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

By

Jan Hendrik Viljoen

Thesis presented in partial fulfilment of the requirements for the degree

DOCTOR OF PHILOSOPHY

To the Faculty of Natural and Agricultural Sciences

Department of Microbiology and Plant Pathology

University of Pretoria

Republic of South Africa

Promotor:

Prof WFO Marasas

Co-promotor:

Prof MJ Wingfield

May 2003
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

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

By

Jan Hendrik Viljoen

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

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 B₁ 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 B₁ 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 ≥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

Jan Hendrik Viljoen

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 statutes 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 deoksinalenol 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. Deoksinalenol kom waarskynlik ook dikwels in plaaslike koring voor. Plaaslike mielies is feitlik totaal vry van aflatoxine, maar aflatoxine kom dikwels teen besorgenswaardige vlakke voor in ingevoerde VSA en Argentynse mielies. Uit die literatuur is dit duidelijk
dat aflatoxsiene akut sowel as chronies giftig is vir mens en dier en die meeste lande handhaaf regulatoriese Maksimum Aanvaarbare Vlakke (MAVe) vir aflatoxsiene in graan en graanprodukte. In verskeie lande is regulatoriese MAVe vir deosinivalenol ook van krag, maar minder wetenskaplike data is beskikbaar as die basis daarvan. Die mees algemene mikotoksien in Suid-Afrikaanse mielies is die fumonisiene B-groep van analogë, waarvan fumonisien B₁ 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 B₁ 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 inhoud, word die volgende nuwe MAVe vir fumonisene (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 ≥3.0 %, droëmassabasis (bv. gesifte en ongesifte mielimeel); en
- 1 µg/g in mielieprodukte van die droëmaalbedryf, met ‘n vetinhoud <3.0 %, droëmassabasis (bv. mieliegruis, brouersgruis, stampmielies, mielierys, super and spesiale mielimeel)

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 \( \mu g/g \) vir graan bestem as voedsel aan die hand gedoen, en 1 \( \mu g/g \) 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

PREFACE i

SUMMARY ii

EKSERP iv

CONTENTS vii

LIST OF TABLES xxx

LIST OF FIGURES xxv

GLOSSARY AND ABBREVIATIONS USED xxvi

1. Introduction 1

1.1. What are mycotoxins? 1

1.2. Where do mycotoxins come from in grain? 2

1.3. Purpose of the study 6

1.4. Objectives 7

2. Literature survey 9

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 9

2.1.1. Explanation of terminology as used 9

2.1.2. Existing limits for aflatoxin 10

2.1.2.1. USA 11

2.1.2.2. Europe 14
2.1.2.3. Canada 14
2.1.2.4. Australia 14
2.1.2.5. Japan 14
2.1.2.6. China 15
2.1.2.7. Other Asian – India 15
2.1.2.8. African countries 15
2.1.3. Existing limits for fumonisins 18
2.1.3.1. Switzerland 18
2.1.3.2. USA 18
2.1.3.3. South Africa - Recommended level for fumonisins in maize 21
2.1.4. Existing limits for deoxynivalenol 21
2.1.5. Existing limits for zearalenone 23
2.1.6. Existing limits for diacetoxyscirpenol 24
2.1.7. Existing limits for T-2 toxin and HT-2 toxin 24
2.1.8. Existing limits for other mycotoxins 24

2.2. Overview of the Groups of carcinogens of the International Agency for Research on Cancer (IARC) and mycotoxins considered carcinogens 26

2.2.1. Classification of carcinogens 26
2.2.2. Common substances and mycotoxins considered carcinogens 27
2.2.2.1. Group 1 - confirmed human carcinogens 27
2.2.2.2. Group 2A - probable human carcinogens 28
2.2.2.3. Group 2B - possible human carcinogens 29
2.2.4. Group 3 – suspected human carcinogens

