 1997).

2.1.2.6. China

MTLs varying between 5 and 20 ng/g AFB_1 apply to cereals, nuts and oils in foods. In cow milk and in milk products, calculated on the basis of milk, a maximum of 0.5 ng/g AFB_1 is allowed. In various feeds and feed components, a maximum varying between 10 and 50 ng/g AFB_1 is allowed (Anonymous, 1997).

2.1.2.7. Other Asian – India

An MTL of 30 ng/g (30 ng/g) of AFB_1 applies to maize, herbs, seeds and groundnuts intended for human consumption in India (Anonymous, 1997). However, according to one study, this level was exceeded in 21% of groundnut samples and 26% of maize samples analysed (Vasanthi & Bhat, 1998). Based on their results, the authors of this report calculated ingestion (PDI) of AFLA by the Indian population to be in the range of 4-100 ng/kg body weight/day, or between 280 and 7 000 ng/day for a 70-kg person. It was therefore obvious that routine monitoring does not take place in India and that consignments in which the legal limit is exceeded, are not removed from use, or redirected to other than human uses.

In peanut meal intended for export as a feed component, an MTL of 120 ng/g AFB_1 applies (Anonymous, 1997).

2.1.2.8. African countries

Only 8 African countries are known to have regulations for AFLA in food and/or feed. These are summarized in the Table 2, adapted from Anonymous (1997):

The FAO compendium (Anonymous, 1997) from which these figures were extracted, aimed to reflect the position as it was in 1995. However, during their survey, no new information could be obtained for a number of countries, and therefore the situation for Kenya as it stood in 1981, and for Malawi, Nigeria and Senegal as it stood in 1987 was given. The MTL in animal feeds in South Africa were not included in the compendium and were obtained from the Animal Feed Manufacturers Association (AFMA) in South Africa.

Table 2 - MTLs for aflatoxins in food and feed in African countries

Country	Commodity	MTL (ng/g)	AFLA type	MTL basis
Ivory Coast	Feedstuffs	100	B_1, B_2, G_1, G_2	Reg[1]
	Mixed feeds	10	B_1, B_2, G_1, G_2	Reg
	Mixed feeds: pigs/poultry	38	B_1, B_2, G_1, G_2	Reg
	Mixed feeds: ruminants	75	B_1, B_2, G_1, G_2	Reg
	Mixed feeds: dairy cattle	50	B_1, B_2, G_1, G_2	Reg
Egypt	Peanuts and products; oil seeds and products; cereals and products (foods)	10	B_1, B_2, G_1, G_2	Reg
		5	B_1	Reg
	Maize (food)	20	B_1, B_2, G_1, G_2	Reg
		10	B_1	Reg
	Starch and derivatives (food)	0	B_1, B_2, G_1, G_2	Reg
		0	B_1	Reg
	Milk, dairy products	0	M_1, M_2, G_1, G_2	Reg
		0	M_1	Reg
	Animal and poultry feeds	20	B_1, B_2, G_1, G_2	Reg
		10	B_1	Reg
Kenya (1981)	Peanuts and products, vegetable oils (food).	20	B_1, B_2, G_1, G_2	Reg

Malawi (1987)	Peanuts for export (food).	5	B_1	?[2]
Nigeria (1987)	All foods	20	B_1	?
	Infant foods	0	B_1	?
	Milk	1	M_1	?
	Feedstuffs	50	B_1	?
Senegal (1987)	Peanut product feeds	50	B_1	Reg
	Peanut product feed components	300	B_1	Reg
South Africa	All foods	10	B_1, B_2, G_1, G_2	Reg
		5	B_1	Reg
	Feed components	50	B_1, B_2, G_1, G_2	Reg
	Mixed feeds for beef cattle, sheep and goats	50	B_1, B_2, G_1, G_2	Reg
	Mixed feeds for lactating cows, swine, calves, lambs	20	B_1, B_2, G_1, G_2	Reg
	Mixed feeds for unweaned piglets, broilers and pullets	10	B_1, B_2, G_1, G_2	Reg
	Mixed feeds for trout	0	B_1, B_2, G_1, G_2	Reg
Zimbabwe	Foods	5	B_1	Reg
		4	G_1	Reg
	Groundnuts, maize, sorghum	5	B_1	Reg

	4	G_1	Reg
Feedstuffs for dairy animals.	?	B_1, B_2, G_1, G_2	?
Poultry feed	10	B_1, B_2	?

Information from Anonymous (1997)

[1]Reg – MTL set by statutory regulation or equivalent

[2]? = Not known

2.1.3. Existing limits for fumonisins

So far, three countries have formulated MTLs of one kind or another for FBs. In Switzerland a regulatory level has been enacted, in the USA, the FDA has recently published guidance (or advisory) levels, and in South Africa a recommended level has been proposed.

2.1.3.1. Switzerland

Switzerland is the only country that has so far adopted a legislative regulatory limit for FBs in food, where an MTL of 1 µg/g (1 000 ng/g) in maize products applies. This level was chosen arbitrarily and is not based on scientific consideration (Zoller *et al*, 1994).

2.1.3.2. USA

The FDA provided guidelines for FB levels in food and feed since 1993 (Anonymous 2000b; 2000c; 2000d). In June 2000 the FDA published the following draft guidance limits for FBs for comment that was to be filed by 7 August 2000 (Anonymous 2000b):

"Human Foods

Product	Total fumonisins (FB_1+FB_2+FB_3)
Degermed dry milled corn products (e.g., flaking grits, corn grits, corn meal, corn flour with fat content of < 2.25 %, dry weight basis)	2 µg/g
Whole or partially degermed dry milled corn products (e.g., flaking grits, corn grits, corn meal, corn flour with fat content of ≥ 2.25 %, dry weight basis)	4 µg/g
Dry milled corn bran	4 µg/g
Cleaned corn intended for masa production	4 µg/g
Cleaned corn intended for popcorn	3 µg/g

Animal Feeds

Corn and corn by-products intended for:	Total FBs (FB_1+FB_2+FB_3)
Equids (horses, donkeys, etc) and rabbits	5 µg/g (no more than 20% of diet)[1]
Swine and catfish	20 µg/g (no more than 50% of diet)[1]
Breeding ruminants, breeding poultry and breeding mink[2]	30 µg/g (no more than 50% of diet)[1]
Ruminants ≥3 months old raised for slaughter and mink being raised for pelt production	60 µg/g (no more than 50% of diet)[1]

Poultry being raised for slaughter	100 µg/g (no more than 50% of diet)[1]
All other species or classes of livestock and pet animals	10 µg/g (no more than 50% of diet)[1]

[1]Dry weight basis

[2]Includes lactating dairy cattle and hens laying eggs for human consumption"

The FDA prepared two background papers (Anonymous, 2001b; 2001c) to support their "Guidance for Industry: Fumonisin Levels in Human Foods and Animal Feeds" (Anonymous, 2001a). The first, entitled "Background Paper in Support of Fumonisin Levels in Corn and Corn Products Intended for Human Consumption" (Anonymous, 2001b) was prepared by the FDA Center for Food Safety and Applied Nutrition (CFSAN). The second, entitled "Background Paper in Support of Fumonisin Levels in Animal Feeds" (Anonymous, 2001c) was prepared by the FDA Centre for Veterinary Medicine (CVM). The contents of these papers will be dealt with in full detail in Section 2.5.3.1. In the paper on human foods (Anonymous, 2001b), the FDA concludes that:

> "Currently, the available information on human health effects associated with FBs is not conclusive. However, based on the wealth of available information on the adverse animal health effects associated with FBs (discussed in this document and in the document entitled "Background Paper in Support of Fumonisin Levels in Animal Feed" prepared by FDA's CVM), FDA believes that human health risks associated with FBs are possible."

The apparent anomalies in the MTLs for humans compared to that for equids and rabbits will be discussed in Section 4.6.3.2.2.

2.1.3.3. South Africa - Recommended level for fumonisins in maize

At the Fifth European *Fusarium* Seminar in Hungary, Prof WFO Marasas of the South African Medical Research Council (MRC) recommended a tolerance level of 0.100 to 0.200 µg/g (100 – 200 ng/g) for FBs in maize in South Africa. This followed a similar recommendation by Gelderblom (1996). Marasas based his recommendation on an assessment of human exposure to FBs and a hazard assessment, using toxicology data on rats. The daily intake of maize products in rural and urban areas in South Africa respectively was taken as 460 g, and 276 g per 70 kg person per day (Marasas, 1997). FB content of maize meal was taken on average as 0.3 µg/g (see Section 4.1 for mycotoxin levels in SA grain and grain products). The no observed adverse effect level (NOAEL) in long term studies in rats has been estimated at 800 µg/kg body weight, to which was applied a safety factor of 1 000. This gave the calculated tolerable daily intake (TDI) of FBs in humans as 0.8 µg/kg body weight/day. This figure translates to an MTL in maize products of 122 ng/g for rural people and to 202 ng/g for urban people (Gelderblom *et al*, 1996; Marasas, 1997). The safety factor of 1 000 was arbitrarily chosen as being the borderline value for differentiating between toxic and carcinogenic effects. As a rule of thumb, a safety factor of 100 to 1 000 is applied to toxins when extrapolating from animal data to humans, and 1 000 to 5 000 to carcinogens. The safety factor is increased if there are many uncertainties about the effects that the substance may have on humans and decreased with less uncertainty (Kuiper-Goodman, 1995; 1999). FBs are considered as being non-genotoxic carcinogens, and 'weak' cancer initiators (Gelderblom *et al*, 1996).

2.1.4. Existing limits for deoxynivalenol

The MTLs of all countries known to have MTLs for DON are listed in Table 3.

The 5 ng/g given for feedstuffs in Romania (Anonymous, 1997) is probably an error, because it is well below the minimum detectable limit for DON and is more likely to be 5 µg/g (5 000 ng/g).

Table 3 - Details of all countries known to have MTLs for deoxynivalenol

Country	Commodity	MTL ng/g	MTL basis
Austria	Wheat, rye (food)	500	Reg[1]
	Durum wheat (food)	750	Reg
Canada	Uncleaned soft wheat	2 000	Reg
	Mixed feeds for cattle, poultry	5 000	Reg
	Mixed feeds for swine, calves, lactating dairy animals	1 000	Reg
Romania	All feedstuffs	5	Reg
Russia	Cereals, flour, wheat bran (food)	1 000	Reg
USA	Finished wheat food products (food)	1 000	Reg
	Grains and grain by-products for cattle older than 4 months and chickens (not more than 50% of diet)	10 000	Reg
	Grains and grain products for dairy cattle (not more than 40% of diet)	5 000	Reg
	Grains and grain products for swine (not more than 20% of diet)	5 000	Reg

Information from Anonymous (1997)

[1]Reg – MTL set by statutory regulation or equivalent

2.1.5. Existing limits for zearalenone

The MTLs of all countries known to have MTLs for ZEA are listed in Table 4.

Table 4 - Details of all countries known to have MTLs for zearalenone

Country	Commodity	MTL ng/g	MTL basis
Austria	Wheat, rye (food)	60	Reg[1]
	Durum wheat (food)	60	Reg
Brazil	Maize (food)	200	?[2]
France	Cereals, vegetable oils (food)	200	Reg
Romania	All foods	30	?
Russia	Cereals, flour, wheat bran (food)	1 000	Reg
	Leguminous, protein isolates and concentrates, vegetable oil (food)	1 000	Reg
	Nuts (kernel) (food)	1 000	Reg

Information from Anonymous (1997)

[1]Reg – MTL set by statutory regulation or equivalent

[2]? = Legal basis not known

2.1.6. Existing limits for diacetoxyscirpenol

Israel is the only country to have enacted a MTL for DAS, where an MTL of 1 000 ng/g applies to grain intended for animal feed (Anonymous, 1997). The legal basis of this MTL is, however, not clear.

2.1.7. Existing limits for T-2 toxin and HT-2 toxin

All countries with MTLs for T-2 toxin (T-2) or HT-2 toxin (HT-2) (Anonymous, 1997), are listed in Table 5. HT-2 is chemically closely related to T-2.

Table 5 - Details of all countries known to have MTLs for T-2, or HT-2 toxin

Country	Commodity	MTL ng/g	MTL basis
Canada	Mixed feeds for cattle and poultry (HT-2)	100	?[1]
	Mixed feeds for swine, calves and lactating dairy animals (HT-2).	25	?
Israel	Grain intended for animal feed (T-2).	100	?
Russia	Cereals, flour, wheat bran (food) (T-2).	100	Reg[2]

Information from Anonymous (1997)

[1]? = legal basis not known

[2]Reg – MTL set by statutory regulation or equivalent

2.1.8. Existing limits for other mycotoxins

No country has MTLs for NIV, MON or AME (Anonymous, 1997).

Mycotoxins not included in this study, but for which one or more countries have MTLs, are listed in Table 6.

Table 6 - Mycotoxins not included in this study for which some countries maintain MTLs

Mycotoxins	Countries	Commodities[1]	MTL - range
OA	Austria, Brazil, Czech Republic, Denmark, France, Greece, Israel, Romania, Sweden, Switzerland and Uruguay	Wheat rye, durum wheat, rice, barley, beans, maize, pig kidneys, raw coffee beans.	2 ng/g-300 ng/g
PAT	Austria, Czech Republic, Finland, France, Greece, Norway, Romania, Russia, South Africa, Sweden, Switzerland, and Uruguay	Apples, apple juice, apple products, fruit juice, canned fruit, canned vegetables	20 ng/g-50 ng/g
Phomopsin	Australia	All foods	5 ng/g
Chetomin	Romania	All feedstuffs	0
Stachyobotryotoxin	Romania	All feedstuffs	0

Information from Anonymous (1997)

[1]Mostly specific commodities are listed here for brevity and non-specific denominations, such as 'infant foods', 'cereal products', 'fruit juice' and 'feedstuffs' have been omitted from the list, except where only one country has an MTL.

2.2. Overview of the Groups of carcinogens of the International Agency for Research on Cancer (IARC) and mycotoxins considered carcinogens

2.2.1. Classification of carcinogens

The International Agency for Research on Cancer of the World Health Organisation (IARC) classifies substances and activities evaluated for carcinogenicity in humans into five groups. The National Toxicology Program (NTP), in the USA Government's Annual Report on Carcinogens makes a similar classification. The categories of carcinogens that are distinguished in these lists are (IARC, 2001; National Toxicology Program, 1991):

Group 1: Substances for which there is sufficient evidence for a causal relationship with cancer in humans (confirmed human carcinogen).

Group 2A: Substances for which there is a lesser degree of evidence in humans but sufficient evidence in animal studies, or degrees of evidence considered appropriate to this Group, e.g. unequivocal evidence of mutagenicity in mammalian cells (probable human carcinogen).

Group 2B: Substances for which there is sufficient evidence of carcinogenicity in animal tests, or degrees of evidence considered appropriate to this Group (possible human carcinogen).

Group 3: Substances which are unclassifiable as to their carcinogenicity to humans, but which are suspected to be carcinogenic in humans and for which assessment evidence is 'limited' (suspected carcinogen).

Group 4: Substances probably not carcinogenic to humans.

2.2.2. Common substances and mycotoxins considered carcinogens

2.2.2.1. Group 1 - confirmed human carcinogens

Listed in this Group are 63 agents and groups of agents, 12 mixtures, and 12 exposure circumstances (activities). Included in the list are, amongst others:

- Alcoholic beverages;
- Benzene;
- Boot and shoe manufacture and repair;
- Coal tar;
- Combined oral contraceptives and sequential oral contraceptives;
- Furniture and cabinet making;
- Iron and steel founding;
- Occupational exposure as a painter;
- Oestrogen replacement therapy;
- Oral contraceptives, combined;
- The rubber industry;
- Salted fish (Chinese style);
- Solar radiation;
- Tobacco smoke; and
- Wood dust.

All of these are used or practiced everyday and many could probably be considered as carrying a very low risk of causing cancer. (See Section 2.2.3 for a list of the factors determining risk). Some well-known potent carcinogens are also included in this list. Inclusion of a substance in any Group is purely on a qualitative basis as is declared in the Preface to the IARC document (IARC, 2001) and quantification of the risk involved is not depicted in any way whatsoever.

AFLA is currently the only mycotoxin included in this Group. See Section 2.5.2.2.3 for a discussion of AFLA as a carcinogen.

2.2.2.2. Group 2A - probable human carcinogens

Listed in this Group are 54 agents and groups of agents, five mixtures, and four exposure circumstances (activities). Everyday substances and activities included in the list are:

- Diesel engine exhaust;
- Glass manufacturing industry (occupational exposure);
- Art glass, glass containers and pressed ware (manufacture of);
- Hairdresser or barber (occupational exposure, probably dyes);
- Insecticide use (occupational);
- Maté drinking (hot);
- Petroleum refining (occupational refining exposures);
- Ultraviolet radiation: A, B and C including sunlamps and sunbeds.

No mycotoxins are included in this Group.

2.2.2.3. Group 2B - possible human carcinogens

Listed in this Group are 219 agents and groups of agents, 12 mixtures, and four exposure circumstances (activities). Some of the more common substances and activities included in the list are:

- Bitumens (extracts of steam-refined and air-refined bitumens);
- Bracken ferns;
- Carbon tetrachloride;
- Carpentry and joinery;
- Coffee (bladder);
- Dichlorvos;
- Diesel fuel (marine);
- Gasoline;
- Gasoline engine exhausts;
- Lead and lead compounds (inorganic);
- Man-made mineral fibres (glasswool, rockwool, slagwool, and ceramic fibres).
- Occupational exposures in dry cleaning;
- Pickled vegetables, traditional Asian;
- Saccharin;
- Textile manufacturing (occupational exposures);
- Welding fumes;
- Wood industries.

Fungus and mycotoxins included in this list are:

- Toxins derived from *Fusarium moniliforme*;
- Fumonisin B_1 (IARC, 2002; JECFA, 2002; see also Marasas *et al*, 2000)
- AFM_1;
- OA;
- Sterigmatocystin.

The inclusion of toxins derived from *Fusarium moniliforme* (=*verticillioides*) and fumonisin B_1 (FB_1) in this Group (IARC, 2002) is for a large part based on extensive work related to FB_1 and fumonisin B_2 (FB_2) by scientists of the South African MRC. Much of this work relate to possible links of the high OC incidence in areas of the Transkei with fungal infections and mycotoxins in maize grown by subsistence farmers, as well as extensive toxicological studies on animals. See Section 2.3.2 for more information.

2.2.2.4. Group 3 – suspected human carcinogens

This Group currently contains 483 agents and groups of agents, mixtures, and four exposure circumstances (activities). Mycotoxins included in this Group are toxins derived from *Fusarium graminearum*, *F. culmorum F. crookwellense* and *F. sporotrichioides*. The mycotoxins involved are not specifically listed and in their evaluation of the carcinogenicity of the mycotoxins concerned, the IARC (1993) previously found that inadequate data were available to do an evaluation.

2.2.2.5. Group 4 – Substances probably not carcinogenic in humans

Only one substance – caprolactam - is currently listed in this Group. Understandably, few studies are ever done with a purpose to establish the non-carcinogenicity of any substance, but the implication of having only one substance listed in this Group seems nonetheless to be that there is little certainty about the non-carcinogenicity in humans of any substance at all.

2.2.3. Determinants of risk

In the IARC Monographs the term 'carcinogenic risk' is taken to mean the probability – on a purely qualitative basis - that exposure to an agent could lead to cancer in humans (IARC, 1993). The determinants of the risk (or probability) are not defined and this could be seen as a shortcoming in the current approach applied by the IARC. However, it could be logically reasoned that the quantitative probability of suffering an adverse effect from exposure to any risk factor is determined by the interactive cumulative effect of a number of considerations. In the case of exposure to a carcinogen it could be reasoned that the quantitative probability of developing cancer is likely to be determined by:

- The **carcinogenic potency** of the substance relative to other carcinogens;
- The **susceptibility** of the species in general and the individual;
- The **intensity** of exposure, i.e. the dose of the substance;
- The **frequency** of exposure; and
- The **duration** of exposure.

In the IARC groupings of suspected human carcinogens quantatative risk is not determined and the Group in which a substance is categorized is meaningless with regard to quantatative risk. Inclusion of any substance in Group 1, for example, means that the listed substance is regarded as having been confirmed as a cause of cancer in (some) humans, but it does not imply anything about the degree of risk involved of it causing cancer in humans.

