TOXINS DERIVED FROM *FUSARIUM GRAMINEARUM*, *F. CULMORUM* AND *F. CROOKWELLENSE*: ZEARALENONE, DEOXYNIVALENOL, NIVALENOL AND FUSARENONE X

The most widely distributed toxigenic *Fusarium* species is *Fusarium graminearum*, which causes disease in wheat and maize all over the world, except in dryland wheat and subtropical maize. This fungus produces the type-B triochothecenes deoxynivalenol and nivalenol (Thrane, 1989) and zearalenone, depending on the strain. The closely related species, *F. culmorum* and *F. crookwellense*, produce the same toxins and occur in cooler and slightly warmer areas, respectively. *F. crookwellense* and some strains of *F. graminearum* also produce the type-B trichothecene fusarenone X.

Zearalenone was considered by a previous working group, in October 1982 (IARC, 1983), and fusarenone X was considered (as fusarenon X) by two groups, in February 1976 (IARC, 1976) and October 1982 (IARC, 1983). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluations.

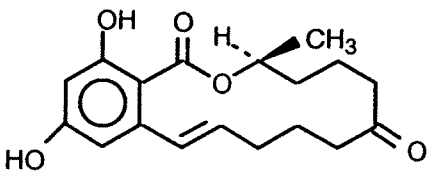
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Synonyms, structural and molecular data*

Zearalenone

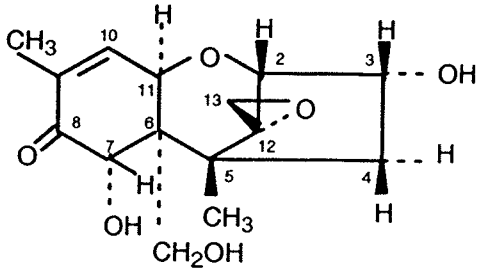
Chem. Abstr. Services Reg. No.: 17924-92-4
Chem. Abstr. Name: 1*H*-2-Benzoxacyclotetradecin-1,7(8*H*)-dione, 3,4,5,6,9,10-hexahydro-14,16-dihydroxy-3-methyl, [*S*-(*E*)]-
IUPAC Systematic Name: (–)-(3*S*,11*E*)-3,4,5,6,9,10-Hexahydro-14,16-dihydroxy-3-methyl-1*H*-2-benzoxacyclotetradecin-1,7(8*H*)-dione
Synonyms: F2; compound F-2; fermentation estrogenic substance; FES; (S)-(–)-3,4,5,6,9,10-hexahydro-14,16-dihydroxy-3-methyl-1*H*-2-benzoxacyclotetradecin-1,7-(8*H*)-dione; mycotoxin F2; toxin F2; (–)-zearalenone; (*S*)-zearalenone; *trans*-zearalenone; (10*S*)-zearalenone; zenone



$C_{18}H_{22}O_5$ Mol. wt: 318.4

Deoxynivalenol

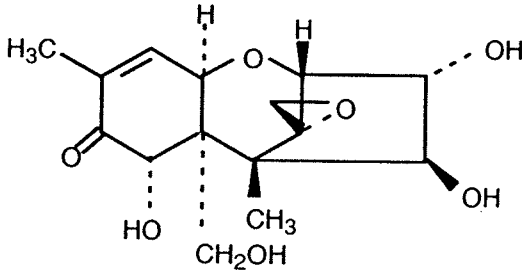
Chem. Abstr. Services Reg. No.: 51481–10–8
Chem. Abstr. Name: Trichothec-9-en-8-one, 12,13-epoxy-3,7,15-trihydroxy-(3α,7α)-
Synonyms: Dehydronivalenol; 4-deoxynivalenol; 12,13-epoxy-3α,7α,15-trihydroxy-9-trichothecen-8-one; Rd toxin; spiro[2,5-methano-1-benzoxepin-10,2′-oxirane], trichothec-9-en-8-one derivative; vomitoxin



$C_{15}H_{20}O_6$ Mol. wt: 296.32

Nivalenol

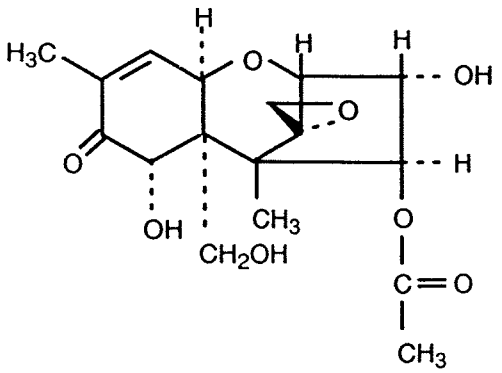
Chem. Abstr. Services Reg. No.: 23282–20–4
Chem. Abstr. Name: Trichothec-9-en-8-one, 12,13-epoxy-3,4,7,15-tetrahydroxy-, (3α,4β, 7α)-
IUPAC Systematic Name: Trichothec-9-en-8-one, 12,13-epoxy-3α,4β,7α,15-tetrahydroxy-
Synonyms: Spiro[2,5-methano-1-benzoxepin-10,2′-oxirane], trichothec-9-en-8-one derivative; 3α,4β,7α,15-tetrahydroxy-12,13-epoxytrichothec-9-en-8-one



$C_{15}H_{20}O_7$ Mol. wt: 312.32

Fusarenone X

Chem. Abstr. Services Reg. No.: 23255–69–8
Chem. Abstr. Name: Trichothec-9-en-8-one, 4-(acetyloxy)-12,13-epoxy-3,7,15-trihydroxy(3α,4β,7β)-
IUPAC Systematic Name: 12,13-Epoxy-3α,4β,7β,15-tetrahydroxytrichothec-9-en-8-one 4-acetate or (2*R*,3*R*,4*S*,5*S*,5a*R*,6*R*,9a*R*,10*S*)-2,3,4,5,5a,9a-hexahydro-3,4,6-trihydroxy-5a-(hydroxymethyl)-5,8-dimethylspiro[2,5-methano-1-benzoxepin-10,2′-oxirane]-7-(6*H*)-one 4-acetate
Synonyms: Fusarenon; fusarenon X; nivalenol 4-*O*-acetate; nivalenol monoacetate; 3,7,15-trihydroxy-4-acetoxy-8-oxo-12,13-epoxy-Δ^9-trichothecene; 3,7,15-trihydroxy-scirp-4-acetoxy-9-en-8-one



$C_{17}H_{22}O_8$ Mol. wt: 354.1

1.1.2 *Chemical and physical properties*

Zearalenone

From Urry *et al.* (1966), Pohland *et al.* (1982) and Budavari (1989), unless otherwise specified

(*a*) *Description*: White crystals
(*b*) *Melting-point*: 164–165 °C
(*c*) *Optical rotation*: $[\alpha]_D^{25}$ −170.5° (c = 1.0 in methanol); $[\alpha]_D^{21}$ −189° (c = 3.14 in chloroform)
(*d*) *Spectroscopy data*: Ultraviolet, infrared, mass spectral and proton nuclear magnetic resonance data have been reported.
(*e*) *Solubility*: Solubilities at 25 °C in percent by weight are: water, 0.002; *n*-hexane, 0.05; benzene, 1.13; acetonitrile, 8.6; dichloromethane, 17.5; methanol, 18; ethanol, 24; and acetone, 58 (Hidy *et al.*, 1977).
(*f*) *Stability*: Stable when heated at 120 °C; 29% decomposed when sample heated at 150 °C and 69% when heated at 200 °C for 60 min (Kuiper-Goodman *et al.*, 1987); stable to hydrolysis in neutral or acid buffer solutions (Müller, 1983)

Deoxynivalenol

From Cole and Cox (1981)

(*a*) *Description*: White needles
(*b*) *Melting-point*: 151–153 °C
(*c*) *Optical rotation*: $[\alpha]_D^{25}$ +6.35° (c = 0.07 in ethanol)
(*d*) *Spectroscopy*: Infrared, ultraviolet, mass spectral and proton nuclear magnetic resonance data have been reported.
(*e*) *Solubility*: Soluble in ethanol, methanol, ethyl acetate, water and chloroform

Nivalenol

From Cole and Cox (1981), unless otherwise specified

(*a*) *Description*: White crystals
(*b*) *Melting-point*: 222–223 °C (decomposition; dried in presence of P_2O_5 at reduced pressure)

(*c*) *Optical rotation*: $[\alpha]_D^{24}$ +21.54° (c = 1.3 in ethanol)

(*d*) *Spectroscopy data*: Ultraviolet, infrared, mass spectral (Brumley *et al.*, 1982) and proton nuclear magnetic resonance data have been reported.

(*e*) *Solubility*: Soluble in methanol, ethanol, ethyl acetate and chloroform; slightly soluble in water; soluble in polar organic solvents (Budavari, 1989)

Fusarenone X

From Ueno *et al.* (1969), Saito and Ohtsubo (1974) and Cole and Cox (1981), unless otherwise specified

(*a*) *Description*: Transparent bipyramid crystals

(*b*) *Melting-point*: 91–92 °C

(*c*) *Optical rotation*: $[\alpha]_D^{25}$ +58° (c = 1.0 in methanol); $[\alpha]_D^{24}$ +56.1° (in ethanol)

(*d*) *Spectroscopy data*: Ultraviolet, infrared, nuclear magnetic resonance and mass spectra have been reported.

(*e*) *Solubility*: Soluble in chloroform, ethyl acetate, methanol and water; insoluble in *n*-hexane and *n*-pentane

(*f*) *Stability*: Generally stable (WHO, 1990); hydrolysed by bases to nivalenol

(*g*) *Reactivity*: Reacts with acetic anhydride to give tetraacetylnivalenol

1.1.3 *Analysis*

Zearalenone

A detailed review of methods for the analysis of zearalenone is provided by Kuiper-Goodman *et al.* (1987). Analysis can be done by thin-layer chromatography, high-performance liquid chromatography (HPLC)–fluorescence detection and gas chromatography after derivatization. Zearalenone and related alcohols can be analysed by gas chromatography with derivatization (Schwadorf & Müller, 1992). Two methods have undergone trials by the Association of Official Analytical Chemists (AOAC) (USA): a thin-layer chromatographic method and an HPLC–fluorescence detection method (Gilbert, 1991). A number of antibody-based methods also exist (e.g., Warner *et al.*, 1986). Sensitive methods exist for the determination of zearalenone and related compounds in animal tissues (e.g., HPLC with fluorescence) (Kuiper-Goodman *et al.*, 1987).

Deoxynivalenol

Detailed reviews of methods of analysis for deoxynivalenol are provided by Scott (1990) and WHO (1990). Deoxynivalenol can be determined by thin-layer chromatography, HPLC with ultraviolet detection and gas chromatography after derivatization. Two methods have undergone trials at the AOAC: a thin-layer chromatography method and a gas chromatography method involving derivatization (Scott *et al.*, 1981; Trucksess *et al.*, 1986; Gilbert, 1991). Materials contaminated with deoxynivalenol are usually co-contaminated with other trichothecenes, and mass spectrometry confirmation is required, at least for some samples (Scott, 1990). Various antibody-based methods are available for the analysis of deoxynivalenol (Scott, 1990; WHO, 1990; Abouzied *et al.*, 1991), but they suffer from problems of cross-reactivity with other trichothecenes.

Nivalenol

A detailed review of methods for the analysis of nivalenol is given by WHO (1990). There is no AOAC-accepted method for the analysis of nivalenol (Gilbert, 1991). This toxin has been analysed by HPLC–ultraviolet detection and gas chromatography with derivatization, to provide useful data on its occurrence (Lauren & Greenhalgh, 1987; Tanaka *et al.*, 1988). Antibody-based assays with reasonable specificity have been developed by at least two groups (Ikebuchi *et al.*, 1990; Teshima *et al.*, 1990; Wang & Chu, 1991).