2.2.5. Group 4 – Substances probably not carcinogenic in humans

2.2.3. Determinants of risk

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

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

2.3.3. World incidence of oesophageal cancer

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

2.4.1. The physiological basis of cancer development

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

2.4.2.1. Exposure to nitrosamines

2.4.2.2. Exposure to tannins

2.4.2.3. Gastro-oesophageal reflux

2.4.2.4. Dry cleaning

2.4.2.5. Smoking and chewing of tobacco

2.4.2.6. Alcohol

2.4.3. Nutritional factors that may affect tumour development

2.4.3.1. General nutritional status

2.4.3.2. Mineral deficiencies or overexposure to certain minerals
2.4.3.3. Vitamins

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

2.4.4.2. Genetic basis

2.4.5. Conclusion

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

2.5.1. Preamble

2.5.2. Toxicology of aflatoxins

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

2.5.2.1.1. Beef Cattle

2.5.2.1.2. Dairy Cattle

2.5.2.1.3. Poultry

2.5.2.1.4. Swine

2.5.2.1.5. Sheep and Goats

2.5.2.1.6. Horses

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

2.5.2.2.1. Acute aflatoxin poisoning

2.5.2.2.2. Sub-acute aflatoxin poisoning

2.5.2.2.3. Aflatoxin and liver cancer

2.5.2.2.4. Evidence contradicting the role of aflatoxins in liver cancer

2.5.2.2.5. Other factors involved in the development of liver cancer

2.5.3. Toxicology of fumonisins

2.5.3.1. The effects of fumonisins on farm animals

2.5.3.2. Co-occurrence of fumonisins and nitrosamines, or aflatoxins
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5.3.3. Physiological effects of fumonisins in rats, mice and monkeys</td>
<td>91</td>
</tr>
<tr>
<td>2.5.3.4. Epidemiological studies of the effect of fumonisins in humans</td>
<td>92</td>
</tr>
<tr>
<td>2.5.4. Toxicology of deoxynivalenol</td>
<td>96</td>
</tr>
<tr>
<td>3. Procedure</td>
<td>99</td>
</tr>
<tr>
<td>3.1. The occurrence of mycotoxins in SA grains and grain products</td>
<td>99</td>
</tr>
<tr>
<td>3.1.1. Preamble</td>
<td>99</td>
</tr>
<tr>
<td>3.1.2. Survey procedure</td>
<td>101</td>
</tr>
<tr>
<td>3.1.2.1. Fungi and mycotoxins in South African maize crops</td>
<td>101</td>
</tr>
<tr>
<td>3.1.2.2. Mycotoxins in white maize products in South Africa</td>
<td>102</td>
</tr>
<tr>
<td>3.1.2.3. Mycotoxins in maize feed mill products</td>
<td>103</td>
</tr>
<tr>
<td>3.1.2.4. Fungi and mycotoxins in imported yellow maize</td>
<td>104</td>
</tr>
<tr>
<td>3.1.2.5. Fungi and mycotoxins in a vessel of exported yellow maize</td>
<td>104</td>
</tr>
<tr>
<td>3.1.3. Fumonisins in foreign maize food products</td>
<td>105</td>
</tr>
<tr>
<td>3.2. An analysis of the correlation of the geographic distribution of</td>
<td>105</td>
</tr>
<tr>
<td>oesophageal cancer in black males and <em>F. verticillioides</em> infection</td>
<td></td>
</tr>
<tr>
<td>rates and fumonisin contamination levels in commercial white maize in</td>
<td></td>
</tr>
<tr>
<td>South Africa</td>
<td></td>
</tr>
<tr>
<td>3.2.1. Estimated usage of commercial maize</td>
<td>105</td>
</tr>
<tr>
<td>3.2.2. Incorporating subsistence maize in the Eastern Cape</td>
<td>116</td>
</tr>
<tr>
<td>3.3. The correlation of oesophageal cancer rates and maize supply in</td>
<td>120</td>
</tr>
<tr>
<td>some African countries</td>
<td></td>
</tr>
<tr>
<td>3.4. Incidence of liver, kidney and brain cancers in Africa in relation</td>
<td>121</td>
</tr>
<tr>
<td>to grain consumption, and in SA in relation to the occurrence of</td>
<td></td>
</tr>
<tr>
<td>fumonisins in maize</td>
<td></td>
</tr>
</tbody>
</table>
3.4.1. Preamble

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

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

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?