Classical risk assessment as applied by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) on the other hand, relies on a human exposure assessment and a hazard assessment to determine risk (WHO, 1987; KuiperGoodman, 1999; Marasas *et al* 2000; see also Section 3.7.1). The exposure assessment assesses the degree to which humans are exposed to a substance and the hazard assessment is based on toxicological studies in experimental animals and on a prediction of the toxicity to humans of the chemical in question from its chemical structure. To

minimize risk to humans when establishing tolerance limits in food for humans, JECFA typically applies a safety factor of 100 to 1 000 for toxins, and 1 000 to 5 000 for carcinogens when extrapolating the no observed adverse effect level (NOAEL) in animal studies (Kuiper-Goodman, 1995; 1999). In the case of carcinogens, this is done regardless of the Group in which the IARC has categorized the substance. In fact, the safety factor currently used depends on the amount of uncertainty remaining about the carcinogenicity of the substance in humans – the greater the uncertainty, the larger the safety factor used (Kuiper-Goodman, 1999). On this basis, uncertainty (JECFA) in Group 4 (IARC) > uncertainty in Group 3 > uncertainty in Group 2B > uncertainty in Group 2A > uncertainty in Group 1. From the point of view of consumers, who may unnecessarily face food shortages or high prices if unreasonably low MTLs are imposed because of greater uncertainty, this may seem illogical. A more appropriate system would be to use a larger safety factor with greater certainty that a substance is carcinogenic to humans. It could be particularly useful to quantify the risk involved.

An attempt to quantify risk is at the basis of the proposed U.S. Environmental Protection Agency carcinogen risk assessment guidelines, which employ a benchmark dose as a point-of-departure (POD) for low-dose risk assessment (Gaylor & Gold, 1998). When information on the carcinogenic mode of action for a chemical supports a nonlinear dose response curve below the POD, this dose may be divided by uncertainty (safety) factors to arrive at a reference dose that is likely to produce no, or at most negligible, cancer risk for humans. According to this approach, a risk index, the Possible Hazard Rodent Potency (HERP) index is calculated as a percentage from average daily human exposure to the substance, the dose equivalent in humans of the dose to rats, and rodent carcinogenic potency (Gold *et al*, 2002).

It could be even more valuable if epidemiological evidence is incorporated in a quantification of the risk. Currently, none of the approaches outlined above assesses carcinogenic risk on the basis of epidemiological indicators, in spite thereof that such indicators are the only available indicators of the effects of actual exposure on humans.

2.3. Overview of the literature on the relationship between the fumonisins and oesophageal cancer

2.3.1. The human oesophagus and carcinoma of the oesophagus

The oesophagus is the part of the gut between the pharynx at the back of the mouth cavity and the stomach. Its only function is to pass food along from the mouth to the stomach. While this is a simple function, progressed carcinoma of the oesophagus is a virtual death sentence. The description below of the structure of the oesophagus and of cancer of the oesophagus has been adapted from Warwick & Harington (1973).

The oesophagus is about 25 cm long and is lined with stratified epithelium beneath which are scattered mucous glands. Gastric type epithelium may be present in the lower portion. Surface cells are shed and replaced by cells in the basal layer. Cell division occurs in the deep layers, and here the cells are small and basophilic. As cells are displaced towards the lumen, they lose the ability to divide. Abnormally active cell division and growth in the basal layer can lead to development of tumours and early detection of such abnormal cell division is important for successful treatment. The oesophagus walls are thin, and although considerably distensible, they can easily be disrupted by certain pathological conditions. The oesophagus is divided into four sections, some portions are narrowed and others more dilated. Unrelated to the 'borders' between the four sections, there are four principal constrictions; foreign bodies can become lodged there and tumours, burns and pathological strictures show predilection for the constricted zones.

Various types of tumours occur in the oesophagus and there are certain differences in the tumour types that occur in the genders. However, squamous cell carcinoma is by far the most common form of cancer arising in the oesophagus in both males and females, although there are gender differences in incidence. Carcinoma of the oesophagus can occur in any part, but is most common in the lower and middle thirds worldwide. More than two-thirds of cases of OC in Africans are found in the middle third, compared to less than half in whites. Certain differences exist between races in the structure of the oesophagus, particularly the epithelial thickness of the oesophagus.

2.3.2. Incidence of oesophageal cancer in South Africa and its linking with fumonisins – a history of events

OC became a focus of scientific interest in South Africa after R J W Burrell reported a high incidence in the East London area. Burrell worked in the area over the period 1952-1956 (Warwick & Harington, 1973). Burrell and a long list of subsequent researchers carried out extensive studies of the problem. Every possible external or environmental cause was investigated, without discovering any clear, unequivocal factor as a cause for the disease. What transpired, was that the Butterworth/Centane area of the Transkei, with about 50 cases annually per 100 000 of the population was the area where the highest incidence rates in South Africa occurred. This area was described as the 'epicenter' of the disease, later called the 'high incidence area', or 'high rate area'. In contrast, in the Bizana area in Pondoland, northern Transkei, the incidence was quite low (see Fig. 1). At fewer than 10 cases per 100 000 of the population, it was considerably lower than the figure for the whole of South and southern Africa. This area became known as the 'low incidence area' in many studies where the Transkei was looked at in relative isolation from other parts of South Africa.

At the time, Burrell and others believed that the disease was of recent origin in the Transkei, with a sudden increase in prevalence in the local community at about the time of World War II. In other parts of Africa with high OC incidence, it was also believed that incidence rates in the 1930's to 1940's were negligible (Cook, 1971). On the other hand, as reported by Warwick & Harington (1973), some researchers recognized that OC has possibly been present at a high rate in parts of the Transkei for a long time, but the high incidence of tuberculosis and other chest diseases probably concealed it. Diseases such as pneumonia are known to be endemic in the area. OC was certainly discovered and correctly diagnosed more often after the fight against tuberculosis in the Transkei was intensified by the introduction of mobile X-ray units, which was made possible by the improvement of roads and health services in the area after World War II. Before, modern infrastructure in the Transkei was almost non-existent, consisting mainly of mission stations and trading posts. Many areas were completely isolated from facilities where the condition could be reasonably well recognized. In this respect, the report by MacCormick (1989) is interesting.

According to him, cancer of the oesophagus has previously been reported as an exceedingly rare tumour in the Kingdom of Lesotho. This is in marked contrast to the extremely high incidence in the neighbouring Transkei. During 1984, gastroscopy was used as a diagnostic tool in determining a more accurate estimation of the incidence of OC in Lesotho, and more specifically in the capital region of Maseru. The results of this study revealed that the incidence of this disease in Lesotho approaches that of the Transkei.

A wide range of possible external causes for OC was investigated in the Transkei over the last three decades, as was the case elsewhere in the world where the incidence is high – see Sections 2.3 and 2.4 for more details. Possible causes investigated included the occurrence of droughts in the area, farming practices, the smoking of tobacco and marijuana, the consumption of alcohol, the exposure of the population to chemicals such as nitrosamines known to produce OC in experimental animals, and many other possible factors. Some of these were found to relate to greater or lesser extent with the incidence of OC, others not (Warwick & Harington, 1973).

In 1971, Paula Cook reported a relationship between cancer of the oesophagus and the consumption of traditional beer brewed from maize (Cook, 1971). The relationship was strengthened by studies in Kenya and Uganda. In west Kenya, where there is a high incidence of OC, maize is used for brewing beer, while in Uganda, where the incidence of OC is low, sorghum, millet, banana and honey are used (Cook *et al*, 1971). Other workers found a relationship between OC and the tannins in red grain sorghums (Oterdoorn, 1985), which is still being followed up, but the maize lead was also followed up by further suggestions and investigations. In the Transkei, as elsewhere in South Africa and the rest of Africa, sorghum was traditionally used for brewing beer, but in the 1960's maize meal was often added, or sometimes used as the main starch component (Warwick & Harington, 1973).

From here on, the chain of events leading to the eventual implication of FBs in OC in Transkei is closely linked with the work of Prof Wally Marasas and his collaborators that commenced with research on a mycotoxicosis in horses.

Between 1971 and 1976, a group of South African scientists, which included Prof Marasas, were renewing investigations of a neurotoxic condition in horses (Kellerman

et al, 1972; Marasas *et al*, 1976) believed to be related to the use of feed components infected by *F. verticillioides*. This fungus is ubiquitous in maize all over the world, and horses in many countries were often affected when fed on maize, or feed containing maize. Maize bran or maize stalks, especially when visibly mouldy, caused symptoms in horses and the disease was generally known as mouldy corn disease, or corn stalk disease. Of course, when grain is visibly mouldy, several fungal species are normally present, and it is not always clear which of them are causing the symptoms, even if one predominates. At the time, little was known about the chemistry of the toxins produced by *F. verticillioides*. In laboratory tests on horses using pure cultures of the fungus as early as in the 1930's and 1950's, conflicting or negative results were obtained. However, the renewed investigations confirmed the work of Wilson & Maronpot (1971) and demonstrated unequivocally that the condition in horses was indeed caused by *F. verticillioides*, when fed experimentally on feed containing large quantities of pure *F. verticillioides* culture material. In particular the brain, but also other organs, such as the liver were affected. In the brain, the myelin sheaths around the axons of nerve cells in the white brain matter were broken down completely in places, leaving void spaces. The myelin sheaths normally contain a fatty material. In the gray brain matter, axons are not enclosed in myelin sheaths and except for one horse, no damage was apparent there (Marasas *et al*, 1976). In some of the horses, the parenchyma in parts of the liver was also destroyed and replaced by fibrous tissue. At the time, the chemicals produced by *F. verticillioides* that caused these aberrations were as yet unidentified. The condition was called leukoencephalomalacia or LEM (Wilson & Maronpot 1971; Kellerman *et al*, 1972; Marasas *et al*, 1976).

In 1975 Prof Marasas joined the National Research Institute for Nutritional Diseases of the South African MRC, and became involved in the investigations on the causes of high OC incidence rates in southern parts of the Transkei. Earlier suggestions by researchers in Africa (Cook, 1971; Cook *et al*, 1971; Cook & Collis, 1972) and elsewhere of a possible link with fungal infections and mycotoxin contamination of maize were then followed up. In their first survey of the area, the team of scientists from the MRC established that it was common practice for people in the Transkei to select apparently uninfected maize ears for making meal for cooking, whilst the visibly mouldy ears were used for feeding animals or brewing beer (Marasas *et al*, 1979b, Marasas *et al*, 1981). The reasons for the presence of so many mouldy maize

ears in the crop that selection became necessary are not mentioned in the published literature. It is not stated whether the main infection occurs in the field or during storage. Very little or no data are available from the literature on the moisture contents of maize at harvest and in storage in Transkei.

A series of surveys of the fungi and mycotoxins in maize grown on subsistence farms in Transkei was carried out, starting in 1976 (Marasas *et al*, 1979a; Marasas *et al*, 1979b; Marasas *et al*, 1981; Thiel *et* al, 1982; Rheeder *et al*, 1992). In the first survey, two 70 kg bags of the 1976 crop intended for human consumption were purchased from farmers, one from the high OC incidence area of Centane and Butterworth, and one from the low incidence area of Lusikisiki and Bizana (Marasas *et al*, 1979b). In their second survey, they collected visibly mouldy 'homegrown' maize ears of the 1977 crop from the storage cribs of about 50 subsistence farmer households, some in the high, and some in the low incidence area. Assumedly these ears would be rejected for grinding and would instead be used for making beer and animal feed.

The fungi in these sets of samples were then identified. In the main, three *Fusarium* species were found: *F. verticillioides*, *F. graminearum* and *F. subglutinans* (at the time, some of these carried different names). In the kernels, very small quantities of DON and somewhat more ZEA were found, but no T-2, nor DAS. There were no statistical differences between the two areas in the fungal infection rates, and in the mycotoxin contamination levels of the pooled maize ears of the 1977 crop, or the bags of the 1976 crop. However, subsamples of hand selected visibly infected kernels, contained statistically highly significantly higher levels of the two mycotoxins in the high incidence area, in spite thereof that the infection rate of the producing fungus, *F. graminearum,* in these subsamples was significantly lower. This means that in the high incidence area the fungus produced more toxins than in the low incidence area. In a follow-up study with these same samples, one of the *F. subglutinans* isolates from the high incidence area was found to be very toxic to experimental animals and produced an extraordinarily large quantity of MON in culture (Thiel *et al*, 1982). In 1984, Fusarin C was also found to occur naturally in a sample of mouldy maize collected in the Butterworth area in 1978 (Gelderblom *et al*, 1984). Fusarin C was found a potent mutagen in the Ames *Salmonella* microsome mutagenicity test, with mutagenic potency comparable to that of AFB_1 and sterigmatocystin. However, in

short-term carcinogenicity assays, as well as long-term trials in rats with *F. verticillioides* culture material that contained high levels of fusarin C, no evidence of the carcinogenicity of fusarin C could be found (Gelderblom *et al*, 1986; Jaskiewicz *et al*, 1987)

Wehner *et al* (1978) found DON, ZEA and MON not mutagenic in the Ames test, and these were therefore thought not to play a role in the occurrence of OC. The results nevertheless suggested that people in the high incidence area might be subjected to greater exposure to these mycotoxins and possibly to some unidentified ones as well.

In a third survey in 1979 (Marasas *et al*, 1979a; Marasas *et al*, 1979b; Marasas *et al*, 1981), samples from low, intermediate and high OC incidence areas in the Transkei were collected as soon as possible after harvest from two households at each of six localities in each of the three areas. From each household, one sample of apparently uninfected maize was collected at random, and one sample was selected from the storage crib of mouldy maize, giving a total of 36 samples of good maize, and 36 samples of mouldy maize. The intermediate incidence area referred to the 'localities with the lowest cancer rates in the Butterworth district'. The samples were analysed and the results were interpreted together with the results of the 1976 and 1977 mycological surveys for fungal infection rates.

The incidence of *F. verticillioides* in the two areas in 1976 and 1977, and in the three areas in 1979, was found to significantly correlate with the OC incidence in the different areas. This finding was emphasized in the report, as well as in subsequent publications (e.g. Rheeder *et al*, 1992). However, in the high incidence area, infection rates of *Geotrichum candidum*, certain members of the Mucorales, *Penicillium* spp and *Phoma sorghina* were also 2-3 times as high as in the low incidence area, but the significance was not analysed. No further comment was offered on these fungi in subsequent surveys. In 1975, in research on the aetiology of OC in north China, the presence of *Geotrichum candidum* was also reported in the food of high-risk groups and some experimental evidence of the co-carcinogenic properties of this fungus was presented (Coordinating Group for Research on Etiology of Esophageal Cancer in North China, 1975).

The FBs produced by *F. verticillioides* were chemically characterized in 1988 (Gelderblom *et al*, 1988), and the maize samples collected in the Transkei in 1985 and 1989 were analysed for the presence of FB_1 and FB_2, the two most abundant of some 28 FBs naturally produced by *F. verticillioides* (Sydenham *et al*, 1990a; 1990b; Rheeder *et al* 1992, Rheeder *et al* 2002).

In 1985/86, samples of maize ears were collected from 12 households in each of the high and low OC incidence areas of the Transkei (Sydenham *et al* 1990a; Rheeder *et al*, 1992). Again, one sample of the 'good' maize ears, and one of the visibly mouldy ears, stored separately at each household, were collected, to a total of 48 samples. The mean levels of FB_1 and FB_2 in the 'good' maize ears were statistically significantly higher in ears from the high incidence area than from the low OC incidence area. FB levels in mouldy maize were significantly higher in the high OC incidence area.

In 1989, eight samples of 'good', and seven of mouldy maize ears were collected from eight households in the low incidence area and six samples each of 'good' and mouldy ears from six households in the high incidence area of the Transkei (Rheeder *et al*, 1992). The fungal infection rates were found significantly higher in the high OC incidence area, but although the FB levels were numerically higher in maize from the high OC incidence area, the difference was not statistically significant. In the mouldy maize, the FB levels were significantly higher in the high incidence area.

To summarize the series of surveys, maize samples were collected from subsistence farmers in areas with high and low rates of OC in the Transkei in six seasons over the period of 1976-1989. The way in which samples were selected suggests a real possibility of bias. The most consistent difference in the mycoflora of the maize kernels was the significantly higher incidence of *F. verticillioides* in maize from the high- vs. the low-rate area. In the 1989 samples, the *F. verticillioides* infection rate of 'good' (apparently free of mould) maize kernels in the high- and low-rate cancer areas was 41.2 and 8.9%, respectively (significant at $P<0.01$), and 61.7 and 21.4% respectively, in visibly mouldy maize. Maize apparently free of mould is used as food, while visibly mouldy maize is used as animal feed and for brewing beer in both the high and low OC incidence areas. Significantly higher levels of both FB_1 and FB_2 were present in the mouldy samples from the high-rate OC areas. Some of the mouldy samples from the high-rate areas contained some of the highest levels of FB_1 (up to

117 520 ng/g, or 117.5 µg/g) and FB_2 (up to 22 960 ng/g, or 22.9 µg/g) yet recorded from naturally infected maize. The FB levels in good maize used for food were significantly higher in the 1985 samples in the high OC incidence area, but not in the 1989 samples. Both 1985 and 1989 when FBs in maize in the high and low incidence areas were determined, were good crop years in Natal (Mielieraad, 1986; 1991), probably high rainfall years.

Sammon (1992) carried out a case-control study of diet and social factors in OC in Transkei on 100 patients with OC and 100 controls matched for sex, age, and educational level. The significant risk factors found were: use of *Solanum nigrum* as a food (relative risk, 3.6), smoking (relative risk, 2.6), and use of traditional medicines (relative risk, 2.1). According to the results of his study, consumption of traditional beer was not a risk factor.

In a recent study, Rheeder & Marasas (1998) found very few isolates of *F. verticillioides* in soil samples and in plant debris from soil from natural grasslands and cultured maize fields in Transkei. Some statistically significant differences were found, including that fewer *F. verticillioides* isolates were found in Transkei soil than in soil samples from commercial maize producing areas in South Africa. However, the data give little indication whether the main fungal infection and mycotoxin production of the high mycotoxin levels in subsistence maize in Transkei might be occurring in the field or during storage.

Meanwhile, toxicological tests with *F. verticillioides* culture material as well as with FBs on horses and other experimental animals continued, which will be reported on in 2.5.3. These showed that FBs caused various serious health conditions in different farm animals, and that it is carcinogenic in rats. Hence, the possibility of health threats to humans is strengthened.

The correlation between the *F. verticillioides* infection rates of subsistence maize and OC incidence in the Transkei is impressive, although there is some doubt about possible bias in the sampling. The correlation between the FB levels in subsistence maize and OC incidence is based on very few samples and is less impressive. These findings remain purely circumstantial because no comparative estimate has been published of the actual quantities of FBs ingested by people in the high and low

incidence areas, as has been done in several countries for AFLA (Van Rensburg *et al*, 1990). For example, it was assumed in these surveys that all the maize in the diet of Transkeians comes from local subsistence farms, but much commercial maize are bought in other parts of South Africa for consumption in Transkei. Commercial mills in East London, about 110 km fom Butterworth, sell maize products throughout the eastern Cape, including Transkei. Furthermore, the method of sampling did not preclude all possibility of bias in the sampling. The number of samples analysed for FBs is extremely small if the results are to be extrapolated to the commercial maize industry. No apparent reason has been offered for *F. verticillioides* infection rates to be so consistently so much higher in the Butterworth/Centane area than in the Lusikisiki/Bizana area. This seems highly unusual compared to the commercial maize production areas of South Africa, where *F. verticillioides* infection rates in white maize (see Tables 22 through 26) vary much more widely between areas. In the commercial maize, where sampling was completely unbiased (see Section 3.1.2), the rank of any area could easily vary 3 to 6 places in only 6 seasons, except for the eastern Free State, which always occupied the lowest or second lowest rank over seasons. Several of these areas are much further apart, and the climate differences between them much larger than those between the north and the south of the Transkei. Nonetheless, similar surveys were conducted, and similar findings made in the LinXian area of China, where there is also an extraordinarily high incidence of OC (Chu & Li, 1994; Yoshizawa *et al*, 1994; Wang *et al*, 2000). Also, Shephard *et al* (2000) showed that 11 maize samples collected randomly in September 1998 from farmers' maize lots in the high OC incidence area of Mazandaran, north-east Iran, had FB levels ranging between 1.270 and 3.980 µg/g FB_1, between 0.190 and 1.175 µg/g FB_2, and between 0.155 and 0.960 µg/g fumonisin B_3 (FB_3). Eight samples from Isfahan - a lower OC incidence area further south - showed lower levels of between 0.010 and 0.590 µg/g FB_1, two samples contained FB_2 at 0.050 and 0.075 µg/g), and two samples contained FB3 at 0.050 and 0.075 µg/g). Of course, fumonisins might be only one of two or more co-factors for OC development. However, if the concerns above are unfounded and the relationship between OC incidence and FB levels in maize products holds true in regions so far apart as the Transkei, Iran and LinXian, the implications are as follows:

- 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 of people is more similar to that of people in the Transkei, than to the lifestyle of people in LinXian. (The recommended MTL for FBs in commercial maize products in South Africa must be at least partly based on a similar premise, since no other specific health effect in humans caused by FBs is evident at present – see Section 2.1.3.3 for details of the recommended MTL).

