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.