TOXINS DERIVED FROM *FUSARIUM SPOROTRICHIOIDES*: T-2 TOXIN

Fusarium sporotrichioides may occur in cereals, particularly in north temperate climates. This species produces T-2 toxin and other metabolites, such as diacetoxyscirpenol, which also occurs naturally but is not considered in this monograph. T-2 toxin is a type-A trichothecene (Thrane, 1989).

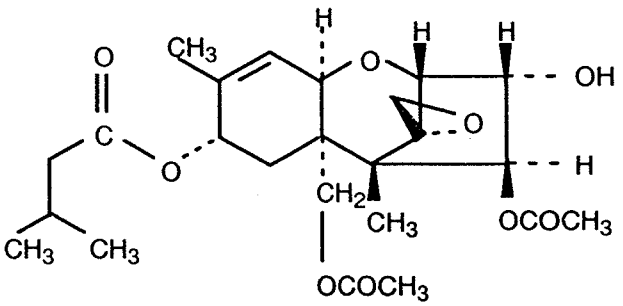
T-2 Toxin was considered by a previous Working Group (as T_2-trichothecene), in October 1982 (IARC, 1983). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Synonyms, structural and molecular data*

Chem. Abstr. Services Reg. No.: 21259-20-1
Chem. Abstr. Name: Trichothec-9-ene-3,4,8,15-tetrol, 12,13-epoxy, 4,15-diacetate 8-(3-methylbutanoate), (3α,4β,8α)-
IUPAC Systematic Name: 12,13-Epoxytrichothec-9-ene-3α,4β,8α,15-tetrol, 4,15-diacetate 8-isovalerate or (2*R*,3*R*,4*S*,5*S*,5a*R*,7*S*,9a*R*,10*S*)-2,3,4,5,7,9a-hexahydro-3,4,7-trihydroxy-5,8-dimethyl spiro[2,5-methano-1-benzoxepin-10,2′-oxirane]-5a(6*H*)-methanol, 4,5a-diacetate 7-isovalerate
Synonyms: Fusariotoxin T2; insariotoxin; 8α(3-methylbutyryloxy)4β,15-diacetoxyscirp-9-en-3α-ol; mycotoxin T2; NSC 138780; T-2 mycotoxin; toxin T2; T_2-toxin; T_2-trichothecene; 3α-hydroxy-4β,15-diacetoxy-8α-(3-methylbutyryloxy)-12,13-epoxy-δ9-tricothecene



$C_{24}H_{34}O_9$ Mol. wt: 466.5

1.1.2 *Chemical and physical properties*

From Bamburg *et al.* (1968) and Wei *et al.* (1971), unless otherwise specified

(*a*) *Description*: White needles

(*b*) *Melting-point*: 151–152 °C

(*c*) *Optical rotation*: $[\alpha]_D^{26}$ + 15° (c = 2.58 in 95% ethanol; $[\alpha]_D^{21}$ -15.5° (c = 2.14 in chloroform) (Pohland *et al.*, 1982)

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

(*e*) *Solubility*: Soluble in acetone, acetonitrile, chloroform, diethyl ether, ethyl acetate, methanol, ethanol and dichloromethane (Yates *et al.*, 1968; Lauren & Agnew, 1991)

(*f*) *Stability*: Stable in the solid state; ester groups are saponified by alkalis, and the epoxide is opened by strong mineral acids (Wei *et al.*, 1971)

1.1.3 *Analysis*

Available methods for the analysis of T-2 toxin include thin-layer chromatography and, after derivatization, gas chromatography, gas chromatography/mass spectrometry, and high-performance liquid chromatography with fluorescence detection (Scott, 1990; Cohen & Boutin-Muma, 1992). Methods involving hydrolysis followed by derivatization and gas chromatography have been developed for screening feedstuffs and biological fluids (Rood *et al.*, 1988; Lauren & Agnew, 1991).

Materials contaminated with T-2 toxin are usually co-contaminated with other trichothecenes, and mass spectrometric confirmation is normally required (Scott, 1990). Methods based on the use of polyclonal antibodies suffer from the same difficulty as those for deoxynivalenol in terms of cross-reactivity to T-2 analogues (Chu *et al.*, 1979; Kawamura *et al.*, 1990; see also Fan *et al.*, 1988).

1.2 Production and use

T-2 Toxin was first isolated by Bamburg *et al.* (1968) from a culture of *Fusarium sporotrichioides*, wrongly identified as *F. tricinctum* (Marasas *et al.*, 1984). This toxin is readily produced in liquid fermentations at yields approaching 1 g/l (Greenhalgh *et al.*, 1988). It can also be produced in large quantities in solid fermentations (Cullen *et al.*, 1982). This species produces a range of toxic metabolites (Marasas *et al.*, 1984; Greenhalgh *et al.*, 1990) and is notorious as the probable cause of the 'alimentary toxic aleukia' (see p. 475) that occurred among men in Russia during the Second World War. The disease was caused by eating over-wintered grain now known to have contained high concentrations of T-2 toxin (Mirocha, 1984).

T-2 Toxin is produced by *F. sporotrichioides*, *F. poae*, *F. equiseti* and *F. acuminatum*. The validity of reports that other species of *Fusarium* produce it has been questioned (Marasas *et al.*, 1984; Thrane, 1989). T-2 Toxin is not produced by any other fungal genus. *F. sporotrichioides* is a saprophyte found primarily on cereals left in the field under wet conditions at harvest.

1.3 Occurrence

T-2 Toxin has been reported in cereals in many parts of the world (Table 1; Scott, 1989; WHO, 1990). It is formed in large quantities under the unusual circumstance of prolonged wet weather at harvest.