These implications are analysed in more detail in Sections 3.2. and 3.3.

2.3.3. World incidence of oesophageal cancer

Table 7 presents OC incidence rates for some of the 174 countries and regions for which data are available from the WHO (Ferlay *et al*, 1999). The following general trends can be observed from Table 7 and provide a good representation of all 174 countries/regions:

- 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. Information about differences in foods, eating habits and the FB content of grains in these regions could help to elucidate the role of extraneous factors in the development of OC;

- In Africa, OC incidence can vary markedly within relatively short distances (Cook, 1971);

- 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.

Table 7 - Age standardised incidence rate (World standard) per 100 000 of oesophageal cancer in 1990 in some countries

Country	Males	Females	M/F Ratio
More developed regions	6.39	1.30	4.92
Less developed regions	10.17	6.18	1.65
Switzerland	6.45	1.49	4.33
United Kingdom	8.01	4.12	1.94
Australia	4.43	2.39	1.85
USA	5.32	1.42	3.75
Kazakhstan	35.38	26.82	1.32
Turkmenistan	51.66	50.36	1.03
Iran	21.74	18.02	1.21
Uruguay[1]	14.76	5.85	2.52
Mexico[1]	3.34	1.33	2.51
Costa Rica[1]	3.99	1.47	2.71

Venezuela[1]	4.07	2.10	1.94
Puerto Rico[1]	9.35	2.46	3.80
Jamaica	8.71	3.27	2.66
France	10.95	1.12	9.78
Northern Africa	2.81	1.75	1.61
Southern Africa[1]	32.60	11.93	2.73
Western Africa	2.10	1.19	1.76
Eastern Africa[1]	12.55	5.35	2.35
Angola[1]	7.93	0.92	8.62
Namibia[1]	8.33	2.29	3.64
Algeria	0.50	0.87	0.57
Kenya[1]	20.17	2.93	6.88
Nigeria	2.32	1.55	1.50
Tanzania[1]	9.50	8.43	1.13
Mali	1.64	0.6	2.73
Malawi[1]	45.37	25.74	1.76
Zambia[1]	7.77	2.99	2.60
Botswana[1]	27.74	11.90	2.33
Lesotho[1]	27.74	11.90	2.33
South Africa[1]	33.73	12.36	2.73
Swaziland[1]	31.47	4.52	6.96

Mozambique[1]	11.65	4.96	2.35
Zimbabwe[1]	23.60	6.08	3.88
Peoples Republic of China	21.58	9.91	2.18
India	8.04	5.43	1.48

Data from Ferlay *et al* (1999)

[1]Countries and regions where maize is a staple

The very large differences between OC rates in African countries are particularly interesting. However, apart from aflatoxins (e.g. Hell *et al*, 2000 in Benin; Udoh *et al*, 1999 in Nigeria) data on the levels of mycotoxins in cereals in the rest of Africa are limited to a handful of reports. In western Kenya, Kedera *et al* (1999) investigated the incidence of *Fusarium* spp. and levels of FB_1 in maize, but they did not comment on the relationship with OC. OC incidence in Kenya is relatively high, particularly in western Kenya near Lake Victoria. To help elucidate the relationship between OC and consumption of staples, the average supply of sorghum, millet and maize per capita per year (calculated over the 4 years 1987 to 1990) can be taken as a rough estimate of consumption of the different grains (FAOSTAT Database – URL: http://apps.fao.org/page/collections?subset=agriculture) and correlated with OC incidence in the various countries. These figures are further analysed in Section 3.3.

2.4. Overview of the literature on other factors implicated in oesophageal cancer

2.4.1. The physiological basis of cancer development

Cherath (1999) describes a tumour as an uncontrolled growth of cells in the tissue of some organ in the body. It occurs where new cells, formed to replace spent tissue cells, fail to become transformed to specialised tissue cells with a specific function, and remain unspecialised cells, themselves forming more unspecialised cells in an uncontrolled fashion. The control over the normal replacement, growth and specialization of cells is lost because of the genetic make-up of the cell governing the physiological processes having become 'confused'. As a result, the formation of specific enzymes and other chemicals at specific stages through the cell formation and specialization process is incorrectly executed at some point in the process, sending inappropriate chemical signals for the next stage, so the process is incorrectly completed. A malignant tumour is one where tumour cells formed within a given tissue can be transferred to other parts of the body where they continue their uncontrolled growth.

The genetic make-up of a cell can be altered by a mutation caused by an extraneous chemical when it, or part of its molecule, binds to a part of the DNA material within the cell. The chemical nature of such extraneous chemicals determines their affinity for specific parts of DNA and hence the types of tumour they cause. Since the physiology of different animal species differs to greater or lesser extent, it appears that the results of tests on animals are not always exactly applicable to humans. For the same reason, it seems likely that the susceptibility of different animal species, including humans, to the effects of a chemical carcinogen will also differ.

The mutagenicity of chemical substances is tested in standardised tests using bacteria such as *Salmonella* sp (e.g. Gelderblom *et al*, 1984). However, not all chemicals that cause mutations in these tests are carcinogens in higher forms of life. Often carcinogens cause mutations and possibly tumours at low doses, but become toxic at higher doses, killing tissue, rather than disrupting the genetic make-up of cells.

A toxic effect might very well be broadly similar to a carcinogenic effect insofar that an extraneous chemical substance interferes with the chemical processes within cells of a tissue, causing malfunctioning of the normal physiological processes. In this case, however, the cells themselves, or cells in another organ, or the animal itself may die as a result of the interference, instead of it leading to uncontrolled cell multiplication taking place with absence of cell specialization.

Much significance has been attached in the literature to the statistical relationships that have been found in the Transkei and China between the OC incidence and the levels of *F. verticillioides* and FBs in maize. However, many other factors have also been found to have a relationship with OC. The following sections briefly overview some of these factors and some interrelationships.

2.4.2. Exposure to toxic/carcinogenic substances in food, water, or the environment

2.4.2.1. Exposure to nitrosamines

Craddock (1992) describes the nitrosamines and the nitrosamides as some of the most potent carcinogens known. These substances can initiate OC as well as various other cancers in experimental animals and several are listed as Group 1 carcinogens. Of the thousands of chemicals tested, the only compounds found potent carcinogens for the oesophagus are the *N*-nitrosamines. Many of these compounds are readily formed from common precursors in the environment (e.g. in food during its storage or preparation) and in vivo in the human stomach. Exposure is therefore likely to be ubiquitous. Although humans may be exposed to other oesophageal carcinogens these have yet to be chemically identified, and at present nitrosamines are the sole contenders for the role of initiators of OC in humans. Evidence suggests strongly that OC is initiated worldwide by nitrosamines, and promoted by secondary factors, the nature of which varies with the population concerned. Notable suspected OC promoters are alcohol in Europe and the USA, dietary deficiencies in China and Iran, and mycotoxins in South Africa. When several risk factors coincide in one locality, the result can be a very high incidence of OC, with no one major cause (Craddock, 1992).

Several nitrosamines such as methylbenzylnitrosamine (MBN) are often used to initiate cancer in experimental animals to test the cancer promoting properties of other substances, including mycotoxins. For example, in liver cells of rainbow trout (*Salmo gairdneri*) and channel catfish (*Ictalurus punctatus*), unscheduled DNA synthesis was induced in hepatocytes after exposure to dimethylnitrosamine, AFB_1, benzo(a)pyrene, and *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (Klaunig, 1984). Trout hepatocytes displayed a decrease in unscheduled DNA synthesis induction with AFB_1 with increased age of the cultures. However, unscheduled DNA synthesis induced by *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine remained constant throughout the culture period.

Toxicological studies (see Section 2.5.3 for references and detail) have found cancer-promoting characteristics by FB_1 in rat liver, where cancer was initiated by a nitrosamine. In oesophageal carcinogenesis, Wild *et al* (1997) tested the hypothesis that nitrosamines and FB_1 would interact by treating male rats with the known oesophageal carcinogen *N*-MBN and FB_1. The treatment groups were: Group 1, *N*-MBN (2.5 mg/kg) intraperitoneally twice per week from week 2 to 4 inclusive; Group 2, as for group 1 but in addition FB_1 (5 mg/kg) daily from weeks 1 to 5 inclusive by gavage; Group 3, FB_1 (5 mg/kg) alone daily from weeks 1 to 5 inclusive by gavage, and Group 4, vehicle treatment from week 1 to 5 inclusive. Two of 12 animals in Group 1 developed oesophageal papillomas and a further two had oesophageal dysplasia. Data were similar in Group 2, animals receiving both *N*-MBN and FB_1, with one of 12 animals having papillomas and three of 12 with dysplasia. Sphingolipid biosynthesis was affected in the kidney and slightly in the liver after FB treatment but not in the oesophagus or lung as determined by sphinganine:sphingosine ratios in urine and tissues. These data show that there is no synergistic interaction between *N*-MBN and FB_1 in the rat oesophagus when the two compounds are administered together. On the other hand, Carlson *et al* (2001) found that FB_1 promotes liver tumours in rainbow trout initiated by N-methyl-N'-nitroso-guanidine (MNNG).

N-MBN is a potent oesophageal carcinogen in rodents, and has been found as a dietary contaminant in certain areas of China where OC in humans is endemic (Morse *et al*, 1999). Human enzymes controlled by the P-450 gene have been found to activate the carcinogenic activity of *N*-MBN. Therefore, physiological studies have

demonstrated a more probable link in humans between nitrosamines and OC than between FB_1 and OC.

It has been suggested that certain alcoholic drinks in countries such as Malawi and Kenya, where OC incidence is high, may be contaminated with nitrosamines, creating a relationship with OC incidence (Warwick & Harington, 1973). 'Malawi gin', distilled from beer brewed from sugar, maize and maize husks, is one of these. The possibility therefore exists that alcoholic drinks could act as carriers for chemicals which may be injurious to the oesophagus, and in this way an explanation may be found for the synergistic effects of drinking and smoking in relation to the development of OC (see Section 2.4.2.6). However, Cook *et al* (1971) found no evidence for the presence of nitrosamines in alcoholic beverages in East Africa, down to a level of 100 ng/g.

In addition, dimethylnitrosamine occurs in the wild apple (bitter apple) *Solanum incanum* used in the Transkei to curdle milk (Du Plessis *et al*, 1969) in cooking, and on the umbilicus of newborns to assist healing (Warwick & Harington, 1973). Ritter (1955) relates the use of poultices or aqueous solutions made from the *umtuma* fruit (*S. incanum*) by Zulu herbalists and witch-doctors to remove external benign tumours. Du Plessis *et al* (1969) state that at least three different sorts of fruit are used in the Transkei as the source of juice to curdle milk. In a case control study in the Transkei, Sammon (1992) found that the significant risk factors associated with OC were use of *Solanum nigrum* as a food (relative risk, 3.6), smoking (relative risk, 2.6), and use of traditional medicines (relative risk, 2.1). Consumption of traditional beer was not a risk factor.

Marasas (2001, personal communication) is sceptical about these findings, and points out that fruit of *S. nigrum* (the common black nightshade - *umsobo*, nastergal), is widely used across South Africa to cook jam. No one has ever suggested that *S. nigrum* is carcinogenic in other parts of South Africa. He also points out that the results of Du Plessis *et al* (1969) are based on uncertain analytical methodology. No published data could be found that confirm or refute the Du Plessis *et al* (1969) results and this is still the only published report on the natural occurrence of nitrosamines in Transkei.

Nitrosamines are easily produced by the action of nitrous acid on secondary amines, and hence many candidates for reaction exist, including peptides and proteins. Nitrosamines are environmental contaminants in many parts of the world, they may be present in cigarette smoke, foodstuffs, constituents of plants, and they may be generated *in vivo* (Craddock, 1992). For example, Yang (1992) analysed a total of 391 gastric juice samples collected from inhabitants of Ji Yuan and An Shi counties, high and medium risk areas of OC in Henan province, China. *N*-nitrosodimethylamine, *N*-nitrosodiethylamine, *N*-methyl-*N*-benzylnitrosamine, *N*-nitrosopiperidine and unknown compounds were assayed in the fasting gastric juice. Among these nitrosamines, *N*-methyl-*N*-benzylnitrosamine, *N*-nitrosopyrrolidine and *N*-nitrosopiperidine were specific in inducing OC in animals. The amount of nitrosamines in the gastric juice collected from Ji Yuan County was higher than that from An Shi County. The exposure level of subjects from these two localities to nitrosamines was significantly different ($P<0.001$). There was a positive relationship between the nitrosamines exposure level and OC mortality rate. The amount of gastric *N*-nitrosamines from An Shi subjects as treated with vitamin C was reduced. Yang (1992) concludes that vitamin C can evidently inhibit *N*-nitrosamine formation in the stomach, thereby reducing the *N*-nitrosamines exposure level.

Case-control studies in Thailand (Mitacek *et al*, 1999) indicate that a high incidence of liver cancer in Thailand has not been associated with common risk factors such as HBV infection, AFLA intake and alcohol consumption. While the infestation by the liver fluke *Opisthorchis viverrini* accounted for the high risk in northeast Thailand, there was no such exposure in the other regions of the country where the incidence of liver cancer is also high. Case-control studies suggest that exposure to exogenous and possibly endogenous nitrosamines in food or tobacco and betel nut may play a role in the development of hepatocellular carcinoma, while *Opisthorchis viverrini* infestation and chemical interaction of nitrosamines may also be aetiological factors in the development of cholangiocarcinoma. Over 1800 samples of fresh and preserved food were systematically collected and tested between 1988 and 1996. All the food items identified by anthropological studies to be consumed frequently in four major regions of Thailand were analysed for volatile nitrosamines using gas chromatography combined with a thermal energy analyser. Relatively high levels of *N*-nitrosodimethylamine, *N*-nitrosopiperidine and *N*-nitrosopyrrolidine were detected in

fermented fish ("Plasalid"). *N*-nitrosodimethylamine was also detected at levels ranging from trace amounts to 66.5 ng/g in several salted and dried fish ("Larb-pla" and "Pla-siu"). *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine were frequently detected in several vegetables, particularly fermented beans ("Tau-chiau") at levels ranging between 1 and 95.1 ng/g and 0-146 ng/g, respectively. There is a distinct possibility that nitrosamines in Thai food play an important role in the aetiology of liver cancer in Thailand (Mitacek *et al*, 1999).

Pickled vegetables are consumed daily in the high-risk areas for OC in China. Ji & Li (1991) analysed the nitrosamine content of LinXian pickles and found trace amounts of six nitrosamines, with the highest concentrations being *N*-nitrosodimethylamine and *N*-nitrosodiethylamine (1.7 and 1.9 ng/g wet weight respectively). The average level of nitrosamine precursors, such as nitrate (111.22 mg/L), nitrite (0.152 mg/L) and secondary amines (4.223 mg/L), in pickled vegetables were also determined, and their pH values ranged from 3 to 5.

Lu *et al* (1980) tested two synthetic *N*-nitrosamines (*N*-3-methylbutyl-*N*-1-methyl acetonylnitrosamine and *N*-methyl-*N*-benzylnitrosamine), in *Salmonella typhimurium* strains TA1535 and TA100 in the presence of a liver postmitochondrial supernatant from Aroclor-treated rats. The two nitrosamines were previously isolated from maize bread which had been inoculated with moulds occurring in Linshien county, Northern China and subsequently nitrosated by sodium nitrite. They observed a concentration-dependent increase in the number of mutant colonies in both bacterial strains. The authors conclude that mutagenic *N*-nitrosamines may be present in foodstuffs that are consumed in Linshien County.

The IARC (1993) found sufficient evidence in humans for the carcinogenicity of Chinese-style salted fish, particularly with regard to nasopharyngeal and stomach cancer, and limited evidence in experimental animals for the carcinogenicity of Chinese-style salted fish. Hence, Chinese style salted fish has been categorised as a Group 1 human carcinogen. The IARC (1993) found inadequate evidence in humans for the carcinogenicity of other salted fish. The IARC cited several studies that investigated the levels of nitrosamines in salted fish. Nitrosamine levels varied from none detected to 388 ng/g in several samples of Chinese-style salted fish.

Subsequent to the IARC review, Lin *et al* (1997) tested 55 food samples in the diets of inhabitants of Nan'ao County in Guangdong Province, a high-risk area for OC in southern China. The food samples were tested for volatile *N*-nitroso compounds and their precursors. Five kinds of *N*-nitrosamines were detected. The average level was 312.0 ng/g (median). The total daily nitrosamines intake was 286.5 μg/70 kg person/day. The authors conclude that their study demonstrated that a relatively high content of volatile *N*-nitrosamines was present in the diet of people in the area.

In his review of the role of nitrosamines and nitrosamides in the aetiology of certain cancers including OC, Mirvish (1995) points out that nitrosamines require activation by cytochrome P-450 enzymes in the endoplasmic reticulum to give α-hydroxynitrosamines. These decompose spontaneously in successive steps to monoalkylnitrosamines, alkyldiazohydroxides and nitrogen-separated ion pairs. Alkyldiazohydroxides can alkylate nucleophiles directly after loss of water to give diazoalkanes. Some of these species alkalate DNA bases, especially at N-7 and O-6 of guanine and O-4 of thymine. O^6-Alkylguanines pair with thymine rather than cytosine and this produces G:C → A:T mutations that are thought to initiate carcinogenesis. Nitrosamides are converted to similar alkylating species by chemical non-enzymatic reactions. He continues that in rodents, nitrosamines principally induce tumours of the liver, oesophagus, nasal and oral mucosa, kidney, pancreas, urinary bladder, lung and thyroid, whereas nitrosamides induce tumours of the lymphatic and nervous systems, and, when given orally, of the glandular stomach and duodenum. The site of tumour induction depends on the *N*-nitroso compound, the rodent species and other factors. The diverse organ specificity of nitrosamines, which is evident even when they are administered at distant sites, suggests they could induce human cancer in these same organs. This specificity probably occurs because tissue-specific P-450 isozymes activate the nitrosamines, which alkylate DNA in the affected organ. With specific reference to OC, Mirvish (1995) says the following observations suggest that nitrosamines initiates squamous OC in humans:

- Squamous papillomas and carcinomas of the oesophagus are induced in rats by intraperitoneal injection of unsymmetrical dialkylnitrosamines such as methyl-*n*-amylnitrosamine, methylbenzylnitrosamine and methylbutylnitrosamine, and

by cyclic nitrosamines, such as *N*-nitrosonornicotine and *N*-nitrosopiperidine, but by almost no other compounds;

- *N*-nitrosonornicotine in tobacco is probably the initiator of OC caused by smoking and drinking;

- Consumption of mould infected maize that may generate OC-specific nitrosamines is associated with OC in South Africa and China; and

- Significant negative associations were found between OC incidence and ascorbic acid (vitamin C) consumption in South Africa, China and elsewhere.

Some studies have found links between infection of maize by *F. verticillioides* and nitrosamines. In a study of the occurrence of FBs in food in the counties of Cixian and LinXian, China, where high incidences of OC have been reported, Chu & Li (1994) analysed 31 maize samples collected from households for FB_1, AFLA, and total trichothecene mycotoxins. High levels of FB_1 (18 to 155 µg/g; mean, 74 µg/g) were found in 16 of the samples that showed heavy mould contamination. FB_1, at lower levels (20 to 60 µg/g; mean, 35.3 µg/g), was also found in 15 samples, collected from the same households that did not show any visible mould contamination. The levels of AFLA in the samples were low (1 to 38.4 ng/g; mean, 8.61 ng/g). High levels of total type-A trichothecenes were also found in the mouldy maize samples (139 to 2 030 ng/g; mean, 627 ng/g). Immunochromatography of selected samples revealed that these samples contained T-2, HT-2, iso-neosolaniol, monoacetoxyscirpenol, and several other type-A trichothecenes. The concentration of total type-B trichothecenes in 15 mouldy maize samples was in the range of 470 to 5 826 ng/g (mean, 2 359 ng/g). Five *F. verticillioides* strains, isolated from the mouldy maize produced high levels (3.7 to 5.0 mg/g) of FB_1 in maize in the laboratory. However, Chu & Li (1994) also found that these fungi were capable of forming various nitrosamines (5 to 16 µg per flask) in the presence of nitrate and precursor amines.

On a similar tack, looking at links between maize and nitrosamines, Singer & Ji (1987) investigated the possible origin of *N*-nitroso-*N*-(1-methylacetonyl)-3-methylbutylamine, a carcinogen identified in mouldy foods in LinXian County, Henan Province, China. They found that it might arise by the interaction of

isoamylamine, a decarboxylation product of leucine, and acetoin (3-hydroxy-2-butanone), a known constituent of maize. In their tests, oxidative nitrosation in dilute sulphuric acid led directly from the amino alcohol to the nitrosamino ketone. Mirvish (1995) states that in the high OC areas of South Africa, China and Italy, where maize products form the staple diet, OC may be initiated by methylalkylnitrosamines formed by in vivo nitrosation of methylalkylamines that may occur in *F. verticillioides*, a fungus common in maize.

