TOX/2019/17

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Review of potential risks from mycotoxins in the diet of infants aged 0 to 12 months and children aged 1 to 5 years

Fusarenon-X

Background

1. The Scientific Advisory Committee on Nutrition (SACN) is undertaking a review of scientific evidence that will inform the Government's dietary recommendations for infants and young children. The SACN is examining the nutritional basis of the advice. The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) was asked to review the risks of toxicity from chemicals in the diet of infants, which has been completed, and young children. The reviews will identify new evidence that has emerged since the Government's recommendations were formulated and will appraise that evidence to determine whether the advice should be revised. The recommendations cover diet from birth to age five years.

2. The Food Standards Agency (FSA) has completed a survey of 36 mycotoxins in the 2014 Total Diet Study (TDS) – mycotoxins analysis (FSA, to be published). The results of the survey provide information on the concentrations of aflatoxins, ochratoxin A, zearalenone, fumonisins, 3-acetyldeoxynalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), deoxynivalenol (DON), diacetoxyscirpenol, fusarenon-X (Fus-X), T-2 toxin, HT-2 toxin, neosolaniol, nivalenol (NIV), sterigmatocystin, citrinin, cyclopiazonic acid, moniliformin, patulin and ergot alkaloids (ergocornine, ergocorninine, ergocristine, ergocristinine, ergocryptine, ergocryptinine, ergometrine, ergometrinine, ergosine, ergosinine, ergotamine, ergotaminine) in relevant foods. Estimates of dietary exposures have been calculated for each mycotoxin for UK infants and young children aged 4 to 60 months using food consumption data taken from the Diet and Nutrition Survey of Infants and Young Children (DNSIYC) and the National Diet and Nutrition Survey (NDNS).

3. A scoping paper (TOX/2015/32)[1] "COT contribution to SACN review of complementary and young child feeding; proposed scope of work for 1-5 years old children" was reviewed by the COT in 2015. A further scoping paper for mycotoxins was presented to the COT in 2017[2]. This discussion paper will provide a review of the available literature and government evaluations for Fus-X.

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[1] COT scoping paper (TOX/2015/32) available at: https://cot.food.gov.uk/sites/default/files/TOX2015-32%20Feeding%20Review%20Scoping%20Paper.pdf

[2]COT mycotoxins scoping paper available at: https://cot.food.gov.uk/sites/default/files/tox2017-30_0.pdf

4. There is currently no evaluation of Fus-X available by the European Food Safety Authority (EFSA) or the Joint FAO/WHO Expert Committee on Food Additives (JECFA), however, an evaluation was performed by The Netherlands National Institute for Public Health and the Environment (RIVM) (Pronk, Schothorst and van Egmond, 2002), which will form the major basis of this discussion paper. Toxicity studies published since the RIVM evaluation are also summarised or described.

5. Fus-X is a type B tricothecene and is predominantly produced by sub-types of the Fusarium species; F. crookwellense, F. poae, F. graminearum, F. culmorum, F. nivale and F. equiseti. Fusarium species are generally field fungi but can continue to grow on crops in storage. Fus-X is predominantly found in cereals such as wheat, barley, oats, rye, rice, sorghum, millet and maize.

Previous evaluation

6. As mentioned, RIVM has previously performed an evaluation of the toxicological effects and occurrence of Fus-X in 2002 (Pronk, Schothorst and van Egmond, 2002).The reviewers concluded that Fus-X incidents were rarely reported, since this mycotoxin was not routinely tested for. Additionally, available toxicology data were too limited to derive a health-based guidance value (HBGV).

7. The International Agency for Research on Cancer (IARC), also provided their evaluation for the carcinogenic potential of Fus-X in 1993, concluding that there was inadequate evidence in experimental animals for the carcinogenicity of the compound and that no human data were available. Therefore, Fus-X was not classifiable as to its carcinogenicity in humans and was classed as Group 3 (IARC, 1993).

8. The International Programme on Chemical Safety (IPCS), also evaluated the toxicological effects of Fus-X in 1990, concluding that from the available long-term in vivo toxicity studies, there was no evidence to indicate that Fus-X is tumorigenic in humans (IPCS, 1990).