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

3.6. Estimated DON content of white maize consumed in SA

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

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

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

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

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

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

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

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

4. Results and Discussion

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

4.1.1. Unprocessed commercial South African maize

4.1.2. Mycotoxins in white maize products

4.1.3. Mycotoxins in maize feed mill products

4.1.4. Fungi and mycotoxins in imported yellow maize

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

4.1.6. Fumonisins in foreign maize food products

4.1.7. Mycotoxins in other grain staples in South Africa

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

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

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 187

4.4.2. Correlation of liver, kidney and brain cancer rates and grain supply in some African countries 188

4.5. Aetiology of NTD in South Africa in relation to the occurrence of fumonisins in maize and maize products 192

4.5.1. The link between NTD and fumonisins 192

4.5.2. Other studies on NTD incidence in South Africa 194

4.5.3. The epidemiological relationship of NTD with fumonisin intake 194

4.5.4. Animal studies on the effect of fumonisins on foetal bone development and NTD 197

4.5.5. Epidemiological studies of NTD in Mexico 198

4.5.6. By what mechanisms could fumonisins induce NTDs? 199

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 201

4.6.1. The current approach to regulation of human exposure to mycotoxins 201

4.6.2. Formulating a proposal for MTLs for aflatoxins in grain and grain products 202

4.6.2.1. Assessment of human exposure to aflatoxins in South Africa 202

4.6.2.1.1. Estimate of direct aflatoxin intake 202

4.6.2.1.2. Estimate of indirect intake through animal products from animals that were fed aflatoxin contaminated feeds 204

4.6.2.1.3. Estimate of food intake and PDI of aflatoxins 204

4.6.2.1.4. Estimate of absorption of aflatoxins in the human gut 205

4.6.2.1.5. Evidence from human tissue of exposure to aflatoxins 206
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

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

4.6.2.3. Other considerations

4.6.2.3.1. Regulations of international trading partners

4.6.2.3.2. Commercial interests

4.6.2.3.3. Sufficiency of food supply

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

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

4.6.3.1.3. Estimate of food intake and PDI of fumonisins

4.6.3.1.4. Estimate of absorption of fumonisins in the human gut

4.6.3.1.5. Evidence from human tissue of exposure to fumonisins

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

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

4.6.3.3. Other considerations

4.6.3.3.1. Regulations of international trading partners related to fumonisins

4.6.3.3.2. Commercial interests

4.6.3.3.3. Sufficiency of food supply

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 219

4.6.4.1.1. Estimate of direct deoxynivalenol intake 219

4.6.4.1.2. Estimate of indirect intake of deoxynivalenol through animal products from animals that were fed deoxynivalenol contaminated feeds 219

4.6.4.1.3. Estimate of food intake and PDI of deoxynivalenol 219

4.6.4.1.4. Estimate of absorption of deoxynivalenol in the human gut 220

4.6.4.1.5. Evidence from human tissue of exposure to deoxynivalenol 220

4.6.4.2. Health hazard assessment of deoxynivalenol 220

4.6.4.2.1. Assessment of the toxicological effects of deoxynivalenol on humans, experimental animals and farm animals 220