In Africa, there is paucity of published information on the occurrence of nitrosamines in food, particularly dried fish, and on the relationship between cancer incidence, including OC, and consumption of cured fish. Sun dried fish is an important part of the diet around the great lakes of the African rift valley and Lake Victoria (*et al*, 1999; Costa-Pierce, 2001; Moelsae *et al*, 1999). Blowfly and other insect infestations, bacterial degradation and moulds are common in dried fish in Africa (Gitonga, 1998) and the author's personal observations).

There also is paucity of modern information on the occurrence of nitrosamines in food and drink in Transkei. For example, a literature search in January 2002 by means of the Cambridge Scientific Abstracts Database Service, of 10 databases using the search terms 'nitrosamine' and 'Transkei' produced no citations from any of the databases, whereas a search using the terms 'fumonisin' and 'Transkei' produced seven citations on the MEDLINE database. This could be interpreted as to indicate that research on nitrosamines as carcinogenic agents of OC in Transkei has been conducted at somewhat lower intensity as that on FBs.

In spite of a large body of evidence supporting the probable role of nitrosamines in cancer in humans, Mirvish (1995) concludes in his review of the role of nitrosamines and nitrosamides in the aetiology of certain cancers that, although he had concentrated on the initiation of cancer, promotion is also important for the cancers discussed and is probably caused by cigarette tar phenols for lung cancer, HBV for liver cancer and Epstein-Barr virus for nasopharyngeal cancer. He says a direct 'smoking gun' link between exposure to N-nitroso compounds and cancer in humans may never be possible. Exposure to several carcinogens is often involved, except for the link between oral cancer and chewing tobacco, where the principal carcinogens are nitrosamines. Exposure levels are especially hard to estimate for endogenous N-

nitroso compounds. Lifetime exposure of smokers to tobacco-specific nitrosamines is not far below the carcinogenic dose in rodents. Exposure to 10 µg dimethylnitrosamine/day, e.g. in 2L/day of beer with 5 ng/g dimethylnitrosamine (the level before 1980), corresponds to 0.2 ng/g per day for a 50-kg man. This dose would induce liver tumours in 0.06% of Wistar rats, according to a dose-response study on 4 000 rats treated daily for life with dimethylnitrosamine. He believes that this incidence can be estimated, because the incidence of dimethylnitrosamine-induced liver tumours in rats was proportional to dimethylnitrosamine dose. He believes that a similar incidence of liver tumours might be induced in humans. In contrast, the OC induction in rats by diethylnitrosamine decreased sharply as its dose was dropped. Finally, he concludes that exposure to N-nitroso compounds is likely to be responsible for a significant proportion of several cancers, some of which are especially important in developing countries.

2.4.2.2. Exposure to tannins

Tannins or tannic acid are water-soluble polyphenols that are present in many plant foods, including sorghum. Sorghum varieties rich in tannins have been specially developed to render them unpalatable to birds (Morton, 1970). In experimental animals, foods rich in tannins have been reported to be responsible for decreased feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility. Therefore, such foods, and particularly sorghum, are generally considered to be of lower nutritional value for farm animals than other grains.

Oterdoorn (1985) does not believe that minerals and vitamins in food play a role in the development of OC and he discounts the association of OC with a zinc deficiency. He cites earlier reports (e.g. Morton, 1970) that implicate tannin-rich sorghum as a cause of OC. According to Oterdoorn, these reports noted a consistency between the four regions of the world with high OC incidence and high intakes of this type of sorghum.

More recently, Chung *et al* (1998) reviewed the role of tannins in human health. They point out that recent findings indicate that the major effect of tannins is not due to their inhibition on food consumption or digestion, but rather the decreased efficiency in converting the absorbed nutrients to new body substances. Many reports indicate

that incidences of certain cancers, such as OC, could be related to consumption of tannin-rich foods such as betel nuts and herbal teas, suggesting that tannins might be carcinogenic. Bogovski (1980) suggests that the occurrence of nasal cancer in woodworkers could probably be better solved if the tannins in wood are taken into account. Chung *et al* (1998) cite reports that indicate that the carcinogenic activity of tannins might be related to components associated with tannins rather than tannins themselves. On the other hand, Chung *et al* (1998) also cite many reports, which indicate a negative association between tea consumption and cancer incidence. Tea polyphenols and many tannin components are suggested to be anticarcinogenic. Many types of tannin molecules have been shown to reduce the mutagenic activity of a number of mutagens. Often, carcinogens and/or mutagens produce oxygen-free radicals, which interact with cellular macromolecules. The anticarcinogenic and antimutagenic potential of tannins may be related to their antioxidative property, which is important to protect cellular oxidative damage, including lipid peroxidation. Tannins and related compounds are reported to inhibit the generation of superoxide radicals. Tannic acid and propyl gallate, but not gallic acid, also inhibit foodborne bacteria, aquatic bacteria, and off-flavor-producing microorganisms. Their antimicrobial properties seem to be associated with the hydrolysis of ester linkage between gallic acid and polyols hydrolyzed after ripening of many fruits.

Mirvish (1995) cites reports that indicate the role of polyphenols such as epigallocatechin, in tea in inhibiting nitrosation and hence the *in vivo* formation of carcinogenic nitrosamines. Tea strongly inhibited formation of *N*-nitrosoproline in humans.

2.4.2.3. Gastro-oesophageal reflux

Gastro-oesophageal reflux is the pushing back of the acidic stomach contents into the oesophagus, causing acidic burns and lesions that can turn into an oesophageal tumour. Certain individuals are predisposed to the condition and heavy alcohol intake can cause motor problems that are implicated in gastro-oesophageal reflux, causing inhibition of oesophageal sphincter function, reduction in the force of oesophageal contraction and modification of oesophageal peristalsis (Anonymous, 1996).

On the basis of a review of available literature, Sammon & Alderson (1998) formulated a hypothesis for the high incidence of OC in parts of Africa. They concluded that a predominantly maize-based diet is high in linoleic acid, a precursor for gastric prostaglandin synthesis. They hypothesize that in combination with low intake of other fatty acids and riboflavin, high levels of prostaglandin E2 are produced in gastric mucosa, leading to reduced gastric acid secretion, relaxation of the pylorus and a reduction in lower oesophageal sphincter pressure. These events result in combined reflux of duodenal and gastric juices low in acidity into the oesophagus. Resulting dysplasia strongly predisposes to local squamous carcinogenesis.

2.4.2.4. Dry cleaning

The relationship between employment in dry cleaning (a Group 2 carcinogenic exposure circumstance) and the occurrence of various cancers has been assessed in proportionate mortality studies, case control studies and four cohort studies (Anonymous, 1985). Two cohort studies restricted to dry-cleaning workers in the United States were given greater weight in the evaluation than were the results of cohort studies of laundry and dry-cleaning workers from Denmark and Sweden. The relative risk for mortality from OC was elevated by a factor of two in both United States cohorts (23 observed deaths in the two studies combined) and increased with increasing duration and/or intensity of employment. This cancer also occurred in slight excess in a proportionate mortality study in the United Kingdom with respect to launderers, dry cleaners and pressers. Risk estimates for OC were not provided in either of the two Nordic studies of laundry and dry cleaning workers. In a case control study of OC in Montreal, Canada, none of the case subjects had worked in dry cleaning, but the study was relatively small. The relative incidence of OC is increased by consumption of alcohol drinking and cigarette smoking, but potential confounding by these exposures could not be explored directly in these studies.

2.4.2.5. Smoking and chewing of tobacco

The highest OC incidence in the world occurs in the Guriev district of Kazakhstan, with about 547 cases per 100 000 males aged 35-64 in the 1960's (Warwick & Harington, 1973). The chewing of *nass*, a mixture of tobacco powder, wood ash, lime and a little vegetable oil, is a common habit among the peasant population in the

region. In the Transkei, the chewing of tobacco, although without alkalic agents used to be a common practice before the 1970's. Tobacco leaves contain nitrosamines. Smoking is a far more universal habit amongst Xhosa OC patients than amongst any other population group in South Africa, and more than 90% of the male Xhosa population smoked in the period 1940 to 1970 (Warwick & Harington, 1973).

OC incidence and mortality among American blacks is over three times the rate for whites (Herbert & Kabat, 1988). Between 1950 and 1977 the age-adjusted OC mortality rate approximately doubled in nonwhites while remaining virtually unchanged in whites. Between World War II and the 1970s menthol cigarette sales dramatically increased, roughly paralleling the increase in OC among black Americans. The authors tested the relationship between the smoking of menthol cigarettes and OC using data from a large hospital-based case-control study. All the OC cases used in the study were current smokers. Controls were matched to the cases on age and sex, had conditions considered not to be related to tobacco use, and were current smokers. It was found that there was no increase in risk for males who have always been smoking menthol cigarettes, compared to those who never smoked menthol cigarettes. For women, however, there was an increased risk and the risk increased with longer menthol use. In women menthol smoking showed about a 5% increase in risk per year, while the smoking of non-menthol cigarettes increased the risk of developing OC at about 2% per year.

2.4.2.6. Alcohol

Alcoholic beverages are listed as Group 1 carcinogens and many forms of home made alcoholic drink have been investigated in the Transkei and elsewhere as possible agents in the development of OC. In the Transkei, where alcohol use is heavy, no clear direct link to OC has been found (Warwick & Harington, 1973). Investigators speculated about the use of tar drums for home brewing traditional beers, as well as the addition of various foreign materials, some of which are known carcinogens, to add 'kick' to the drink. Unfortunately, it was not possible to scientifically investigate the role these factors played.

Chronic alcohol abuse is the main factor in OC in the western world, mainly adenocarcinoma as opposed to squamous cell carcinoma. The risk of cancer is

considerably increased where there is combined alcohol and tobacco addiction: it is 35 times greater in alcohol-tobacco addicted patients than in non-smokers who do not drink (Anonymous, 1996). Not only is alcohol use in the Transkei heavy, many people also are smokers, often using the traditional Xhosa pipe, sometimes lined with lead to make it last longer (Warwick & Harington, 1973). In Johannesburg and Durban males, a reduced risk of developing OC was found when neither drinking nor smoking is practised, and also when there is only drinking without smoking (Warwick & Harington, 1973).

The involvement of alcohol in cancer of the upper respiratory and digestive tracts (tongue, pharynx, mouth, and oesophagus) is well known. By causing motor problems in intestinal transit and modifying the permeability of the mucous membranes, alcohol prolongs the presence and promotes the entry of carcinogenic substances contained in some alcoholic drinks, such as polycyclic hydrocarbons and nitrosamines (Anonymous, 1996).

2.4.3. Nutritional factors that may affect tumour development

2.4.3.1. General nutritional status

Van Rensburg *et al* (1983) chemically assessed nutritional status indicators in blood and urine taken from 625 Transkeians drawn from 3 age-groups in each of 2 regions: 1 with a moderate incidence of OC and 1 with a very high incidence. Aggregate mean values for protein, albumin, vitamin A, and phosphorus were generally acceptable, but many subjects had inadequate (though not necessarily deficient) values for nicotinic acid (74% of subjects), magnesium (60%), vitamin C (55%), carotene (53%), riboflavin (41%), calcium (35%), and zinc (27%). Groups at highest risk for OC had markedly lower serum magnesium and carotene concentrations and mildly depressed hemoglobin and hematocrit values, but such findings are not necessarily associated with esophageal cancer aetiology. Possible intestinal malabsorption in the populations at highest risk may be associated with the unusually high fiber and phytate intake of the high-risk populations as well as with exposure to necrotizing mycotoxins. Thus, while protein and energy nutrition seem generally adequate, both the high- and moderate-risk populations had high incidences of multiple micronutrient malnutrition, which may play a role in susceptibility to OC.

Two randomized nutrition intervention trials were conducted in LinXian, an area of north central China with some of the world's highest rates of oesophageal and stomach cancer. This is also a population with a chronically low intake of several nutrients (Blot *et al*, 1995; Y Zhang *et al*, 1995). One trial used a factorial design that allowed assessment of the effects in nearly 30 000 participants of daily supplementation with four nutrient combinations: retinol and zinc; riboflavin and niacin; vitamin C and molybdenum; and beta-carotene, alpha-tocopherol, and selenium. The second trial provided daily multiple vitamin-mineral supplementation or placebo in 3 318 persons with oesophageal dysplasia, a precursor to OC. After supplements were given for 5.25 y in the general population trial, small but significant reductions in total [relative risk (RR) = 0.91] and cancer (RR = 0.87) mortality were observed in subjects receiving beta-carotene, alpha-tocopherol, and selenium but not the other nutrients. The reductions were greater in women than men, and in those under, compared with over, the age of 55; however, differences by sex or age were not significant. After multiple vitamin and mineral supplements were given for 6 y in the smaller dysplasia trial, reductions in total (RR = 0.93) and cancer (RR = 0.96) mortality were observed but these were not significant. The largest reductions were for cerebrovascular disease mortality, but the effects differed by sex: a significant reduction was observed in men (RR = 0.45) but not women (RR = 0.90). In individuals with oesophageal dysplasia, micronutrient supplementation had little effect on T lymphocyte responses. In contrast, male participants in the larger trial who were supplemented with beta-carotene, vitamin E, and selenium showed significantly ($P<0.05$) higher mitogenic responsiveness of T lymphocytes in vitro than those not receiving these micronutrients. Restoring adequate intake of certain nutrients may help to lower the risk of cancer and other diseases in this high-risk population.

In Iran, Siassi *et al* (2000) also investigated the possible contribution of different dietary nutrients in the development of OC in the Caspian littoral of northeast Iran. Forty-one cases and 145 members of their households were matched for age and gender with 40 non-blood-relative controls and 130 members of their households for their nutrient intake. They used a standard 24-hour dietary recall questionnaire to estimate the daily intake of energy, protein, P, Fe, Na, K, vitamins C and A, thiamin, riboflavin, and niacin. Dietary nutrient deficiency was defined as less than 75% of the World Health Organization human nutritional requirements, except for P, Na, and K,

for which the United States Recommended Dietary Allowances were followed. They found that:

- The mean daily intake of all nutrients, except for riboflavin, was significantly lower in OC cases than in control subjects ($P<0.05$);
- With the exception of protein, riboflavin and phosphorus, significant correlation was observed between the pattern of nutrient intake and health status of the study subjects ($P<0.05$); and
- Dietary deficiency of niacin and phosphorus was associated significantly with the risk of OC development among case and control households ($P<0.01$-0.001), indicating that persons living in case households with dietary deficiencies of these nutrients have more than twice the risk of developing OC tumours than those living in control households.

They conclude that some nutrients, such as P and niacin, may play a role in the aetiology of OC, and that the status of these nutrients may eventually be used as an epidemiological predictive marker for OC in the Caspian littoral of Iran and perhaps in other regions.

2.4.3.2. Mineral deficiencies or overexposure to certain minerals

In Iran, Azin *et al* (1998) measured the levels of four 'carcinogenic' (Ni, Fe, Cu, Pb) and four 'anticarcinogenic' (Zn, Se, Mn, Mg) trace elements in hair samples from OC patients, their unaffected family members, and members of families with no history of cancer. They also measured these levels in patients without OC. They found that Ni and Cu concentrations were significantly higher and Mg and Mn concentrations significantly lower in all cancer cases. Levels of Zn, Fe, Se, and Pb were not significantly different in these groups. In addition, they found the serum albumin fraction, which is reported to have antioxidant activity, to be significantly lower among OC patients.

In Norway, Serck-Hanssen & Stray (1994) diagnosed histological oesophageal injury in the form of ulcers, with deposition of iron salts, in 12 elderly patients over a 3-year period. One patient died following perforation of the oesophagus. The use of iron

tablets was not thought of clinically as a possible cause of the lesions, but this appeared to be the most likely explanation as 10 of the 12 patients reported the use of iron sulphate tablets of the sustained release type.

2.4.3.3. Vitamins

Folic acid (vitamin B_c) deficiency could be involved in the development of many types of cancer, including OC. Lower erythrocyte levels of folic acid and higher prevalence of cellular features compatible with folic acid deficiency were found in areas of the Transkei in individuals at high risk for OC (Jaskiewicz *et al*, 1988; Jaskiewicz, 1989). Folic acid deficiency could be the result of low intake, but it can also be caused by several other factors. For instance, smoking could contribute towards deficiency in folic acid, which has been found in the epithelium of areas of the aerodigestive system (Heimburger, 1992). Smoking and alcohol use have both been implicated in development of cancer. Intake of alcohol reduces folic acid levels in the blood. Folic acid deficiency is related to neural tube defects and it has been speculated that folic acid deficiency could be caused by intake of FBs (Hendricks, 1999; see Section 4.4).

Stevens & Tang (1997) investigated the importance of sphingolipids for folate receptor function in Caco-2 cells using FB_1 to inhibit the biosynthesis of functional lipids in these processes. They found that folate receptor-mediated transport of 5-methyltetrahydrofolate was almost completely blocked in cells in which sphingolipids had been reduced by approximately 40%. Wolf (1998) also found that the folate receptor in the cell membrane, bound to the plasma membrane through a glycosylphosphatidylinositol anchor, requires both sphingolipids and cholesterol in the membrane for full activity. Treatment of cells in culture with FB_1, inhibits sphingolipid synthesis, and virtually abolishes uptake of 5-methyltetrahydrofolate, thus confirming the results of Stevens & Tang (1997). Stevens & Tang (1997) further found that inhibition of the transport of 5-methyltetrahydrofolate was dependent on the concentration and duration of the treatment with FB and was mediated by the sphingolipid decrease. FB_1 treatment inhibited neither receptor-mediated, nor facilitative transport, indicating that the effect of sphingolipid depletion was specific for folate receptor-mediated vitamin uptake. A concurrent loss in the total amount of folate binding capacity in the cells was seen as sphingolipids were depleted,

suggesting a causal relationship between folate receptor number and vitamin uptake. These findings suggest that dietary exposure to FB_1 could adversely affect folate uptake and potentially compromise cellular processes dependent on this vitamin. Stevens & Tang conclude that, because folate deficiency causes neural tube defects, some birth defects unexplained by other known risk factors may be caused by exposure to FB_1.

Dietary intake and blood serum levels of vitamin A were assessed in 681 rural Transkeians who had moderately low or very high risk for OC (Van Rensburg *et al*, 1981). Deficient intakes of vitamin A in 2-4 and 6-9 year old children and nursing mothers were generally 2 or 3 times more frequent for the low risk groups. Serum levels were lower in low risk than in high-risk 2-4 year old children (28 vs. 34 µg/100 ml), as well as in 6-9 year old children (29 and 39 µg/100 ml). All lactating mothers had adequate-to-high serum levels.

In a follow-up study (Van Rensburg *et al*, 1981), the authors maintained inbred male rats on diets either deficient or not deficient at two levels of vitamin A, for 160 days. All rats were dosed with the oesophageal carcinogen MBN between the 40th and 60th day. Vitamin A deficient rats failed to develop any tumours following MBN treatment; 40 and 80% of the rats in two not vitamin A deficient groups developed oesophageal papillomas, respectively. The authors conclude that vitamin A probably promotes carcinogenesis in epithelia, which are normally squamous.

2.4.4. Genetic predisposition towards, and ethnicity in development of cancer

2.4.4.1. Ethnicity and areas of the world with high cancer incidence

In their overview of OC risk factors, Ribeiro *et al* (1996) state that cancer of the oesophagus has great diversity in geographical distribution and incidence, with the rate of OC increasing in some areas. Cook (1971) also points out that OC incidence rates in Africa vary widely within areas less than 100 miles apart. The reasons for this are not clear. In the developed world the effects of alcohol and tobacco are substantial preconditions, while in the developing world factors such as diet, nutritional deficiencies, environmental exposure and infectious agents (especially

papillomavirus and fungi), play a significant role (Ribeiro *et al*, 1996). Chronic irritation of the oesophagus appears to participate in the process of carcinogenesis, particularly in patients with thermal and/or mechanical injury, achalasia, oesophageal diverticulum, chronic lye stricture, radiation therapy, injection sclerotherapy and gastric resection before the appearance of oesophageal tumour. The authors also reviewed association of Plummer-Vinson syndrome, coeliac disease, tylosis and scleroderma with OC.

In South Africa, different ethnic groups show large differences in their disposition towards developing different kinds of cancer (Table 8). Not all of these differences can be accounted for by differences in lifestyle, eating habits etc. In spite of the more sophisticated lifestyle and better nutrition that whites enjoy, the life risk to contract cancer of some kind or another in white males in South Africa, is 1 in 3, compared to 1 in 9 in black males.