9. Fus-X has been categorised as a Category. 1 acute toxicant for the oral route under Regulation EC No. 1272/2008, with a hazard statement of H300 (fatal if swallowed), although the dose as to which this adverse effect occurs was not provided (ECHA, 2018).

ADME

10. Fus-X is a highly lipid soluble compound which is rapidly absorbed from the gastrointestinal tract. Uniformly labelled 3H-Fus-X was administered subcutaneously (sc) at 4 mg/kg bw to mice. After 30 minutes, activity was found at the highest level, in the liver (3%), as well as in the kidneys, intestines, stomach, spleen, bile and plasma. None was detected in heart, brain or testes. After 12 hours, there was no dose in any of the organs and 25% had been recovered in metabolised forms of Fus-X, in the urine. Fus-X is deacetylated to nivalenol (NIV) by rat and rabbit liver carboxy deesterases (Pronk, Schothorst and van Egmond, 2002).

11. In another rodent study, mice were orally administered 18 µg Fus-X/kg bw.

Plasma radioactivity reached a peak at 30 minutes after administration and was mainly excreted as NIV in the urine (Poapolathep et al., 2003).

12. Broilers and ducks were orally administered 2.2 mg Fus-X/kg bw. Fus-X plasma level in broilers and ducks was detected up to 2 and 3 hours, respectively. The elimination half-life was longer in ducks than in broilers, 2.20 hours compared to 1.20 hours. Fus-X was found to be more efficiently absorbed in ducks than in broilers, however, excretion of Fus-X in the form of NIV occurs faster in broilers than ducks (Poapolathep et al., 2008).

13. Toxicokinetic studies performed in piglets revealed similar observations to that in the mice. Rapid absorption was found following oral administration of Fus-X at 1 mg/kg bw, the oral bioavailability was 74.4%. The peak plasma concentration of NIV following oral administration was 518.35 ng/mL, which indicates that Fus-X is rapidly absorbed and metabolised. Both Fus-X and NIV were detectable from 1 hour to 24 hours in various tissues, including the liver, kidney, bile, intestine and muscle, post oral administration. Fus-X is mainly excreted as NIV in the urine and faeces of piglets (Saengtienchai et al., 2014).

14. Goats were orally administered 1 mg Fus-X/kg bw, with an oral bioavailability of 15.8%. A larger proportion of NIV was quantifiable in plasma, urine and faeces up to 8 hours post-administration (HPA) in comparison to Fus-X; Fus-X/NIV ratio was less than one after 5 minutes. The maximum concentration of Fus-X was 413.39 ng/mL, conversion of Fus-X to NIV mainly occurs in the liver microsomal fraction. Fus-X was also found to be largely excreted as NIV in the faeces of goats (Phruksawan et al., 2018).

15. To summarise, Fus-X is metabolised to NIV (Fig. 1). NIV is a separate mycotoxin, which was brought to the COT in the 2017 mycotoxin scoping paper[2]. EFSA established a tolerable daily intake (TDI) of 1.2 µg/kg bw in 2013, based on a benchmark dose response of 5% ($BMDL_{05}$) of 0.35 mg NIV/kg bw/day for the reduction in white blood cell counts in a 90-day rat study (two uncertainty factors were applied; 3 for gaps in the database and 100 for inter- and intra-species differences to the $BMDL_{05}$) (EFSA, 2013).