Fusarenone X

Most methods for the analysis of fusarenone X were developed for its simultaneous determination with other trichothecenes. They involve thin-layer chromatography, polarographic methods, HPLC with ultraviolet detection and gas chromatography or gas chromatography/mass spectrometry after derivatization (Bata *et al.*, 1983; Bottalico *et al.*, 1983; Visconti & Bottalico, 1983; Karppanen *et al.*, 1985; Visconti *et al.*, 1984).

1.2 Production and use

Zearalenone

Zearalenone was first isolated in 1962 from cultures of the fungus *Fusarium graminearum* (Stob *et al.*, 1962). The structure was determined in 1966 (Urry *et al.*, 1966), and a total synthesis was published two years later (Taub *et al.*, 1968). Zearalenone can be produced by culturing strains of the species that produce it in solid-state fermentations on autoclaved rice or maize or on a nutrient medium absorbed on vermiculite (Hidy *et al.*, 1977; Greenhalgh *et al.*, 1983; Kuiper-Goodman *et al.*, 1987).

Zearalenone is produced commercially as an intermediate in the preparation of zeranol (α-zearalenol) by submerged fermentation (Hidy *et al.*, 1977). Zeranol is used as a growth promoter in beef cattle, feedlot lambs and suckling beef calves (US Food and Drug Administration, 1980).

Zearalenone is produced by *F. graminearum*, *F. crookwellense*, *F. culmorum* and *F. semitectum*. The validity of reports that other species produce it has been questioned (Marasas *et al.*, 1984; Thrane, 1989). Cereals are infected by *F. graminearum* and related species when susceptible cultivars and inbred strains are planted. Disease epidemics occur when wet weather occurs at anthesis or silking (Sutton, 1982). Zearalenone can be produced in maize stored in open cribs if the maize does not dry quickly.

Deoxynivalenol

Deoxynivalenol was first isolated by Japanese workers as 'Rd-toxin', and shortly thereafter as 'vomitoxin' in the USA, from barley infected with *F. graminearum* (Morooka *et al.*, 1972; Vesonder *et al.*, 1973; Yoshizawa & Morooka, 1973; Miller *et al.*, 1983) and *F. culmorum* (Greenhalgh *et al.*, 1986). Deoxynivalenol can be produced by culturing strains of the species that produce it on sterilized rice or maize (e.g., Greenhalgh *et al.*, 1983). It can also be produced as the monoacetate in liquid culture followed by a simple hydrolysis step (Greenhalgh *et al.*, 1986).

Deoxynivalenol is produced by strains of *F. graminearum* and *F. culmorum* (Marasas *et al.*, 1984; Thrane, 1989), which are pathogens of cereals, particularly wheat and maize. The

disease is favoured by the planting of susceptible cultivars, and wet weather at anthesis or silking results in epidemic conditions of the disease (Sutton, 1982). *F. graminearum* and *F. culmorum* produce complex mixtures of toxins that vary by region of isolation. Most North American strains of *F. graminearum* produce deoxynivalenol, 15-acetyldeoxynivalenol, zearalenone and many additional metabolites, some of which occur in grains. In contrast, most Japanese, Australian and Italian strains produce either nivalenol, fusarenone X and zearalenone or deoxynivalenol, 3-acetyldeoxynivalenol and zearalenone plus the common metabolites. Strains of *F. graminearum* that produce nivalenol have never been found in North or South America (Miller *et al.*, 1991). An extensive discussion of the major and minor metabolites of *F. graminearum* and related species isolated in different countries is provided by Miller *et al.* (1991).

Nivalenol

Nivalenol was first isolated from '*Fusarium nivale*' Fn2B (Tatsuno *et al.* 1968, 1969; Ueno *et al.*, 1970a); this strain was subsequently shown to be an atypical strain of *F. sporotrichioides* (Marasas *et al.*, 1984). Most naturally occurring nivalenol is produced by strains of *F. graminearum* and *F. crookwellense* (Miller *et al.*, 1991). Nivalenol can be produced by culturing strains of the species that produce it on sterilized rice or maize (Ueno *et al.*, 1970a). It can also be produced in liquid cultures (Ueno *et al.*, 1970a; Lauren *et al.*, 1987).

Nivalenol-producing strains of *F. graminearum* appear to occur primarily in Japan, Australia and New Zealand (Blaney, 1991), although they have also been reported in Italy (Logrieco *et al.*, 1988; Miller *et al.*, 1991). Such strains do not appear to occur in North America. *F. crookwellense* is cosmopolitan, and minor amounts of nivalenol reported in Canadian grain come from this species. *F. graminearum* is a pathogen of cereals, and the disease is favoured by planting susceptible cultivars. Wet weather at anthesis or silking results in epidemic conditions of the disease (Sutton, 1982). *F. crookwellense* is a weak pathogen of cereals and is favoured under warmer conditions than those optimal for *F. graminearum*. Both species produce many other metabolites that occur in contaminated crops (Miller *et al.*, 1991).

Fusarenone X

Fusarenone X was first isolated from '*Fusarium nivale*' Fn2B (Ueno *et al.*, 1969), which was subsequently shown to be an atypical strain of *F. sporotrichioides* (Marasas *et al.*, 1984). In general, only *F. crookwellense* and some strains of *F. graminearum* produce fusarenone X (Ichinoe *et al.*, 1983; Goliński *et al.*, 1988). It has been produced by growing the fungus in liquid culture or on sterilized rice or maize (Ueno *et al.*, 1969; Bottalico *et al.*, 1990).

1.3 Occurrence

Zearalenone

Zearalenone is among the most widely distributed *Fusarium* mycotoxins. It is associated primarily with maize but occurs in modest concentrations in wheat, barley and sorghum, among other commodities (Table 1; Kuiper-Goodman *et al.*, 1987). Concentrations in food in North America, Japan and Europe are generally low; however, in some developing

countries, exposures can be high, particularly where maize is grown under north-temperate conditions (including highlands). Zearalenone is not transmitted from feed to milk to any significant extent (Prelusky *et al.*, 1990a). Milling of cereals contaminated with zearalenone concentrates the toxin in the bran fractions (Kuiper-Goodman *et al.*, 1987).

Table 1. Natural occurrence of zearalenone

Country or region	Product	Year	Positive samples/ total no.	Content (mg/kg)	Reference
North America					
Canada	Feed	1972–78	266/2022	0.01–141	Funnell (1979)
Canada	Feed	1975–79	3/493	0.05–0.2	Prior (1981)
Canada	Maize	1978–80	23/81	0.01–0.5	Williams (1985)
USA	Maize	1968–69	5/293	0.45–0.8	Shotwell *et al.* (1971)
USA	Maize	1972	38/223	0.1–5.0	Eppley *et al.* (1974)
USA	Maize	1972	3/3	0.9–7.8	Bennett *et al.* (1976)
USA	Maize	1974	23/372	0.04–10.4	Stoloff *et al.* (1976)
USA	Maize	NR	8/8	0.43–7.62	Shotwell *et al.* (1976)
USA	Maize	1986	17/19	0.2–13.2	Abbas *et al.* (1988)
USA	Feed	1968–70	28/65	0.1–2909	Mirocha & Christensen (1974)
USA	Feed	1981	40/342	0.1–8	Côté *et al.* (1984)
USA	Food	1985	17/19	0.003–0.13	Warner & Pestka (1987)
USA	Feed	NR	9/11	0.01–0.07	Ware & Thorpe (1978)
South America					
Argentina	Maize	NR	16/55	0.2–0.75	López & Tapia (1980)
Argentina	Maize	1981–82	6/94	0.03–0.91	Bean & Echandi (1989)
Argentina	Maize	1987	9/150	0.04–0.35	Chulze *et al.* (1989)
Argentina	Wheat	1983	20/20	Mean, 0.01	Tanaka *et al.* (1988)
Argentina	Barley	1983	13/20	Mean, 0.005	Tanaka *et al.* (1988)
Brazil	Maize	1985–86	16/328	0.26–9.8	Sabino *et al.* (1989)
Europe					
Austria	Maize	1978–79	3/6	0.42–1.0	Bottalico *et al.* (1981)
Austria	Maize	1988–89	39/67	< 40.0	Lew *et al.* (1991)
Bulgaria	Wheat	1983	0/2	ND	Tanaka *et al.* (1988)
France	Maize	1974	62/75	< 170	FAO (1979)
France	Wheat	1984	0/2	ND	Tanaka *et al.* (1988)
Hungary	Maize	1968	NR	70–80	FAO (1979)
Hungary	Wheat	1984	0/2	ND	Tanaka *et al.* (1988)
Norway	Wheat, barley	1984	20/102	0.001–0.023	Sundheim *et al.* (1988)
Poland	Maize	1988	5/5	0.7–350	Visconti *et al.* (1990)
Spain	Maize	1983–86	18/209	0.8–9.6	Muñoz *et al.* (1990)
Yugoslavia	Maize	1972	23/54	0.7–37.5	FAO (1979)

Table 1 (contd)

Country or region	Product	Year	Positive samples /total no.	Content (mg/kg)	Reference
Africa					
Egypt	Cereal, feed	NR	36/64	0.002–0.43	Abdelhamid (1990)
Swaziland	Beer	1976	6/55	0.8–5.3 mg/l	FAO (1979)
Transkei	Maize	1976–77	10/12	0.45–10.0	Marasas *et al.* (1979)
Transkei	Maize	1979	37/72	< 0.02–5.36	Thiel *et al.* (1982)
Zambia	Maize	1973–74	1	12.8	Marasas *et al.* (1977)
Zambia	Beer	1974	23/23	0.01–4.6 mg/l	Lovelace & Nyathi (1977)
Zambia	Maize	1974	17/17	0.1–0.8	Lovelace & Nyathi (1977)
Zambia	Maize	1986	19/33	0.08–6.0	Siame & Lovelace (1989)
Zambia	Feed	1986	16/97	0.05–0.5	Siame & Lovelace (1989)
Australasia					
Australia	Maize	1983	148/174	0.01–0.8	Blaney *et al.* (1986)
China	Wheat	1984–85	18/38	0.004–0.078	Tanaka *et al.* (1988)
China	Maize		17/47	0.01–0.17	Luo *et al.* (1990)
India	Maize	1989	9/86	0.76–1.5	Sinha (1991)
Indonesia	Maize	NR	7/26	0.001–0.01	Widiastuti *et al.* (1988)
Japan	Barley	1983	13/17	0.004–0.009	Tanaka *et al.* (1988)
Japan	Wheat	1984	18/18	0.01–0.71	Tanaka *et al.* (1985a)
Korea, Republic of	Wheat	1983	2/10	0.01–0.04	Lee *et al.* (1985)
Korea, Republic of	Wheat	1984	5/9	0.003–1.25	Lee *et al.* (1986)
Korea, Republic of	Wheat	1985	2/2	0.001–2.05	Lee *et al.* (1987)
Korea, Republic of	Barley	1983	21/28	0.003–1.6	Lee *et al.* (1985)
Korea, Republic of	Barley	1984	29/31	0.001–0.39	Lee *et al.* (1986)
Korea, Republic of	Barley	1987, 1989	18/57	0.03–1.13	Park & Lee (1990)
Korea, Republic of	Malt	1983	4/4	0.002–0.04	Lee *et al.* (1985)
Korea, Republic of	Malt	1984	5/5	0.003–0.05	Lee *et al.* (1986)
Nepal	Maize	1984	5/9	Mean, 0.82	Tanaka *et al.* (1988)
Nepal	Barley	1984	4/4	Mean, 0.02	Tanaka *et al.* (1988)
New Zealand	Maize	1984	15/20	0.1–16	Hussein *et al.* (1989)

[a]NR, not reported; ND, not detected

Deoxynivalenol

Deoxynivalenol is probably the most widely distributed *Fusarium* mycotoxin. Representative values reported in various crops and processed grains are given in Table 2. Occurrence in foods in North America, Japan and Europe is common, but the concentrations are low (Tanaka *et al.*, 1988; Scott, 1989, 1990; WHO, 1990); its occurrence in cereals in some developing countries, however, particularly in southern China (Luo, 1988) and parts of South America and Africa, is relatively high in some years. Acute mycotoxicoses affecting fairly large numbers of people and caused by ingestion of deoxynivalenol have been reported in

China, India and some other countries (Luo, 1988; Bhat *et al.*, 1989; WHO, 1990; Miller, 1991).