Similar observations have been made in other parts of the world. Regional and temporal patterns of variation in the incidence of cancer of the oesophagus were analysed in the Central Asian republic of Karakalpakstan (Zaridze *et al*, 1992). Karakalpakstan (population about 1 200 000) is an area with high OC. Incidence data within regions (data from 1988-1989), ethnic groups (data from 1987-1989) and calendar periods (data from 1973-1987) were available for analysis, with corresponding official population estimates. No significant difference was observed between rates in urban and rural environments, although significant regional variation was observed ($P<0.05$). The highest rate observed was in the Muinak, the northern region, with world age standardised incidence rates (ASIR) of 125.96 for males and 150.65 for females. There was a highly significant difference among ethnic groups ($P<0.001$). The ethnic group with the highest incidence was the Kazakh people, with an ASIR of 68.0 in males and 86.3 in females. Incidence in the republic as a whole declined in the period from 1973 to 1987. Incidence of cancer of the oesophagus is still high in Karakalpakstan, despite the decline. The authors conclude that incidence is likely to be strongly related to factors associated with region of residence and with ethnicity.

Percesepe & Ponz De Leon (1996) carried out epidemiological studies on high-risk cancer populations of China and Iran and found a strong family relationship for OC.

Up to 60% of the affected patients reported a family history of OC. About 10-15% of gastric cancer patients showed a positive family history. Gastric cancer belongs to the neoplastic spectrum of hereditary nonpolyposis colorectal cancer, a genetic disease with an autosomal dominant pattern of inheritance. Familial polyposis coli and hereditary nonpolyposis colorectal cancer are the two main hereditary colon cancer syndromes. Familial aggregation has been observed in about 10% of colorectal cancer cases. As for pancreatic cancer, anecdotal reports and one case control study have shown an increased risk of pancreatic carcinoma in patients with a positive family history both for all cancers (relative risk, RR, 2), and specific for pancreatic cancer.

Table 8 - Lifetime risks of the top five cancers, excluding basal and squamous cell skin cancers, per population group in South Africa, 1993 – 1995

Population group	Males		Females	
	Cancer	Life risk (0-74 y) 1 in:	Cancer	Life risk (0-74 y) 1 in:
Asian	Colorectal	43	Breast	21
	Prostate	47	Cervix	54
	Bladder	51	Uterus	68
	Stomach	51	Colorectal	79
	Lung	62	Stomach	120
	All cancers	6	All cancers	5
Black	Oesophagus	59	Cervix	34
	Prostate	61	Breast	81
	Lung	67	Oesophagus	141
	Liver, bile duct	227	Uterus	238

	Larynx	204	Lung	313
	All cancers	9	All cancers	11
Coloured	Prostate	50	Cervix	52
	Lung	68	Breast	63
	Stomach	78	Lung	172
	Oesophagus	101	Uterus	189
	Bladder	147	Stomach	250
	All cancers	8	All cancers	11
White	Prostate	14	Breast	13
	Bladder	29	Colorectal	44
	Colorectal	34	Melanoma	56
	Lung	34	Lung	61
	Melanoma	45	Cervix	93
	All cancers	3	All cancers	4
All	Prostate	31	Breast	36
	Lung	52	Cervix	41
	Oesophagus	71	Colorectal	130
	Bladder	83	Lung	147
	Colorectal	94	Oesophagus	169
	All cancers	6	All cancers	7

Data from Sitas *et al* (1998)

Zaridze *et al* (1993) also examined cancer incidence rates in the native peoples of the far northeast of Siberia for the years 1977-1988. Particularly high rates of cancers of the stomach, lung, oesophagus and cervix were observed. For stomach cancer, the male and female age-standardised (to the world population) rates were 103.9 per 100 000 and 50.0 per 100 000 respectively. The corresponding lung cancer rates were 109.4 and 45.7, and for OC 83.9 and 35.0. The age-standardised cervical cancer rate was 38.5 per 100 000. Rates of these cancers were considerably higher than in native Alaskan peoples, although the latter had higher rates of breast and colorectal cancers. The rates were also much higher than those of migrant people from Russia and elsewhere who have settled in the same area over the past 3 centuries, particularly at younger ages. Male rates of stomach and lung cancer were highest in the paleo-Asiatic peoples of the north, whereas male oesophageal rates were highest in the Taiga people. In females, rates of stomach cancer and OC were highest in the paleo-Asiatic peoples, and rates of lung cancer were highest in the Taiga nationalities. Cervical cancer rates were highest in the Amuro-Sakhalin nationalities of the south.

Ethnicity and familial relationship in the occurrence of cancer suggest a genetic basis of susceptibility to cancer. The highest world incidence rates of OC occur in remote areas where people live a secular life; for example, Du Plessis *et al* (1969) state that in the Transkei women - and men up to the age of about 20 – spend most of their lives within about 2 km of their homes. Hence it seems likely that they choose marriage partners from a relatively small local population. Inbreeding under such conditions could very well contribute towards increased expression of a genetic susceptibility factor.

2.4.4.2. Genetic basis

Cytochrome P-450 1A2 (CYP1A2) has been identified as a key factor in the metabolic activation of numerous chemical carcinogens, including AFB_1, various heterocyclic and aromatic amines, and certain nitro-aromatic compounds. In addition, CYP1A2 contributes to the inactivation of several common drugs and dietary constituents, including acetaminophen and caffeine. Two xenobiotic-responsive-element (XRE)-like sequences and an antioxidant response element (ARE) have been

identified in the regulatory region of the CYP1A2 gene; however, the functionality of the ARE remains to be demonstrated. Based on in vivo phenotyping assays, substantial variability between individuals in CYP1A2 activity has been reported. Some population-based studies have reported either bi- or tri-modal distributions in CYP1A2 phenotype, suggesting a genetic basis for the large differences between individuals in CYP1A2 activity. However, despite the polymodal distributions reported for CYP1A2 activity, a distinct functional genetic polymorphism in the gene has not been identified. Several possible mechanisms exist contributing to the large variability in CYP1A2 activity. A thorough understanding of the functions and regulation of the CYP1A2 gene may ultimately lead to new methods for preventing or intervening in the development of certain chemically-related human cancers (Eaton *et al*, 1995).

Lin *et al* (1998) studied genetic polymorphisms in enzymes involved in carcinogen metabolism that have been shown to influence susceptibility to cancer. Cytochrome P450 2E1 is primarily responsible for the bioactivation of many low molecular weight carcinogens, including certain nitrosamines, whereas glutathione S-transferases are involved in detoxifying many other carcinogenic electrophiles. OC, which is prevalent in China, is hypothesized to be related to environmental nitrosamine exposure. Thus, these authors conducted a pilot case-control study to examine the association between Cytochrome P450 2E1, glutathione S-transferases M1, T1, and P1 genetic polymorphisms and OC susceptibility. DNA samples were isolated from surgically removed oesophageal tissues or scraped oesophageal epithelium from cases with cancer (n = 45), cases with severe epithelial hyperplasia (n = 45), and normal controls (n = 46) from a high-risk area, LinXian County, China. RFLPs in the Cytochrome P450 2E1 and the glutathione S-transferase P1 genes were determined by PCR amplification followed by digestion with RsaI or DraI and Alw26I, respectively. Deletion of the glutathione S-transferase M1 and glutathione S-transferase T1 genes was examined by a multiplex PCR. The Cytochrome P450 2E1 polymorphism detected by RsaI was significantly different between controls (56%) and cases with cancer (20%) or severe epithelial hyperplasia (17%; $P<0.001$). Persons without the RsaI variant alleles had more than a 4-6-fold risk of developing severe epithelial hyperplasia (adjusted odds ratio, 6.0; 95% confidence interval, 2.3-16.0) and cancer (adjusted odds ratio, 4.8; 95% confidence interval, 1.8-12.4). Polymorphisms in the

glutathione S-transferases were not associated with increased OC risk. These results indicate that Cytochrome P450 2E1 may be a genetic susceptibility factor involved in the early events leading to the development of OC.

On a different tack, Song *et al* (2001) examined the relationship between two genetic methylenetetrahydrofolate reductase polymorphisms and susceptibility to OC in 240 OC cases and 360 age- and sex-matched controls in northern China. Methylenetetrahydrofolate reductase plays a central role in folate metabolism that affects DNA methylation and synthesis. Germ-line mutations at nucleotides 677 (C→T) and 1298 (A→C) in the methylenetetrahydrofolate reductase gene cause diminished enzyme activity, and aberrant DNA methylation is oncogenic. They found that the allele frequency of methylenetetrahydrofolate reductase 677T was significantly higher among cases than among controls (63% *versus* 41%, $P<0.001$). Subjects with the 677TT genotype had a more than 6-fold increased risk of developing OC (adjusted odds ratio 6.18; 95% confidence interval 3.32–11.51) compared with those who had the 677CC genotype. Furthermore, the elevated OC risk associated with the 677 polymorphism was in an allele-dose relationship (trend test, $P=0.0001$) with odds ratios of 1.00, 3.14 (95% confidence interval 1.94–5.08), and 6.18 (95% confidence interval 3.32–11.51) for the *CC, CT*, and *TT* genotype, respectively, after adjustment for age, sex, smoking status, and the methylenetetrahydrofolate reductase 1298 polymorphism. The allele frequency for the methylenetetrahydrofolate reductase 1298C was 14% among cases and 17% among controls. The 1298CC genotype was extremely rare in both controls (1.4%) and cases (2.9%) and was also associated with an elevated risk of OC (adjusted odds ratio 4.43; 95% confidence interval 1.23–16.02) compared with the 1298AA genotype, whereas the 1298AC genotype had no effect on the risk of OC. Thus, their findings support the hypothesis that genetic polymorphisms in the methylenetetrahydrofolate reductase gene may contribute to susceptibility to carcinogenesis of the oesophagus in the at-risk Chinese population.

In order to explore the mode of inheritance of OC in a moderately high-incidence area of northern China, W Zhang *et al* (2000) conducted a pedigree survey on 225 patients affected by OC in Yangquan, Shanxi Province, Peoples' Republic of China. Segregation analysis showed that Mendelian autosomal recessive inheritance of a major gene that influences susceptibility to OC provided the best fit to the data. In the

best-fitting recessive model, the frequency of the disease allele was 0.2039. There was a significant sex effect on susceptibility to the disease. The maximum cumulative probability of OC among males with the AA genotype was 100%, but, among females, it was 63.5%. The mean age at onset for both men and women was 62 years. The age-dependent penetrances for males with the AA genotype by the ages of 60 and 80 years were 41.6% and 95.2%, respectively, whereas, for females, they were 26.4% and 60.5%, respectively. Incorporating environmental risk factors such as cigarette smoking, pipe smoking, alcohol drinking, eating hot food, and eating pickled vegetables into the models did not provide significant improvement of the fit of the models to these data. The results suggest a major locus underlying susceptibility to OC with sex-specific penetrance.

2.4.5. Conclusion

From all these studies it is clear that a single cause for OC is highly unlikely. Like liver cancer and many other cancers, environmental circumstances that contribute to, or cause OC are multi-factorial. In addition, there is clear evidence of large variations in the susceptibility of groups of humans to the condition. Exposure of one group of humans with high tolerance to a set of causative factors may therefore have little effect, while exposure of a group with low tolerance, or high genetic predisposition towards OC to the same, or even a lesser set of causative or contributory factors may result in a much higher OC incidence. Thus the scene is set for a complex aetiology, which indeed is the case.

2.5. Overview of toxicological studies on mycotoxins in humans and animals

2.5.1. Preamble

Ever since the very early days of grain trading, the grading regulations that were made applicable to traded grain in all the important grain producing countries, invariably discriminated against the presence of visibly mouldy grain kernels in general, and against some specific moulds like ergot in wheat in particular. In each country, a maximum tolerance level for such grain kernels is strictly enforced. Grain which cannot meet the tolerances for mouldy kernels is classed or graded as sample class or sample grade in most grading systems and is not allowed to enter the normal trading channels. Effectively, such grain is declared unsuitable for food and instead is often utilised as animal feed. Anyone who buys such grain, even for use as animal feed, is therefore by implication forewarned about the possible risks involved in using the grain. Worldwide, the limits on mouldy kernels restrict to a considerable extent the levels of mycotoxins that can be present in commercial grain. As a result, the levels of mycotoxins found in some maize produced on subsistence farms, like FBs in maize in the Transkei (see Section 2.3.2), is highly unlikely to ever occur in commercial maize that meets the grading specifications.

However, as will be shown in Section 4.1 for commercial South African (RSA maize), Argentinean (ARG maize) and USA maize, certain mycotoxins nevertheless do occur in commercial grain and grain products, sometimes at levels that could be detrimental to the health of some of the more sensitive animal species. The same applies to all grain all over the world. Also, screenings and other milling by-products derived from commercial grain can contain damaging levels of certain mycotoxins, because screenings contain most of the mouldy kernels removed during cleaning and many mycotoxins are located mainly in the bran and germ, which are removed during commercial milling. These by-products are usually used as feed components in animal feed milling. While the grading system is helpful to limit the number of mouldy kernels in grain, a nil tolerance is impractical, so some infected kernels and some ocurrence of mycotoxins in commercial grain is inevitable. In addition, certain fungal

infections, notably *F. verticillioides*, which produces FBs in maize, very often leave no visible indication of infection. Therefore, these cannot sufficiently be discriminated against through the grading system, even if it was possible to apply a nil tolerance or a very low tolerance for mouldy kernels in grain.

If all the different kinds of grains and all possible environmental conditions over the whole world are taken into account, several mycotoxins could occur in grain that could be a threat to human health. However, from a South African perspective, and from what already has, or still will transpire in the coming sections of this thesis, only three mycotoxins need to be singled out as possible mycotoxin contaminants of significance in locally produced or imported commercial grains. These are AFLA, FBs and DON. AFLA are rarely found in local grain apart from groundnuts, but are important in maize imported from the USA and Argentina. FBs are important in locally produced, as well as imported maize, particularly from the USA, and DON occurs at moderately low levels in locally produced maize. DON could probably also be found at significant levels in locally produced wheat at times when head blight (scab) occurs, and it certainly can be present at damaging levels in imported wheat, as well as in imported maize. In Section 4.1, it will be shown that all the other mycotoxins covered in this study, with the possible exception of MON, are rare or occur only at insignificant levels in South African commercial maize. Unfortunately, inadequate data are available on MON in maize, as well as the levels of all mycotoxins in locally produced wheat and sorghum, to form a representative picture of the mycotoxin scene in these grains. Once more complete information becomes available, another look may need to be taken at these grains. For the present, the toxicology of AFLA, FBs and DON will be overviewed in the following sections.

Toxins can have varying effects on humans and animals, depending on the nature of the toxin, the dose, the susceptibility of the exposed species and the nature of the exposure. Thus, acute toxicity results from exposure to relatively large doses of a potent toxin, whilst chronic toxicity is the result of exposure over an extended period to sub-acute doses of a toxin, more often a less potent toxin. Some toxins are restricted to a toxic action, where some essential biochemical procedure in the affected species is disrupted; others are also carcinogenic, disrupting the genetic code in some locus in the body. This then results in uncontrolled growth of cells and

development of tumour in particular body tissues. In the following sections, the acute and chronic toxicity and the carcinogenic activity where applicable, of the three mycotoxins in experimental animal tests, in farm animals and in human exposures will be covered.

2.5.2. Toxicology of aflatoxins

Voluminous data on experimental animals have established a lucid molecular-biological basis for the toxic action of AFLA - mycotoxins produced by certain *Aspergillus* species, mainly *Aspergillus flavus* and *A. parasiticus* (IARC, 1993). These account for many of the effects observed in experimental animals. In experiments on primates, the symptoms and pathology closely resemble some forms of human liver disorders probably caused by AFLA. In addition to data on experimental animals, sufficient epidemiological data are available of the effect of human exposure to AFLA to reasonably quantify the acute, chronic and carcinogenic effects of AFLA on humans. Therefore, these will be covered in some detail here, while the effects on farm animals will be covered briefly, and the scores of data on laboratory animals will be largely omitted.

2.5.2.1. Toxicology of aflatoxins in farm animals (adapted from Krausz, 1998)

2.5.2.1.1. Beef Cattle

Early indications of AFLA toxicity include reduced feed intake followed by reduced weight gain or weight loss. Often, there is reduced feed efficiency, increased susceptibility to stress, and decreased reproductive performance. Chronic aflatoxicosis is characterized by unthriftiness, anorexia, prolapse of the rectum, liver and kidney damage, depression of the immune system, and oedema in the abdominal cavity. Feeds containing as little as 60 - 100 ng/g AFLA, fed over an extended period, may depress performance in cattle. Chronic symptoms of aflatoxicosis can result from the continued intake of 700 - 1000 ng/g of AFLA in feed of young cattle. Death of steers has been reported from an intake of 1000 ng/g of AFLA in feed during a 59-day trial. Once damage has been done, the animals do not fully recover.

2.5.2.1.2. Dairy Cattle

AFLA affects dairy cow health and performance in a similar manner to beef cattle. AFLA is excreted in the milk in the form of AFM1 at approximately 1 to 2 percent of the dietary level. Generally, levels of 50 ng/g AFLA in the feed produce levels over 0.5 ng/g in the milk. Once the contaminated feed is removed, AFLA levels in the milk will disappear in 48 to 72 hours.

2.5.2.1.3. Poultry

AFLA affects all poultry species. Young poultry, especially ducks and turkeys, are very susceptible to aflatoxicosis. Growing poultry should not receive more than 20 ng/g AFLA in the diet. However, feeding levels lower than 20 ng/g may still reduce their resistance to disease, decrease their ability to withstand stress and bruising, and generally make them unthrifty. Laying hens usually can tolerate higher levels of AFLA than young birds, but AFLA levels still should be less than 100 ng/g. Aflatoxicosis can reduce the birds' ability to tolerate stress and other diseases by inhibiting the immune system. Stunted growth, increased mortality, reduced egg size and production, liver and kidney disorders, leg and bone problems, suppression of the immune system with increased susceptibility to infections such as *Salmonella* are common symptoms of aflatoxicosis in poultry. Decreased blood-clotting results in greater downgrading and rejection of birds at slaughter due to bleeding within tissues and bruises.

2.5.2.1.4. Swine

Swine are sensitive to AFLA levels of 100 to 400 ng/g, causing reduced growth rate and lower feed efficiency. AFLA primarily causes liver damage and can result in reductions in feed intake and growth performance. Breeding stock, nursing, and growing pigs are more sensitive than finishing swine (greater than 50 kg). AFLA levels of 400 to 800 ng/g cause liver damage, bleeding disorders, immune system suppression, abortions and death.

2.5.2.1.5. Sheep and Goats

Sheep and goats are affected by AFLA like other ruminants. Aflatoxicosis causes liver damage, kidney damage, anemia, and other symptoms similar to those found in cattle. Early symptoms may include depression, loss of appetite, weakness and slow movement.

2.5.2.1.6. Horses

Based on field observations, it has been suggested that the maximum AFLA level for mature, non-breeding horses should not exceed 50 ng/g, and that growing horses (less than 2 years old), breeding horses, and workhorses, should receive only AFLA-free rations.

2.5.2.2. Toxicology of aflatoxins in humans (adapted from Angsubhakorn, 1998)

2.5.2.2.1. Acute aflatoxin poisoning

Taiwan Outbreak

In 1967, there was an outbreak of apparent poisoning of 26 persons in two Taiwan rural villages (Ling *et al*, 1967). The victims had consumed moldy rice for up to 3 weeks; they developed oedema of the legs and feet, abdominal pain, vomiting, and palpable livers, but no fever. The three fatal cases were children between 4 and 8 years. Autopsies were not done, and the cause of death could not be established. In a retrospective analysis of the outbreak, a few rice samples from affected households were assayed for AFLA. Two of the samples contained up to 200 ng/g AFB_1.

Kenya Case

In 1982, an acute hepatitis was reported in Kenya (Bulato-Jayme *et al*, 1982). There were 12 of 20 cases that died with malaise, abdominal discomfort, with subsequent appearance of dark urine and jaundice. Local dogs that shared the food were affected, with many deaths. Stored grain appeared to be the cause of the outbreak. AFLA was

detected in two liver samples (39 and 89 ng/g). Histologically, there was centrolobular necrosis.

Uganda Case

AFB_1 was circumstantially associated with the death of a 15-year-old African boy in Uganda (Serck-Hanssen, 1970). The youth, his younger brother, and his sister became ill at the same time; the young sibling survived, but the older boy died 6 days later with symptoms resembling the victims in the Taiwan outbreak.

An autopsy revealed pulmonary oedema, flabby heart and diffuse necrosis of the liver. Histology demonstrated centrolobular necrosis with a mild fatty liver, in addition to the oedema and congestion in the lungs.