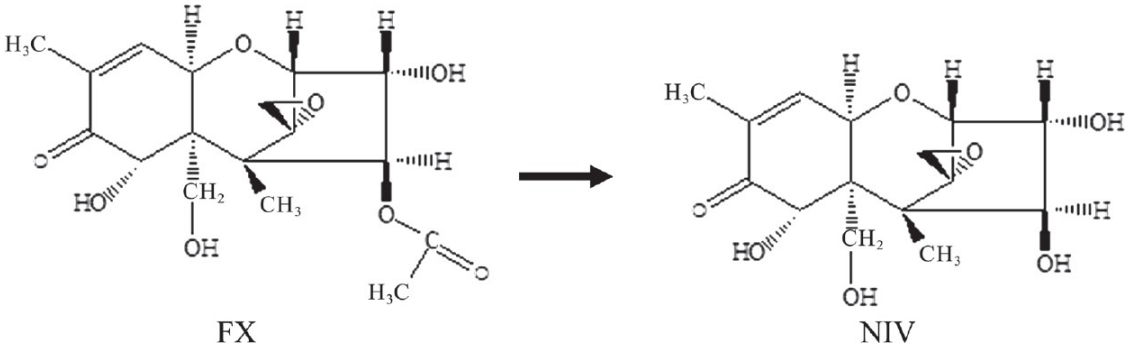

Figure 1 schematic 2D metabolic conversion of Fusarenon-X (FX) to nivalenol (NIV) in mammalian systems (reproduced from Aupanun et al., 2017).

Mode of Action (MOA)

16. The mechanism of toxicity for Fus-X is yet to be fully elucidated, however, it is generally accepted and known to evoke a ribotoxic response; targeting the 60s subunit organelle of eukaryotic ribosomes. Fus-X binds to peptidyl transferase and causes inhibition of initiation of protein synthesis, as evidenced in in silico (Dellafiora et al., 2017) and in vitro (Ohtsubo et al., 1972; Ueno et al., 1973 and Mizuno 1975) studies.

17. Carter & Cannon (1984) carried out inhibition studies to determine the MOA of Fus-X. At 2 µg/mL, Fus-X was shown to cause extensive "run off" of polyribosomes in reticulocyte free systems, at concentrations approaching 100 µg/mL inhibition of protein synthesis was evident. The authors concluded that the ribosomal target is the peptidyl transferase catalytic centre, however, the extent of binding to the site is dependent on the concentration of Fus-X present.

18. It has been suggested that the toxic potency of the trichothecenes varies depending on the modification of side chains in the molecule (Pronk, Schothorst and van Egmond, 2002). Fus-X has a ketone side chain (Fig. 2).

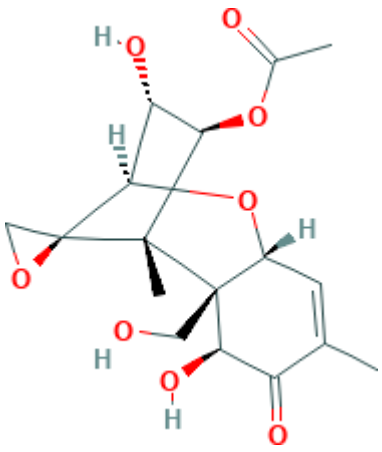

Figure 2 schematic 2D structure of Fusarenon-X (reproduced from PubChem, 2018).

Summary of toxic effects

Acute and subacute toxicity

19. A range of lethal dose 50 (LD_{50})[3] values in mice, rats, guinea pigs and cats (0.1 to 5.6 mg/kg bw) were obtained for oral, sc, intraperitoneal (ip) and intravenous (iv) administrations. Oral administration of Fus-X has been found to be acutely toxic to rats and mice, with a LD_{50} value of 4.4 mg/kg bw and 4.5 mg/kg bw, respectively (Pronk, Schothorst and van Egmond, 2002). A lower LD_{50} was reported in mice by Ciegler

[3] LD_{50}: Lethal Dose 50 is the dose required to kill half the members of a population in a set timeframe.

(1978) at 3.3 mg/kg, however, the route of exposure was not mentioned. Ueno et al. (1971) carried out a comparative study on the routes of exposure to Fus-X. The LD_{50} (mg/kg) in mice were; 3.4 (iv), 3.4 (ip), 4.2 (sc), and 4.5 (oral).

20. General pharmacokinetic studies of Fus-X were carried out by Matsuoka et al., (1979). Ip injections of 0.7 (n=3), 3 (n=6) and 15 (n=3) mg Fus-X/kg were administered to male DD mice. Iv injections of 0.3, 0.5, 1 or 3 mg Fus-X/kg (n=3; per dose) were administered to male Wistar rats and Nihon-albino rabbits. Oral exposures were not studied by the authors.

