AFLATOXINS

These substances were considered by previous working groups, in December 1971 (IARC, 1972), October 1975 (IARC, 1976) and March 1987 (IARC, 1987a). Since that time, new data have become available, and these have been incorporated into the monograph and taken into account in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Synonyms, structural and molecular data*

Aflatoxin B_1

Chem. Abstr. Services Reg. No.: 1162–65–8
Chem. Abstr. Name: (6aR-*cis*)(2,3,6a,9a)Tetrahydro-4-methoxycyclopenta[*c*]furo[3′,2′:4,5]furo[2,3-*h*][*l*]benzopyran-1,11-dione
Synonyms: 6-Methoxydifurocoumarone; 2,3,6aα,9aα-tetrahydro-4-methoxycyclopenta-[*c*]furo[3′,2′:4,5]furo[2,3-*h*][*l*]benzopyran-1,11-dione

Aflatoxin B_2

Chem. Abstr. Services Reg. No.: 7220–81–7
Chem. Abstr. Name: (6aR-*cis*)(2,3,6a,8,9,9a)Hexahydro-4-methoxycyclopenta[*c*]furo-[3′,2′:4,5]furo[2,3-*h*][*l*]benzopyran-1,11-dione
Synonyms: Dihydroaflatoxin B_1; 2,3,6aα,8,9,9aα-hexahydro-4-methoxycyclopenta[*c*]-furo[3′,2′:4,5]furo[2,3-*h*][*l*]benzopyran-1,11-dione

Aflatoxin G_1

Chem. Abstr. Services Reg. No.: 1165–39–5
Chem. Abstr. Name: (7aR-*cis*)(3,4,7a,10a)Tetrahydro-5-methoxy-1*H*,12*H*-furo[3′,2′:4,5]furo[2,3-*h*]pyrano[3,4-*c*][*l*]benzopyran-1,12-dione
Synonym: 3,4,7aα,10aα-Tetrahydro-5-methoxy-1*H*,12*H*-furo[3′,2′:4,5]furo[2,3-*h*]pyrano[3,4-*c*][*l*]benzopyran-1,12-dione

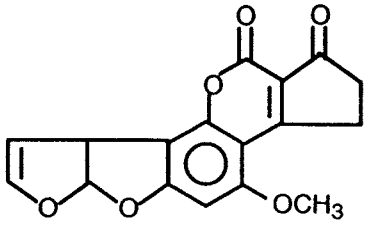
Aflatoxin G_2

Chem. Abstr. Services Reg. No.: 7241–98–7
Chem. Abstr. Name: (7aR-*cis*)(3,4,7a,9,10,10a)Hexahydro-5-methoxy-1*H*,12*H*-furo-[3′,2′:4,5]furo[2,3-*h*]pyrano[3,4-*c*][*l*]benzopyran-1,12-dione

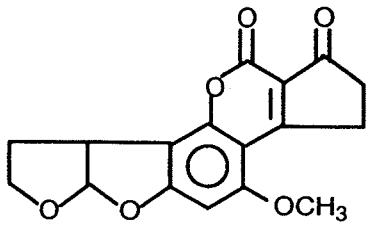
Synonyms: Dihydroaflatoxin G_1; 3,4,7aα,9,10,10aα-hexahydro-5-methoxy-1*H*,12*H*-furo[3′,2′:4,5]furo[2,3-*h*]pyrano[3,4-*c*][*l*]benzopyran-1,12-dione

Aflatoxin M_1

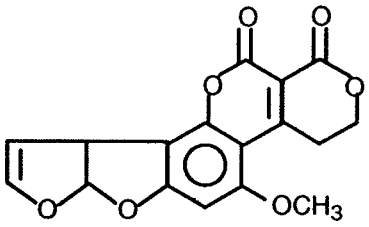
Chem. Abstr. Services Reg. No.: 6795-23-9
Chem. Abstr. Name: 2,3,6a,9a-Tetrahydro-9a-hydroxy-4-methoxycyclopenta[*c*]furo-[3′,2′:4,5]furo[2,3-*h*][*l*]benzopyran-1,11-dione
Synonym: 4-Hydroxyaflatoxin B_1



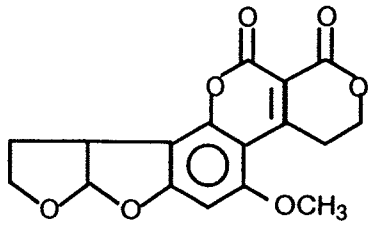
B_1: $C_{17}H_{12}O_6$ Mol. wt: 312.3



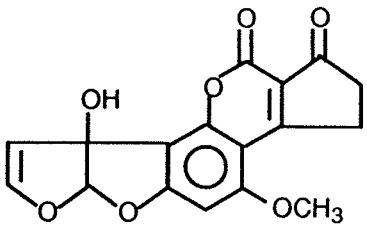
B_2: $C_{17}H_{14}O_6$ Mol. wt: 314.3



G_1: $C_{17}H_{12}O_7$ Mol. wt: 328.3



G_2: $C_{17}H_{14}O_7$ Mol. wt: 330.3



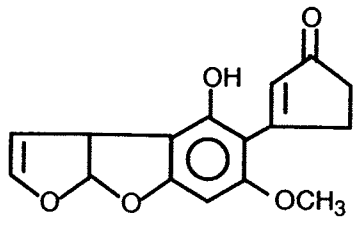
M_1: $C_{17}H_{12}O_7$ Mol. wt: 328.3

The structures of other metabolites and degradation products mentioned in the monographs are shown in Figure 1 and given by Castegnaro *et al.* (1980).

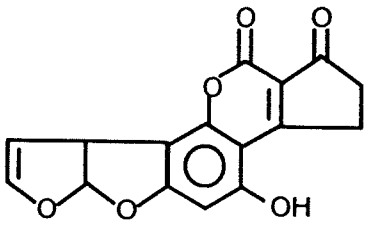
1.1.2 *Chemical and physical properties of aflatoxins* (from Castegnaro *et al.*, 1980, 1991; Budavari, 1989, unless otherwise stated)

(*a*) *Description*: Colourless to pale-yellow crystals. Intensely fluorescent in ultraviolet light, emitting blue (aflatoxins B_1 and B_2) or yellow-green (aflatoxins G_1, G_2) fluorescence, from which the designations B and G were derived, or blue-violet fluorescence (aflatoxin M_1)

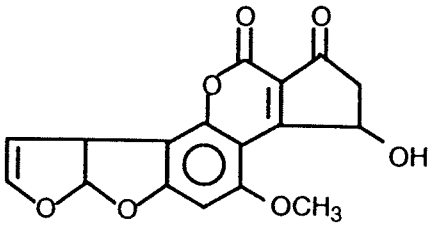
Fig. 1. Structures of other metabolites and degradation products of aflatoxins



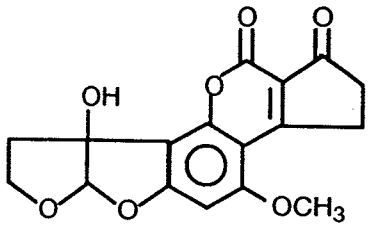
Aflatoxin D_1



Aflatoxin P_1



Aflatoxin Q_1



Aflatoxin M_2

(*b*) *Melting-point and (c) Absorption spectroscopy*:

Table 1. Melting-points and ultraviolet absorption of aflatoxins

Aflatoxin	Melting-point (°C)	Ultraviolet absorption (ethanol)	
		λ_{max} (nm)	ϵ
B_1	268–269 (decomposition) (crystals from chloroform)	223 265 362	25 600 13 400 21 800
B_2	287–289 (decomposition) (crystals from chloroform–pentane)	222 265 363	17 000 11 700 23 400
G_1	244–246 (decomposition) (crystals from chloroform–methanol)	243 257 264 362	11 500 9 900 10 000 16 100
G_2	237–239 (decomposition) (crystals from ethyl acetate)	214 265 363	28 100 11 600 21 000
M_1	299 (decomposition) (crystals from methanol)	226 265 357	23 100 11 600 19 000

From IARC (1976); Castegnaro *et al.* (1980); Cole & Cox (1981); Budavari (1989)

(*d*) *Solubility*: Very slightly soluble in water (10–30 μg/ml); insoluble in non-polar solvents; freely soluble in moderately polar organic solvents (e.g., chloroform and methanol) and especially in dimethyl sulfoxide (Cole & Cox, 1981)

(*e*) *Stability*: Unstable to ultraviolet light in the presence of oxygen, to extremes of pH (< 3, > 10) and to oxidizing agents

(*f*) *Reactivity*: The lactone ring is susceptible to alkaline hydrolysis. Aflatoxins are also degraded by reaction with ammonia or sodium hypochlorite.

1.1.3 *Trade names, technical products and impurities*

No data were available to the Working Group.

1.1.4 *Analysis*

The numerous methods for the determination of aflatoxins B and G in maize (known as 'corn' in the USA), groundnuts (peanuts) and cottonseed meal (Egan *et al.*, 1982) and of aflatoxin M_1 in milk products (Scott, 1989) have been reviewed. Those that have been verified in collaborative studies and have been proposed as official methods by the Association of Official Analytical Chemists (Scott, 1990) are shown in Table 2.

Table 2. Analytical methods validated by the Association of Official Analytical Chemists

Method no.	Aflatoxin	Food	Method[a]	Detection limit (μg/kg)
975.36	All	Foods and feeds (screening)	MC	10
979.18	All	Maize and groundnuts (screening)	MC	10
990.31	All	Maize and groundnuts (Aflatest screening)	IC	10
990.34	All	Maize, cottonseed, groundnuts (screening)	ELISA	20–30
989.06	B_1	Cottonseed products and mixed feed (screening)	ELISA	15
990.32	B_1	Maize and groundnuts (screening)	ELISA	20
968.22	B_1, B_2, G_1, G_2	Groundnuts and groundnut products	TLC	5
970.45	B_1, B_2, G_1, G_2	Groundnuts and groundnut products	TLC	10
971.23	B_1, B_2, G_1, G_2	Cocoa beans	TLC	10
971.24	B_1, B_2, G_1, G_2	Coconut, copra and copra meal	TLC	50
972.26	B_1, B_2, G_1, G_2	Maize	TLC	5
980.20	B_1, B_2, G_1, G_2	Cottonseed products	TLC, HPLC	10, 5
970.46	B_1, B_2, G_1, G_2	Green coffee	TLC	25
974.16	B_1, B_2, G_1, G_2	Pistachio nuts	TLC	15
972.27	B_1, B_2, G_1, G_2	Soya beans	TLC	10
990.33	B_1, B_2, G_1, G_2	Maize and groundnut butter	HPLC	5
978.15	B_1	Eggs	TLC	0.1
982.24	B_1 and M_1	Liver	TLC	0.1
974.17	M_1	Dairy products	TLC	0.1
980.21	M_1	Milk and cheese	TLC	0.1
986.16	M_1 and M_2	Fluid milk	HPLC	0.1

From Scott (1990)

[a]MC, minicolumn; IC, immunoaffinity column, ELISA, enzyme-linked immunosorbent assay; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography

Analytical quality assurance for the analysis of aflatoxins B_1, B_2, G_1, G_2 and M_1 in foods is available for laboratories through the International Mycotoxin Check Sample Programme organized by IARC (Friesen, 1989).

As contamination may not occur in a homogeneous way throughout a sample of maize or groundnuts, good sampling and sample preparation procedures must be used to obtain accurate quantitative results. Sampling and sample preparation procedures for aflatoxin analysis have been reviewed (Egan *et al.*, 1982; van Egmond, 1984), and the Association of Official Analytical Chemists' method (977.16) for sampling aflatoxin-containing commodities has been published (Scott, 1990).

A number of approaches have been used to analyse for aflatoxins and their metabolites in human tissues and body fluids. These include immunoaffinity purification, immunoassay (Wild *et al.*, 1987), high-performance liquid chromatography with fluorescence or ultraviolet detection and synchronous fluorescence spectroscopy (Groopman & Sabbioni, 1991). Molecular biomarkers, such as urinary markers, metabolites in milk and parent compounds in blood, are used for determining exposure to aflatoxins (Groopman, 1993).

1.2 Production and use

1.2.1 *Production*

Aflatoxins were first identified in 1961 in animal feed contaminated by *Aspergillus parasiticus* (Sargeant *et al.*, 1961). They are known to be produced by three species: *A. flavus*, *A. parasiticus* and the rare species *A. nomius* (Kurtzman *et al.*, 1987). It is generally considered that *A. flavus* produces aflatoxins B_1 and B_2, whereas *A. parasiticus* produces aflatoxins B_1, B_2, G_1 and G_2 (Dorner *et al.*, 1984). In the USA and Africa, *A. flavus* and *A. parasiticus* are widely distributed; in southeast Asia, *A. flavus* occurs to the virtual exclusion of the other species (Pitt *et al.*, 1993).

Aflatoxins are produced, in small quantities for use in research only, by large-scale fermentation on solid substrates or liquid media, from which the aflatoxins are extracted and purified by chromatography. Total annual production probably does not exceed 100 g.

1.2.2 *Use*

Aflatoxins are not used commercially other than in research.

1.3 Occurrence

The aflatoxin-producing *Aspergillus* species, and consequently dietary aflatoxin contamination, are ubiquitous in areas of the world with hot, humid climates, including sub-Saharan Africa and Southeast Asia. Exposure in those countries results from contamination of dietary staples and is therefore likely to be chronic. Since countries in colder climatic areas import foods from areas where aflatoxin levels are high, however, aflatoxins are of worldwide concern. Data on occurrence are available predominantly from importing countries, where regulation of contamination should contribute to ensuring relatively low exposure in those countries.

The relative proportions of aflatoxin B_1, aflatoxin G_1, aflatoxin B_2 and aflatoxin G_2 on crops depend on the particular *Aspergillus* species present. Aflatoxin B_1 is the most frequent type present in contaminated samples, and aflatoxin G_1 has never been reported in the absence of aflatoxin B_1. Aflatoxin B_2 and aflatoxin G_2 are typically present in much lower quantities. Aflatoxin M_1 is a metabolic hydroxylation product of aflatoxin B_1; it can occur in the absence of other aflatoxins.

1.3.1 *Diet*

Samples of most dietary staples have been shown to be contaminated with aflatoxins at one time or another; however, the frequency with which different dietary items are contaminated and the level of contamination differs. High levels have been found notably in groundnuts and maize in African regions, Southeast Asia and southern China, where these foods are dietary staples. Contamination of raw commodities in the USA also occurs at high levels in some years. Foodstuffs may be contaminated with aflatoxin both pre- and post-harvest.

An extensive review of the levels of aflatoxins in foods and feeds from 16 countries in North America, South America, Europe, Asia and Africa was made for the period 1976–83 (Jelinek *et al.*, 1989). Data were presented as total aflatoxins. In maize and maize products from nine countries, median levels ranged from < 0.1 to 80 μg/kg; in several countries, more than 10% of grain samples contained levels above 5 μg/kg (Table 3). Data from the USA demonstrated annual and geographical fluctuations in aflatoxin levels and increased levels in maize in conditions of drought. Levels in other grains and cereal products were lower than those in maize in all the countries surveyed. Extensive surveys of groundnuts in North America, Central America, South America, Europe and Australia indicated that both median and 90% levels were almost always below 20 μg/kg. Groundnuts imported into the USA in 1981 from India, the Sudan and Brazil contained much higher levels, more than 50% of samples containing over 26 μg/kg (Table 4). Other nuts, including almonds, cashews, filberts, hazel-nuts, mixed nuts, pecans and walnuts, were contaminated to a lower extent, but higher levels were occasionally seen in surveys of pistachios, pumpkin seeds and Brazil-nuts.

Table 3. FAO/WHO/UNEP Monitoring Program: aflatoxins in maize and maize products

Country	Year	No. of samples	Median[a] (μg/kg)	90th percentile[b] (μg/kg)
Maize				
Brazil	1981	228	< 8.0	< 8.0
Canada	1976	25	< 4.0	< 4.0
Guatemala	1976–79	231	< 4.0	4–360
Kenya	1978–79	78	< 0.1–70	30–1920
Mexico	1979–80	96	< 2.5–< 10	< 2.5–30
United Kingdom	1978	29	5.0	8.0
USA	1978–83	2633	< 1–80	10–700
USSR	1981–82	219	< 1.0	< 1–662

Table 3 (contd)

Country	Year	No. of samples	Median[a] (μg/kg)	90th percentile[b] (μg/kg)
Maize products				
Canada[c]	1983	20	4.0	6.0
Guatemala[c]	1977–79	22	< 4.0	10–20
Kenya[d]	1978–79	283	< 10.0	< 10.0
Mexico[e]	1978	217	< 2.0	Not reported
Switzerland[c]	1978	40	< 0.5	2.0
United Kingdom[d]	1978	13	< 0.1	< 0.1
USA[f]	1978–83	1174	< 1.0	< 1–56
USSR[c]	1976	87	< 1.0	5.0

From Jelinek *et al.* (1989)
[a]Many of the median values were below the detection limits of the assay.
[b]The level below which 90% of the findings occurred in a given survey; when more than one value is given, they refer to separate surveys in different years.
[c]Maize meal
[d]Maize flour
[e]Tortillas
[f]Ground or dry-milled maize

Table 4. Aflatoxins in raw, shelled groundnuts imported into the USA, 1981

Origin	No. of lots	Lots (%)	
		Determinable	> 26 μg/kg
China	2 585	15	2.5
India	1 453	92	58.0
Sudan	932	94	78.0
Argentina	446	40	4.5
South Africa	112	41	21.0
Malawi	80	60	10.0
Australia	52	10	4.0
Brazil	44	100	95.0
Egypt	41	14	2.0
Taiwan	37	27	0.0
USA[a]	172 669	20	3.0

From Jelinek *et al.* (1989)
[a]For comparison, levels in US domestic groundnuts for the combined crop years 1973–79

Rice exposed during cyclones in India had levels up to 1130 μg/kg. This commodity is otherwise rarely contaminated at levels > 20 μg/kg (Choke, 1990). High levels of aflatoxins (> 100 μg/kg) occurred in cottonseed and groundnut and in sunflower seeds. Data from other food surveys similarly showed high levels of aflatoxins in groundnuts and maize (Stoloff, 1982; Choke, 1991).

Exposure can occur due to the presence of aflatoxin and aflatoxin metabolites in milk and milk products from animals that have consumed contaminated feed (Applebaum *et al.*, 1982). Some data on the occurrence of aflatoxin M_1 in the late 1960s, 1970s and 1980s in several countries are reported in Table 5. High proportions of positive samples were found in some surveys, usually at levels of less than 0.5 μg/kg.

Table 5. Occurrence of aflatoxin M_1 in milk

Country or region	Period of sampling	No. positive/ no. samples	Proportion positive (%)	Range of concentrations (μg/kg)[a]
Austria	1983–86	0/65	0	< 0.03
Belgium	1960s, 1970s	42/68	62	0.02–0.2
	1980–86	135/809	16.7	< 0.01–0.5
China	1981, 1983	173/319	54.2	0.02–0.5
Finland	1982, 1986	0/17	0	< 0.1
France	1981–85	580/3634	16.4	0.02–0.5
Germany	1960s, 1970s	229/788	29.1	0.04–6.5[b]
India	1960s, 1970s	3/21	14	up to 13.3
Ireland	1981–82	0/36	0	< 0.015
Italy	1982–84	213/537	39.7	0.001–0.15
Netherlands	1960s, 1970s	74/95	82	0.03–0.5
	1985–86	964/1241	77.7	0.01–0.09
South Africa	1960s, 1970s	5/21	24	0.02–0.2
Spain	1983	7/95	7	0.02–0.04
Sweden	1983–86	384/647	59.4	0.005–0.3
Taiwan	1986	0/217	0	< 0.1
United Kingdom	1960s, 1970s	85/278	31	0.03–0.52
	1981–83	59/686	8.6	0.01–0.78
USA	1960s, 1970s	191/302	63	Trace–> 0.7

Compiled from van Egmond (1989a)

[a]For milk powder, calculated on the basis of reconstituted milk (dilution factor, 10 ×)

[b]Western part, 0.04–0.54; eastern part, < 0.1–6.5

The dietary exposure resulting from intake of aflatoxin-contaminated food has been estimated for a number of populations by integrating the level of aflatoxins in the dietary constituents and estimating consumption (see Table 6). High levels of exposure in the USA were calculated by Stoloff (1983) for the years prior to the surveys included in Table 6. Exposures to aflatoxin B_1 in Africa and Southeast Asia are usually in the range 3–200 ng/kg bw per day (Hall & Wild, 1993).

Table 6. Population exposures estimated on the basis of analyses of aflatoxins in food

Country or region	Period of sampling	Food source[a]	Estimated exposure to aflatoxin B_1 (ng/kg bw/day)	Reference
Kenya	1969	P (diet and beer)	3.5–14.8[b]	Peers & Linsell (1973)
Swaziland	1972–73	H (wet diet)	5.1–43.1[c]	Peers *et al.* (1976)
	1982–83	H	[11.4–158.6]	Peers *et al.* (1987)
Mozambique	1969–74	P	38.6–183.7	van Rensburg *et al.* (1985)
Transkei	1976–77	P	16.5	van Rensburg *et al.* (1985)
Gambia	1988	P	[4–115][d]	Wild *et al.* (1992a)
Southern Guangxi, China	1978–84	M	[11.7–2027]	Yeh *et al.* (1989)
Thailand	1969–70	P	5–55	Shank *et al.* (1972a)
USA	1960–79	M	2.7	Bruce (1990)

[ ]Calculated by Working Group, assuming 70-kg weight per person
[a]P, samples of cooked food from the plate; H, uncooked food samples from the home; M, samples from the market
[b]B_1 and B_2
[c]Aflatoxin, unspecified
[d]B_1, B_2, G_1, G_2

Aflatoxins in foods are not readily degraded under normal cooking conditions (Goldblatt, 1969; Müller, 1982).

Methods for the decontamination and detoxification of agricultural commodities contaminated with mycotoxins, including aflatoxins, have been reviewed (Jemmali, 1990). Ammoniation of aflatoxin B_1-contaminated animal feeds has been studied extensively as a potential method of detoxification. This process, when applied in combination with heat, leads to conversion of aflatoxin B_1 to aflatoxin D_1 (see Fig. 1) and a further degradation product, both reported to be non-toxic, whereas without heat, this process may be reversible and regenerate aflatoxin B_1 upon acidification. The process has been used commercially on a limited basis; however, there is still some controversy about the safety of the detoxified feedstuffs (Müller, 1983).

1.3.2 *Occupation*

Occupational exposures can occur through the handling and processing of aflatoxin-contaminated crops. Low-level respiratory exposure to aflatoxin-contaminated dust particles was reported in workers processing imported maize (Silas *et al.*, 1987) and in workers extracting oil from linseeds and groundnuts (van Nieuwenhuize *et al.*, 1973). A study of occupational exposure of Danish workers in animal feed production showed that seven of 45 workers exposed to aflatoxin B_1-contaminated feeds (0–26 μg/kg) had detectable levels of aflatoxin B_1 bound to serum albumin (none detected [< 5 pg/mg] to 100 pg/mg albumin). Dust samples collected at different sites showed none detectable to 8 μg/kg dust (Olsen *et al.*, 1988; Autrup *et al.*, 1991).

1.3.3 *Human biological fluids*

Aflatoxins have been detected in human urine, milk and blood samples. An initial study reported the presence of aflatoxin M_1 in human urine from the Philippines (Campbell *et al.*, 1970); early observations from a number of other laboratories have been reviewed (Garner *et al.*, 1985).

In Zimbabwe, 4.3% of 1228 urine samples collected throughout the country contained aflatoxins; the most common metabolite detected was aflatoxin M_1 (Nyathi *et al.*, 1987). Aflatoxin metabolites were detected in urine samples from the Gambia, the Philippines, Singapore and France (Wild *et al.*, 1988). Aflatoxin B_1–*N*7-guanine adducts were found in 12.6% of 983 urine samples from Kenya (Autrup *et al.*, 1987).

In a study of 252 urine samples from 32 households in Guangxi Autonomous Region in China, a good correlation was seen between urinary excretion of aflatoxin M_1 over a three-day period and dietary intake of aflatoxin B_1. Between 1.2 and 2.2% of dietary aflatoxin B_1 was found as urinary aflatoxin M_1 (Zhu *et al.*, 1987). A more detailed study (Groopman *et al.*, 1992a) of the pattern of urinary metabolites in the same urine samples confirmed the findings on aflatoxin M_1, revealed the presence of aflatoxin P_1 (see Fig. 1) as a major metabolite and demonstrated the presence of aflatoxin B_1, aflatoxin Q_1 (see Fig. 1; rarely found) and aflatoxin B_1–*N*7-guanine adducts. The total mean percentage of dietary aflatoxin B_1 excreted as the above metabolites was calculated to be 4.4% for women and 7.6% for men. This study also established that the levels of aflatoxin M_1 and of aflatoxin B_1–*N*7-guanine adducts were correlated with intake, while those of aflatoxin P_1 and aflatoxin B_1 were not.

In the Gambia, urinary excretion of total aflatoxin metabolites and of aflatoxin B_1–*N*7-guanine adducts over a four-day period in 20 individuals was correlated with their dietary intake of aflatoxins. In contrast to the study in China, where no aflatoxin G_1 was detected, aflatoxin G_1 was present in the diet and was also found to be the major urinary aflatoxin in this study (Groopman *et al.*, 1992b).

Several studies have demonstrated the presence of aflatoxins in human milk: aflatoxins M_1, M_2, B_1, B_2, G_1 and G_2 have been reported in the Sudan, Zimbabwe and Ghana (Coulter *et al.*, 1984; Wild *et al*, 1987; Lamplugh *et al.*, 1988). Aflatoxin M_1 was the type most frequently present, at levels up to 64 pg/ml in the Sudan (Coulter *et al.*, 1984), up to 50.5 pg/ml in Zimbabwe (Wild *et al.*, 1987) and 1.8 pg/ml in Ghana (Lamplugh *et al.*, 1988). In one report on five lactating women in the Gambia, 0.09–0.43% of dietary intake was excreted in milk as aflatoxin M_1 (Zarba *et al.*, 1992).

Aflatoxins have been detected in blood samples in Nigeria (Denning *et al.*, 1988), the Sudan (Hendrickse *et al.*, 1982) and Japan (Tsuboi *et al.*, 1984). Sera from the United Kingdom were found to contain less than 20 pg/ml (Wilkinson *et al.*, 1988). Aflatoxins were also detected in 17 of 35 umbilical cord blood samples in southern Thailand (Denning *et al.*, 1990), in nine of 78 samples in Nigeria and in 63 of 188 samples in Ghana (Lamplugh *et al.*, 1988).

The biologically effective dose of aflatoxin for individuals can be determined in assays for covalent binding to albumin in peripheral blood (Gan *et al.*, 1988; Wild *et al.*, 1990a) and for aflatoxin B_1–*N*7-guanine adducts (Groopman *et al.*, 1992b,c). The level of urinary adducts reflects dietary exposure to aflatoxin over the previous 24–48 h (Groopman *et al.*,

1992b,c), while the level of aflatoxin–albumin adducts is assumed to reflect exposure over the previous two to three months (Wild *et al.*, 1992a). The urinary aflatoxin B_1–*N*7-guanine adduct is by definition a specific indicator of exposure to aflatoxin B_1, whereas the aflatoxin–albumin adduct, determined by immunoassay, is only predominantly a measure of such exposure. An aflatoxin G_1–albumin adduct has also been identified (Sabbioni & Wild, 1991).

In a study of 42 residents of Guangxi, China, the level of aflatoxin–albumin adducts was shown to be correlated with both dietary aflatoxin B_1 intake and urinary aflatoxin M_1 excretion at the individual level (Gan *et al.*, 1988). A later report on the same subjects demonstrated a correlation between the level of urinary aflatoxin B_1–*N*7-guanine adducts and serum aflatoxin–albumin adducts (Groopman *et al.*, 1992a).

The levels of aflatoxin–albumin adducts in subjects from several countries in Africa, Southeast Asia and Europe have been reviewed (Wild *et al.*, 1992b; Table 7). Although the subjects in the different studies were not matched, the data illustrate the quantitative differences in exposure that can occur worldwide. Exposure was significantly higher in the Gambia, Kenya and the Guangxi region of China than in Thailand, while in Europe the levels were below the detection limit. In the Gambia, only seven of 390 individuals (< 2%) had no detectable adduct. The same adduct was detected in 21 of 30 samples of cord blood from the Gambia, and there was a good correlation with the level of adduct in matched maternal venous blood (Wild *et al.*, 1991). Exposure levels did not vary with age or sex in the Gambian population (Allen *et al.*, 1992; Wild *et al*, 1992a), but seasonal variations in exposure occurred (May compared to November).

Table 7. Aflatoxin–albumin adducts in human sera

Country	No. of subjects	No. of subjects with different adduct levels (pg aflatoxin B_1–lysine eq./mg albumin)					
		< 5	5–25	26–50	51–75	76–100	> 100
Gambia							
May	323	7	53	76	49	40	98
November	67	0	39	13	7	3	5
Senegal	29	0	20	6	2	1	0
Kenya	91	48	26	5	1	5	6
China							
Guangxi	93	28	35	13	6	2	9
Shandong	69	69	0	0	0	0	0
Thailand	84	73	10	1	0	0	0
France	44	44	0	0	0	0	0
Poland	30	30	0	0	0	0	0

From Wild *et al.* (1992b); limit of detection, 5 pg aflatoxin B_1–lysine eq./mg albumin

Aflatoxins have also been reported in human tissues, either in the free form, e.g., in liver (Wray & Hayes, 1980; Stora *et al.*, 1981; Lamplugh & Hendrickse, 1982; Onyemelukwe *et al.*, 1982; Stora & Dvořáčková, 1986, 1987), or bound to DNA in liver (Hsieh *et al.*, 1988; Zhang

et al., 1991). Aflatoxins were also reported in a case of colon adenocarcinoma (Deger, 1976) and in two cases of lung cancer (Dvořáčková *et al.*, 1981).

1.4 Regulations and guidelines

It is probably not possible to eliminate completely exposure of humans to aflatoxins. In 1987, at least 50 countries had existing or proposed regulations for aflatoxins in foodstuffs (van Egmond, 1989b, 1992; Stoloff *et al.*, 1991). The maximum limits range from none detectable to 50 μg/kg of food for either the sum of aflatoxins B_1, B_2, G_1 and G_2 or for aflatoxin B_1 alone; 5 μg/kg is the commonest maximal limit.

In 1987, aflatoxin M_1 levels in dairy products were regulated in 14 countries (van Egmond, 1989b). The tolerances in infants' and children's food were 0.05–0.5 μg/kg milk.

Aflatoxins were reviewed by a joint FAO/WHO Expert Committee on Food Additives in 1987 (WHO, 1987). No acceptable daily intake was given; it was recommended that human intake be reduced to the lowest practicable level.

2. Studies of Cancer in Humans

2.1 Descriptive studies

In a number of studies, estimates of the incidence of primary liver cancer were correlated with the intake of aflatoxins in the same population groups. Apart from methodological limitations of correlation studies, two specific problems related to liver cancer hinder the interpretation of the results of these studies. Firstly, diagnosis and registration of liver cancer are likely to be incomplete, especially in some developing areas of the world, and inaccurate. Secondly, most of the studies have not evaluated the rate of chronic infection with hepatitis B or hepatitis C virus, which are potential confounders for aflatoxins. Studies have been conducted in Swaziland (Keen & Martin, 1971; Peers *et al.*, 1976), Uganda (Alpert *et al.*, 1971), Thailand (Shank *et al.*, 1972a,b,c), Kenya (Peers & Linsell, 1973), Mozambique (van Rensburg *et al.*, 1974), China (Armstrong, 1980; Wang *et al.*, 1983; Sun & Chu, 1984; Wu *et al.*, 1984; Yu *et al.*, 1989), the USA (Stoloff, 1983) and the Transkei, South Africa (van Rensburg *et al.*, 1985, 1990). The substantial differences in the incidence of liver cancer between as well as within these areas generally are strongly correlated with differences in the estimated intake of aflatoxin B_1 within the same population groups. The intra-country correlation was first demonstrated by Linsell and Peers (1977) and was corroborated by data from the Transkei (van Rensburg *et al.*, 1985) and from Swaziland (Peers *et al.*, 1987; Bosch & Muñoz, 1988). In an early study in Kenya, there appeared to be little correlation between liver tumour incidence and an estimate of the prevalence of hepatitis B surface antigen (HBsAg), analysed using a radioimmunoassay method (Bagshawe *et al.*, 1975); in contrast, the estimated consumption of aflatoxin B_1-contaminated food in the same areas was correlated with liver tumour incidence (Peers & Linsell, 1973).

Two correlation studies have been reported in which attempts were made to evaluate differences in liver tumour incidence in areas of Africa simultaneously with data on exposure

to aflatoxins and hepatitis B infection. A study in Swaziland was based on estimates of aflatoxin consumption from a defined sampling of foods in 11 designated areas in the country, subsequently analysed for aflatoxin content, and surveys of locally grown crops between harvesting and consumption (Peers *et al.*, 1987); hepatitis B virus markers were assessed in blood donors and primary liver cancer incidence from a cancer registration system that operated from 1979 to 1983. In this study, 120 of 2583 food samples were contaminated with aflatoxin, and groundnuts were the food item most commonly contaminated. Of the 120 contaminated samples, 97% contained aflatoxin B_1, 73% aflatoxin G_1 and 33% contained either B_2 or G_2. Aflatoxin B_1 constituted 19.5–57% of the estimated average daily exposure to total aflatoxin in the 11 areas. The correlation between liver cancer incidence and aflatoxin exposure was reported to be significant for both total aflatoxin and aflatoxin B_1. [The Working Group noted that the relatively high proportion of aflatoxin G_1, G_2 and B_2 in these samples could probably have contributed to the reported correlation with total aflatoxins.] In the analysis, a model incorporating daily consumption of total aflatoxins and the proportion of people positive for HBsAg provided the best fit to the variation in liver cancer incidence. It was not significantly better than a model that included only total aflatoxin consumption; however, it provided a significantly better fit to the liver cancer rates than a model that included only the proportion of people with HBsAg. Thus, at an area or ecological level, evidence of hepatitis B infection did not confound the association of liver cancer incidence with aflatoxin consumption, and although both were associated with liver cancer incidence, aflatoxin exposure appeared to be more important in explaining the variation in liver cancer incidence.

In a second study, conducted in Kenya (Autrup *et al.*, 1987), the incidence of liver cancer by area was determined from the records of one large hospital. Specimens of urine and blood were collected from patients attending the out-patient departments of various hospitals; urinary excretion of aflatoxin B_1–guanine adducts and serological markers of hepatitis B infection were determined. Of the 983 tested individuals, 12.4% excreted aflatoxin B_1–guanine adducts and 10.6% had chronic infection with hepatitis B virus. There were wide variations in the prevalences of these exposures by area: Among Bantus, there was a significant correlation between liver cancer 'incidence' [as estimated from hospital records] and the prevalence of exposure to aflatoxin (r, 0.75) but not with evidence of hepatitis B infection (r, 0.19). There was no evidence of interaction between the two exposures. [The Working Group noted that the associations were 'limited to certain ethnic groups', but no information was provided on the size of those groups in relation to the total number of subjects in the study.]

Stoloff (1983), in evaluating the role of aflatoxin ingestion in the prevalence of primary liver cancer in the USA, also did not perform a formal correlation analysis. Data from many published sources were used to estimate levels of exposure in the USA, which was found to be much higher in southeastern than in northern or western USA. [The Working Group noted that these estimates included extrapolations to time periods before the early 1960s that preceded structural identification of aflatoxins.]

In China, a number of studies have been performed to correlate liver cancer incidence or mortality with estimates of aflatoxin exposure by area, and several have attempted to take hepatitis B infection into account. Armstrong (1980) reported on data from Chinese

investigators that had been presented at an international workshop. In an analysis which included a number of different factors suspected of being associated with liver cancer, including the prevalence of hepatitis B infection, only the product of proportion of mould-producing days in the year and proportion of maize in food was correlated significantly with liver cancer mortality (r, 0.71).

In a factor analysis that included data on climate, grain-oil contamination by aflatoxins and HBsAg positivity rates in relation to liver cancer mortality in China, Wang *et al.* (1983) found no association with HBsAg positivity rates (although they noted the absence of data from some areas) but did find an association with aflatoxin exposure, which accounted for 60.7% of the variation in liver cancer mortality in males and 44.0% in females.

Sun and Wang (1983) and Sun and Chu (1984) reviewed previous studies that showed differences in liver cancer rates which do not seem to be explained by differences in hepatitis B carrier status. The additional factor appeared to be differences in aflatoxin exposure.

Yeh *et al.* (1989) performed a geographical analysis within a cohort study of 7917 men aged 25–64 in Guangxi, China. The results showed a 3.5-fold difference in liver cancer mortality according to place of residence, which was linearly associated with dietary aflatoxin contamination (r, 1.00; $p = 0.004$), estimated for each commune after twice yearly sampling of foods. The four communes that contributed to the correlation with aflatoxins had a range of mean aflatoxin B_1 intakes of 0.3–51.8 mg/person per year. In the same four communes, the range in age-adjusted prevalence of HBsAg was 21.6–24.8%, and there was little relationship between prevalence of HBsAg positivity by area and liver cancer mortality (r, 0.28; $p = 0.65$). At the individual level, however, the cohort study showed a strong association between HBsAg positivity at the time of enrolment and liver cancer mortality.

In a large cross-sectional survey performed in 48 sites in China with an estimated 600-fold variation in aflatoxin exposure, a 28-fold range in HBsAg prevalence and a 39-fold range in mortality from primary liver cancer, no association with aflatoxin exposure but a strong correlation with HBsAg prevalence was noted (Campbell *et al.*, 1990). Liver cancer mortality was determined in 1973–75, and biochemical data were obtained in 1983. Aflatoxin exposure was estimated from measures of aflatoxin metabolites in age-specific pooled urine samples from each region. [The Working Group noted that the method provided a mean intake but no estimate of standard deviation. Furthermore, the time difference between estimates of aflatoxin exposure and liver cancer incidence and mortality was substantial.]

Another correlation study was performed in Thailand, which was based on more sensitive markers of aflatoxins in serum and urine than those used in most previous studies (Srivatanakul *et al.*, 1991a). Hepatocarcinoma incidence in five areas correlated poorly with these markers and with markers of hepatitis B infection.

2.2 Cohort studies

Hayes *et al.* (1984) reported on mortality between 1963 and 1980 in a cohort of 71 Dutch workers involved in extracting oil from linseeds and groundnuts, who had been exposed by inhalation to aflatoxin-containing dust during the period 1961–69 and who had been employed in the study plant for at least two years. A comparison group of 67 plant workers

not exposed to aflatoxin-contaminated dust was also studied, and for each group standardized mortality ratios (SMR) with the Dutch male population as standard were computed. In the aflatoxin-contaminated group, but not in the comparison group, the observed numbers of all cancer deaths were above expectation (16 in the exposed and 7 in the controls; SMR (95% confidence intervals [CI]), 2.50 (1.40–4.00) and 1.13 (0.40–2.30), respectively). In particular, there were seven cases of respiratory cancer among aflatoxin-exposed workers (SMR, 2.53; 95% CI, 1.00–5.00). There was no death in either group from primary liver cancer. This study was an extension of a follow-up, with inclusion of younger workers from the same plant, documented in a previous report (van Nieuwenhuize *et al.*, 1973), which gave levels of aflatoxins in residues from groundnut cakes of 75–1320 μg/kg and reported one case of primary liver cancer in the aflatoxin-exposed workers. Hayes *et al.* (1984) reported, however, that re-examination of the pathological material had shown the case to have been a metastasis in the liver from a tumour of undefined endocrine origin.

Yeh *et al.* (1985) summarized a study in China in which HBsAg-positive and -negative individuals were stratified into those who lived in villages with 'light' or 'heavy' aflatoxin contamination of foods and were followed up to 1982 (total years of follow-up, five to eight). Maize was the major source of aflatoxin contamination. The average annual intake of aflatoxins in the heavily contaminated villages was 6.016 mg per person, and that in the lightly contaminated villages, 0.638 mg per person. The mortality rate for liver cancer was [372 per 100 000] in the heavily contaminated villages, based on 15 deaths, and [32.8 per 100 000] in the lightly contaminated villages, based on one death [$p < 0.05$]. There was a multiplicative effect between contamination with aflatoxins and HBsAg status, but misclassification of HBsAg status could have affected the findings.

Olsen *et al.* (1988) reported on cancer risk among employees of 241 livestock feed processing companies, using a system based on record linkage between the Danish Supplementary Pension Fund and the Danish Cancer Registry, with occupational data dating back to 1964. Exposure to aflatoxins from imported crops intended for animal consumption had been documented by government surveys; average concentrations in imported groundnut oilcakes were around 1000 μg/kg in 1976–78. When mixed with other products, the concentrations of aflatoxins in prepared cattle feed were ~ 140 μg/kg. Because dust levels in the industry were high, it was estimated that workers may have inhaled 170 ng aflatoxin B_1 per working day. Aflatoxin G_1 was not measured. As population denominators were not available, the analysis was based on standardized proportional incidence ratios (SPIRs), all employees with cancer in the data base serving as the referent. All males with cancer diagnosed in 1970–84, whose longest work experience had been at one of the 241 feed processing companies in the period 1 April 1964 to the date of diagnosis, were included in the analysis. There was no excess of respiratory cancer. For liver cancer, the SPIR was 1.41 (95% CI, 0.57–2.93; six observed cases); for gall-bladder and extrahepatic bile-duct cancer, the SPIR was 2.19 (0.89–4.55; six observed cases, including one incorrectly classified as an intrahepatic cholangiocarcinoma). In an analysis restricted to the period 10 or more years before diagnosis, workers who had had major exposure to aflatoxin-contaminated feed had an SPIR for liver cancer of 2.46 (1.08–4.86; seven observed cases), while that for gall-bladder and extrahepatic bile-duct cancers was 2.98 (1.09–6.59; five cases). [The Working Group noted

that although possible confounding factors were not evaluated in this study, they were not likely to have affected the results.]

Ross *et al.* (1992) conducted a cohort study of 18 244 male residents of Shanghai, China, 96% of whom were aged 45–64 years on entry to the study. The men were recruited by invitation from four geographically defined areas and responded to questionnaires administered by nurses, usually in their homes, on life style (including smoking and alcohol consumption) and on food frequency. Blood and urine specimens were collected. The men were followed up by identification of death records in the same or neighbouring areas and through linkage with the Shanghai Cancer Registry (estimated to be 85% complete). An attempt was also made to contact each cohort member annually. The cohort was established between January 1986 and September 1989 and was followed to 1 March 1990 for the current analysis, resulting in 35 299 person-years of follow-up. Only 24 members were lost to follow-up. Of 176 cancer cases identified, 22 were primary liver cancers, although only five were confirmed by biopsy. The first six cases were each matched for age, sample collection date and area of residence to 10 controls randomly selected from cohort members who had not been diagnosed with liver cancer at the time of diagnosis of the case. The remaining 16 cases were similarly matched to five controls each. For each case and control, the urine samples were analysed for aflatoxin B_1 and its principal metabolites, aflatoxins P_1 and M_1, as well as for aflatoxin B_1–*N*7-guanine adducts. HBsAg was measured by radioimmunoassay. The study was analysed as a nested case–control study within the cohort. Each of the four markers of exposure was more frequently present among cases than controls. For 13 of the 22 liver cancer cases and 53 of 140 controls, results were positive in at least one of the four assays (relative risk (RR), 2.4; 95% CI, 1.0–5.9). The RR for HBsAg positivity was 7.8 (95% CI, 3.0–20.6). When this and other risk factors for liver cancer (including smoking and alcohol use) were included in a multivariate logistic regression analysis, the RR for detectable urinary aflatoxins or aflatoxin–guanine adducts became 3.8 (95% CI, 1.2–12.2). Among individuals who did not have HBsAg, the RR for urinary aflatoxins was 1.9 (0.5–7.5); however, among those who had HBsAg, the RR was 60.1 (6.4–561.8), although the latter estimate was based on only seven cases and two controls who had both factors. Urinary aflatoxin metabolites and guanine adducts reflect recent dietary exposure. It was considered unlikely, however, that individuals with early symptoms could have changed their diet and somehow increased consumption of aflatoxin-contaminated food. Given the short follow-up period (less than two years as a mean), some of the observed tumours might already have been present, in a clinically silent phase, at the time of urine collection. Moreover, chronic liver disease, which frequently underlies hepatocellular carcinoma, might affect metabolism of aflatoxin. [The Working Group noted that no analysis was presented by interval between urine collection and liver cancer diagnosis and that the proportion of liver cancer cases known to be accompanied by cirrhosis was not given. It was considered, however, that to explain the observed case–control differences in terms of some metabolic effect of preclinical liver conditions would imply a substantial enhancement of all the different enzymatic metabolic pathways leading to, respectively, aflatoxin P_1, aflatoxin M_1 and aflatoxin B_1–*N*7-guanine adducts, and that the data do not support such effects. (See also Hall & Wild, 1992.)]