During milling, deoxynivalenol is concentrated in the bran, and cooking flour-based products contaminated with this toxin does not reduce the level appreciably (Scott, 1990; WHO, 1990). It is not transferred into milk, meat or eggs (Prelusky *et al.*, 1984; Trenholm *et al.*, 1989).

Table 2. Natural occurrence of deoxynivalenol

Country or region	Product	Year	Positive samples/ total no.	Content (mg/kg)	Reference
North America					
Canada	Wheat (soft)	1979–88	NR/667	Mean, 0.03–0.74	Scott (1990)
Canada	Wheat (hard)	1979–88	NR/1072	Mean, 0.02–3	Scott (1990)
Canada	Maize	1980–88	NR/203	Mean, 0.2–1.2	Scott (1990)
Canada	Food	1982–89	NR/783	Mean, 0.1–0.2	Scott (1990)
USA	Wheat	1982	54/57	0.2–9.0	Eppley *et al.* (1984)
USA	Wheat	1982	156/157	0.2–43.0	Shotwell *et al.* (1985)
USA	Wheat	1984	NR/123	Trace–2.3	Jelinek *et al.* (1989)
USA	Wheat	1982, 1984–85	163/280	Mean, 0.6–1.4	Scott (1990)
USA	Food	1983/84	NR/132	Trace–0.5	Jelinek *et al.* (1989)
USA	Food	NR	14/21	< 0.5	Scott (1989)
USA	Food	1989	46/92	1.2–19.0	Abouzied *et al.* (1991)
USA	Maize	1977, 1984–85	117/250	Mean, 0.4–5.0	Scott (1990)
USA	Maize	NR	24/52	0.5–11	WHO (1990)
USA	Maize	1981	274/342	0.1–42	Côté *et al.* (1984)
USA	Maize	1970–77	33/66	0.001–28	Scott (1989)
USA	Maize	1972–73	18/20	0.4–65.8	Abbas *et al.* (1988)
Europe					
Austria	Maize	1988–89	36/67	< 500	Lew *et al.* (1991)
Austria	Feed	1979–85	1053/1913	< 0.1–> 1.0	Scott (1989)
Austria	Feed	NR	179/389	0.03–22	Scott (1989)
Finland	Feed	1984	11/167	0.001–0.12	Karppanen *et al.* (1985)
France	Wheat	1982–84	30/43	< 0.27	Snijders (1990)
France	Maize	NR	2/3	0.14, 0.6	Jemmali *et al.* (1978)
Germany	Wheat	1987	42/44	Mean, 0.13	Scott (1990)
Germany	Oats	1979–80, 1982	35/399	0.01–2.0	Scott (1989)
Germany	Rye	1984	4/22	Mean, 0.40	Tanaka *et al.* (1988)
Hungary	Maize	NR	2/11	0.2, 1.3	Scott (1989)
Italy	Wheat	1984	1/120	0.12	Tanaka *et al.* (1988)

Table 2 (contd)

Country or region	Product	Year	Positive samples/ total no.	Content (mg/kg)	Reference
Europe (contd)					
Netherlands	Wheat	1982–84	33/51	< 0.51	Snijders (1990)
Norway	Wheat	1984	32/53	0.008–3.2	Sundheim *et al.* (1988)
Poland	Wheat	1984	13/48	Mean, 0.1	Tanaka *et al.* (1988)
Spain	Maize	1984–86	9/209	0.04–0.3	Muñoz *et al.* (1990)
Sweden	Wheat	1984	8/14	Mean, 0.40	Snijders (1990)
United Kingdom	Wheat	NR	57/148	0.02–> 0.5	WHO (1990)
United Kingdom	Wheat	1984	20/31	Mean, 0.03	Tanaka *et al.* (1988)
United Kingdom	Barley	1980	34/85	0.01–0.36	Gilbert *et al.* (1983)
South America					
Argentina	Wheat	1983	3/20	Mean, 0.015	Tanaka *et al.* (1988)
Argentina	Wheat	1986	7/7	1–20.0	Marpegan *et al.* (1988)
Argentina	Feed	1986	3/3	1.7–8.0	Marpegan *et al.* (1988)
Argentina	Maize	1983	2/20	Mean, 0.11	Tanaka *et al.* (1988)
Argentina	Maize	NR	14/58	0.20–0.40	Chulze *et al.* (1989)
Africa					
South Africa	Maize	1982–85	50/50	0.007–7.4	Gilbert (1989)
Egypt	Feed	NR	31/64	0.07–4.0	Abdelhamid (1990)
Nigeria	Acha	NR	3/6	0.01–0.06	Scott (1989)
Transkei	Maize	1976–77	8/12	0.07–4.0	Marasas *et al.* (1979)
Transkei	Maize	NR	43/72	0.01–15.8	Thiel *et al.* (1982)
Zambia	Maize	1985–86	2/51	Mean, 1.0	Siame & Lovelace (1989)
Zambia	Maize	1985–86	16/33	0.5–16.0	Siame & Lovelace (1989)
Australasia					
Australia	Wheat	1983	11/12	< 6.7	Tobin (1988)
Australia	Triticale	1983	3/3	1.1–11.0	Tobin (1988)
China	Wheat	NR	49/49	Mean, 2.82	Tanaka & Ueno (1989)
China	Wheat	1984	4/4	Mean, 4.28	Tanaka *et al.* (1988)
China	Wheat	1985	19/19	1.0–40.0	WHO (1990)
China	Wheat	1986	79/150	Mean, 0.03–0.81	Gang *et al.* (1988)
China	Wheat	1986	77/135	0.01–20.0	Luo (1988)
China	Wheat	1989	14/30	0.01–0.30	Luo *et al.* (1990)
China	Flour	1984	5/5	0.01–0.69	Ueno *et al.* (1986)
China	Maize	NR	5/5	0.3–92.8	WHO (1990)
China	Maize	1984–86	29/29	0.36–12.67	Hsia *et al.* (1988)
China	Maize	1989	34/47	0.01–3.51	Luo *et al.* (1990)
India	Wheat	NR	1/58	0.31	Ramakrishna *et al.* (1990)
India	Flour	NR	13/56	0.35–8.38	Ramakrishna *et al.* (1990)
India	Maize	NR	2/86	0.41, 2.02	Sinha (1991)
Japan	Wheat	1976–80	28/39	0.1–12.4	Yoshizawa (1983)

Table 2 (contd)

Country or region	Product	Year	Positive samples/ total no.	Content (mg/kg)	Reference
Australasia (contd)					
Japan	Wheat	1984	18/18	0.70–6.92	Tanaka *et al.* (1985a)
Japan	Flour	1982–85	36/36	0.002–0.24	Gilbert (1989)
Japan	Barley	1970–80	73/89	0.05–49.6	Yoshizawa (1983)
Japan	Grain food	NR	14/51	0.02–0.23	Scott (1989)
Korea, Republic of	Wheat	1983	2/10	0.02–0.10	Lee *et al.* (1985)
Korea, Republic of	Wheat	1984	5/9	0.01–0.17	Lee *et al.* (1986)
Korea, Republic of	Barley	1983	26/28	0.004–0.51	Lee *et al.* (1985)
Korea, Republic of	Barley	1984	31/31	0.001–0.90	Lee *et al.* (1986)
Korea, Republic of	Barley	1987	17/18	0.008–0.50	Park & Lee (1990)
Korea, Republic of	Barley	1989	9/20	0.01–0.16	Park & Lee (1990)
Nepal	Maize	1984	3/9	Mean, 0.54	Tanaka *et al.* (1988)
Nepal	Wheat	1984	1/10	0.06	Tanaka *et al.* (1988)
New Zealand	Maize	1984	11/20	0.02–0.3	Hussein *et al.* (1989)
Taiwan	Wheat	1984–85	12/22	0.03–2.45	Ueno *et al.* (1986)

NR, not reported

Nivalenol

Nivalenol has been reported extensively in Japanese and Korean grain samples and has been found as a minor contaminant in Europe. It has also been reported in samples from southern Africa (Scott, 1989; WHO, 1990) and Australia (Blaney & Dodman, 1988). It is virtually unknown in grains in North and South America (Table 3).

Little is known about the effects of milling and baking on levels of nivalenol or about its transmission into milk, meat and eggs (WHO, 1990).

Table 3. Natural occurrence of nivalenol

Country or region	Product	Year	Positive sample/ total no.	Content (mg/kg)	Reference
North America					
Canada	Wheat	1980–84	4/10	av. 0.02	Tanaka *et al.* (1988)
Canada	Maize	1984	1	1.0	Foster *et al.* (1986)
Europe					
Austria	Maize	1988–89	3/39	< 10.0	Lew *et al.* (1991)
Austria	Wheat	NR	3/4	0.01–0.04	Scott (1989)
Finland	Feed	1982	1/167	0.01	Karppanen *et al.* (1985)
France	Wheat	NR	2/2	0.02, 0.06	Ueno *et al.* (1985)

Table 3 (contd)

Country or region	Product	Year	Positive sample/total no.	Content (mg/kg)	Reference
Europe (contd)					
Germany	Food	NR	27/67	< 0.94	Scott (1989)
Germany	Wheat	NR	16/42	0.01–0.12	Scott (1989)
Hungary	Wheat	NR	1/2	0.004	Ueno *et al.* (1985)
Italy	Feed	NR	7/7	0.08–0.20	Scott (1989)
Norway	Barley	1984	49/49	0.01–0.25	Sundheim *et al.* (1988)
Norway	Wheat	1984	53/53	0.02–0.89	Sundheim *et al.* (1988)
Poland	Wheat	1985	43/48	0.003–0.35	Ueno *et al.* (1985)
Poland	Maize	1988	2/5	33.2, 42.5	Visconti *et al.* (1990)
United Kingdom	Wheat	1984	17/31	0.004–0.67	Tanaka *et al.* (1986)
United Kingdom	Barley	1984	3/8	0.007–1.1	Tanaka *et al.* (1986)
South America					
Argentina	Cereals	1983	15/60	Mean, 0.03	Tanaka *et al.* (1988)
Africa					
Transkei	Maize	1985	20/72	0.01–1.41	Thiel *et al.* (1982)
Transkei	Maize	1985	24/24	0.88–15.2	Sydenham *et al.* (1990)
Australasia					
China	Wheat	NR	45/49	Mean, 0.04	Tanaka & Ueno (1989)
China	Wheat	1984	1/5	6.66	Ueno *et al.* (1986)
China	Wheat	1989	7/30	0.01–0.02	Luo *et al.* (1990)
China	Maize	1985	28/28	0.05–4.05	Hsia *et al.* (1988)
China	Maize	NR	100%	0.4–12.7	WHO (1990)
India	Flour	NR	2/37	0.03–0.1	Ramakrishna *et al.* (1990)
Japan	Barley	1970–80	73/89	0.06–22.9	Yoshizawa (1983)
Japan	Barley	1977–82	46/50	< 0.05–11.4	Scott (1989)
Japan	Wheat	1984	7/18	0.05–0.44	Tanaka *et al.* (1985a)
Japan	Wheat flour	1982–85	12/36	0.004–0.08	Tanaka *et al.* (1985b)
Korea, Republic of	Wheat	1983	9/10	0.03–0.63	Lee *et al.* (1985)
Korea, Republic of	Barley	1983	28/28	0.02–3.0	Lee *et al.* (1985)
Korea, Republic of	Barley	1984	31/31	0.18–1.15	Lee *et al.* (1986)
Korea, Republic of	Barley	1987	17/18	0.03–1.11	Park & Lee (1990)
Nepal	Maize	1984	6/9	Mean, 0.89	Tanaka *et al.* (1988)
Taiwan	Barley	1985	4/4	0.29–0.98	Ueno *et al.* (1986)
Taiwan	Wheat	1984–85	10/22	0.005–0.17	Ueno *et al.* (1986)

NR, not reported

Fusarenone X

Fusarenone X is the acetylated precursor of nivalenol, and small amounts (10–20%) can occur in nivalenol (Miller *et al.*, 1991).