Table 1. Natural occurrence of T-2 toxin

Country or region	Product	Year	Positive samples/ total no.	Content (mg/kg)	Reference
North America					
Canada	Barley	1973	1/1	25	Puls & Greenway (1976)
Canada	Feed	1979–82	1/51	2.5	Abramson *et al.* (1983)
Canada	Maize	1984	1/1	3.0	Foster *et al.* (1986)
USA	Maize	1971	1/1	2.0	Hsu *et al.* (1972)
			9/118	0.08–0.7	Scott (1989)
South America					
Argentina	Maize	NR	22/100	ND	Scott (1989)
Europe					
Czechoslovakia	Grain	1980	3/100	0.1–1.0	Scott (1989)
Finland	Feed	1976–77	3/230	0.01–0.05	Scott (1989)
Finland	Feed	1984	8/167	0.02–0.71	Karppanen *et al.* (1985)
France	Maize	NR	1/3	0.02	Jemmali *et al.* (1978)
France	Maize	1976–78	19/301	< 0.2–1.4	Scott (1989)
France	Feed	NR	4/8	< 1–2.0	Scott (1989)
France	Maize flour	NR	2/38	Trace, 0.03	Scott (1989)
Germany	Feed	NR	1/188	0.065	Scott (1989)
Germany	Oats	NR	2/19	0.08, 0.09	Scott (1989)
Germany	Wheat	NR	1/26	0.1	Scott (1989)
Hungary	Maize	1976–78	5/491	ND	Scott (1989)
Hungary	Maize	1980–81	8/23	0.1–4.4	Bata *et al.* (1983)
Hungary	Wheat	1980–81	2/23	0.2, 1.9	Bata *et al.* (1983)
Hungary	Feed	1980–81	2/23	4.1, 5.8	Bata *et al.* (1983)
Italy	Feed	NR	9/9	0.05–0.3	Scott (1989)
Norway	Barley	1984	2/49	0.02, 0.04	Sundheim *et al.* (1988)
United Kingdom	Feed	NR	4/ > 2000	Trace	Scott (1989)
Australasia					
India	Wheat	NR	3/58	0.55–4.0	Ramakrishna *et al.* (1990)
India	Wheat flour	NR	1/37	0.8	Ramakrishna *et al.* (1990)
India	Maize	NR	1/1	2.0	Ueno (1986)
India	Rice	1981	1/32	0.03	Reddy *et al.* (1983)
India	Groundnuts	1984–85	6/56	0.17–38.9	Bhavanishankar & Shantha (1987)

Table 1 (contd)

Country or region	Product	Year	Positive samples/ total no.	Content (mg/kg)	Reference
India	Sorghum	1981	2/20	0.01–0.05	Reddy *et al.* (1983)
India	Sorghum	1984–85	4/67	1.67–15.0	Bhavanishankar & Shantha (1987)
New Zealand	Maize	1984	13/20	0.01–0.2	Hussein *et al.* (1989)

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

1.4 Regulations and guidelines

An official tolerance level of 0.1 mg/kg was established for T-2 toxin in grains in the USSR in 1984 (van Egmond, 1989).

2. Studies of Cancer in Humans

Ecological studies of the relationship between exposure to *Fusarium* toxins and oesophageal cancer are summarized in the monographs on toxins derived from *F. graminearum*, *F. culmorum* and *F. crookwellense* and from *F. moniliforme*, pp. 409 and 450.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female CD-1 mice, six weeks of age, were fed a semi-synthetic diet containing 0, 1.5 or 3.0 mg/kg T-2 toxin (> 99% pure) for 71 weeks. Mice found moribund (up to 70 weeks) and those at termination of the study (71 weeks) were killed and tissues examined. There was no difference in food consumption or weight gain among the groups. More than 50% of male mice and 75% of females in each group survived to the end of the study; controls had the poorest survival. The incidence of pulmonary adenomas was increased only in males: controls, 4 (10%); low-dose, 7 (15%); and high-dose, 11 (23%); two control males and three high-dose males also had pulmonary adenocarcinomas. Male mice also had an increased incidence of hepatocellular adenomas: controls, 3 (7%); low-dose, 3 (6%); and high-dose, 10 (21%) [actual effective numbers not given]; one mouse in each group also had a hepatocellular carcinoma. The incidences of both hepatocellular and pulmonary adenomas in male mice fed the high dose were reported to be significantly greater than that in the control group ($p < 0.05$). Hyperplasia of the squamous mucosa of the forestomach was increased in a dose-related fashion in both males and females (Schiefer *et al.*, 1987).

A group of 50 male Kunming mice, six to eight weeks of age, received oral administrations of 100 μg/kg bw T-2 toxin in ethanol:saline solution three times a week for 25 weeks. A control group of 30 mice received 10 ml/kg bw ethanol solution. Small numbers of mice were killed at 1, 2, 3, 4, 5, 6, 12, 20 and 25 weeks. Forestomach papillomas occurred in 5/35 treated animals that were available for analysis: one killed at week 6, one killed at week 20 and three killed at week 25. No papilloma of the forestomach was seen in controls (Yang & Xia, 1988a). [The Working Group noted the small number of animals.]

3.1.2 *Rat*

Approximately 40 weanling male and female Wistar-Porton rats [sex distribution unspecified] were administered one to eight doses of 0.2–4 mg/kg bw T-2 toxin [purity unspecified] intragastrically at approximately monthly intervals [duration unspecified]. Another 30 rats of the same strain received the same treatment but were also given intraperitoneal injections of 200–250 mg/kg bw nicotinamide 10 min before and 2 h after each dose of T-2 toxin. Ten rats were given nicotinamide only, and another 10 rats served as untreated controls. About 65% of the rats treated with T-2 toxin alone or with nicotinamide died within a few days after the T-2 toxin treatment; 25 rats given 1–3 mg/kg bw T-2 toxin alone or with nicotinamide survived for 12–27.5 months, and the authors reported increased incidences of pancreatic and other tumours in these rats (Schoental *et al.*, 1979). [The Working Group noted the lack of detail concerning the experimental protocol.]