4.6.4.2.2. An epidemiological assessment of possible effects of deoxynivalenol on humans 220

4.6.4.3. Other considerations 221

4.6.4.3.1. Regulations of international trading partners related to deoxynivalenol 221

4.6.4.3.2. Commercial interests 221

4.6.4.3.3. Sufficiency of food supply 221

4.6.5. Summary of proposed MTLs for certain mycotoxins in grain and grain products intended for human consumption 222

4.6.5.1. Aflatoxins 222

4.6.5.2. Fumonisins 222

4.6.5.3. Deoxynivalenol 222

4.6.6. The basis for determination of compliance of grain with MTLs 222

4.7. Overview of available test methods for the mycotoxins included in this study in grains and grain products 223

4.7.1. Categories of analytical tests (After Duncan & Hagler, Undated; Woloshuk, 2000) 223

4.7.1.1. Ultraviolet light 223
4.7.1.2. Minicolumn method 224
4.7.1.3. Fluorometric-iodine method (Genter *et al.*, 2000) 224
4.7.1.4. Thin layer chromatography (TLC) 226
4.7.1.5. High performance liquid chromatography (HPLC) 227
4.7.1.6. Mass Spectrometry 227
4.7.1.7. Immunoaffinity columns (ELISA, or antibody test kits) (Scott & Trucksess, 1997) 227
   4.7.1.7.1. The Vicam Test Kits 230
   4.7.1.7.2. FumoniTest™ from Vicam 230
   4.7.1.7.3. The Neogen Test Kit 232
4.7.2. Infrastructure and labour for on-site immuno-affinity testing 233
4.8. Recommendations of test methods, sampling methods and testing procedures to be adopted together with MTLs for fumonisins, aflatoxins and deoxynivalenol 234
   4.8.1. Preamble 234
   4.8.2. Sampling grain for mycotoxin analysis 234
      4.8.2.1. General principles 234
      4.8.2.2. Specific sampling procedures 236
         4.8.2.2.1. Sampling from bulk rail or road trucks 236
         4.8.2.2.2. Sampling bulk grain in silo bins and ships holds 236
         4.8.2.2.3. Sampling from a grain conveyor 237
         4.8.2.2.4. Sampling bagged grain 237
         4.8.2.2.5. Sampling packaged products in stacks 237
   4.8.2.3. Sample preparation 238
   4.8.3. Practical application of MTLs for aflatoxins, fumonisins and deoxynivalenol in grain and grain products 238
| 4.8.3.1 | Options for consideration | 238 |
| 4.8.3.2 | Routine testing at harvest intake | 239 |
| 4.8.3.3 | Routine testing after harvest intake | 241 |
| 4.8.3.4 | Sampling and testing of truckloads on dispatch to mills | 241 |
| 4.8.3.5 | Sampling and testing of individual silo bins before grain is outloaded | 242 |
| 4.9 | Possible implications of MTLs for mycotoxins in South Africa and major grain trading partners on international trade in grains and grain products | 244 |
| 4.9.1 | General considerations | 244 |
| 4.9.1.1 | Difficulty of harmonization between countries | 245 |
| 4.9.1.2 | Effects of MTLs on desirability of grain from specific sources and on price | 246 |
| 4.9.1.3 | Need for, and cost of testing, supervision and control | 246 |
| 4.9.1.3.1 | Elevated cost of imported grain that can meet local MTLs | 247 |
| 4.9.2 | Specific considerations | 248 |
| 4.9.2.1 | Summary of existing/recommended and proposed MTLs | 248 |
| 4.9.2.2 | Aflatoxins | 249 |
| 4.9.2.2.1 | Implications for millers of the existing MTL | 249 |
| 4.9.2.2.2 | Implications for millers of the newly proposed MTLs for aflatoxins | 249 |
| 4.9.2.3 | Fumonisins | 250 |
| 4.9.2.3.1 | Implications for millers of the MTL for fumonisins recommended by the MRC | 250 |
| 4.9.2.3.2 | Implications for millers of the proposed MTLs for fumonisins | 253 |
| 4.9.2.4 | Deoxynivalenol | 254 |
| 5 | Conclusions | 255 |
5.1. Existing regulatory, advisory and recommended MTLs for mycotoxins in grain and grain products in various countries

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

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

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

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

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

5.7. Neural tube defects and mycotoxins

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

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

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

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

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

6. References
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1</td>
<td>FDA action levels for aflatoxins in food and feed in the USA</td>
<td>12</td>
</tr>
<tr>
<td>Table 2</td>
<td>MTLs for aflatoxins in food and feed in African countries</td>
<td>16</td>
</tr>
<tr>
<td>Table 3</td>
<td>Details of all countries known to have MTLs for deoxynivalenol</td>
<td>22</td>
</tr>
<tr>
<td>Table 4</td>
<td>Details of all countries known to have MTLs for zearalenone</td>
<td>23</td>
</tr>
<tr>
<td>Table 5</td>
<td>Details of all countries known to have MTLs for T-2, or HT-2 toxin</td>
<td>24</td>
</tr>
<tr>
<td>Table 6</td>
<td>Mycotoxins not included in this study for which some countries maintain MTLs</td>
<td>25</td>
</tr>
<tr>
<td>Table 7</td>
<td>Age standardised incidence rate (World standard) per 100,000 of oesophageal cancer in 1990 in some countries</td>
<td>43</td>
</tr>
<tr>
<td>Table 8</td>
<td>Lifetime risks of the top five cancers, excluding basal and squamous cell skin cancers, per population group in South Africa, 1993 – 1995</td>
<td>65</td>
</tr>
<tr>
<td>Table 9</td>
<td>Hepatoma incidence (per 100,000) and frequency (%) of aflatoxin contamination of foodstuffs in Uganda</td>
<td>80</td>
</tr>
<tr>
<td>Table 10</td>
<td>Hepatoma incidence and aflatoxin ingestion in Kenya</td>
<td>82</td>
</tr>
<tr>
<td>Table 11</td>
<td>Summarized results of studies measuring primary liver cancer incidence rate and aflatoxin intake</td>
<td>83</td>
</tr>
<tr>
<td>Table 12</td>
<td>Percentage <em>F. verticillioides</em> 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)</td>
<td>108</td>
</tr>
<tr>
<td>Table 13</td>
<td>Total fumonisn 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)</td>
<td>108</td>
</tr>
</tbody>
</table>
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