21. The effects of ip exposures are hereby summarised. At 15 mg Fus-X/kg, observed adverse toxicological effects included; diarrhoea, haemorrhage from the proximal nail fold, as well as death in 2 out of three mice. Other effects such as haemoptysis, hyperaemia of the intestine, lung and kidney and expansion of the intestine was observed in the surviving mice. At 3 mg Fus-X/kg, hyperaemia of the intestine and lungs were observed in all six mice, whilst in two out of six; hyperaemia of the kidney and expansion of the intestine were additionally reported. The lowest administered dose of 0.7 mg Fus-X/kg caused no visceral change in mice. At all doses, Fus-X induced slight ataxic gait and fall in body temperature of mice within 3 hours.

22. The effects of iv exposures are hereby summarised. Exposure through the iv route at 3 mg Fus-X/kg exhibited the same clinical signs of that of the ip route. Concentrations of 1 or 3 mg Fus-X/kg decreased the respiratory rate at an early stage, which returned to control levels in ~90 minutes post-administration. Fus-X was also found to cause blood pressure changes. The maximum was reached at 80 mmHg at a concentration of 3 mg Fus-X/kg at ~60 minutes. A gradual fall was observed until death. Iv injection at 0.3 or 0.5 mg Fus-X/kg was not found to induce detectable effects on the respiratory rate in rats, however, the higher dose induced a decrease in respiration and a gradual decrease in heart rate, and blood pressure 15 minutes post-administration in rabbits.

23. Fus-X was found to induce apoptosis (9 hours after treatment) in the liver, kidney and spleen of male and female mice (14 groups of 5 animals each, with 10 treated groups and 4 control groups) after a single oral exposure of 4 mg Fus-X/kg bw. Apoptosis was observed, particularly in hepatocytes surrounding the central lobular zone of the liver, in proximal tubular cells of the kidney and haematopoietic cells in the red pulp area of the spleen (Sutjarit & Poapolathep, 2016).

24. ICR male mice were orally administered Fus-X at 0, 0.1, 0.3 and 0.5 mg/kg bw (one daily treatment for 14 days) (n=25; per dose). Body and organ weight were not affected, however, at the higher doses (0.3 and 0.5 mg/kg bw), nuclear condensation and fragmentation of lymphocytes in the cortical thymus and germinal center of Peyer's patches were observed (Aupanun et al., 2015).

25. Li & Shimizu (1997), studied the course of apoptotic changes in the rat gastric mucosa caused by oral administration of Fus-X. Two groups of male Wistar rats (n=40 each) were administered with 0 or 1.5 mg Fus-X/kg and were sacrified 0, 0.5, 1, 1.5, 2, 3, 4, 6, 12, 24 and 48 HPA. Compared to controls, rats administered 1.5 mg Fus-X showed significant dilatation of the stomach with increased fluid contents at 1-24 HPA.

Karyopyknosis[4] of the chief cells in the basal region of the gastric glands appeared at 1 hour, and nuclear fragments were seen in the neck cell region at 1.5 HPA. Apoptotic cells appeared diffusely in the neck region and focally in the basal region (2-4 HPA). Electron microscopy revealed that cells phagocytosing apoptotic bodies were the surface epithelia, undifferentiated neck cells, parietal cells and chief cells. The authors concluded that, chief cells appeared to be the main target of Fus-X induced apoptosis.

26. The same group, studied the same effects as above caused by ip administration of 1.5 mg Fus-X/kg (Li et al., 1997). Two groups of male Wistar rats (n=24 each) were administered with 0 or 1.5 mg Fus-X/kg and were sacrified 0, 0.5, 1, 2, 3, and 4 HPA. Ip treated rats showed similar effects to that of orally exposed rats, with dilation ofthe stomach and increased fluid contents. Apopototic karypyknosis in chief cells was observed in the basal region.