In an extension of the analysis (Qian *et al.*, 1993), the risk for hepatocellular carcinoma was higher among people who excreted aflatoxin B_1–guanine adducts in their urine: the RR

(adjusted for hepatitis B, smoking and alcohol use) was 7.8 (95% CI, 1.5–40.0). No association was found with dietary aflatoxin levels, as determined by an in-person interview and a survey of market foods in the survey region. There was no association between dietary and urinary aflatoxin levels among the subjects for whom both levels were determined.

2.3 Case–control studies

Bulatao-Jayme *et al.* (1982) reported a case–control study based on 90 hospital cases (66% non-paying) of confirmed primary liver cancer from three hospitals in the Philippines. The controls were non-paying patients from surgical and medical wards who showed normal liver function after a battery of biochemical tests and who were individually matched by age and sex to the cases [response rates for cases and controls not reported]. Recent aflatoxin exposure was estimated from 24-h urine samples collected on or before the first day of admission and analysed by thin-layer chromatography for aflatoxin B_1 and aflatoxin M_1. Of the samples from 76 cases, 51% were positive for aflatoxin B_1 and/or M_1; this result was significantly different from the 35% in 89 controls. Aflatoxin contamination of the diet was estimated by measuring the aflatoxin B_1 and M_1 content of food samples, mostly from the greater Manila area but some (maize) from 'practically' all regions of the country. These measurements (usually covering at least 20 samples cooked in various ways) were applied to a usual dietary food intake history collected from each subject by a professional nutritionist, who was given only the patient's name and bed number before the interview. A separate dietary record was taken for each period of life in which there had been a different residence for one year or more; the overall mean aflatoxin load per day from these separate periods was used in the analysis. Aflatoxin intake was generally categorized as heavy or light, heavy being defined as a total estimated aflatoxin load of 4 μg or more per day, although for some analyses the heavy category was divided into moderately heavy (4–6 μg) and very heavy (≥ 7 μg). For all aflatoxin-containing food items except rice, the aflatoxin load was significantly higher in the cases than in the controls, with correspondingly elevated odds ratios (ORs), including 4.2 for maize, 7.7 for groundnuts, 11.0 for sweet potatoes and 22.5 for cassava. When aflatoxin intake was categorized into three levels, the OR for very heavy exposure was 17.0 and that for moderately heavy, 13.9 (both significant). The OR for heavy aflatoxin and heavy alcohol consumption relative to light aflatoxin and light alcohol consumption was 35.0, that for heavy aflatoxin and light alcohol was 17.5, and that for light aflatoxin and heavy alcohol was 3.9. A similar finding was reported when the analysis was restricted to 35 pairs of subjects who consumed only aflatoxin-free alcoholic beverages. [The Working Group noted that no data were available on hepatitis B infection; however, there would have to be an unusually close correlation between estimates of aflatoxin consumption and hepatitis B infection status for the ORs reported to be fully explained by confounding. The dietary data on which the estimates of aflatoxin intake were based presumably involve substantial misclassification, and generally it would be expected that this would bias the ORs towards the null.]

In a hospital-based case–control study in Hong Kong of primary liver cancer, involving 107 pairs of Chinese subjects, Lam *et al.* (1982) evaluated a number of risk factors, including hepatitis B virus infection and ingestion of aflatoxin-containing foods. The cases interviewed comprised 72% of those initially ascertained. The controls were selected from among

patients admitted with trauma to the orthopaedic ward of the same hospital. Frequency of consumption of a number of dietary food items was ascertained currently and 20 years previously; there was little difference in these estimates, and current frequency of consumption was used in the analysis. Aflatoxin contamination of food was based on a market survey that had been performed 10 years before the study. Maize was the most frequently contaminated food. The analysis of aflatoxin intake was based on a comparison of frequency of consumption of different foods, with no significant difference between cases and controls in these consumption frequencies. The results were similar when adjusted for HBsAg status. [The Working Group noted that no quantitative estimate of aflatoxin consumption at the individual level was made and that, given the low reported frequency of consumption of presumed aflatoxin-contaminated foods, the power of the study to detect an association was low.]

Two hospital-based case–control studies of liver cancer were carried out in patients aged less than 75 when recruited during 1987–88 from three hospitals in north-east Thailand—one on cholangiocarcinoma (Parkin *et al.*, 1991) and one on hepatocellular carcinoma (Srivatanakul *et al.*, 1991b). The studies included assessment of both hepatitis B virus infection status and consumption of foods possibly contaminated with aflatoxins as well as measurement in blood of aflatoxin–albumin adducts by the method of Wild *et al.* (1990a), which is believed to provide an indication of integrated aflatoxin exposure over recent weeks or months. Controls were selected from among individuals who were visiting clinics or who had been admitted to the same hospital as the cases and were matched to the cases on age and sex. Dietary data were obtained from responses on a food-frequency questionnaire directed to the period one year prior to interview. Several food groups were characterized, one of which was foods liable to aflatoxin contamination on the basis of previous studies in northern Thailand. Frequency of consumption was dichotomized on the basis of consumption frequency among controls. In the study of cholangiocarcinoma (Parkin *et al.*, 1991), 113 patients were interviewed, but 10 cases that did not fulfil the diagnostic criteria and their matched controls were excluded. In the analysis of aflatoxin–albumin adducts, 21 pairs were considered, with only one discordant pair in each category and an OR of 1.0 (95% CI, 0.1–16.0). The OR for consumption of aflatoxin-contaminated foods was 1.4 (0.8–2.7). In the study of hepatocellular carcinoma, 73 cases were interviewed, and 65 pairs that fulfilled the diagnostic criteria were included in the analysis (Srivatanakul *et al.*, 1991b). Forty-five pairs were tested for albumin-bound aflatoxins, with 16 discordant pairs—eight in each category—for an OR of 1.0 (0.4–2.7). The prevalence of detectable aflatoxins in the 99 specimens examined was 22.2%. Of the food groups considered, those potentially associated with aflatoxin contamination gave the highest OR (1.9), but it was not significant [confidence interval not given].

Sera from 100 patients with lung cancer in Italy were tested for the presence of aflatoxins, as were sera from 150 healthy donors (Cusumano, 1991). Positive results were obtained in seven and one, respectively. Only one of the seven cases was a smoker. Of the seven, five were positive for aflatoxin B_1 and two for aflatoxin B_2; the one in the control group was positive for aflatoxin G_1. [The Working Group noted that the controls were not strictly comparable to the cases.]

3. Studies of Cancer in Experimental Animals

3.1 Mixtures of aflatoxins

3.1.1 *Oral administration*

Rat: Since the first report of the induction of hepatocellular tumours in rats by groundnut meal (Lancaster *et al.*, 1961), many studies in rats have demonstrated the carcinogenic potency of aflatoxins (IARC, 1976, 1987a).

Groups of rats [strain, sex, age and initial number unspecified] were fed diets containing 5, 200, 1000, 3500 or 5000 μg/kg of diet aflatoxins for 294–384 days. A linear dose–response relationship was observed in the incidence of hepatomas: 0/10, 3/20, 13/24, 18/25 and 14/15, respectively (Newberne, 1965).

A group of six young rats [strain and sex unspecified] was fed a diet containing groundnut meal providing aflatoxins at a concentration of 3000–4000 μg/kg of diet for three weeks and then returned to a normal diet. Another group of six male rats [strain unspecified], one year old, was fed the same diet for more than 39 weeks. Of the six rats treated with aflatoxins for three weeks early in life, one animal, which lived for 106 weeks after return to the normal diet, developed a carcinosarcoma of the stomach and another animal a hepatocellular carcinoma. Of the six rats fed the diet containing aflatoxins after one year of age, five survived more than 39 weeks; anaplastic hepatocellular carcinomas were found in 3/5 animals and adenocarcinomas of the stomach and rectum in 1/5 animals each (Butler & Barnes, 1966).

Groups of six male rats [strain and age unspecified] were administered 10 or 100 μg of a mixture of aflatoxins (containing 37.7% aflatoxin B_1, 56.4% aflatoxin G_1 and traces of aflatoxins B_2 and G_2) in 100 ml drinking-water for 64 weeks. At the high dose of aflatoxins (approximately 300 μg/week), 6/6 rats treated for more than 39 weeks developed hepatocellular tumours. At the low dose (approximately 35 μg/week), 1/5 rats developed a hepatocellular tumour, which appeared at 66 weeks (Dickens *et al.*, 1966).

Groups of 16–19 female Wistar rats, weighing 50–96 g, were administered 0, or a single dose of 0.5 mg/animal aflatoxin B_1 (approximately 7650 μg/kg bw) in 0.1 ml dimethylformamide, or a mixture of aflatoxins (40% aflatoxin B_1, 60% aflatoxin G_1; approximately 5.1 mg/kg bw in terms of aflatoxin B_1) in 0.1 ml dimethylformamide by stomach tube. Survival after 10 months was 18/19 vehicle controls, 15/16 rats treated with aflatoxin B_1 and 16/18 rats receiving the mixture. The remaining animals died or were killed between 15 and 32 months after aflatoxin administration. Increased incidences of hepatic tumours (mainly hepatocellular carcinomas) were found in 7/15 rats treated with aflatoxin B_1, in 7/16 rats that received the mixture and in 0/18 vehicle controls. The first hepatic tumours were found after 21 months, and the mean time to tumour induction was approximately 26 months. In addition, nodular hepatic hyperplasia was observed in 3/15 rats treated with aflatoxin B_1, in 5/16 rats that received the mixture and in 0/18 vehicle controls. In some animals treated with aflatoxin B_1 and G_1, cystic bile-duct hyperplasia was seen (Carnaghan, 1967).

Groups of 6–36 male and female Porton rats, 40–90 days of age, were fed diets (prepared from a basic diet containing 10 000 μg/kg aflatoxin B_1 and 200 μg/kg aflatoxin B_2) containing

aflatoxin at approximately 0, 100, 500 and 5000 μg/kg of diet for one to nine weeks or for lifetime. Animals were necropsied when they became moribund or died. In male rats fed 5000 μg/kg of diet aflatoxin B_1 for one to nine weeks, the incidence of hepatocarcinomas (based on the number of animals alive when the first tumour appeared) was related to the time of treatment (control, 0/46; one week, 0/13; three weeks, 3/20; six weeks, 12/19; nine weeks, 6/6). In male rats fed 0, 100 or 500 μg/kg of diet aflatoxin for lifetime, the incidences of hepatocarcinomas were 0/46 controls, 17/34 low-dose and 25/25 high-dose animals. The incidences of hepatocarcinomas in female rats were: control, 0/34; low-dose, 5/30; high-dose, 26/33. A small number of neoplasms at other sites, including kidney (adenomas and carcinomas of the renal pelvis in 5/53 rats), stomach (carcinomas of the glandular stomach in 2/53 rats after 86 weeks following feeding for 1–9 weeks), lung and salivary glands, was found in some rats given aflatoxin but not in the control rats (Butler & Barnes, 1968). In a similar experiment in male Fischer rats, histochemical findings indicated that all of the carcinomas had a hepatocellular origin (Butler & Hempsall, 1981). Various types of hepatic nodules were found which were composed of vacuolated (lipid-rich), basophilic (ribosome-rich) or eosinophilic cells (containing abundant smooth endoplasmic reticulum, frequently arranged in whorls) (Pritchard & Butler, 1988). In male Fischer rats treated with the same regimen, foci of altered hepatocytes (as defined by increased basophilia, starvation-resistant glycogen and a decrease in the activity of various enzymes) were seen in the liver parenchyma, for the first time at three weeks (Butler *et al.*, 1981).

Groups of 60 male and 30 female Sprague Dawley rats, each weighing about 40 g, were fed a diet containing aflatoxins (aflatoxin B_1 was present at a level of 5–7.5 μg/kg of diet) derived from contaminated groundnut oil or a diet prepared with aflatoxin-free corn oil for 22 months and sacrificed thereafter. Survival at that time was 90/90 controls and 76/90 animals treated with aflatoxins. Sarcomas were found in 3/76 treated animals (one sarcoma each in the liver, colon and subcutaneous tissue). In addition, 18/76 rats had parenchymal liver damage (swelling of cytoplasm, fatty infiltration) and 1/76 had a premalignant liver lesion. Five benign mammary tumours were reported to have occurred in 90 controls (Fong & Chan, 1981).

Duck: A group of 37 Khaki Campbell ducklings, seven days of age, were fed a diet containing aflatoxins derived from contaminated groundnut meal at a level of 35 μg/kg diet aflatoxin B_1. Sixteen control ducklings were fed the same diet without added groundnut meal. The survivors of both groups were killed 14 months after commencement of the experiment. Liver tumours classified as hepatomas were found in 6/11 and liver tumours classified as cholangiomas in 2/11 treated animals. No liver tumour occurred in 10 control animals (Carnaghan, 1965).

Trout: Groups of 300 hatched rainbow trout were fed various diets containing aflatoxins (values were calculated as aflatoxin B_1) at levels of 3.7–42 μg/kg of diet or a purified test diet to which aflatoxin B_1 had been added. Subsamples of fish were selected randomly at multiple time periods for evaluation. The diet containing the highest dose level of aflatoxins (42 μg/kg of diet) produced a high incidence of hepatomas (six months, 10/20; nine months, 20/20; 12 months, 20/20), and the level of 3.7 μg/kg of diet produced hepatomas in 5/10 fish by 12 months. A lower incidence of hepatomas developed in trout that received the purified test diet to which aflatoxin B_1 had been added: after 12 months, the incidence at 7.9 μg/kg of diet

was 5/12, that at 4.0 μg/kg, 2/13, and that at 0.8 μg/kg, 0/12; after 15 months, the incidence was 24/30 in those given the high dose; after 20 months, the incidences were 3/21 in those given the medium dose and 3/31 in those receiving the low dose. No liver tumour was observed in a group that received the control diet (Sinnhuber *et al.*, 1968a).

Groups of 20 rainbow trout fry, 60 days old, were fed a commercial diet containing 42 μg/kg aflatoxins for two, four, six and eight weeks and then switched to control diet until the end of the experiment at nine months. There was a high incidence of hepatomas even after the shortest treatment period (two weeks, 12/20; four weeks, 15/20; six weeks, 14/20; eight weeks, 15/20) (Sinnhuber *et al.*, 1968b).

3.1.2 *Oral administration of ammoniated samples* (see also pp. 253 and 307)

Rat: Groups of 12 male and 12 female Fischer 344 rats, three weeks old, were fed diets derived from maize with no detectable aflatoxin and from maize naturally contaminated with aflatoxins, in both cases with and without ammoniation. The diets contained 0 and 176 μg/kg of a mixture of aflatoxins (150 μg B_1, 18 μg B_2 and 8 μg G_1) or the corresponding ammoniated aflatoxin by-products. The experiment was terminated at 92 weeks. The first liver tumours were found in rats treated with aflatoxin-contaminated diet after 80 weeks, when 6/12 males and 2/12 females had died. The overall incidence of hepatocellular carcinomas in rats receiving the aflatoxin-contaminated diet was 12/12 males and 11/12 females; the incidence of neoplastic hepatic nodules was 6/12 males and 10/12 females. In addition, preneoplastic foci of altered hepatocytes (clear-cell foci) were observed in this group. No neoplastic or preneoplastic liver lesion was found in rats fed diets free of aflatoxins or diets containing aflatoxins after ammoniation (Norred & Morrissey, 1983).

Groups of 20 male and 20 female Fischer 344 rats, three to five weeks of age, were fed diets naturally contaminated with aflatoxins (derived from groundnut meal) with and without ammoniation. The unammoniated diets contained 0 or 590.7 (375.0 μg B_1, 36.2 μg B_2, 156.5 μg G_1 and 23.0 μg G_2) and the ammoniated diets 0 or 37.6 (26.4 μg B_1, 6.5 μg B_2, 3.5 μg G_1 and 1.2 μg G_2) μg/kg aflatoxins. The diets were fed for up to 90 days, when the experiment was terminated. Preneoplastic foci of altered hepatocytes (as defined by γ-glutamyltransferase-positive [GGT^+] staining) were found in rats receiving the unammoniated aflatoxin-contaminated diet but not in rats receiving the ammoniated diet or an uncontaminated control diet (Manson & Neal, 1987).

Groups of 15–31 male and 10–20 female Fischer 344 or Wistar WAG rats, four to five weeks old, were fed diets containing mixtures of aflatoxins B_1 and G_1: 60, 140 or 1000 μg/kg of diet aflatoxin B_1 and 10, 20 or 170 μg/kg of diet aflatoxin G_1; the two lower dose levels of aflatoxin B_1 and G_1 were obtained by treating the diet containing the highest dose with ammonia gas at pressures of 3 and 2 bar (300 and 200 kPa), respectively, for 15 min at 95 °C. Control groups of 20 male and 10 female rats were fed a diet which was found to be contaminated with 50 μg/kg aflatoxin B_1. The experiment was terminated after 18 months. In males, 40/40 controls, 46/46 low-dose, 40/40 medium-dose, and 36/40 high-dose animals survived to 18 months; in females, survival was 20/20 controls, 39/40 low-dose, 20/20 medium-dose and 22/30 high-dose animals. The incidence of hepatocellular carcinomas (which were associated with cholangiocellular carcinomas in some animals) showed a dose-dependent increase in animals of each sex: males, 0/40 controls, 0/46 low-dose, 2/40

medium-dose and 35/36 high-dose; females, 0/20 controls, 0/40 low-dose, 1/20 medium-dose and 14/22 high-dose (Frayssinet & Lafarge-Frayssinet, 1990).

3.1.3 *Intratracheal administration*

Rat: Groups of six male rats [strain and age unspecified] were administered a mixture of aflatoxins (37.7% B_1, 56.4% G_1 and traces of B_2 and G_2) at doses of 0 or 300 μg suspended in 30 μl arachis oil intratracheally by intubation twice weekly for 30 weeks. The rats were then held without further treatment for up to 100 weeks. Three of the six treated animals developed squamous-cell carcinomas of the trachea, four of six developed hepatomas and one had a renal-cell adenoma and a carcinoma of the pylorus. No tumour was found in the vehicle controls (Dickens *et al.*, 1966).

3.1.4 *Subcutaneous administration*

Mouse: A group of 20 Tuck No. 1 strain mice [sex unspecified], weighing 20–25 g, were injected subcutaneously with 10 μg of a mixture of aflatoxins (37.7% B_1, 56.4% G_1 and traces of B_2 and G_2) suspended in arachis oil twice weekly for up to 65 weeks. Surviving animals which did not develop tumours were killed 106 weeks after the first injection. Fifteen of 17 animals which were alive when the first tumour appeared developed subcutaneous sarcomas at 23 and 76 weeks (Dickens & Jones, 1965).

Rat: Groups of six male rats [strain unspecified], weighing about 100 g, were injected subcutaneously with 0, 2, 10, 50 or 500 μg of a mixture of aflatoxins (37.7% B_1, 56.4% G_1 and traces of B_2 and G_2) suspended in arachis oil twice weekly for up to 65, 65, 60 and eight weeks, respectively. After injections at the highest dose level had ceased, the animals were kept under observation for a total of 30 weeks. A high incidence of subcutaneous sarcomas developed in all treated groups: 0 μg, 0/6; 2 μg, 5/6; 10 μg, 6/6; 50 μg, 6/6; 500 μg, 5/5. The time at which tumours first appeared was dose-dependent: 2 μg, 44 weeks; 10 μg, 24 weeks; 50 μg, 21 weeks; 500 μg, 20 weeks (Dickens & Jones, 1963, 1965).

3.1.5 *Intramuscular and oral administration*

Monkey: A male rhesus monkey was injected intramuscularly on five days a week with mixed aflatoxins (44% B_1, 44% G_1, 2% B_2 and G_2) at 50 μg for one month followed by 100 μg for 11 months, and then received them orally by gavage at 200 μg per day for 4.5 years. A hepatocellular carcinoma was found 2.5 years after the end of treatment (Gopalan *et al.*, 1972). A female rhesus monkey treated identically, except that the oral dose was 100 μg per day, developed a metastasizing intrahepatic bile-duct carcinoma 5.25 years after the end of treatment (Tilak, 1974).

3.1.6 *Pre- and postnatal exposure*

Rat: Six groups of 10 female Wistar rats [age unspecified] were fed a diet containing 25 or 50% groundnut meal containing 10 000 μg/kg aflatoxin B_1 and 200 μg/kg aflatoxin B_2 from day 10 of pregnancy to parturition, from day 1 to day 10 post-partum, or from day 10 of pregnancy to day 10 post-partum. Among 113 male and 95 female offspring, observed for up

to 36 months, one male exposed *in utero* from day 10 of pregnancy and one female exposed *via* the milk for 10 days post-partum developed cholangiocarcinomas, and two females exposed *in utero* from day 10 of pregnancy and *via* the milk for 10 days post-partum developed liver-cell carcinomas. No liver tumour was reported in 50 male or 50 female controls obtained from mothers fed 25–50% soya bean meal in the diet during pregnancy (Grice *et al.*, 1973).

3.2 Aflatoxin B_1

3.2.1 *Oral administration*

Mouse: Wogan (1969a) reported in a review a series of experiments in which aflatoxin B_1 was fed at a level of 1000 μg/kg of diet to three strains of mice (Swiss, C3HfB/HEN, C57Bl/6NB) [number and sex unspecified] for 70 weeks. No tumour was reported.

Rat: Groups of 25 male and 25 female Fischer rats, weighing 80 and 70 g, respectively, were fed a semi-synthetic diet containing 0, 15, 300 or 1000 μg/kg of diet aflatoxin B_1 (purity, > 99.5%) for 52 weeks or until tumours developed. Additional groups of 25 male and 25 female rats were fed 1000 μg/kg of diet aflatoxin B_1 for 14 days and then returned to control diet for the remainder of the experiment. The incidence of hepatocellular carcinomas was increased at all dose levels of aflatoxin B_1 in rats of each sex; in addition, time to tumour decreased with increasing dose: males, 0/25 controls, 12/12 low-dose after 68 weeks, 6/20 medium-dose between 35 and 52 weeks, 18/22 high-dose between 35 and 41 weeks; females, 0/25 controls, 13/13 low-dose after 80 weeks, 11/11 medium-dose between 60 and 70 weeks and 4/4 high-dose after 64 weeks. In addition to hepatocellular carcinomas, hepatocellular adenomas and/or preneoplastic liver lesions (transitional-cell foci) were frequently found. One male animal in the low-dose group had a colonic mucinous adenocarcinoma at 68 weeks. Administration of aflatoxin B_1 at 1000 μg/kg of diet for only 14 days resulted in very low incidences of hepatocellular carcinomas in animals of each sex at 82 weeks: males, 1/16; females, 1/13 (Wogan & Newberne, 1967).

Groups of 30 male and 30 female Fischer rats, weighing 80 and 70 g, respectively, were administered aflatoxin B_1 in dimethyl sulfoxide (DMSO) at doses of 0 or 80 μg/day orally by gavage for five days (total dose, 400 μg/rat). All treated male animals died within 14 days after the last dose; mortality at 35 weeks in treated females was 11/30. Among the 16 animals surviving to 82 weeks, two had hepatocellular adenomas and three had preneoplastic liver lesions (transitional-cell foci). When the total dose of aflatoxin B_1 (400 μg per rat) was given in 10 consecutive doses of 40 μg per day, no mortality attributable to acute toxicity occurred. Following this treatment, an increase in the incidence of hepatocellular carcinomas was observed in males (4/20) but not in females (0/20) that survived for 35 or 82 weeks; in addition, hepatocellular adenomas were found in 1/19 males and 6/17 females by 82 weeks. Preneoplastic lesions (transitional-cell foci) were observed after this treatment in animals of each sex. In addition, groups of 20 and 22 male rats received a single dose of 5 mg/kg bw aflatoxin B_1 (the LD_{50}). Of five animals that survived to 69 weeks, one developed a hepatocellular adenoma and three had preneoplastic lesions (transitional-cell foci). No tumour or preneoplastic liver lesion was found in 60 vehicle controls (Wogan & Newberne, 1967).

Groups of 10–15 male and 15 female MRC rats, eight to nine weeks old, were administered 0 or 20 μg/animal aflatoxin B_1 in the drinking-water (using dark bottles to avoid photolysis) on five nights a week for 10 weeks (total dose of aflatoxin B_1, 1000 μg/animal) or 20 weeks (total dose, 2 mg/animal) and were followed until they were moribund or dead. Survival at 90 weeks was 26/30 (both sexes combined) controls, 4/10 animals (males only) treated with aflatoxin B_1 for 10 weeks and 12/30 animals (both sexes combined) treated for 20 weeks. Aflatoxin B_1 produced liver neoplasms (predominantly hepatocellular tumours, two cholangiocarcinomas) in 8/15 males and 11/15 females treated for 20 weeks and in 3/10 males treated for 10 weeks. In addition, hyperplastic hepatic nodules and cystadenomas were frequently found in the livers of treated animals. A variety of neoplasms, including two renal-cell tumours, were observed in other organs of treated rats but not in untreated controls (Butler *et al.*, 1969).

Groups of 10–20 male Fischer rats [age unspecified] were administered 0, 25, 37.5 or 70 μg/animal aflatoxin B_1 (purified by chromatography and recrystallization) in 0.05 ml DMSO by gastric intubation four or five times a week for two to eight weeks (total doses of aflatoxin B_1, 0, 500, 630, 1000 and 1500 μg/animal) and were sacrificed between 25 and 78 weeks. A high incidence of hepatocellular carcinomas was found at all dose levels: vehicle control, 0/10 at 59 weeks; total dose of 500 μg, 7/7 at 74 weeks; 630 μg, 2/4 at 75 weeks; 1000 μg, 18/18 between 42 and 58 weeks; 1500 μg, 17/17 between 42 and 46 weeks. In addition, preneoplastic liver lesions (foci of hyperplasia and transitional cells) were often observed (Wogan *et al.*, 1971).

Groups of male Fischer rats [initial number unspecified], weighing approximately 80 g, were fed a semi-synthetic diet containing 0, 1, 5, 15, 50 or 100 μg/kg of diet aflatoxin B_1 (purity, > 99.5%) until clinical deterioration of animals was observed, at which time all survivors in that treatment group were killed. A dose- and time-related increase in the incidence of hepatocellular carcinomas and of preneoplastic liver lesions (foci of hyperplasia and transitional cells) was observed (Table 8) (Wogan *et al.*, 1974).

Table 8. Incidences of hyperplastic foci and hepatocellular carcinomas in rats fed aflatoxin B_1

Dietary aflatoxin B_1 (μg/kg of diet)	Time of earliest tumour (weeks)	Numbers of rats with	
		Hyperplastic foci	Hepatocellular carcinomas
0	–	1/18	0/18
1	104	7/22	2/22
5	93	5/22	1/22
15	96	13/21	4/21
50	82	15/25	20/25
100	54	12/28	28/28

From Wogan *et al.* (1974)

Groups of 16–26 male Wistar rats, weighing 150–200 g, were fed a diet containing aflatoxin B_1 (highly purified) at 0, 250 500 or 1000 μg/kg for 147 days and thereafter

maintained on basal diet until death. Survival beyond 100 days was 24/26 control, 13/16 low-dose, 18/18 medium-dose and 14/17 high-dose animals. An increased incidence of hepatocellular carcinomas (control, 0/24; low-dose, 8/13; medium-dose, 13/18; high-dose, 12/14) was seen, which were diagnosed after mean induction periods of 742, 622 and 611 days, respectively. In addition, hyperplastic liver nodules were often observed in animals treated with aflatoxin B_1 that survived 100 days. There was also an increased incidence of renal-cell tumours (control, 0/24; low-dose, 3/13; medium-dose, 5/18; high-dose, 8/14), which were diagnosed after mean induction periods of 783, 696, and 603 days, respectively. The renal-cell tumours showed papillary, tubular and acinar formations consisting of various cell types, including clear and granular cells. Basophilic hyperplastic tubules were seen in some kidneys (Epstein *et al.*, 1969).

Groups of 18 and 36 male Wistar R rats, weighing 80–120 g, were administered 0 and 50 μg/animal aflatoxin B_1 in 0.1 ml DMSO by gastric intubation twice a week for four weeks and then 0 and 75 μg/animal aflatoxin B_1 in 0.15 ml DMSO twice a week for 10 weeks. Groups of one to three animals were killed at various times between three and 86 weeks after the beginning of treatment. Administration of aflatoxin B_1 induced hepatocellular and hepatocholangiocellular carcinomas in 70% of rats [number unspecified] that received a total dose of 1900 μg aflatoxin B_1, the first carcinoma appearing after 44 weeks. Foci of altered hepatocytes (clear, acidophilic and hyperbasophilic cell GGT^+ foci) appeared after 15 weeks and increased in number and size with time, to form hyperplastic nodules (Kalengayi *et al.*, 1975).

Groups of about 30 male Charles River rats, 63 days old, were fed aflatoxin B_1 at 0 or 1000 μg/kg of diet for 15 weeks, and unspecified numbers were killed 8 and 16 weeks after beginning of exposure. The 16 treated and 16 control surviving animals were kept on control diet until week 88. Small foci of vacuolated hepatocytes were observed in treated animals for the first time after 16 weeks, and hepatocellular carcinomas after 68 weeks; the cumulative incidence of hepatocellular carcinomas reached 40% at 88 weeks (Nishizumi *et al.*, 1977).

Groups of 25 male and 25 female Wistar rats, seven weeks old, were administered 0, 100 (males) or 75 (females) μg/animal aflatoxin B_1 in DMSO intragastrically twice a week for five weeks, followed by 0, 20 (males) or 15 (females) μg/animal aflatoxin B_1 in DMSO twice a week for 10 weeks. Treated rats died or were killed between 184 and 486 days after cessation of aflatoxin B_1 treatment, and vehicle controls were killed between 250 and 500 days. Malignant hepatomas, sometimes accompanied by cholangiocellular adenomas, appeared for the first time after 386 days in males and after 417 days in females treated with aflatoxin B_1. The incidences of malignant hepatomas at those times were 8/8 males and 5/8 females. Benign hepatomas appeared for the first time after 265 days in males and 295 days in females, and their incidences at those times were 14/22 males and 10/26 females. Precancerous focal lesions (designated as hyperplastic) were observed in all treated rats starting from 184 days after the cessation of aflatoxin B_1 administration. No neoplastic or preneoplastic lesion was observed in vehicle controls. The progress of hepatocarcinogenesis induced by aflatoxin B_1 was accompanied by an increased level of porphyrins in liver and faeces and by an increased concentration of GGT in liver, especially in hepatomas, and in serum (Zawirska & Bednarz, 1981).

Groups of 66 and 120 female Wistar rats, weighing about 200 g, were administered a single dose of 0 or 5000 μg/kg bw aflatoxin B_1 in olive oil by gavage. Of the treated rats, 29 died in the first few days and eight between 52 and 104 weeks after dosing. Groups of five aflatoxin B_1-treated rats and two to five untreated control rats were killed at two days and at various times between one and 104 weeks after the beginning of the experiment. Thirteen additional aflatoxin B_1-treated rats which had survived 52 weeks were killed when moribund. Hepatocellular adenomas (designated as neoplastic nodules) were found in 10/26 animals that survived 78 weeks but not in controls. Foci of altered hepatocytes (predominantly tigroid-cell foci) were detected for the first time eight weeks after administration of aflatoxin B_1; these increased in number and size up to 104 weeks. Rarely, foci of altered hepatocytes were also observed in controls that survived 52 weeks (Bannasch *et al.*, 1985).

Groups of 15 and 30 male Wistar rats, weighing 100 g, were administered 0 or 50 μg/animal aflatoxin B_1 in 0.1 ml DMSO by gastric intubation twice a week for four weeks and then 0 and 75 μg/animal in 0.15 ml DMSO for 10 weeks [number of times per week unspecified]. Randomly selected animals were sacrificed 4–78 weeks after the beginning of treatment. Preneoplastic hepatic foci (clear, mixed, diffusely basophilic and tigroid-cell foci) appeared in the livers of aflatoxin B_1-treated rats from 22 weeks onward but were not observed in untreated controls (Gil *et al.*, 1988).

Groups of 56 male Fischer 344/CDF rats, six weeks old, were exposed [route not clearly specified but probably gavage] to 0 or 25 μg/animal aflatoxin B_1 in 0.1 ml DMSO five times a week for eight weeks, or to 0 or 70 μg/animal in 0.15 ml DMSO nine times in two weeks. Six animals per group were sacrificed 2, 6, 10, 14, 22, 32 and 47 weeks after the last treatment with aflatoxin B_1, and all surviving rats were killed after 66 weeks. After treatment with 25 μg/animal aflatoxin B_1, hepatocellular carcinomas were observed in 1/6 rats after 47 weeks and in 3/10 rats after 66 weeks; neoplastic hepatic nodules were found in 3/6 rats after 32, 3/6 rats after 47 and in 6/10 rats after 66 weeks; preneoplastic hepatic foci (as defined by glycogen storage, cytoplasmic basophilia or GGT^+ staining) were found for the first time two weeks after the last exposure to aflatoxin B_1, and their number and size increased with time. In rats exposed to 70 μg/animal aflatoxin B_1 for two weeks only, hepatocellular carcinomas occurred in 1/13 and neoplastic hepatic nodules in 3/13 rats after 66 weeks but not earlier; preneoplastic hepatic foci were first observed 6–14 weeks after the last administration of aflatoxin B_1 and increased in number and size with time. No neoplastic or preneoplastic hepatic lesion was observed in vehicle controls (Soffritti & McConnell, 1988).

Hamster: Male Syrian golden hamsters [initial number not specified], four weeks old, were given 2000 μg/kg bw aflatoxin B_1 (dissolved in DMSO and diluted with an equal volume of trioctanoin) by gavage on five days a week for six consecutive weeks. Starting 24 h after the last dose of aflatoxin B_1, some animals [number unspecified] were given 0.1% phenobarbital in the drinking-water. Control animals received only the vehicle (2 ml/kg bw DMSO–trioctanoin per day). At least six hamsters per group were killed at 6, 14 and 46 weeks, and groups of 30 hamsters were observed for tumour development; the 15 surviving animals were killed at 78 weeks. The hamsters killed at or after 23 weeks included those given phenobarbital in the drinking-water and groups given only tap-water. In hamsters that were treated with aflatoxin B_1 and were killed at or survived to 46 weeks, a high incidence of

cholangiocellular tumours was observed: cholangiocarcinomas in 8/30 hamsters given aflatoxin B_1 and phenobarbital and in 9/33 without phenobarbital, and cystic cholangiomas in 19/30 given aflatoxin B_1 and phenobarbital and 21/33 without phenobarbital. Focal bile-duct proliferation and foci of altered hepatocytes were also frequently observed in aflatoxin-treated hamsters. Hepatocellular carcinomas were found in two aflatoxin-treated hamsters killed at 78 weeks, one of which had been given phenobarbital. No notable change was seen in control livers (Moore *et al.*, 1982).

Monkey: A total of 47 monkeys (rhesus, cynomolgus and African green) [sex and age unspecified] received aflatoxin B_1 dissolved in > 0.2 ml/kg DMSO by intraperitoneal injection at 125–250 μg/kg bw and/or orally at 100–800 μg/kg bw for two months or longer. Thirteen of 35 necropsied monkeys had developed one or more malignant neoplasms: two hepatocellular carcinomas, three liver angiosarcomas, two osteogenic sarcomas, six adenocarcinomas of the gall-bladder or bile duct, two pancreatic adenocarcinomas, one undifferentiated pancreatic tumour and one papillary grade-I carcinoma of the urinary bladder. The tumours developed at an average total dose of 709 mg (range, 99–1354 mg) aflatoxin B_1 after an average period of 114 months (range, 47–147 months). Fifteen of the 22 necropsied monkeys without tumours showed histological evidence of liver damage, including toxic hepatitis, cirrhosis and hyperplastic nodules. These animals had received an average total dose of 363 mg (range, 0.35–1368 mg) for an average of 55 months (range, 2–141 months). No liver tumour, but one gall-bladder carcinoma and three malignant lymphomas, were found in 68 necropsied, untreated breeder and vehicle-treated (0.2 ml/kg DMSO) monkeys which had died in the colony during the observation period (Sieber *et al.*, 1979).

Tree shrew: Groups of 10 female and 8 male tree shrews (*Tupaia glis*), weighing 95–140 g, were intermittently given aflatoxin B_1 at a dietary concentration of 2000 μg/kg. Groups of three female and five male animals were fed control diet. The experiment was terminated after 172 weeks. Of 12 animals that survived, 6/6 female and 3/6 male tree shrews developed hepatocellular carcinomas after 74–172 weeks of treatment (total dose of aflatoxin B_1, 24–66 mg). None of the eight control animals developed liver cancers. In two tree shrews, the liver tumours were associated with severe post-necrotic scarring; in the other seven tumour-bearing livers, only mild to moderate portal fibrosis was seen (Reddy *et al.*, 1976).

Guppy: Groups of 50 male and female guppies (*Lebistes reticularis*), four weeks old, were fed 0 or 6000 μg/kg of diet aflatoxin B_1 and were killed at intervals of 2–4 months between 4 and 13 months or were followed until death. Aflatoxin B_1 induced hepatic tumours in 9/16 fish between nine and 11 months. No liver tumour was observed in five untreated fish that survived nine months (Sato *et al.*, 1973).

Salmon and trout: Groups of hatched coho salmon and rainbow trout [initial numbers unspecified] were fed aflatoxin B_1 at 0, 20, 40 or 5000 μg/kg of diet for four (20 and 40 μg) or three (5000 μg) weeks and were sacrificed at 12 months of age. The incidence of liver lesions was much lower in salmon (control, 0/118; 40 μg aflatoxin B_1, 0/116; 5000 μg aflatoxin B_1, 9/176—one basophilic focus and eight hepatic adenomas) than in rainbow trout (control, 1/121; 20 μg aflatoxin B_1, 74/120—predominantly hepatocellular carcinomas). Higher doses were lethal to the trout (Bailey *et al.*, 1988).

Trout: Groups of rainbow trout [number and age unspecified] were fed aflatoxin B_1 at 0 or 8 μg/kg of diet for up to 12 months, when the experiment was terminated. A total of

95 liver lesions, ranging from small preneoplastic foci to hepatocellular, cholangiocellular and mixed neoplasms, were observed in treated trout (Kirby *et al.*, 1990).

3.2.2 *Subcutaneous administration*

Rat: Groups of six male rats [strain unspecified], weighing about 100 g, were injected subcutaneously with 0 or 20 μg aflatoxin B_1 suspended in 0.5 ml arachis oil twice weekly for up to 65 weeks. Subcutaneous sarcomas were found in 6/6 treated rats within 18–37 weeks and in 0/6 controls (Dickens & Jones, 1965).

Groups of male Fischer rats [initial number and age not clearly specified] were administered 0 or 10 μg/rat aflatoxin B_1 suspended in 0.2 ml trioctanoin subcutaneously twice weekly for 20 weeks (total dose of aflatoxin B_1, 400 μg/rat). Subcutaneous sarcomas were found by 58 weeks at the injection site in 9/9 treated rats but not in vehicle controls (Wogan *et al.*, 1971).

3.2.3 *Intraperitoneal administration*

Mouse: Groups of 16 female strain A/He mice, two months old, were administered 0 or 2000 μg/kg bw aflatoxin B_1 (purity, > 99.5%) in 0.1 ml DMSO intraperitoneally three times a week for four weeks (total average dose of aflatoxin B_1, 5600 μg/animal). A group of 16 females served as untreated controls. The experiment was terminated at 24 weeks after the start of treatment. Survival was similar in all three groups: untreated controls, 14/16; vehicle control, 15/16; aflatoxin B_1, 14/16. Aflatoxin B_1 produced an average of 5.6 pulmonary adenomas/mouse in 14/14 animals. No tumour occurred in untreated controls. Of the vehicle controls, 4/15 had lung adenomas, with an average of 0.3 tumours/mouse (Wieder *et al.*, 1968).

Groups of eight male and eight female strain A/J mice, six to eight weeks old, were administered 5000, 12 500 or 25 000 μg/kg bw aflatoxin B_1 in 0.1 ml DMSO intraperitoneally once a week for six weeks. A group of 16 male and 16 female DMSO controls was available, and groups of 136 males and 131 females served as untreated controls. The experiment was terminated 24 weeks after the start of treatment, at which time survival was 135/136 male and 131/131 female untreated controls; survival was reduced in vehicle controls and in animals treated with aflatoxin B_1 at the highest dose level: males, 12/16 vehicle controls, 9/9 low-dose, 8/8 medium-dose, 5/8 high-dose; females, 14/16 vehicle controls, 7/7 low-dose, 8/8 medium-dose and 5/8 high-dose animals. Treatment with aflatoxin B_1 produced lung adenomas in 100% of the surviving animals at all three dose levels, and the average number of lung adenomas per mouse was dose-related: males, low-dose, 6.56; medium-dose, 15.75; high-dose, 20.20; females, low-dose, 11.57; medium-dose, 16.13; high-dose, 28.80. The proportion of untreated controls with lung tumours was 38% in males and 25% in females, and that in vehicle controls was 17% in males and 50% in females. The average number of lung tumours in untreated and vehicle controls of each sex ranged between 0.29 and 0.57 (Stoner *et al.*, 1986).

Groups of newborn male and female inbred (C57Bl × C3H)F_1 mice [initial numbers unspecified], one to seven days old, were administered 0, 250, 1000, 2000 or 6000 μg/kg bw aflatoxin B_1 in 10 ml/kg bw trioctanoin intraperitoneally once, three or five times at

three-day intervals between one and 16 days after birth (total doses of aflatoxin B_1, 1250, 2000, 3000 or 6000 μg/kg bw. Of the mice treated at day 1 with a single dose of 2000 μg/kg bw aflatoxin B_1, 55% died within four days. Seventy animals [sex unspecified] selected at random (10–20 per group) were sacrificed at 52 weeks, and 105 animals (16–29 per group) at 82 weeks of age. An increased incidence of hepatomas (total, 31/70) was found at 52 weeks in all aflatoxin B_1-treated groups except that given a single dose of 2000 μg/kg bw. No hepatoma was observed in 100 vehicle controls at that time. By 82 weeks, all aflatoxin-treated groups, including the group that received a total dose of 1250 μg/kg bw, showed increased incidences of hepatomas (total, 82/105). The incidence of hepatomas in the control group at 82 weeks was 3/100 (Vesselinovitch *et al.*, 1972).

Transgenic mouse: Groups of 10 female transgenic mice of C57Bl/6 background, which overexpress the hepatitis B virus large envelope polypeptide, two to three months of age, and 9–10 female, age-matched nontransgenic littermates were given intraperitoneal injections of aflatoxin B_1 in tricaprylin at doses of 0 or 250 μg/kg bw as a single dose or five doses at approximately monthly intervals or 2000 μg/kg bw in three weekly doses. At 15 months, when the experiment was terminated, seven to nine animals in each group were still alive. After three doses of 2000 μg/kg bw, two hepatocellular carcinomas and 10 hepatocellular adenomas were found in transgenic mice. At that time, four hepatocellular adenomas and no carcinoma were observed in transgenic mice that received 250 μg/kg bw as five doses and six adenomas but no carcinoma in those that received 250 μg/kg bw as a single dose. No liver neoplasm was described in nontransgenic mice with or without aflatoxin B_1 treatment; however, multiple liver nodules of different sizes (which were not regarded as neoplasms) were observed in control transgenic mice, and four such nodules of < 2 mm per group were seen in nontransgenic mice exposed to aflatoxin B_1 (Sell *et al.*, 1991).