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

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

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

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

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

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

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

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
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 143

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 146

Table 25 - Mean incidence of fungi (% kernels infected) in white (W) and yellow (Y) RSA maize of the 1992 crop from different production areas 148

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 151

Table 27 - Summary of mean mycotoxin content (ng/g) of white maize of the 1989 to 1994 crops in different production areas 156

Table 28 - Mycotoxin content (ng/g) of white maize products in South Africa (1990/91 marketing season) 160

Table 29 - Mycotoxin content (ng/g) of white maize products in South Africa (1991/92 marketing season) 162

Table 30 - Mycotoxin content (ng/g) of white maize products in South Africa (1994/95 marketing season) 165

Table 31 - Mycotoxin content (ng/g) of yellow maize and other maize products used in feed milling in South Africa (1994/95 marketing season) 168

Table 32 - Mean fumonisin and aflatoxin levels in South African (SA) and imported USA (1991 and 1992 crops), and Argentinean (ARG) maize (1992 crop) 171

Table 33 - Mean incidence of fungi in twelve bulk shipments of imported USA maize after arrival in South Africa 174

Table 34 - Fumonisin B₁ levels in commercial maize-based human foodstuffs in the USA, South Africa and Switzerland (from Marasas et al, 1993) 178
Table 35 - Fumonisin B₂ levels in commercial maize-based human foodstuffs (from Marasas et al, 1993)

Table 36 - The OC incidence rates in black males in 1990 and 1991¹, the estimated total FB (FB₁+FB₂+FB₃) content (ng/g) of commercial white maize and subsistence maize consumed², the estimated average percentage of *F. verticillioides* infected kernels of commercial white maize³, the estimated per capita maize consumption⁴ and the estimated PDI of total FBs⁵ in areas of South Africa

Table 37 - The average supply of sorghum, millet and maize in kg per capita per year¹ (calculated over the 4 years 1987 to 1990) in each of 23 African countries², and the OC rate (ASIR world population per 100 000) in males and females in each of the countries³

Table 38 - Incidence of liver, kidney and brain cancer incidence in black males in 1990 and 1991 in different geographic areas of South Africa¹, the estimated total FB (FB₁+FB₂+FB₃) content (ng/g)² of commercial white maize and of subsistence maize in the Eastern Cape, the estimated average percentage of *F. verticillioides* infected kernels³, the estimated per capita maize consumption⁴ and the estimated PDI of total FBs⁵ in areas of South Africa

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

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

Table 41 - AFB₁ concentration in autopsy specimens from Reye's syndrome cases poisoned with AFB₁ (Shank et al, 1971)
Table 42 - Some of the commercially available antibody test kits (Anonymous 2000e)

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

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

Figure 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 100

Figure 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 155
GLOSSARY AND ABBREVIATIONS USED

AFMA – Animal Feed Manufacturers Association in South Africa

AFB₁, AFB₂, AFG₁, AFG₂, AFM₁, AFM₂ - Aflatoxin B₁, B₂, G₁, G₂, M₁ & M₂ 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 - Diacetoxyseirpenol

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 B₁, B₂, B₃, B₄
FB₁, FB₂, FB₃, B₄ – fumonisin B₁, B₂, B₃ and B₄ 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