Rats were reported to have developed papillomas and carcinomas of the forestomach after prolonged gavage with T-2 toxin. Tumours were also seen in other organs (Li *et al.*, 1988). [The Working Group noted the lack of detail given.]

3.1.3 *Trout*

Groups of 1000 (reduced to 400 after nine months) rainbow trout, seven to eight months of age, were given 0.2 or 0.4 mg/kg T-2 toxin [purity unspecified] in the diet. Five fish from each group were killed every three months and the livers examined. The experiment was terminated after 12 months of treatment. No evidence of neoplasia was found (Marasas *et al.*, 1969). [The Working Group noted the short duration of the experiment.]

3.2 Administration with known carcinogens

Mouse

Groups of 20 white mice [strain, sex and age unspecified] each received single applications of 10 or 20 μg T-2 toxin [purity unspecified] on the dorsal skin. Two weeks later, the mice received topical applications of croton oil (two drops of a 0.5% solution) twice a week for 10 weeks. The treatment induced no skin papilloma. In a second set of experiments, groups of 20 white mice received an application of 25 μg 7,12-dimethylbenz[*a*]anthracene (DMBA), followed two weeks later by applications of 10 μg T-2 toxin once a week for 10 weeks. Skin papillomas were observed in 2/20 mice (Marasas *et al.*, 1969). [The Working Group noted the incomplete reporting.]

Groups of eight female CD-1 mice, six weeks old, received single applications of 50 μg DMBA on the shaved back, followed four days later by topical skin applications of T-2 toxin

at 10 μg weekly or 25 μg every three weeks for 22 weeks. Positive (DMBA and croton oil), T-2 toxin-treated and solvent controls were also available. One of eight mice administered DMBA followed by 25 μg T-2 toxin developed a skin papilloma. All the positive controls developed papillomas. No such tumour was observed in the solvent controls or in the T-2 toxin-treated mice (Lindenfelser *et al.*, 1974).

Groups of 15–35 male strain 615 mice, 8–10 weeks of age, were given a single skin application of 100 μg DMBA, followed one week later by applications of acetone (15 mice), 2 μg 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (20 mice) or 0.5 μg T-2 toxin (45 mice) three times a week for 26 weeks. One skin papilloma was observed among the mice treated with DMBA alone; in the mice treated with DMBA plus T-2 toxin, skin papillomas were observed in 8/45 and one skin carcinoma was observed. No skin tumour was observed in mice given DMBA and TBA. In a second part of the experiment, mice were treated with six daily doses of 5 μg T-2 toxin followed one week later by applications of 2 μg TPA (35 mice) or acetone (30 mice) three times a week for 26 weeks. No skin tumour occurred in either group. Similar experiments were carried out for 20 weeks with groups of 6–23 male and female BALB/c mice (about twice as many females as males), 8–12 weeks of age. No skin papilloma occurred in five mice treated with DMBA or in 21 mice treated with T-2 toxin alone; however, skin papillomas were found in 2/22 mice treated with DMBA and T-2 toxin, in 9/9 mice treated with DMBA and TPA and in 4/21 mice treated with T-2 toxin followed by TPA. One skin carcinoma was observed in the group given DMBA and T-2 toxin (Yang & Xia, 1988b).

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

^{3}H-T-2 Toxin given orally to mice and rats was distributed rapidly to tissues and eliminated in faeces and urine. Maximal levels of radiolabel were found after 30 min in plasma of mice after oral administration (Matsumoto *et al.*, 1978) and of guinea-pigs after intramuscular injection (Pace *et al.*, 1985). In chicks administered ^{3}H-T-2 toxin in the diet, maximal levels were reached by 4 h in blood, plasma, abdominal fat, heart, kidneys, gizzard, liver and the remainder of the carcass and by 12 h in muscle, skin, bile and gall-bladder (Chi *et al.*, 1978a). The distribution of T-2 toxin in tissues of swine was similar to that in chickens (Robison *et al.*, 1979a).

Following intravascular administration, the plasma elimination half-time of T-2 toxin and its metabolites (total radiolabel) in swine was approximately 90 min (Corley *et al.*, 1986). After intravenous administration to swine and calves, T-2 toxin was rapidly metabolized (mean elimination half-times of 13.8 and 17.4 min, respectively). A negligible fraction of the dose was recovered unmetabolized in urine. Detectable amounts were present in the spleen

and mesenteric lymph nodes 3 h after administration. No T-2 toxin was detected in the liver (Beasley *et al.*, 1986).

In dogs, T-2 toxin administered intravenously was biotransformed rapidly to HT-2 toxin (4-deacetyl-T-2 toxin; see Fig. 1), with a mean plasma elimination half-time of 5.3 min. The mean half-time of HT-2 toxin was four times longer than that of T-2 toxin (19.6 min). Both toxins had high total body clearance (Sintov *et al.*, 1986). The urinary metabolites of T-2 toxin were HT-2 toxin, T-2-triol and T-2-tetraol (free and conjugated forms) (Sintov *et al.*, 1987).