A sample of the cassava eaten by these children contained 1.7 µg/g AFLA, which Alpert & Serck-Hanssen (1970) suggest may be lethal if such a diet is consumed over a few weeks. This estimate is based on the acute toxicity of AFB_1 in monkeys.

Reye's Syndrome

Reye's syndrome is an acute and often fatal childhood illness, which is characterized by encephalopathy and fatty degeneration of viscera (EFDV). Reye and his co-workers in Australia first described the syndrome in 1963 (Reye *et al*, 1963).

Clinically, the main features of Reye's syndrome are vomiting, convulsions and coma. Hypoglycemia, corrhachia and elevated serum transaminases are the most constant biochemical abnormalities. Fatty degeneration in the liver and kidneys, and cerebral oedema are the major autopsy findings. Various cases of Reye's syndrome are discussed below:

In Thailand, Bourgeois *et al* (1971) reported in some detail on the case of a 3-year-old Thai boy who was brought to a northeast provincial hospital after a 12-hr illness of fever, vomiting, coma and convulsions. The child died 6 hours later, and an autopsy revealed marked cerebral symptoms with neuronal degeneration, severe fatty metamorphosis of the liver, kidneys, and heart, and lymphocytolysis in the spleen, thymus, and lymphnodes.

Upon admission of the child to the hospital, a small sample of steamed glutinous rice that had been the only food the family had for the past 2 days was obtained. The small size of the sample precluded an accurate measurement of the amount of AFLA present but clinical assay indicated the amount was in the parts per million (ppm) range. The rice examined also contained toxigenic strains of *A. flavus*, *A. clavatus*, *A. ochraceous*, and *A. niger* (Angsubhakorn *et al*, 1978).

AFB_1 was found in one or more autopsy specimens from 22 of the 23 Reye's syndrome cases studied in Thailand by Shank *et al* (1971). In several instances, these AFLA concentrations were as high as those seen in specimens from monkeys poisoned with AFLA (Bourgeois *et al*, 1971). However, Shank *et al* (1971) also found trace amounts of AFLA in tissue specimens from control cases. These are thought to reflect chronic low-level ingestion of the mycotoxin in that area of Thailand.

In New Zealand, Becroft & Webster (1972) analysed liver specimens from two children who died of Reye's syndrome, and suggested that contamination of foods by AFLA may have a role in the aetiology of Reye's syndrome. The amount of AFB_1 present was estimated to be in the range of 5 to 50 ng/g in each specimen of liver analysed (5-50 ng/g).

In the United States, Chaves-Carballo *et al* (1976) found fluorescing material chromatographically similar to AFG2 in the formaldehyde fixed-liver of a 15-year-old Reye's syndrome patient. However, similar material could not be found in seven other cases or in 12 controls.

In Germany, Rosenberg (1972) described the case of a 45-year old man, who died a short time after an apparent gastric illness. He had eaten an unusually large amount of nuts, which were apparently quite mouldy. The illness was diagnosed as acute yellow atrophy of the liver, but analysis of the liver revealed the presence of a blue fluorescing material that co-chromatographed with AFB_1 on a thin layer chromatographic (TLC) plate. The author suggests the case may be one of acute AFLA poisoning.

2.5.2.2.2. Sub-acute aflatoxin poisoning

There are reports that suggest that some outbreaks of sub-acute poisonings resulted from ingestion of AFLA over an extended period of time. Most of those outbreaks involve children.

Possible association with Indian hepatitis

In October 1974, unseasonal rains in 150 villages in Gujerat and Rajasthan western India resulted in extensive mould damage to standing maize crops. The people in these rural areas were poor and were forced to eat the contaminated grain for lack of alternate foodstuffs. After a few weeks of consuming the mouldy maize, many people became ill with symptoms of liver injury (Krishnamachari *et al*, 1975). One hundred and six of 397 patients died. The disease mainly affected male adults and spared infants and children (ages of 6 and 30 years). Patients suffered sub-acute poisoning with anorexia, vomiting, jaundice and ascites.

Dogs that shared food of affected households also developed ascites and jaundice and died a few weeks after onset. Other domestic animals, which did not share the family food, were not affected.

Five specimens of mouldy maize were collected from affected households and chemical analysis revealed AFLA contents ranging from 6.25 to 15.6 mg/kg maize which is a very high level of contamination. AFB_1 was detected in 2 of 7 serum samples collected from patients. Histopathologically, liver specimens revealed extensive bile duct proliferation, periportal fibrosis, and occasional multinucleated giant cells. The authors estimated that the patients had ingested 2 to 6 mg of AFLA each day for several weeks.

Possible association with Indian Childhood Cirrhosis

In India, liver cirrhosis is the third most common cause of death in hospitals among children under the age of 5 years. With its characteristically insidious onset, involving low grade fever, mild abdominal distension followed by enlarged liver with a characteristic leafy border, the disease may progress to jaundice, ascites, fibrosis, cirrhosis, and hepatic coma (Yadgiri *et al*, 1970, Amla *et al*, 1971). In one episode,

children suffering from kwashiorkor were given peanut flour supplement for several weeks until it was discovered that the peanut flour contained 300 ng/g AFLA. Liver biopsies taken 1-2 months after consumption of the toxic meal showed fatty liver while after some 4 months fibrosis and cirrhosis were apparent.

According to newspaper reports, levels of 271.63 ng/g of total AFLA and 165.05 ng/g AFB_1 were reported in peanut butter given to school children in the Eastern Cape, South Africa in the course of a primary school nutritional program (Anonymous, 2001d). These levels are approximately 30 times higher than the legal limits in South Africa and appear to be the result of poor or no application of statutory regulations by the health authorities in that country.

2.5.2.2.3. Aflatoxin and liver cancer

Geographic distribution of liver cancer

Primary liver cancer is not a common disease in most areas of the world. There are particular geographic areas, however, where the annual liver cancer rate is reported to be well above 2 cases per 100 000 people. Certain populations in Africa, southern India, Japan, and Southeast Asia have unusually high incidences of liver cancer (see Ferlay *et al*, 1999; Yu *et al*, 1997; 2000).

The hazards from chronic exposures to mycotoxins are potential rather than documented. The evidence for the association of AFLA in the cause of liver cancer has been considered strong enough to justify intervention in the food contamination cycle, and many countries maintain MTLs for AFLA in food – see Section 2.1.2. However, other factors such as the part played by HBV, must also be assessed.

Several field studies, which have associated consumption of AFLA with human liver cancer, have been documented. The studies took place from 1966 to 1973 in Uganda, the Philippines, Thailand, Kenya, Mozambique and Swaziland.

Uganda

Alpert *et al* (1971) at Harvard Medical School Massachusetts Institute of Technology undertook the pioneering effort in the field associations. Food samples were collected during the nine-month period from September 1966 to June 1967 from village

markets and home granaries throughout Uganda by staff and medical students on vacation leave from Kampala. All food specimens were sealed upon collection and kept in cold storage until shipped by airfreight to Boston, for chemical assay for AFLA.

Of a total of 480 food samples, 29% contained more than 1 ng/g AFLA and 4% more than 1 µg/g. AFLA occurred most frequently in beans (72% of samples), whereas maize (45%) peanuts (18%) and cassava (12%) were contaminated less frequently.

At the time the AFLA survey was being conducted, local cancer registry records covering 1964 to 1966 were studied to estimate the geographical distribution of liver cancer in Uganda. Table 9 gives the relationship between the incidence of liver cancer and the AFLA contamination in foodstuffs in Uganda.

Table 9 - Hepatoma incidence (per 100 000) and frequency (%) of aflatoxin contamination of foodstuffs in Uganda

Area	Hepatoma incidence	% Conta-mination	Aflatoxin contamination of foodstuffs (%)		
			Total aflatoxin content (ng/g)		
			1-100	100-1000	1000
Toro	No data	79	10	31	38
Karamoja	6.8	44	24	15	5
Buganda	2.3	29	23	4	1
West Nole	2.7	23	19	4	0
Acholi	2.7	15	15	0	0
Busoga	2.4	10	5	5	0
Ankole	1.4	11	11	0	0

Data from Alpert *et al* (1971)

Thailand and South East Asia

Over a 23 - month period from September 1967 through July 1969, mycological studies (Shank *et al*, 1972) on cereals, beans, cassava, dried fish, dried and fresh vegetables and prepared foods showed *Aspergillus flavus* to be the most common contaminating fungus. *Penicillium*, *Fusarium*, and *Rhizopus* fungi were also prevalent.

The consumption of AFLA was determined by three separate surveys, each of 2-day duration, over a period of 1 year. Within the three survey areas of Thailand (Singburi, Ratchaburi and Songkhla), samples of food served were collected, and the amounts of each food eaten by the family were measured. Daily AFLA ingestion, expressed as nanograms of total AFLA consumed per kilogram body weight on family, rather than individual basis, was highest in Singburi (73 to 81 ng/kg body weight), intermediate in Ratchaburi (45 to 77 ng/kg body weight), and lowest in Songkhia. (5 to 8 ng/kg body weight).

Incidence of liver cancer, as measured in this survey, was two new cases per year in Songkhla and 6 new cases/100 000/year in Ratchaburi. National health records indicated that the incidence of primary liver cancer in Singburi area was 14 deaths/100 000/year, but this rate could not be measured directly as part of the AFLA study due to the unavailability of a key figure in the study.

Kenya

Another investigation was conducted in Kenya at the time of the Thailand study (Peers & Linsell, 1973; 1977). The main evening meal was sampled over 24 times in sample clusters of individuals distributed in 132 sub-locations in the district. The collection period was 21 months. Estimation of the incidence of primary liver cancer in the district was based on data from the Kenya Cancer Registry (Table 10).

Table 10 - Hepatoma incidence and aflatoxin ingestion in Kenya

Altitude area	Liver cancer incidence cases/100 000/year (1967-1970)		Average daily AFB1 intake (ng/kg body weight/day)	
	Male	Female	Male	Female
Low	12.9	5.4	14.81	10.03
Middle	10.8	3.3	17.84	5.86
High	3.1	2.5	4.88	3.46

Data from the Kenya Cancer Registry – Peers & Linsell (1973; 1977)

Mozambique

Van Rensburg *et al* (1974) reported results in measuring AFLA consumption in Mozambique, in particular the Inhambane district, which showed a liver cancer rate of 35.5 and 25.4/100 000/year for the periods 1964-68 and 1969-71, respectively, with more than twice as many cases in males as in females.

AFLA contamination of prepared foods consumed by the study population was measured by chemical assay of 880 meals. The mean daily per capita consumption of AFLA was calculated to be 222.4 ng/kg body weight. Thus, the highest primary liver cancer rate correlates with the highest known AFLA intake in the world.

Swaziland

Two studies on AFLA and human liver cancer have been performed in Swaziland. In 1971, Keen & Martin (1971a; 1971b) found an association between the geographical distribution for AFLA in peanut samples from the lowveld, middleveld, and highveld with the distribution of liver cancer cases.

In 1972, the International Agency for Research on Cancer (IARC) and Tropical Products Institute (TPI) of London initiated a study in Swaziland that was modeled on

their earlier study in Murang's district of Kenya (Van Rensburg *et al*, 1974; Van Rensburg, 1977). AFLA determinations were made from 1 056 samples of the main meal and 455 samples of beer, etc. The result showed a clear correlation between estimated AFLA consumption and liver cancer rates.

The Philippines

Peanut butter and maize have been shown to be contributors of AFLA to the Philippines food products (Campbell & Salamat, 1971). AFLA were found in almost all of the 149 samples of peanut butter, with an average concentration of AFB_1 of 213 ng/g. The most heavily contaminated sample of peanut butter contained 8.6 µg/g AFB_1 whereas 95 of 98 maize samples analysed contained an average of 110 ng/g AFB_1.

Much of Angsubhakorn's (1998) overview above have been summarized before by Van Rensburg (1977) – see Table 11 - and the correlation between cancer incidence and AFLA intake calculated. A statistically highly significant correlation was found, but Van Rensburg points out that the majority of primary liver cancer cases have been shown to have HBV surface antigen and antibody against HBV core antigen in their sera. He asks the question if hepatitis infection might be a result, rather than a cause of liver cancer.

Table 11 - Summarized results of studies measuring primary liver cancer incidence rate and aflatoxin intake

Locality	Cancer rate (100 000/year)	Aflatoxin intake ng/kg body weight/day
Kenya – high altitude	0.7	3.5
Thailand – Songkhla	2.0	5.0
Swaziland – highveld	2.2	5.1
Kenya – middle altitude	2.9	5.8
Swaziland – middleveld	4.0	8.9

Kenya – low altitude	4.2	10.0
Thailand – Ratburi	6.0	45.0
Swaziland – lowveld	9.7	43.1
Mozambique - Inhambane	13.0	222.4

Correlation r = 0.9683 (*P*<0.01)

Data from Van Rensburg (1977)

From Table 11, it appears that the NOAEL of AFLA for liver cancer in humans is an intake of 3.5 - 5.0 ng per kg body weight per day, or 245 - 350 ng per 70-kg person per day. If the total intake at this level came from maize meal, it would translate to a dietary level of 0.53 - 0.76 ng/g (µg/kg) of AFLA for consumers eating 460 g of maize meal per person per day.

2.5.2.2.4. Evidence contradicting the role of aflatoxins in liver cancer

Costa Rica

In Costa Rica, where white maize is consumed as a staple, a 1985 to 1988 study (Mora, 1990) found average AFLA levels in white maize for the country as a whole to be as high as 147 ng/g (µg/kg). The average per region varied between 18 and 289 ng/g (µg/kg). The MTL for AFB_1, AFB_2, AFG_1, and AFG_2 in food maize in Costa Rica is 35 ng/g, and in feed maize, it is 50 ng/g (Mora, 1990). Costa Rica, with an incidence rate in males of 6.57 and in females of 3.85 per 100 000 (Ferlay *et al*, 1999) in 1990, is not a country with an extraordinarily high incidence of liver cancer (hepatocellular carcinoma -HCC) – the type of cancer most likely to result from exposure to AFLA. Unfortunately, figures on the actual amounts of AFLA ingested in Costa Rica are not available. However, if the intake of maize product is taken as a moderate 100 g per day, an average AFLA intake of 14.7 µg per person per day is implied.

India

In India, the incidence of liver cancer in males is 2.63, and in females it is 1.22 cases per 100 000 of the age standardised world population (Ferlay *et al*, 1999), some of the lowest incidence rates for liver cancer in the world in 1990. This is in spite of the fact that about 5% of people on the Indian sub-continent are carriers of HBV or HCV virus. Moreover, in more than 2 000 samples analysed in one study (Dhir & Mohandas, 1998), the regulatory limit for AFLA in India of 30 ng/g was exceeded in 21% of the peanut samples, and in 26% of the maize samples. The PDI of AFLA by the Indian population was estimated to be in the range of 4-100 ng/kg body wt/day (Vasanthi & Bhat, 1998), which translates to between 280 and 7 000 ng per 70 kg person per day, which is considerably higher than the 245 - 350 ng/person per day, which in other countries appears to be about the NOAEL. In a country like South Africa, where rural people are estimated to take in about 460 g of maize products per day (Gelderblom *et al*, 1996), this would indicate that in grain products up to 15 ng/g (µg/kg) mean AFLA level would not be harmful to consumers, in spite of a high incidence rate of HBV infection. This is higher than the existing South African regulatory level of 10 ng/g in grains and groundnuts for human consumption.

The USA

From death certificate records compiled by the National Centre for Health Statistics in the USA, Stoloff (1983) computed the primary liver cell cancer mortality ratios for the periods 1968 to 1971 and 1973 to 1976. He sorted the data by race, sex, urbanization and region. He then selected the data on rural white males from the Southeast and the North-and-West regions respectively for comparison of mortality ratios and past dietary exposure to AFLA. He calculated the expected average daily ingestion of AFB_1 for each group, based on projections of recent AFLA contamination information, back to the 1910 to 1960 period, and estimates of maize and groundnut consumption obtained from household food consumption surveys relevant to the period. The expected average ingestion of AFB_1 for the Southeast group came to between 13 and 197 ng/kg bodyweight per day, and to 0.2 to 0.3 ng/kg bodyweight per day for the North-and-West group. When the age-adjusted mortality ratios for the two groups were compared, the Southeast group showed a 10% excess

for all ages, and 6% excess for the 30 to 49 year age group. Stoloff (1983) concludes that the difference was in the expected direction in relation to the projected past exposure to AFLA, but it was far from the manifold difference that would have been anticipated from experiments with rats and from earlier epidemiological studies in Africa and Asia. Moreover, he believes that the remaining major portion of the mortality in the Southeast may be attributed to many unidentified causes for which the two populations that were compared were not controlled, leaving in doubt the validity of any attribution of the excess primary liver cell mortality to AFLA ingestion. The primary liver cell mortality ratios for Orientals living in the USA and for urban black males were in considerable excess over the USA average.

2.5.2.2.5. Other factors involved in the development of liver cancer

From many other studies, it is clear that, in addition to exposure to AFLA in the diet and HBV and HCV viral infection, various other factors may also contribute towards the development of liver cancer in humans. These include exposure to nitrosamines, certain other carcinogenic chemicals, alcohol, infection by liver fluke, and other mycotoxins, such as sterigmatocystin. Liver cancer, like many other cancers, has multifactorial aetiology, but in spite of some contradicting evidence, it is clear from both animal experiments and human case studies that sub-acute exposure to AFLA often plays an important role in the development of liver cancer. In addition, AFLA are acutely toxic to humans at a dietary level of about 1.7 µg/g.

2.5.3. Toxicology of fumonisins

In spite thereof that FBs are ubiquitous in maize and maize products in all parts of the world where maize is consumed as a staple, no cases of acute or chronic toxicity of FBs to humans have been recorded in the literature, with the possible exception of an outbreak of a syndrome in India attributed to FBs (Bhat *et al*, 1997). Therefore greater use of toxicity studies with FBs on experimental animals, as well as clusters of acute toxicity to farm animals will have to be made to arrive at some indication of the hazards, if any, that FBs may pose to human health. Following is an overview of toxicity studies on various animal species. This will be used to pinpoint the loci of main damage caused by FBs in animals. Since FBs do not appear to be acutely toxic to humans at naturally occurring levels, which in the Transkei have been recorded as

high as 140 µg/g total FB_1 and FB_2 on maize produced on subsistence farms, an epidemiological overview of possible chronic effects, specifically any possible carcinogenic effects in the sensitive loci, as established through the animal studies, will be attempted in Section 3.4 as an indication of the possible hazard of FBs to human health. Epidemiological overviews concerning human OC have already been done in Sections 2.3 and 2.4, and an epidemiological overview concerning neural tube defects will be done in Section 3.5.

2.5.3.1. The effects of fumonisins on farm animals

The FDA's Centre for Veterinary Medicine prepared a 'Background Paper in Support of Fumonisin Levels in Animal Feed', which offers a convenient overview in a single document of toxicological studies with FBs on a variety of farmed animals. This provides a concept of the relative sensitivity of different farmed animals to fumonisins. The document has been published on the Internet (Anonymous, 2001c) and the summary is reproduced here:

"**SUMMARY of RECOMMENDED LEVELS for TOTAL FUMONISINS in FEED**

Table I. Summary of Recommended Levels for Total Fumonisins (FB_1 + FB_2 + FB_3) in Corn, Corn By-products, and the Total Ration in Various Animal Species.

Animal or Class	Recommended Maximum Level of Total Fumonisins in Corn and Corn By-Products (ppm[1])	Feed Factor[2]	Recommended Maximum Level of Total Fumonisins in the Total Ration (ppm[1])
Horse[3]	5	0.2	1
Rabbit	5	0.2	1
Catfish	20	0.5	10
Swine	20	0.5	10
Ruminants[4]	60	0.5	30
Mink[5]	60	0.5	30

Poultry[6]	100	0.5	50
Ruminant, Poultry & Mink Breeding Stock[7]	30	0.5	15
All Others[8]	10	0.5	5

[1] total fumonisins = $FB_1 + FB_2 + FB_3$.
[2] fraction of corn or corn by-product mixed into the total ration.
[3] includes asses, zebras and onagers.
[4] cattle, sheep, goats and other ruminants that are $\geq$ 3 months old and fed for slaughter.
[5] fed for pelt production.
[6] turkeys, chickens, ducklings and other poultry fed for slaughter.
[7] includes laying hens, roosters, lactating dairy cows and bulls.
[8] includes dogs and cats.

The purpose of this document is to provide the scientific support behind our (CVM's) recommended maximum levels for fumonisins in animal feed (Table I). Fumonisins are environmental toxins produced by molds and found primarily in corn. The major types of fumonisins are B_1 (FB_1), B_2 (FB_2) and B_3 (FB_3).