Haematological effects

27. Aoki & Toyama (1983), observed that Fus-X markedly suppressed the growth of colony forming unit cells and colony forming unit erythroid in adjuvant-treated rats when injected with 0.5 and 1.0 g/kg of Fus-X.

28. Shortly after injection of mice with Fus-X at 0.1-0.5 mg/kg, a rapid increase of leukocytes and lymphocytes was observed along with increased β-globulin and decreased γ-globulin. At autopsy, cellular degeneration and karryorrhexis were marked in bone marrow and small intestine (Pronk, Schothorst, van Edmond, 2002).

Effects on the inflammatory responses

29. Wu et al., (2014) observed that Fus-X was more potent in inducing IL-1β mRNA[5] in comparison to DON, whilst also having the same ability to induce TNFα[6] and CCL-2[7] mRNAs in mice orally dosed with 2.5 µg Fus-X/kg bw. Both cytokine and chemokine responses to Fus-X were still detected 6 HPA.

Cytotoxicity

30. Ohtsubo & Saito (1970) tested Fus-X for its growth inhibiting activity and suppressive effects on DNA, RNA and protein synthesis. Fus-X exhibited complete inhibition of growth of HeLa S3 cells at a concentration of 0.5 µg/mL. The effective

[4] Karyopyknosis; is the irreversible condensation of chromatin in the nucleus of cells undergoing necrosis or apoptosis.
[5] IL-1β; Interleukin 1β, is a cytokine that mediates the inflammatory response, and is involved in a variety of cellular activities e.g. cell proliferation, differentiation, and apoptosis.
[6] TNFα; Tumour necrosis alpha, is an inflammatory cytokine produced by marcrophages/monocytes during acute inflammation and is responsible for a diverse range of signalling events; leading to necrosis or apoptosis.
[7] CCL-2; C-C motif chemokine ligand 2 is a cytokine that displays chemotactic activity for monocytes and basophils.

dose 50 (ED_{50})[8] for ^{3}H-thymidine incorporation into DNA and ^{3}H-leucine into protein were 0.1 and 0.13 µg Fus-X/mL, respectively.

31. Abbas & Mirocha (1988) reported lethal concentration 50 (LC_{50})[9] values for human fibroblast cell lines: GM 498 at 1 µg Fus-X/mL and GM 3349 at 0.50 µg Fus-X/mL.

32. Miura et al., (2002) investigated the mode of apoptosis induced by Fus-X in HL-60 cells. At 0.5 µg Fus-X/mL cell degradation after 5 hours of exposure was observed, with DNA fragmentation into 180-basepair multimers. Furthermore, most of the cells had undergone apoptosis 24 hours after exposure. Activities of caspase-3, -8, and -9 were found to be elevated within 2 hours of the exposure. The authors concluded that, Fus-X induces apoptosis in HL-60 cells by stimulating cytochrome c release and subsequent activation of the aforementioned caspases.

33. Eriksen et al., (2004) assessed the cytotoxicity of Fus-X in 3T3 fibroblast cells using the 5-bromo-2'-deoxyuridine incorporation assay, which assesses DNA-synthesis. The concentration inhibiting 50% of the DNA synthesis (IC_{50}) was 0.72 µM.

34. Alassane-Kpembi et al., (2013) has measured the cytotoxicity of Fus-X on the intestinal epithelial cell; Caco-2. The measured IC_{50} was 0.04 µM for the MTT assay[10], whilst for the Neutral Red assay[11] the IC_{50} was 0.02 µM. Bony et al., (2007) also assessed the genotoxic potential of Fus-X in Caco-2 cells. Fus-X was found to increase DNA strand breaks at the range of 0.01-0.05 µM range in dividing cells after 72 hours of exposure.

35. Ueno (1984) studied the effect of Fus-X on mast cells of rat peritoneal membranes and the stability of lysosomal particles isolated from rat livers. The authors stated that 10 µg of Fus-X caused no significant changes in the morphology of mast cells within 1-2 hours of incubation, however, the data was not provided. The spontaneous release of acid phosphatase from lysosomes was not accelerated in the presence of 4 µg/mL of Fus-X within 4 hours of incubation at 37°C.