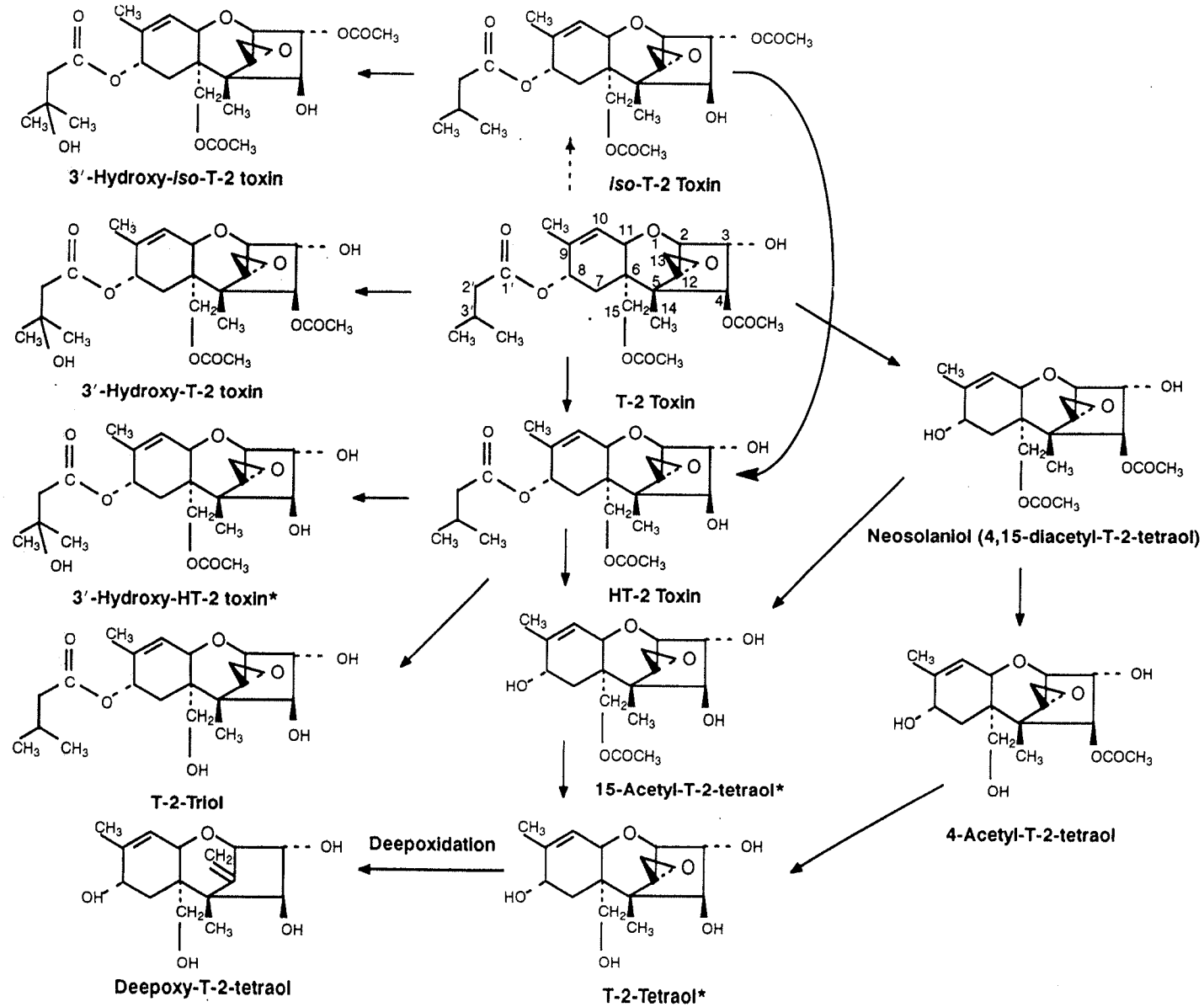
The hepatobiliary system is the major route for the metabolism, detoxification and elimination of T-2 toxin; the metabolized compounds are found mainly in bile (Matsumoto *et al.*, 1978). During a single pass through perfused rat liver, 93% of the delivered ^{3}H-T-2 toxin label was extracted and metabolized by the liver and 55% appeared in bile (Pace, 1986). Oral administration of T-2 toxin to rabbits over 10 days at a subtoxic dose of 1 mg/kg bw per day resulted in a gradual decrease in the toxin-metabolizing capacity of the liver (Ványi *et al.*, 1988). Quantitative and qualitative differences exist between species in the hepatic microsomal metabolism of T-2 toxin (Kobayashi *et al.*, 1987). Human liver enzymes deacetylate T-2 toxin to HT-2 toxin *in vitro* (Ellison & Kotsonis, 1974). Other tissues, in particular intestinal tissues, can also metabolize T-2 toxin (Conrady-Lorck *et al.*, 1989).

After intravascular administration of T-2 toxin to swine, 21 metabolites were detected by reverse-phase high-performance liquid chromatography and radiochromatography, but the structures of some are not known (Corley *et al.*, 1986).

The metabolism of T-2 toxin *in vivo* has been studied in chickens, rats, swine and cows and found to involve (i) deacylation, (ii) hydroxylation, (iii) glucuronide conjugation, (iv) acetylation and (v) de-epoxidation (for a review, see Sintov *et al.*, 1987). The C4 acetyl residue of T-2 toxin is removed rapidly to give HT-2 toxin, which is then deacylated to T-2-tetraol *via* 4-deacetylneosolaniol (15-acetyl T-2-tetraol) (Yoshizawa *et al.*, 1980a,b). Additional deacetylation of HT-2 toxin at C15 gives T-2-triol (Ványi *et al.*, 1988). Another pathway is hydroxylation of the C8 isovaleroxy residue of T-2 toxin and HT-2 toxin to 3′-hydroxy T-2 and 3′-hydroxy-HT-2 toxins (Yoshizawa *et al.*, 1982; Visconti & Mirocha, 1985). Deepoxidation of 3′-hydroxy-HT-2 toxin and T-2-tetraol has been shown to occur *in vivo*, leading to the formation of the deepoxy derivatives 3′-hydroxydeepoxy-HT-2 toxin, 3′-hydroxydeepoxy-T-2-triol, 15-acetyldeepoxy-T-2-tetraol (deepoxy-4-diacetylneosolaniol) and deepoxy-T-2-tetraol (the only one of these structures shown in Fig. 1), which were identified in rat excreta (Yoshizawa *et al.*, 1985). Deepoxy T-2-tetraol was also found in cow blood and urine as a metabolite of T-2 toxin (Chatterjee *et al.*, 1986). Neosolaniol (4,15-diacetyl-T-2-tetraol) is produced when isovaleric acid (3-methylbutanoic acid) is removed from the ester group at the C8 position of T-2 toxin (Chi *et al.*, 1978a). In addition, acetylation of T-2 toxin followed by deacetylation to HT-2 toxin may occur *via iso*-T-2 toxin (in which the hydroxy in C3 and the acetyl in C4 of T-2 toxin are reversed) (Visconti *et al.*, 1985; Sintov *et al.*, 1986). 4-Acetyl-, 8-acetyl- and 15-acetyl-T-2-tetraols have also been identified (Visconti & Mirocha, 1985). Glucuronic acid conjugates represented 63% of total metabolites in urine and 77% of those in bile of swine (Corley *et al.*, 1985).