Rat: Groups of male Fischer rats [initial number and age not clearly specified] were administered 0 or 32.5 μg aflatoxin B_1 in 0.05 ml DMSO intraperitoneally five times a week for eight weeks (total dose of aflatoxin B_1, 1300 μg/animal). By 46 weeks, the incidence of hepatocellular carcinomas was 9/9 in rats treated with aflatoxin B_1 and 0/10 in vehicle controls (Wogan *et al.*, 1971).

Toad: Groups of 50 male and 50 female Egyptian toads (*Bufo regularis*) [age unspecified] were injected into the dorsal lymph sac with 0 or 200 μg/kg bw aflatoxin B_1 in 1 ml of commercial corn oil once a week for 15 weeks. Experimental and control animals were autopsied at various times between two and 15 weeks; four males and seven females died spontaneously but were also autopsied. Hepatocellular carcinomas were found in 12 male and seven female treated toads. Tumours observed in the kidneys of three males and one female were diagnosed by the authors as metastases of the hepatic tumours. No tumour was detected in the controls (El-Mofty & Sakr, 1988).

3.2.4 *Intraperitoneal administration after partial hepatectomy*

Mouse: Two groups of 16 male $STCF_1$ mice, nine weeks of age, were administered 0 or 6000 μg/kg bw aflatoxin B_1 in 10 ml/kg bw tricaprylin intraperitoneally 46 h after a two-thirds partial hepatectomy. A group of 24 mice received the same single dose of aflatoxin B_1 without partial hepatectomy, and 38 intact mice served as untreated controls. The experiment was terminated at 104 weeks. At that time, survival was 13/38 in intact, untreated

controls, 4/16 in controls that had a partial hepatectomy, 8/24 in the group treated with aflatoxin B_1 without partial hepatectomy and 4/16 in those given aflatoxin B_1 and a partial hepatectomy. In mice exposed to aflatoxin B_1, hepatocellular carcinomas were observed in 9/16 animals with and in 0/24 animals without preceding partial hepatectomy. In untreated controls, one hepatocellular carcinoma each was observed in the groups with and without partial hepatectomy (in animals that died at 98 and 103 weeks, respectively). In the mice that received aflatoxin B_1 after partial hepatectomy, the first hepatocellular carcinoma was observed after 55 weeks. Hepatocellular adenomas were observed in 4/38 controls without partial hepatectomy, in 3/16 controls with partial hepatectomy, in 8/16 mice exposed to aflatoxin B_1 with preceding partial hepatectomy and in 2/24 treated mice without partial hepatectomy. Statistical analyses revealed a significantly increased risk for the development of hepatocellular carcinomas ($p < 0.001$) and hepatocellular adenomas ($p < 0.01$) in mice exposed to aflatoxin B_1 with but not without partial hepatectomy. There was no significant increase in risk for lung tumour development in mice following a single exposure to aflatoxin B_1 (Dix, 1984).

Rat: Groups of 10–15 male Fischer 344 rats, six to eight weeks old, were administered a single intraperitoneal dose of 0, 75 or 250 μg/kg bw aflatoxin B_1 in DMSO 6, 24 and 31 h after partial hepatectomy, sham operation or no operation. Survival of rats that received the high dose of aflatoxin B_1 after partial hepatectomy was markedly reduced at 62 weeks: untreated control, 10/12; high-dose aflatoxin B_1 without prior operation, 12/12; high-dose 24 h after sham operation, 8/10; high-dose 6 h after partial hepatectomy, 2/12; high-dose 24 h after partial hepatectomy, 0/12; high-dose 31 h after partial hepatectomy, 2/12. All surviving animals were killed at 62 weeks. No hepatocellular neoplasm was found, but there was an increased incidence of preneoplastic foci of altered hepatocytes (basophilic and clear-cell foci) at both dose levels of aflatoxin B_1 following partial hepatectomy, and an increased incidence of clear-cell foci after the high dose of aflatoxin B_1 following sham operation (Neal & Cabral, 1980).

Groups of 20–40 male inbred AS_2 rats, weighing 120–150 g, were administered a single intraperitoneal dose of 250 μg/kg bw aflatoxin B_1 in DMSO 24 h after two-thirds partial hepatectomy or sham operation. At 40 weeks, 11/40 hepatectomized and 12/20 non-hepatectomized rats were still alive; those that had undergone hepatectomy [number unspecified], but not non-hepatectomized animals, had preneoplastic hepatic foci consisting of clear, vacuolated, acidophilic or basophilic cells. A high incidence of hepatocellular carcinomas (9/10) and neoplastic hepatic nodules (10/10) was found in hepatectomized rats 55–65 weeks after aflatoxin B_1 administration; none of the 10 non-hepatectomized rats killed at that time had hepatocellular carcinomas or neoplastic hepatic nodules, although there were small preneoplastic hepatic foci. One hepatectomized and 1/2 non-hepatectomized rats followed until 90 weeks after administration of aflatoxin B_1 had both hepatocellular carcinomas and neoplastic hepatic nodules (Rizvi *et al.*, 1989).

3.2.5 *Pre- and postnatal exposure*

Rat: Female Fischer 344 rats were fed aflatoxin B_1 at 2000 μg/kg of diet throughout pregnancy and lactation, and then 26 males and 21 females of their weaned offspring were fed the same diet until they died. An additional six male and 19 female weaned offspring of

untreated Fischer 344 rats were fed aflatoxin B_1 at the same dose from six to seven weeks of age until death. A control group of male and female offspring of untreated Fischer 344 rats [numbers unspecified] remained untreated until death or were killed at 75 weeks, at which time the majority [numbers unspecified] had survived. Mean survival of groups exposed to aflatoxin B_1 both *in utero* and postnatally (males, 45.6 weeks of age; females, 61.6) and groups exclusively exposed postnatally (males, 50.2 weeks of age; females, 63.1) did not differ significantly ($p > 0.1$), but mean survival was significantly ($p < 0.01$) shortened in males of both treatment groups as compared to females. More than 75% [detailed data not given] of animals in both treatment groups [groups exposed *in utero* and postnatally and exclusively postnatally were not separated; sex unspecified] developed malignant liver neoplasms (haemangiosarcomas, hepatocellular carcinomas), which were the main cause of death. The numbers of rats still alive after the first malignant liver neoplasms were detected in males (34 weeks) and females (49 weeks) were 20/26 males and 14/21 females exposed to aflatoxin B_1 from conception and 5/6 males and 14/19 females fed aflatoxin B_1 from six to seven weeks of age. In addition to malignant liver neoplasms, hyperplastic hepatic nodules and two types of areas of hyperplasia (consisting either of small dark-staining or large pale-staining liver cells) were found frequently. An increased incidence of colonic tumours (predominantly small polypoid tumours without metastases, one adenocarcinoma) was observed in rats of each sex which had been exposed to aflatoxin B_1 from conception (males 2/20; females, 5/14) and in rats of each sex that had been fed aflatoxin B_1 from weeks six to seven of age (males, 2/5; females, 3/14). A few treated rats had tumours at other sites, including two renal-cell tumours. No tumour was reported in controls (Ward *et al.*, 1975). [The Working Group noted the limited reporting of the data.]

Groups of 15 female Sprague-Dawley rats, 12 weeks old, were administered 500 μg/kg bw aflatoxin B_1 intraperitoneally from days 15 to 18 or 18 to 21 of pregnancy, and then 872 offspring of each sex received additional intraperitoneal injections of 0 or 500 μg/kg bw on days 2–5 or 14–17 after birth. A control group of 10 pregnant rats received daily intraperitoneal injections of saline on days 15–21 of pregnancy, and their neonatal offspring were given intraperitoneal injections of saline on days 2–5 or 14–17 after birth. The mean survival time of dams was 834 days in controls, 791 days in those treated with aflatoxin B_1 on days 15–18 and 792 days in those treated on days 18–21 of pregnancy. Of the offspring treated with aflatoxin B_1, 483/717 treated and 113/113 control animals survived the lactation period; the median survival times were 834 in male and 839 days in female controls and 728–844 days in male and 744–842 days in female animals treated with aflatoxin B_1. In dams treated with aflatoxin B_1, increased incidences of benign and malignant neoplasms (other than leukaemia) were observed in various organs: benign tumours, liver and gastrointestinal tract, 34/90; endocrine organs, 49/90; mammary gland, 30/90; other organs, 11/90; malignant tumours, liver and gastrointestinal tract, 6/90; endocrine organs, 6/90; mammary gland, 5/90; other organs, 10/90. No malignant neoplasm was detected in controls; the incidences of benign tumours in controls were 0/10 in liver and gastrointestinal tract, 3/10 in endocrine organs, 6/10 in mammary gland and 0/10 in other organs. In offspring that had been exposed to aflatoxin B_1 *in utero* (with and without additional aflatoxin B_1 treatment after birth), a significant increase was seen in the total number of malignant tumours, except for mammary gland tumours and leukaemias. The location and number of benign and malignant tumours

in offspring that had been exposed to aflatoxin B_1 *in utero* (with and without additional aflatoxin B_1 treatment after birth) are given in Table 9. Significant differences in the incidences of benign tumours between control and treated offspring were found in the trachea (control, 1/113; treated, 17/483), Harderian gland (control, 2/113; treated, 27/483) and liver (control, 14/113; treated, 207/483). Benign and malignant tumours were also observed in the central and peripheral nervous systems of offspring exposed to aflatoxin B_1 but not in controls; prenatal exposure alone was sufficient to produce these tumours (Goerttler *et al.*, 1980).

Trout: Groups of 200 rainbow trout eggs, 14 days after fertilization, were exposed to 0 or 500 μg/l aflatoxin B_1 in water for 1 or 2 h. After birth, the trout were fed a semipurified diet for up to 12 months, when the experiment was terminated. Aflatoxin exposure for 1 h resulted in hepatocellular carcinomas in 42/100 fish, and exposure for 2 h resulted in hepatocellular carcinomas in 45/100 fish at 12 months. No control fish kept in well water developed a hepatocellular carcinoma (Wales *et al.*, 1978).

In another experiment, groups of 200 rainbow trout eggs were exposed to aflatoxin B_1 at 1000 μg/l of water for 1 h/day on either day 1, 3, 4, 7, 9, 11, 13, 15, 17, 19, 21 or 23 after fertilization. A control group was not exposed to aflatoxin B_1. Random samples of fish were taken at 10 and 12 months. The incidence of hepatocellular carcinomas increased with increasing age of the embryo at the time of exposure to aflatoxin B_1 and was 20/35 at 10 months and 35/60 at 12 months in fish exposed on day 23 after fertilization. None of the control fish developed tumours. The earliest indication of neoplastic development was occasional foci of basophilic cells in the livers of fish at four months, and the first hepatocellular carcinomas were seen at six months after birth (Wales *et al.*, 1978).

Salmon and trout: Ninety-nine coho salmon eggs, 20 days before hatching, were administered 90 ng/egg (400 μg/kg) aflatoxin B_1 in DMSO by microinjection, and hatched fish were killed eight months after exposure to aflatoxin B_1. A further 199 rainbow trout eggs, < 3 days before hatching, were injected similarly with 100 ng/egg (1400 μg/kg), and hatched fish were killed nine months after exposure. The mortality of trout eggs was: uninjected control, 13/199; vehicle control, 78/200; and 100 ng aflatoxin B_1, 145/199; and that of salmon eggs was: uninjected control, 14/117; vehicle control, 107/153; and 90 ng aflatoxin B_1, 53/99. Hepatocellular neoplasms (carcinomas and neoplastic nodules combined) occurred in 5/20 rainbow trout and 3/18 coho salmon exposed as embryos to aflatoxin B_1 and in none of 48 rainbow trout or 21 coho salmon exposed as embryos to the vehicle only (Black *et al.*, 1985).

Salmon: Groups of 300 coho salmon eggs were administered 50 or 100 ng/egg (217 or 434 μg/kg) aflatoxin B_1 in 1 μl DMSO by microinjection, and all surviving hatched fish were killed 14 months later. None of the fish that had been exposed as embryos to the high dose of aflatoxin B_1 survived to the end of the experiment; the effective numbers at 14 months were 38 in the low-dose group and 64 vehicle controls. Neoplastic hepatic nodules were found in 4/38 and focal bile-duct proliferation in 16/38 fish at the low dose; no liver lesion was observed in vehicle controls (Black *et al.*, 1988).

Table 9. Location and numbers of malignant and benign tumours in offspring treated with aflatoxin B_1 *in utero* and in control rats

Group	Sex	No.	Liver, stomach and intestines		Endocrine organs		Other organs		Mammary gland		Leukaemias (malignant)
			Benign[a]	Malignant[b]	Benign[c]	Malignant[d]	Benign[e]	Malignant[f]	Benign	Malignant	
Treated	M	228	68	16	143	12	48	49	13	–	22
	F	255	129	21	198	15	34	29	134	14	19
Control	M	53	–	–	18	1	–	–	3	–	2
	F	60	–	–	41	–	1	–	30	4	3

From Goerttler *et al.* (1980)

[a]Mainly hepatocellular and cholangiocellular adenomas, some papillomas and adenomas of the stomach and intestines

[b]21 tumours in liver; 16 in stomach and intestines

[c]Pituitary gland, adrenal glands, ovaries, testes, thyroid gland, parathyroid glands and endocrine pancreas: treated, 170 pituitary gland tumours (129 F, 41 M), 82 adrenal gland tumours (22 F, 60 M), 65 thyroid gland tumours (39 F, 26 M) and 24 others; control, 39 pituitary gland tumours (31 F, 8 M), 10 adrenal gland tumours (4 F, 6M), nine thyroid gland tumours (6 F, 3M) and two others

[d]Treated, 11 tumours of the adrenal gland, six tumours of the thyroid gland, six tumours of the pituitary gland and four others; control, one papilloma of the thyroid gland

[e]Treated, 30 central and peripheral nervous system tumours, 21 skin tumours, nine urogenital tract tumours (except ovaries and testes), four skeletal tumours, three nasal cavity tumours and 15 others; control, one adenoma of the exocrine pancreas

[f]16 skin tumours (3 F, 13 M), 11 skeletal tumours (3 F, 8 M), 12 tumours of uterus or vagina, 14 central or peripheral nervous system tumours (5 F, 9 M), eight nasal cavity tumours (2 F, 6 M) and 17 others

3.3 Carcinogenicity of metabolites

3.3.1 *Aflatoxin M_1*

Oral administration

Rat: Groups of 10–30 male weanling Fischer rats were administered 0 or 25 μg/rat of synthetic aflatoxin M_1 (purity, > 95%) or purified aflatoxin B_1 suspended in 0.5 ml distilled water by gastric intubation on five days a week for eight consecutive weeks and held thereafter without treatment. Only 1/29 animals treated with aflatoxin M_1 had developed a hepatocellular carcinoma by 96 weeks. The remaining animals in that group were killed after 100 weeks and, of these, eight had preneoplastic liver lesions (hyperplasia or transitional cells). Nine of nine rats treated with aflatoxin B_1 developed hepatocellular carcinomas between 47 and 53 weeks. No liver tumour was observed in untreated controls (Wogan & Paglialunga, 1974).

Groups of 42 and 62–63 male Fischer rats, seven weeks old, were fed 50 μg/kg of diet aflatoxin B_1 and 0, 0.5, 5 or 50 μg/kg of diet of naturally occurring aflatoxin M_1 for up to 21 months. Serial sacrifices were carried out between three and 21 months. Liver neoplasms were found only after 16 months in rats administered aflatoxin B_1 and aflatoxin M_1 at dose levels of 50 μg/kg of diet and in one control animal. The incidence of liver tumours (diameter > 2 mm; hepatocellular carcinomas and neoplastic nodules combined) was lower after administration of aflatoxin M_1 (16 months, 1/6; 17 months, 0/6; 19 months, 2/19; 21 months, 6/18) than after treatment with aflatoxin B_1 (16 months, 9/9; 17 months, 19/20; no surviving animals later). After administration of aflatoxin M_1, 2/6 tumours found after 21 months were hepatocellular carcinomas; in contrast, in animals treated with aflatoxin B_1 and killed after 16 and 17 months all liver tumours (28/29) were hepatocellular carcinomas (Hsieh *et al.*, 1984).

Groups of 42 and 62 male Fischer 344 rats, seven weeks of age, were fed a semisynthetic diet containing 0, 0.5, 5 or 50 μg/kg of naturally occurring aflatoxin M_1 or 50 μg/kg of diet aflatoxin B_1 up to 21 months of age. Animals were killed at different times between 3 and 22 months. After 21 months, the incidence of hepatocellular carcinomas induced by the high dose of aflatoxin M_1 was 2/18, which was significantly ($p < 0.01$) lower that that (19/20) in rats administered aflatoxin B_1 at the same dose level for 17 months. As in rats administered aflatoxin B_1, the induction of hepatocellular carcinomas by aflatoxin M_1 was preceded by the appearance of foci of altered hepatocytes (predominantly eosinophilic-cell foci) and neoplastic hepatic nodules (composed of basophilic, eosinophilic and clear cells). The number and size of foci of altered hepatocytes induced by the high dose of aflatoxin M_1 were significantly ($p < 0.05$) less than those produced by the same dose of aflatoxin B_1. Intestinal adenocarcinomas were found in three animals treated with the high dose of aflatoxin M_1 after 9 and 18 months, but were not observed in any other group. No hepatocellular carcinoma or neoplastic hepatic nodule (but a very low incidence of foci of altered hepatocytes at 21 months) was found in rats that received the lower doses of aflatoxin M_1 or in controls (Cullen *et al.*, 1987).

Trout: Groups of 250 rainbow trout, four months old, were fed diets containing 0, 5.9 or 27.3 μg/kg of naturally occurring aflatoxin M_1 (the diet also contained aflatoxin M_2) or 5.8 μg/kg of diet aflatoxin B_1 for 16 months. Groups of fish were killed 5, 9, 12 and 16 months

after the beginning of treatment. The hepatocarcinogenic effect of aflatoxins M_1 and M_2 was much weaker than that of aflatoxin B_1, the incidence of hepatocellular carcinomas at 16 months being 0/49 low-dose aflatoxins M_1 and M_2-treated animals, 1/48 high-dose aflatoxins M_1 and M_2-treated animals and 6/48 aflatoxin B_1-treated animals. A similar trend was observed in the incidence of hyperplastic (eosinophilic, basophilic and mixed cell) nodules (Canton *et al.*, 1975).

3.3.2 *Aflatoxin Q_1*

Oral administration

Trout: Two duplicate groups of 80 rainbow trout, four months old, were fed diets containing 0 or 100 μg/kg aflatoxin Q_1 (produced by incubation of aflatoxin B_1 with monkey liver microsomes) for 10 months or 4 μg/kg of diet aflatoxin B_1 for 12 months. Groups of fish were taken at six and nine months, and the remaining animals were killed at 12 months. Aflatoxin Q_1 induced hepatocellular carcinomas in 12/113 fish by 12 months; aflatoxin B_1 at a dose of 4 μg/kg of diet induced hepatocellular carcinomas in 55/115 fish by 12 months. No hepatocellular tumour was observed in untreated controls (Hendricks *et al.*, 1980a).

3.3.3 *Aflatoxicol*

Oral administration

Rat: Groups of 20 male Fischer 344 rats, four weeks of age, were fed diets containing 0, 50 or 200 μg/kg aflatoxicol or 50 μg/kg aflatoxin B_1 for 12 months and were observed up to 24 months, when the experiment was terminated. Survival at 24 months was 11/20 controls, 9/20 aflatoxin B_1-treated rats, 5/20 low-dose aflatoxicol-treated animals and 0/20 high-dose aflatoxicol-treated animals. The incidence of hepatocellular carcinomas in animals treated with aflatoxicol was about one-half of that in rats treated with aflatoxin B_1 at the same dose level: controls, 0/20; aflatoxin B_1, 8/20; low-dose aflatoxicol, 4/20; high-dose aflatoxicol, 14/20. The incidence of neoplastic hepatic nodules showed a similar trend: controls, 0/20; aflatoxin B_1, 7/20; low-dose aflatoxicol, 4/20; high-dose aflatoxicol, 2/20 (Nixon *et al.*, 1981). [The Working Group noted the poor survival of animals treated with aflatoxicol.]

Trout: Two duplicate groups of 120 rainbow trout fingerlings [sex unspecified] were fed a semi-synthetic diet alone or containing (per kg of diet) 20 μg aflatoxin B_1, 29 μg aflatoxicol or 61 μg of the diastereomer of aflatoxicol. Groups of 40 trout were selected randomly and killed at four, eight and 12 months. The incidences of hepatocellular carcinomas were 0/80 controls, 45/80 aflatoxin B_1-treated, 20/80 aflatoxicol-treated and 0/80 animals treated with the diastereomer at eight months after initiation of the experiment, and 0/76 controls, 62/75 aflatoxin B_1-treated, 46/57 aflatoxicol-treated and 17/70 diastereomer-treated animals 12 months after beginning of treatment (Schoenhard *et al.*, 1981).

3.4 Aflatoxin B_2

3.4.1 *Oral administration*

Rat: Groups of 30 and 10 male MRC rats, eight to nine weeks old, were administered 0 or 20 μg/animal aflatoxin B_2 in the drinking-water (using dark bottles to avoid photolysis) on

five nights a week for 10 weeks (total dose of aflatoxin B_2, 1 mg/animal). Eight treated and 26 control rats were still alive at week 90; no treated rat survived after week 100. Hepatic nodules (designated as atypical hyperplastic) [incidence unspecified] were found, but neither hepatocarcinomas nor renal-cell tumours were observed in rats treated with aflatoxin B_2. No neoplasm was seen in untreated controls (Butler *et al.*, 1969). [The Working Group noted the lack of detailed reporting.]

Groups of 10 male Fischer rats [age not clearly specified] were administered 0, 50 or 100 μg/animal aflatoxin B_2 (purified by column chromatography and recrystallization) in 0.05 ml DMSO by gastric intubation on five days a week for 10 weeks (total doses of aflatoxin B_2, 0, 500 and 1000 μg/animal) and were sacrificed between 62 and 78 weeks. No hepatocellular carcinoma was observed after treatment with aflatoxin B_2 or in vehicle controls, but there was an increased incidence of preneoplastic liver lesions (foci of hyperplasia) in treated animals of each sex at 78 weeks: vehicle control, 0/10; low-dose, 6/9; high-dose, 5/7 (Wogan *et al.*, 1971).

Trout: Duplicate groups of 100 rainbow trout, 60 days old, were fed a semi-synthetic diet containing 0 or 20 μg/kg aflatoxin B_2 and 0, 4, 8 or 20 μg/kg aflatoxin B_1. Groups of fish were taken 9, 12 and 16 months after beginning of treatment for evaluation. Aflatoxin B_2 had little hepatocarcinogenic effect at the dose level used. Hepatoma incidence at 12 months was: control, 0/20; 4 μg aflatoxin B_1, 10/40; 8 μg aflatoxin B_1, 40/57; 20 μg aflatoxin B_1, 62/80; 20 μg aflatoxin B_2, 1/20. Hepatoma incidence at 16 months was: control, 0/40; 4 μg aflatoxin B_1, 14/40; 8 μg aflatoxin B_1, 32/40; 20 μg aflatoxin B_2, 0/40 (Ayres *et al.*, 1971).

3.4.2 *Subcutaneous administration*

Rat: Groups of male Fischer rats [initial number and age not clearly specified] were administered 0 or 300 μg/rat aflatoxin B_2 suspended in 0.2 ml trioctanoin subcutaneously twice weekly for 20 weeks (total dose of aflatoxin B_2, 12 000 μg/rat). Neither aflatoxin B_2 or the vehicle alone induced tumours in 20 rats that survived to 78 or 86 weeks (Wogan *et al.*, 1971).

3.4.3 *Intraperitoneal administration*

Rat: Groups of male Fischer rats [initial number and age not clearly specified] were administered 0 or 3750 μg aflatoxin B_2 in 0.05 ml DMSO intraperitoneally five times a week for eight weeks (total dose of aflatoxin B_2, 150 mg/animal). By 57–59 weeks, the incidence of hepatocellular carcinomas was 2/9 in rats treated with aflatoxin B_2 and 0/10 in vehicle controls (Wogan *et al.*, 1971).

3.5 Aflatoxins G_1 and G_2

3.5.1 *Oral administration*

Rat: Groups of 10–15 male and 15 female MRC rats, eight to nine weeks old, were administered 0, 20 or 60 μg/animal aflatoxin G_1 in the drinking-water (using dark bottles to avoid photolysis) on five nights a week for 10 weeks (low-dose only; total dose of aflatoxin G_1, 1000 μg/animal) or 20 weeks (total doses, 2000 and 6000 μg/animal), and followed until

they were in poor condition or died. Survival at 90 weeks was 26/30 (the two sexes combined) controls, 6/10 low-dose (males only) treated for 10 weeks, 17/30 (the two sexes combined) low-dose groups treated for 20 weeks and 9/28 (the two sexes combined) high-dose groups treated for 20 weeks. Aflatoxin G_1 produced a dose-dependent increase in the incidence of hepatic neoplasms in animals of each sex: males, controls, 0/15; low-dose, 10 weeks' treatment, 1/10; low-dose, 20 weeks' treatment, 2/15; high-dose, 9/11; females, control, 0/15; low-dose, 1/15; high-dose, 12/15. In males treated with aflatoxin G_1, there was also a dose-dependent increase in the incidence of renal-cell tumours: control, 0/15; low-dose treated for 20 weeks, 5/15; high-dose, 6/11. A variety of neoplasms was observed in other organs of treated rats but not in untreated controls (Butler *et al.*, 1969).

Groups of up to 30 male Fischer rats [age not clearly specified] were administered 0, 50 or 100 μg/animal aflatoxin G_1 (purified by column chromatography and recrystallization) in 0.05 ml DMSO by gastric intubation on four days a week for 2.5 or 8 weeks (total doses of aflatoxin G_1, 0, 700, 1400 and 2000 μg/animal) and were sacrificed between 4 and 68 weeks. Increased incidences of hepatocellular carcinomas were observed after treatment with aflatoxin G_1 with the two higher total doses: vehicle control, 0/10 at 59 weeks; low-dose, 0/3 at 68 weeks; medium-dose, 3/5 at 68 weeks; high-dose, 18/18 between 45 and 64 weeks. Preneoplastic liver lesions (foci of hyperplasia and transitional cells) were observed in the majority of animals treated with aflatoxin G_1 at all dose levels and sacrificed between 4 and 20 weeks. A total of 4/26 animals dosed with aflatoxin G_1 developed renal adenocarcinomas within 68 weeks. No neoplasm or preneoplastic lesion was observed in untreated controls (Wogan *et al.*, 1971).

Trout: Duplicate groups of 100 rainbow trout, 60 days old, were fed a semi-synthetic diet containing 0 or 20 μg/kg aflatoxin G_1 or 20 μg/kg aflatoxin G_2 [purity unspecified] and 0, 4, 8 or 20 μg/kg aflatoxin B_1 [purity unspecified]. Groups of fish were taken 9, 12 and 16 months after beginning of treatment for evaluation. Aflatoxin G_1 had a weaker hepatocarcinogenic effect than aflatoxin B_1, and aflatoxin G_2 had no hepatocarcinogenic effect at the dose level used. Hepatoma incidence at 12 months was: control, 0/20; 4 μg aflatoxin B_1, 10/40; 8 μg aflatoxin B_1, 40/57; 20 μg aflatoxin B_1, 62/80; 20 μg aflatoxin G_1, 1/20; 20 μg aflatoxin G_2, 0/20. Hepatoma incidence at 16 months was: control, 0/40; 4 μg aflatoxin B_1, 14/40; 8 μg aflatoxin B_1, 32/40; 20 μg aflatoxin G_1, 7/40; 20 μg aflatoxin G_2, 0/40 (Ayres *et al.*, 1971).

3.5.2 *Subcutaneous administration*

Rat: A group of six male rats [strain unspecified], weighing about 100 g, were injected subcutaneously with 20 μg aflatoxin G_1 suspended in arachis oil twice weekly for up to 65 weeks. Subcutaneous sarcomas were found in 4/6 rats within 30–50 weeks (Dickens & Jones, 1965).

3.6 Administration with known carcinogens and other modifying factors

3.6.1 *Mixtures of aflatoxins*

Rat: Groups of 6–10 rats of each sex [strain unspecified], 28 days old, were fed diets containing 5, 10 or 100 μg/kg aflatoxins [type unspecified] for 232, 225 and 10 days,

respectively, and received either a high-protein (20% casein) or a low-protein diet (5% casein) for up to two years, when the experiment was terminated. Two control groups of six male rats each received the high- and low-protein diet but no aflatoxins. Survival in rats treated with aflatoxins at the different dose levels was 12/22 in those on the low-protein diet and 20/22 in those on the high-protein diet at 52 weeks; the majority of the remaining animals died between 52 and 104 weeks due to massive pneumonia. there was a high incidence of hepatomas (6/20) and precancerous liver lesions (10/20; described as generalized cytoplasmic vacuolization, abundant cytoplasmic eosinophilia or increase in cytoplasmic ribonucleoprotein) in aflatoxin-treated rats on the high-protein diet. No hepatoma or precancerous liver lesion but one renal-cell carcinoma was observed in 12 aflatoxin-treated rats on the low-protein diet (Madhavan & Gopalan, 1968).

Groups of 12–25 male Fischer rats, weighing about 80 g, were fed synthetic diets containing 0 or 1 mg/kg of diet aflatoxins (approximately 1:1 mixture of aflatoxin B_1 and G_1; traces of aflatoxin B_2 and G_2) plus low (5%) or high (20%) casein with and without addition of 0.5 μg/kg of diet vitamin B_{12} for up to 33 weeks, when the experiment was terminated. In rats treated with aflatoxins, the incidence of hepatocellular carcinomas was 2/23 when the aflatoxins were combined with the low-protein diet without vitamin B_{12}, 1/24 when combined with the low-protein diet plus vitamin B_{12}, 1/24 when combined with the high-protein diet without vitamin B_{12} and 6/25 when combined with high-protein diet plus vitamin B_{12}. Administration of aflatoxins at a concentration of 1 mg/kg of low-protein diet with and without vitamin B_{12} produced a higher incidence of various other types of liver lesions (designated as hyperplastic nodules, cirrhosis, cholangiofibrosis and cysts) than treatment with the same dose of aflatoxins in the high-protein diet with and without vitamin B_{12} (Temcharoen *et al.*, 1978).

Groups of 10–15 male Long-Evans rats, weighing 50–60 g, were fed diets containing aflatoxin B_1 at concentrations of 0, 10, 100, 300 and 400 μg/kg of diet plus 0, 8.4, 84, 252 and 336 μg/kg of diet aflatoxin G_1. Similar groups received aflatoxins B_1 and G_1 plus 220 μg/kg cyclopropenoid fatty acids. In the combined experiment, another group received 100 μg/kg of diet aflatoxin B_1 and 84 μg/kg of diet aflatoxin G_1 together with 400 μg/kg of diet cyclopropenoid fatty acids. All the groups were treated for up to 18 months. Some animals were killed after 12 months or when ill, and the remaining animals were sacrificed after 18 months. Administration of aflatoxins resulted in an increased total incidence of renal-cell adenomas (control, 0/7; all aflatoxin treatments, 18/41; cyclopropenoid fatty acid, 1/10; all aflatoxins plus cyclopropenoid fatty acid treatment, 11/43). The incidences of liver-cell tumours were 0/7, 6/41, 0/10 and 2/43, respectively (Lee *et al.*, 1969).

Groups of 20–40 male Charles River rats, three weeks of age, were fed diets containing 0 or 200 μg/kg aflatoxins (added as groundnut meal containing 600 μg/kg) and 0 or 4000 μg/kg of diet diethylstilboestrol [duration unspecified]. Survival time was not appreciably affected by any of the treatments. Diethylstilboestrol alone or in combination with aflatoxins, but not aflatoxins alone, markedly decreased body weights at all recorded times. Rats fed diethylstilboestrol in addition to aflatoxins had a lower incidence of liver carcinomas than aflatoxin-treated animals (control, 0/20; diethylstilboestrol alone, 1/28; aflatoxins alone, 25/35; aflatoxins plus diethylstilboestrol, 8/40) and a lower incidence of liver nodules (control, 0/20; diethylstilboestrol alone, 3/28; aflatoxins alone, 10/35; aflatoxins plus diethylstilboestrol,

19/40). Paired-feeding and feeding-to-weight indicated that the decreased tumour incidence associated with diethylstilboestrol was not the result of decreased food intake (Newberne & Williams, 1969).

Groups of 20 intact and 37 hypophysectomized male MRC rats, eight weeks old, were fed aflatoxins (containing about 45% aflatoxin B_1, 45% aflatoxin G_1, 5% aflatoxin B_2 and 3% aflatoxin G_2) in the diet at approximately 4000 μg/kg and were killed when moribund. The mean quantity of diet consumed by the intact rats was calculated to have been approximately 10 g/rat per day and that by the hypophysectomized rats, 8 g/rat per day. The mean body weight of the hypophysectomized animals was only 30% that of intact rats. Mortality was significantly greater in hypophysectomized than in intact rats treated with aflatoxins at every time up to 60 weeks. Survival at 49 weeks was 14/37 hypophysectomized and 14/20 intact rats treated with aflatoxins. Liver carcinomas developed after treatment with aflatoxins in 14/14 intact rats and in 0/14 hypophysectomized rats (Goodall & Butler, 1969). [The Working Group noted the poor survival and the lack of exact food consumption data.]

Groups of 10 and 20 male Porton rats [age unspecified] were fed 0 or approximately 5000 μg/kg of diet (as calculated from aflatoxin B_1 content) of a mixture of aflatoxins from weeks 1–10 and received 0 or 1 g/l of sodium phenobarbital in the drinking-water during weeks 0–12. After interim sacrifices, the remaining animals were observed for up to 24 months, when the experiment was terminated. Survival after 11 months was the same in animals treated with aflatoxin B_1 alone and with aflatoxin B_1 plus phenobarbital. At 24 months, the incidence of liver tumours (predominantly carcinomas with mixed hepatocellular and cholangiocellular appearance) was significantly higher (17/18) in animals treated with aflatoxin B_1 alone than in animals administered aflatoxin B_1 plus phenobarbital (12/18). In the groups killed at earlier times, the incidences of liver tumours induced by the combined treatment with aflatoxin B_1 and phenobarbital were also lower than those with aflatoxin B_1 alone, and the tumours had a higher degree of differentiation (McLean & Marshall, 1971).

Groups of 15 male Fischer 344 rats, eight weeks old, were fed a diet free of or naturally contaminated with aflatoxins (4 mg/kg of diet aflatoxin B_1) for six weeks, together with 0, 500 or 5000 mg/kg of diet ethoxyquin [stability not measured] either two weeks before, six weeks during or two weeks after aflatoxin. The rats were kept for 48 weeks after the start of treatment. Ethoxyquin produced a dose-related inhibition of aflatoxin-induced liver carcinogenesis. The most effective reduction in the incidence of liver-cell tumours was found after simultaneous administration of aflatoxin and ethoxyquin (aflatoxin B_1 alone, 3/11; aflatoxin plus ethoxyquin, 0/9). Similar trends were observed in the incidence of neoplastic hepatic nodules (aflatoxin B_1 alone, 7/11; aflatoxin plus ethoxyquin, 0/9) and of preneoplastic hepatic foci (aflatoxin B_1 alone, 8/11; aflatoxin plus ethoxyquin, 1/9). No liver lesion was found in rats given control diet or ethoxyquin alone. In another experiment, groups of 21 rats of the same strain, sex and age were fed the same aflatoxin-contaminated diet for six weeks after administration of 0 or 5000 mg/kg of diet ethoxyquin for two weeks and a treatment-free interval of four weeks. The authors stated that there was no difference between the two groups in the incidence of liver lesions at 52 weeks (Cabral & Neal, 1983).

3.6.2 *Aflatoxin B_1*

(*a*) *Viruses*

Marmoset: Groups of 12 young marmosets (*Saguinus oedipomidas*) of each sex, weighing 370–430 g, were given 2000 μg/kg aflatoxin B_1 in chow or fruit diet (200 μg/kg bw), either alone or in combination with hepatitis virus (0.25 ml of a 1/50 dilution containing 100 or greater infectious doses of the G. Baker strain of hepatitis agent), which was injected six weeks after the start of aflatoxin diet. Groups of seven marmosets receiving the hepatitis virus alone and five marmosets on chow and fruit diet served as controls. Nine of 12 marmosets treated with aflatoxin B_1 alone died between 9 and 55 weeks after the beginning of the experiment; 5/12 marmosets that received both aflatoxin B_1 and the hepatitis virus and 2/7 marmosets injected with the hepatitis virus alone were still alive at the time of reporting; 3/5 animals in the untreated control group died. Hepatocellular carcinomas developed in 1/9 marmosets fed aflatoxin B_1 alone and in 2/7 animals injected with hepatitis virus during aflatoxin exposure and which survived 3–94 weeks of treatment. No tumour was found in three animals infected with hepatitis virus alone which died 28–67 weeks after the start of the experiment (Lin *et al.*, 1974). [The Working Group noted the high mortality and that some animals were still alive at the time of reporting.]

Duck: Groups of 2–22 newly hatched ducklings of each sex, free of infection or infected with duck hepatitis virus (DHBV), were administered 0 or 100 μg/kg bw aflatoxin B_1 in DMSO by gastric intubation twice a week for 54 weeks, continuously for five weeks followed by 49 weeks without aflatoxin B_1, or for 25 weeks after an interval of 16 weeks without aflatoxin B_1. After continuous administration of aflatoxin B_1, 16/22 ducks inoculated with the virus and 8/16 ducks without virus died within 10 weeks of hatching due to extensive liver-cell necrosis. After administration of aflatoxin B_1 following an interval of 16 weeks without aflatoxin treatment, 3/5 DHBV-positive ducks died between 20 and 25 weeks. The remaining ducks were sacrificed when ill or 54 weeks after the beginning of the experiment. All ducks (except one) that had been inoculated with the virus showed the presence of DHBV, and all those that were not inoculated did not show the virus, as demonstrated by analysis for DHBV DNA in the sera or tissues and/or immunohistochemistry. After continuous administration of aflatoxin B_1, hepatocellular carcinomas were found in 2/8 DHBV-negative ducks and in 0/8 DHBV-positive ducks that survived 10 weeks. In addition, long-term administration of aflatoxin B_1 often produced cirrhotic changes and nodular liver lesions in ducks both positive and negative for DHBV. Administration of aflatoxin B_1 for five weeks induced one hepatocellular carcinoma in five DHBV-positive ducks by 52 weeks and in 0/3 DHBV-negative ducks kept for 54 weeks. DHBV did not significantly accelerate the induction of hepatic disorders by aflatoxin B_1 (Uchida *et al.*, 1988). [The Working Group noted the high mortality among aflatoxin-treated ducks and the limited reporting.]

Groups of 8–12 Pekin ducks [sex unspecified], three days of age, congenitally infected or free of infection with DHBV, were administered 0 or 200 μg/kg bw aflatoxin B_1 (dissolved in chloroform and added to corn oil) by gavage for 60 days (total dose of aflatoxin B_1, 1740–2040 μg/animal) and kept up to 28 months. Survival at 12 months was 13/22 (DHBV-positive, 9/12; DHBV-negative, 4/10) in the group treated with aflatoxin B_1 and 13/17 (DHBV-positive, 6/8; DHBV-negative, 7/9) in vehicle controls. DHBV core antigen

was demonstrated by immunohistochemistry in the liver and several other tissues of most virus-infected ducks but in none of the uninfected ducks. Hepatic neoplasia occurred only in ducks treated with aflatoxin B_1; the incidence of hepatocellular and cholangiocellular carcinomas was 3/8 DHBV-positive ducks and 3/4 DHBV-negative ducks; hepatocellular adenomas occurred in 3/8 DHBV-positive and 0/4 DHBV-negative birds. In addition, foci of altered hepatocytes (as defined by morphology and changes in glycogen content) were often observed in ducks treated with aflatoxin B_1 but not in congenitally DHBV-infected or uninfected ducks that did not receive aflatoxin B_1. The authors concluded that persistent congenital DHBV infection does not contribute significantly to the emergence of hepatic neoplasia induced in ducks by aflatoxin B_1 (Cullen *et al.*, 1990). [The Working Group noted the high mortality.]

Groups of 13–16 male and female Peking ducklings congenitally infected or free of infection with DHBV were administered 0, 20 or 80 μg/kg bw ^{3}H-aflatoxin B_1 in DMSO intraperitoneally once a week every week from the third month after hatching until they were sacrificed 2.3 years later (total doses of aflatoxin B_1/year, 1050 and 4150 μg/kg bw. Of the control ducks, which did not receive aflatoxin B_1, approximately 80% survived to 17 months, whether infected or not. Aflatoxin B_1 treatment resulted in a lower survival by 17 months (high-dose—with infection, approximately 10%; without infection, 40%; low-dose—with infection, 70%; without infection, 50%). Seven hepatocellular carcinomas and two hepatocellular adenomas were observed in eight ducks that were treated with aflatoxin B_1, regardless of the presence of DHBV infection, but not in infected ducks treated with the lower dose of aflatoxin B_1: high-dose with infection, 3/6; without infection, 3/10; low-dose with infection, 0/13; without infection, 2/10. No liver tumour was observed in ducks that were not given aflatoxin B_1 (Cova *et al.*, 1990). [The Working Group noted the poor survival.]

(*b*) *Parasites*

Rat: Groups of 72–83 male Buffalo rats, weighing 80–100 g, were fed diets containing 0 or 2000 μg/kg of diet aflatoxin B_1 for 10 weeks, beginning 12 days after intraperitoneal inoculation of 10^6 erythrocytes infected with *Plasmodium berghei* or 0.5 ml of heparinized blood. Three animals in each group were killed at 3, 6 and 12 days after inoculation with parasite or injection of heparinized blood, and 4–25 animals in each group 1, 5, 10, 20, 40, 60 and 82 weeks after beginning of the treatment with aflatoxin B_1. Survival at 11 weeks was 72/80 rats injected with heparinized blood, 79/83 rats given infected erythrocytes, 62/73 rats given infected erythrocytes and treated with aflatoxin B_1 and 67/72 rats treated with aflatoxin B_1 alone. In rats treated with aflatoxin B_1 alone, the incidence of hepatocellular carcinomas was 13/19 after 60–82 weeks, and that of neoplastic hepatic nodules was 5/12 after 20 weeks, 12/23 after 40 weeks and 13/19 after 60–82 weeks. In rats that received infected erythrocytes and aflatoxin B_1, the incidence of hepatocellular carcinomas was 5/14 after 60–82 weeks, and that of neoplastic hepatic nodules was 0/15 after 20 weeks, 5/24 after 40 weeks and 5/14 after 60–82 weeks. Only 1/22 rats inoculated with infected erythrocytes developed a neoplastic hepatic nodule at week 82. No animal in the control group developed a hepatocellular carcinoma or a neoplastic nodule (Angsubhakorn *et al.*, 1988).

Groups of 25 male Wistar rats, 8–10 weeks old, were fed diets containing 0 or 1000 μg/kg aflatoxin B_1 (pure) for 12 weeks and were given a single dose of 0 or 50 metacercariae of

Clonorchis sinensis by intubation at the beginning of the experiment. Three rats from each group were killed at four-week interval up to 28 weeks. Two hepatocellular carcinomas were found at 28 weeks in rats treated with aflatoxin B_1 and infected with *C. sinensis*, and mild to moderate cirrhotic changes were seen after 24 weeks. No liver tumour or cirrhotic change was observed in animals treated with aflatoxin B_1 or *C. sinensis* alone (Park, 1989).

(*c*) *Chemicals and dietary factors*

(i) *Oral administration*

Rat: Groups of 10–40 male CD rats, three weeks of age, were subjected to a number of additional liver insults (ethionine treatment, choline deficiency, partial hepatectomy, repeated biopsies) before, during or after feeding of 0, 70, 400, 1000 or 1500 μg/kg of diet aflatoxin B_1 (purified). Aflatoxin and ethionine appeared to have syncarcinogenic effect on the liver: The incidences of liver tumours in the various groups suggested a potentiating effect of cirrhosis on the hepatocarcinogenic effect of aflatoxin B_1, particularly when the animals were returned to an adequate diet after the induction of cirrhosis (Newberne *et al.*, 1964, 1966).