Reports of the occurrence of fusarenone X are limited to a few samples of maize naturally infected in the field in Europe, with a maximal concentration of 1.8 mg/kg (Bottalico *et al.*, 1983; Scott, 1989; Visconti *et al.*, 1990). It is found together with other *Fusarium* toxins produced by the same fungal species, i.e., nivalenol and zearalenone (Visconti *et al.*, 1990).

1.4 Regulations and guidelines

Zearalenone

An official tolerance level of 1 mg/kg zearalenone in grains, fats and oils was established in the USSR in 1984. Proposed levels in other countries are 0.2 mg/kg in maize in Brazil and 0.03 mg/kg in all foods in Romania (van Egmond, 1989).

Deoxynivalenol

Deoxynivalenol is apparently not subject to regulation in any country; however, guidelines, advisory levels and 'official tolerance levels' exist in some countries. In Canada, a guideline of 2 mg/kg is given for the occurrence of deoxynivalenol in uncleaned soft wheat, except in infant foods for which a guideline of 1 mg/kg is given; a guideline of 1.2 mg/kg is given for uncleaned non-staple foods calculated on the basis of flour or bran. In Romania, there is a tolerance of 0.005 mg/kg in all feeds. In the USA, advisory levels of 2 mg/kg in wheat and wheat products for milling and 1 mg/kg in finished wheat products were established; a level of 4 mg/kg is advised for wheat and wheat products for feed ingredients. Official tolerance levels in the USSR in 1984–85 were 1 mg/kg for durum wheat and 0.5 mg/kg for other wheats (van Egmond, 1989). In China, the suggested tolerance limit in wheat is 1 mg/kg (Luo, 1988).

Nivalenol and fusarenone X

No regulation or guideline exists for these compounds (van Egmond, 1989).

2. Studies of Cancer in Humans

A number of ecological studies have addressed the relationship between exposure to *Fusarium* toxins and oesophageal cancer. Most of the studies refer to mixtures of many toxins from many species of fungi on maize.

Cancer incidence among the Bantu of the Transkei, South Africa, has been reported in a number of surveys covering the period 1955–84 (Rose, 1967, 1973; Rose & Fellingham, 1981; Jaskiewicz *et al.*, 1987). Annual age-standardized incidence (African standard) for all cancer sites combined (1965–69) was 60 and 42 per 100 000 per year for men and women, respectively (Rose & Fellingham, 1981); oesophageal cancer accounted for approximately half of the cases (35 and 17–19 per 100 000) in 1955–69, based on 5095 cases (Rose, 1973). Inci-

dence varied markedly over time and among sub-districts of the 26 districts of the Transkei. The incidence was higher in people of each sex in south-western sub-districts than in north-eastern sub-districts (Jaskiewicz *et al.*, 1987). Similarly high rates for oesophageal cancer are reported from areas in northern China. The age-adjusted mortality rate in the two sexes combined was 100 per 100 000 in the Linxian registry for oesophageal cancer (1959–70), ranging from 140 per 100 000 in high-risk areas to approximately 2 per 100 000 in low-risk areas (Yang, 1980).

Marasas *et al.* (1979) examined the amounts of deoxynivalenol and zearalenone in samples of mouldy maize from randomly selected areas in the high-risk and low-risk oesophageal cancer regions of the Transkei, where maize is the main dietary staple. The level of contamination of maize kernels with each of these two *Fusarium* mycotoxins was apparently higher in the pooled samples from the high-risk than the low-risk region. [The Working Group noted that the actual number of kernels infected with *Fusarium* is not specified; statistical evaluation of the data was not possible.]

In an extension of this study, Marasas *et al.* (1981) included an area of the Transkei with an intermediate rate of oesophageal cancer and collected visibly healthy maize samples at random from each of the three study areas. The proportion of kernels in both mouldy and healthy maize samples infected by *F. graminearum*, which was responsible for contamination of the crops with deoxynivalenol and zearalenone, was not correlated with oesophageal cancer rates.

Marasas *et al.* (1988) studied the prevalence of three *Fusarium* species and other fungi in home-grown maize harvested in 1985 by 12 households situated in a district of high incidence of oesophageal cancer in the Transkei and by 12 households in a low-incidence district. Households in the high-incidence area were identified during a preliminary cytological screening for oesophageal cancer as having one or more adult occupant who showed mild to severe oesophageal abnormalities; households in the other study area was chosen at random. The ears of maize were sorted by the housewife at each domicile into 'good' ears intended for making porridge and 'mouldy' ears intended for brewing beer. No correlation was found between the occurrence of *F. graminearum* in healthy maize and the risk for oesophageal cancer, but an inverse correlation was seen with the occurrence of this fungus in mouldy maize.

Sydenham *et al.* (1990) found high concentrations of various *Fusarium* mycotoxins in the same samples of mouldy home-grown maize collected during 1985 and examined by Marasas *et al.* (1988). The mean levels of nivalenol and zearalenone, produced by *F. graminearum*, were significantly higher in mouldy maize samples from the low-risk than in those from the high risk area.

In a cross-sectional study, Hsia *et al.* (1988) examined the amounts of five *Fusarium* mycotoxins in 109 samples of maize and maize meal stocked by the families of 24 oesophageal cancer patients in 1985–86 and at 68 farms in five villages in Linxian, Henan, China, in 1985, where the death rate from cancer of the oesophagus is excessive and where maize has constituted the main staple food for the past few decades. Nivalenol and deoxynivalenol were found in all of the maize samples from the families of the 24 oesophageal cancer patients, at mean levels of 757 ng/g and 5376 ng/g, respectively. According to the authors, these levels are much higher than those seen in various cereals in foodstuffs in Japan, the United

Kingdom and the USA, where oesophageal cancer rates are low or moderate. The levels were apparently not, however, higher than those observed in all samples of maize from randomly selected farms in the area (1964 ng/g and 4543 ng/g, respectively).

No analytical epidemiological study was available that addressed the carcinogenicity of *Fusarium* toxins.

3. Studies of Cancer in Experimental Animals

No data were available to the Working Group on deoxynivalenol, but they were aware of a study in progress by oral administration to mice (Ghess *et al.*, 1992).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F$_1$ mice, seven weeks old, were fed diets containing 0, 50 or 100 mg/kg (maximum tolerated dose) *zearalenone* (purity, > 99%) for 103 weeks. All survivors were killed 105–108 weeks after the start of treatment. No significant difference in survival was observed between groups; 64–88% of the mice survived to termination. Hepatocellular adenomas were found in 3/50 (6%) low-dose and 7/49 (14%) high-dose males and in 4/50 (8%) male controls; and in 2/49 (4%) low-dose and 7/49 (14%) high-dose females and 0/50 female controls. The incidence in high-dose females was statistically significantly different ($p < 0.006$) in individual comparisons with the control group. The incidence of hepatocellular adenomas in untreated historical control female B6C3F$_1$ mice at the institute where the study took place was 14/498 (2.8%). A statistically significant positive trend was observed in the incidence of pituitary adenomas in both males (control, 0/40; low-dose, 4/45 (9%); high-dose, 6/44 (14%); $p < 0.022$) and females (control, 3/46 (7%); low-dose, 2/43 (5%); high-dose, 13/42 (31%); $p < 0.001$). The increased incidence of pituitary adenomas was statistically significant in high-dose males ($p < 0.032$) and in high-dose females ($p < 0.003$). The incidence of pituitary adenomas and carcinomas in untreated historical control B6C3F$_1$ mice at the institute where the study was undertaken was 21/428 (4.9%) in females and 0/399 in males (US National Toxicology Program, 1982).

Groups of 42 seven-week-old female C57BL/6CrSlc SPF mice were fed diets containing 0, 6, 12 or 30 mg/kg *nivalenol* (containing less than 100 μg/kg fusarenone X) for two years, to give a daily exposure to nivalenol of 0, 0.66, 1.38 or 3.49 mg/kg bw in the four groups, respectively. Body weights were reduced in all treated groups during the study, but terminal weights were reduced only in the high-dose group (26.4 g *versus* 34.0 g in controls; $p < 0.01$). After two years, the surviving mice were killed, and the liver, thymus, spleen, kidneys and brain examined. The numbers of mice still alive at that time were 22/42 controls, 22/42 fed 6 mg/kg, 20/42 fed 12 mg/kg and 29/42 fed 30 mg/kg. No significant increase in tumour incidence was reported (Ohtsubo *et al.*, 1989). [The Working Group noted the limited number of tissues studied.]

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, five weeks old, were fed diets containing 0, 25 or 50 mg/kg (maximum tolerated dose) *zearalenone* (purity, > 99%) for 103 weeks.

All survivors were killed 104–106 weeks after the start of treatment. Mean body weight gains of treated rats were lower than those of controls, and the depression in mean body weight was dose related. Survival rates of treated and control rats were similar; 74–82% of the animals were still alive at termination of the study. No increase in tumour incidence was observed (US National Toxicology Program, 1982).

A group of 20 male Donryu rats, eight weeks old, were given 0.4 mg/kg bw *fusarenone X* [purity unspecified] weekly by oral intubation for 50 weeks. Of 12 rats that survived 50 weeks, one developed a hepatoma. No tumour occurred in 10 male controls during the experimental period of over 400 days (Saito & Ohtsubo, 1974). [The Working Group noted the incomplete reporting of the experiment.]

Groups of 25 or 49 male Donryu rats, six weeks old, were given diets containing either 3.5 or 7 mg/kg *fusarenone X* [purity unspecified] (isolated from a culture filtrate of *F. nivale*, which is in fact *F. sporotrichioides* (see p. 402)) for two years; a third group of 26 animals was given 7 mg/kg diet for only one year. There were 48 controls. All animals were given a restricted volume of feed (15 g/day, to provide 50 and 105 μg fusarenone X per day per animal). Survivors were killed at 24 months. The mean body weights of the treated animals were in general lower than those of the respective controls, and a treatment-related effect on survival was noted: after 18 months of treatment, 50% of the controls were alive, compared with 15/49 (31%) and 4/25 (16%) in the low- and high-dose groups, respectively; survival at that time in the group receiving the 7 mg/kg diet for one year was 9/52 (17%). The major cause of death was chronic bronchopneumonia. No increase in the incidence of tumours was noted in treated rats (Saito *et al.*, 1980). [The Working Group noted the poor survival of the treated animals.]

3.2 Pre- and postnatal exposure

Rat

Groups of 50 male and 50 female FDRL-Wistar-derived rats, six to eight weeks of age, were fed diets that resulted in daily intakes of 0.1, 1.0 or 3.0 mg/kg bw *zearalenone* (> 96% pure). Groups of 70 rats served as controls. Diets were adjusted weekly according to the previous week's body weights and food consumption. F_0 generation rats were fed the diet for five weeks before mating, during mating (approximately two weeks) and during gestation but not during lactation. After weaning, the F_0 generation was killed. In the F_1 generation, 90 rats per sex were fed zearalenone (0.1, 1.0 and 3.0 mg/kg bw) at 28 days of age; 140 of each sex served as controls. Ten animals per sex per group (selected randomly) were killed at 13, 26, 64 and 104 weeks after initiation of zearalenone feeding. All surviving male and female animals were killed at 108 and 111 weeks, respectively. Male rats fed diets containing 1.0 and 3.0 mg/kg bw of zearalenone had decreased terminal body weights. There was no difference in the incidence of tumours in any of the zearalenone-exposed groups as compared to controls in the F_1 generation (Becci *et al.*, 1982a).