Robison *et al.* (1979b) observed T-2 toxin at 10–160 μg/l in the milk of a pregnant cow which had been intubated with 182 mg of the toxin on 15 consecutive days. In a lactating cow given a single oral dose of 157 mg ^{3}H-T-2 toxin, maximal levels (37 μg/l) of radiolabel were

Fig. 1. Proposed pathways for the metabolism of T-2 toxin



*Undergoes deepoxidation

reached in milk after 16 h; 3′-hydroxy-T-2 toxin and 3′-hydroxy-HT-2 toxin were present at concentrations of 2.5–8.5 µg/l after 12–24 h (Yoshizawa *et al.*, 1981, 1982). *iso*-3′-Hydroxy-T-2 toxin, an isomer in which the hydroxy and acetyl groups at the C3 and C4 positions are reversed, was found as the main metabolite of T-2 toxin in cow's urine 13.5 h after oral administration of 200 mg (Visconti *et al.*, 1985). An unknown metabolite was present at a level of 2 mg/l 48 h after administration of the toxin (Ueno, 1987).

The toxicity of T-2 toxin in one-day-old broiler chick diminished gradually as a result of deacetylations: T-2 toxin (oral LD_{50}, 5 mg/kg bw) was 1.5 times more toxic than HT-2 toxin (LD_{50}, 7 mg/kg bw), which was 3.4 times more toxic than neosolaniol (LD_{50}, 25 mg/kg bw), which in turn was 1.4 times more toxic than T-2-tetraol (LD_{50}, 34 mg/kg bw) (Chi *et al.*, 1978b). After intraperitoneal injection to mice, 3′-hydroxy-T-2 toxin (LD_{50}, 4.6 mg/kg bw) was slightly more toxic than the parent compound (LD_{50}, 5.3 mg/kg bw), whereas 3′-hydroxy-HT-2 toxin (LD_{50}, 22.8 mg/kg bw) was approximately 3.5 times less toxic than HT-2 toxin (LD_{50}, 6.5 mg/kg bw) (Yoshizawa *et al.*, 1982).

T-2 Toxin and its metabolites were eliminated in faeces and urine at ratios of 3:1 in mice, 5:1 in rats (Matsumoto *et al.*, 1978) and 1:4 in guinea-pigs (Pace *et al.*, 1985).

4.2 Toxic effects

The toxicology and pharmacology of trichothecenes have been reviewed (Ueno, 1983a,b, 1987).

4.2.1 *Humans*

Little definitive information is available on the toxic effects in humans of specific trichothecenes. A disease known as 'alimentary toxic aleukia' first identified in Siberia in the former USSR, is, however, believed to be due to the consumption of grain contaminated with T-2 toxin. The aleukia usually occurs in four stages: In the first stage, hyperaemia of the oral mucosa occurs, accompanied by weakness, fever, nausea and vomiting. In more severe cases, the high fever continues and is accompanied by acute oesophagitis, gastritis and gastroenteritis. In rare cases, circulatory failure and convulsions occur. In a second stage, leukopenia, granulopenia and progressive lymphocytosis occur. A third stage is characterized by severe haemorrhagic diathesis, severe necrotic pharyngitis and laryngitis, which causes death in some cases by total closure of the larynx, described by the authors as strangulation. At this stage, exposed individuals have marked leukopenia, the leukocyte count being as low as 100–200/mm^3 or less, platelet diminution and anaemia, resulting in anoxia. The fourth stage is recovery, during which exposed individuals are susceptible to secondary infections; convalescence lasts several weeks. In severe outbreaks of poisoning, fatality rates have been as high as 50% (Joffe, 1974). Mirocha and Pathre (1973) identified T-2 toxin in a grain sample from the USSR that was associated with an episode of alimentary toxic aleukia.

In the outbreak of poisoning in Kashmir, India, in 1987 (Bhat *et al.*, 1989), described on p. 416, T-2 toxin was detected in flour samples at 0.55–0.8 mg/kg.

Bamburg and Strong (1971) reported that accidental contact of laboratory workers with crude extracts containing T-2 toxin (about 200 mg/l) caused severe irritation, loss of sensitivity and desquamation of the skin of the hands. Normal sensitivity was restored 18 days after contact.

4.2.2 *Experimental systems*

Signs somewhat similar to those in human alimentary toxic aleukia were observed in rhesus monkeys fed T-2 toxin at 0.5 mg/kg bw for 15 days (Rukmini *et al.*, 1980).

The oral LD_{50}s for T-2 toxin are (mg/kg bw): chickens, 4; mice, 10.5; rats, 5.2; guinea-pigs, 3.1 (WHO, 1990); and swine, 4.0 (Cole & Cox, 1981). The intraperitoneal LD_{50} in mice is 5.2 mg/kg bw. After subcutaneous injection, newborn animals (LD_{50}, 0.15 mg/kg bw) were more sensitive than adults (LD_{50}, 1.6 mg/kg bw) to the toxic effects of T-2 toxin (Ueno, 1984).