Groups of 20 male and 20 female weanling Wistar rats and 16 male and 16 female weanling Fischer rats were fed diets containing 0, 20 or 100 μg/kg aflatoxin B_1 (spectrally pure), with and without 0.02 and 0.035% cyclopropenoid fatty acids for 24 months, and were killed when moribund or showing tumour development. The fatty acids had little, if any, modifying effect on the response to aflatoxin B_1 (Nixon *et al.*, 1974).

Groups of male Sprague-Dawley and male Fischer rats [initial numbers unspecified], about six weeks of age, were fed marginal lipotrope diets (3% casein, 2% corn oil) or a control diet (23% casein, 16% corn oil) and were given 25 μg/animal aflatoxin B_1 in 0.1 ml DMSO intragastrically for 15 days. Hepatocarcinomas [number not given] were found from six months onwards in the deficient rats (6 months, 25%; 9 months, 50%; 12 months, 60%; 18 months, 85%) but not until 12 months in rats on a normal diet (12 months, 30%; 18 months, 60%). Hyperplastic hepatic nodules were present in significant numbers in deficient rats immediately after treatment with aflatoxin B_1 but not in rats fed the normal diet until six months after administration of aflatoxin B_1. The early appearance of preneoplastic focal liver lesions in animals fed the marginal lipotrope diet correlated well with the short tumour induction time (Rogers & Newberne, 1971).

Groups of 50 male weanling Sprague-Dawley rats were fed a purified basal diet containing 50 μg vitamin A palmitate (controls), a purified diet with 100 μg/kg of diet aflatoxin B_1 (purity, > 99.5%) and 5 μg/day vitamin A palmitate (low dose), 100 μg/kg aflatoxin B_1 with 50 μg/day vitamin A (medium dose) or 100 μg/kg aflatoxin B_1 plus 5000 μg/day vitamin A palmitate (high dose). Survival of rats at 24 months was 35/50 controls, 23/50 low-dose, 34/50 medium-dose and 31/50 high-dose animals. The incidence of liver-cell carcinomas was increased by aflatoxin B_1, and the dietary concentration of vitamin A had no significant effect: control, 0/50; low-dose, 11/50; medium-dose, 24/50; high-dose, 19/50. In addition, colon carcinomas were observed in rats treated with aflatoxin B_1 plus low dietary vitamin A: control, 0/50; low-dose, 6/50; medium-dose, 0/50; high-dose, 0/50. In another experiment, 49 male Sprague-Dawley rats were fed diets containing enough vitamin A (added as acetate) to provide 50 μg/day and were administered aflatoxin B_1 (in DMSO) by

gastric intubation at a dose of 25 μg for 15 days. The incidence of hepatocellular carcinomas was 27/49 at 24 months (Newberne & Rogers, 1973).

Groups of 60 male Sprague-Dawley rats, about four weeks old, were each given 25 μg aflatoxin B_1 in DMSO by gastric intubation for 15 days and simultaneously or subsequently received diets containing either 28% beef fat and 2% corn oil or no beef fat and 30% corn oil. Animals fed beef fat had a lower incidence of hepatocellular carcinomas (32/60) than those fed the corn oil diet during and after exposure to aflatoxin B_1 (60/60) at 96 weeks. No difference in the incidence of liver tumours was observed between groups fed beef fat throughout exposure to aflatoxin B_1 and afterwards (32/60) and those fed beef fat diet only after exposure to aflatoxin B_1 (28/60) (Newberne *et al.*, 1979).

Groups of 28–35 male Sprague-Dawley CD rats, weighing 40–50 g, were each administered 15 μg aflatoxin B_1 in DMSO by gavage on three to five days per week for seven weeks (total dose, 375 μg/animal) and received semi-synthetic diets containing different amounts of lipids and lipotropes (complete or deficient) throughout the experiment. The animals were killed when moribund or when they reached 90 weeks. All rats fed lipotrope-deficient diets (lipotrope-deficient, deficient plus control vitamin mix, deficient plus lipotropes, deficient plus amino acids) were dead by 82 weeks, while 4/27 of rats fed the lipotrope-complete diet and 10/29 of rats receiving complete diet with high fat content were still alive at that time. The incidences of hepatocellular carcinomas were significantly higher in rats treated with aflatoxin B_1 and fed the various deficient diets: control, 4/27; control plus high fat, 2/29; lipotrope-deficient, 12/31; deficient plus control vitamin mix, 28/34; deficient plus lipotropes, 19/33; deficient plus amino acids, 16/33 (Rogers *et al.*, 1980).

Groups of 10 male Sprague-Dawley rats, weighing 100 g, were administered 0 or 380 μg/kg bw aflatoxin B_1 in tricaprylin by gastric intubation five times a week for up to two weeks and, after an interval of five days, were fed low-fat (11% of calories) or high-fat (35% of calories) diets without or with ethanol (35% of calories) for 12 weeks, when the experiment was terminated. Preneoplastic foci of altered hepatocytes (as defined by morphology and GGT^+ staining) were found in livers of all rats pretreated with aflatoxin B_1; their development was not affected by ethanol consumption, but their number and size were increased significantly ($p < 0.01$) by the high-fat diet as compared with the low-fat diet (Misslbeck *et al.*, 1984).

Groups of 10–18 male Fischer rats, weighing 80 g, were each administered 0 or 25 μg aflatoxin B_1 (in 0.5 ml saline emulsion containing 2.5 μl each of DMSO and propylene glycol) by intubation on five days a week for eight weeks and were fed 0 or 0.015% β-naphthoflavone one week before, during and one week after exposure to aflatoxin B_1. The experiment was terminated 42 weeks after the beginning of aflatoxin B_1 administration. Survival was 10/10 vehicle controls without β-naphthoflavone, 18/18 vehicle controls with β-naphthoflavone, 15/17 rats receiving aflatoxin B_1 without β-naphthoflavone and 16/18 rats receiving aflatoxin B_1 with β-naphthoflavone. Administration of aflatoxin B_1 alone resulted in hepatomas in 15/15 rats and in neoplastic hepatic nodules in 11/15 rats. Combined treatment with aflatoxin B_1 and β-naphthoflavone markedly reduced the incidence of hepatomas (5/16) and neoplastic hepatic nodules (3/16). No neoplastic or preneoplastic liver lesion was found in rats receiving vehicle with or without β-naphthoflavone (Gurtoo *et al.*, 1985).

A total of 267 male Fischer rats, eight weeks of age, were divided into nine groups [numbers of animals per group unspecified, except that there were 21 untreated controls] and were administered 0 or 25 μg/kg bw aflatoxin B_1 in 0.05 ml DMSO by gavage three times a week for 20 weeks (total dose of aflatoxin B_1, 1500 μg/kg bw) and a diet containing 0, 1000 or 6000 mg/kg of either butylated hydroxyanisole or butylated hydroxytoluene one week before, during and one week after administration of aflatoxin B_1. Rats that received aflatoxin B_1 alone were killed every two weeks from weeks 4 to 20 after the beginning of treatment; the four remaining rats in that group and animals in all other groups were killed 12 and 24 weeks after cessation of treatment with aflatoxin B_1. The additional administration of butylated hydroxytoluene and butylated hydroxyanisole resulted in a dose-related reduction in the combined incidence of hepatocellular carcinomas and neoplastic hepatic nodules: aflatoxin B_1 alone, 17/27; plus 1000 mg/kg of diet butylated hydroxytoluene, 3/25; plus 6000 mg/kg of diet butylated hydroxytoluene, 0/26; plus 1000 mg/kg of diet butylated hydroxyanisole, 3/23; plus 6000 mg/kg of diet butylated hydroxyanisole, 2/25. No hepatocellular neoplasm was observed in rats receiving basal diet, DMSO (vehicle control) or 6000 mg/kg of diet butylated hydroxyanisole or butylated hydroxytoluene alone. The number of foci of altered hepatocytes (as defined by iron exclusion and GGT^+ staining) in rats given aflatoxin B_1 alone increased with time from week 4 to week 20. In groups given butylated hydroxytoluene or butylated hydroxyanisole, there was a significant dose-related reduction in the number of foci of altered hepatocytes at the end of exposure to aflatoxin B_1 and 12 and 24 weeks thereafter (Williams *et al.*, 1986).

Groups of 6 and 12 male Fischer 344 rats, weighing 80 g, were administered 0 or 250 μg/kg bw aflatoxin B_1 in tricaprylin by gastric intubation on five days a week for two weeks and were then fed diets containing high (20%) or low (5%) levels of casein one week prior, during and one week after treatment with aflatoxin B_1; they then remained on the same diet or were switched from high to low or low to high casein for another 12 weeks. There was a significant ($p < 0.05$) increase in the number and volume of aflatoxin B_1-induced foci of altered hepatocytes (as defined by GGT^+ staining) in rats given the high-casein diet compared to those given the low-casein diet in the period after aflatoxin B_1 treatment (Appleton & Campbell, 1983a). In a similar experiment, the same treatment schedule was used up to one week after administration of aflatoxin B_1, but in addition to groups receiving diets containing high (20%) and low (5%) levels of casein throughout the subsequent 12 weeks, two groups received the high- or low-casein diet for six weeks and were then switched to low- or high-casein diet for another six weeks. As in the previous experiment, animals fed 5% casein in the diet throughout the 12-week period after treatment with aflatoxin B_1 had a marked reduction in the development of GGT^+ foci. Animals fed 20% casein in the diet throughout the same period had the greatest response. Groups fed 5% casein in the diet for half of the period after treatment and 20% for the other half had intermediate responses (Appleton & Campbell, 1983b).

Groups of 8 and 14 male Fischer 344 rats, weighing 40 g, were administered 0 or 250 μg/kg aflatoxin B_1 in tricaprylin by gastric intubation five times per week for two weeks. One week after completion of the aflatoxin treatment, the animals were given diets containing 4, 8, 10, 12, 15, 20 or 30% casein for 12 weeks and then sacrificed. Preneoplastic hepatic foci were observed in all animals treated with aflatoxin B_1; their number and volume

were small in rats fed low levels of casein (4, 6, 8 and 10%) but larger in those receiving higher levels of casein (12, 20 and 30%) (Dunaif & Campbell, 1987a).

Groups of 12 male Fischer 344 rats, weighing about 40 g, were administered 0, 40, 100, 150, 200, 250, 300, 350 or 400 μg/kg bw aflatoxin B_1 in tricaprylin by gavage five days a week for two weeks and then received a diet containing 20% casein. The experiment was terminated 15 weeks after beginning of treatment with aflatoxin B_1. The incidence of preneoplastic hepatic foci (as defined by GGT^+ staining) was increased after treatment with aflatoxin B_1 at the higher doses (control, 0/12; 40, 0/12; 100, 0/12; 150, 8/12; 200, 11/12; 250, 9/12; 300, 11/11; 350, 8/8; 400 μg/kg bw, 5/5) (Dunaif & Campbell, 1987b). In another experiment, groups of 18–19 male Fischer 344 rats were administered 0, 200, 235, 270, 300 or 350 μg/kg bw aflatoxin B_1 in tricaprylin by gavage five days a week for two weeks; and, one week after the last treatment, the rats were placed on diets containing 4, 8, 12, 16 or 20% casein for 12 weeks and then sacrificed. Increasing the dose of aflatoxin B_1 from 200 to 350 μg increased the incidence and volume fraction of preneoplastic hepatic foci (as defined by GGT^+ staining) when the diet contained 20% casein but not when the diet contained 4, 8, 12 or 16% casein (Dunaif & Campbell, 1987b).

Groups of 46 and 51 male and 40 and 51 female Sprague-Dawley rats, 12 weeks old, were fed diets containing 0 or 2000 μg/kg of diet aflatoxin B_1, and a low level of protein (10.32%) and riboflavin or a normal content of proteins (24.2%) and vitamins. Four animals from each group were sacrificed at different times between 15 and 400 days after the beginning of treatment, when the experiment was terminated. Hepatocellular carcinomas were found in 18/23 males and 8/23 females fed aflatoxin B_1 in the diet with normal protein and vitamin content and which survived more than 320 days. No liver tumour was observed by 400 days in rats fed aflatoxin B_1 in a diet deficient in protein and riboflavin, or in rats on high- or low-protein diets with or without administration of aflatoxin B_1 (Stora *et al.*, 1987).

Groups of 11 weanling male Fischer 344 rats were administered 250 μg/kg bw aflatoxin B_1 in tricaprylin intragastrically five times per week for two weeks and were then fed diets containing 20% casein or 20% gluten (low-quality protein) one week prior, during and one week after administration of aflatoxin B_1, followed by diets containing either 20% gluten, 20% casein, 5% casein or 20% gluten plus lysine for 11 weeks, when the experiment was terminated. Preneoplastic foci of altered hepatocytes (as defined by GGT^+ staining) were observed in all rats treated with aflatoxin B_1; the number and size of the foci was significantly greater ($p < 0.05$) in rats fed high-protein diets (20% casein; 20% casein followed by 20% gluten plus lysine) than in rats fed high-protein diets during aflatoxin B_1 treatment but afterwards switched to low-protein diet (20% gluten; 5% casein) or in rats fed low-protein diet throughout the experiment (20% gluten) (Schulsinger *et al.*, 1989).

Groups of 10 weanling male Fischer rats were fed semi-purified diets containing 0 or 1000 μg/kg aflatoxin B_1 with or without 25% freeze-dried green beans, beetroot or squash for eight weeks. All animals were killed after eight weeks, and their livers were examined for the presence of preneoplastic hepatic foci (as defined by γ-glutamyltransferase positivity). Among aflatoxin B_1-treated groups, both the percentage of the liver area that was GGT^+ (aflatoxin B_1 alone, 17.9 ± 3.9; plus bean, 33.0 ± 3.8; plus beetroot, 28.5 ± 2.8; plus squash, 30.0 ± 5.0) and the number of foci per square centimetre (aflatoxin B_1 alone, 3.6 ± 3.0; plus bean, 12.8 ± 1.1; plus beetroot, 16.9 ± 6.5; plus squash, 12.8 ± 6.6) were significantly

($p \leq 0.05$) higher in each of the groups given vegetables than in the group given aflatoxin B_1 alone (Boyd *et al.*, 1983).

Groups of 33–42 male Buffalo rats, weighing about 50 g, were fed a semi-synthetic diet containing 1000 μg/kg aflatoxin B_1, 25 mg/kg *N*-nitrosodimethylamine (NDMA) or a combination of the two for six months and were killed 3, 6, 9 and 12 months after the onset of the experiment. The number of effective animals ranged between 26 and 34. At 12 months, hepatocellular carcinomas were found in 9/20 rats treated with aflatoxin B_1, in 1/21 rats administered NDMA and in 11/14 rats given the combined treatment. The incidence of neoplastic hepatic nodules at this time was 7/20 in rats treated with aflatoxin B_1, 0/21 in animals administered NDMA and 11/14 after the combined treatment. Preneoplastic hepatic foci (clear-, acidophilic- and basophilic-cell foci) were observed in all treatment groups, but their incidence was higher in rats that received the combination of aflatoxin B_1 and NDMA then in those treated with aflatoxin B_1 or NDMA separately. In addition, multiple liver cysts were found at all times in animals fed aflatoxin B_1 plus NDMA, whereas rats fed aflatoxin B_1 and NDMA separately had developed only a few cysts by the end of the experiment. Neither neoplastic nor preneoplastic lesions were found in untreated controls (Angsubhakorn *et al.*, 1981a).

Groups of about 25–28 male Buffalo rats, weighing 40–50 g, were fed diets containing 0 or 1000 μg/kg aflatoxin B_1, 500 mg/kg α-benzene hexachloride or a combination of aflatoxin B_1 and α-benzene hexachloride for 35 weeks and then placed on basal diet for 30 weeks. Three to 10 animals from each group were killed at intervals of 5, 10, 15, 35 and 65 weeks after the beginning of the experiment. Administration of aflatoxin B_1 resulted in both hepatocellular carcinomas and neoplastic hepatic nodules in 6/6 rats by 65 weeks. After additional administration of α-benzene hexachloride, no liver-cell carcinoma and only one neoplastic hepatic nodule was observed in 10 animals by 65 weeks. No change in the livers of rats fed either α-benzene hexachloride or the basal diet was noted. Foci of altered hepatocytes (predominantly acidophilic-cell foci) were found for the first time at 10 weeks (1/4 rats) and regularly (10/10 rats) at 35 and 65 weeks after treatment with aflatoxin B_1; they did not appear in rats that received aflatoxin B_1 plus α-benzene hexachloride up to 35 weeks, but were observed in 2/10 animals after the combined treatment by 65 weeks (Angsubhakorn *et al.*, 1981b).

Groups of 10–12 male Fischer 344 rats, weighing 75–100 g, were administered 0 or 250 μg/kg bw aflatoxin B_1 in 0.1 ml tricaprylin by gavage five times a week for two weeks and then fed a diet containing 4000 mg/kg ethoxyquin one week prior to, during and one week after treatment with aflatoxin B_1. The experiment was terminated 16 weeks after the beginning of ethoxyquin administration. Preneoplastic foci of altered hepatocytes (as defined by GGT^+ staining) were found in all 12 rats treated with aflatoxin B_1 alone. Only 3/10 rats that also received ethoxyquin had preneoplastic hepatic foci, but a significantly lower number per square centimetre of liver tissue and volume fraction of total liver parenchyma ($p < 0.01$) as compared to those in rats that received aflatoxin B_1 alone (Kensler *et al.*, 1986).

Groups of 8–10 male Fischer 344 rats, seven to eight weeks of age, were given a single intraperitoneal injection of 250 μg/kg bw aflatoxin B_1 and were then fed diets containing 0 or 5000 mg/kg of diet ethoxyquin four weeks prior to and two weeks after administration of

aflatoxin B_1; they were then placed on diets containing 0 or 1000 μg/kg aflatoxin B_1 plus 0 or 5000 mg/kg ethoxyquin for another 17 weeks. Twenty-three weeks after the beginning of the experiment, all animals were killed. Preneoplastic foci of altered hepatocytes (as defined by morphology and several histochemical parameters) were often found in animals receiving aflatoxin alone but were not observed in animals additionally treated with ethoxyquin or in controls. In rats that received ethoxyquin, with or without aflatoxin B_1, many altered renal tubules (designated as hyperplastic and putatively preneoplastic) were found (Manson *et al.*, 1987).

An unspecified number of male Fischer 344 rats, weighing 75–100 g, were administered 250 μg/kg bw aflatoxin B_1 by gavage on five days a week for two weeks and were then fed 0, 100, 200, 400 or 750 mg/kg of the schistosomicidal drug 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (oltipraz) one week prior to, during and one week after treatment with aflatoxin B_1. The experiment was terminated at the end of the fourth month after the beginning of treatment with oltipraz. All rats administered aflatoxin B_1 alone developed preneoplastic hepatic foci (as defined by GGT^+ staining); the average number of foci per square centimetre of liver tissue was 1.41 ± 0.29. Oltipraz induced a significant ($p < 0.01$), dose-dependent reduction in the number and size of preneoplastic hepatic foci (0, 1.41 ± 0.29; 100, 0.44 ± 0.11; 200, 0.21 ± 0.10; 400, 0.17 ± 0.06; 750, 0.03 ± 0.01). Preneoplastic hepatic foci were not found in 40, 20 and 30% of rats receiving 750, 400 and 200 mg oltipraz, respectively (Kensler *et al.*, 1987).

Groups of 55 and 56 male Fischer 344 rats, five weeks of age, were each administered 25 μg aflatoxin B_1 (purity, > 97%) by gavage in 0.1 ml tricaprylin on five days a week for two weeks (total dose of aflatoxin B_1, approximately 2000 μg/kg bw) and then received a purified diet containing 0 or 0.075% [750 mg/kg of diet] of oltipraz one week before, during and one week after the beginning of treatment with aflatoxin B_1. Ten rats from each group were killed 15 weeks after the first dose of aflatoxin B_1. At 23 months after the first dose of aflatoxin B_1, survival of the remaining animals was 10/45 in the group treated with aflatoxin B_1 alone and 24/46 in the group receiving aflatoxin B_1 plus oltipraz. Oltipraz significantly decreased the incidence of hepatocellular tumours (aflatoxin B_1 alone: 9/45 (five carcinomas, four adenomas); aflatoxin B_1 plus oltipraz: 0/45). The difference in the incidence of hepatocellular carcinomas and adenomas between the two groups was statistically significant ($p < 0.05$, χ^2-test). Foci of altered hepatocytes (detected by GGT^+ staining) were found in both groups, but their number and size were significantly smaller in rats treated with aflatoxin B_1 plus oltipraz than in those treated with aflatoxin B_1 alone (Roebuck *et al.*, 1991).

Groups of 24–34 female Wistar rats, weighing 100 g, were each administered 0.25 μg aflatoxin B_1 in 1.0 ml water (diluted after dissolution in DMSO) by gavage five times a week for eight weeks, followed, after an interval of 16 months, by water (control) or by 100 mg/animal reduced glutathione, 5 mg/animal butylated hydroxytoluene, 80 mg/animal methionine or 50 mg/animal ascorbic acid in 2.5 ml water for an additional eight months, when all remaining animals were sacrificed. None of the additional treatments increased the percentage of aflatoxin-treated animals alive at the terminal sacrifice date, but after the methionine and ascorbic acid treatments significantly ($p < 0.05$) fewer animals were alive at termination of the experiment. No treatment had any effect on the number or size of hepatic

nodules, but the incidence of cystic cholangiomas was reduced by ascorbic acid treatment (Iverson *et al.*, 1987).

Groups of 25 female Buffalo rats, weighing 100 g, were fed a semi-synthetic diet, a diet containing 1000 μg/kg aflatoxin B_1 for 15 weeks, a diet containing 100 mg/kg lindane for 15 weeks or a diet containing aflatoxin B_1 and lindane for 1, 3, 5, 10 or 15 weeks and then the semi-synthetic diet until week 15. All animals then received basal diet for 67 weeks. Survival at the end of the experiment (82 weeks) ranged from 13/25 in the group that received both aflatoxin B_1 and lindane for 15 weeks to 23/25 in the untreated control group. Administration of aflatoxin B_1 alone for 15 weeks resulted in hepatocellular carcinomas in 6/19 rats by the end of the experiment. Administration of lindane in the diet appeared to prevent the induction of liver tumours by aflatoxin B_1 at 82 weeks: one hepatocellular carcinoma was seen in the 19 animals that received aflatoxin B_1 and lindane for one week, and no liver tumour occurred in any other treatment group (Angsubhakorn *et al.*, 1989).

Groups of 28–34 Fischer 344 rats of each sex, weighing 75–100 g, were each given 25 μg aflatoxin B_1 in the drinking-water five times a week for 20 weeks and administered either 2 units of adrenocorticotropin subcutaneously twice a week for 20 weeks, 0.25 units of insulin twice a week for 20 weeks or 0.5 units of growth hormone twice a week for 10 weeks. Groups of four rats were killed at various times between 7 and 77 weeks after the beginning of the experiment. Hepatocellular carcinomas were induced by 77 weeks in all 17 animals given aflatoxin B_1 alone, in all 12 animals given aflatoxin B_1 and growth hormone, in 6/10 animals given aflatoxin B_1 and insulin and in none of eight untreated controls. The development of hepatocellular carcinomas was preceded by focal and nodular liver lesions (designated as hyperplastic or neoplastic) in all groups, the incidence of which was correlated with the incidence of liver carcinomas in the different groups. Six animals that received aflatoxin B_1 and adrenocorticotropin simultaneously failed to develop hepatocellular carcinomas by 77 weeks, but 3/6 animals had malignant lymphomas at 56 weeks and 8/8 animals had lymphoid hyperplasia at 35 weeks; these lesions were not seen in other groups (Chedid *et al.*, 1977).

Groups of 10–14 male and 7–14 female Wistar rats, 12 weeks of age, were administered 0 and 50 μg/animal aflatoxin B_1 in 0.1 ml DMSO intragastrically twice a week for four weeks, followed by 0 or 70 μg/animal aflatoxin B_1 in 0.15 ml DMSO twice a week for an additional six weeks, with or without thyroidectomy six days before the beginning of aflatoxin B_1 treatment. Further groups received 0 or 0.5 mg/animal 1-methyl-2-mercaptoimidazole in 0.5 ml water intragastrically twice weekly from the beginning of aflatoxin B_1 treatment to the end of the experiment and were given 0 or 0.05 g calcium pantothenate in the drinking-water throughout the experiment. The experiment was terminated 58 weeks after its start. At this time, the incidence of hepatomas [histology not further specified] in animals treated with aflatoxin B_1 alone was 11/12 in males and 10/11 in females, and that of cholangiocellular adenomas 5/12 in males and 3/11 in females. After combined treatment with aflatoxin B_1 and 1-methyl-2-mercaptoimidazole, hepatomas were observed in only 1/11 males and 0/9 females, and no cholangiocellular tumour occurred. There was also a much lower incidence of precancerous liver lesions (designated as focal and nodular hyperplasia) after the combined treatment than after administration of aflatoxin B_1 alone. No hepatoma but discrete focal hyperplastic changes and a single focus of nodular hyperplasia were observed

when the combined treatment with aflatoxin B_1 and 1-methyl-2-mercapatoimidazole was preceded by thyroidectomy. No neoplastic or preneoplastic liver lesion was found in other groups, including the untreated controls (Bednarz, 1989).

Groups of 18 and 42 male Wistar rats, nine weeks of age, were each given 0 or 42 μg aflatoxin B_1 in DMSO in drinking-water three times a week for 27 weeks (total dose of aflatoxin B_1, 3.4 mg/animal) and 0, 3 or 6 mg/kg of water sodium selenite up to 79 weeks after the beginning of the experiment. Seven to 10 animals in each group were sacrificed at 18, 30, 38, 51 and 79 weeks. Malignant liver tumours (predominantly hepatocellular carcinomas) developed in 11/18 rats treated with aflatoxin B_1 alone but not in animals that received the combined treatment with aflatoxin B_1 and sodium selenite or in untreated controls. At each interim sacrifice, the number and size of aflatoxin B_1-induced preneoplastic foci of altered hepatocytes (basophilic-, acidophilic- and clear-cell foci) was reduced by additional administration of selenium. The inhibitory effect on the development of preneoplastic liver lesions was more pronounced at the low than at the high dose of selenium (Lei *et al.*, 1990).

Groups of 10–30 male Wistar rats, nine weeks old, were each given 0 or 42 μg aflatoxin B_1 (dissolved in DMSO prior to dilution in water) in drinking-water three times a week for 27 weeks (total dose of aflatoxin B_1 per rat, 3.4 mg at 27 weeks) with and without cultured extracts of *Rhizopus delemar* (an edible yeast). Unspecified numbers of rats were sacrificed 18, 30, 38 and 52 weeks after the beginnng of the experiment [number of surviving animals unspecified]. At 52 weeks, 5/7 rats that had received aflatoxin B_1 alone had liver neoplasms (two hepatocellular carcinomas, three combined hepatocellular–cholangiocellular carcinomas). No liver neoplasm was observed in rats treated with aflatoxin B_1 and extracts of *R. delemar*. Various types of foci of altered hepatocytes (as defined histologically or by histochemical demonstration of adenosinetriphosphatase, glucose-6-phosphatase and GGT) were seen in rats treated with aflatoxin B_1 for more than 18 weeks; the number and size of such foci were significantly lower ($p < 0.01$) in rats that received aflatoxin B_1 with extracts of *R. delemar* (Zhu *et al.*, 1989). [The Working Group noted the incomplete reporting.]

Female Wistar rats, 13 weeks of age, received a single intragastric dose of 5 mg/kg aflatoxin B_1 dissolved in 96.6% olive oil:3.5% DMSO. Three weeks later, 25 treated and 28 untreated rats were fed a diet containing nafenopin, the concentration of which was adjusted to provide a daily dose of 100 mg/kg bw; 24 aflatoxin-B_1 treated and 28 untreated rats were fed normal diet. Subgroups of nine rats killed after 55 weeks had hepatocellular adenomas at incidences of 0 (nafenopin only), 11% (aflatoxin only) and 44% (aflatoxin plus nafenopin); hepatocellular carcinomas occurred in 11% (nafenopin only), 0 (aflatoxin only) and 11% (aflatoxin plus nafenopin). Subgroups of 10–14 rats killed at week 70 had hepatocellular adenomas at incidences of 43% (nafenopin only), 0 (aflatoxin only) and 72% (aflatoxin plus nafenopin); hepatocellular carcinomas occurred at incidences of 29% (nafenopin only), 0 (aflatoxin only) and 45% (aflatoxin plus nafenopin). In a similar experiment, male Wistar rats, weighing 260–300 g, received a single dose of 2000 μg/kg aflatoxin B_1. At termination, after 55–59 weeks, the following liver tumour incidences were obtained: adenoma, 41% (nafenopin only, 17 rats), 0 (aflatoxin only, 22 rats), 80% (aflatoxin plus nafenopin, 10 rats); carcinoma, 29% (nafenopin only), 0 (aflatoxin only), 60% (aflatoxin plus nafenopin). No hepatic adenoma or carcinoma was found in 20 female or 18 male control rats. Foci of altered hepatocytes were checked at various times between 3 and

70 weeks in haematoxylin–eosin-stained sections from animals of each sex. Clear eosinophilic and tigroid foci were found in all aflatoxin-treated groups, the numbers and size increasing with time, but were rarely seen in controls. Nafenopin treatment had little effect on the prevalence of eosinophilic foci but decreased that of tigroid foci; it strongly (approximately 20 fold) enhanced the appearance of a further subpopulation of weakly basophilic foci, which were very rare in rats treated only with aflatoxin and in untreated controls (Kraupp-Grasl *et al.*, 1990).

Trout: Duplicate groups of 150 rainbow trout fingerlings were fed diets containing 0, 4, 8 or 20 μg/kg aflatoxin B_1 or 4 μg/kg of diet aflatoxin B_1 (purified) plus either 250 mg/kg gossypol, 50 μg/kg 3-methylcoumarin, 0.5% polymerized corn oil or 220 mg/kg cyclopropenoid fatty acids. Samples representing 10% of the fish in each group were taken after 3, 6, 9, 12 and 15 months. After administration of aflatoxin B_1 alone, the incidence of hepatomas increased with increasing dose and time (4 μg aflatoxin B_1: 6 months, 0/30; 9 months, 4/20; 12 months, 3/20; 15 months, 4/20; 8 μg aflatoxin B_1; 6 months, 2/30; 9 months, 5/20; 12 months, 8/20; 15 months, 17/20; 20 μg aflatoxin B_1: 6 months, 1/30; 9 months, 11/20; 12 months, 13/20; 15 months, 16/17). Cyclopropenoid fatty acids fed in the diet containing 4 μg/kg aflatoxin increased the incidence and growth of the hepatomas to 27/30 at 6 months, 20/20 at 9 months and 9/10 at 12 months. Gossypol and 3-methylcoumarin did not promote the early development of tumours induced by the diet containing 4 μg/kg aflatoxin B_1 but resulted in a higher incidence of hepatomas after 12 months (gossypol: 12 months, 6/20; 15 months, 12/20; 3-methylcoumarin: 12 months, 8/20; 15 months, 11/20). Polymerized corn oil did not enhance the carcinogenicity of aflatoxin B_1. No hepatoma was found in fish fed control diet or control diet plus 3-methylcoumarin (Sinnhuber *et al.*, 1968b).

Groups of 120 rainbow trout, 10 weeks of age, were fed diets containing 0 or 6 μg/kg aflatoxin B_1 and received 0 or 100 mg/kg of diet of a polychlorinated biphenyl (Aroclor 1254) for 12 months. Samples of fish were taken at different times from one to nine months, and the remaining animals were killed 12 months after the beginning of the experiment. The first liver neoplasms were observed at nine months (untreated controls, 0/10; aflatoxin B_1, 1/8; Aroclor 1254, 0/6; aflatoxin B_1 plus Aroclor 1254, 1/10). After 12 months, the incidence of hepatocellular carcinomas was significantly ($p < 0.001$) reduced in trout treated with aflatoxin plus the Aroclor 1254 compared to aflatoxin B_1 alone (controls, 0/68; aflatoxin B_1, 26/37; Aroclor 1254, 0/39; aflatoxin B_1 plus Aroclor 1254, 14/46). In addition, fewer tumours per liver were produced in trout on the combined treatment, and the tumours were smaller than those of trout treated with aflatoxin B_1 alone (Hendricks *et al.*, 1977).

Duplicate groups of 250 rainbow trout, six weeks old, were fed diets containing 0 or 6 μg/kg aflatoxin B_1 and received 0 or 5 mg/kg of diet dieldrin (purity, 99%) for 12 months. The first hepatocellular carcinomas were observed after nine months (untreated controls, 0/80; aflatoxin B_1, 19/80; dieldrin, 0/51; aflatoxin B_1 plus dieldrin, 31/80). There was no significant difference in the incidence of hepatocellular carcinomas induced by aflatoxin B_1 alone and by the combined treatment with aflatoxin B_1 and dieldrin (untreated controls, 0/147; aflatoxin B_1, 102/146; dieldrin, 0/149; aflatoxin B_1 plus dieldrin, 113/145) (Hendricks *et al.*, 1979).

Duplicate groups of 100 rainbow trout [age unspecified] were fed semi-purified diets containing 0, 1, 4 or 8 μg/kg aflatoxin with or without 50 mg/kg Aroclor 1254. In each group,

51–74 fish were sacrificed at nine months and 118–126 at 12 months. The presence of 50 mg/kg Aroclor 1254 at 9 and 12 months significantly ($p \leq 0.001$) inhibited the dose-dependent incidence of aflatoxin B_1-induced hepatocellular carcinomas (Shelton *et al.*, 1984).

Duplicate groups of 100 rainbow trout fingerlings were fed diets containing 0 or 20 μg/kg aflatoxin B_1 for two weeks and 0, 50 or 500 mg/kg β-naphthoflavone, 1000 mg/kg flavone, 1000 mg/kg tangeretin-nobilitin mixture, 1000 mg/kg indol-3-carbinol, 1000 mg/kg β-ionone or 2000 mg/kg of diet quercetin during and six weeks after the treatment with aflatoxin B_1. The fish were kept on basal diet for another 52 weeks. Samples of 20 fish were taken from each group at 32 and 45 weeks, and the remaining animals were killed 58 weeks after the end of aflatoxin B_1 exposure. At 58 weeks, the incidence of hepatic neoplasms (predominantly hepatocellular carcinomas, some basophilic hepatic foci included) induced by aflatoxin B_1 was significantly reduced by additional feeding with indol-3-carbinol ($p < 0.01$) and β-naphthoflavone (high-dose, $p < 0.01$; low-dose, $p < 0.05$); the other compounds were less effective (Nixon *et al.*, 1984).

Duplicate groups of 100 rainbow trout, 19 weeks old, were fed diets containing 0 or 20 μg/kg aflatoxin B_1 for four weeks and 0, 500 or 2000 mg/kg indol-3-carbinol either eight weeks before and during or 12 weeks after aflatoxin exposure. Samples of fish were taken 30, 40 and 52 weeks after cessation of aflatoxin B_1 exposure. The high dose of indol-3-carbinol given before and during aflatoxin exposure did not affect the incidence of hepatocellular carcinomas, whereas the same dose given after aflatoxin B_1 exposure significantly ($p < 0.0001$) increased the tumour incidence compared to that in fish treated with aflatoxin B_1 alone (Bailey *et al.*, 1987).

Triplicate groups of 150 rainbow trout fingerlings were administered ^{3}H-aflatoxin B_1 at 0, 10, 20, 40, 80, 160 and 320 μg/l of water and fed a diet containing 0, 1000, 2000, 3000 or 4000 mg/kg indol-3-carbinol four weeks prior to and during aflatoxin B_1 treatment. Groups of 15 trout were selected randomly and killed after 7 and 14 days of treatment with aflatoxin B_1; the remaining 120 fish from each group were observed up to 52 weeks after cessation of treatment. Administration of aflatoxin B_1 alone at doses of 10, 20, 40 and 80 μg/l of water resulted in incidences of liver tumours [histology unspecified] ranging from 10.7 to 68.4%. Tumour incidences in fish treated with aflatoxin B_1 plus indol-3-carbinol showed a dose-related reduction (Dashwood *et al.*, 1989). In another experiment, groups of 100 trout fingerlings were exposed to aflatoxin B_1 at 12.5, 25, 50, 100, 200 or 400 μg/l of water for 30 min and subsequently fed diets containing 0, 750, 1500 or 2000 mg/kg indol-3-carbinol for 24 weeks. The incidences of tumours [histological types unspecified] in fish treated with aflatoxin B_1 followed by indol-3-carbinol were increased compared to those treated with aflatoxin B_1 alone (Dashwood *et al.*, 1990).

Duplicate groups of 100 rainbow trout fry were exposed to aflatoxin B_1 at 12.5 μg/l of water, followed by a diet containing 0 or 2000 μg/kg indol-3-carbinol for three, six and nine months, beginning zero, three and six months after treatment with aflatoxin B_1 or administered on alternating months after aflatoxin B_1 administration. In addition, triplicate groups of 100 rainbow trout fry were exposed to aflatoxin B_1 at 50 μg/l of water, followed by a diet containing 0 or 2000 μg/kg indol-3-carbinol administered on alternating weeks or on two days each week. The experiment was terminated after 36 weeks. Trout exposed to

aflatoxin B_1 at 12.5 μg/l of water had a liver tumour incidence of 19.1%; fish exposed to the same dose of aflatoxin B_1 immediately followed by 2000 μg/kg indol-3-carbinol for 12, 24 and 36 weeks had average tumour incidences of 27.3, 41.7 and 53.9%, respectively, the incidence in the latter two groups being significantly increased ($p < 0.05$) compared to that in trout treated with aflatoxin B_1 alone. Trout exposed to aflatoxin B_1 at 12.5 μg/l of water, followed by 2000 μg/kg indol-3-carbinol after a delay of 4, 12 or 24 weeks had average liver tumour indidences, respectively, of 56.8, 53.9 and 33.3%, the incidence being significantly increased ($p < 0.05$) in the two groups with longer treatment. Trout exposed to aflatoxin B_1 at 12.5 μg/l of water, followed by 2000 μg indol-3-carbinol administered for alternating one- or three-month periods had tumour incidences of 35 and 43.6%, only the latter being significantly increased ($p < 0.05$). Exposure to aflatoxin B_1 at 50 μg/l of water followed by 0 or 2000 μg/kg indol-3-carbinol either on alternating weeks or on two days each week induced liver tumour incidences of 40.4, 63.9 and 62.0%, respectively. The liver tumour incidence in trout treated with aflatoxin B_1 alone was significantly ($p < 0.05$) higher than that in fish that received indol-3-carbinol (Dashwood *et al.*, 1991).

(ii) *Intraperitoneal administration*

Rat: Groups of 30 male Fischer 344 rats, four weeks old, were each given 0 or 100 μg aflatoxin B_1 in 0.2 ml saline (after dissolution in dimethylformamide) intraperitoneally and were then administered 0 or 1.5 mg corticosterone or hydrocortisone or 2 units of adrenocorticotropin intramuscularly twice a week for 10 weeks, and were sacrificed 65 weeks after beginning of treatment. The additional treatment with hormones resulted in a significant reduction in the incidence of aflatoxin B_1-induced hepatocellular carcinomas at 65 weeks: aflatoxin B_1 alone, 12/13; plus adrenocorticotropin, 9/27; plus hydrocortisone, 15/24; plus corticosterone, 12/23. In addition to hepatocellular carcinomas, neoplastic hepatic nodules and basophilic lesions were observed in several animals (Chedid *et al.*, 1980).

Groups of 20 male Fischer 344 rats, weighing 150 g, were administered a single intraperitoneal dose of 0, 62.5, 250 or 1000 μg/kg bw aflatoxin B_1 in DMSO; after an interval of two weeks, they were fed 200 mg/kg of diet 2-acetylaminofluorene for two weeks and were then partially hepatectomized at the end of the third week of the experiment. All rats were killed at the end of week four. Administration of aflatoxin B_1 resulted in a dose-dependent increase in the number (and size) of hyperplastic liver lesions (composed of acidophilic and basophilic cells) (Imaida *et al.*, 1981).

Groups of 100 well-fed and 120 malnourished (by food restriction and increased litter size during suckling up to 21 days after birth, resulting in a 60% decrease in body weight) male newborn Holtzmann rats were administered 500, 250 and 250 μg/kg bw of aflatoxin B_1 in DMSO intraperitoneally on days 10, 14 and 18, respectively, and were killed at between 30 and 65 weeks. The effective numbers of animals were 34/100 in the well-fed and 43/120 in the malnourished group. Neoplastic hepatic nodules were observed only in malnourished rats treated with aflatoxin B_1 between 46 and 55 weeks (malnourished, 3/13; well-fed, 0/8) and between 56 and 65 weeks (malnourished 6/17; well-fed, 0/15). The differences in the incidence of neoplastic hepatic nodules were significant ($p = 0.006$; Fisher's exact probability test). Phenotypically altered preneoplastic hepatic lesions (consisting of clear, acidophilic,

basophilic or vacuolated cells) were observed in both groups of aflatoxin B_1-treated rats but appeared earlier and progressed more rapidly in the malnourished rats (Rizvi *et al.*, 1987).

Groups of 10–15 male ACI/N rats, six weeks of age, were administered 0 or 1500 μg/kg bw aflatoxin B_1 in 0.2 ml DMSO intraperitoneally twice a week for 10 weeks (total dose of aflatoxin B_1, 30 mg/kg bw), followed, after an interval of one week, by oral administration of ethanol (10% in drinking-water) or plain water for 56 weeks. In order to demonstrate iron-excluding (preneoplastic) liver lesions, all rats were given subcutaneous injections of 125 mg/kg bw elemental iron three times a week for two weeks prior to killing at 67 weeks. The incidence of hepatocellular neoplasms was 3/15 (one hepatocellular carcinoma, two neoplastic nodules) in rats exposed to aflatoxin B_1 followed by ethanol and 0/14 in animals treated with aflatoxin B_1 alone. No hepatocellular tumour was observed in 15 vehicle controls given 10% ethanol in the drinking-water or in 10 vehicle controls. The number of iron-excluding hepatic foci per square centimetre of liver tissue section was 6.98 in rats treated with aflatoxin B_1 alone, 26.39 in rats treated with aflatoxin B_1 followed by ethanol, 0.11 in the vehicle controls treated with ethanol and 0.09 in the vehicle controls. Similar results were obtained by morphometric evaluation of foci of altered hepatocytes as detected in haematoxylin–eosin-stained tissue sections. The increase in the number (and total area) of preneoplastic hepatic foci in rats treated with aflatoxin B_1 followed by ethanol as compared to those treated with aflatoxin B_1 alone was significant ($p < 0.001$) (Tanaka *et al.*, 1989).

Groups of 13 and 18 male Fischer 344 rats, eight weeks old, were administered 150 μg/kg bw aflatoxin B_1 intraperitoneally five times a week for two weeks, submitted to partial hepatectomy three weeks after the last dose of aflatoxin B_1, were given 0 or 75 mg/kg bw phenobarbital by gavage during week 9 of the experiment and were sacrificed one day after the last dose of phenobarbital. The size and area of aflatoxin-induced GGT^+ hyperplastic hepatic nodules were not significantly affected by additional treatment with phenobarbital, which, however, induced the expression of the cytochrome P450 isozyme 2B1/2 in all nodules (Chen & Eaton, 1991).

Groups of 7–16 male Wistar rats, 60 days of age, were administered [route unspecified but assumed to be intraperitoneal] a single dose of 0 or 500 μg/kg bw aflatoxin B_1 in saline, were fed a diet containing 5, 15 or 40% casein 28 days prior to and four days after exposure to aflatoxin B_1, then fed the 15% casein diet for an additional 24 days, followed by 200 mg/kg of diet 2-acetylaminofluorene for a period of two weeks, in the middle of which a two-thirds partial hepatectomy was performed. All animals were killed 10 days after completion of the administration of 2-acetylaminofluorene. The number of foci of altered hepatocytes (as defined by GGT^+ staining or the placental form of glutathione *S*-transferase and calculated per liver) was increased significantly ($p < 0.05$) with increasing levels of casein in the diet following aflatoxin, while increasing levels of casein without aflatoxin did not increase the number of foci (Blanck *et al.*, 1992).