Our goal was to identify fumonisin levels in feed that are adequate to protect animal and human health and that are achievable with the use of good agricultural and good manufacturing practices. We wish to emphasize that the recommended levels are intended to provide guidance that may change following public input and are not to be considered tolerances. Future research and/or different interpretations of existing research could change the recommended values.

These recommendations are the result of reviewing the published literature to determine the effects of fumonisins when fed to various animals, including horses, rabbits, catfish, ruminants, poultry and mink. There were many gaps in the literature regarding the feeding of low levels of fumonisins to animals. Although this compelled some extrapolation of the data to establish draft guidance levels for fumonisins in the diets of various species, all calculations are derived from factors found in the literature.

In six instances, we grouped species together because the animals seemed to have a similar sensitivity to fumonisins. This is an attempt to avoid a multitude of guidance levels and does not necessarily imply that the species are biologically similar.

Horses and rabbits were grouped together as the most sensitive species. Corn and corn by-products used in rations of horses and rabbits should contain less than 5 ppm of $FB_1 + FB_2 + FB_3$ and comprise no more than 20% of the dry weight of

the total ration (Table I). The total ration should contain less than 1 ppm of FB_1 + FB_2 + FB_3 (0.2 x 5 ppm FB_1 + FB_2 + FB_3 = 1 ppm of FB_1 + FB_2 + FB_3).

Catfish and swine were grouped together as intermediate in sensitivity to fumonisins. Corn and corn by-products used in rations of catfish and swine should contain less than 20 ppm of FB_1 + FB_2 + FB_3 and comprise no more than 50% of the dry weight of the total ration (Table I). The total ration should contain less than 10 ppm of FB_1 + FB_2 + FB_3 (0.5 x 20 ppm of FB_1 + FB_2 + FB_3 = 10 ppm of FB_1 + FB_2 + FB_3).

Ruminants, mink and poultry were considered more resistant than horses, rabbits, catfish and swine to fumonisin; however, there was no data found in ruminants and mink at total dietary levels between 25 and 100 ppm of total fumonisins, while the data in poultry at these levels was more robust. Due to this data gap, we were more conservative in our recommendations for ruminants and mink than in poultry.

Corn and corn by-products used in rations of ruminants that are at least 3 months old and fed for slaughter and in rations of mink fed for pelt production should contain less than 60 ppm of FB_1 + FB_2 + FB_3 and comprise no more than 50% of the dry weight of the total ration (Table I). The total ration should contain less than 30 ppm of FB_1 + FB_2 + FB_3 (0.5 x 60 ppm of FB_1 + FB_2 + FB_3 = 30 ppm of FB_1 + FB_2 + FB_3).

Corn and corn by-products used in rations of poultry fed for slaughter should contain less than 100 ppm of FB_1 + FB_2 + FB_3 and comprise no more than 50% of the dry weight of the total ration (Table I). The total ration should contain less than 50 ppm of FB_1 + FB_2 + FB_3 (0.5 x 100 ppm of FB_1 + FB_2 + FB_3 = 50 ppm of FB_1 + FB_2 + FB_3).

The National Center for Toxicological Research (NCTR in Jefferson, AR) recently completed a chronic dietary bioassay with purified FB_1. This study showed clear evidence of kidney tumors in male rats and of liver tumors in female mice at dietary levels of 50 ppm and above.

We believe 15 ppm of FB_1 + FB_2 + FB_3 in the total ration of mink, ruminant and poultry breeding stock should provide adequate protection against any potential carcinogenic effects in these animals. This recommendation is based upon the NCTR chronic study where 15 ppm FB_1 produced the same or fewer kidney and liver tumors compared to the controls. Corn and corn by-products used in the rations of mink, ruminant and poultry breeding stock should contain less than 30 ppm of FB_1 + FB_2 + FB_3 and comprise no more than 50% of the dry weight of the total ration (Table I). If the recommended total fumonisin level in the total ration for a species was less than 15 ppm, we did not believe that the breeding stock of the species needed additional protection from possible carcinogenic effects.

The last grouping was of animal species/classes not mentioned above (e.g. dogs, cats). Often there was no published dietary study with fumonisins in these animals and no historical indication/association of problems from feeding corn. We believe 5 ppm of FB_1 + FB_2 + FB_3 in the total ration should provide adequate

protection against any potential acute and/or carcinogenic effects in these animals. This recommendation is based largely upon the NCTR chronic study where 5 ppm FB_1 appeared to be the no-observed-adverse-effect level. Corn and corn by-products used in the rations of these animals should contain less than 10 ppm of $FB_1 + FB_2 + FB_3$ and comprise no more than 50% of the dry weight of the total ration (Table I).

We acknowledge that extensively validated "quick" or confirmation tests are not commercially available for total rations. However, the Association of Official Analytical Chemists International has established an official method (995.15) for determining fumonisins B_1, B_2 and B_3 in corn. In addition, the United States Department of Agriculture's Grain Inspection, Packers and Stockyards Administration (GIPSA) announced on June 5, 2001, that two test kits have been approved for official testing of fumonisins in the national grain inspection system. GIPSA authorized the use of the Veratox Quantitative Fumonisin Test kit, manufactured by Neogen Corporation, to determine fumonisins in corn, corn meal, popcorn, rough rice, corn/soy blend, and wheat; and RIDASCREEN® FAST Fumonisin test kit, manufactured by r-Biopharm Inc., for fumonisins in corn, corn meal, sorghum, corn gluten meal, corn germ meal, and corn/soy blend. We believe that the recommended fumonisin levels will stimulate additional interest in developing and certifying/validating confirmatory tests and "quick tests" for determining fumonisins in corn, corn by-products, and complete animal feed rations."

2.5.3.2. Co-occurrence of fumonisins and nitrosamines, or aflatoxins

Wild *et al* (1997) tested the hypothesis that nitrosamines and FBs would interact in oesophageal carcinogenesis by treating male rats with the known oesophageal carcinogen *N*-MBN, and FB_1. The results showed that there is no synergistic interaction between *N*-MBN and FB_1 in the rat oesophagus when the two compounds are administered together.

On the other hand, Gelderblom *et al* (2002) reported a significant synergistic carcinogenic interaction between FB_1 and AFB_1. When utilising a short-term carcinogenesis model in rat liver, both the compounds exhibited slow cancer initiating potency by increasing gluthatione-S-transferase lesions. However, when rats were treated in a sequential manner with AFB_1 and FB_1 the number and size of these lesions significantly increased as compared to the separate treatments. Histopathological analyses indicated that the individual treatments showed far less toxic effects, including occasional hepatocytes with dysplastic nuclei, oval cell proliferation and, in the case of FB_1, a few apoptotic bodies in the central vein

regions. The sequential treatment regimen induced numerous foci and dysplastic hepatocyte nodules, and with oval cells extending from the periportal regions into the centrilobular regions. This would imply that, in addition to the cancer promoting activity of FB_1 of AFB_1-initiated hepatocytes, the AFB_1 pre-treatment enhanced the FB_1 initiating potency, presumably by rendering the liver more susceptible to the toxic effects of FB_1. The authors conclude that the co-occurrence of AFB_1 and FB_1 in maize consumed as a staple diet could pose an increased risk and should be included in establishing risk assessment parameters in humans.

2.5.3.3. Physiological effects of fumonisins in rats, mice and monkeys

FBs have produced liver damage and changes in the levels of certain classes of lipids, especially sphingolipids, in all animals studied (Merrill *et al*, 1997). Kidney lesions were also found in many animals (Merrill *et al*, 1997; Norred *et al*, 1998). Feeding of *Fusarium* culture material containing FBs has also been associated with heart failure in baboons (Kriek *et al*, 1981) and swine (Smith *et al*, 2000; Haschek *et al*, 2001), with atherogenic effects in vervet monkeys (Fincham *et al*, 1992), and with medial hypertrophy of pulmonary arteries in swine (Casteel *et al*, 1994).

Chronic feeding of purified FB_1 (at levels of 50 µg/g or more) produced liver cancer and decreased life span in female $B6C3F_1$ mice and kidney cancer in male F344/N rats without decreased life spans (National Toxicology Program, 1999). At lower exposures, no carcinogenic effect was observed. However, in the first study on the carcinogenicity of pure FB_1, the feeding of similar levels of FBs (50 µg/g) to BD IX male rats resulted in liver cancer (Gelderblom *et al*, 1991). FB was negative in genotoxicity assays (Gelderblom *et al,* 1992, Norred *et al*, 1998). See also the papers on the hepatocarcinogenicity in rats of *F. verticillioides* MRC826 (Marasas *et al*, 1984b) and purified FB_1 (Gelderblom *et al*, 1991).

FB_1 and FB_2 are known to be potent inhibitors of sphingosine *N*-acyltransferase (ceramide synthase) and hence to disrupt *de novo* sphingolipid biosynthesis. The sphingoid bases, sphingosine and sphinganine (and hence their ratio), were measured (Shephard *et al*, 1996b) at varying intervals over a period of 60 weeks in the serum of non-human primates (vervet monkeys; *Cercopithecus aethiops*) which were consuming diets containing 'low' and 'high' amounts of *F. moniliforme* culture

material, such that their total daily FB intake was approximately 0.3 and 0.8 mg/kg body weight/day, respectively. In humans in rural areas of South Africa, where average 70 kg persons consume about 460 g of maize products per person per day (Gelderblom *et al*, 1996), these levels would translate to dietary levels of approximately 45 and 121 µg/g respectively of FBs in maize products. Such levels would be fatal within a few weeks to horses and pigs. No significant differences were found in the monkey serum levels of sphingosine compared to controls, but serum sphinganine levels in the experimental groups (mean of 219 nM and 325 nM, respectively) were significantly ($P = 0.02$) elevated above the levels in controls (mean 46 nM). As a consequence, the ratio sphinganine (Sa)/sphingosine (So) was significantly ($P = 0.003$) elevated from a mean of 0.43 in the control group to 1.72 and 2.57 in the experimental groups, respectively. Similar changes in sphingolipid profiles were also measured in urine with an increase of the ratio from 0.87 in controls to 1.58 and 2.17 in the experimental groups, although the differences were not statistically significant. Hence, the disruption of sphingolipid biosynthesis in vervet monkeys by FBs in culture material added to their diet can effectively be monitored in the serum as an elevation of the Sa/So ratio.

These high FB intakes over an extended period of 60 weeks raises the question whether primates, which include humans, might be much more resistant to FBs than many other species. Sewram *et al* (2001) describes the accumulation of FB_1 levels as high as 5.98 mg FB_1, 33.77 mg FB_1, and 65.93 mg FB_1/kg (of hair) in the hair of vervet monkeys, *Cercopithecus aethiops* respectively receiving control, low-dose, and high-dose fumonisin contaminated diets. Hair of rats given either single gavage doses (1 and 10 mg FB_1/kg body weight), or contaminated feed (50 mg FB_1/kg - approximately 4.25 mg FB_1/kg body weight/day) by the fourth week contained mean levels of up to 34.50 mg/kg (rats treated by gavage at 10 mg FB_1/kg body weight) and 42.20 mg/kg (rats receiving contaminated feed).

2.5.3.4. Epidemiological studies of the effect of fumonisins in humans

With the possible exception of one report in India (Bhat *et* al, 1997), there is currently no direct evidence that FBs cause adverse health effects in humans (Anonymous, 2001b). FBs are ubiquitous in maize worldwide, but with the possible exception of a case in India, no cases of either acute, or chronic toxicity to humans have been

recorded in any country where maize is a staple food. This also applies to South American countries such as Mexico, where maize is processed through alkali cooking (nixtamalization) to produce masa for tortillas and other products. During this process, FBs are hydrolyzed, but hydrolyzed FB_1 is less toxic to the brine shrimp (Hartl & Humpf, 2000) and to rat embryos (Flynn *et al*, 1997) than the original FB_1. No incidents of acute intoxication of humans by FBs have been recorded in the Transkei, where total FB levels as high as >140 µg/g (Rheeder *et al*, 1992) were found in some mouldy maize samples. Mouldy maize is reportedly used to make traditional beer, of which some Transkeieans consume large quantities (Warwick & Harington, 1973), but it should be noted that Sammon (1992) in a case control study in Transkei on 100 OC patients matched for age sex and education level, found that consumption of traditional beer was not a risk factor. Marasas (1997) estimated the FB levels in mouldy and 'healthy' Transkeian maize at respectively 54 and 7.1 µg/g. He estimated FB intake in the Transkei at between 46.6 and 354.9 µg/kg body weight/day. Such levels would be acutely toxic to horses and pigs respectively, but there are no reports of human fatalities or disease other than a high incidence of OC.

The studies currently available demonstrate inconclusively a statistical association between FBs and human OC. Investigators at the MRC suggested an association between high levels of FB-producing moulds on maize grown on subsistence farms in a part of the Transkei, with a high OC incidence (Rheeder *et al*, 1992). However, these studies are limited by the lack of controlled conditions and have not been substantiated through fully-fledged epidemiological studies. Particularly, confounding risk factors e.g. alcohol consumption, and exposure to nitrosamines were not established. Shephard *et al* (2002) recently estimated FB levels in maize porridge compared to uncooked maize meal, but data on FB levels in traditional beer, and the actual levels of ingestion of FBs are still lacking, as well as estimates of absorption of FBs in the human gut. There may be other, as yet unidentified factors linked with maize consumption that play a role in the development of OC. For example, Sammon (1999a; 1999b) and Sammon & Alderson (1998) found high levels of non-esterified fatty acids (11 to 42% of contained fatty acids) in maize meal and in foods prepared from it. In food prepared from maize meal, 49 to 363mg non-esterified linoleic acid per 100g sample was found. The authors reason that high levels of non-esterified linoleic acid in the diet may create a predisposition to oesophageal

carcinogenesis, by causing raised intragastric production of prostaglandin E2 and by profoundly affecting the normal pH and fluid content of the oesophagus. High levels of prostaglandin E2 in the gastric mucosa lead to reduced gastric acid secretion, relaxation of the pylorus and a reduction in lower oesophageal sphincter pressure. These events result in combined reflux of duodenal and gastric juices low in acidity into the oesophagus. Resulting dysplasia strongly predisposes to local squamous carcinogenesis. Production of prostaglandin E2 also causes inhibition of the proliferation and cytokine production of Th1 cells, mediators of cellular immunity. Tuberculosis, measles, hepatoma, secondary infection in HIV and kwashiorkor are all favoured by this reduction in cellular immunity. Diet-associated inhibition of the Th1 subset is a major contributor to the high prevalence of these diseases found in areas of sub-Saharan Africa where maize is the staple. In addition, *Solanum nigrum*, beans, and pumpkin, foods commonly consumed in areas of southern Africa with high OC prevalence, all contain protease inhibitors. Sammon (1998) believes that suppression of protease inhibitors can lead to overexpression of growth factors in the oesophagus, resulting in a proliferative and oncogenic drive.

Therefore, the existing studies do not allow any definitive conclusions to be made about cancer causation in humans. Other studies associating high levels of FB-producing moulds on maize with OC also lacked controls (Chu & Li, 1994), or did not measure FB levels (Franceschi *et al*, 1990) – see Sections 2.3 and 2.4 for detail. Further, in an area of China with high incidence of gastric cancer, Groves *et al* (1999) observed a lack of association between consumption of FB contaminated maize with gastric or any other human cancer, including OC.

In a limited epidemiological study in India, an association between high levels of FBs (but not other mycotoxins) in mouldy sorghum and maize damaged by unseasonal rains beginning in a few villages of the Deccan plateau in India, and gastrointestinal symptoms (e.g., cramping and diarrhea) was noted (Bhat *et al*, 1997). However, this study also lacked control of established risk factors. In addition, contaminants other than mycotoxins cannot be eliminated as causative factors, and a similar association was not detected in studies conducted in other countries.

Other factors that make it difficult to extrapolate the results of these studies are the differences in agricultural and nutritional conditions in the areas where these studies

were conducted compared to those in the commercial maize areas in South Africa. For example, commercial maize in South Africa contains much lower levels of FBs than has been reported in subsistence maize from the high OC area of the Transkei. In commercial maize, FB levels appear to be similar to those in subsistence farm produced maize in parts of the Transkei with a moderately low incidence of OC. Maize as visibly mouldy as has been reported from the Transkei could never make a grade and can therefore not enter the commercial grain trading system. FB levels in maize as high as in some Transkeian samples would be fatal to horses and swine (see Marasas *et al*, 2000), resulting in claims for damages from stock farmers against feed manufacturers, if such maize was used in feeds. In addition, maize processed for consumption on subsistence farms is processed whole and contains all the mouldy material and all parts of the kernel, whereas in commercial milling, mouldy and broken kernels are removed during cleaning. The bran and germ, the kernel parts that contain most of the mycotoxins, are also removed to greater or lesser extent in the various grades of milled product. Furthermore, in most instances the human populations under study were significantly malnourished in comparison with the sections of the population consuming commercial maize products in South Africa and consequently might have been more susceptible to adverse influences.

Van der Westhuizen *et al* (1999) conducted a study on human volunteers in Transkei and KwaZulu-Natal in South Africa, and in the Bomet district, western Kenya. They determined the sphinganine (Sa)/sphingosine (So) ratios in the plasma and urine of males and females consuming a staple diet of maize produced on subsistence farms (referred to as home grown maize, as opposed to commercial maize). In Transkei, the ratios were 0.34 $\pm$ 0.36 (mean $\pm$ standard deviation) (n = 154) and 0.41 $\pm$ 0.72 (n = 153), in plasma and urine respectively and in plasma samples from KwaZulu-Natal it was 0.44 $\pm$ 0.23 (n = 26). In Kenya, the ratios in plasma and urine were 0.28 $\pm$ 0.07 (n = 29) and 0.34 $\pm$ 0.20 (n = 27), respectively. Mean total FB level in Transkeian maize, randomly collected from the same region where the human volunteers lived, was 580 ng/g (n = 40). This is similar to the long-term averages in commercial maize in South Africa (see Table 27). In the KwaZulu-Natal province, no FB (n = 17) was detected (< 10 ng/g) in the maize. In Kenya, only one of seven samples was contaminated with 60 ng/g FBs. No significant differences were found in the Sa/So ratios of males and females, neither within, nor between the different regions ($P > 0.05$). The authors

conclude that the ratio is possibly not sensitive enough to act as a biomarker for FB exposure in humans at these FB levels. However, it could also be concluded that levels of FBs up to about 600 ng/g and perhaps considerably higher, have no observable effect on the Sa/So ratios in humans.

In another study, Qiu & Liu (2001) monitored over one month the Sa/So ratio in urine of humans exposed to FB_1 in maize diets. Twenty-eight healthy adult volunteers consumed for one month a normal diet containing their homegrown maize potentially contaminated with FB_1. The daily FB_1 intakes were estimated and used to assess the relationship between FB_1 intake and the urinary Sa/So ratios. All the maize samples contained FB_1 at levels between 0.08 to 41.1 µg/g. Estimated daily FB_1 intakes ranged from 0.4 to 740 µg/kg body weight/day. The results suggest that sphingolipid metabolism of humans could be affected by FB_1 intake, and that the urinary Sa/So ratio may be useful for evaluating FB_1 exposure when the contamination of maize with FB_1 is high.

Based on these results, the recommended MTLs for FBs in maize of 100 – 200 ng/g appear very low.

2.5.4. Toxicology of deoxynivalenol

Trichothecene mycotoxins are a group of structurally similar fungal metabolites that are capable of producing a wide range of toxic effects. DON, a trichothecene, is prevalent worldwide in crops used for food and feed production, including in Canada (Scott, 1997), the United States, Europe and Argentina (Pacin *et al*, 1997). Although DON is one of the least acutely toxic trichothecenes, it should be treated as an important food safety issue because it is a very common contaminant of grain. In a review of the toxicology of DON, Rotter *et al* (1996) focus on the ability of DON to induce toxicological and immunotoxic effects in a variety of cell systems and animal species. At the cellular level, the main toxic effect is inhibition of protein synthesis via binding to the ribosome. In animals, moderate to low ingestion of toxin can cause a number of as yet poorly defined effects associated with reduced performance and immune function. The main overt effect 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. The serotoninergic system

appears to play a role in mediation of the feeding behavior and emetic response. Animals fed low to moderate doses are able to recover from initial weight losses, while higher doses induce more long-term changes in feeding behavior. At low dosages of DON, hematological, clinical, and immunological changes are also transitory and decrease as compensatory/adaptation mechanisms are established. Swine are more sensitive to DON than mice, poultry, and ruminants, in part because of differences in metabolism of DON, with males being more sensitive than females.