Once it enters the systemic circulation, by any route of exposure, T-2 toxin rapidly affects proliferating cells in the thymus, lymph nodes, testes and ovaries, spleen, bone marrow and crypts of the small intestine (Ueno, 1977a,b). Oral, parenteral and cutaneous exposures produced gastric and intestinal lesions, haematopoietic and immunosuppressive effects (Paucod *et al.*, 1990) described as radiomimetic (Saito *et al.*, 1969), central nervous system toxicity resulting in anorexia, reduced food intake, lassitude and nausea, suppression of reproductive organ function and acute vascular effects leading to hypotension and shock (Smalley, 1973; Parker *et al.*, 1984, Abstract).

Gross morphological lesions were observed in pigs given a single intravenous injection of T-2 toxin at 1.2 mg/kg bw or more, resulting in oedema, congestion and haemorrhage in lymph nodes and pancreas; congestion and haemorrhage in the gastrointestinal mucosa, sub-endocardium, adrenal glands and meninges; and oedema in the gall-bladder. Degeneration and necrosis of the lymphoid tissues and gastrointestinal mucosa were seen. Scattered foci of necrosis were present in the pancreas, myocardium, bone marrow, adrenal cortex and the tubular epithelium of the renal medulla (Pang *et al.*, 1987a). Similar effects were observed after exposure by inhalation (Pang *et al.*, 1987b).

Fatty degeneration and enlargement of the liver were observed after administration of T-2 toxin to rats (Suneja *et al.*, 1983) and cattle (Kosuri *et al.*, 1970). No effect was detected in swine fed 8 mg/kg of feed for eight weeks (Weaver *et al.*, 1978).

Vomiting is one of the commonest symptoms of T-2 toxin toxicosis. It induced vomiting at doses of 0.1–10 mg/kg bw in cats, dogs, pigs and ducklings (WHO, 1990).

In rats, the minimal effective dose of T-2 toxin for dermal toxicity, which is characterized by red spots and inflammation on painted skin, was 10 ng (Ueno, 1987). Rabbits are more sensitive to the dermal toxicity of this compound than rats (Hayes & Schiefer, 1979). The no-observed-effect level for weight gain and oral lesions in broiler chicks was 0.2 mg/kg in the diet for nine weeks (WHO, 1990).

Transient leukocytosis due to increased numbers of both neutrophils and lymphocytes shortly after a single injection of T-2 toxin in mice, rats and cats is believed to be caused by a sudden release of leukocytes from lymph nodes (Sato *et al.*, 1975, 1978). The effect seems to involve the action of inflammatory mediators, since it can be prevented by some anti-inflammatory steroids. Repeated administration of T-2 toxin to chickens, mice, guinea-pigs, cats and monkeys, however, severely decreased the number of leukocytes (reviewed by Ueno, 1983a).

Detailed studies of the effects of T-2 toxin on coagulation in several species have revealed multiple mechanisms for haemorrhage, including tissue necrosis, thrombocyto-

penia, platelet dysfunction, decreased activity of coagulation factors and (possibly) altered vascular integrity (for review, see WHO, 1990).

T-2 Toxin affects the immune system and thereby modifies the immune response in experimental animals. The impairment comprises the following functions: antibody formation, allograft rejection, delayed hypersensitivity and blastogenic response to lectins; it results in decreased resistance to microbial infection. The impairment of the immune system is thought to be linked to the inhibitory effect of T-2 toxin on macromolecule synthesis (reviewed in WHO, 1990).

Increased blood pressure and severe vascular damage were reported in a study of rats administered four doses of 1–3 mg/kg bw T-2 toxin intragastrically over 12 months (Wilson *et al.*, 1982).

Sirkka *et al.* (1992) observed acute behavioural effects, such as decreased motor activity and performance in the passive avoidance test, and reduced body weight gain in rats given a single oral dose of 2 mg/kg bw T-2 toxin, but not in those given 0.4 mg/kg bw. Fitzpatrick *et al.* (1988) found elevated concentrations of indoleamines in rat brain 24 h after an oral dose of 2.5 mg/kg bw T-2 toxin. MacDonald *et al.* (1988) found that T-2 toxin increased the concentrations of tryptophan, serotonin and dopamine in the brain of rats, but decreased those of 3,4-dihydroxyphenylacetic acid.

Feeding of 1.5 mg/kg bw T-2 toxin to young male albino rats daily for four days significantly decreased liver protein and DNA and intestinal mucosal protein content (Suneja *et al.*, 1983). The compound inhibits DNA and protein synthesis in a variety of cell types (reviewed by Ueno, 1983a,b). After HeLa cells were cultured with 30 ng/ml for three days, they underwent complete cytolysis (Saito & Ohtsubo, 1974). DNA synthesis in spleen cells *in vitro* was inhibited by 73% with a dose of 0.25 ng/ml, and protein synthesis was inhibited by 55% at a dose of 0.5 ng/ml (Rosenstein & Lafarge-Frayssinet, 1983).

The IC_{50} for T-2 toxin in rat hepatoma cells and dog kidney cells was 1–5 ng/ml (Mirocha *et al.*, 1992). The ID_{50} for protein synthesis was 7 ng/ml in reticulocytes from guinea-pigs and 30 ng/ml in reticulocytes from rabbits. T-2 Toxin induced rapid disaggregation of the polysomes, indicating inhibition at the initiation step of protein synthesis. In rabbit reticulocytes *in vitro*, using poly-U as messenger RNA, the ID_{50} for polyphenylalanine synthesis was 0.15 μg/ml (Ueno *et al.*, 1973). The molecular mechanism of inhibition of protein synthesis may be the high affinity of T-2 toxin for the 60S ribosomal subunit (Hobden & Cundliffe, 1980). The binding affinity of T-2 toxin for yeast ribosomes was 0.03 μM [14 μg]; the binding was reversible at 37 °C and specific (Middlebrook & Leatherman, 1989).