Trout: Groups of 25 rainbow trout fingerlings were given 0 or 50 μg/kg bw aflatoxin B_1 in propylene glycol and (3 h prior to each injection of aflatoxin B_1) 0 or 5 mg/kg bw of β-diethylaminoethyldiphenyl propylacetate hydrochloride (SKF-525A) intraperitoneally twice weekly for 25 weeks (total doses: aflatoxin B_1, 25 mg/kg bw; SKF-525A, 250 mg/kg bw). The experiment was terminated after 50 weeks. Survival after 25 weeks was 20/25 in the group treated with aflatoxin B_1 alone and 17/25 in the group that received the combined treatment.

Treatment with aflatoxin B_1 alone induced hepatocellular carcinomas in 16/20 fish. When SKF-525A was administered prior to each injection of aflatoxin B_1, the incidence of hepatocellular carcinomas was 1/17. Of the animals that did not develop hepatocellular carcinomas, 3/20 given aflatoxin B_1 alone and 2/17 given the combined treatment had proliferative basophilic hepatic foci (Scarpelli, 1976).

(iii) *Skin application*

Mouse: In a two-stage protocol, groups of eight female CD-1 mice, six weeks old, received a topical application to the skin of aflatoxin B_1 as a single dose of 25, 50 and 100 μg (dissolved in benzene) followed by 1000 μg croton oil (dissolved in acetone) twice weekly for 22 weeks. Two additional groups of eight mice received topical applications of doses of 100 and 500 μg twice weekly for 22 weeks after initiation with a single dose of 50 μg 7,12-dimethylbenz[*a*]anthracene (DMBA) dissolved in acetone. Animals treated with DMBA followed by croton oil served as positive controls and mice treated with the solvents alone as negative controls. Five to six of the eight animals that received aflatoxin B_1 as an initiator, followed by croton oil, developed skin papillomas. No animal receiving DMBA as an initiator, followed by aflatoxin B_1, developed a skin papilloma. All of the positive controls and none of the negative controls developed skin papillomas (Lindenfelser *et al.*, 1974).

(iv) *Exposure of fish eggs*

Trout: Groups of 200 rainbow trout eggs, 21 days after fertilization, were exposed to aflatoxin B_1 (added in 0.5 ml of 95% ethanol) at 0 or 500 μg/l of water for 1 h. After hatching, duplicate groups of 100 fry were fed semi-purified diet with or without 100 mg/kg Aroclor 1254 for one year. Samples of fish were taken at 9 and 12 months. At the end of 12 months, the incidences of aflatoxin B_1-induced hepatocellular carcinomas were essentially the same in fish additionally treated with Aroclor 1254 and those that were not: untreated controls, 0/107; aflatoxin B_1, 79/120; Aroclor 1254, 1/108; aflatoxin B_1 plus Aroclor 1254, 69/108 (Hendricks *et al.*, 1980b).

Duplicate groups of 100 rainbow trout embryos were exposed to a single dose of aflatoxin B_1 at 500 μg/l of water 21 days after fertilization; after hatching, they were fed diets containing 0, 0.03 or 0.3% butylated hydroxyanisole or 0.005 or 0.05% β-naphthoflavone for eight weeks. Fish were kept for up to one year after exposure to aflatoxin B_1. In trout treated with aflatoxin B_1 alone, the incidence of hepatocellular carcinomas was 83/191, and that of cholangiocellular carcinomas was 5/191. There was no significant difference in the incidence of liver tumours in trout treated with aflatoxin B_1 plus butylated hydroxyanisole at either dose level and in trout treated with aflatoxin B_1 alone. Administration of β-naphthoflavone at the high dose level to aflatoxin B_1-treated trout significantly ($p < 0.01$) enhanced the incidence of hepatocellular carcinomas (104/171) (Goeger *et al.*, 1988).

Groups of 200 rainbow trout eggs were exposed 21 days after fertilization to aflatoxin B_1 at 0, 5, 25 or 125 μg/l of water for 30 min. Six weeks later, duplicate groups of 60 trout from each group received 0 or 20 mg/kg of diet 17β-oestradiol for five weeks and, after a treatment-free interval of four weeks, were fed 0 or 10 mg/kg of diet 17β-oestradiol for up to nine months after the beginning of oestradiol treatment. At nine months, a low incidence of hepatocellular and/or mixed hepatocellular/cholangiocellular carcinomas (6/119) and of

hepatocellular adenomas (1/119) was observed in trout exposed as embryos to aflatoxin B_1 alone at the highest dose, but no liver neoplasm was found in the medium- and low-dose aflatoxin B_1 group at nine months. 17β-Oestradiol increased the incidence of liver carcinomas in fish exposed to aflatoxin B_1 as embryos at all dose levels (5 μg/l, 1/75; 25 μg/l, 8/90; 125 μg/l, 53/89). This increase was significant ($p < 0.001$) in the groups treated with the medium and high doses of aflatoxin B_1. Additional treatment with 17β-oestradiol of trout exposed as embryos to the highest dose level of aflatoxin B_1 also resulted in an increased incidence of hepatocellular adenomas and/or basophilic hepatic foci (aflatoxin B_1 alone, 1/119; aflatoxin B_1 plus 17β-oestradiol, 9/89). No neoplastic or preneoplastic liver lesion was observed in untreated controls or in fish treated with 17β-oestradiol alone (Núñez *et al.*, 1989).

In another experiment, groups of 200 rainbow trout eggs were exposed 21 days after fertilization to aflatoxin B_1 at 0 or 0.25 μg/l of water for 30 min. Five weeks later, duplicate groups of 70 fish were fed 0, 5, 10 or 15 mg/kg of diet 17β-oestradiol on alternate weeks up to nine months after the beginning of oestradiol treatment. 17β-Oestradiol significantly ($p < 0.05$) increased the incidence of hepatocellular carcinomas induced by aflatoxin B_1 (no oestradiol, 1/130; low-dose oestradiol, 7/136; medium-dose oestradiol, 7/140; high-dose oestradiol, 10/132) (Núñez *et al.*, 1989).

3.6.3 *Aflatoxin M_1*

Trout: Duplicate groups of 60 rainbow trout, 50 days old, were fed diets containing 0, 4, 16, 32 or 64 μg/kg aflatoxin M_1 or 4 μg/kg aflatoxin B_1 plus 0 or 100 mg/kg of diet cyclopropenoid fatty acid for 12 months, and then placed on basal diet. Random samples of fish were taken at 4, 8, 12 and 16 months. Administration of aflatoxin M_1 increased the incidence of hepatomas after 12 and 16 months at dose levels of 4 μg (12 months, 6/46; 16 months, 8/20), 16 μg (12 months, 30/43; 16 months, 13/20), 32 μg (12 months, 28/46; 16 months, 19/20) and 64 μg/kg of diet aflatoxin M_1 (12 months, 30/50; 16 months, 14/20). Treatment with aflatoxin M_1 at a dose of 4 μg/kg of diet produced a lower incidence of hepatomas than aflatoxin B_1 at 4 μg/kg of diet (12 months, 22/46; 16 months, 12/20). Additional administration of cyclopropenoid fatty acid increased the hepatocarcinogenic effect of both aflatoxin M_1 and aflatoxin B_1 given at a dose level of 4 μg/kg of diet. No neoplasm was found in fish fed control diet (Sinnhuber *et al.*, 1974).

In another experiment, groups of 50 rainbow trout, 50 days old, were fed diets containing 20 μg/kg aflatoxin M_1 and 0 or 100 mg/kg cyclopropenoid fatty acid for 5, 10, 20 or 30 days and then placed on basal diet. Samples of 20 fish were taken from each group at 8 months, and the remaining trout were sacrificed at 12 months. The incidence of hepatomas at 12 months was markedly enhanced by additional administration of cyclopropenoid fatty acid: treatment with aflatoxin M_1 plus cyclopropenoid fatty acid for five days, 3/28; 10 days, 10/27; 20 days, 16/32; 30 days, 20/26 (Sinnhuber *et al.*, 1974).

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Aflatoxins can cross the placental barrier in humans. In a study carried out in Songkhla, Thailand, during the dry season, Denning *et al.* (1990) showed that the concentrations of aflatoxins (B_1, G_1 and Q_1) were higher in cord sera obtained at birth than in maternal sera obtained immediately after birth. Seventeen of the 35 cord sera samples contained aflatoxin at concentrations between 0.064 and 13.6 nmol/ml. The sera of two of the mothers contained aflatoxin, the highest value being 1.22 nmol/ml. All of the women stated that they had eaten groundnuts, maize and mouldy food frequently, but the relatively low proportion who had aflatoxin in their sera reflects the fact that the dry season in this area is associated with low levels of aflatoxin contamination.

Aflatoxins M_1, M_2, B_1, B_2, G_1 and G_2 have been detected in cord blood samples from Ghana (63/188, 34%) and aflatoxins M_1, M_2 and B_2 in cord blood from Nigeria (9/78, 12%). Higher levels of aflatoxins M_1 and M_2 were detected in cord blood than in maternal blood samples collected simultaneously in Nigeria, suggesting that aflatoxins may accumulate in fetuses exposed to these toxins *in utero*; aflatoxin B_1 was found only in maternal blood in Nigeria (Lamplugh *et al.*, 1988).

Lactating African women (in the Sudan, Ghana, Kenya and Nigeria) exposed to aflatoxins in the diet secrete principally aflatoxin M_1 in their milk, less frequently aflatoxin M_2 and even less frequently aflatoxins B_1, B_2, G_1 and G_2 (Maxwell *et al.*, 1989). In Ghana, aflatoxin M_1 was detected in mothers' milk most frequently at concentrations ranging from 20 to 1816 ng/l and more often in the wet season (41%) than in the dry season (28%); the mean concentration of aflatoxin M_1 was higher in the wet season (445 ng/l) than in the dry season (293 ng/l). Aflatoxins M_2, B_1 and B_2 were also detected (Lamplugh *et al.*, 1988).

Aflatoxin M_1 is present in the urine of humans exposed to aflatoxin B_1, at levels correlated with that of intake of aflatoxin B_1 (Campbell *et al.*, 1970; Zhu *et al.*, 1987).

Evidence that humans activate aflatoxin B_1 *in vivo* is provided by the detection of bound aflatoxins in serum albumin samples (Gan *et al.*, 1988) and by the excretion of aflatoxin B_1-guanine adducts (Autrup *et al.*, 1983; Groopman *et al.*, 1985; Autrup *et al.*, 1987). In the Gambia, 30 pregnant women were recruited, and sera from umbilical cord and maternal venous blood were assayed for aflatoxin–albumin adducts immediately after delivery. There was a highly significant correlation between adduct levels in maternal venous blood and matched cord sera ($r = 0.52, p = 0.001$) (Wild *et al.*, 1991). In one study, hepatitis B virus carriers had higher levels of aflatoxin–serum albumin adducts (mean, 4.41) than non-carriers (mean, 4.04) or uninfected people in the same village in the Gambia (Allen *et al.*, 1992). Human urine samples from exposed Chinese populations contained aflatoxin B_1–*N*7-guanine at a range of 0.1–10 ng/ml, in addition to aflatoxin M_1 and P_1 (Groopman *et al.*, 1985); the adduct has also been detected in the urine of African populations (Autrup *et al.*, 1983, 1987).

Several studies (Gan *et al.*, 1988; Groopman *et al.*, 1992a; Wild *et al.*, 1992a) have reported dose–response characteristics for aflatoxin–albumin and –guanine adducts. Dose

was calculated from individuals' foods, and specific adduct markers were measured (see also section 4.4.1). Although aflatoxin P_1 is a major human urinary metabolite of aflatoxin B_1, its level of excretion correlated poorly with aflatoxin B_1 ingested by 30 men and 12 women in a study of exposed Chinese individuals, whereas aflatoxin B_1–guanine and aflatoxin M_1 excretion showed strong positive correlations (Groopman *et al.*, 1992a). Correlations have been demonstrated between aflatoxin B_1–serum albumin adduct levels, aflatoxin B_1 intake and the urinary excretion of aflatoxin M_1 (Gan *et al.*, 1988).

Aflatoxin G_1 was present in the urine of people in the Gambia who were exposed to this aflatoxin in the diet; no aflatoxin G_1 metabolites were found (Groopman *et al.*, 1992b).

4.1.2 *Experimental systems*

Aflatoxin B_1 penetrated isolated human epidermis (stratum corneum plus viable epidermis) *in vitro*. The rate of penetration was low under non-occluded conditions but was approximately 40 times greater under conditions of occlusion (Riley *et al.*, 1985).

Human liver microsomes activate aflatoxin B_1 *in vitro* to DNA binding species; hydrolysis of the adducts to yield aflatoxin B_1 8,9-dihydrodiol indicates the formation of aflatoxin B_1 8,9-epoxide as an intermediate (Swenson *et al.*, 1974). Metabolism of aflatoxin B_1 *in vitro* by human liver microsomes produced aflatoxin Q_1 (70–90% of the soluble metabolites) and aflatoxin B_1 8,9-dihydrodiol (10–30%). Aflatoxin M_1 was also detected in traces. Human liver cytosols have a low capacity to catalyse the formation of an aflatoxin B_1–glutathione conjugate (Moss & Neal, 1985). Human liver microsomes activate aflatoxin G_1 at a rate two to three times less than aflatoxin B_1 to form aflatoxin G_1–*N*7-guanine adducts (Baertschi *et al.*, 1989).

Human liver cytosol fractions from 24 healthy individuals with μ class glutathione *S*-transferases are more effective in reducing aflatoxin B_1–DNA binding than are corresponding fractions deficient in this class of enzyme, assayed using *trans*-stilbene oxide as substrate (Liu *et al.*, 1991). Activity in lymphocytes was found to be correlated with that in the liver. In the population studied, about 65% had higher glutathione *S*-transferase activity ($>$ 100 pmol/min per 10^7 cells) and 40% had lower activity ($<$ 100 pmol/min per 10^7 cells).

The cytochrome P450IIIA4 (nifedipine oxidase) plays a major role in the activation of aflatoxin B_1 (Shimada & Guengerich, 1989); other cytochromes P450 are also active (Forrester *et al.*, 1990).

The metabolic activation of aflatoxin B_1 in human adult and fetal liver to a mutagenic metabolite has been examined using homogenates of fetal liver and microsomes from adult liver. Mutation in the *umu* gene in a plasmid-containing strain of *Salmonella typhimurium* was induced by both adult and fetal systems (Kitada *et al.*, 1990). Antibody inhibition studies indicated the involvement of cytochrome P450IIIA4 in the adult system and of a homologous isozyme (P450 HFLa) in the fetal system.

Human bronchus and colon in culture metabolize aflatoxin B_1 to a DNA binding species, bronchus being more active than colon; the adducts formed are aflatoxin B_1–*N*7-guanine (8,9-dihydro-8-(*N*7-guanyl)-9-hydroxyaflatoxin B_1) and the open-ring aflatoxin B_1 (8,9-dihydro-8-(*N*5-formyl-2′,5′,6′-triamino-4′-oxo-*N*5-pyrimidyl)-9-hydroxyaflatoxin B_1) (Autrup *et al.*, 1979) (see Fig. 2).

Aflatoxin B_1 was absorbed via the skin in rats (Wei *et al.*, 1970). Aflatoxins are absorbed from the gut of sheep (Wilson *et al.*, 1985) and rats (Kumagai, 1989) and are transported *via* blood, and not by the lymphatic system. Absorption after intratracheal instillation in rats is more rapid than after an oral dose, but the body distribution and excretion patterns are not affected by route of administration (Coulombe & Sharma, 1985). If the tracheally administered dose is adsorbed onto dust, binding to lung and tracheal DNA is increased and retention of aflatoxin B_1 in the trachea is prolonged (Coulombe *et al.*, 1991).

The patterns of aflatoxin B_1 metabolic activation, DNA adduct formation and excretion in liver and kidneys of rats have been reviewed (Essigmann *et al.*, 1982).

Aflatoxin B_1 incubated with rat plasma or administered intraperitoneally binds noncovalently, primarily to albumin which is probably the major transport protein for aflatoxin B_1 (Dirr & Schabort, 1986). A low, non-toxic intraperitoneal dose (0.7 μg/kg bw) of aflatoxin B_1 given to rats is taken up into blood, plasma and liver in a biphasic manner (rapid, 0–2 h, followed by slow, 2–12 h) (Ewaskiewicz *et al.*, 1991).

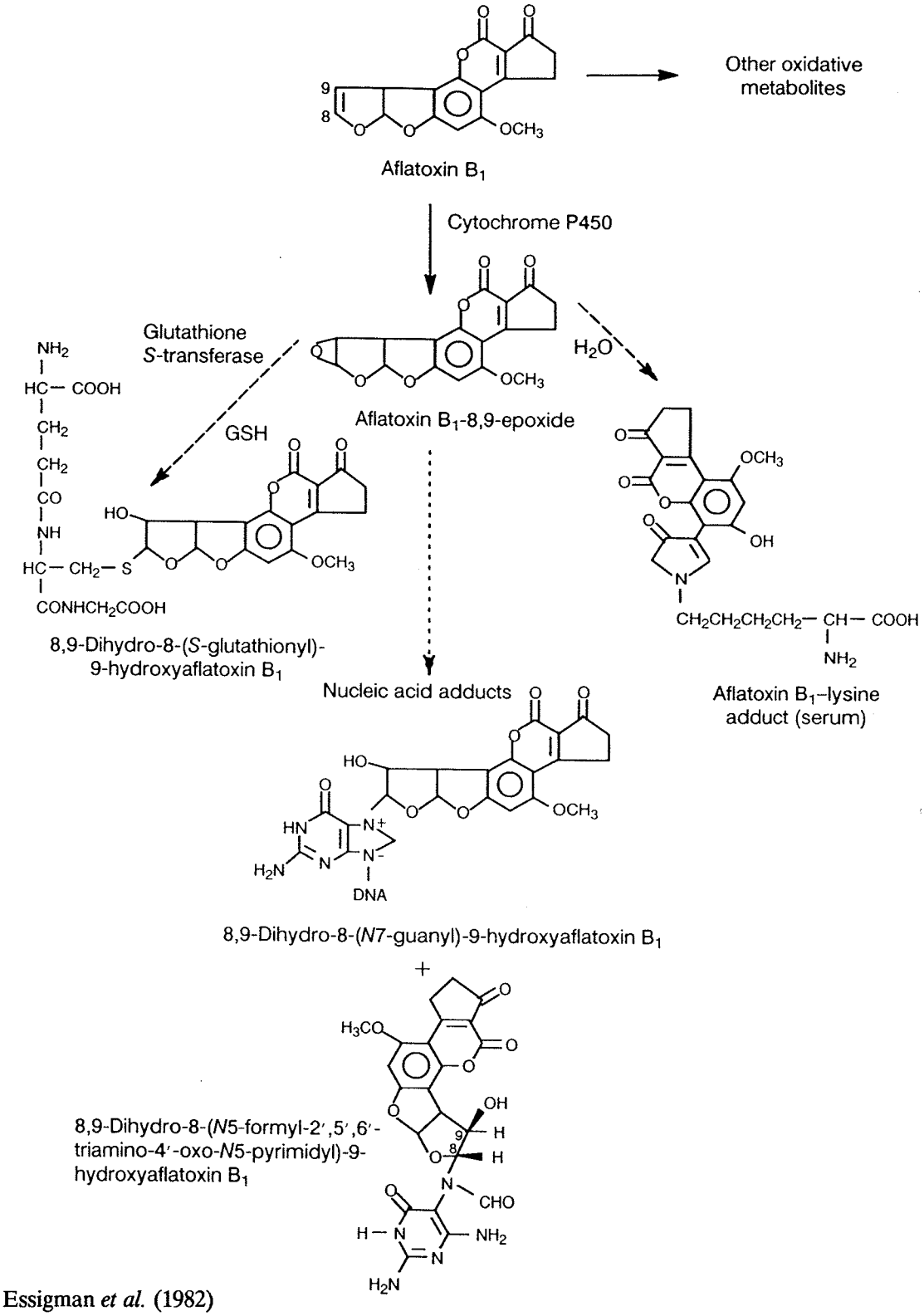
Multiple binding proteins for aflatoxin B_1 exist in rat liver cytosol (Taggart *et al.*, 1986), some of which may facilitate movement of aflatoxin B_1 within the cell (Mainigi & Sorof, 1977). Rats exposed to ^{14}C-aflatoxin B_1 (2 μCi; 20 μg) intraperitoneally secrete principally ^{14}C-aflatoxin M_1 in the milk. Aflatoxin M_1 accumulates in the liver and lung of offspring, accompanied by macromolecular binding to protein and RNA. Binding to DNA was not detected by radiochemical assay (Allameh *et al.*, 1989).

Aflatoxin M_1 is excreted in the milk of a range of mammalian species (sheep, goats, cows) exposed to aflatoxin B_1 within the order of a 1% conversion level of the toxin (Nabney *et al.*, 1967; Wogan, 1969b; Applebaum *et al.*, 1982; Goto & Hsieh, 1985).

Aflatoxin B_1 as well as aflatoxin M_1 have been shown to be concentrated in the liver of rats 30 min after an intraperitoneal or oral dose of 7 mg/kg bw ^{14}C-aflatoxin B_1; at 24 h, both aflatoxins were detected only as traces (Wogan, 1969b). Whole-body autoradiographic studies in mice have shown that aflatoxin B_1 and/or its metabolites are concentrated in nasal glands (Larsson *et al.*, 1990), in pigmented eye cells (Larsson *et al.*, 1988) and in the pigment of the Harderian glands (Arora *et al.*, 1978). In-vitro studies using bovine melanin have shown that unmetabolized aflatoxin B_1 binds reversibly to the pigment (Larsson *et al.*, 1988).

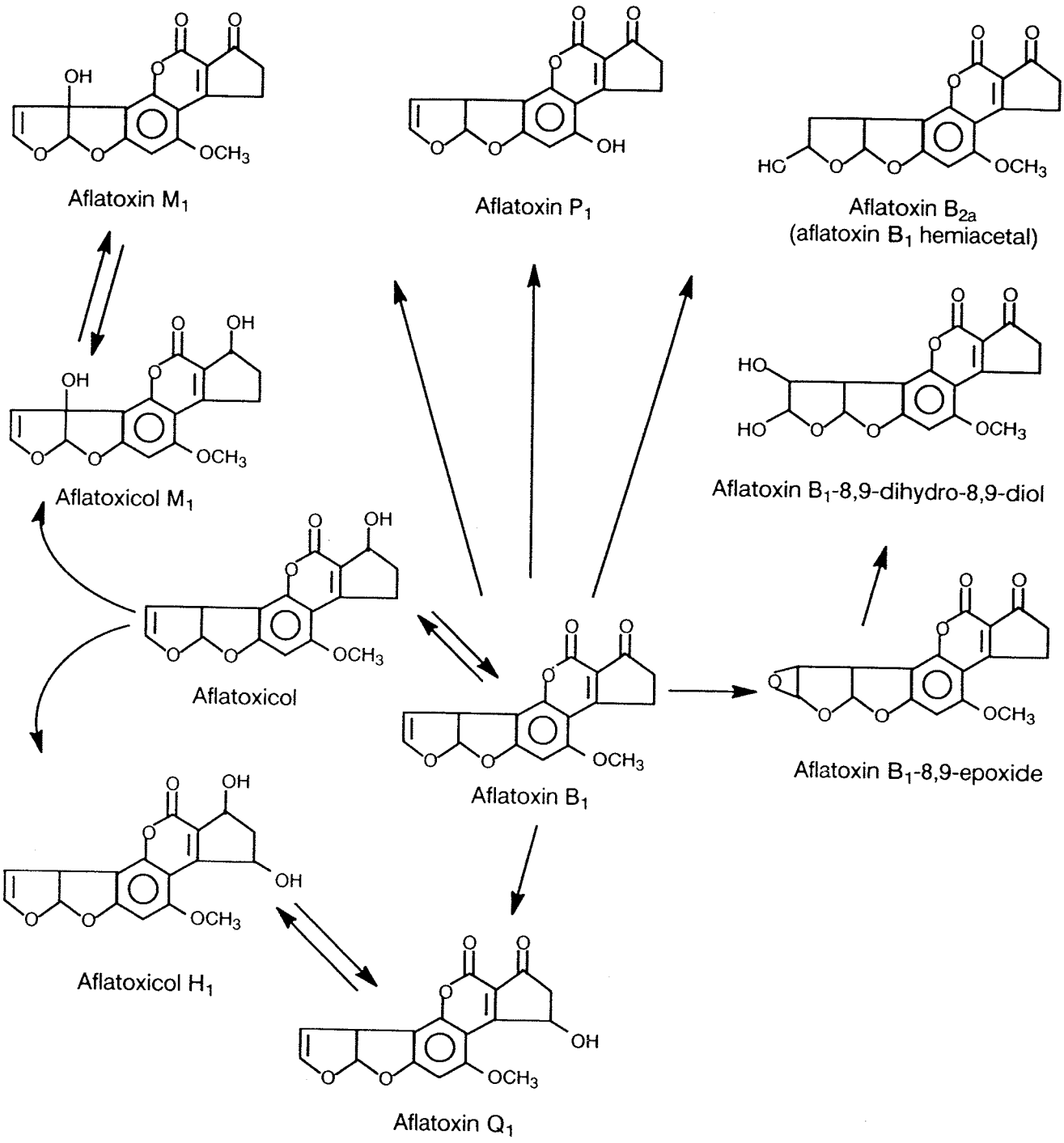
Aflatoxin B_1 is metabolized to a range of metabolites by microsomal systems (Figs 2 and 3), including aflatoxins P_1, M_1 and Q_1 and the presumed active metabolite aflatoxin B_1 8,9-epoxide, but there is considerable variation in the spectrum of metabolites between species. Aflatoxin P_1 is the major liver microsomal metabolite in mouse but not rat microsomal systems *in vitro*, whereas aflatoxin Q_1 shows the reverse association (Dahms & Gurtoo, 1976). Aflatoxicol is produced by cytosolic enzymes, whereas aflatoxicol H_1 and M_1 are produced by a combination of cytosolic and microsomal enzymes (Wogan, 1973; Essigmann *et al.*, 1982; Ueno *et al.*, 1985). Species that are more resistant to the carcinogenic effect of aflatoxin B_1 produce more aflatoxin P_1 (which is often excreted in conjugated form (Dalezios *et al.*, 1971)) and have less aflatoxicol in their plasma. Resistant mice excrete ten times more aflatoxin P_1 than sensitive rats. Aflatoxicol was a major aflatoxin B_1 metabolite detected in the serum of rats 50 min after intravenous injection of ^{14}C-aflatoxin B_1, whereas the metabolite was not detected in mouse or monkey serum (Wong & Hsieh, 1980).

Fig. 2. Metabolic activation of aflatoxin B_1 to the 8,9-epoxide, leading to binding to glutathione, DNA and serum albumin



From Essigman *et al.* (1982)

Fig. 3. Major metabolites of aflatoxin B_1



From Essigmann *et al.* (1982)

Susceptibility to acute toxicity has been suggested to be correlated with glutathione conjugating activity (O'Brien *et al.*, 1983; Lotlikar, 1989), which depends on the presence of appropriate glutathione *S*-transferases (Neal *et al.*, 1987; Ramsdell & Eaton, 1990; Hayes *et al.*, 1991). The majority of the preneoplastic focal liver lesions (as defined by morphology and immunohistochemical demonstration of glutathione *S*-transferases) induced by aflatoxin B_1 in rainbow trout showed deficient (67%) or normal (12%) expression of glutathione *S*-transferases; in the remaining foci of altered hepatocytes (21%), increased expression of glutathione *S*-transferase was observed. Advanced neoplasms were either deficient or normal in their expression of glutathione *S*-transferases (Kirby *et al.*, 1990). The relative levels of DNA binding of aflatoxin B_1 in rats *in vivo* correlate inversely with glutathione–aflatoxin B_1 conjugating activity in the cytosol (Kensler *et al.*, 1986). Primary metabolites can also be conjugated as sulfates (Dalezios *et al.*, 1971) or glucuronides (Rohrig & Yourtee, 1983). The production of primary microsomal metabolites, including activated epoxide, can be influenced by the induction of cytochrome P450 or P448 (Metcalfe *et al.*, 1981). Mitochondrial P450s can also activate aflatoxin B_1 (Niranjan *et al.*, 1984). Aflatoxin B_1 dihydrodiol is formed by the hydration of aflatoxin B_1 epoxide and at neutral pH forms a protein-binding species by Schiff's base reaction (Neal & Colley, 1979). *In vivo*, aflatoxin B_1 is adducted to the lysine of serum albumin by Schiff's base reaction (Sabbioni *et al.*, 1987). The hemiacetal aflatoxin B_{2a} has been reported as a microsomal metabolite, but this result may have been due in part to misidentification of aflatoxin B_1–8,9-dihydrodiol (Neal *et al.*, 1981a).

In many species—rats (Groopman *et al.*, 1985), sheep (Masri *et al.*, 1967), pigs (Lüthy *et al.*, 1980) and cows (Allcroft *et al.*, 1968)—aflatoxin M_1 is the main unconjugated metabolite of aflatoxin B_1 found in the urine and accounts for 2–9% of the dose. In the urine of rhesus monkeys, aflatoxin M_1 was reported to account for 2.3% of an intraperitoneal dose of aflatoxin B_1 and conjugated aflatoxin P_1 for > 20% of the injected dose (60% of the urinary metabolites: 50% as glucuronide, 10% as sulfite and 3% unconjugated) (Dalezios *et al.*, 1971).

In the DNA of exposed rats, aflatoxin B_1 forms a major adduct, aflatoxin–*N*7-guanine, following metabolic epoxidation. It is released from the DNA by depurination and is excreted in the urine, predominantly over 24 h after exposure, in a dose-dependent manner (Groopman *et al.*, 1992c). Rats administered 1 mg/kg bw aflatoxin B_1 by intraperitoneal injection excreted this adduct in 48 h, which accounted for 30–40% of the initial DNA binding present in the liver (Bennett *et al.*, 1981).

More aflatoxin B_1 metabolites are usually excreted in rat faeces than in urine after intraperitoneal injection of ^{14}C-ring-labelled aflatoxin B_1 (Wogan, 1969b). Excretion of glutathione conjugates of aflatoxin B_1 in rats occurs almost exclusively through the bile; 14% of an intraperitoneally injected dose was disposed of by this route (Emerole, 1981). Degradation of aflatoxin B_1–glutathione conjugate by enzymes of the mercapturic acid pathway has been described in rat kidney preparations *in vitro* (Moss *et al.*, 1985), and the level of urinary excretion of aflatoxin B_1–mercapturate, together with the sulfate and glucuronide conjugates, correlates with species sensitivity to aflatoxin B_1 (Raj & Lotlikar, 1984).

Aflatoxin B_2 is metabolically converted to aflatoxin B_1 in rats following an intraperitoneal dose of 1 mg/kg bw aflatoxin B_2. The resulting aflatoxin B_1 can then be activated to form aflatoxin B_1–*N*7-guanine adducts in the liver (Groopman *et al.*, 1981a).

In-vitro studies using rat-derived metabolizing systems indicate a decreased capacity to induce DNA binding, overall decreased metabolism but increased aflatoxicol production with age (Jayaraj *et al.*, 1985).

4.2 Toxic effects

4.2.1 *Humans*

Attempted suicide by ingestion of a mixture of aflatoxins (a total of 1.5 mg/kg over two weeks), containing 15–45% aflatoxin B_1 caused no major symptoms of poisoning (Willis *et al.*, 1980).

Cell-mediated resistance to malaria is increased in individuals exposed to aflatoxins (Hendrickse & Maxwell, 1989).

Aflatoxicosis (jaundice, fever, ascites, oedema of the feet and vomiting) in India was associated with consumption of maize heavily contaminated with *Aspergillus flavus* and containing 6.25–15.6 ppm (mg/kg) aflatoxin. Fatalities occurred in individuals who may have consumed 2–6 mg aflatoxin daily over a period of one month; 106 people died out of a total of 397 patients. Men appeared to be more susceptible than women; no infant was affected. As many as 200 villages were involved in the episode (Krishnamachari *et al.*, 1975). Three- and five-year follow-ups, involving liver biopsies, showed almost complete recovery from acute poisoning (Tandon & Tandon, 1989).

In Kenya, an outbreak of jaundice, accompanied by fatalities, was associated with the consumption of maize which contained up to 12 mg/kg aflatoxin B_1. Livers showed centrolobular necrosis (Ngindu *et al.*, 1982).

A case report of fatal hepatic toxicity in a 15-year-old boy was linked to the consumption of mouldy cassava. Histological changes in the liver were similar to those observed in monkeys treated with aflatoxin (Alpert *et al.*, 1970) and consisted of centrilobular lesions in the liver characterized by loss of retainable cytoplasm, with areas of liver cell necrosis, polymorphonuclear neutrophil infiltration and mild fatty changes in midzonal cells. Two siblings were taken ill at the same time as the affected boy. Analysis of the mouldy cassava in the family's store showed a high aflatoxin content (1.7 mg/kg), and it was concluded that 3.1 kg of cassava was sufficient to produce a lethal dose of aflatoxin (Serck-Hanssen, 1970).

Ingestion of aflatoxin B_1 has been suggested as a possible cause of cirrhosis in Indian children (Amla *et al.*, 1971), but other studies of liver cirrhosis using similar thin-layer chromatographic methods in urine and blood assays failed to detect the fluorescent compounds reported in the earlier studies (Bhandari & Bhandari, 1980). Aflatoxin B_1 was detected in the urine of only 2/35 children with cirrhosis and in 1/35 controls (Dhatt *et al.*, 1982).

Exposure to aflatoxins has been implicated in the etiology of kwashiorkor (Hendrickse & Maxwell, 1989); 5 of 16 liver biopsy samples from children with kwashiorkor contained aflatoxins B_1, B_2 or aflatoxicol; none of 10 samples from children with marasmus did so. The levels of aflatoxins detected were: aflatoxin B_1, 32 and 33 ng/g liver; aflatoxin B_2, 2 ng/g liver; and aflatoxicol, 1 and 4 ng/g liver (Coulter *et al.*, 1986).

Blood and urine samples from 252 Sudanese children were investigated for aflatoxin content. Aflatoxins were detected more frequently (36%) and at higher levels (706 pg/ml) in sera from children with kwashiorkor than in controls (16% and 77 pg/ml); aflatoxicol was frequently found in these samples (14%), but rarely in children who did not have kwashiorkor. A higher proportion of urine samples from kwashiorkor patients contained detectable levels of aflatoxin (33%) than controls (20%), but the mean concentration was lower than in the other children in the study (143 pg/ml, controls 191 pg/ml) (Hendrickse *et al.*, 1982). Children with kwashiorkor placed on aflatoxin-free diet excreted large amounts of aflatoxins in the faeces for up to nine days (Hendrickse & Maxwell, 1989).

The etiology of the development of symptoms of encephalitis and fatty degeneration of the viscera (EFDV), which involves a high level of mortality (similar to Reye's syndrome), was studied in Thai children. Samples were obtained at autopsy from 23 children who had died with symptoms of EFDV and from 15 children who had died from other causes. For the 23 children who had died with EFDV, the presence of aflatoxin B_1 was confirmed in 2/21 liver samples (at levels of 47 and 93 μg/kg), and blue fluorescent spots in chromatograms similar to those produced by aflatoxin B_1 were seen in 15 other samples, suggesting the presence of trace amounts (1–4 μg/kg) of aflatoxin B_1. Of the 15 children who had died from other causes, eight had similar trace amounts of aflatoxin B_1. In a separate study, the urine of eight of 51 children with EFDV contained trace amounts of aflatoxin B_1. A spot similar to that produced by aflatoxin B_2 was seen in one sample, and a spot similar to that of aflatoxin M_1 was detected in two samples. No compound similar to aflatoxins was detected in urine samples from 39 healthy children (Shank *et al.*, 1971).

4.2.2 *Experimental systems*

The order of potency for both the acute and chronic toxicity of aflatoxins is $B_1 > G_1 > B_2 > G_2$ (Busby & Wogan, 1984).

Species that are more sensitive to the acute toxic effects of aflatoxin B_1 (LD_{50}: monkey, 2.2 mg/kg bw; rat, 5.5–17.9 mg/kg bw) show higher distribution volumes, higher levels of aflatoxins in plasma and liver and longer plasma half-lives than less sensitive species (mouse, 9.0–60.0 mg/kg bw) (Wong & Hsieh, 1980).

Liver lesions occurred in rabbits after percutaneous absorption of aflatoxins B_1 and B_2 at doses of 160–1250 μg/kg bw, and severe glycogen depletion was seen. After topical administration of aflatoxins at > 1400 μg/kg bw, midzonal necrosis was seen, accompanied by fatty changes, in 8/10 treated rabbits, and hyaline-acidophilic changes in the cytoplasm were seen in liver cells. No lesion was observed in the livers of rabbits treated with the vehicle alone or with a dose of aflatoxins < 50 μg/kg bw (Ungar & Joffe, 1969).

The liver is the usual target organ for both acute and chronic toxicity, but lesions in the kidney and glandular stomach have been reported in rats (Butler & Barnes, 1966, 1968). Ethanol ingestion has been found to potentiate aflatoxin B_1 hepatotoxicity (Toskulkao *et al.*, 1986). Ammoniation of aflatoxin B_1-contaminated maize or groundnut meal fed to rats was effective in reducing hepatotoxicity (Norred & Morrissey, 1983; Manson & Neal, 1987). Low-protein diets increased the acute toxic effects of aflatoxin B_1 (Madhavan & Gopalan, 1965). For data on the effects of dietary constituents on the formation of aflatoxin–guanine adducts, see p. 336.

Aflatoxin B_1 has been reported to affect both humoral and cell-mediated immune systems (for reviews, see Pier & McLoughlin, 1985; Richard, 1991). Direct and complex effects of aflatoxins on lymphocytes have been reported in mice, with differing sensitivities in various sub-sets. Inhibitory effects noted on T and natural killer cells, which would compromise cell-mediated immunity (Reddy & Sharma, 1989), were correlated with inhibition of ^{3}H-thymidine uptake in lymphocyte cultures (Reddy *et al.*, 1987) and inhibition of the humoral immune system by aflatoxin B_1 in mice (Reddy *et al.*, 1983) and in a range of other species, such as birds, pigs, horses and rabbits (Ray *et al.*, 1991). Aflatoxins B_1 and M_1 are particularly potent in reducing phagocytosis and superoxide production in rat peritoneal macrophages (Cusumano *et al.*, 1990). Exposure to aflatoxin B_1 resulted in an exaggerated response to mitogens in mouse lymphocytes (Hendrickse & Maxwell, 1989).

Symptoms similar to those of Reyes syndrome were observed in adult macaque monkeys given aflatoxin B_1; fatty degeneration of the liver was the most consistent pathological finding (Bourgeois *et al.*, 1971).

Protein kinase C and phospholipase C in human platelets are activated by aflatoxin B_1 *in vitro* (Van Den Heever & Dirr, 1991).

4.3 Reproductive and developmental toxicity

4.3.1 *Humans*

Aflatoxins cross the placental barrier, and there is some evidence that concentrations in cord blood are higher than in maternal blood (see section 4.1.1).

4.3.2 *Experimental systems*

Sixteeen intraperitoneal injections of approximately 60 μg/kg bw aflatoxin B_1 to rats induced pronounced testicular degeneration and impaired spermatogenesis (Egbunike *et al.*, 1980).

Groups of 8–12 pregnant JCl-ICR mice were treated intraperitoneally with 16 or 32 mg/kg bw aflatoxin B_1 for various two-day period during days 6–13 of gestation. Fetuses were examined on day 18 of gestation. Maternal death, decreased body weight and increased kidney weight were observed in the group given 32 mg/kg bw. Reduced fetal weight and external malformations, such as cleft palate and open eyelids, and skeletal malformations, such as wavy ribs and bent long bones, were also seen in this group (Tanimura *et al.*, 1982).

Groups of 19–36 NMRI mice were treated with doses of 0, 15, 45 or 90 mg/kg bw aflatoxin B_1 intraperitoneally or with 45 mg/kg bw orally on days 12 and 13 of pregnancy. The intraperitoneal treatment with 45 or 90 mg/kg bw aflatoxin B_1 produced retardation in fetal development; incidences of cleft palate were higher than in controls (control, 1.5%; 45 mg/kg, 4.1%; 90 mg/kg, 5.6%). No corresponding effect was seen after oral treatment. At a dose of 45 mg/kg bw, diaphragmatic malformations were observed after either intraperitoneal (18%) or oral (13%) administration. Intraperitoneal injection of aflatoxin G_1 at 90 mg/kg bw induced malformations of the diaphragm (14.7% of fetuses) and kidney (5.5%). No such anomaly was seen in controls (Roll *et al.*, 1990).

A group of eight CBA mice was treated by oral intubation on day 8 of pregnancy and seven mice on day 9 with 4 mg/kg bw aflatoxin B_1. Fetal anomalies—exencephaly (4), open

eyes (3) and protrusion of intestines (2)—were observed in 7/61 fetuses exposed on day 8 and in none of the 51 fetuses exposed on day 9 (Arora *et al.*, 1981).

Groups of 10 pregnant rats (*Rattus norvegicus*) were treated subcutaneously with 0.7, 1.4, 3.5 or 7.0 mg/kg bw aflatoxin (75% B_1, 25% B_2) on either day 8 or day 16 of pregnancy. Average fetal weight was decreased, and fetuses showed wrinkled skin and slightly enlarged heads in comparison with controls. Malformations were not observed (Sharma & Sahai, 1987).

A significant increase in liver triglyceride content was found in one- and two-month-old Fischer rat offspring of dams that had been treated intraperitoneally with 2 mg/kg bw aflatoxin B_1 during days 8–10 of pregnancy. Treatment on days 8–10 or on days 15–17 induced a decrease in motor activity in one-month-old offspring. At the age of two or three months, behaviour became normal, but there was persistent neuronal degeneration in the brains of the offspring (Chentanez *et al.*, 1986).

In day-10 rat embryos exposed *in vitro* to aflatoxin B_1 at 15 μM [4.7 mg] or more, neural tube defects occurred. The presence of hepatic fractions from induced adult male rats had no effect on the ability of the compound to produce dysmorphogenesis, but it enhanced the embryolethal effects (Geissler & Faustman, 1988).

4.4 Genetic and related effects (see also Tables 10–19 and Appendices 1 and 2)

4.4.1 *Humans*

(*a*) *Urinary excretion of guanine adducts*

Aflatoxin B_1–guanine adducts were reported to have been detected in human urine in a previous IARC monograph (IARC, 1987a). Further studies have been published since then.

The diets of 30 men and 12 women (age range, 25–64 years) living in Guangxi Autonomous Region, China, were monitored for one week and aflatoxin intake levels determined daily. Starting on the fourth day, total urine volumes were obtained in consecutive 12-h fractions for three or four days. Each urine sample was analysed by high-performance liquid chromatography (HPLC) and radioimmunoassay, and the relationships between excretion of total aflatoxin metabolites, aflatoxin–*N*7-guanine, aflatoxin M_1, aflatoxin P_1 and aflatoxin B_1, and aflatoxin B_1 intake levels were determined. The average intake of aflatoxin B_1 was 48.4 μg/day by men and 77.4 μg/day by women, giving total mean exposures over seven days of 276.8 and 542.6 μg aflatoxin B_1, respectively. Linear regression analysis of the association between daily aflatoxin B_1 intake and daily total aflatoxin metabolite excretion gave a non-significant correlation coefficient (r) of 0.26. Aflatoxin–*N*7-guanine excretion and aflatoxin B_1 intake from the previous day, however, gave $r = 0.65$ ($p < 0.000001$). Comparison of total aflatoxin–*N*7-guanine excretion throughout the collection period with total dietary aflatoxin B_1 for each individual, smoothing day-to-day variations, gave $r = 0.80$ ($p < 0.0000001$) (Groopman *et al.*, 1992a).