[8] ED_{50}: Effective Dose 50 is the half dose that produces a biological response.
[9] LC_{50} values: Lethal Concentration of a toxin which results in 50% reduction in cell density.
[10] MTT assay; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay, assess the cell's metabolic activity, and therefore its viability indirectly.
[11] Neutral Red assay; 3-amino-7dimethyl-2-methylphenazine hydrochloride assay, assess cellular membrane integrity, and therefore the cell's viability indirectly.

36. Fus-X was found to induce the transcription of COX-2[12] mRNA expression, and activation of three mitogen-activated protein kinases (MAPK)[13] families in the RAW 264.7 murine macrophage cell line at a concentration of 0.20 mg Fus-X/mL (Moon et al., 2003).

(Sub)Chronic toxicity/Carcinogenicity

37. A series of experiments were described in the RIVM 2002 evaluation, in which male Donryu rats and male dopamine-deficient dopamine transporter- knockout (DDD) mice received repeated oral or subcutaneous administrations of Fus-X (Pronk, Schothorst and van Egmond, 2002). The RIVM reviewers noted that the description of the experiments was rather limited, which had also been observed by and commented on by IARC (1993).

38. In the first experiment 20 rats (sex not reported) were administered weekly oral 0.4 mg Fus-X/kg bw by oral intubation for 50 weeks. Twelve rats survived the 50 weeks. Of these, one had developed a hepatoma. Histopathology showed that approximately 50% of the animals had hypoplasia and atrophy of the bone marrow, thymus and spleen and some rats showed intrahepatic bile duct hyperplasia and atypical hyperplasia in the gastric and intestinal mucosa (Pronk, Schothorst and van Egmond, 2002).

39. The same dose was given weekly via sc injection in 20 rats for 22 weeks; similar histopathologic results were observed. Eighteen rats survived for more than 1 year, however, one animal developed lung adenoma (Pronk, Schothorst and van Egmond, 2002).

40. Two groups of mice (16 or 18 animals) were administered weekly subcutaneous injections of 2.5 mg Fus-X/kg bw for 10 or 20 weeks. Most of the animals survived. Alopecia was observed locally to the injection site, but hair regrew within a few months. Minimal pathological changes were noted except for moderate thymus atrophy. One case of leukaemia was observed (Pronk, Schothorst and van Egmond, 2002).

41. In 20 control rats and 11 control mice no tumours occurred during the experimental periods of over 57 weeks. Atrophy of the organs was mild and only observed in a few cases (Pronk, Schothorst and van Egmond, 2002).

42. Male Donryu rats were administered 3.5 (n = 49) or 7 (n = 25) mg FUS-X/kg in the diet for 2 years. Two additional groups of animals (n = 26, each) were given 7 mg Fus-X/kg bw for 1 year and then a control diet for 1 year with one of the groups also receiving 20 oral administrations of penicillic acid (50 mg/animal) in the first year.

[12] COX-2; Cyclooxygenase (COX) is an enzyme that catalyses the oxygenation of arachidonic acid to prostaglandin endoperoxides. These metabolites are converted enzymatically into prostaglandins and thromboxane A_2, which play both physiologic and pathologic roles at diverse inflammatory sites.
[13] The three MAPKs were; c-Jun N-terminal kinase 1 and 2, extracellular signal-related protein kinase 1 and 2, and p38. Transcription of COX-2 is modulated all the MAPKs.

Control animals received no treatment (n = 48) or 20 administrations of penicillic acid (n = 25). All animals were given restricted feed (15 g/day) to provide 50 and 105 µg Fus-X/day/animal. Survivors were killed at 24 months (Pronk, Schothorst and van Egmond, 2002).

43. Effects included generally lower mean bodyweights of treated animals than control animals, however, bodyweight gain recovered after removal of the experimental diet. Survival was poor and treatment-related. The major cause of death in the controls and treated groups was bronchopneumonia, but the incidence was higher in treated animals. No increase in the incidence of tumours was observed in treated rats (Pronk, Schothorst and van Egmond, 2002).