3.3 Subcutaneous administration

3.3.1 *Mouse*

Two groups of 16 or 18 DDD male mice, eight weeks of age, received 10 or 20 weekly subcutaneous injections of 2.5 mg/kg bw *fusarenone X*. A group of 11 mice served as controls. Most of the animals survived the treatment. No increase in tumour incidence was noted in treated animals when compared with controls; one case of leukaemia was observed (Saito & Ohtsubo, 1974). [The Working Group noted the incomplete reporting of the experiment.]

3.3.2 *Rat*

A group of 18 male Donryu rats, eight weeks of age, were given weekly subcutaneous injections of 0.4 mg/kg bw *fusarenone X* for 22 weeks; most of the rats survived more than one year, and one developed a lung adenoma. No tumour was seen in 10 controls (Saito & Ohtsubo, 1974). [The Working Group noted the incomplete reporting of the experiment.]

3.4 Skin application

Mouse

Groups of 10 female ICR mice [age unspecified] each received topical applications of 2 or 20 μg (0.4 μg for the first six weeks) *fusarenone X* twice a week for 25 weeks. Other mice each received applications of 51.2 μg 7,8-dimethylbenz[*a*]anthracene (DMBA) and, two weeks later, fusarenone X at the same doses as the first groups, twice a week for 23 weeks. Groups of 10 positive controls each received DMBA plus 10.5 μg 12-*O*-tetradecanoylphorbol 13-acetate (TPA) twice a week for 20 weeks. Eight of 10 mice receiving DMBA plus TPA developed skin papillomas on their backs 4–8 weeks after the beginning of treatment with DMBA [unspecified whether gross or microscopic examinations], but no skin papilloma was found in mice receiving 2 or 20 μg fusarenone X alone or 20 μg fusarenone X in combination with DMBA. One mouse treated with DMBA plus 2 μg fusarenone X developed multiple small skin papillomas at 23 weeks (Ueno, 1984).

3.5 Administration with known carcinogens

Mouse

Groups of 38–56 male and female C57Bl/6 × C3H F_1 mice, one week of age, were given single doses of 6 mg/kg bw aflatoxin B_1 by intraperitoneal injection and seven weeks later were fed diets containing 0, 6 or 12 mg/kg diet *nivalenol* for one year. They were sacrificed at 71 weeks of age. All treated male mice developed liver tumours (mostly hepatocellular carcinomas). In females, the incidences were 0/21 in controls, 8/26 in mice given aflatoxin B_1 alone, 3/15 in mice given aflatoxin B_1 plus 6 mg/kg nivalenol and 0/19 in mice given aflatoxin B_1 plus 12 mg/kg nivalenol (Ueno *et al.*, 1991).

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Zearalenone

An adult man was given a single oral dose of 100 mg zearalenone, and his urine was collected over the following 24 h. Urinary analysis showed the presence of parent compound and α-zearalenol in approximately equal amounts together with lower levels of the isomeric β-zearalenol, all in the form of glucuronic acid conjugates (Mirocha *et al.*, 1981).

No data were available to the Working Group on deoxynivalenol, nivalenol or fusarenone X.

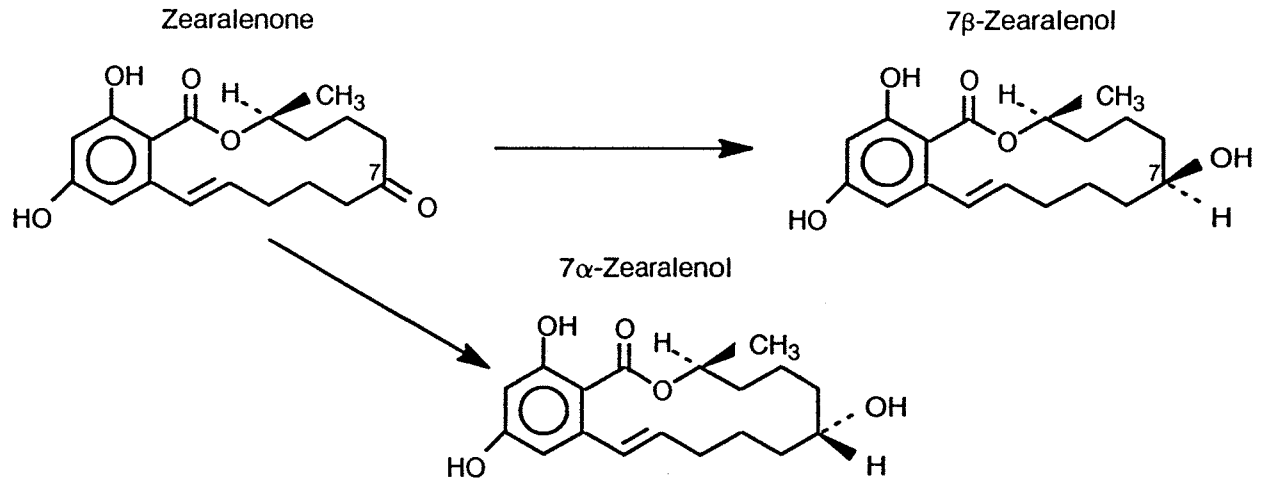
4.1.2 *Experimental systems*

Zearalenone

Zearalenone is fairly rapidly absorbed in several species following oral administration (Mirocha *et al.*, 1981; Olsen *et al.*, 1985). In mice injected with ^{3}H-zearalenone, specific localization was found in oestrogen target organs such as the uterus, interstitial cells of the testicles and the follicles of the ovary (Appelgren *et al.*, 1982). Some is concentrated in adipose tissue of rats (Ueno *et al.*, 1977), and fat-soluble metabolites accumulate in egg yolk (Dailey *et al.*, 1980).

The primary metabolites of zearalenone are the reduced products, α- and β-zearalenol (Fig. 1), and the glucuronic acid conjugates of both the parent compound and metabolites. The reduction is catalysed by microsomal and cytoplasmic fractions. Species differ in the distribution of the two epimers (Mirocha *et al.*, 1981; Farnworth & Trenholm, 1983). In prepubertal gilt, α-zearalenol was found in plasma at levels exceeding those of zearalenone by three to four times (Olsen *et al.*, 1985). The metabolites were also found in milk produced five days after discontinuation of administration of zearalenone to cows, sheep and pigs (Hagler *et al.*, 1980; Palyusik *et al.*, 1980).

Fig. 1. Metabolism of zearalenone



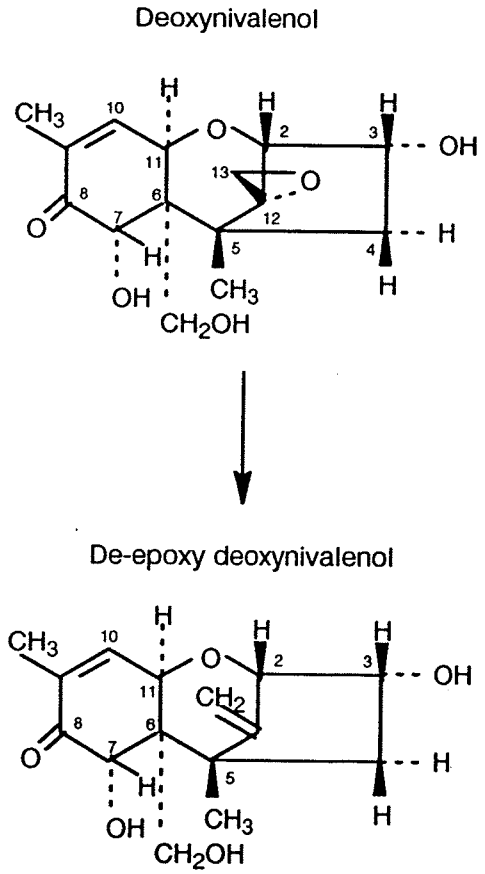
Deoxynivalenol

Following a single intravenous injection (1 mg/kg bw) of deoxynivalenol to swine, the mycotoxin was rapidly distributed to all tissues; the highest concentrations were found in kidney and liver and, correspondingly, in urine and bile. The elimination half-life was estimated at 3.9 h. Trace levels of the toxin were still detectable after 24 h (Prelusky & Trenholm, 1991). In cows, 24 h after an oral dose of 920 mg, trace levels of deoxynivalenol were detectable in blood, and extremely low levels of free and conjugated deoxynivalenol (< 4 ng/ml) were present in the milk (Prelusky *et al.*, 1984).

In rats, 96 h after a single oral dose of 10 mg/kg bw ^{14}C-deoxynivalenol, most of the radiolabel was excreted in the faeces (64%) and urine (25%). Some radiolabel was retained in the liver and very little in other tissues (Lake *et al.*, 1987).

Deoxynivalenol is metabolized by rodents *in vivo* by an apparently novel reaction involving loss of the epoxide oxygen function (referred to as de-epoxidation) to give de-epoxy deoxynivalenol (Fig. 2). This is the predominant metabolite in faeces after oral administration of deoxynivalenol to rats (Yoshizawa *et al.*, 1983); it was also found in urine, faeces, plasma and milk of lactating cows (Côté *et al.*, 1986; Yoshizawa *et al.*, 1986).

Fig. 2. Metabolism of deoxynivalenol



From Yoshizawa *et al.* (1983)

After oral administration of 5 mg/kg bw deoxynivalenol to two sheep, 54 and 75% of the unmetabolized compound was recovered in the faeces and 7% in the urine of each animal (Prelusky *et al.*, 1986). After intravenous administration to sheep of 4 mg/kg bw, the metabolites were excreted in urine in the order: conjugated deoxynivalenol, conjugated de-epoxy deoxynivalenol, deoxynivalenol and de-epoxy deoxynivalenol. After a single oral administration of deoxynivalenol at 1.32 g for three days or a single intravenous injection at 4 mg/kg to sheep, only trace amounts of deoxynivalenol or its metabolites were found in milk (Prelusky *et al.*, 1987).

Nivalenol

Nivalenol is metabolized to de-epoxy nivalenol. After long-term oral administration of nivalenol to male rats, the dose was recovered as faecal nivalenol (7%), faecal de-epoxy nivalenol (80%), urinary nivalenol (1%) and urinary de-epoxy nivalenol (1%) (Onji *et al.*, 1989).

Fusarenone X

Thirty minutes after subcutaneous administration of uniformly labelled ^{3}H-fusarenone X at 4 mg/kg bw to mice, activity was found in liver, kidneys, intestines, stomach, spleen, bile and plasma; none was detected in heart, brain or testis. The highest activity, corresponding to 3% of the dose, was observed in the liver. Twelve hours after administration, no label was present in the organs, and 25% of the dose was recovered as metabolized forms of fusarenone X in the urine (Ueno *et al.*, 1971).

Fusarenone X was deacetylated by rat and rabbit liver carboxy esterases to nivalenol (Ohta *et al.*, 1978).

4.2 Toxic effects

4.2.1 *Humans*

Two outbreaks of disease related to trichothecenes have been well described—one in China in 1984–85 (Luo, 1988) and one in India in 1987 (Bhat *et al.*, 1989). Each involved several hundred cases.

During the first incident, outbreaks of poisoning from mouldy maize and scabby wheat were reported. After about 600 persons consumed mouldy cereals, 463 cases of poisoning (77% of the total) were reported. The latent period for onset of symptoms was 5–30 min, and these included nausea, vomiting, abdominal pain, diarrhoea, dizziness and headache. No death occurred. Pigs and chicks fed the same mouldy cereals were also affected. *Deoxynivalenol* was detected within a range of 0.34–92.8 mg/kg and *zearalenone* within a range of 0.004–0.587 mg/kg; neither T-2 toxin nor nivalenol was found (WHO, 1990).