Daily aflatoxin intake levels were measured for one week in the diets of 10 men and 10 women (age range, 15–56 years) living in a village in the Gambia. Starting on the fourth day, total 24-h urines were obtained for four days. The subjects were also tested for hepatitis B virus carrier status. Preparative monoclonal antibody affinity chromatography/HPLC and

competitive enzyme-linked immunosorbent assays (ELISA) were performed on each urine sample, and the relationship between aflatoxin intake levels and urinary excretion of all aflatoxin metabolites and of aflatoxin–*N*7-guanine was determined. The average intake of all aflatoxins during the one-week collection period was 8.2 μg for men and 15.7 μg for women [these are almost certainly underestimates of the true intake (Wild *et al.*, 1992a)]. There was considerable day-to-day variation in exposures, from zero to 29.6 μg aflatoxins. Linear regression analysis of the association between urinary excretion of aflatoxins and average daily aflatoxin intake gave $r = 0.65$ ($p < 0.001$). HPLC revealed a preponderance of aflatoxin G_1 in many of the urine samples, in addition to the metabolites aflatoxin P_1 and aflatoxin Q_1 and to aflatoxin–*N*7-guanine adducts. Regression of total urinary excretion of aflatoxin–*N*7-guanine by all subjects over the collection period on total dietary aflatoxin B_1 intake gave $r = 0.82$ ($p < 0.0001$). Separation of the population into hepatitis B virus carriers and non-carriers revealed no difference in urinary aflatoxin–guanine adduct levels for a given dietary exposure (Groopman *et al.*, 1992b).

(*b*) *Covalent binding to DNA*

Consistent with the presence of aflatoxin B_1–*N*7-guanine in human urine are reports of the presence of aflatoxin–guanine adducts in human liver tissues, as determined by immunoassay on DNA. One study in Taiwan, China, of nine hepatocellular carcinoma patients showed antibody inhibition in a competitive ELISA by tumour DNA from all seven samples tested and from two of eight DNA samples from 'adjacent-normal' tissue (Hsieh *et al.*, 1988). In a study of 27 further hepatocellular carcinoma patients in Taiwan, a positive signal was seen after immunofluorescent staining in eight (30%) of the tumour and seven (26%) of the non-tumour liver tissues. Some of these samples gave positive results in ELISA (Zhang *et al.*, 1991). Reports of aflatoxin–guanine adducts in human tissues have been made from Czechoslovakia (Garner *et al.*, 1988) and California, USA (Lee *et al.*, 1989), on the basis of studies of antibodies; however, the specificity of all the available assay methods is dependent on the specificity of the antibody used, and no other confirmatory analytical approach was employed.

Studies of DNA binding of aflatoxin B_1 *in vitro* and in fish and mammals *in vivo* are summarized in Tables 10 and 11, respectively.

(*c*) *Modulation of DNA binding* (Table 12)

Many factors have been identified that modulate aflatoxin B_1-induced DNA binding, including several antioxidants and dietary factors. The available data are summarized in Table 12. In studies *in vitro*, retinoid analogues, indol-3-carbinol and allixin inhibited binding of aflatoxin B_1 to DNA.

Antioxidants such as butylated hydroxytoluene (see IARC, 1986), butylated hydroxyanisole (see IARC, 1986) and ethoxyquin and the dithiolthiones, oltipraz and 1,2-dithiol-3-thione, reduced aflatoxin B_1–DNA binding *in vivo*. Pretreatment of trout and salmon with β-naphthoflavone resulted in reduced activation and subsequent binding to DNA. Both pretreatment and concomitant dietary treatment with indol-3-carbinol in trout reduced covalent aflatoxin B_1–DNA binding. Dietary administration of broccoli and brussels sprouts to rats and pretreatment with crocetin or geniposide reduced hepatic aflatoxin B_1–guanine

adduct formation, as did concomitant phenobarbital treatment of rats (Holeski *et al.*, 1987). In one study, covalent binding of aflatoxin B_1 to DNA was increased following pretreatment with ethanol 6 h previously (Wang *et al.*, 1990). No such effect was found in another study (Marinovich & Lutz, 1985).

Neonatal treatment of rats with diethylstilboestrol, followed by exposure to aflatoxin B_1 in adulthood resulted in significantly higher levels of DNA adducts in females but not in males (Lamartiniere, 1990). Feeding rats with a lipotrope-deficient diet resulted in significantly lower levels of covalent binding of aflatoxin B_1 to DNA (Campbell *et al.*, 1978). Rats fed a choline-deficient, low-methionine diet had significantly higher levels of total adducts following exposure to multiple doses of aflatoxin B_1 than controls (Schrager *et al.*, 1990).

Treatment of rats with low levels of aflatoxin B_1 followed by exposure to a higher dose resulted in reduced levels of covalent binding to hepatic DNA (Neal *et al.*, 1981b).

Partial reduction of hepatic glutathione levels slightly increased covalent binding of aflatoxin B_1 to DNA; however, when glutathione depletion was almost complete, a 30-fold increase in covalent aflatoxin B_1–DNA binding was found (Monroe & Eaton, 1988). In a large majority of the studies, modulation of aflatoxin B_1–DNA binding was associated with changes in the activity of various enzyme systems involved in the biotransformation of aflatoxin B_1. Protective effects were strongly associated with induction of conjugating enzyme systems, and especially the glutathione *S*-transferase isozymes.

(*d*) *Mutations in* p53 *tumour-suppressor gene in human hepatocellular carcinoma* (Table 13)

Mutations of the *p53* tumour suppressor gene—mainly missense (transitions and transversions)—in those regions of the gene that are highly conserved during evolution (exons 5–8) have been found at high frequency in a variety of common human cancers (Hollstein *et al.*, 1991a,b; Jones *et al.*, 1991; Levine *et al.*, 1991). A high frequency of mutations at a mutational 'hot-spot' (Challen *et al.*, 1992) (the third nucleotide of codon 249 in exon 7) has been found in hepatocellular carcinomas from patients resident in areas where they are considered to be at high risk of exposure to aflatoxins and where there is a high incidence of hepatocellular carcinoma. The data are given in detail in Table 13. Of 101 tumours from patients in high-exposure areas (for example, Qidong in China, Mozambique, Viet Nam and India), 40 contained a G to T transversion (AGG to AGT) in codon 249 in exon 7 of the *p53* gene, and one contained a G to C transversion at that position (Hsu *et al.*, 1991). In contrast, only one of 205 hepatocellular carcinomas from regions where there is low exposure to aflatoxins (including Taiwan, Australia, Japan, southern Africa, Germany, Spain, Italy, Turkey, Israel, Saudi Arabia, the United Kingdom and the USA) contained this mutation. The uncertainties associated with classification of tumours across different studies and the small number of studies in which such data are provided preclude a judgement as to whether tumour size or stage is correlated with the presence or absence of the codon-249 mutation. [The Working Group noted that some of the G to T transversions described by Hsu *et al.* (1991) may have been included in the study of Scorsone *et al.* (1992), as the patients were from the same institute. Similarly, the patients studied by Bressac *et al.* (1991) were included in the study of Ozturk *et al.* (1991).]

Point mutations of *p53* in hepatocellular carcinomas have been documented at sites other than codon 249. [The Working Group noted that those studies in which restriction analysis alone was used to detect mutations would not have have picked up such mutations.] Murakami *et al.* (1991) detected eight different mutations in 7/22 advanced hepatocellular carcinomas in patients in Tokyo, Japan, where exposure to aflatoxins is low (Table 13). Six were base-pair substitutions in codons other than 249, and two were deletions. There appeared to be an association between tumour size and degree of differentiation, as there were more mutations in larger, poorly differentiated tumours; however, this conclusion was based on small numbers. No mutation was seen in 21 early hepatocellular carcinomas. In a study in another region of low exposure to aflatoxins—the United Kingdom—Challen *et al.* (1992) found *p53* mutations in 2/19 hepatocellular carcinomas, neither of them at codon 249 (Table 13).

Whether there is any relationship between hepatitis B virus (HBV) status and specific *p53* mutations, especially at codon 249, is difficult to establish from the data presently available. Investigators have used various methods of assigning HBV status, and numbers within individual studies are small. What evidence is available does not strongly suggest a direct relationship between codon-249 mutation and HBV status. For example, Ozturk *et al.* (1991) found that of seven patients in Mozambique positive for HBsAg, four had a tumour with a codon-249 *p53* mutation; and four of eight patients negative for HBsAg had a tumour with a codon-249 *p53* mutation and one had a *p53* mutation at codon 157. Of 16 patients in Australia with hepatocellular carcinoma, eight had been exposed to HBV and eight had not; none carried a codon-249 *p53* mutation in their tumour (Hayward *et al.*, 1991). Similarly, 6/19 patients in the United Kingdom had serological evidence of past or present HBV infection, and 14/19 did not; none carried a codon-249 *p53* mutation in their tumour (Challen *et al.*, 1992).

(*e*) *Loss of heterozygosity and hepatitis B virus*

Although epidemiological studies show that HBV infection is intimately linked with the development of hepatocellular carcinoma in high-risk populations, the underlying molecular mechanism is unknown. HBV–DNA sequences integrated into host DNA, seemingly at random, are commonly found in hepatocellular carcinomas in patients from areas endemic for HBV infection. The finding that gross chromosomal abnormalities (including deletions) are frequent at sites of viral integration raises the possibility that integration of HBV–DNA and the chromosomal damage that ensues may cause allele loss at tumour suppressor gene loci (Fujimori *et al.*, 1991; Slagle *et al.*, 1991; Walker *et al.*, 1991; Scorsone *et al.*, 1992).

Loss of the second allele of a tumour-suppressor gene in a cell that has already sustained a mutation in the first allele is considered to be a critical step in carcinogenesis (Harris, 1991). Allele loss, as measured by loss of heterozygosity for restriction fragment-length polymorphism markers, is common in hepatocellular carcinomas and occurs in several chromosomes (including 17p, which contains *p53*), suggesting the involvement of several tumour-suppressor genes in the development of liver cancers. No specific association has been found, however, between HBV status and HBV integration (Fujimori *et al.*, 1991; Walker *et al.*, 1991).

Table 10. Studies of aflatoxin B_1 and DNA binding *in vitro*

Reference	Details of study	DNA binding
DNA binding *in vitro*		
Puisieux *et al.* (1991)	Covalent binding of AF-8,9-epoxide to plasmid pC53SN3 containing a full-length human wild-type *p53* complementary DNA insert *in vitro*. *p53* nucleotide residues specifically targeted by AFB_1 were identified by DNA polymerase fingerpring analysis. Percentage of *p53* nucleotides in exons 5–8 targeted by AFB_1: G, 62%; C, 6%; T, 2%; A, 3%	0.3–2 adducts/1000 bases
Garner *et al.* (1979)	Covalent binding to calf thymus DNA *in vitro* in presence of liver microsomes from phenobarbital-induced male Wistar rats	2.83 μg AFB_1/mg DNA
	Covalent binding to calf thymus DNA *in vitro* with chloroperoxybenzoic acid oxidation	16.03 μg AFB_1/mg DNA
Groopman *et al.* (1981b)	Covalent binding to calf thymus DNA *in vitro* in presence of liver microsomes from phenobarbital-induced rats. Study of chemical stability of AFB_1-modified DNA *in vitro*	1 adduct/60 or 1500 nucleotides
Pestka *et al.* (1982)	Covalent binding to DNA *in vitro* in presence of liver microsomes from male Fischer rats. Study of AFB_{2a} antiserum for detecting AFB_1-modified DNA and related metabolites	[190 μg AFB_1/ml] 8 μg AFB_1/mg DNA at 1 h
Misra *et al.* (1983)	Covalent binding to ΦX174 and pBR322 DNA *in vitro* using chloroperoxybenzoic acid oxidation or photoactivation at 350 nm. Study of covalent and noncovalent interactions of AFB_1 with defined DNA sequences: sequence-specific effects abolished in single-stranded DNA, and reactivity of guanine strongly suppressed	
Israel-Kalinsky *et al.* (1984)	Covalent binding to calf thymus DNA *in vitro* using photoactivation at 365 nm and 390 nm	2 nmol AFB_1/μmol DNA-P at 20 μM AFB_1
	Covalent binding to DNA of *Salmonella typhimurium* TA100 in presence of liver microsomes from Aroclor 1254-induced male Sprague-Dawley rats	2 μM [0.62 μg/ml/15 min]; 5.3 pmol AFB_1/μmol DNA-P
	Covalent binding to DNA of *S. typhimurium* TA100 photoactivated at 365 nm	5 μM [1.55 μg/ml/120 min]; 0.37 pmol AFB_1/μmol DNA-P
	AFB_1 binding to DNA induced *in vitro* by mammalian microsomes or by photoactivation proportional to mutagenicity and lethality	
Amstad *et al.* (1984)	Covalent binding to human lymphocytes *in vitro*. Clastogenic effects observed at this dose of AFB_1 considered to be caused by indirect effects on cell membrane	0.3 μM [0.09 μg/ml] causing ca. 20% cells with aberrations; 2 adducts/10^8 DNA-P (not certain to be DNA adducts)
Yang *et al.* (1985)	Covalent binding *in vitro* to DNA from normal human liver, primary hepatocellular carcinoma cells, Mahlavu hepatocellular carcinoma cells, in presence of liver microsomes from rats or chloroperoxybenzoic acid oxidation. Kinetics of AFB_1 binding to these DNAs similar initially but extents of binding to carcinoma cell. DNA slightly higher	100–400 fmol/μg DNA
Jayaraj *et al.* (1985)	Covalent binding to calf thymus DNA *in vitro*, in presence of liver microsomes from male Fischer 344 rats 4, 12 and 26 months old. Formation of AFB_1–DNA adducts with microsomes from 4- and 12-month-old rats was similar but significantly lower [about half] with those from 26-month-old rats	

Table 10 (contd)

Reference	Details of study	DNA binding
Mariën *et al.* (1987)	Covalent binding of chloroperoxybenzoic acid-oxidized AFB_1 to purified λDNA; 195-bp fragment of φX174 of known sequence; 195-bp fragment of pLV405. Activated AFB_1 reacts primarily with guanyl residues to form *N*7 adducts; ≤10-fold variation in binding of AFB_1 in 33 different guanyl residues in two different 195-bp DNA fragments	
Benasutti *et al.* (1988)	Covalent binding to M13mp19 DNA fragments of known sequence *in vitro* following chloroperoxybenzoic acid oxidation of AFB_1. Study of reaction of binding of AFB_1 to 190 guanyl residues in different pentanucleotide contexts confirmed that *N*7 of guanine is major adduct. In a pentanucleotide 5′-WXGYZ-3′, the influence of neighbouring bases (X and Y) most influential in determining reactivity of G are: X = G > C > A > T and Y = G > T > C > A	
Loechler *et al.* (1988)	Molecular modelling study of interaction of AFB_1 binding site in double-stranded DNA fragments of different sequences. Two potential binding modes proposed in which AFB_1 moiety is either intercalated or bound externally in the major groove. One particular external binding site could account for the preference that the reactive G has for another G on its 5′ side.	
Cole *et al.* (1988)	Covalent binding to DNA of mouse, rat and human hepatocytes *in vitro*. Binding in male but not female rat hepatocytes declined over one week	0.2 μm for 24 h: male rat (203 pmol/mg DNA), human (42) > female rat (38); mouse (1.4)
Delcuve *et al.* (1988)	Covalent binding of 2,3-dichloro-AFB_1 to DNA of mature and immature chicken erythrocytes *in vitro*	
Loveland *et al.* (1988)	Covalent binding to *Salmo gairdneri* (rainbow trout) hepatocyte DNA *in vitro*. Binding (pmol bound AF/μg DNA)/(μmol dose) *versus* time fitted a linear function passing close to origin for each AF	DNA binding at 1 h relative to AFB_1: aflatoxicol, 0.53; AFM_1, 0.81; aflatoxicol M_1, 0.83; significantly less than that of AFB_1
Bailey *et al.* (1988)	Covalent binding to hepatocyte DNA of *S. gairdneri in vitro* Covalent binding to hepatocyte DNA of *Oncorhynchus kisutch* (coho salmon) *in vitro*	0.2 μg/ml; 667 pmol AFB_1/mg DNA 0.2 μg/ml; 34 pmol AFB_1/mg DNA
Stone *et al.* (1988)	Non-covalent binding to calf thymus DNA, synthetic polydeoxyribonucleotides and the oligodeoxynucleotide $d(ATGCAT)_2$ *in vitro*	
Stark *et al.* (1988)	Covalent binding to calf thymus and supercoiled pBR322 DNA *in vitro* using photoactivation at 365 nm. Authors concluded that photoactivated aflatoxins bind to guanines, some of which are released, creating apurinic sites which are converted to DNA chain breaks at physiological pH and temperature.	
Baertschi *et al.* (1989)	Covalent binding of AFB_1 to calf thymus DNA *in vitro*, in presence of human liver microsomal cytochrome $P450_{NF}$; comparison with AFG_1	Guanyl-*N*7 adduct formation in pmol/min/nmol cytochrome P protein: 28 (50 μM AFB_1), 12 (50 μM AFG_1)

Table 10 (contd)

Reference	Details of study	DNA binding
Harris *et al.* (1989)	Covalent binding of AFB_1 epoxide to synthetic oligodeoxynucleotides $d(ATCGAT)_2$ and $d(ATGCAT)_2$ *in vitro*. Synthetic AFB_1-epoxide reacts with $d(ATCGAT)_2$ with 1 adduct/duplex and 2 per $d(ATGCAT)_2$ duplex. Both react to give the *N*7 adduct on guanine. Difference in stoichiometry explained by geometrical constraints, such that two sites of intercalation are allowed in $d(ATGCAT)_2$ but only one in $d(ATCGAT)_2$.	
Lipsky *et al.* (1990)	Covalent binding to calf thymus DNA *in vitro* in presence of microsomes, with and without cytosols, from mouse, rat and human livers. Binding mediated by liver microsomes from 13 humans varied from 0.87 to 14.58 nmol/mg microsomal protein. Binding mediated by mouse and rat liver microsomes was 4.68 (mouse) and 3.14 (rat) nmol/mg microsomal protein.	
	Covalent binding to DNA in human, rat and mouse hepatocytes *in vitro*	AFB_1 bound (pmol/mg DNA) hepatocyte DNA: human, 53, 69, 25; rat, 203; mouse, 1.4
Ball *et al.* (1990)	Covalent binding to DNA in tracheal explants from hamsters, rats and rabbits *in vitro*. Repair rates in hamster and rat constant over time, with removal of *N*7-guanine adduct accounting for majority of adduct loss. All adduct types removed rapidly during first 12 h after treatment, followed by a slower removal phase	Binding of 0.5 μM AFB_1 (pmol/mg DNA): rabbit, 78; hamster, 28; rat, 3
Stark *et al.* (1990)	Covalent binding to calf thymus DNA *in vitro* using photoactivation at 365 nm, under aerobic and anaerobic conditions in H_2O or D_2O. Photoactivated binding decreased markedly under anaerobic conditions and with compounds known to quench or scavenge singlet oxygen ($^1O_2$)	Binding 68–330 pmol/μmol nucleotide in dark; 1–7 nmol/μmol nucleotide after irradiation
Shaulsky *et al.* (1990)	Covalent binding to nick-translated DNA labelled with ^{14}C in each DNA base *in vitro*, using:	
	liver microsomes from phenobarbital-induced male Sprague-Dawley rats	2626 pmol/μmol nucleotide
	photoactivation at 365 nm	2304 pmol/μmol nucleotide
	The only AFB_1–DNA adducts detected in significant amounts were guanine adducts; no stable adduct with A, C or T detected	
Crespi *et al.* (1990)	Covalent binding to DNA of human lymphoblastoid AHH-1 $TK^{+/-}$ cells transfected with and expressing human CYP1A2 gene *in vitro*. Binding not detectable in cells not carrying or expressing CYP1A2	Exposure to 3 ng/ml: 16.3 dpm/μg DNA
Crespi *et al.* (1991)	Covalent binding to DNA of human lymphoblastoid AHH-1 $TK^{+/-}$ cells transfected with and expressing either human CYP1A2, human CYP2A3 or human CYP3A4 genes *in vitro*. Binding not detected in cells carrying and expressing CYP2A3, 3A4; detected in CYP1A2 cells	Exposed to 3 ng/ml: 1.16 pmol/mg DNA
Stark & Liberman (1991)	Covalent binding to calf thymus DNA *in vitro* using photoactivation at 365 nm, under aerobic and anaerobic conditions in H_2O or D_2O. DNA binding enhanced in D_2O, and a singlet-oxygen scavenger inhibited mutagenesis. DNA photobinding of AFB_1 increased in presence of AFB_2	[10 μM, 30 min irradiation] 128 pmol/μmol nucleotide

[a]AF, aflatoxin

Table 11. Studies of DNA binding in fish and mammals exposed to aflatoxin B_1 *in vivo*

Reference	Details of study	Route and dose	DNA binding
Toledo *et al.* (1987)	Covalent binding to liver DNA of *Oryzias latipes* (medaka fish) *in vivo*; dose–response linear over the range 70–550 μg AFB_1/kg; maximum binding within the first 24 h after injection, followed by rapid loss of adducts	i.p.; 70, 140, 275, 550 μg/kg bw	1.0–22.0 pmol/μmol DNA at 24 h
Bailey *et al.* (1988)	Covalent binding to liver DNA of *S. gairdneri* (rainbow trout) *in vivo*	i.p.; 10 μg/kg bw	48 pmol AFB_1–*N*7-guanine/mg DNA at 1 day
		Oral; 80 ppb, 3 weeks	29 pmol/mg DNA
		Embryos; 0.5 μg/ml, 1 h	1.4 pmol/mg DNA at 1 day
	Covalent binding to liver DNA of *Oncorhynchus kisutch* (coho salmon) *in vivo*	i.p.; 10 μg/kg bw	4 pmol AFB_1–*N*7-guanine/mg DNA at 1 day
		Oral; 80 ppb, 3 weeks	1.6 pmol/mg DNA
		Embryos; 0.5 μg/ml, 1 h	0.07 pmol/mg DNA at 1 day
	DNA binding 7.56 times greater in trout than salmon liver at various times after injection, 20 times greater in embryos, and 18 times greater in trout liver after a three-week dietary (80 ppb) exposure. Major DNA adduct, 8,9-dihydro-8-(*N*7-guanyl)-9-hydroxyAFB_1 in both species		
Nakatsuru *et al.* (1989)	Covalent binding to liver DNA of *Salmo mykiss* (a trout species) *in vivo*	i.p.; 0.1, 0.5 mg/kg bw, 24 h	35, 150 fmol AFB_1/μg DNA
	Covalent binding to liver DNA of *O. kisutch in vivo*	i.p.; 0.1, 0.5 mg/kg bw, 24 h	Not detected, 22 fmol AFB_1/μg DNA
	Covalent binding to liver DNA of male Fischer 344 rats *in vivo*	i.p.; 1, 2 mg/kg bw, 2 h	268, 420 fmol AFB_1/μg DNA
Garner *et al.* (1979)	Covalent binding to kidney and liver DNA of Wistar rats *in vivo*; comparison of AFB_1 and AFG_1 binding to macromolecules; binding to RNA > DNA > protein	i.p.; 0.6 mg/kg bw	Kidney: 10 ng AFB_1/mg DNA at 2 h Liver: 15 ng AFB_1/mg DNA at 2 h

Table 11 (contd)

Reference	Details of study	Route and dose	DNA binding
Bennett *et al.* (1981)	Covalent binding to DNA *in vivo*: detection of urinary AFB_1-guanine adducts in male Fischer rats. Administration of AFB_1 resulted in urinary excretion of major DNA adduct formed *in vivo*. Measurement of urinary adducts in rats injected with different doses of AFB_1 showed that excretion occurred in a dose-dependent manner. Comparison of dose–response curve for adduct excretion with that previously observed for adduct formation in rat liver DNA *in vivo* revealed close similarity	i.p.; 0.125, 0.25, 0.5, 1 mg/kg	
Groopman *et al.* (1982)	Covalent binding to liver DNA of male CDF Fischer rats *in vivo*. Monoclonal antibodies specific for modified DNA, containing both the 2,3-dihydro-2-(*N*7-guanyl)-3-hydroxy-AFB_1 and putative 2,3-dihydro-2-(*N*5-formyl-2′,5′,6′-triamino-4′-oxo-*N*5-pyrimidyl)-3-hydroxy-AFB_1, suggesting that these DNA adducts share a common antigenic determinant	i.p.; 0.01–1 mg/kg bw	1 AFB_1 residue per 1.35×10^6, 2.5×10^5, 3×10^4 nucleotides at 2 h
Irvin & Wogan (1984)	Covalent binding to nuclear liver DNA and ribosomal liver DNA sequences of male Fischer rats *in vivo*. Over a dose range of 0.25–2.0 mg AFB_1/kg bw, ribosomal DNA contained 4–5 times more AFB_1 residues than nuclear DNA, showing that ribosomal DNA is preferentially accessible to carcinogen modification *in vivo*.	i.p.; 0.25, 0.5, 1.0, 2.0 mg/kg bw	90–640 pmol AFB_1/mg nuclear DNA (30–240 AFB_1 adducts/10^6 nucleotides)
Irvin & Wogan (1985)	Covalent binding to liver nuclear DNA and ribosomal DNA of male Fischer rats *in vivo*: 12 h after injection, ribosomal DNA contained 4-5 times more AFB_1 residues than did total nuclear DNA. AFB_1-ribosomal DNA residues removed 5.7 times more rapidly than total nuclear DNA residues over 12 h. Levels of the major adduct, *N*7-guanyl-AFB_1 and its stable formamidopyrimidine derivatives also determined: no difference in proportion of these adducts in ribosomal and total nuclear DNA	i.p.; 1 mg/kg bw	288, 227, 158 pmol AFB_1/mg nuclear DNA; 1212, 800, 590 pmol AFB_1/mg ribosomal DNA, 2, 6, 12 h after dosing
Wild *et al.* (1986)	Covalent binding to liver DNA of male Wistar rats *in vivo* given a single dose 24 h before killing; 24 h after single dose, constant ratio found between levels of AFB_1 bound to plasma protein and that bound to liver DNA; 0.98–2.15% of dose bound to plasma protein at 24 h	Intra-gastric; 3.5, 10, 100, 200 μg/kg bw	100–2500 pg AFB_1/mg DNA
	Covalent binding to liver DNA of male Wistar rats *in vivo* given multiple doses for 23 days before killing Binding of AFB_1 to plasma protein rose three times higher than after single dose; reached plateau after 7–14 days of treatment and remained stable. Binding to DNA accumulated 2.5-fold and in parallel to plasma protein. All detectable plasma protein-bound AFB_1 associated with a single peak corresponding to albumin.	Intra-gastric, 0.5 μg/rat, twice daily, 5 days/week, 2, 3, 7, 11, 21, 24 days before killing	108.4 pg AFB_1/mg at 2 days to peak of 267 pg AFB_1/mg DNA at 14 days

Table 11 (contd)

Reference	Details of study	Route and dose	DNA binding
Yu *et al.* (1988)	Covalent binding to liver DNA fractions (nuclear, nucleolar and transcriptionally active subnucleolar fraction rich in ribosomal RNA DNA) *in vivo*, in male Sprague-Dawley rats. Binding of AFB_1 to nuclear and nucleolar DNA reached plateau at 3000 μg AFB_1/kg bw; binding to protein linear, although with different slopes, for nuclear and nucleolar fractions up to highest dose used	i.p.; 100, 500, 1000, 3000, 5000 μg /kg bw	Binding (pmol AFB_1/mg) linear to 3000 μg /kg dose. Nuclear DNA, ~ 100 DNA; nucleolar DNA, ~ 400; P3 DNA, ~ 580
Buss *et al.* (1990)	Covalent binding to liver DNA *in vivo* in male Fischer F344 rats given AFB_1:		Adducts/10^9 nucleotides:
	One dose level by oral gavage once or 10 times in 10 days	Gavage, 0.1 μg/kg bw	Single dose: ~ 4, falling to ~ 1.2 after 10 days Multiple doses: ~ 20, falling to ~ 10 after 10 days
	Six dose levels by oral gavage once or 10 times in 10 days	Gavage, 1, 10, 100 ng/kg bw; 1, 10, 100 μg/kg bw	Single dose: $> 10^{-2}$ to $> 10^3$ Multiple doses: $> 10^{-1}$ to $> 10^3$
	Three dose levels in drinking-water for 4, 6 and 8 weeks	Oral; 2.2, 73, 2110 ng/kg bw per day	At 8 weeks, 0.9, 32, 850
	AFB_1-DNA adduct levels measured after 4, 6 and 8 weeks; did not increase after 4 weeks, indicating steady-state for adduct formation and removal had been reached; at 8 weeks, adduct levels 0.91, 32 and 850 AFB_1 adducts per 10^9 nucleotides, i.e., directly proportional to dose		
Wild *et al.* (1990b)	Covalent binding to DNA *in vivo* in various organs of male Sprague-Dawley rats exposed to single and multiple doses of AFB_1. Marked intercellular variation in adduct levels observed in kidney and lung, in contrast to liver, where binding was more homogeneous. No adduct detected in oesophagus, forestomach, colon, spleen or testis (detection limit, ~ 300 pg AFB_1/mg DNA)	Gavage, [^{3}H]AFB_1, 19, 32, 75 μg/kg bw	Single dose: 83, 146, 444 pg AFB_1/mg DNA at 24 h
		Gavage, AFB_1 daily up to 14 days (165 μg/kg bw on day 1)	Multiple doses: rapid accumulation in liver over 3 days to plateau

Table 11 (contd)

Reference	Details of study	Route and dose	DNA binding
Groopman *et al.* (1992c)	Covalent binding to liver DNA *in vivo* in male Fischer 344 rats. Measurement of AFB_1–*N*7-guanine excretion in rat urine, using combined monoclonal antibody immunoaffinity chromatography/HPLC method, showed dose-dependent excretion in urine. Comparison of dose-dependent residual levels of AFB_1 binding to liver DNA with amount of AFB_1–*N*7-guanine excreted in urine showed a correlation coefficient of 0.98. Urinary AFM_1 and AFP_1 evaluated as molecular dosimeters: AFM_1 found to be excellent marker	i.p.; 1 mg/kg bw p.o.; 0.03, 0.07, 1.0 mg/kg	Not possible to deduce from log-log plot

AF, aflatoxin

Table 12. Modulation of aflatoxin B_1–DNA binding

Reference	Details of study	Route and dose	DNA binding
DNA binding *in vitro*			
Fukayama & Hsieh (1984)	Covalent binding to calf thymus DNA *in vitro* in presence of liver microsomes from untreated male Fischer 344 rats		2.11 nmol AFB_1/mg DNA
	Covalent binding to calf thymus DNA *in vitro* in presence of liver microsomes from rats given dietary BHT (0.5%) for 10 days before killing. Microsomes from BHT-treated rats mediated same level of DNA binding as those from untreated rats, despite significantly lower bacterial mutagenicity in presene of BHT microsomes		2.2 nmol AFB_1/mg DNA
Firozi *et al.* (1987)	Covalent binding to calf thymus DNA *in vitro* in presence of liver microsomes from phenobarbital-induced male Wistar rats. Study of effect of vitamin A and derivatives on microsome-mediated AFB_1–DNA binding. Concentrations that induced 50% inhibition of binding were: retinol, 40 nM; retinyl acetate, 50 nM; all-*trans*-retinoic acid, 75 nM; retinyl palmitate, 170 nM		
Jhee *et al.* (1988, 1989a,b)	Covalent binding to calf thymus DNA or endogenous hepatocyte nuclei *in vitro* in presence of liver microsomes with or without cytosol from untreated male Fischer 344 rats or rats given dietary BHA (0.75%) for 2 weeks before killing. Cytosol from untreated rats produced more DNA binding than cytosol from BHA-treated rats. G5H conjugation of AFB_1 higher in presence of BHA-treated rat cytosol		
Fong *et al.* (1990)	Covalent binding to calf thymus DNA *in vitro* in presence of liver microsomes from *Salmo gairdneiri* and rats; I3C under acid conditions caused dose-dependent reduction in DNA binding mediated by rat or trout microsomes		132 pmol AFB_1 bound/mg DNA (trout microsomes)
Yamasaki *et al.* (1991)	Covalent binding to calf thymus DNA *in vitro* in presence of liver post-mitochondrial supernatant from Aroclor 1254-induced male Sprague-Dawley rats: allixin (a phenolic from garlic) inhibited DNA binding and adduct formation by AFB_1 (by ~30%)	500 pmol AFB_1/ml [0.16 μg/ml] for 1 h	106 pmol/mg DNA
		With 75 μg allixin/ml for 1 h	71 pmol/mg DNA
DNA binding *in vivo*			
Whitham *et al.* (1982)	Covalent binding to liver DNA of *S. gairdneri* (rainbow trout) *in vivo*	i.p.; 5, 25, 100, 300 μg/kg bw	9.2, 56, 315 and 1240 pmol/mg DNA at 24 h
	Covalent binding to liver DNA of *Oncorhynchus kisutch* (coho salmon) *in vivo*	i.p.; dose not given in a comparative study with trout	10.6 pmol/mg DNA at 24 h in salmon; 243 pmol/mg DNA at 24 h in trout
	In trout, almost linear increase in binding with increasing dose; time-course parameters showed no difference in binding between 4 h and 48 h after ^{3}H-AFB_1 treatment. Compared with AFB_1-treated controls, binding was two-fold lower in β-naphthoflavone-treated trout and 20-fold lower in salmon. AFB_1 binding not significantly altered by dietary protein or cyclopropenoid fatty acids		

Table 12 (contd)

Reference	Details of study	Route and dose	DNA binding
Dashwood *et al.* (1988)	Covalent binding to liver DNA of *S. gairdneri in vivo*. Fish exposed to AFB_1 and to I3C by concomitant dietary exposure. Linear increases in DNA binding with increasing dose of AFB_1 and with time of inhibitor/carcinogen co-treatment, at each I3C dose level. Successive increases in I3C dose resulted in corresponding dose-related decreases in DNA binding, suppressed by almost 95% at highest I3C dose tested (4000 ppm)	Dietary AFB_1: 10, 20, 40, 80, 160, 320 ppb Dietary I3C; 1000–4000 ppm	80 ppb AFB_1, ~ 1800 pmol/mg DNA reduced to 90 pmol/mg DNA by 4000 ppm dose
Goeger *et al.* (1988)	Covalent binding to liver and red-blood cell DNA of *S. gairdneri in vivo*	i.p.; 180 nmol/kg bw [56 μg/kg]	~ 150 nmol AFB_1/g liver DNA in control and BHA-treated at 1 day
		i.p.; 64 nmol/kg bw [20 μg/kg]	~ 45 nmol AFB_1/g liver DNA in control reduced to 25 nmol AFB_1/g liver DNA with β-naphthoflavone at 1 day
Dashwood *et al.* (1989)	Covalent binding to liver DNA of *S. gairdneri in vivo*. About 10 000 fish pretreated with one of five doses of I3C. After 4 weeks, they received the same dietary level of I3C for a further two weeks, with ^3H-AFB_1 in dose range 10–320 ppb. 15 fish randomly selected to assess liver DNA binding levels; remaining animals returned to control diet for determination of tumour response at 1 year. Linear increases in DNA binding with dose of AFB_1 at each I3C dose level. Successive increases in I3C dose gave dose-related decreases in DNA binding, resulting in a series of curves of decreasing slope. At I3C doses of ≤2000 ppm, inhibitor-altered tumour response predicted precisely by changes in levels of DNA adducts formed in liver	(see Dashwood *et al.*, 1988)	(see Dashwood *et al.*, 1988)
Campbell *et al.* (1978)	Covalent binding to liver DNA in male Sprague-Dawley rats *in vivo* given nutritionally complete synthetic diet (1) or marginal lipotrope-deficient diet (2). Value obtained with diet 2 claimed to be significantly lower ($p < 0.05$) than that obtained with diet 1; with both diets, binding to RNA > DNA > protein	i.p.; 1 mg/kg bw	Diet 1: 19.2 ng AFB_1/mg DNA at 6 h Diet 2: 15.6 ng AFB_1/mg DNA at 6 h
Neal *et al.* (1981a)	Covalent binding to liver DNA of male Fischer 344 rats *in vivo*; controls and rats fed diet with 4 ppm unlabelled AFB_1 10 weeks before injection of ^3H-AFB_1 Study of effect of pre-feeding low level of AFB_1 before measuring DNA binding at high single dose. Increased levels of reduced GSH and GSH *S*-transferase accompanied reduction in binding in rats fed diet with AFB_1, suggesting that decreased binding is due to increased detoxification of AFB_1 metabolites	i.p.; 0.5 mg/kg bw	Controls, ~ 12.5 ng AFB_1/mg DNA Treated, ~ 2 ng AFB_1/mg DNA

Table 12 (contd)

Reference	Details of study	Route and dose	DNA binding
Appleton *et al.* (1982)	Covalent binding to liver DNA of male Fischer F344 *in vivo*; treated rats given single i.p. injection of 2.7 nmol/0.5 ml/kg bw diethyl maleate 1 h before injection of AFB_1, to reduce GSH content In dose range 10–1000 ng/kg, radiolabelled AFB_1 produced measurable covalent binding of AFB_1 to DNA, RNA, and protein, which increased linearly over dose range. Adduct formation observed at lowest dose used (10 ng/kg), which is within human exposure range. Although diethyl maleate reduced hepatic GSH from 5 to 2.3 μmol/g of liver and slightly increased binding, the dose-response curve for macromolecular adduct formation remained linear in both pretreated and control groups; binding to RNA > DNA > protein	i.p. 10, 25, 65, 160, 390 and 1000 ng/kg bw	Controls: 0.37, 0.48, 1.47, 3.93, 8.54, 16.48 pg AFB_1/mg DNA at 1 h Treated: 0.35, 0.48, 1.39, 4.70, 11.47, 19.43 pg AFB_1/mg DNA at 1 h
Loury & Hsieh (1984)	Covalent binding to liver DNA *in vivo* in male Fischer F344 rats fed, for 41 weeks before single dose of radiolabelled AFB_1, Control semi-synthetic diet 50 ppb AFB_1 in semi-synthetic diet 0.5 ppb AFM_1 in semi-synthetic diet 50 ppm AFM_1 in semi-synthetic diet In animals pre-exposed to 50 ppb AFB_1, binding to DNA, RNA and protein decreased by 72%, 74% and 61%, respectively. Pre-exposure to AFM_1 resulted in a small reduction in binding to nucleic acids. GSH *S*-transferase activity increased by 133% in animals fed 50 ppb AFB_1, by 48% in those fed 50 ppb AFM_1, and remained at control values in rats fed 0.5 ppb AFM_1	Gavage, 5 μg/kg bw	~ 3.6 pmol/mg DNA at 6 h ~ 1.0 pmol/mg DNA at 6 h ~ 2.2 pmol/mg DNA at 6 h ~ 2.1 pmol/mg DNA at 6 h
Marinovich & Lutz (1985)	Covalent binding to liver DNA *in vivo* in male Fischer F344 rats given ethanol (0.44–3.4 g/kg bw) in drinking-water for 10 days or by single oral administration. Level of binding not affected by any type of ethanol pretreatment	Oral; 100 ng/kg bw	13 500 μmol AFB_1/mol DNA nucleotide/mmol AFB_1 dose, mg/kg bw (CBI units) at 1 day

Table 12 (contd)

Reference	Details of study	Route and dose	DNA binding
Kensler *et al.* (1986)	Covalent binding to liver and kidney DNA in male Fischer F344 rats fed semi-purified diet: Alone	I.p.; 1 mg/kg bw	Liver: 859; kidney: 94 pmol AFB_1/mg DNA
	Containing 0.45 % BHA for 2 weeks		Liver: 304; kidney: 36 pmol AFB_1/mg DNA
	Containing 0.45% BHT for 2 weeks		Liver: 129; kidney: 61 pmol AFB_1/mg DNA
	Containing 0.5% ethoxyquin for 2 weeks		Liver: 77; kidney: 19 pmol AFB_1/mg DNA
	Containing 0.1% 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (oltipraz) for 2 weeks		Liver: 202; kidney: 34 pmol AFB_1/mg DNA
	Several AFB_1 metabolite–DNA adducts formed in both tissues. Principal and related adducts of 8,9-dihydro-8-(*N*7-guanyl)-9-hydroxy-AFB_1 represented 80–90% of all adducts in both tissues and in all treatment groups. Antioxidants ethoxyquin, BHT, BHA and oltipraz reduced binding of AFB_1 to liver DNA by 91, 85, 65 and 76% and to kidney DNA by 80, 35, 62 and 64%, respectively. Concordantly, the specific activities of three hepatic enzymes involved in AFB_1 detoxification were significantly elevated by all antioxidants. Correlation (r = 0.95) between degree of inhibition of DNA binding and induction of hepatic GSH *S*-transferase activities by antioxidants		
Fukayama & Hsieh (1985)	Covalent binding to liver DNA of male Fischer F344 rats fed control diet or 0.5% BHT in diet for 10 days	Intra-gastric; 62.5 μg/kg bw	Control: ~ 7 pmol AFB_1/mg DNA at 6 h
	Radioactivity bound to hepatic nuclear DNA six times less in BHT-pretreated rats than in controls 6 h after administration of AFB_1. Half-lives of DNA binding, 30 and 46 h for control and BHT-pretreated rats, respectively		Treated: ~ 13 pmol AFB_1/mg DNA at 6 h

Table 12 (contd)

Reference	Details of study	Route and dose	DNA binding
Mandel *et al.* (1987)	Covalent binding to kidney and liver DNA in male Fischer 344 rats fed: Control diet	i.p.; 1 mg/kg bw	Kidney: 12.2; liver: 126.4 pg/μg DNA at 2 h
	Diet containing 0.5% ethoxyquin 2 days before dosing with AFB_1		Kidney, 5.4; liver, 29.5 pg/μg DNA at 2 h
	Diet containing 0.5% ethoxyquin 14 days before dosing with AFB_1		Kidney, 3.7; liver, 3.9 pg/μg DNA at 2 h
	Formation of presumed detoxified metabolites AFM_1 and AFQ_1 enhanced to a greater extent than formation of active metabolite, AFB_1-8,9-epoxide. Ethoxyquin reduced binding of AFB_1 to DNA of liver and kidney		
Holeski *et al.* (1987)	Covalent binding to liver DNA *in vivo* in male Sprague-Dawley, either intact or injected i.p. daily for 4 days with 75 mg/kg bw phenobarbital	i.p.; 0.25 mg/kg bw	Controls: 78 pmol AFB_1/mg DNA at 2 h
	Phenobarbital treatment had no significant effect on amount of AFB_1 remaining in liver but decreased amount of binding to liver DNA by 55%. For individual animals from each group, correlation between increase in excretion of AFB_1-GSH and decrease in covalent binding was significant ($r = 0.77$)		Treated: 35 pmol AFB_1/mg DNA at 2 h
Monroe *et al.* (1986)	Covalent binding to liver DNA in male Sprague-Dawley rats given: Propylene glycol (vehicle)	i.p.; 0.25 mg/kg bw	97.5 pmol/mg DNA
	Single oral dose (500 mg/kg bw) of BHA		77.8 pmol/mg DNA
	Corn oil (vehicle)		95.8 pmol/mg DNA
	Daily s.c. BHA (500 mg/kg bw) for 9 days		16.4 pmol/mg DNA
	In order to differentiate between enzyme induction and direct antioxidant effects, BHA was given for 9 days or as a single dose. Repeated treatment enhanced biliary excretion of both the GSH conjugate of AFB_1 and the AFB_1 glucuronide to 200% of control values, and reduced covalent binding of AFB_1 to liver DNA to 17% of control. A single BHA treatment had no effect on biliary excretion or binding to liver macromolecules, despite high concentrations of BHA in liver during period of AFB_1 metabolism		

Table 12 (contd)

Reference	Details of study	Route and dose	DNA binding
Monroe & Eaton (1987)	Covalent binding to liver DNA *in vivo* in	0.25 mg/kg bw	
	Female CD-1 mice fed control diet		~ 4.5 AFB_1 adducts/10^7 nucleotides
	Female CD-1 mice fed diet containing 0.75% BHA		~ 3.1 AFB_1 adducts/10^7 nucleotides
	Male Sprague-Dawley rats fed control diet		~ 375 AFB_1 adducts/10^7 nucleotides
	Male Sprague-Dawley rats fed diet containing 0.75% BHA		~ 70 AFB_1 adducts/10^7 nucleotides
	BHA treatment resulted in a decrease in hepatic AFB_1-guanine adduct formation in mice to 68% of control and, in rats, to 18% of control. AFB_1-DNA binding in control mice 1.2% of that in control rats		
Monroe & Eaton (1988)	Covalent binding to liver DNA in male Swiss-Webster mice:	i.p.; 0.25 mg/kg bw	
	Controls		6.24 AFB_1 adducts/10^7 nucleotides
	Controls fed diet containing 0.75% BHA for 13 days		3.39 AFB_1 adducts/10^7 nucleotides
	Given D,L-buthionine-*S*-sulfoximine (0.6 g/kg) and diethyl maleate (0.75 ml/kg) i.p. 2 and 1.5 h before AFB_1 injection		184.9 AFB_1 adducts/10^7 nucleotides
	Fed diet containing 0.75% BHA for 13 days and given buthionine-*S*-sulfoxime and diethyl maleate as above		5.51 AFB_1 adducts/10^7 nucleotides
	Depletion of GSH accomplished with buthionine-*S*-sulfoxime and diethyl maleate before injection of AFB_1, giving 97% and 70% in control and BHA-treated mice, respectively. In control mice, GSH depletion associated with 30-fold increase in covalent AFB_1-DNA binding. DNA binding in mice treated with dietary BHA alone was reduced to 54% of control. In BHA-treated mice, pretreatment with buthionine-*S*-sulfoxime and diethyl maleate increased DNA binding by 62%. Dietary BHA increased hepatic microsome-mediated activation of AFB_1 to the AFB_1-epoxide by eight-fold in both control and pretreated mice		