44. Sakai et al., (2007) examined the initiation and promotion activity of Fus-X using v-Ha-ras-transfected BALB/3T3 cells, which can mimic the two-stage process of chemical carcinogenesis. Fus-X concentrations of 0-0.1 µg/mL did not exhibit any activity for initiation or promotion after 21 days of incubation.

45. The Carcinogenic Potency Database informs that there is no positive test available for the carcinogenic potential of Fus-X, in either rat or mouse rodent models (CPDB, 2007).

Genotoxicity

46. The IARC identified three studies which concluded in positive genotoxic results for Fus-X. It was found to elicit petite mutation in yeast cells (Saccharomyces cerevisiae) at 250 µg Fus-X/mL without metabolic activation. Fus-X was tested in vitro in Chinese hamster V79-E cells and was classed as weakly clastogenic for induction of chromosomal aberrations and sister chromatid exchanges at 3 µg Fus-X/mL (the activity of Fus-X was not affected by metabolic activation). DNA single-strand breaks were observed in human HeLa cells at 32 µg Fus-X/mL without metabolic activation.

47. However, negative genotoxicity results were reported. Fus-X did not induce DNA damage in the Rec-assay using Bacillus subtilis at 100 µg Fus-X/plate, it was not mutagenic to Salmonella typhimurium; TA100 and TA98 strains at 250 µg Fus-X/mL and did not induce gene mutations in cultured mouse mammary carcinoma FM3A cells at 1 µg Fus-X/mL (IARC, 1993).

Reproductive and developmental toxicity

48. Fus-X was administered to groups of female DDD mice (n=4) by sc injection. Single doses of 0.63, 1, 1.6, 2.6 or 4.1 mg Fus-X/kg bw were given on day 10 of gestation, or at a single dose of 1.6 mg Fus-X/kg bw on day 6, 8 or 13 of gestation, or at multiple doses of 0.63, 1 or 1.6 mg Fus-X/kg bw on days 8-12 or 8-14 of gestation (Ito et al., 1980).

49. Dams given 4.1 mg Fus-X/kg bw died within 24 hours of the injection and all dams given 2.6 mg Fus-X/kg bw aborted 1 day after the injection. At lower doses,

abortion occurred less frequently (16-20 %) with longer intervals after injection (2 - 7 days), but embryotoxicity as evidenced by a dose-dependent increase in the number of resorbed and dead fetuses. A single administration of 1.6 mg Fus-X/kg bw induced more abortions when given on day 6 (75 %) or 13 (50 %) than on day 10, but none when given on day 8. More fetal absorptions occurred the later the day of administration during gestation. Multiple doses of 1 and 1.6 mg Fus-X/kg bw caused all animals to abort, but none aborted with multiple administrations of 0.63 mg Fus-X/kg bw. Body weight and body length of the surviving fetuses were reduced compared with control fetuses, especially when given a single dose of 1.6 mg Fus- X/kg bw on day 6 or 8 of gestation, or multiple doses of 0.63 mg Fus-X/kg bw. Teratogenic effects were not observed (Ito et al., 1980).

50. Pregnant mice (n=20) were orally administered 3.5 mg Fus-X/kg bw at the 14th day of pregnancy, and were assessed at 0, 12, 24 and 48 hours after treatment. Observations from histopathology revealed that apoptotic cells in the telencephalon of mouse foetus peaked at 12 hours and decreased at 24 and 48 hours. Gene expression studies carried out by microarray and real time-reverse transcription polymerase chain reaction, confirmed that Fus-X induced the upregulation of Bax, Trp53, and caspase-9 and down-regulated Bcl2 mRNA. The authors suggested that from the data Fus-X induces apoptosis in the developing mouse brain in treated Fus-X dams (Sutjarit et al., 2014).

Immunotoxicity

51. Blastogenesis in cultured human lymphocytes was inhibited by Fus-X. A concentration of 18 ng Fus-X/mL inhibited [^{3}H] thymidine uptake by mitogen-stimulated human lymphocytes by 50% (Forsell & Petska, 1985).