T-2 Toxin at 0.2–1.2 ng/ml induced proliferation in cultured human fetal oesophagus, including focal basal-cell hyperplasia, dysplasia and an increased number of mitoses (Hsia *et al.*, 1983). The changes were reported to be similar to the premalignant lesions seen in epithelium adjacent to human oesophageal carcinomas. At higher doses of T-2 toxin (2–4 ng/ml for six days), the cultured epithelium became necrotic.

T-2 toxin at low concentrations (0.4 pg/ml to 4 ng/ml) appears to have multiple effects on cell membrane function, which are independent of inhibition of protein synthesis. It may act on amino acid, nucleotide and glucose transporters or calcium and potassium channel activities (Bunner & Morris, 1988).

4.3 Reproductive and developmental toxicity

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Pregnant mice [strain unspecified] were injected intraperitoneally with T-2 toxin at 0.5, 1.0 or 1.5 mg/kg bw on one of days 7–11 of gestation. With the two higher doses, there was significant maternal mortality and decreased prenatal survival. In eight litters evaluated from the group that received 1.0 mg/kg bw and in four litters from the group that received 1.5 mg/kg bw on day 10, fetal weight was significantly decreased, and 38 and 29% of the fetuses, respectively, had gross malformations, including missing tails, limb malformations, exencephaly, open eyes and retarded jaw development (Stanford *et al.*, 1975).

A dose of 0.5 mg/kg bw T_2-toxin given intraperitoneally to pregnant CD-1 mice on day 10 of gestation induced tail and limb anomalies in 12.5% of the offspring. Additional treatment with 4 mg/kg bw ochratoxin A induced a reduction in fetal weight and a higher incidence of malformations (Hood *et al.*, 1976, 1978). In a similar experiment, T-2 toxin induced tail and limb anomalies in 8.4% of offspring, but additional treatment with 0.4 mg/kg bw rubratoxin B did not significantly increase the incidence of malformations (Hood, 1986).

CD-1 mice were treated orally on day 9 of gestation with 0, 0.5, 1.0, 2.0, 3.0, 3.5 or 4.0 mg/kg bw T-2 toxin. The two highest doses caused fetal and maternal deaths; skeletal defects occurred at low incidences with the 3.0-mg/kg bw dose. In a second experiment, treatment with 3.0 mg/kg bw on days 6, 7, 8, 10, 11 or 12 of gestation caused less fetal mortality; treatment on day 7 significantly reduced litter size (Rousseaux & Schiefer, 1987). Continuous feeding of 1.5 or 3.0 mg/kg T-2 toxin to CD-1 mice in the diet for two generations was neither embryo- nor fetotoxic and had only minimal effects on growth rates of mice (Rousseaux *et al.*, 1986).

Intraperitoneal injection of T-2 toxin to pregnant WAG rats at doses of 0.1, 0.2 or 0.4 mg/kg bw daily or feeding of the toxin at doses of 0.1 or 0.4 mg/kg bw on days 14–20 of gestation produced a decrease in thymus weight in newborn rats, which lasted for about one week (Bertin *et al.*, 1978).

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 Table 2 and Appendices 1 and 2)

The genotoxicity of T-2 toxin has been reviewed (Haschek, 1989).

Table 2. Genetic and related effects of T-2 toxin

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS spot test, *Escherichia coli* PQ37	–	–	0.0000	Auffray & Boutibonnes (1986)
PRB, SOS chromotest test, *Escherichia coli* PQ37	–	–	1.0000	Krivobok *et al.* (1987)
BSD, *Bacillus subtilis rec* strains, differential toxicity	–	0	100 μg/plate	Ueno & Kubota (1976)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–		Wehner *et al.* (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	50.0000	Kuczuk *et al.* (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	50.0000	Wehner *et al.* (1978)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	50.0000	Kuczuk *et al.* (1978)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	50.0000	Wehner *et al.* (1978)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	50.0000	Kuczuk *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	50.0000	Wehner *et al.* (1978)
SCH, *Saccharomyces cerevisiae* D-3, mitotic crossing over, *ade2* locus	–	–	100 μg/plate	Kuczuk *et al.* (1978)
SCF, *Saccharomyces cerevisiae*, petite forward mutations	–	0	50.0000	Schappert & Khachatourians (1986)
ACC, *Allium cepa*, polyploidy induction	+	0	20.0000	Linnainmaa *et al.* (1979)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	(+)	0	63.0000	Sorsa *et al.* (1980)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–	–	100-1000 ppm (2–3 days in feed)	Sorsa *et al.* (1980)
DMN, *Drosophila melanogaster*, sex chromosome loss, adult feeding	+	0	20 ppm, 48 h	Sorsa *et al.* (1980)
DIA, DNA single-strand breaks, BALB/c mouse primary hepatocytes *in vitro*	(+)	0	0.0005 (single dose)	Lafarge-Frayssinet *et al.* (1981)
DIA, DNA single-strand breaks, BALB/c mouse spleen lymphocytes *in vitro*	+	0	0.0005	Lafarge-Frayssinet *et al.* (1981)
DIA, DNA single-strand breaks, BALB/c mouse thymic lymphocytes *in vitro*	+	0	0.0005 (single dose)	Lafarge-Frayssinet *et al.* (1981)
G9H, Gene mutation, Chinese hamster V79 fibroblasts, thioguanine^r *in vitro*	–	+[b]	0.1000	Zhu *et al.* (1987)
SIC, Sister chromatid exchange, Chinese hamster V79 fibroblasts *in vitro*	(+)	(+)	2.3000	Thust *et al.* (1983)
SIC, Sister chromatid exchange, Chinese hamster V79 fibroblasts *in vitro*	–	(+)[c]	0.1000	Zhu *et al.* (1987)