Table 12 (contd)

Reference	Details of study	Route and dose	DNA binding
Ramsdell & Eaton (1988)	Covalent binding to liver DNA in male Sprague-Dawley rats fed 21 or 22 days:	i.p., 0.25 mg/kg bw	
	Purified diet		162 pmol AFB_1/mg DNA at 2 h
	Purified diet containing 25% freeze-dried broccoli		72.1 pmol AFB_1/mg DNA at 2 h
	Proprietary rodent diet		142 pmol AFB_1/mg DNA at 2 h
	Binding of AFB_1 to DNA significantly lower in the group given broccoli but not in rats fed rodent diet		
Lotlikar *et al.* (1989)	Covalent binding to liver DNA in male Sprague-Dawley rats fed:	i.p., 400 μg/kg bw	
	Control diet and water		29.8 pmol AFB_1/mg DNA
	Control diet with 0.1% phenobarbital in drinking-water for 1 week		8.6 pmol AFB_1/mg DNA
	Phenobarbital treatment caused significant reduction in binding *in vivo*		
Salbe & Bjeldanes (1989)	Covalent binding to liver DNA in male Sprague-Dawley rats fed:		
	Basal diet	i.p., 3 μg/kg bw; intra-gastric, 3 μg/kg bw	1.5 pmol AFB_1/mg DNA at 2 h; 0.98 pmol AFB_1/mg DNA at 2 h
	Basal diet containing 25% dry weight freeze-dried brussels sprouts for 2 weeks before dosing with AFB_1	i.p., 3 μg/kg bw; intra-gastric, 3 μg/kg bw	0.59 pmol AFB_1/mg DNA at 2 h; 0.45 pmol AFB_1/mg DNA at 2 h
	Basal diet containing 250 ppm I3C for 2 weeks before dosing with AFB_1	i.p., 3 μg/kg bw; intra-gastric, 3 μg/kg bw	1.11 pmol AFB_1/mg DNA; 0.82 pmol AFB_1/mg DNA at 2 h
	Basal diet with 0.1% phenobarbital in drinking-water for 1 week before dosing with AFB_1	i.p., 3 μg/kg bw; intra-gastric, 3 μg/kg bw	0.83 pmol AFB_1/mg DNA at 2 h; 0.37 pmol AFB_1/mg DNA at 2 h
	Brussels sprouts caused significant 50–60% decrease in AFB_1–DNA binding and increased hepatic and intestinal GSH-*S*-transferase activities. Route of administration did not alter inhibition of binding compared with control rats in either treatment group, suggesting that small intestine may not play significant role in metabolism of AFB_1. In a second experiment, rats were dosed either i.p. or intragastrically with AFB_1 and killed 2, 6, 12, 24 or 48 h later. Brussels sprouts significantly decreased hepatic AFB_1–DNA binding		

Table 12 (contd)

Reference	Details of study	Route and dose	DNA binding
Jhee *et al.* (1989b)	Covalent binding to liver DNA in male Fischer F344 rats fed:	i.p.; 400 μg/kg bw	
	Control diet		440 pmol AFB_1/mg DNA at 2 h
	Diet containing 0.75% BHA for 2 weeks		69 pmol AFB_1/mg DNA at 2 h
	BHA treatment reduced DNA binding to 15% of controls with concomitant increase in biliary excretion of AFB_1-reduced GSH conjugate		
Lamartiniere (1990)	Covalent binding to liver DNA in:	i.p.; 1 mg/kg bw	*pmol total AFB_1 adducts/mg DNA*
	Male Sprague-Dawley CD control rats, given AFB_1 at 6 months. Groups of these rats also given corn oil (CO) and 0.1% phenobarbital (PB) in drinking-water for 5 days or 20 mg/kg 3-methylcholanthrene (MC) for 3 days before AFB_1		CO, 255; PB, 28; 3 MC, 94
	Male Sprague-Dawley CD rats given 1.45 μmol diethylstilboestrol s.c. 2, 4 and 6 days after birth and AFB_1 at 6 months. Groups of these rats also given CO or 0.1% PB in drinking-water for 5 days or 20 mg/kg MC for 3 days before AFB_1		CO, 291; PB, 46; 3MC, 52
	Female Sprague-Dawley CD control rats, given AFB_1 at 6 months. Groups of these rats also given CO or 0.1% PB in drinking-water for 5 days or 20 mg/kg MC for 3 days before AFB_1		CO, 250; PB, 49; 3 MC, 39
	Female Sprague-Dawley CD rats given 1.45 μmol diethylstilboestrol s.c. 2, 4 and 6 days after birth and AFB_1 at 6 months. Groups of these rats also given CO or 0.1% PB in drinking-water for 5 days or 20 mg/kg MC for 3 days before AFB_1		CO, 920; PB, 49; 3MC, 36
	Female rats exposed to diethylstilboestrol had significantly higher DNA adduct levels (3 to 6 fold) than adult female rats treated neonatally with vehicle. DNA adduct levels were not significantly higher in control males than in diethylstilboestrol-treated males. PB and MC treatment followed by AFB_1 injection resulted in significantly decreased AFB_1-guanine adduct levels in all rats.		
Wang *et al.*, 1990	Covalent binding to liver DNA in male Wistar rats:	i.p.; 10 μg/kg bw	
	Controls		1.97 pmol AFB_1/mg DNA
	Given a single oral dose of 100 mmol/kg ethanol 6 h before AFB_1		2.90 pmol AFB_1/mg DNA
	Given a single oral dose of 100 mmol/kg ethanol 18 h before AFB_1		2.22 pmol AFB_1/mg DNA
	Binding increased by 47% and hepatotoxicity potentiated in rats treated with ethanol 6 h (time of maximal GSH depletion) before administration of AFB_1; binding not increased by treatment with ethanol 18 h (time for approximately normal GSH levels) before AFB_1, and no potentiation of hepatotoxicity observed		

Table 12 (contd)

Reference	Details of study	Route and dose	DNA binding
Schrager *et al.* (1990)	Covalent binding to liver DNA in male rats fed:	i.p.; 25 μg/rat [~ 0.8 mg/kg Fischer 344 rats; ~ 0.6 mg/kg Sprague-Dawley rats]	pmol AFB_1/mg DNA:
	Control diet for 21 days then AFB_1 5 days/week for 2 weeks (Fischer 344 rats)		21 at 2 h , day 2
	Control diet for 21 days then AFB_1 once (Sprague-Dawley rats)		1.3 at 2 h, 6 at 24 h
	Choline-deficient/methionine-low diet for 21 days then dosed AFB_1 5 days/week for 2 weeks (Fischer 344 rats)		49 at 2 h, day 2
	Choline-deficient/methionine-low diet for 21 days then AFB_1 once (Sprague-Dawley rats)		16 at 2h, 4 at 24 h
	Choline-deficient/methionone-low diet increased AFB_1 hepatocarcinogenesis and reduced time to first tumours. Total adduct levels in choline-deficient animals increased significantly during multiple-dose schedule. When total adduct levels were integrated over 10-day dose period, 41% increase in adduct burden apparent in choline-deficient animals		
Wang *et al.* (1991a)	Covalent binding to liver DNA in male Wistar rats:	i.p.; 6 μg/kg bw	
	Controls		142.3 pg AFB_1/mg DNA
	Given 6 mg crocetin/kg bw by gastric gavage for 3 days before AFB_1		116.6 pg AFB_1/mg DNA
	Given 10 mg crocetin/kg bw by gastric gavage for 3 days before AFB_1		88.1 pg AFB_1/mg DNA
	Crocetin raised hepatic GSH, GSH *S*-transferase and GSH peroxidase levels and significantly decreased AFB_1-guanine adduct formation		
Wang *et al.* (1991b)	Covalent binding to liver DNA in male Wistar rats:	i.p.; 33.9 μg/kg bw	
	Controls		3.3 pg AFB_1/mg DNA
	Given 6 mg geniposide/kg bw by gastric gavage for 3 days before AFB_1		1.7 pg AFB_1/mg DNA
	Given 10 mg geniposide/kg bw by gastric gavage for 3 days before AFB_1		1.8 pg AFB_1/mg DNA
	Geniposide raised hepatic GSH, GSH *S*-transferase and GSH-peroxidase levels and significantly decreased AFB_1-guanine adduct formation		
Groopman *et al.* (1992c)	Covalent binding to liver DNA in male Fischer rats:	Gavage; 250 μg/kg bw	
	Controls		51 pmol AFB–*N*7-guanine per mg DNA at 2 days
	Given 0.03% 1,2-dithiol-3-thione in the diet		9 pmol AFB–*N*7-guanine per mg DNA at 2 days
	Reduction in adduct levels in serum and urine of 1,2-dithiol-3-thione-treated rats was similar to that seen in liver		

AF, aflatoxin; BHT, butylated hydroxytoluene; BHA, butylated hydroxyanisole; I3C, indole-3-carbinol; GSH, glutathione; CBI, carcinogen binding index

Table 13. Mutations in the *p53* tumour suppressor gene in human hepatocellular carcinomas

Reference	Details of patients					Details of tumours		Details of mutations				Remarks
	Sex/age	Ethnic origin/ country	Exposure to aflatoxins	HBV status	Cirrhosis	Grade/ presentation	Number analysed	Loss of *p53* allele	*p53* alteration			
									Codon	Base change (nucleotide)	Amino acid change	
Murakami *et al.* (1991)	No data; no relationship between *p53* aberrations, age, sex or chronic liver disease	Tokyo, Japan	Low	No data	See column 1	Early	21	0/21		Wild type 21/21	0/21	9 DNA fragments containing 1 or 2 exons 2–11 examined by PCR and SSCP; mutations detected by SCCP sequenced after asymmetric PCR. Of 22 advanced tumours, 0/3 well-differentiated, 4/11 moderately differentiated, 4/8 poorly differentiated tumours contained *p53* aberrations; seen in 0/18 tumours < 2 cm, 1/6 2–3 cm and 7/19 > 3 cm
						Advanced	22	+		Wild type 15/22		
								+	246	ATG to GTG	Met to Val	
								+	244	GGC to AGC	Gly to Ser	
								+	110	CGT to TGT	Arg to Cys	
								+	381-382	One base-pair deletion	Frameshift	
								+	168-178	31 base-pair deletion	Frameshift	
								-	175, 272	CGC to AGC, GCC to GAC	Arg to Ser, Lys to Asp	
								+	276	GTG to ATG	Val to Met	
								7/22		8/22		
						Early + advanced		7/43		8/43		No codon-249 mutation in 43 tumours
Hayward *et al.* (1991)		*Australia*					16					Study to detect changes in bases 2 or 3 of codon 249 in exon 7 of *p53* gene. Assay depends on abolition of *Hae*III restriction site. 125 base-pair fragment containing all of exon 7 and part of flanking introns amplified by PCR. All 16 samples cleaved at this site by *Hae*III, showing that none of the tumours had a mutation at that position
	F/44	Caucasian	Low	-	-	2[a], early[b]		Not assayed for	249	Wild type		
	F/36	Caucasian	Low	-	-	1[a], early			249	Wild type		
	M/71	Caucasian	Low	-	-	2, late			249	Wild type		
	M/67	Caucasian	Low	-	+	2, late			249	Wild type		
	M/68	Caucasian	Low	-	+	1–3, late			249	Wild type		
	M/76	Caucasian	Low	-	+	3, late			249	Wild type		
	M/79	Caucasian	Low	-	+	2, late			249	Wild type		
	M/68	Caucasian	Low	-	+	2–3, late			249	Wild type		
	M/51	Melanesian	Low	+	-	2, early			249	Wild type		
	M/52	Aboriginal	Low	+	+	1, early			249	Wild type		
	M/52	Caucasian	Low	+	+	1, early			249	Wild type		
	M/29	Melanesian	Low	+	+	3, early			249	Wild type		
	M/57	Vietnamese	Low	+	+	1, late			249	Wild type		
	M/35	Melanesian	Low	+	-	2, late			249	Wild type		
	F/69	Caucasian	Low	+	+	1, late			249	Wild type		
	M/36	Causasian	Low	+	+	2, late			249	Wild type		
										Total: 0/16		No codon-249 mutation in 16 tumours

Table 13 (contd)

Reference	Details of patients					Details of tumours		Details of mutations				Remarks
	Sex/age	Ethnic origin/ country	Exposure to aflatoxins	HBV status	Cirrhosis	Grade/ presentation	Number analysed	Loss of *p53* allele	*p53* alteration			
									Codon	Base change (nucleotide)	Amino acid change	
Challen *et al.* (1992)	9 M, 10 F; mean age, 62; range, 21–76	17/19 British	Low	+ 6/19	+14/19	No data	19	Not assayed	17/19 no mutation	17/19 wild type		Exons 5, 6, 7, 8 of *p53* from 19 tumours amplified by PCR and analysed by SSCP and direct sequencing
									1/19 codons 158, 159	6 base-pair deletion		
									1/19 codon 286	GAA to AAA	Glu to Lys	No codon-249 mutation in 19 tumours
Hosono *et al.* (1991)	No data	Taiwan, China	Low	Most tumours contained integrated HBV DNA	No data	No data	18 2	1/18 0/2		0/9	0/9	Steady-state levels of *p53*-specific RNA in tumours did not differ from those in normal adult liver. Exons 5, 6, 7, 8 of *p53* from 9 tumours amplified by PCR, subcloned and sequenced. No structural alteration seen. Synthesis of *p53*-specific mRNA or protein in HepG2 human hepatoblastoma cell line not affected by gene expression and replication of human HBV
								Total: 1/20	Total: 0/9			No codon-249 mutation in 9 tumours

Table 13 (contd)

Reference	Details of patients					Details of tumours		Details of mutations				Remarks
	Sex/age	Ethnic origin/ country	Exposure to aflatoxins	HBV status	Cirrhosis	Grade/ presentation	Number analysed	Loss of *p53* allele	*p53* alteration			
									Codon	Base change (nucleotide)	Amino acid change	
Hsu *et al.* (1991)	M/44	Qidong, China	High	High-risk area for HBV	No data	II, 5 x 4 cm			249	Wild type		Sections of *p53* gene containing exons 5, 6, 7, 8 and nearby intron regions amplified by PCR from genomic DNA from tumour samples and sequenced. Each mutation confirmed by sequencing both a second PCR sample and coding and non-coding strands of products. No other mutation found in exons 5, 6 or 8 or remainder of exon 7
	F/33		High			II, 14 x 10 cm			249	Wild type		
	M/49		High.			III, 11 x 10 cm II, 4 x 4 cm		+ (?)	249	AGG to AGT	Arg to Ser	
	M/60		High			III, 10 x 7 cm			249	Wild type		
	F/58		High			II, 11 x 8 cm		+ (?)	249	AGG to AGT	Arg to Ser	
	F/39		High			II, 5 x 5 cm			249	Wild type		
	F/35		High			II, 11 x 5 cm		+ (?)	249	AGG to AGC	Arg to Ser	
	M/65		High			II, 13 x 6 cm			249	Wild type		
	M/54[c]		High			II, 10 x 15 cm			249	AGG to AGG/AGT[d]	Arg to Ser	
	M/37[c]		High			II, 3 x 3 cm			249	AGG to AGG/AGT[d]	Arg to Ser	
	M/31		High			II, 5 x 4 cm		+ (?)	249	AGG to AGT	Arg to Ser	
	M/52		High			II, 6 x 4 cm			249	Wild type		
	M/35		High			III, 5 x 5 cm		+ (?)	249	AGG to AGT	Arg to Ser	
	M/39		High			III, 9 x 10 cm			249	Wild type		
	M/40		High			III, 6 x 7 cm			249	Wild type		
	M/38		High						249	AGG to AGG/AGT[d]	Arg to Ser	
										Total: 8/16		8 Codon-249 mutations in 16 tumours
Bressac *et al.* (1991)	No data	Southern Africa	High or low	-	-	Late[f]	10	*17p LOH*[e] NI[j]				Exons 5, 6, 7, 8 were amplified by PCR from tumour DNA and sequenced
			High	NT[g]	-	Late		-				
				+	-	Early		-				
				+	-	Early		+	249	AGG to AGT	Arg to Ser	
				+	-	Late		+	286	8 base-pair deletion	Frameshift	
				+	-	Late		NT	157	GTC to TTC	Val to Phe	
				+	-	Late		NT				
				+	+	Early		NI				
				+	+	Late		NI	249	AGG to AGT	Arg to Ser	
				+	+	Late		+	249	AGG to AGT	Arg to Ser	
								Total: 3/8		Total: 5/10		3 Codon-249 mutations in 10 tumours

Table 13 (contd)

Reference	Details of patients					Details of tumours		Details of mutations				Remarks
	Sex/age	Ethnic origin/ country	Exposure to aflatoxins	HBV status	Cirrhosis	Grade/ presentation	Number analysed	Loss of *p53* allele	*p53* alteration			
									Codon	Base change (nucleotide)	Amino acid change	
Ozturk *et al.* (1991)				*HBsAG*								167 specimens collected from patients in 14 countries. RNA (reversed transcribed to cDNA) used for 18 tumours, genomic DNA for rest. Exon 7 (including codon 249) amplified by PCR; separate PCR products analysed by restriction analysis with 2 enzymes. *Hae*III digestion of exon 7 of normal *p53* results in 2 fragments; mutation at codon 249 abolishes *Hae*III site: only one fragment detected after *Hae*III digestion. G to T mutation at base 3 of codon 249 creates new *Ple*I recognition site in exon 7; digestion with *Ple*I yields 3 fragments; with no codon-249 mutation, *Ple*I digestion yields only 2 fragments. Restriction analysis with *Hae*III used for initial screening. All identified mutations confirmed by additional restriction analysis with *Hae*III and *Ple*I
	M/23	Mozambique	High	+	No data	No data	15	No data	249	AGG to AGT	Arg to Ser	
	M/27		High	+					249	AGG to AGT	Arg to Ser	
	M/27[g]		High	+					249	AGG to AGT	Arg to Ser	
	M/36[g]		High.	+					249	AGG to AGT	Arg to Ser	
	M/40		High	-					249	AGG to AGT	Arg to Ser	
	M/27[g]		High	-					249	AGG to AGT	Arg to Ser	
	M/32		High	-					249	AGG to AGT	Arg to Ser	
	M/37		High	-					249	AGG to AGT	Arg to Ser	
	M/28		High	+					249	Wild type		
	M/28		High	+					249	Wild type		
	M/38[g]		High	+					249	Wild type		
	M/26		High	-					249	Wild type		
	M/41		High	-					249	Wild type		
	M/50		High	-					249	Wild type		
	M/39[g]		High	-					157	GTC to TTC	Val to Phe	
				Total: 7/15						Total: 8/15		
	M/28		Low	+	No data	No data	12	No data	249	AGG to AGT	Arg to Ser	
	M/29		Low	+					249	Wild type		
	M/35		Low	+					249	Wild type		
	M/46		Low	+					249	Wild type		
	M/49		Low	+					249	Wild type		
	M/24		Low	+					249	Wild type		
	M/48[g]		Low	+					249	Wild type		
	M/58[g]		Low	+					249	Wild type		
	M/32[g]		Low	+					286	8 base-pair deletion	Frameshift	
	M/27		Low	-					249	Wild type		
	M/50		Low	-					249	Wild type		
	M/45[g]		Low	NT					249	Wild type		
				Total: 9/11						Total: 1/12		

Table 13 (contd)

Reference	Details of patients					Details of tumours		Details of mutations				Remarks
	Sex/age	Ethnic origin/ country	Exposure to aflatoxins	HBV status	Cirrhosis	Grade/ presentation	Number analysed	Loss of *p53* allele	*p53* alteration			
									Codon	Base change (nucleotide)	Amino acid change	
Ozturk *et al.* (1991) (contd)	No data			*HBsAg*								
		Viet Nam	High	10%			3		249	AGG to AGT 1/3	Arg to Ser	12 Codon-249 mutations in 167 tumours
		China	High	10%			30		249	AGG to AGT 2/30	Arg to Ser	
		S. Africa[h]	Low	10%			24		249	AGG to AGT 1/24	Arg to Ser	
		Korea (Republic of)	Low	5%			5		249	Wild type 5/5		
		Japan	No data	2%			12		249	Wild type 12/12		
		USA	Low	0.3%			27		249	Wild type 27/27		
		Germany	Low	0.5%			20		249	Wild type 20/20		
		Spain	Low	1%			12		249	Wild type 12/12		
		Turkey	Low	1-5%			8		249	Wild type 8/8		
		Saudi Arabia	Low	1-5%			4		249	Wild type 4/4		
		Israel	Low	1-5%			3		249	Wild type 3/3		
		Italy	Low	1-5%			3		249	Wild type 3/3		
		India	High	1-5%			1		249	Wild type 1/1		
Scorsone *et al.* (1992)				*HBV integration sites*				*17p marker loss*[j]				DNA extracted from normal and tumour tissue from each patient. Separate PCR products of exon 7 (including codon 249) analysed by restriction with 2 enzymes. *Hae*III digestion described above (Ozturk *et al.*, 1991). AG to T transversion at codon 249 produced *Hinf*I site
	M/48	Qidong, China[i]	High	+	No data	No data	36	+i	249	Wild type		
	M/52			+				+	249	AGG to AGT	Arg to Ser	
	F/49			-				+	249	Wild type		
	M/42			+				+i	249	AGG to AGT	Arg to Ser	
	M/38			+				+	249	Wild type		
	M/38			-				+	249	Wild type		
	M/43			+				-	249	Wild type		
	M/54			+				+	249	AGG to AGT	Arg to Ser	
	M/37			+				+i	249	AGG to AGT	Arg to Ser	
	M/56			+				+i	249	Wild type		
	F/41			+				+i	249	Wild type		
	M/50			+				-	249	AGG to AGT	Arg to Ser	
	M/51			+				-	249	Wild type		
	m/38			+				+i	249	AGG to AGT	Arg to Ser	
	M/48			+					249	Wild type		

Table 13 (contd)

Reference	Details of patients					Details of tumours		Details of mutations				Remarks
	Sex/age	Ethnic origin/ country	Exposure to aflatoxins	HBV status	Cirrhosis	Grade/ presentation	Number analysed	Loss of *p53* allele	*p53* alteration			
									Codon	Base change (nucleotide)	Amino acid change	
Scorsone *et al.* (1992) (contd)	M/40			+				+	249	AGG to AGT	Arg to Ser	All 21 tumour DNAs showing loss of *Hae*III site showed gain of *Hinf*I site not present in DNA from normal tissue of same patient; loss of *Hae*III site thus due to specific mutation in base 3 of codon 249
	M/37			+				-	249	AGG to AGT	Arg to Ser	
	M/36			+				+	249	Wild type		
	F/31			+				-	249	Wild type		
	M/56			+				-	249	Wild type		
	M/36			+				+	249	AGG to AGT	Arg to Ser	
	M/26			+				+i	249	AGG to AGT	Arg to Ser	
	M/44			+				+	249	AGG to AGT	Arg to Ser	
	M/64			+				+	249	AGG to AGT	Arg to Ser	
	No data			+				NI	249	Wild type		
	No data			+				-	249	Wild type		
	M/48			+				-	249	AGG to AGT	Arg to Ser	
	M/45			+				-	249	AGG to AGT	Arg to Ser	
	M/33			+				-	249	AGG to AGT	Arg to Ser	
	F/50			+				+i	249	AGG to AGT	Arg to Ser	
	M/55			+				+	249	AGG to AGT	Arg to Ser	
	M/41			+				NI	249	Wild type		
	F/32			+				-	249	AGG to AGT	Arg to Ser	
	M/37			+				NI	249	AGG to AGT	Arg to Ser	
	M/52			+				-	249	AGG to AGT	Arg to Ser	
	F/40			+				+	249	AGG to AGT	Arg to Ser	
								Total: 12/20/36[k]		Total: 21/36		21 Codon-249 mutations in 36 tumours

HBV hepatitis B virus; PCR, polymerase chain reaction, SSCP, single-strand conformation polymorphism; NT, not tested; NI, not informative

[a]Grade 1, well differentiated; grade 2, moderately well differentiated; grade 3, poorly differentiated

[b]Early, found incidentally at transplantation or screening for α-fetoprotein and tumour < 2 cm diameter.

[c]See Scorsone *et al.* (1992)

[d]Heterozygote with both wild-type and mutant bands present for the third base-pair of codon 249.

[e]Loss of heterozygosity on chromosome 17p, determined using pYNZ22.1 probe or by analysis of *p53* gene alleles by Southern blotting of *Ban*II- or ScaI-digested genomic DNA using a human p53 cDNA probe

[f]Tumours designated as early or late on basis of size (small, < 5 cm diameter: moderately small, 5–10 cm: large, > 10 cm) and patient prognosis; late tumours large (or massive) and patients died within days or a few weeks after admission to hospital; early tumours small or moderately small and surgically resected

[g]Mentioned in Bressac *et al.* (1991) who give data on cirrhosis and presentation

[h]Includes Transkei, above

[i]From same liver cancer institute as Hsu *et al.* (1991)

[j]+, i, indirect evidence of loss of p53 allele

[k]No. direct p53 allele losses/all 17p allele losses/total no. of tumours analysed [Authors stated that 22 patients had 17p loss; only 20 found]

Aflatoxin B_1, not only induces point mutations but is also a potent clastogen, inducing chromosomal aberrations in several species *in vivo*. It is possible, therefore, that the allele loss seen in hepatocellular carcinomas is due in part to exposure to aflatoxin.

Some insight into the temporal relationship between codon-249 mutation and loss of the second allele of *p53* in hepatocellular carcinoma was gained in the study of Scorsone *et al.* (1992). Of 21 patients whose tumours had codon-249 mutations, 12 showed evidence of allele loss [numbers different in table and text of paper]. This suggests that the first step in *p53* inactivation in hepatocellular carcinoma is a point mutation in the allele, followed by loss of the wild-type allele.

(f) Point mutations in oncogenes in human hepatocellular carcinoma

Of the limited numbers of human liver tumours examined, only a few have exhibited mutations in proto-oncogenes. Of 34 tissue specimens surgically resected from 30 patients and of five cell lines of human hepatocellular carcinomas in Japan, only two had *ras* point mutations. In one, there was GGT to GTT transversion in codon 12 of c-Ki-*ras*; in the other, there was a CAA to AAA transition in codon 61 of N-*ras* (Tsuda *et al.*, 1989).

In another study conducted in Japan, 23 malignant hepatic tumours were examined. Point mutations at K-*ras* codon 12 or K-*ras* codon 61 were found in six of nine cholangiocarcinomas, but no point mutation around codon 12, 13 or 61 of the *ras* genes was found in 12 hepatocellular carcinomas or two hepatoblastomas (Tada *et al.*, 1990).

Expression of c-N-*ras* proto-oncogene was one to four times greater in tumour tissue from 12 hepatoma patients (in Shanghai, China) than in tissue from two normal livers. Increased expression of c-N-*ras* was also observed in tissue surrounding the tumours. Expression of c-*myc* was highly enhanced to various degrees in all 12 hepatoma patients. No point mutation was seen in c-N-*ras* or c-*myc* in any of the tumours (Zhang *et al.*, 1990).

4.4.2 *Experimental systems*

Aflatoxin B_1

Following its metabolic conversion to the 8,9-epoxide, aflatoxin B_1 reacts almost exclusively at *N*7 of guanine to form 8,9-dihydro-2-(*N*7-guanyl)-9-hydroxyaflatoxin B_1 (Autrup *et al.*, 1979; Shaulsky *et al.*, 1990). No other adduct has been identified.

The genetic effects of aflatoxin B_1 have been reviewed (Stark, 1986; IARC, 1987b). It has been tested extensively for genetic effects in a wide variety of tests *in vivo* and *in vitro*, giving positive results in the majority of assays. It is mutagenic and induces DNA damage in bacteria and binds covalently to isolated DNA. In fungi, aflatoxin B_1 is mutagenic and induces gene conversion and mitotic recombination. It induces sex-linked recessive lethal mutations and somatic mutation and recombination in *Drosophila*. It binds covalently to DNA of fish and chicken cells *in vitro*. It induces cell transformation in several test systems and chromosomal aberrations, sister chromatid exchange, mutation, unscheduled DNA synthesis and DNA strand breaks in rodent cells *in vitro*. In human cells *in vitro*, aflatoxin B_1 induces chromosomal aberrations, micronucleus formation, sister chromatid exchange, mutation and unscheduled DNA synthesis and binds covalently to DNA. It induces DNA damage and mutation in bacteria in host-mediated assays employing mice. It binds covalently to DNA of several species of fish and to DNA of chicken embryos.

Aflatoxin B_1 induces chromosomal aberrations, micronucleus formation, sister chromatid exchange, unscheduled DNA synthesis and DNA strand breaks, and binds covalently to DNA in cells of rodents treated *in vivo*; in one study, it was reported to be weakly active in a dominant lethal mutation assay in mice, but it was inactive in another study. In a single study, a mixture of 75% aflatoxin B_1 and 25% aflatoxin B_2 induced dominant lethal mutations in rats. Aflatoxin B_1 induced chromosomal aberrations in bone marrow of rhesus monkeys treated *in vivo*.

The 8,9-dihydro-2-(*N*7-guanyl)-9-hydroxyaflatoxin B_1 adduct has been isolated and identified in mammals treated *in vivo*. This adduct has also been identified in human urine (Autrup *et al.*, 1987; Groopman *et al.*, 1992a,b).

(*a*) *Modulating factors in the genetic activity of aflatoxins*

The genetic activity of aflatoxin B_1 can be inhibited by dietary components, including vitamin A (Qin & Huang, 1986), phenolic compounds (gallic acid, chlorogenic acid, caffeic acid, dopamine, eugenol, *para*-hydroxybenzoic acid) (San & Chan, 1987; Francis *et al.*, 1989b), plant flavonoids (kaempferol, morin, fisetin, biochanin A, rutin) (Francis *et al.*, 1989a), allixin (Yamasaki *et al.*, 1991) and penta-acetyl geniposide (Tseng *et al.*, 1992).

Aflatoxin B_1 induced fewer sister chromatid exchanges in mice fed a diet supplemented with retinyl acetate (vitamin A) than in mice fed a diet deficient in retinyl (Qin & Huang, 1986). The number of chromosomal aberrations induced in bone marrow of Chinese hamsters was reduced when they were given sodium selenite at 2 mg/l in drinking-water for 14 days (Petr *et al.*, 1990), and in mice given ascorbic acid at 10 mg/kg bw for 6 and 12 weeks (Bose & Sinha, 1991).

(*b*) *Mutations in proto-oncogenes and tumour-suppressor genes in tumours induced by aflatoxins in animals* in vivo (Table 19)

In rainbow trout fed aflatoxin B_1 at 80 ppb (μg/kg) in the diet for two weeks, 8/14 liver tumours of mixed histology contained mutations (seven GGA to GTA transversions, one GGA to AGA transition) in codon 12 of the c-Ki-*ras* proto-oncogene, and 2/14 contained GGT to GTT transversions at codon 13 of that gene (Chang *et al.*, 1991).

Male CF1 mice from the Shell Toxicology Laboratory, seven days old, were given single intraperitoneal injections of aflatoxin B_1 at 6 μg/g bw. Of eight liver tumours that developed 24 weeks or more after injection, one contained a CAA to CTA transversion at codon 61 of c-Ha-*ras* and two contained a CAA to AAA transversion at that codon (Bauer-Hofmann *et al*, 1990).

DNA samples from liver tumours induced in male Fischer 344 rats fed 1 ppm (mg/kg) aflatoxin B_1 and 0.3 ppm (mg/kg) aflatoxin G_1 and from two cell lines derived from the liver tumours were transfected into NIH 3T3 mouse cells, and primary transformants were selected by injection into nude athymic mice; secondary and tertiary transformants were obtained *in vitro* by focus assay. Of four tumours and two cell lines analysed, all contained exogenous *ras* sequences. Activation was found in 1/7 Ha-*ras*, 1/7 Ki-*ras* and 5/7 N-*ras* genes, but only one mutation (a G to A transition at codon 12) was identified in Ki-*ras* (Sinha *et al.*, 1988).

In an assay similar to that used by Sinha *et al* (1988), McMahon *et al.* (1990) showed that 3/8 liver carcinomas that had developed in male Fischer 344 within one to two years after intraperitoneal injection of 25 μg aflatoxin B_1 on five days a week for eight weeks contained c-Ki-*ras* mutations at codon 12. One was a GGT to TGT transversion, and two were GGT to GAT transitions.

In a retrospective study of four rhesus monkeys and four cynomolgus monkeys treated with aflatoxin B_1 by a variety of schedules, no codon 249 mutation was detected in exons 5, 7 or 8 of the *p53* gene in four hepatocellular carcinomas (two from one rhesus monkey), two cholangiocarcinomas, one spindle-cell carcinoma, one haemangioendothelial sarcoma or one osteogenic sarcoma. One hepatocellular carcinoma carried a G to T transversion at codon 175 (Fujimoto *et al.*, 1992).

Aflatoxin B_2

No data were available on the genetic and related effects of aflatoxin B_2 in humans. Aflatoxin B_2 binds to DNA *in vitro* after photoactivation by exposure to radiation at 365 nm. It induces mutation and DNA damage in bacteria but is not mutagenic to fungi in the absence of a metabolic system and does not induce gene conversion or mitotic recombination in yeast. It transforms Syrian hamster embryo cells and induces sister chromatid exchange in Chinese hamster cells and unscheduled DNA synthesis in rat hepatocytes but not in human fibroblasts *in vitro*. It inhibits intercellular communication between Syrian hamster cells *in vitro*. Aflatoxin B_2 binds covalently to DNA in hepatocytes of rats treated *in vivo*.

Aflatoxin G_1

No data were available on the genetic and related effects of aflatoxin G_1 in humans. Aflatoxin G_1 induces mutation and DNA damage in bacteria and binds covalently to isolated DNA. It induces mutation in *Neurospora crassa* but neither mutation nor gene conversion in *Saccharomyces cerevisiae*. It induces unscheduled DNA synthesis in human fibroblasts and rat hepatocytes *in vitro* and causes chromosomal aberrations and sister chromatid exchange in Chinese hamster cells *in vitro*. Aflatoxin G_1 induces chromosomal aberrations in bone-marrow cells of Chinese hamsters and mice treated *in vivo* and binds to DNA in kidney and liver cells of treated rats.

Aflatoxin G_2

No data were available on the genetic and related effects of aflatoxin G_2 in humans. Aflatoxin G_2 gave conflicting results for mutation in bacteria and does not cause DNA damage. It does not induce mutation in cultured rodent cells or in fungi in the absence of a metabolic system. It induces sister chromatid exchange in Chinese hamster cells and unscheduled DNA synthesis in rat and hamster hepatocytes *in vitro*. Aflatoxin G_2 does not induce unscheduled DNA synthesis in human fibroblasts *in vitro*.

Aflatoxin M_1

No data were available on the genetic and related effects of aflatoxin M_1 in humans. Aflatoxin M_1 is mutagenic to bacteria and binds to DNA *in vitro*. It binds to DNA of cultured trout hepatocytes and induces unscheduled DNA synthesis in rat hepatocytes *in vitro*.

Table 14. Genetic and related effects of aflatoxin B_1

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage induction/SOS/DNA strand breaks/cross-links	-	+	68.0000	Goze *et al.* (1975)
PRB, Prophage induction/SOS/DNA strand breaks/cross-links	0	+	25.0000	Sarasin *et al.* (1977)
PRB, Prophage induction/SOS/DNA strand breaks/cross-links	0	+	0.0500	Elespuru & Yarmolinsky (1979)
PRB, Prophage induction/SOS/DNA strand breaks/cross-links	(+)	+	0.0500	Wheeler *et al.* (1981)
PRB, Prophage induction/SOS/DNA strand breaks/cross-links	0	+	0.0050	Ho & Ho (1981)
PRB, Prophage induction/SOS/DNA strand breaks/cross-links	+	0	0.0300	Affolter *et al.* (1983a)
PRB, SOS repair, *Escherichia coli* PQ37	?	+	0.0100	Krivobok *et al.* (1987)
PRB, SOS repair, *Escherichia coli* PQ37	0	+	0.0100	Auffray & Boutibonnes (1987)
PRB, *umu* expression, *Salmonella typhimurium* TA1535/pSK1002	0	+	1.5000	Shimada *et al.* (1987)
PRB, *umu* expression, *Salmonella typhimurium* TA1535/pSK1002	0	+	3.1000[b]	Shimada *et al.* (1989)
PRB, *umu* expression, *Salmonella typhimurium* TA1535/pSK1002	0	+	3.1000[b]	Kitada *et al.* (1990)
PRB, *umu* expression, *Salmonella typhimurium* TA1535/pSK1002	0	+	3.0000	Baertschi *et al.* (1989)
ECB, *Escherichia coli*, DNA strand breaks/cross-links/repair	-	0	300.0000	Thielmann & Gersbach (1978)
ECD, *Escherichia coli pol*A, differential toxicity (spot test)	0	+	2.5000	Rosenkranz & Leifer (1980)
ERD, *Escherichia coli rec* strain, differential toxicity	-	+	0.0200	Ichinotsubo *et al.* (1977)
ERD, *Escherichia coli rec* strain, differential toxicity	0	+	0.8000	Mamber *et al.* (1983)
BSD, *Bacillus subtilis rec* strains, differential toxicity	+	0	10.0000	Ueno & Kubota (1976)
BSD, *Bacillus subtilis rec* strains, differential toxicity	0	+	13.4000	McCarroll *et al.* (1981)
BSD, *Bacillus subtilis rec* strains, differential toxicity	0	+	1.0000	Hirano *et al.* (1982)
SAF, *Salmonella typhimurium*, forward mutation	0	+	0.0300	Stark *et al.* (1979)
SAF, *Salmonella typhimurium*, forward mutation	0	+	0.0900	Skopek & Thilly (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	+	5.0000	von Engel & von Milczewski (1976)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	+	0.0250	Coles *et al.* (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0500	Tang & Friedman (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	0.0500	Ueno *et al.* (1978)

Table 14 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	0.0500	Wehner *et al.* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	0.0060	Malaveille *et al.* (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0130	Baker *et al.* (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0050	Booth *et al.* (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0010	Matsushima *et al.* (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	0.0250	Coles *et al.* (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0500	Decloitre & Hamon (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0100	Booth *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0100	Nishioka *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0250	Wheeler *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	0.0300	Dobiáš *et al.* (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	0.0050	Dunn *et al.* (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0060	Sizaret *et al.* (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0300	Stark & Giroux (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0400	Affolter *et al.* (1983b)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0100	Distelrath *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0500	Dorange *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.3000	Malaveille *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.3700	Södeerkvist *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–[c]	+	0.6000	Israel-Kalinsky *et al.* (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	0.2500	Yourtee & Kirk-Yourtee (1986)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.1500[b]	Ishii *et al.* (1986)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	0.5000	Yourtee *et al.* (1987a)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.2000[b]	Francis *et al.* (1989a)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0250[b]	Yamasaki *et al.* (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	0.0250	Turmo *et al.* (1991)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5.0000	Commoner (1976)

Table 14 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	0.5000	Wehner *et al.* (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	0	–	0.0500	Wheeler *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	0.0500	Dunn *et al.* (1982)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	0	–	0.0500	Dorange *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	0.5000	Commoner (1976)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	+	0.0500	Wehner *et al.* (1978)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	0.0500	Dunn *et al.* (1982)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	(+)	5.0000	von Engel & von Milczewski (1976)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	+	0.2500	Commoner (1976)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	0	+	0.1300	Stott & Sinnhuber (1978)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	0.0500	Dunn *et al.* (1982)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	0	+	0.0500	Dorange *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	(+)	5.0000	von Engel & von Milczewski (1976)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	0.0250	Coles *et al.* (1977)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	0.0050	Ueno *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	0.0500	Wehner *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.3100	Norpoth *et al.* (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0500	Booth *et al.* (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0100	Busk & Ahlborg (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0100	Booth *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0500	Gayda & Pariza (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.1000	Jayaraj & Richardson (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0100	Nishioka *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.5000	Nix *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0050	Robertson *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0500	Wheeler *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	3.1000	Chan *et al.* (1982)

Table 14 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0250	Chernesky *et al.* (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	+	0.0200	Dobiáš *et al.* (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	-	0.0500	Dunn *et al.* (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.1200	Friedman *et al.* (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	2.5000	Higashi *et al.* (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0500	Dorange *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0100	Israels *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0500	Kawajiri *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0050	Robertson *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.5000	Södeerkvist *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	+	0.0050	Walters & Combes (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0120	Coulombe *et al.* (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.5000	Yourtee & Kirk-Yourtee (1986)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0500	Qin & Huang (1986)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	9.0000[b]	San & Chan (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.2000	Francis *et al.* (1989a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	(+)	+	0.0250	Turmo *et al.* (1991)
SAS, *Salmonella typhimurium* (other strains), reverse mutation	0	+	3.100	Stark & Giroux (1982)
SCG, *Saccharomyces cerevisiae*, gene conversion	+	0	166.0000	Callen & Philpot (1977)
SCG, *Saccharomyces cerevisiae*, gene conversion	-	+	50.0000	Callen *et al.* (1977)
SCG, *Saccharomyces cerevisiae*, gene conversion	+	0	84.2400	Callen *et al.* (1978)
SCH, *Saccharomyces cerevisiae*, homozygosis	+	0	165.0000	Callen & Philpot (1977)
SCH, *Saccharomyces cerevisiae*, homozygosis	-	+	50.0000	Kuczuk *et al.* (1978)
SCH, *Saccharomyces cerevisiae*, homozygosis	-	-	1000.0000	Simmon (1979a)
NCF, *Neurospora crassa*, forward mutation	+	0	40.0000	Ong (1970)
NCF, *Neurospora crassa*, forward mutation	+	0	10.0000	Ong (1971)
NCF, *Neurospora crassa*, forward mutation	+	0	41.0000	Ong & de Serres (1972)

Table 14 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
NCF, *Neurospora crassa*, forward mutation	+	–	137.0000	Matzinger & Ong (1976)
DMG, *Drosophila melanogaster*, genetic crossing over/ recombination	+	0	50.0000	Graf *et al.* (1983)
DMM, *Drosophila melanogaster*, somatic mutation	+	0	12.5000	Fahmy & Fahmy (1983a)
DMM, *Drosophila melanogaster*, somatic mutation	+	0	6.2400	Fahmy & Fahmy (1983b)
DMM, *Drosophila melanogaster*, somatic mutation	+	0	50.0000	Graf *et al.* (1983)
DMX, *Drosophila melanogaster*, sex–linked recessive lethal mutation	+	0	20.0000	Lamb & Lilly (1971)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+	0	15.6000	Fahmy *et al.* (1978)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+	0	0.5000	Nix *et al.* (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+	0	6.2400	Fahmy & Fahmy (1983b)
DIA, DNA strand breaks/cross-links, animal cells *in vitro*	–	0	1.0000	Casto (1983)
DIA, DNA strand breaks/cross-links, rat hepatocytes *in vitro*	+	–	0.9400	Sina *et al.* (1983)
DIA, DNA strand breaks, Chinese hamster ovary cells *in vitro*	–	0	10.0000	Štětina & Votava (1986)
DIA, DNA strand breaks, AWRF rat fibroblasts *in vitro*	+	0	10.0000	Štětina & Votava (1986)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	0	3.1200	Williams (1976)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	0	0.0300	Michalopoulos *et al.* (1978)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	0	0.3000	Probst *et al.* (1981)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	0	31.0000	Ito (1982)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	0	0.0030	Loury & Byard (1983)