The other outbreak occurred in Kashmir, India, in 1987 (Bhat *et al.*, 1989) and was ascribed to the consumption of bread made from mouldy flour. Of 224 randomly selected persons investigated, 97 had symptoms that included abdominal pain (100%), throat irritation (63%), diarrhoea (39%), blood in stools (5%) and vomiting (7%). Symptoms developed 15 min to 1 h after consumption of locally baked bread. Trichothecene toxins were detected in refined and ordinary wheat flour samples at the following ranges of concen-

trations: *deoxynivalenol*, 0.35–8.38 mg/kg; *nivalenol*, 0.03–0.1 mg/kg; T-2 toxin, 0.55–0.8 mg/kg; and acetyl deoxynivalenol, 0.6–2.4 mg/kg.

4.2.2 *Experimental systems*

Zearalenone

The toxicology of zearalenone has been reviewed (Kuiper-Goodman *et al.*, 1987).

Zearalenone given in the diet of mice for 13 weeks caused atrophy of seminal vesicles and testes, squamous metaplasia of the prostate gland, osteopetrosis, myelofibrosis of the bone marrow, cytoplasmic vacuolization of the adrenal glands, hyperkeratosis of the vagina and endometrial hyperplasia. Rats are about 10 times more sensitive than mice, as osteopetrosis was found in 5/10 female rats given 30 mg/kg in their diet (US National Toxicology Program, 1982). Swine are the most sensitive domestic animals; for example, dietary levels of zearalenone as low as 5 mg/kg induce pseudopregnancy with a failure to cycle (Etienne & Jemmali, 1982).

The visible signs of zearalenone-induced hyperoestrogenism in female swine are swollen vulva and mamma, enlargement of the uterus, ovarian changes and infertility (Mirocha & Christensen, 1974; Bauer *et al.*, 1987). the no-observed-adverse-effect level is less than 5 mg/kg bw (Farnworth & Trenholm, 1983).

Zearalenone binds to oestrogen receptors in human breast cancer cells; however, its relative binding affinity is only 5% of that of 17β-oestradiol (Martin *et al.*, 1978). In oestrogen-sensitive cell lines exposed to zearalenone, oestrogen-specific proteins were expressed. In this system, zearalenone was suggested to activate the oestrogen receptor (Mayr, 1988), inducing expression of oestrogen-controlled genes.

Zearalenone inhibits mitogen-induced blastogenesis in rat and human peripheral blood lymphocytes. The amount of zearalenone necessary to inhibit proliferation by 50% was 13 μg/ml, i.e., 250 times more than the dose required for the action of deoxynivalenol (see below) (Atkinson & Miller, 1984).

The relative binding affinity of α-zearalenol for cytosolic oestrogen receptors was 10–20 times greater than that of zearalenone and some 100 times greater than that of β-zearalenol. Thus, reduction to α-zearalenol is an activation process, while the production of β-zearalenol is a deactivation process: the observed interspecies variations in sensitivity to dietary zearalenone may be due to differences in metabolism and the relative binding activity of these metabolites for oestrogen receptors (Fitzpatrick *et al.*, 1989).

Deoxynivalenol

Deoxynivalenol is one of the least acutely toxic compounds of the trichothecene class of mycotoxins. LD_{50}s for this compound in $B6C3F_1$ mice were reported to be 78 mg/kg bw by gavage and 49 mg/kg bw intraperitoneally (Forsell *et al.*, 1987). In male DDY mice, the LD_{50} was 46 mg/kg bw by oral administration and 70 mg/kg bw by intraperitoneal administration. The subcutaneous LD_{50} in 10-day-old Peking ducklings was 27 mg/kg bw (Yoshizawa & Morooka, 1974), and an oral LD_{50} of about 140 mg/kg bw was found for broiler chickens (Huff *et al.*, 1981).

Intravenous administration of 0.5 mg/kg bw deoxynivalenol to swine induced vomiting, diarrhoea, muscular weakness, tremors and twilight coma. Hypoglycaemia and pancreatic islet cell lesions were observed (Coppock *et al.*, 1985).

Acute intraperitoneal doses of deoxynivalenol (10–1000 mg/kg bw) resulted in extensive necrosis of the gastrointestinal tract, bone marrow and lymphoid tissues and focal lesions in kidney and cardiac tissue in B6C3F$_1$ mice (Forsell *et al.*, 1987). Engorgement of the testes was reported by Yoshizawa and Morooka (1974). In broiler chickens, acute deoxynivalenol toxicosis is characterized by extensive ecchymotic haemorrhaging throughout the carcass, disturbance of the nervous system and irritation of the upper gastrointestinal tract (Huff *et al.*, 1981). Fitzpatrick *et al.* (1988) found elevated concentrations of indoleamines, serotonine and 5-hydroxy-3-indolacetic acid in rat brain 24 h after oral dosing with 2.5 mg/kg bw.

The minimal doses that caused vomiting after subcutaneous administration of deoxynivalenol were 10 mg/kg bw in ducklings and 0.1 mg/kg bw in dogs (Yoshizawa & Morooka, 1974).

Swine fed a diet containing 5 mg/kg deoxynivalenol for nine weeks developed vomiting and depleted hepatic glycogen (Schuh *et al.*, 1982). In young pigs, a dietary level of 20 mg/kg caused vomiting, 12 mg/kg caused almost complete feed refusal, and 1.3 mg/kg induced significant depression in feed intake and rate of weight gain (Forsyth *et al.*, 1977; Young *et al.*, 1983). Feed refusal appears to occur at the level of the central nervous system (for review, see Prelusky *et al.*, 1990b). After intravenous administration (1.0 mg/kg bw), deoxynivalenol was detected in the cerebral spinal fluid of sheep and swine. An analysis of the area under curves for body compartment distribution indicated that about 2.5 times as much toxin eventually reaches the cerebral spinal fluid in pigs as in sheep (Prelusky *et al.*, 1990b).

In rats fed 20 mg/kg deoxynivalenol in the diet for 90 days, no significant effect was seen on serum enzyme levels, haematological parameters or histopathological lesions. There was no sign of feed refusal, but the treated rats were less efficient at converting feed into body mass than controls (Morrissey *et al.*, 1985). Body weight gain was, however, completely inhibited in rats fed diets containing 150 mg/kg deoxynivalenol (Yoshizawa *et al.*, 1978).

Hunder *et al.* (1991) found impairment of intestinal transfer and uptake of nutrients such as glucose and 5-methyltetrahydrofolic acid in mice fed 10 mg/kg deoxynivalenol in the diet.

Feeding studies in poultry demonstrated only minor adverse effects on growth, food consumption and fertility, even at levels up to 38 mg/kg in the diet (Hulan & Proudfoot, 1982; Kubena *et al.*, 1987; Moran *et al.*, 1987). In growing lambs given a wheat diet containing 15 mg/kg deoxynivalenol, no change in body weight gain or food consumption was observed (Harvey *et al.*, 1986). Feeding trials with farm animals showed no serious adverse effect of deoxynivalenol at dietary concentrations of 2 mg/kg in swine, 5 mg/kg in poultry and 6 mg/kg in dairy cattle when fed at a rate of 1% body weight per day (Trenholm *et al.*, 1984).

Deoxynivalenol inhibited protein synthesis at the ribosomal level in an in-vitro system from rabbit reticulocytes and in a suspension of reticulocytes. The inhibition took place at the elongation–termination step of protein synthesis; most other trichothecenes (T-2 toxin and its metabolites, nivalenol and fusarenone X) inhibit the initial step of protein synthesis

(Ueno, 1983). It also inhibits DNA synthesis in murine splenic lymphocytes and human peripheral blood lymphocytes (Mekhancha-Dahel *et al.*, 1990).

At low doses, deoxynivalenol causes immunotoxicity, with a no-observed-effect level in mice of 0.25–0.50 mg/kg bw per day (Tryphonas *et al.*, 1986). Of particular interest is the capacity of dietary deoxynivalenol to induce extremely high levels of immunoglobulin A (IgA) in mice, owing to alteration of IgA production at the mucosal and systemic levels. Dietary deoxynivalenol enhances terminal differentiation of IgA-secreting cells in Peyer's patches. This and resultant migration of IgA-secreting cells into the systemic compartment favour a shift from IgG to IgA as the primary serum isotype (Bondy & Pestka, 1991).

Deoxynivalenol inhibits phytohaemaglutinin-induced lymphocyte proliferation, reducing ^{3}H-thymidine incorporation into DNA by 50% at a concentration of 90 ng/ml in rat lymphocytes and at a concentration of 220 ng/ml in human lymphocytes (Atkinson & Miller, 1984).

Nivalenol

LD_{50}s for nivalenol in six-week-old male DDY mice were found to be 38.9 (oral), 7.4 (intraperitoneal), 7.2 (subcutaneous) and 7.3 (intravenous) mg/kg bw. Post-mortem examination revealed marked congestion and haemorrhage in the intestine (Ryu *et al.*, 1988). Tatsuno (1968) determined the intraperitoneal LD_{50} for nivalenol in ddS mice to be 4.0 mg/kg bw; pathological changes included cell degeneration of bone marrow, lymph nodes, intestines, testes and thymus. Nivalenol induced radiomimetic damage in animal cells, and newborn mice were found to be much more sensitive than adult mice, having a subcutaneous LD_{50} of 0.16 mg/kg bw (Ueno, 1987). In Fischer 344 rats, the oral LD_{50} was 19.5 mg/kg bw. Sedation, eyelid closure, staggering gait, diarrhoea and congestion of the lungs and digestive tract were observed (Kawasaki *et al.*, 1990). The subcutaneous LD_{50} in rats was found to be 0.9 mg/kg bw (Ueno, 1983).

Subacute toxicity studies were performed for 24 days, during which female mice were given 30 mg/kg nivalenol in the diet. Significant erythropenia and slight leukopenia were observed, but no marked change was seen in other haematological parameters, feed consumption, body weight gain or weights of liver, spleen or thymus. Ultrastructural studies revealed polyribosomal breakdown of bone-marrow cells. No effect was seen in groups receiving 10 mg/kg nivalenol or less in the feed (Ryu *et al.*, 1987).

In another subacute toxicity test, nivalenol was given orally at daily doses of 0.4 and 2.0 mg/kg bw to rats for 30 days. No significant change was observed in biological or haematological parameters. Liver and spleen weights were slightly increased with the dose of 2.0 mg/kg bw, but no histological change was seen (Kawasaki *et al.*, 1990).

The long-term toxicity of nivalenol was studied for one (Ryu *et al.*, 1988) and two (Ohtsubo *et al.*, 1989) years in female mice fed diets containing 0, 6, 12 or 30 mg/kg nivalenol. Body weight gain and feed consumption showed dose-dependent decreases throughout the study period, indicating that nivalenol retards growth. The absolute weight of the liver in the group given 30 mg/kg and that of the kidneys in the groups given 12 and 30 mg/kg were significantly reduced compared with those of controls. When kidney weight was expressed relative to brain weight, a reduction was seen only in the group given 12 mg/kg. Some leukopenia was seen in nivalenol-treated animals, and dose-dependent increases in the serum

concentrations of alkaline phosphatase and non-esterified fatty acids were observed. No ultrastructural change in the bone marrow was noted after one year.

Mice given nivalenol developed changes in proliferating cells of the small intestine and germ centres of lymph follicles in spleen, lymph node, thymus and bone marrow; they also developed testicular lesions: the spermatogenic cells were reduced in number, some of the blastic cells were necrotic, and multinucleated spermatic giant cells were present in the seminiferous tubules (Saito *et al.*, 1969).

The lowest subcutaneous dose that caused vomiting in ducklings was 1.0 mg/kg bw (Ueno, 1987).

Skin necrotization occurred in guinea-pigs and mice painted with 100 μg nivalenol, showing that this compound is much less active than diacetoxyscirpenol or fusarenone X (Ueno *et al.*, 1970b).