The capacity of DON to alter normal immune function has been of particular interest (Rotter *et al*, 1996). There is extensive evidence that DON can be immunosuppressive or immunostimulatory, depending upon the dose and duration of exposure. While immunosuppression can be explained by the inhibition of translation, immunostimulation can be related to interference with normal regulatory mechanisms. In vivo, DON suppresses normal immune response to pathogens and simultaneously induces autoimmune-like effects, which are similar to human immunoglobulin A nephropathy. Other effects include superinduction of cytokine production by T helper cells (in vitro) and activation of macrophages and T cells to produce a proinflammatory cytokine wave that is analogous to that found in lipopolysaccharide-induced shock (in vivo). To what extent the elevation of cytokines contributes to metabolic effects such as decreased feed intake remains to be established. Although these effects have been largely characterized in the mouse, several investigations with DON suggest that immunotoxic effects are also likely in domestic animals. The authors conclude that further toxicological studies and an assessment of the potential of DON to be an etiologic agent in human disease are warranted.

Hughes *et al* (1999) conducted studies to determine the dietary amounts of DON in dog and cat food that are required to produce overt signs of toxicity (e.g., vomiting or reduced food intake). Wheat naturally contaminated with 37 mg of DON/kg was used to manufacture pet foods containing 0, 1, 2, 4, 6, 8, and 10 mg of DON/kg. DON concentration in pet food following manufacture was unchanged, indicating that the toxin was stable during conventional extrusion processing. Dogs previously fed DON-contaminated food were able to preferentially select uncontaminated food. Dogs not previously exposed to DON-contaminated food consumed equal quantities of contaminated and uncontaminated food. There was no effect of 6 mg of DON/kg on

dog food digestibility. Food intake of dogs was significantly reduced by DON concentrations greater than 4.5 $\pm$ 1.7 mg/kg, and DON greater than 7.7 $\pm$ 1.1 mg/kg reduced cat food intake. Vomiting by dogs and cats was commonly observed at the 8 and 10 mg DON/kg levels.

When DON was tested as a skin tumour initiator in experimental mice (Lambert *et al*, 1995), there were no statistically significant differences in the number of cumulative tumours or the number of tumour-bearing mice between the DON-initiated/PMA-promoted group and its control, the vehicle-initiated/PMA-promoted group. When DON was administered as a tumour promoter, no tumours were observed. Histopathology of the skin revealed that DON induced a mild diffuse squamous hyperplasia, but there was no progression of the lesion to neoplasia.

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.

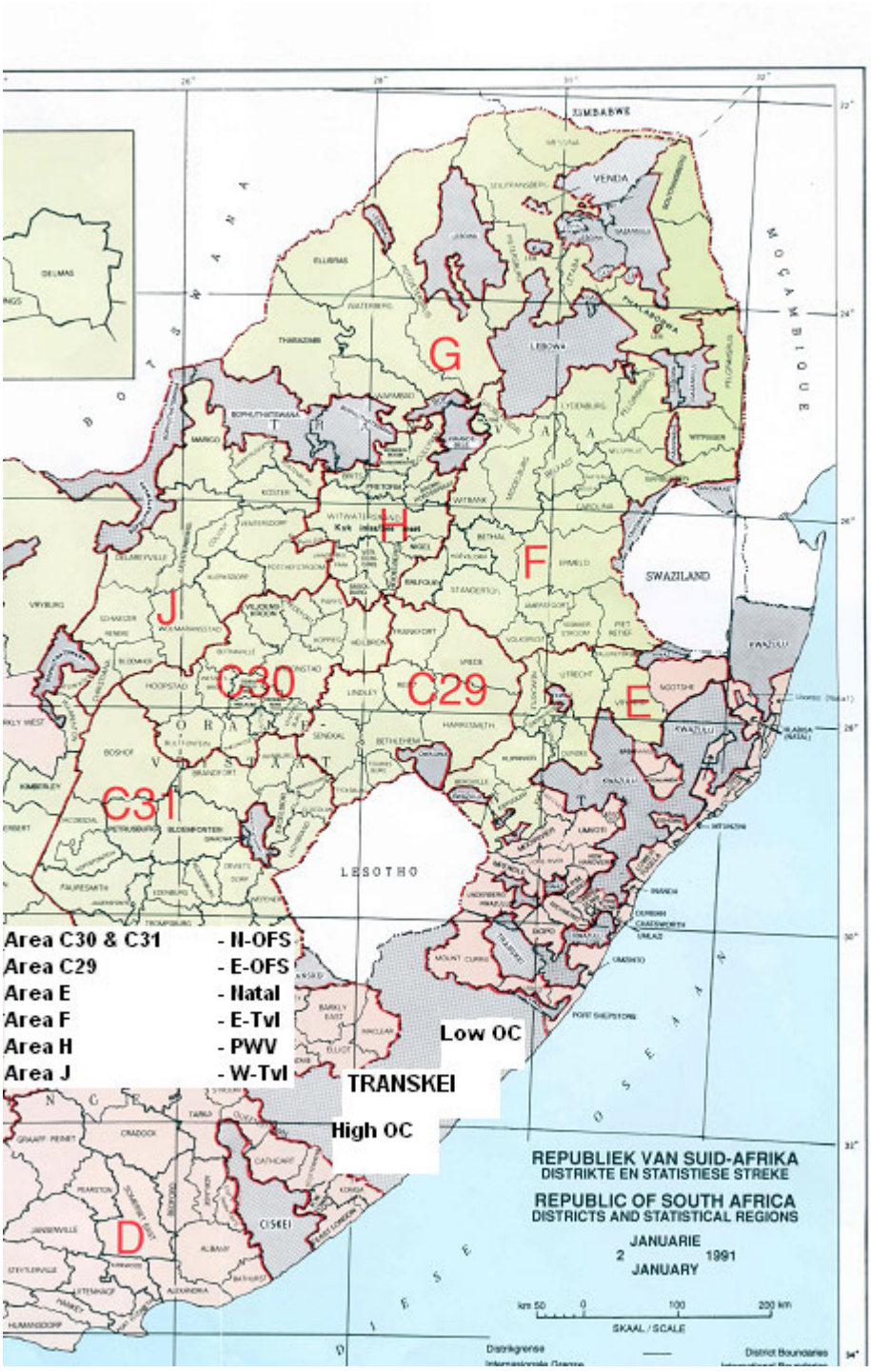

Fig. 1 – Map of the eastern parts of South Africa, showing the maize production areas in 1991 referred to in the text and the 'high' and 'low' OC incidence areas in Transkei referred to in the literature

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 FB_1, FB_2, DON, NIV, ZEA, MON, and AFLA in both years, and additionally in 1990, for FB_3. 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, FB_1, FB_2, FB_3, 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, FB_1, FB_2 and FB_3 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. Fumonisins 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 (FB_1+ FB_2+ FB_3) 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 (FB_1+FB_2+FB_3) (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

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

[3]The 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 % *F. verticillioides* 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

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

[2]Total 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 consquently 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 above 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 Mpumulanga 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 date 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 µg/g levels, regulatory MTLs in the high ng/g's, or low µg/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 µg/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 µg/g in horse rations.

FBs are also acutely toxic to pigs at dietary levels around 50 to 90 µg/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 µg/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 µg/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 µg/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 year[1] (calculated over the 4 years 1987 to 1990) in each of 23 African countries[2], and the cancer rates (ASIR world population per 100 000 per year) in males and females[3] in each of the countries

Country	Brain		Kidney		Liver		Maize	Sorghum	Millet
	F	M	F	M	F	M	kg/person		
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

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

[1]Per capita supplies in terms of product weight are derived from the total supplies available for human consumption (i.e. food) by dividing the quantities of food by the total population actually partaking of the food supplies during the reference period, i.e. the present in-area (de facto) population. 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 (Delport *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

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.

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.

4. Results and Discussion

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

4.1.1. Unprocessed commercial South African maize

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

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

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

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

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

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

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

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

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

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

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

Detection limits of mycotoxins were as follows:

FB_1, FB_2, – 20 ng/g; and

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Detection limits of mycotoxins were as follows:

DON - 100 ng/g;

NIV, MON and ZEA - 50 ng/g;

FB_1, FB_2, FB_3 – 20 ng/g; and

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

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

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

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

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

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

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

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

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

Detection limits of mycotoxins were as follows:

DON - 100 ng/g;

NIV - 50 ng/g;

FB_1, FB_2, FB_3 – 20 ng/g; and

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

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

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

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

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

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

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

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

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

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

Detection limits of mycotoxins were as follows:

DON - 100 ng/g;

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

DAS, T-2 – 250 ng/g

FB_1, FB_2, FB_3 – 20 ng/g; and

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

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

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

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

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

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

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

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

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

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

Detection limits of mycotoxins were as follows:

DON - 100 ng/g;

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

DAS, T-2 – 250 ng/g

FB_1, FB_2, FB_3 – 20 ng/g; and

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

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

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

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

[3] Means were not compared statistically

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

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

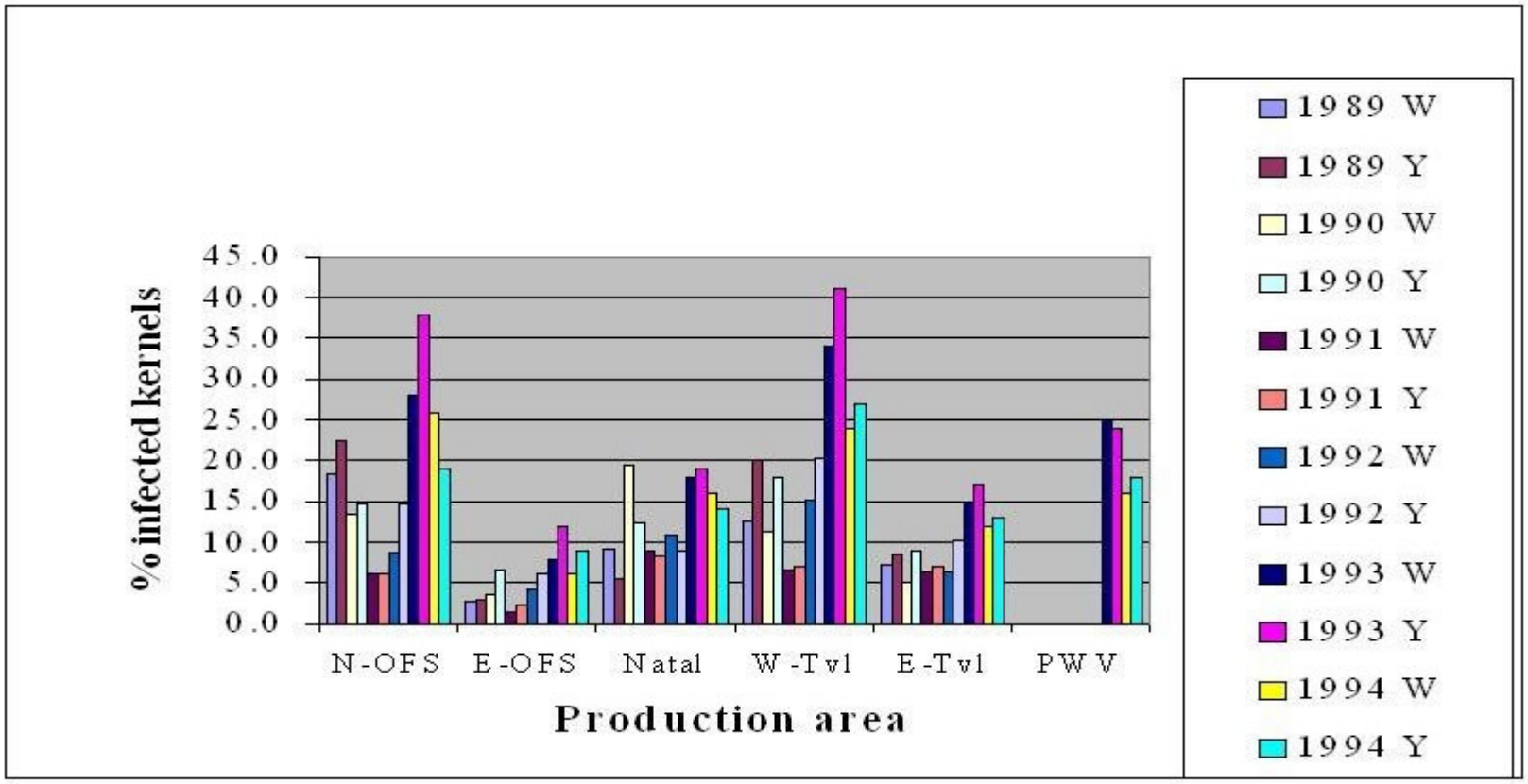

Fig. 2 – Mean percentage white and yellow maize kernels infected by *F. verticillioides* in representative samples of each of six crop years in the main maize production areas of South Africa

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

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

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

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

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

4.1.2. Mycotoxins in white maize products

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

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

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

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

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

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

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

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

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

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

Detection limits of mycotoxins were as follows:

DON - 100 ng/g;

NIV, MON and ZEA - 50 ng/g;

FB_1, FB_2, FB_3 – 20 ng/g; and

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

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

[2] n = number of samples

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

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

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

Detection limits of mycotoxins were as follows:

DAS – 250 ng/g – none detected

DON - 100 ng/g;

NIV, MON and ZEA - 50 ng/g;

FB_1, FB_2, FB_3 – 20 ng/g; and

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

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

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

[3] n = number of samples

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

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

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

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

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

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

Detection limits of mycotoxins were as follows:

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

OA and AME – 50 ng/g – none detected

DON - 100 ng/g;

NIV, MON and ZEA - 50 ng/g;

FB_1, FB_2, FB_3 – 20 ng/g; and

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

[1] n = number of samples

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

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

4.1.3. Mycotoxins in maize feed mill products

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

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

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

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

No 2 Straightrun yellow maize meal

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

Unsifted crushed yellow maize

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

Sifted crushed yellow maize

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

Defatted maize germ meal (from white maize milling)

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

Maize bran (from white maize milling)

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

Screenings (from white maize milling)

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

Detection limits of mycotoxins were as follows:

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

OA and AME – 50 ng/g – none detected

DON - 100 ng/g;

NIV, MON and ZEA - 50 ng/g;

FB_1, FB_2, FB_3 – 20 ng/g; and

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

[1] n = number of samples

4.1.4. Fungi and mycotoxins in imported yellow maize

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

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

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

Detection limits of mycotoxins were as follows:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4.1.6. Fumonisins in foreign maize food products

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

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

4.1.7. Mycotoxins in other grain staples in South Africa

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

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

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

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

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

[3] NT = None tested

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

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

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

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

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

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

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

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

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

[3] NT = None tested

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

Mean/Pos = mean for all the positive samples

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

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

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

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

[2, 3,] See Tables 16 and 17

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

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

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

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

[8] The value of r, the correlation coefficient

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[2]FAO, 2000

[3]Ferlay *et al*, 1999

[4]The value of r, the correlation coefficient

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

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

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

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

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

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

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

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

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

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

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

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

maize consumed in the area

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

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

[2,3]See Tables 16 and 17

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

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

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

[7] The value of r, the correlation coefficient

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

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

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

4.5.1. The link between NTD and fumonisins

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

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

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

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

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

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

4.5.2. Other studies on NTD incidence in South Africa

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

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

4.5.3. The epidemiological relationship of NTD with fumonisin intake

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

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

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

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

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

[1] µg/70-kg person/day

[2] The value of r, the correlation coefficient

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

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

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

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

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

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

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

4.5.5. Epidemiological studies of NTD in Mexico

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

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

4.5.6. By what mechanisms could fumonisins induce NTDs?

Hendricks (1999) speculates as follows:

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

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

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

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

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

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

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

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

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

4.6.2.1. Assessment of human exposure to aflatoxins in South Africa

4.6.2.1.1. Estimate of direct aflatoxin intake

Local maize and grain sorghum

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

Local wheat

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

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

Nuts and groundnuts

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

Imported maize

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

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

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

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

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

4.6.2.1.3. Estimate of food intake and PDI of aflatoxins

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

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

4.6.2.1.4. Estimate of absorption of aflatoxins in the human gut

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

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

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

4.6.2.1.5. Evidence from human tissue of exposure to aflatoxins

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

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

4.6.2.2. Health hazard assessment

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

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

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

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

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

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

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

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

4.6.2.3. Other considerations

4.6.2.3.1. Regulations of international trading partners

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

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

4.6.2.3.2. Commercial interests

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

4.6.2.3.3. Sufficiency of food supply

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

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

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

4.6.3.1. Assessment of human exposure to fumonisins in South Africa

4.6.3.1.1. Estimate of direct fumonisin intake

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

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

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

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

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

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

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

4.6.3.1.3. Estimate of food intake and PDI of fumonisins

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

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

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

4.6.3.1.4. Estimate of absorption of fumonisins in the human gut

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

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

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

4.6.3.1.5. Evidence from human tissue of exposure to fumonisins

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

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

4.6.3.2. Health hazard assessment of fumonisins

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

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

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

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

Acute toxicity, oesophageal cancer, and liver or kidney damage

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

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

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

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

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

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

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

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

Neural tube defects

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

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

4.6.3.3. Other considerations

4.6.3.3.1. Regulations of international trading partners related to fumonisins

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

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

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

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

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

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

4.6.3.3.2. Commercial interests

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

4.6.3.3.3. Sufficiency of food supply

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

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

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

4.6.4.1. Assessment of human exposure to deoxynivalenol in South Africa

4.6.4.1.1. Estimate of direct deoxynivalenol intake

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

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

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

4.6.4.1.3. Estimate of food intake and PDI of deoxynivalenol

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

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

4.6.4.1.4. Estimate of absorption of deoxynivalenol in the human gut

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

4.6.4.1.5. Evidence from human tissue of exposure to deoxynivalenol

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

4.6.4.2. Health hazard assessment of deoxynivalenol

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

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

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

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

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

4.6.4.3. Other considerations

4.6.4.3.1. Regulations of international trading partners related to deoxynivalenol

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

4.6.4.3.2. Commercial interests

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

4.6.4.3.3. Sufficiency of food supply

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

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

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

4.6.5.1. Aflatoxins

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

4.6.5.2. Fumonisins

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

4.6.5.3. Deoxynivalenol

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

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

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

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

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

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

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

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

4.7.1.1. Ultraviolet light

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

4.7.1.2. Minicolumn method

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

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

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

Sample preparation:

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

Extraction:

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

Affinity Chromatography:

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

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

4.7.1.4. Thin layer chromatography (TLC)

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

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

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

4.7.1.5. High performance liquid chromatography (HPLC)

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

4.7.1.6. Mass Spectrometry

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

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

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

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

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

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

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

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

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

4.7.1.7.1. The Vicam Test Kits

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

4.7.1.7.2. FumoniTest™ from Vicam

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

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

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

Measure

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

Ordering Information

Cat. No. Description

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

Each item available individually.

Cat. No. Description

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

33060 FumoniTest™ calibration standards

34000 Cuvettes, 250/pack

35016 Methanol, HPLC Grade, 4 x 4 L bottles

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

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

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

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

4.7.1.7.3. The Neogen Test Kit

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

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

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

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

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

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

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

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

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

4.8.1. Preamble

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

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

4.8.2. Sampling grain for mycotoxin analysis

4.8.2.1. General principles

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

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

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

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

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

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

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

4.8.2.2. Specific sampling procedures

4.8.2.2.1. Sampling from bulk rail or road trucks

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

4.8.2.2.2. Sampling bulk grain in silo bins and ships holds

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

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

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

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

4.8.2.2.3. Sampling from a grain conveyor

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

4.8.2.2.4. Sampling bagged grain

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

4.8.2.2.5. Sampling packaged products in stacks

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

4.8.2.3. Sample preparation

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

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

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

4.8.3.1. Options for consideration

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

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

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

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

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

4.8.3.2. Routine testing at harvest intake

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

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

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

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

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

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

The disadvantages are that:

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

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

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

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

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

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

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

4.8.3.3. Routine testing after harvest intake

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

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

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

4.8.3.4. Sampling and testing of truckloads on dispatch to mills

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

The advantages of this option are as follows:

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

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

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

The disadvantages are that:

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

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

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

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

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

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

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

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

The disadvantages are that:

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

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

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

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

4.9.1. General considerations

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

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

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

A country without an MTL, exporting grain

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

A country with an MTL, exporting grain

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

4.9.1.1. Difficulty of harmonization between countries

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

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

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

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

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

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

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

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

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

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

4.9.2. Specific considerations

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

4.9.2.1. Summary of existing/recommended and proposed MTLs

Aflatoxins:

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

Fumonisins:

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

DON:

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

4.9.2.2. Aflatoxins

4.9.2.2.1. Implications for millers of the existing MTL

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

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

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

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

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

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

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

4.9.2.3. Fumonisins

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

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

Availability of maize and maize products

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

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

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

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

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

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

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

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

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

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

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

4.9.2.3.2. Implications for millers of the proposed MTLs for fumonisins

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

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

4.9.2.4. Deoxynivalenol

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

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

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.

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