Table 14 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	0	0.0030	McQueen & Way (1991)
UIA, Unscheduled DNA synthesis, other animal cells *in vitro*	+	0	5.0000	Casto *et al.* (1976)
UIA, Unscheduled DNA synthesis, other animal cells *in vitro*	-	0	0.3000	Ide *et al.* (1981)
UIA, Unscheduled DNA synthesis, other animal cells *in vitro*	0	+	0.0300	McQueen *et al.* (1983)
UIA, Unscheduled DNA synthesis, other animal cells *in vitro*	+	0	10.0000	Tsutsui *et al.* (1984)
GCL, Gene mutation, Chinese hamster lung cells *hprt* locus *in vitro*	-	0	80.0000	Sawada *et al.* (1992)
GCL, Gene mutation, Chinese hamster lung cells *hprt* locus *in vitro*	+	0	1.2500	Sawada *et al.* (1992)
GCO, Gene mutation, Chinese hamster ovary cells *in vitro*	0	+	0.3000	Bermudez *et al.* (1982)
GCO, Gene mutation, Chinese hamster ovary cells *in vitro*	0	+	0.0400	Thompson *et al.* (1983)
G9H, Gene mutation, Chinese hamster lung V79 cells *hprt* locus *in vitro*	-	+	0.1300	Krahn & Heidelberger (1977)
G9H, Gene mutation, Chinese hamster lung V79 cells[d] *hprt* locus *in vitro*	0	+	0.1600	Kuroki *et al.* (1979)
G9H, Gene mutation, Chinese hamster lung V79 cells *hprt* locus *in vitro*	-	0	9.4000	Doehmer *et al.* (1988)
G9H, Gene mutation, Chinese hamster lung V79 cells[e] *hprt* locus *in vitro*	+	0	6.2000	Doehmer *et al.* (1988)
G9O, Gene mutation, Chinese hamster lung V79 cells ouabain^r *in vitro*	0	+	0.3000	Langenbach *et al.* (1978a)
G9O, Gene mutation, Chinese hamster lung V79 cells ouabain^r *in vitro*	0	+	0.0620	Langenbach *et al.* (1978b)
GML, Gene mutation, mouse lymphoma cells exclusive of L5178Y, *in vitro*	0	+	0.6000	Friedrich & Nass (1983)
GIA, Gene mutation, other animal cells *in vitro*	+	0	0.3000	Tong & Williams (1978)
GIA, Gene mutation, other animal cells *in vitro*	+	0	0.1000	Billings *et al.* (1983)
GIA, Gene mutation, other animal cells *in vitro*	+	0	0.0200	Link *et al.* (1983)
GIA, Gene mutation, other animal cells *in vitro*	+	0	0.3000	Ved Brat *et al.* (1983)

Table 14 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
GIA, Gene mutation, other animal cells *in vitro*	+	0	0.3000	Tong *et al.* (1984)
SIC, Sister chromatid exchange, Chinese hamster cells *in vitro*	(+)	+	0.3100	Wolff & Takehisa (1977)
SIC, Sister chromatid exchange, Chinese hamster cells *in vitro*	+	+	0.3100	Thomson & Evans (1979)
SIC, Sister chromatid exchange, Chinese hamster cells *in vitro*	-	+	0.1000	Batt *et al.* (1980)
SIC, Sister chromatid exchange, Chinese hamster cells *in vitro*	+	+	0.3100	Ray-Chaudhuri *et al.* (1980)
SIC, Sister chromatid exchange, Chinese hamster cells *in vitro*	0	+	0.0250	Huang *et al.* (1982)
SIC, Sister chromatid exchange, Chinese hamster cells *in vitro*	+	+	0.0300	Baker *et al.* (1983)
SIC, Sister chromatid exchange, Chinese hamster cells *in vitro*	+	0	0.3000	Kroeger-Koepke *et al.* (1983)
SIR, Sister chromatid exchange, rat cells *in vitro*	+	0	0.1600	Ved Brat *et al.* (1983)
CIC, Chromosomal aberrations, Chinese hamster cells *in vitro*	0	+	0.5000	Batt *et al.* (1980)
CIC, Chromosomal aberrations, Chinese hamster cells *in vitro*	-	+	1.6000	Stich & Stich (1982)
CIC, Chromosomal aberrations, Chinese hamster cells *in vitro*	-	+	3.1200	Whitehead *et al.* (1983)
CIT, Chromosomal aberrations, transformed cells *in vitro*	+	0	1.0000	Umeda *et al.* (1977)
TBM, Cell transformation, BALB/c 3T3 mouse cells *in vitro*	+	0	0.5000	DiPaolo *et al.* (1972)
TBM, Cell transformation, BALB/3T3 clone A31-1-1 mouse cells *in vitro*	+	0	0.0600	Cortesi *et al.* (1983)
TCM, Cell transformation, C3H 10T½ mouse cells *in vitro*	-	0	15.6000	Boreiko *et al.* (1982)
TCM, Cell transformation, C3H 10T½ mouse cells *in vitro*	+	0	1.0000	Nesnow *et al.* (1982)
TCM, Cell transformation, C3H 10T½ mouse cells *in vitro*	+	0	0.5000	Oshiro & Balwierz (1982)
TCM, Cell transformation, C3H 10T½ mouse cells *in vitro*	+	0	1.0000	Billings *et al.* (1983)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay *in vitro*	+	0	0.5000	DiPaolo *et al.* (1972)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay *in vitro*	+	0	0.1000	Pienta *et al.* (1977)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay *in vitro*	+	0	0.1250	DiPaolo (1980)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay *in vitro*	+	0	0.0300	Poily *et al.* (1980)

Table 14 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
TFS, Cell transformation, Syrian hamster embryo cells, focal assay *in vitro*	+	0	0.1200	Casto *et al.* (1977)
TCS, Cell transformation, other cell lines *in vitro*	+	0	15.6000	Shimada *et al.* (1983)
T7S, Cell transformation, SA7/Syrian hamster embryo cells *in vitro*	+	0	0.2500	Casto *et al.* (1976)
T7S, Cell transformation, SA7/Syrian hamster embryo cells *in vitro*	+	0	0.0600	Casto (1981)
DIH, DNA strand breaks/cross-links, human cells *in vitro*	-	0	10.0000	Casto (1983)
RIH, DNA repair exclusive of unscheduled DNA synthesis, human cells *in vitro*	0	+	0.0800	Leadon *et al.* (1981)
UHF, Unscheduled DNA synthesis, human fibroblasts *in vitro*	+	+	3.1200	San & Stich (1975)
UHF, Unscheduled DNA synthesis, human fibroblasts *in vitro*	(+)	+	18.7000	Stich & Laishes (1975)
UHF, Unscheduled DNA synthesis, human fibroblasts *in vitro*	+	0	31.2000	Mitchell (1976)
UHL, Unscheduled DNA synthesis, human lymphocytes *in vitro*	+	0	31.6000	Lake *et al.* (1980)
UHT, Unscheduled DNA synthesis, transformed human cells *in vitro*	(+)	+	0.3000	Martin *et al.* (1977)
UHT, Unscheduled DNA synthesis, transformed human cells *in vitro*	-	+	0.3000	Yu *et al.* (1983)
UIH, Unscheduled DNA synthesis, other human cells *in vitro*	+	0	9.4000	Freeman & San (1980)
UIH, Unscheduled DNA synthesis, other human cells *in vitro*	+	0	0.3000	Butterworth *et al.* (1982)
GIH, Gene mutation, human lymphoblastoid AHH-1 $tk^{+/-}$ cells *in vitro*	+	0	5.0000	Crespi *et al.* (1990)
GIH, Gene mutation, human lymphoblastoid AHH-1 $tk^{+/-}$ cells *in vitro*	+	0	1.0000	Crespi *et al.* (1991)
GIH, Gene mutation, human lymphoblastoid AHH-1 $tk^{+/-}$ cells[f] *hprt* locus *in vitro*	+	0	0.0030	Crespi *et al.* (1990)
GIH, Gene mutation, human lymphoblastoid AHH-1 $tk^{+/-}$ cells[g] *hprt* locus *in vitro*	+	0	0.0100	Crespi *et al.* (1991)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	0	0.0100	El-Zawahri *et al.* (1977)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	+	0.3000	Thomson & Evans (1979)

Table 14 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	-	0	6.0000	Fabry & Roberfroid (1981)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	+	0.3000	Inoue *et al.* (1983)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	(+)	0	0.0094	Amstad *et al.* (1984)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	0	0.0400	Li *et al.* (1989)
SHT, Sister chromatid exchange, transformed human cells *in vitro*	+	0	7.8000	Huh *et al.* (1982)
SHT, Sister chromatid exchange, transformed human cells *in vitro*	+	0	0.1600	Abe *et al.* (1983)
MIH, Micronucleus formation, human cells *in vitro*	+	+	0.0300	Iskandar & Vijayalaxmi (1981)
CHF, Chromosomal aberrations, human fibroblasts *in vitro*	-	+	18.7000	Stich & Laishes (1975)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	0	0.0100	El-Zawahri *et al.* (1977)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	(+)	+	0.0600	Fabry & Roberfroid (1981)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	0	0.0300	Amstad *et al.* (1984)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	-	+	0.6000	Ferguson *et al.* (1986)
BFA, Body fluids from animals, microbial mutagenicity	-	+	3.0000	Suit *et al.* (1977)
HMM, Host-mediated assay, microbial cells in animal hosts	+		10 × 1 ip	Brennan-Craddock *et al.* (1990)
HMM, Host-mediated assay, microbial cells in animal hosts	+		5 × 1 ip	Zeilmaker *et al.* (1991)
HMM, Host-mediated assay, microbial cells in mouse hosts	+		33 × 1 ip	Simmon *et al.* (1979)
HMM, Host-mediated assay, microbial cells in animal hosts	+		3.1000	Wei & Chang (1982)
DVA, DNA strand breaks/cross-links, rats *in vivo*	+		1 × 1 ip	Petzold & Swenberg (1978)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	+		2 × 1 po	Mirsalis *et al.* (1982)
UVA, Unscheduled DNA synthesis, chick embryo liver cells *in vivo*	+		0.3 (injection into egg)	Hamilton & Bloom (1984)
SVA, Sister chromatid exchange, mouse bone-marrow cells *in vivo*	+		20 × 1 iv	Nakanishi & Schneider (1979)
SVA, Sister chromatid exchange, chick embryo cells *in vivo*	+		0.30 (0.01 µg/ embryo)	Todd & Bloom (1980)
SVA, Sister chromatid exchange, bone marrow, male and female NMRI mice *in vivo*	+		100 × 1 po	Madle *et al.* (1986)

Table 14 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SVA, Sister chromatid exchange, bone marrow, male and female Chinese hamsters *in vivo*	–		25 × 1 po	Madle *et al.* (1986)
SVA, Sister chromatid exchange, bone marrow, male Wistar rats *in vivo*	+		6.25 × 1 po	Madle *et al.* (1986)
SVA, Sister chromatid exchange, bone marrow, female Wistar rats *in vivo*	–		6.25 × 1 po	Madle *et al.* (1986)
SVA, Sister chromatid exchange, bone marrow, male C57/6J mice *in vivo*	+		16.00 × 1 sc	Qin & Huang (1986)
SVA, Sister chromatid exchange, leukocytes, male Wistar rats *in vivo*	+		1.00 × 1 ip	Li & Lin (1990)
MVM, Micronucleus test, mouse bone-marrow cells *in vivo*	+		20.00 × 1 ip	Friedman & Staub (1977)
MVM, Micronucleus test, mice *in vivo*	–		100.00 × 1 ip	Bruce & Heddle (1979)
MVM, Micronucleus test, mice *in vivo*	+		5.00 × 1 ip	Fabry & Roberfroid (1981)
MVM, Micronucleus test, male and female NMRI mice *in vivo*	+		100.00 × 1 po	Madle *et al.* (1986)
MVR, Micronucleus test, rats *in vivo*	+		2.50 × 1 ip	Trzos *et al.* (1978)
MVR, Micronucleus test, male and female Wistar rats *in vivo*	+		3.13 × 1 po	Madle *et al.* (1986)
MVC, Micronucleus test, hamster bone-marrow cells *in vivo*	–		3.00 × 1 ip	Friedman & Staub (1977)
MVC, Micronucleus test, male and female Chinese hamsters *in vivo*	–		25.00 × 1 po	Madle *et al.* (1986)
CBA, Chromosomal aberrations, Chinese hamster bone-marrow cells *in vivo*	+		12.50 × 2 po	Korte & Rückert (1980)
CBA, Chromosomal aberrations, animal bone-marrow cells *in vivo*	+		5.00 × 1 ip	Fabry & Roberfroid (1981)
CBA, Chromosomal aberrations, bone marrow, male and female Swiss albino mice *in vivo*	+		8.00 × 1 ip	Krishnamurthy & Neelaram (1986)
CBA, Chromosomal aberrations, bone marrow, male and female Swiss albino mice *in vivo*	+		0.05 µg/kg bw diet	Kumari & Sinha (1990)
CBA, Chromosomal aberrations, bone marrow, male Wistar rats	+		5.00 × 1 ip	Ito *et al.* (1989)

Table 14 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CBA, Chromosomal aberrations, bone marrow, male Chinese hamsters (*Cricetulus griseus*) *in vivo*	+		0.001 × 1 ip	Bárta *et al.* (1984)
CBA, Chromosomal aberrations, bone marrow, male Chinese hamsters (*Cricetulus griseus*) *in vivo*	+		5.00 × 1 po	Petr *et al.* (1990)
CBA, Chromosomal aberrations, bone marrow, male Chinese hamsters (*Cricetulus griseus*) *in vivo*	+		0.0001 × 1 ip	Bárta *et al.* (1990)
CBA, Chromosomal aberrations, bone marrow, Chinese hamsters (*Cricetulus griseus*) *in vivo*	+		12.50 × 1 po	Roll *et al.* (1990)
CBA, Chromosomal aberrations, bone marrow, male Chinese hamsters (*Cricetulus griseus*) *in vivo*	+		1 × 1 ip	Petr *et al.* (1991)
CBA, Chromosomal aberrations, bone marrow, rhesus (*Macaca mulatta*) monkeys *in vivo*	+		0.1 × 1 ip	Bárta *et al.* (1984)
CLA, Chromosomal aberrations, leukocytes, male Wistar rats *in vivo*	+		1 × 1 ip	Li & Lin (1990)
CGC, Chromosomal aberrations, mouse spermatogonia *in vivo*, spermatocytes	-		5 × 1 ip	Leonard *et al.* (1975)
DLM, Dominant lethal mutation, mice	(+)		75 × 1 ip	Epstein *et al.* (1972)
DLM, Dominant lethal mutation, NMRI mice	-		45 × 1 ip	Roll *et al.* (1990)
DLR, Dominant lethal mutation, rats (strain unspecified)	+[h]		0.7 × 1 sc	Sharma *et al.* (1988)
ICR, Inhibition of intercellular communication, animal cells *in vitro*	-	0	50.0000	Jone *et al.* (1987)

Table 14 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SPM, Sperm morphology, mice *in vivo*	–		100	Bruce & Heddle (1979)
SPR, Sperm morphology, rats *in vivo*	–		0.3000	Egbunike (1979)

+, positive; (+), weakly positive; –, negative; 0, not tested
[a]In-vitro tests, μg/ml; in-vivo tests, mg/kg bw
[b]Only dose tested
[c]Photoactivated aflatoxin B_1 positive
[d]Transfected with and stably expressing cynomolgus monkey cytochrome P450IA1 cDNA (not on profile)
[e]Transfected with and stably expressing rat cytochrome P450IIB1 cDNA (not on profile)
[f]Transfected with and expressing human CYP1A2 gene (not on profile)
[g]Transfected with and expressing human CYP3A4 gene (not on profile)
[h]75% aflatoxin B_1, 25% aflatoxin B_2

Table 15. Genetic and related effects of aflatoxin B_2

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage induction/SOS/DNA strand breaks/cross-links	-	-	5.0000	Wheeler *et al.* (1981)
PRB, SOS repair, *Escherichia coli* PQ37	?	+	3.0000	Krivobok *et al.* (1987)
ECB, *Escherichia coli*, DNA strand breaks/cross-links/repair	-	0	24.0000	Thielmann & Gersbach (1978)
ECL, *Escherichia coli pol*A, differential toxicity (liquid)	0	-	250.0000	Rosenkranz & Poirier (1979)
BSD, *Bacillus subtilis rec* strains, differential toxicity	-	0	100.0000	Ueno & Kubota (1976)
SAF, *Salmonella typhimurium*, forward mutation	-	+	0.1800	Xu *et al.* (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	-	5.0000	von Engel & von Milczewski (1976)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	50.0000	McCann *et al.* (1975)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	-	3.0000	Gurtoo *et al.* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	-	0.2000	Dahl *et al.* (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	-	5.0000	Wheeler *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+[b]	0	40 μM	Israel-Kalinsky *et al.* (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	-	25.0000	Yourtee *et al.* (1987b)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	-	250.0000	Rosenkranz & Poirier (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	-	125.0000	Simmon (1979b)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	-	5.0000	Wheeler *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	-	-	125.0000	Simmon (1979b)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	-	5.0000	von Engel & von Milczewski (1976)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	-	250.0000	Rosenkranz & Poirier (1979)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	-	5.0000	Wheeler *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	-	5.0000	von Engel & von Milczewski (1976)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	50.0000	McCann *et al.* (1975)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0250	Hsieh *et al.* (1977)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	37.5000	Kleinwächter & Koukalová (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	-	0.2000	Dahl *et al.* (1980)

Table 15 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5.0000	Wheeler *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	0.5000	Coulombe *et al.* (1982)
SAS, *Salmonella typhimurium* other strains, reverse mutation	–	–	125.0000	Simmon (1979b)
SCH, *Saccharomyces cerevisiae*, homozygosis	–	–	100.0000	Simmon (1979a)
NCF, *Neurospora crassa*, forward mutation	–	0	38.0000	Ong & de Serres (1972)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	(+)	0	3.1400	Williams (1976)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	0	30.0000	Williams (1977)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	0	3.1400	Probst *et al.* (1981)
G9H, Gene mutation, Chinese hamster lung V79 cells *hprt* locus *in vitro*	0	–	14.0000	Krahn & Heidelberger (1977)
SIC, Sister chromatid exchange, Chinese hamster cells *in vitro*	–	+	3.1400	Batt *et al.* (1980)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay *in vitro*	–	+	0.5000	Pienta *et al.* (1977)
UHF, Unscheduled DNA synthesis, human fibroblasts *in vitro*	–	–	314.0000	Stich & Laishes (1975)
HMM, Host-mediated assay, microbial cells in mouse hosts	–		2 × 1 po	Simmon *et al.* (1979)
DLR, Dominant lethal mutation, rats	+[b]		0.7 × 1 sc	Sharma *et al.* (1988)
BID, Binding to calf thymus DNA *in vitro*[c]	+	0	30.0000	Israel-Kalinsky *et al.* (1984)
BID, Binding to calf thymus and supercoiled pBR322 DNA *in vitro*[c]	+	0	90.0000	Stark *et al.* (1988)
BID, Binding to ^{14}C-labelled nick-translated DNA *in vitro*[c]	+	0	31.2000	Shaulsky *et al.* (1990)
BID, Binding to calf thymus DNA *in vitro* (aerobic or anaerobic)[c]	+	0	0.6000	Stark *et al.* (1990)
BID, Binding to calf thymus DNA *in vitro* (aerobic or anaerobic)[c]	+	0	3.2000	Stark & Liberman (1991)
BVD, Binding to liver DNA of rats *in vivo*	(+)		2 × 1 ip	Swenson *et al.* (1977)
BVD, Binding to liver DNA of male CDF Fischer rats *in vivo*	+		1 × 1 ip	Groopman *et al.* (1981a)
ICR, Inhibition of gap-junctional intercellular communication, Chinese hamster V79 lung cells *in vitro*	–	0	50.0000	Jone *et al.* (1987)

+, positive; (+), weakly positive; –, negative; 0, not tested

[a] In-vitro tests, μg/ml; in-vivo tests, mg/kg bw

[b] 75% aflatoxin B_1, 25% aflatoxin B_2

[c] Photoactivated

Table 16. Genetic and related effects of aflatoxin G_1

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage induction/SOS/DNA strand breaks/cross-links	0	+	10.0000	Mamber *et al.* (1984)
PRB, SOS repair, *Escherichia coli* PQ37	?	+	1.0000	Krivobok *et al.* (1987)
PRB, SOS repair, *Salmonella typhimurium* TA1535/pSK1002	0	+	6.2000	Shimada *et al.* (1989)
PRB, *umu* expression, *Salmonella typhimurium* TA1535/pSK1002	0	+[b]	3.0000	Baertschi *et al.* (1989)
ECB, *Escherichia coli*, DNA strand breaks/cross-links/repair	-	0	300.0000	Thielmann & Gersbach (1978)
ERD, *Escherichia coli rec* strains, differential toxicity	0	+	0.8000	Mamber *et al.* (1983)
ERD, *Escherichia coli rec* strains, differential toxicity	0	+	0.8000	Mamber *et al.* (1984)
BSD, *Bacillus subtilis rec* strains, differential toxicity	+	0	100.0000	Ueno & Kubota (1976)
SAF, *Salmonella typhimurium*, forward mutation	-	+	0.0200	Xu *et al.* (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	+	5.0000	von Engel & von Milczewski (1976)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	1.0000	Gurtoo *et al.* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	-	0.0250	Booth *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	2.5000	Yourtee *et al.* (1987b)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	-	5.0000	von Engel & von Milczewski (1976)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	-	5.0000	von Engel & von Milczewski (1976)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0500	Hsieh *et al.* (1977)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	+	0.5000	Ueno *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	1.0000	Kleinwächter & Koukalová (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	1.0000	Booth *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	+	0.2500	Coulombe *et al.* (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.8000	Mamber *et al.* (1984)
SCG, *Saccharomyces cerevisiae*, gene conversion	-	-	200.0000	Callen *et al.* (1977)
SCR, *Saccharomyces cerevisiae*, reverse mutation	-	-	300.0000	Callen *et al.* (1977)
NCF, *Neurospora crassa*, forward mutation	(+)	0	40.0000	Ong (1971)

Table 16 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
NCF, *Neurospora crassa*, forward mutation	+	0	39.0000	Ong & de Serres (1972)
NCF, *Neurospora crassa*, forward mutation	-	+	219.7600	Matzinger & Ong (1976)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	0	3.2800	Williams (1977)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	0	0.2000	Probst *et al.* (1981)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	-	+	32.0000	Ito (1982)
SIC, Sister chromatid exchange, Chinese hamster cells *in vitro*	-	+	2.5000	Batt *et al.* (1980)
CIC, Chromosomal aberrations, Chinese hamster cells *in vitro*	0	+	29.8000	Batt *et al.* (1980)
UHF, Unscheduled DNA synthesis, human fibroblasts *in vitro*	-	+	32.8000	San & Stich (1975)
UHF, Unscheduled DNA synthesis, human fibroblasts *in vitro*	-	+	20.0000	Stich & Laishes (1975)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	0	30.0000	El-Zawahri *et al.* (1990)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	0	1.0000	El-Zawahri *et al.* (1990)
CBA, Chromosomal aberrations, Chinese hamster bone-marrow cells *in vivo*	+		50 × 2 po	Korte & Rückert (1980)
CBA, Chromosomal aberrations, mouse bone-marrow cells *in vivo*	+		50 × 1 po or ip	Roll *et al.* (1990)
BID, Binding to calf thymus DNA, DNA adducts (guanyl-*N*7)	0	+	15.0000	Baertschi *et al.* (1989)
BID, Binding to DNA *in vitro*	+	0	79.0000	Garner *et al.* (1979)
BVD, Binding to DNA, rats *in vivo*	+		0.6 × 1 ip	Garner *et al.* (1979)
BVD, Binding to DNA, rats *in vivo*	+		0.5 × 1 ip	Wild *et al.* (1990b)

+, positive; (+), weak positive; -, negative; 0, not tested

[a]In-vitro tests, μg/ml; in-vivo tests, mg/kg bw

[b]With rat and human microsomes

Table 17. Genetic and related effects of aflatoxin G_2

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS repair, *Escherichia coli* PQ37	-	-	60.0000	Krivobok *et al.* (1987)
ECB, *Escherichia coli*, DNA strand breaks/cross-links/repair	-	0	25.0000	Thielmann & Gersbach (1978)
BSD, *Bacillus subtilis rec* strains, differential toxicity	-	0	100.0000	Ueno & Kubota (1976)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	-	5.0000	von Engel & von Milczewski (1976)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	-	1.0000	Gurtoo *et al.* (1978)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	-	5.0000	von Engel & von Milczewski (1976)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	-	5.0000	von Engel & von Milczewski (1976)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0500	Hsieh *et al.* (1977)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	-	25.0000	Kleinwächter & Koukalová (1979)
NCF, *Neurospora crassa*, forward mutation	-	0	40.0000	Ong & de Serres (1972)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	-	0	3.8000	Williams (1977)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	0	0.0300	Probst *et al.* (1981)
URP, Unscheduled DNA synthesis, female rat primary hepatocytes *in vitro*	-	0	30.0000	McQueen & Way (1991)
UIA, Unscheduled DNA synthesis, Syrian hamster primary hepatocytes *in vitro*	+	0	0.0300	McQueen *et al.* (1983)
G9H, Gene mutation, Chinese hamster lung V79 cells *hprt* locus *in vitro*	-	-	3.3000	Kuroki *et al.* (1979)
G9O, Gene mutation, Chinese hamster lung V79 cells ouabain^r *in vitro*	0	-	1.1000	Langenbach *et al.* (1978a)
G9O, Gene mutation, Chinese hamster lung V79 cells ouabain^r *in vitro*	-	-	3.3000	Kuroki *et al.* (1979)
GIA, Gene mutation, other animal cells *in vitro*	-	0	3.3000	Tong & Williams (1978)
GIA, Gene mutation, other animal cells *in vitro*	-	0	165.0000	Ved Brat *et al.* (1983)
GIA, Gene mutation, other animal cells *in vitro*	-	0	3.3000	Tong *et al.* (1984)

Table 17 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SIC, Sister chromatid exchange, Chinese hamster cells *in vitro*	-	+	3.3000	Batt *et al.* (1980)
SIC, Sister chromatid exchange, Chinese hamster cells *in vitro*	-	+	0.3000	Ray-Chaudhuri *et al.* (1980)
SIR, Sister chromatid exchange, rat cells *in vitro*	(+)	0	3.3000	Ved Brat *et al.* (1983)
UHF, Unscheduled DNA synthesis, human fibroblasts *in vitro*	-	-	330.0000	Stich & Laishes (1975)

+, positive; (+), weak positive; -, negative; 0, not tested
[a]In-vitro tests, μg/ml; in-vivo tests, mg/kg bw

Table 18. Genetic and related effects of aflatoxin M_1

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	5.0000	von Engel & von Milczewski (1976)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	–	0.5000	Gurtoo *et al.* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.1000	Uwaifo & Bababunmi (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	0.1000	Uwaifo *et al.* (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	0.1500	Uwaifo *et al.* (1979)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	+	0.1500	Uwaifo *et al.* (1979)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	5.0000	von Engel & von Milczewski (1976)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	+	0.1500	Uwaifo *et al.* (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5.0000	von Engel & von Milczewski (1976)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0250	Hsieh *et al.* (1977)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	0.1500	Uwaifo *et al.* (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	0.5000	Coulombe *et al.* (1982)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	0	0.0125	Green *et al.* (1982)
BID, Binding to λDNA *in vitro*	+	0	20.0000	Mariën *et al.* (1987)
BID, Binding to *Salmo gairdneri* (rainbow trout) hepatocyte DNA *in vitro*	+	0	0.6200	Loveland *et al.* (1988)

+, positive; (+), weak positive; –, negative; 0, not tested
[a]In-vitro tests, μg/ml; in-vivo tests, mg/kg bw

Table 19. Mutations in oncogenes or tumour-suppressor genes found in animals treated *in vivo* with aflatoxin B_1

Species	Aflatoxin B_1 treatment	Type of tumours examined	Method of analysis	No. mutated genes/ no. tumours analysed	Details of mutations		Reference
					Gene, codon	Base change	
Oncorhynchus mykiss (Rainbow trout)	80 ppb in diet for 2 weeks; fish killed 9 months after treatment	25% pure hepatocellular carcinomas, 75% mixed hepatocellular/ cholangiocellular carcinomas; not examined histologically and consequently not identified	PCR from tumour DNA of 111 base-pair fragment that included codons 12 and 13 of c-Ki-*ras* gene. PCR fragments analysed by oligonucleotide hybridization with probes carrying different codon 12 and 13 base-pair substitutions. Four tumour DNAs were also cloned and sequenced.	10/14[a]	c-Ki-*ras*, 12 c-Ki-*ras*, 13	GGA to GTA (7/10) GGA to AGA (1/10) GGT to GTT (2/10)	Chang *et al.* (1991)
STCF1 male mice (derived from CF1 mice)	7-day-old mice injected i.p. with 6 µg/g bw aflatoxin B_1 and killed sequentially after start of treatment	First liver tumour found in control animals at 78 weeks and in treated mice at 24 weeks. 48% treated mice over 40 weeks had liver tumours; 77% adenoma, 23% adenoma and carcinoma or carcinoma alone	Fragments around codon 61 of c-Ha-*ras* exon 2 and codon 12 amplified by PCR from DNA isolated from formalin-fixed paraffin-embedded tissues. PCR fragments analysed by oligonucleotide hybridization with probes carrying different base-pair substitutions	3/8[b]	c-Ha-*ras*, 61	CAA to CTA, 1/3 CAA to AAA, 2/3[c]	Bauer-Hofmann *et al.* (1990)
Male Fischer 344 rats	Dietary groundnut meal naturally contaminated with aflatoxins to final concentrations of 1 ppm aflatoxin B_1 and 0.3 ppm aflatoxin G_1. Rats, 3 weeks old at start of feeding, given diet for 8 weeks and killed after 1 year	Yield not given; treatment produces liver tumours of mixed types. Tumours analysed not examined histologically	Co-transfection of DNA from each of 4 liver tumours and 2 tumorigenic liver tumour cell-lines with a gene for antibiotic resistance, followed by selection for antibiotic resistance; tumorigenicity testing in nude mice demonstrated DNA-mediated transfer of neoplastic phenotype. DNA extracted from nude mouse tumours used in secondary round of transfection with NIH 3T3 cells, which gave positive results in focus assays. DNA from transfectants analysed by oligonucleotide hybridization with probes carrying different *ras* base-pair substitutions	4/4 tumours Hepatoma cell lines JB1 BL10 Immortalized, non-transformed rat liver-cell lines BL8 BL9 BL8 exposed to aflatoxin B_1 and transformed L6	c-Ki-*ras*, codon 12 in 1/4 tumours c-N-*ras* in 3/4 tumours c-N-*ras* c-N-*ras* None None c-Ha-*ras*	GGA to GAA	Sinha *et al.* (1988)

Table 19 (contd)

Species	Aflatoxin B_1 treatment	Type of tumours examined	Method of analysis	No. mutated genes/ no. tumours analysed	Details of mutations		Reference
					Gene, codon	Base change	
Male Fischer 344 rats	Weanling rats given daily i.p. injections of 25 µg aflatoxin B_1 5 days/week for 8 weeks; killed at 12–18 months	9/9 rats developed liver carcinomas within 1–2 years after treatment	DNA from excised tumours transfected into NIH 3T3 mouse cells, which were assayed for ability to form morphologically transformed foci or to induce tumours in nude mice. c-Ki-*ras* and N-*ras* fragments amplified by PCR of DNA from transformed foci or s.c. nude mouse tumours and analysed by oligonucleotide hybridization with probes carrying different *ras* base-pair substitutions. Primary liver tissue from control and treated rats also analysed	3/8[d]	c-Ki-*ras*, 12	GGT to TGT (1/8) GGT to GAT (2/8)	McMahon *et al.* (1990)
Macaca mulatta (rhesus monkey) *Macaca fascicularis* (cynomolgus monkey)	Aflatoxin B_1 administered by a variety of schedules from 1964 to 1978	*4 rhesus monkeys* 3 hepatocellular carcinomas (2 in 1 animal); 2 cholangiocarcinomas *4 cynomolgus monkeys* 1 hepatocellular carcinoma; 1 spindle-cell carcinoma; 1 haemangioendothelial sarcoma; 1 osteogenic sarcoma	DNA extracted from paraffin blocks and analysed for *p53* mutations. At least 6 clones from PCR-amplified exons 5,7, 8 of each of 9 tumours sequenced. Restriction analysis of exon 7 using *Hae*III fragments used to look for codon-249 mutations	1/9	No codon-249 mutation. Exons 5,7, 8 of *p53* gene at codon 175	CGC to CTC substitution at codon 175 of exon 5	Fujimoto *et al.* (1992)

PCR, polymerase chain reaction

[a]Similar analysis of exon 1 of c-*ras*-2 gene revealed no mutation

[b]No mutation at codon 12 of c-Ha-*ras* gene

[c]Also seen in 2/8 tumours from phenobarbital-treated mice

[d]A GGC to GTT mutation at codon 13 of N-*ras* seen in livers from controls and from aflatoxin B_1-treated rats at equal frequencies (3/3 and 5/5, respectively) [suggesting that Fischer F344 rats carry a germ-line mutation that incurs high sensitivity to liver carcinogenesis by aflatoxin B_1 and other chemical carcinogens]

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Aflatoxins are a group of relatively stable toxins produced mainly by two *Aspergillus* species that are ubiquitous in areas of the world with hot, humid climates. Whether exposure is predominantly to aflatoxin B_1 or to mixed B_1 and G_1 depends on the geographical distribution of the *Aspergillus* strains. *Aspergillus flavus*, which produces aflatoxins B_1 and B_2, occurs worldwide; *A. parasiticus*, which produces aflatoxins B_1, B_2, G_1 and G_2, occurs principally in the Americas and in Africa. Exposure occurs primarily through dietary intake of maize and groundnuts. Exposure to aflatoxin M_1 occurs mainly through consumption of milk, including mother's milk. Life-time exposure to aflatoxins in some parts of the world, commencing *in utero*, has been confirmed by biomonitoring.

5.2 Human carcinogenicity data

One cohort study of a small number of Dutch oilpress workers exposed to aflatoxin-containing dusts indicated increased mortality from cancer, but no death from hepatocellular carcinoma was observed. A cohort study in China found significant excess mortality from liver cancer among individuals in villages where foods were heavily contaminated with aflatoxins. A cohort study of Danish workers exposed to aflatoxin from imported feed found an excess of hepatocellular carcinoma among those who had had major exposure to aflatoxin-contaminated feed in the period 10 or more years before diagnosis. In a cohort study in China, a significant elevation in risk for hepatocellular carcinoma was found among people with aflatoxin metabolites in the urine, after adjustment for hepatitis B surface antigen positivity. The elevation in risk was particularly high among those excreting aflatoxin B_1–guanine adducts; however, there was no association between dietary and urinary aflatoxin levels among subjects in whom both were detected.

Of three hospital-based case–control studies in which an attempt was made to evaluate exposure to aflatoxin B_1, one (in the Philippines) found a significantly greater risk for hepatocellular carcinoma among people whose intake of aflatoxin was estimated to be heavy than in those with light aflatoxin intake. The other two studies, one in Hong Kong and one in Thailand, gave negative results. In Thailand, one study on hepatocellular carcinoma and another on cholangiocarcinoma also found no association with the presence of aflatoxin B_1–albumin adducts in sera.

The two cohort studies in China addressed combined exposure to hepatitis B virus and aflatoxins and suggested that each has an independent effect.

Several correlation studies have been performed, the majority showing a strong association between estimated aflatoxin intake and incidence of hepatocellular carcinoma. In only a few was it possible to evaluate simultaneously any correlation with the prevalence of hepatitis infection. Of those that did so, two—one in Swaziland and one in China—showed a stronger correlation with exposure to aflatoxin B_1 than with hepatitis B viral infection. The largest such study, in China, did not show an association with the presence of aflatoxin B_1

metabolites in urine. The study from Swaziland was the only one in which it was shown that subjects had concomitant exposure to aflatoxin B_1 and G_1.

5.3 Carcinogenicity in experimental animals

Mixtures of aflatoxins and aflatoxin B_1 have been tested extensively for carcinogenicity by various routes of administration in several strains of mice and rats, in hamsters, several strains of fish, ducks, tree shrews and monkeys. Following their oral administration, mixtures of aflatoxins and aflatoxin B_1 caused hepatocellular and/or cholangiocellular liver tumours, including carcinomas, in all species tested except mice. In rats, renal-cell tumours and a low incidence of tumours at other sites, including the colon, were also found. In monkeys, liver angiosarcomas, osteogenic sarcomas and adenocarcinomas of the gall-bladder and pancreas developed, in addition to hepatocellular and cholangiocellular carcinomas. In adult mice, aflatoxin B_1 administered intraperitoneally increased the incidence of lung adenomas. Intraperitoneal administration of aflatoxin B_1 to infant mice, adult rats and toads produced high incidences of liver-cell tumours in all of these species. Subcutaneous injection of aflatoxin B_1 resulted in local sarcomas in rats. Exposure of fish embryos to aflatoxin B_1 induced a high incidence of hepatocellular adenomas and carcinomas. Intraperitoneal administration of aflatoxin B_1 to rats during pregnancy and lactation induced benign and malignant tumours in mothers and their progeny in the liver and in various other organs, including those of the digestive tract, the urogenital system and the central and peripheral nervous systems. In several species, aflatoxin B_1 administered by different routes induced foci of altered hepatocytes, the number and size of which was correlated with later development of hepatocellular adenomas and carcinomas.

Aflatoxin B_2 induced foci of altered hepatocytes and hepatocellular adenomas following its oral administration to rats. A low incidence of hepatocellular carcinomas was observed after intraperitoneal administration of aflatoxin B_2 to rats.

Oral administration of aflatoxin G_1 induced foci of altered hepatocytes, hepatocellular adenomas and carcinomas and renal-cell tumours in rats and liver-cell tumours in fish. The hepatocarcinogenic effect of aflatoxin G_1 was weaker than that of aflatoxin B_1. Subcutaneous injection of aflatoxin G_1 in rats resulted in local sarcomas, which developed at a lower incidence and at later times than those induced by aflatoxin B_1 at the same dose level and by the same route. Oral administration of aflatoxin G_2 to trout had no hepatocarcinogenic effect in one experiment.

Aflatoxin M_1, a hydroxy metabolite of aflatoxin B_1, produced fewer hepatocellular carcinomas following its oral administration to rats and fish than aflatoxin B_1 given at the same dose level and by the same route. Aflatoxin Q_1, another metabolite of aflatoxin B_1, produced a high incidence of hepatocellular carcinomas following its oral administration to fish. Administration to rats and fish of aflatoxicol, yet another metabolite of aflatoxin B_1, induced hepatocellular carcinomas in both species; the tumour incidence was lower than that in animals treated with aflatoxin B_1 at the same dose level.

A large number of experiments have been carried out in which aflatoxins were administered in combination (prior to, during and following) with diets, viruses, parasites, known carcinogens and a number of different chemicals in order to study the modulating effects,

including chemoprevention, of the agents on aflatoxin-induced carcinogenesis. Enhancing and inhibitory effects on the carcinogenicity of aflatoxins have been observed.

5.4 Other relevant data

Aflatoxin B_1 is consistently genotoxic, producing adducts in humans and animals *in vivo* and chromosomal anomalies in rodents and, in a single study, in rhesus monkeys *in vivo*. In human and animal cells in culture, it produces DNA damage, gene mutation and chromosomal anomalies; in animal cells *in vitro*, it also induces cell transformation. In insects and lower eukaryotes, it induces gene mutation and recombination. In bacteria, it produces DNA damage and gene mutation.

Aflatoxin B_1 is hepatoxic in humans and animals and is nephrotoxic and immunosuppressive in animals.

Aflatoxin B_2 has not been studied extensively, and most data are derived from single reports. Aflatoxin B_2 becomes bound to DNA of rats treated *in vivo*, after metabolic conversion to aflatoxin B_1. In rodent cells, it induces DNA damage, sister chromatid exchange and cell transformation, but not gene mutation. In fungi, it produces neither gene mutation nor recombination, whereas it produced gene mutation in bacteria.

Aflatoxin G_1 binds to DNA and produces chromosomal aberrations in rodents treated *in vivo*. In cultured human and animal cells, it induces DNA damage, and, in single studies, it induced chromosomal anomalies. It induces mutation in fungi and DNA damage and gene mutation in bacteria.

There are few published genetic studies on **aflatoxin G_2** and **aflatoxin M_1**. Aflatoxin G_1 produced DNA damage and sister chromatid exchange in animal cells in culture. Aflatoxin M_1 produced DNA damage in cultured rodent cells and gene mutation in bacteria.

Humans metabolize aflatoxin B_1 to an 8,9-epoxide, forming DNA and albumin adducts by the same activation pathways as susceptible animal species. Humans metabolize aflatoxin B_1 to the major aflatoxin B_1–*N*7-guanine and -serum albumin adduct at levels comparable to those in susceptible animal species (rat).

Glutathione *S*-transferase-mediated conjugation of glutathione to the 8,9-epoxide reduces DNA damage, and this mechanism is important in reducing the tumour burden in experimental animals. Animal species, such as the mouse, that are resistant to aflatoxin carcinogenesis have three to five times more glutathione *S*-transferase activity than susceptible species, such as the rat. Humans have less glutathione *S*-transferase activity for 8,9-epoxide conjugation than rats or mice, suggesting that humans are less capable of detoxifying this important metabolite.

Studies of human microsomal activation of aflatoxin B_1 show that at non-saturating concentrations of aflatoxin B_1 the rate of formation of the 8,9-epoxide is similar to that found in sensitive species (rat and monkey).

The value of aflatoxin B_1–*N*7-guanine as an indicator of risk for developing tumours is demonstrated by experiments with chemoprotective agents that show concordance between reduction of levels of DNA adduct formation and reduced incidence of liver tumours in rats and trout.

The presence of DNA- and protein–aflatoxin adducts in humans, the urinary excretion of aflatoxin B_1–*N*7-guanine adducts by humans, and the ability of human tissues to activate aflatoxin B_1 to form DNA adducts *in vitro* provide evidence that humans have the biochemical pathways required for aflatoxin-induced carcinogenesis. The following evidence is consistent with those biochemical mechanisms.

Studies with bacteria show that activated aflatoxin B_1 specifically induces G to T transversions. On the basis of experiments conducted *in vitro*, aflatoxin B_1 specifically targets the third and not the second nucleotide of codon 249 (AG*G*) of the human *p53* gene, an effect not seen with benzo[*a*]pyrene-7,8-diol-9,10-epoxide when tested at the same level of binding.

A high frequency of mutations at a mutational 'hot-spot' (the third nucleotide of codon 249 in exon 7) has been found in *p53* tumour suppressor genes in hepatocellular carcinomas from patients resident in areas considered to offer a high risk of exposure to aflatoxins and where there is a high incidence of hepatocellular carcinoma. In contrast, this mutation is rare in hepatocellular carcinomas from regions of low exposure to aflatoxins (including Australia, Japan, southern Africa, Germany, Spain, Italy, Turkey, Israel, Saudi Arabia, the United Kingdom and the USA).

5.5 Evaluation[1]

There is *sufficient evidence* in humans for the carcinogenicity of naturally occurring mixtures of aflatoxins.

There is *sufficient evidence* in humans for the carcinogenicity of aflatoxin B_1.

There is *inadequate evidence* in humans for the carcinogenicity of aflatoxin M_1.

There is *sufficient evidence* in experimental animals for the carcinogenicity of naturally occurring mixtures of aflatoxins and aflatoxins B_1, G_1 and M_1.

There is *limited evidence* in experimental animals for the carcinogenicity of aflatoxin B_2.

There is *inadequate evidence* in experimental animals for the carcinogenicity of aflatoxin G_2.

Overall evaluations

Naturally occurring aflatoxins *are carcinogenic to humans (Group 1).*

Aflatoxin M_1 *is possibly carcinogenic to humans (Group 2B).*

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[1]For definitions of the italicized terms, see Preamble, pp. 26-29.

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