Nivalenol inhibited multiplication of HeLa cells at every phase of the growth cycle. By means of autoradiography, nivalenol at concentrations higher than 0.5 μg/ml was shown to block G1 about 2 h before the beginning of S phase and to block G2 just before mitosis (Ohtsubo *et al.*, 1968). It did not inhibit RNA synthesis in HeLa cells, but at a dose of 15 μg/ml it caused complete breakdown of polyribosomes in HeLa cells after only 1 min (Cundliffe *et al.*, 1974).

Nivalenol was also highly toxic to other cells of human origin—uterine carcinoma, embryonic kidney and lymphocytes—at ID_{50}s between 0.3 and 1.0 μg/ml (Tanaka *et al.*, 1978).

Nivalenol inhibited protein synthesis in rabbit reticulocytes at an ID_{50} of 2.5 μg/ml. It also inhibited poly U-directed synthesis of polyphenylalanine in a rabbit reticulocyte cell-free system at the ribosomal level, at an ID_{50} of 0.5 μg/ml (Ueno *et al.*, 1968). It inhibited protein synthesis (ID_{50}, 6 μg/ml) and DNA synthesis (ID_{50}, > 10 μg/ml) in Ehrlich ascites tumour cells (Ueno *et al.*, 1973).

Fusarenone X

The LD_{50} of fusarenone X in mice was 3–5 mg/kg bw, irrespective of the route of administration or the sex of the animal used. The survival time of mice injected intraperitoneally with the toxin was usually three to four days; when a dose several times higher than the LD_{50} was given, mice survived for less than 24 h, regardless of the route of administration. Newborn mice were highly sensitive, succumbing to lethal intoxication at doses of less than 0.1 mg/kg bw. Of the species tested, guinea-pigs are the most sensitive. In cats and ducklings, vomiting is a major symptom (Ueno *et al.*, 1971).

In mice administered fusarenone X, severe cellular destruction and karyorrhexis were induced in actively dividing cells of the gastrointestinal tract, thymus, lymph nodes, spleen, bone marrow, ovary and testes. These effects were independent of the route of administration (Ueno *et al.*, 1971).

Administration of fusarenone X to mice, rats and guinea-pigs induced multiple symptoms, including diarrhoea, food refusal, vomiting and hyperaemia in the intestine (Matsuoka & Kubota, 1981, 1985). Fusarenone X was highly irritating to the skin of mice, rabbits and guinea-pigs, causing haemorrhage and necrosis of the epidermis and degeneration and necrosis of hair follicles and dermis. Medium to severe toxicity (depending on the animal

species) was observed after application of 10–100 μg of the toxin for two days (Ueno *et al.*, 1970b). Foot oedema was induced in rats after subplantar injection of 10–100 μg (Matsuoka *et al.*, 1979).

Fusarenone X caused diarrhoea in mice and rats by increasing the permeability of intestinal epithelial cells, resulting in exudation of plasma contents and an eventual increase in the volume of intestinal fluid (Matsuoka & Kubota, 1981, 1987a,b). Fusarenone X (intraperitoneally injected; 1, 2, 4 mg/kg bw) caused a dose-related increase in capillary permeability in mice (Matsuoka & Kubota, 1987b).

Fusarenone X is a potent inhibitor of protein and DNA syntheses in rat hepatocytes, rabbit and guinea-pig reticulocytes, Ehrlich ascites tumour cells, HeLa cells, guinea-pig splenic cells, mouse fibroblasts and *Escherichia coli* (Ohtsubo & Saito, 1970; Ohtsubo *et al.*, 1972; Ueno *et al.*, 1973; Ito *et al.*, 1982). The ID_{50} for protein synthesis was 0.25 μg/ml in whole rabbit reticulocytes, 0.2 in a cell-free rabbit reticulocyte system and 8 μg/ml in a cell-free rat liver system. The toxin binds to the 80 S eukaryotic ribosomes and polysomes (Ueno, 1977). No inhibition of RNA synthesis was found. The ID_{50}s for incorporation of ^{3}H-thymidine into DNA and of ^{3}H-leucine into protein of HeLa S3 cells were 0.1 and 0.13 μg/ml (2.8 and 3.6 $\times$ 10^{-7} M), respectively (Ohtsubo & Saito, 1970).

Fusarenone X binds *in vitro* to active SH groups of creatine phosphokinase, lactate dehydrogenase and alcohol dehydrogenase, inhibiting their activities (Ueno & Matsumoto, 1975).

Daily intraperitoneal administration of fusarenone X from 25 μg for seven days had an immunosuppressive effect in BALB/c mice. IgE and IgG1 antibody formation *in vivo*, as well as in-vitro antibody formation by splenic lymphocytes raised by T-dependent and independent mitogens, was suppressed. Strongly adherent, phagocytic, suppressive cellular elements—possibly activated macrophages—were found in the spleens of treated animals (Obara *et al.*, 1984).

Fusarenone X injected intraperitoneally to guinea-pigs several times at a dose of 0.75 mg/kg bw inhibited the responses of splenic cells towards mitogenic stimulation with lipopolysaccharide of *E. coli* and concanavalin A *in vitro*. *In vivo*, it did not appreciably affect the antibody response to 2,4-dinitrophenyl-bovine serum albumin. The levels of IgG_1 and IgG_2 were not significantly reduced after four weeks (Ito *et al.*, 1982).

4.3 Reproductive and developmental toxicity

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Zearalenone

Groups of 10 Wistar rats received 1, 5 or 10 mg/kg bw zearalenone by oral intubation on days 6–15 of gestation. Mean fetal weight was significantly reduced in the high-dose group, and there was a higher prevalence of minor skeletal anomalies in the fetuses (Ruddick *et al.*, 1976).

Groups of 50 male and 50 female Wistar rats received 0.1, 1.0 or 10.0 mg/kg bw zearalenone daily in the diet. Fertility was greatly impaired in females receiving the high dose: only 26 pregnancies resulted from 50 matings in the F_1 generation, and the number of resorptions and stillbirths was greatly increased; none of the 12 females in the F_2 generation that were mated became pregnant. No teratogenic response was seen at any dose (Becci *et al.*, 1982b).

Newborn female mice were injected daily for five days with 1 μg zearalenone. At eight months of age, corpora lutea were absent from 25/34 treated mice, indicating ovarian dysfunction; 56% of the exposed mice had dense collagen deposition in the uterine stroma and lacked uterine glands. Altered vaginal epithelium was found in 32% of the zearalenone-treated mice (Williams *et al.*, 1989). Similar effects were induced in rats after a single subcutaneous injection of 1 mg zearalenone to three- or five-day-old animals (Kumagai & Shimizu, 1982).

Deoxynivalenol

Groups of 15–19 pregnant Swiss-Webster mice were treated daily on days 8–11 of pregnancy with deoxynivalenol (purity, 96%; containing 4% 4,7-*d*-dideoxynivalenol as an impurity) at 0.5–15 mg/kg bw by oral intubation. Doses of 2.5 mg/kg bw and higher significantly increased the resorption rate; doses of 10 mg/kg bw or more induced complete resorption of all embryos. Low incidences of several anomalies (lumbar vertebrae, ribs, sternebrae) were observed in fetuses from the 1.0-, 2.5- and 5.0-mg/kg bw groups. No increase in the incidence of adverse effects was observed in the 0.5-mg/kg bw dose group (Khera *et al.*, 1982).

Male and female Swiss-Webster mice were fed diets resulting in daily doses of 0.375–2.0 mg/kg bw deoxynivalenol before mating and during pregnancy, and progeny (F_{1a}) were examined up to 21 days of age. Mice were then rebred to produce F_{1b} litters, which were evaluated on day 19 of gestation for gross, visceral and skeletal malformations. The highest dose decreased food consumption and reduced body weight in animals of each sex in the F_0 generation; transient body weight reduction during the last week of pregnancy was also observed in female mice exposed to 1.5 mg/kg bw. Fertility was not impaired by the treatment. Pronounced postnatal mortality was observed in the highest-dose group only. No major malformation was found (Khera *et al.*, 1984).

Male and female Sprague-Dawley rats were fed diets containing deoxynivalenol at 0.25, 0.5 or 1.0 mg/kg bw for six weeks prior to mating; females were treated throughout pregnancy. Dilatation of the renal pelvis (15, 9 and 29% compared to 0 in controls) and urinary bladder (24, 39 and 34% compared to 11% in controls) was observed in exposed fetuses; no other adverse effect was noted (Khera *et al.*, 1984).

Fischer 344 rats were fed a diet containing deoxynivalenol at 0, 0.5, 2.0 or 5.0 mg/kg during pregnancy. At the end of the experiment, the body weights of females (without fetuses and uterus) in the two highest-dose groups were significantly lower than those in controls. Fetal weight was unaffected by treatment; and no significant adverse effect on the incidence of gross, skeletal or visceral abnormalities was noted (Morrissey, 1984).

Administration of a diet containing 20 mg/kg deoxynivalenol to male and female Sprague-Dawley rats before mating and throughout pregnancy induced a slight decrease in fertility of females, but there was no significant difference from the control group with

respect to postnatal survival of pups, number of pups born, sex ratio, mean pup weight or percentage of animals alive at four days that survived the 21-day lactation period (Morrissey & Vesonder, 1985).

New Zealand White rabbits were fed diets containing deoxynivalenol from day 0 to day 30 of gestation to give daily intakes of 0, 0.3, 0.6, 1.0, 1.6, 1.8 and 2.0 mg/kg bw. A decrease in mean fetal body weight was observed after daily intakes of 1.0 and 1.6 mg/kg bw. Complete resorption of fetuses was noticed in the two highest-dose groups. Doses that had no maternal toxic effect (0.3 and 0.6 mg/kg bw) had no adverse effect on fetuses at term. No teratogenic effect was observed (Khera *et al.*, 1986).

Nivalenol

Nivalenol was injected intraperitoneally into pregnant ICR mice at doses of 0, 0.1, 0.5 or 1.5 mg/kg bw daily from day 7 to day 15 of gestation. The highest dose caused stillbirths after vaginal haemorrhage in 6 of 10 animals. High percentages of embryolethality (48.4 and 87.8%) were recorded in the two highest-dose groups. No fetal malformation was observed. A single administration of 3 mg/kg bw on day 7 affected embryos within 10 h, damaged the placenta within 24 h and induced stillbirths by 48 h (Ito *et al.*, 1986).

Fusarenone X

All of a group of four DDD female mice treated with a single subcutaneous injection of 2.6 mg fusarenone X [purity unspecified] on day 10 of gestation aborted the following day. Abortion occurred less frequently (16–20%) at doses of 0.63–1.6 mg/kg bw and at longer intervals after injection. The weight and length of surviving fetuses from dams given 1.6 mg/kg bw on day 6 or 8 of gestation were significantly reduced as compared with those of controls. All mice fed diets containing 10 or 20 mg/kg fusarenone X (approximately 50 or 100 μg/animal per day) aborted in early pregnancy or throughout pregnancy. Feeding of diets containing 10 and 20 mg/kg fusarenone X for seven days during the middle of pregnancy caused 40 and 100% of dams to abort, respectively. No teratogenic effect was observed (Ito *et al.*, 1980).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see also Tables 4–7 and Appendices 1 and 2)

Zearalenone

The genotoxic effects of zearalenone and some of its derivatives have been reviewed (Kuiper-Goodman *et al.*, 1987).

Zearalenone did not induce SOS error-prone DNA repair in *Escherichia coli*, although the *rec* assay indicated that the compound induced differential toxicity in repair-deficient and -proficient *Bacillus* strains. Zearalenone did not induce mutation in *Salmonella typhimurium* or mitotic crossing over in *Saccharomyces cerevisiae*. In Chinese hamster cells *in vitro*,

zearalenone induced sister chromatid exchange, chromosomal aberrations and polyploidy; sister chromatid exchange was weakly induced in cultured human lymphocytes.