Table 2 (contd)

Test system	Result		Dose (LED/HID)[a]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CIC, Chromosomal aberrations, Chinese hamster V79 fibroblasts *in vitro*	+	(+)	0.5000	Thust *et al.* (1983)
CIC, Chromosomal aberrations, Chinese hamster V79 fibroblasts *in vitro*	+	0	0.0005	Hsia *et al.* (1986)
CIC, Chromosomal aberrations, Chinese hamster V79 fibroblasts *in vitro*	(+)	(+)[b,c]	0.0500	Zhu *et al.* (1987)
CIC, Chromosomal aberrations, Chinese hamster V79 fibroblasts *in vitro*	+	0	0.0010	Hsia *et al.* (1988)
MIA, Micronucleus formation, Chinese hamster V79 fibroblasts *in vitro*	+	+[b,c]	0.0500	Zhu *et al.* (1987)
UHF, Unscheduled DNA synthesis, human fibroblasts *in vitro*	+	0	0.0050	Oldham *et al.* (1980)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	-	-[b]	0.0030	Cooray (1984)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+[d]	0	0.0001	Hsia *et al.* (1986)
DVA, DNA single-strand breaks, BALB/c mouse liver *in vivo*	-		3.00 × 1 ip	Lafarge-Frayssinet *et al.* (1981)
DVA, DNA single-strand breaks, BALB/c mouse spleen *in vivo*	+		3.00 × 1 ip	Lafarge-Frayssinet *et al.* (1981)
DVA, DNA single-strand breaks, BALB/c mouse thymus *in vivo*	(+)		3.00 × 1 ip	Lafarge-Frayssinet *et al.* (1981)
MVC, Micronucleus test, Chinese hamster bone marrow *in vivo*	-		3.00 × 1 ip	Norppa *et al.* (1980)
CBA, Chromosomal aberrations, Chinese hamster bone marrow *in vivo*	(+)		1.70 × 1 ip	Norppa *et al.* (1980)
ICR, Inhibition of intercellular communication, Chinese hamster V79 cells *in vitro*	+	0	0.0030	Jone *et al.* (1987)
T-2-Tetraol				
BSD, *Bacillus subtilis rec* strains, differential toxicity	-	0	5000.0000	Boutibonnes et al. (1984)
UHF, Unscheduled DNA synthesis, human fibroblasts *in vitro*	+	0	0.1000	Oldham *et al.* (1980)

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

[a]In-vitro tests, μg/ml; in-vivo tests, mg/kg bw; 0.0000, dose not given

[b]Activation by rat hepatocytes

[c]Activation by rat oesophageal epithelium

[d]Control aberration frequency extremely low (6/3000)

T-2 Toxin did not induce DNA damage in bacteria, mutation in *Salmonella typhimurium* or mitotic crossing over or mitochondrial petite mutations in yeast. In *Allium cepa*, T-2 toxin increased the number of polyploid root-tip cells. In *Drosophila melanogaster*, it induced sex chromosome loss, but the results for sex-linked recessive lethal mutations were inconclusive. T-2 Toxin inhibited gap-junctional intercellular communication in Chinese hamster V79 cells *in vitro* and induced DNA single-strand breaks in mouse spleen and thymic lymphocytes and [marginally] hepatocytes *in vitro*. In cultured Chinese hamster V79 cells, T-2 toxin induced gene mutation, sister chromatid exchange and chromosomal aberrations. After treatment with T-2 toxin, unscheduled DNA synthesis was induced in cultured human fibroblasts, and chromosomal aberrations, but not sister chromatid exchange, were induced in cultured human lymphocytes. Administration of T-2 toxin to mice *in vivo* resulted in single-strand breaks in spleen and thymus, but not in liver. In Chinese hamsters treated *in vivo*, a slight increase in the frequency of chromosomal aberrations, but not [one dose only] of micronuclei, was observed in bone marrow.

The genotoxic effects of T-2 toxin were observed only at very low doses, since toxic effects interfered at higher doses.

T-2-Tetraol, a metabolic hydrolysis product of T-2 toxin, induced unscheduled DNA synthesis in cultured human fibroblasts.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

T-2 Toxin is produced primarily by *Fusarium sporotrichioides*, which occurs rarely on cereals such as wheat and maize. The toxin is considered to have played a role in large-scale human poisonings in Siberia during this century.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

T-2 Toxin was tested for carcinogenicity in mice and in trout by oral administration in the diet and in rats by intragastric administration. In mice, it increased the incidences of pulmonary and hepatic adenomas in males. The studies in trout and rats were inadequate for evaluation.

5.4 Other relevant data

T-2 Toxin causes outbreaks of haemorrhagic disease in animals and has been associated with alimentary toxic aleukia in humans.

No data were available on the genetic and related effects of T-2 toxin in humans.

Experimental data were drawn mainly from single studies. T-2 Toxin induced DNA damage and chromosomal aberrations in rodents *in vivo*, in cultured human cells and in cultured rodent cells. It inhibited protein synthesis in various mammalian and human cell types *in vitro*. Chromosomal aberrations were also induced in insects. It induced gene mutation in cultured rodent cells but not in bacteria. It did not induce DNA damage in bacteria.

5.5 Evaluation[1]

No data were available on the carcinogenicity to humans of toxins derived from *Fusarium sporotrichioides*.

There is *limited evidence* in experimental animals for the carcinogenicity of T-2 toxin.

Overall evaluation

Toxins derived from *Fusarium sporotrichioides are not classifiable as to their carcinogenicity to humans (Group 3).*

6. References

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

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