β-Zearalenol, but not α-zearalenol, induced differential toxicity in *Bacillus subtilis* strains.

Deoxynivalenol

Deoxynivalenol was not mutagenic to *Salmonella typhimurium*. In Chinese hamster V79 cells *in vitro*, it inhibited gap-junctional intercellular communication and induced chromosomal aberrations, but it did not produce gene mutation at the *hprt* locus. A sample of maize from Linxian County, China, was extracted with acetonitrile and water and fractionated by HPLC. The fraction that co-eluted with deoxynivalenol induced chromosomal aberrations in V79 cells (Hsia *et al.*, 1988). Deoxynivalenol did not induce unscheduled DNA synthesis in rat primary hepatocyte cultures, but it enhanced cell transformation in mouse embryo cells *in vitro*. A precursor, 3-acetyldeoxynivalenol, also induced chromosomal aberrations in mammalian cells *in vitro* (Hsia *et al.*, 1988).

Nivalenol

In Chinese hamster V79 cells *in vitro*, nivalenol slightly increased the frequencies of chromosomal aberrations and sister chromatid exchange. The fraction of maize described above that co-eluted with nivalenol induced chromosomal aberrations in V79 cells (Hsia *et al.*, 1988).

Fusarenone X

Fusarenone X did not induce DNA damage and was not mutagenic to *Salmonella typhimurium* in one study; another study, which was considered to provide positive results by a previous working group (IARC, 1983), was considered to be inadequate. Fusarenone X increased the frequency of petite mutations in yeast. It did not induce gene mutation in cultured mouse carcinoma cells, but it induced chromosomal aberrations and (weakly) sister chromatid exchange in Chinese hamster cells *in vitro*. Weak induction of DNA single-strand breaks was described in cultured human cells.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

The mycotoxins considered are produced by *Fusarium* species that occur primarily on wheat, barley and maize. The toxins occur whenever these cereals are grown under humid conditions. Exposure occurs through dietary consumption of contaminated cereals. Deoxynivalenol has been held responsible for large-scale human poisonings this century in China and India. Chronic exposures to deoxynivalenol, zearalenone and nivalenol occur in several parts of the world; humans are rarely exposed to fusarenone X.

5.2 Human carcinogenicity data

A few ecological studies that considered *F. graminearum* suggested no correlation with the incidence of oesophageal cancer.

Table 4. Genetic and related effects of zearalenone and α- and β-zearalenol

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS spot test, *Escherichia coli*	-	-	0.0000	Auffray & Boutibonnes (1986)
PRB, SOS chromotest test, *Escherichia coli* PQ37	-	-	30.0000	Krivobok *et al.* (1987)
BSD, *Bacillus subtilis rec* strains, differential toxicity	+	0	100 μg/plate	Ueno & Kubota (1976)
BSD, *Bacillus subtilis rec* strains, differential toxicity	+	0	500.0000	Boutibonnes *et al.* (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	-	200.0000	Wehner *et al.* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	-	25.0000	Boutibonnes & Loquet (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	-	100.0000	Bartholomew & Ryan (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	-	250.0000	Ingerowski *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	-	167.0000	Mortelmans *et al.* (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	-	50.0000	Kuczuk *et al.* (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	-	200.0000	Wehner *et al.* (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	-	25.0000	Ingerowski *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	-	167.0000	Mortelmans *et al.* (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	-	-	50.0000	Kuczuk *et al.* (1978)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	-	-	200.0000	Wehner *et al.* (1978)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	-	-	25.0000	Ingerowski *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	-	-	167.0000	Mortelmans *et al.* (1986)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	-	50.0000	Kuczuk *et al.* (1978)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	-	25.0000	Bartholomew & Ryan (1980)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	-	250.0000	Ingerowski *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	-	200.0000	Wehner *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	-	25.0000	Boutibonnes & Loquet (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	-	100.0000	Bartholomew & Ryan (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	-	250.0000	Ingerowski *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	-	167.0000	Mortelmans *et al.* (1986)

Table 4 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SCH, *Saccharomyces cerevisiae* D-3, mitotic crossing over, *ade2* locus	–	–	100 µg/plate	Kuczuk *et al.* (1978)
GML, Gene mutation, mouse lymphoma L5178Y tk^+/tk^- cells *in vitro*	–	–	60.0000	McGregor *et al.* (1988)
SIC, Sister chromatid exchange, Chinese hamster V79 lung cells *in vitro*	–	–	32.0000	Thust *et al.* (1983)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	–	10.0000	Galloway *et al.* (1987)
CIC, Chromosomal aberrations, Chinese hamster V79 lung cells *in vitro*	–	–	32.0000 (no concurrent control)	Thust *et al.* (1983)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	(+)	15.0000	Galloway *et al.* (1987)
PIA, Polyploidy, Chinese hamster ovary cells *in vitro*	+	–	10.0000	Galloway *et al.* (1987)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	(+)	0	3.0000	Cooray (1984)
Zearalenol (α and β-mixture or unidentified)				
PRB, SOS chromotest test, *Escherichia coli* PQ37	–	–	60.0000	Krivobok *et al.* (1987)
BSD, *Bacillus subtilis rec* strains, differential toxicity	–	0	500.0000 (single dose)	Boutibonnes *et al.* (1984)
α-Zearalenol				
BSD, *Bacillus subtilis rec* strains, differential toxicity	–	0	100 µg/plate	Ueno & Kubota (1976)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	50.0000	Bartholomew & Ryan (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	250.0000	Ingerowski *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	250.0000	Ingerowski *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	250.0000	Ingerowski *et al.* (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	250.0000	Ingerowski *et al.* (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	250.0000	Bartholomew & Ryan (1980)

Table 4 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
α-Zearalenol (contd)				
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250.0000	Bartholomew & Ryan (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250.0000	Ingerowski *et al.* (1981)
β-Zearalenol				
BSD, *Bacillus subtilis rec* strains, differential toxicity	+	0	100 μg/plate	Ueno & Kubota (1976)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250.0000	Ueno *et al.* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	50.0000	Bartholomew & Ryan (1980)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	250.0000	Bartholomew & Ryan (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250.0000	Bartholomew & Ryan (1980)

+, positive; (+), weakly positive; –, negative; 0, not tested; ?, inconclusive (e.g., variable response in several experiments within an adequate study)
[a]In-vitro tests, μg/ml; 0.0000, not given

Table 5. Genetic and related effects of deoxynivalenol

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	200.0000	Wehner *et al.* (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	200.0000	Wehner *et al.* (1978)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	200.0000	Wehner *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	200.0000	Wehner *et al.* (1978)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	1000.0000	Bradlaw *et al.* (1985)
G9H, Gene mutation, Chinese hamster V79 cells, thioguanine^r *in vitro*	–	–[b]	3.0000	Rogers & Héroux-Metcalf (1983)
CIC, Chromosomal aberrations, Chinese hamster V79 cells *in vitro*	+	0	0.1 µg/l[c]	Hsia *et al.* (1988)
CIC, Chromosomal aberrations, Chinese hamster V79 cells *in vitro*	+	0	0.1 µg/l	Hsia *et al.* (1988)
TBM, Cell transformation, BALB/c 3T3 A31-1-1 mouse embryo cells *in vitro*	+	0	0.2000	Sheu *et al.* (1988)
ICR, Inhibition of gap-junctional intercellular communication, Chinese hamster V79 cells *in vitro*	+	0	0.3000	Jone *et al.* (1987)

+, positive; –, negative; 0, not tested

[a]In-vitro tests, µg/ml

[b]Hepatocyte-mediated activation

[c]HPLC fraction of maize sample that co-eluted with deoxynivalenol

Table 6. Genetic and related effects of nivalenol

Test system	Result		Dose (LED/HID)[a]		Reference
	Without exogenous metabolic system	With exogenous metabolic system			
SIC, Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	(+)[b]	(+)	3.0000		Thust *et al.* (1983)
CIC, Chromosomal aberrations, Chinese hamster V79 cells *in vitro*	–	(+)[c]	15.0000	(5×10^{-5}M) (no concurrent control)	Thust *et al.* (1983)
CIC, Chromosomal aberrations, Chinese hamster V79 cells *in vitro*	+	0	0.001[d]		Hsia et al. (1988)

+, positive; (+), weakly positive; –, negative; 0, not tested; ?, inconclusive (e.g., variable response in several experiments within an adequate study)

[a]In-vitro tests, μg/ml

[b]Toxic doses not reached

[c]All are chromatid exchanges

[d]HPLC fraction of maize sample that co-eluted with nivalenol

Table 7. Genetic and related effects of fusarenone X

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage induction, *Escherichia coli* K-12	–	0	100.0000	Ueno *et al.* (1971)
BSD, *Bacillus subtilis rec* strains, differential toxicity	–	0	100 μg/plate	Ueno & Kubota (1976)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	250.0000	Ueno *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250.0000	Ueno *et al.* (1978)
SCF, *Saccharomyces cerevisiae*, petite mutation	+	0	250.0000	Ueno *et al.* (1971)
GIA, Gene mutation, mouse mammary carcinoma FM3A cells, 8-azaguanine^r *in vitro*	–	0	1.0000	Umeda *et al.* (1977)
SIC, Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	(+)	(+)	3.0000	Thust *et al.* (1983)
CIC, Chromosomal aberrations, Chinese hamster V79 cells *in vitro*	+	+	3.0000[b]	Thust *et al.* (1983)
DIH, DNA single-strand breaks, human HeLa cells *in vitro*	(+)	0	32.0000	Umeda *et al.* (1972)

+, positive; (+), weakly positive; –, negative; 0, not tested
[a]In-vitro tests, μg/ml
[b]No concurrent control

5.3 Animal carcinogenicity data

Zearalenone was tested for carcinogenicity by administration in the diet in one experiment in mice and in two experiments in rats. An increased incidence of hepatocellular adenomas was observed in female mice and of pituitary adenomas in mice each sex. No increase in the incidence of tumours was observed in rats.

No data were available to the Working Group on the carcinogenicity in experimental animals of deoxynivalenol.

Nivalenol was tested for carcinogenicity in one experiment in female mice by oral administration in the diet. No increase in tumour incidence was observed.

Fusarenone X was tested for carcinogenicity in two studies in male rats by oral administration and in male mice and male rats by subcutaneous injection. The studies were inadequate for evaluation.

5.4 Other relevant data

In episodes of food poisoning in humans caused by deoxynivalenol, severe gastrointestinal involvement was the primary sign.

Zearalenone has oestrogenic effects in domestic pigs and experimental animals. Deoxynivalenol causes outbreaks of feed refusal and vomiting in domestic pigs. Deoxynivalenol and fusarenone X cause immunosuppression in mice. Nivalenol causes bone-marrow toxicity in experimental animals.

No data were available on the genetic and related effects of zearalenone, deoxynivalenol, nivalenol or fusarenone X in humans.

Zearalenone induces chromosomal anomalies in cultured rodent cells. It does not induce recombination in yeast or gene mutation or DNA damage in bacteria.

Deoxynivalenol induces cell transformation, chromosomal aberrations and inhibition of gap-junctional intercellular communication in cultured mammalian cells. It does not induce unscheduled DNA synthesis or mutation in cultured mammalian cells and does not induce mutation in bacteria.

Nivalenol and fusarenone X have not been studied adequately for genetic effects.

5.5 Evaluation[1]

There is *inadequate evidence* in humans for the carcinogenicity of toxins derived from *Fusarium graminearum*.

No data were available on the carcinogenicity to humans of toxins derived from *F. crookwellense* and *F. culmorum*.

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

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

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[1]For definition of the italicized terms, see Preamble, pp. 26–29.

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

There is *inadequate evidence* in experimental animals for the carcinogenicity of fusarenone X.

Overall evaluation

Toxins derived from *Fusarium graminearum, F. culmorum* and *F. crookwellense are not classifiable as to their carcinogenicity to humans (Group 3).*

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