52. T and B cell proliferation were significantly and dose-dependently inhibited by Fus-X in an in vitro test with human peripheral blood mononuclear cells at the concentrations of 0.2-1800 ng Fus-X/mL (Berek et al., 2001).

53. Fus-X caused 50% apoptosis at 7.5 µg/mL in human Jurkat T-cell lines. IL-2 upregulation was suppressed at 0.625 – 5 µg Fus-X/mL (Petska et al., 2005).

54. Immunosuppressive effects were observed in BALB/c mice (n=4) after repeated daily ip administration of Fus-X (50 µg for 7 days). In vivo IgE and IgG1 antibody formation was suppressed, as was in vitro antibody formation by splenic lymphocytes raised by T-dependent and independent mitogens (Masuda et al., 1982).

55. Mice received three ip injections of Fus-X at 3.0 mg/kg bw (n=3 for each sex), pathologic investigations showed that Fus-X caused severe atrophy and disappearance of thymocytes in the thymic cortex. CD_4^+ and CD_8^+ T cells were also found to be depleted (Miura et al., 1998).

56. Male ICR mice (n=25) were orally dosed at 0.5 mg Fus-X/kg bw (one treatment per day for 14 days). The upregulation of Bax, Bid, Trp53, and caspase-9 mRNA were observed, but the relative expression of Fas, tumour necrosis factor, and caspase-8 proteins remained unchanged in lymphoid tissues. Fus-X exhibited concentration and

time dependent inhibition of cell viability. The authors suggested that Fus-X at low doses can induce apoptosis in lymphocytes through effects on the proteins mentioned above, and therefore the mitochondrial apoptotic pathway (Aupanun et al., 2016).

57. Ito et al., (1982) studied the effect of Fus-X at 0.75 mg/kg (ip; administered twice in 5 days) for antibody responses in guinea pigs (n=10). Fus-X was not found to exhibit any immunosuppressive activity at the sublethal dose tested, however, it inhibited the in vitro blast transformation of guinea pig splenic cells stimulated with a B cell mitogen (lipopolysaccharide of Escherichia coli) and a T cell mitogen (concanavalin A).

58. Terao et al., (1978) studied the effects of Fus-X on the Bursa of Fabricius[14] in day-old chicks. Repeated injections of Fus-X at 5 mg/kg into the residual yolk sac caused cellular injury that was primarily limited to the epithelial cells; located in the central portion of the follicle-associated epithelium. Necrosis then spread out to the peripheral epithelial cells. Degeneration and necrosis were then observed in the lymphoid cells in the lymphoid follicles.

Other effects

Gastrointestinal toxicity

59. Shimizu et al., (1979) reports that Fus-X disrupts glycolysis and induced intestinal malabsorption by causing hypoglycaemia and inhibiting mitosis of intestinal crypt cells in male ddY mice (n=36) when administered intraperitoneally with 60 µg of Fus-X in 0.4 mL saline.

60. Ip injection of Fus-X caused a decrease in the intake of food and water, and induced watery diarrhoea in rats (n=6) at 1mg Fus-X/kg. Two out of six rats showed mild diarrhoea 12 HPA, the remaining four 24 HPA. In 3/6 rats violent watery diarrhoea was observed 36-48 HPA. Two rats died. The small intestine was distended, and no blood was found in the lumen of the intestine (Matsuoka & Kubota, 1981). A dose-dependent effect was observed regarding the permeability of abdominal blood vessels, not found to be mediated by; serotonin, histamine, norepinephrine, prostaglandins, leukotrienes, or thromboxanes. Therefore, the adverse effect was not mediated by the cyclic nucleotide system (Matsuoka & Kubota, 1987).

61. Kumagai & Shimuzu (1988) studied the effects of Fus-X on intestinal absorption of monosaccharides in male Wistar rats. Fus-X treatment was injected into the jejunal lumen (30 or 60 µg Fus-X/mL) for the intestinal route, or via iv treatment (45 or 90 µg Fus-X/mL in 90 µL saline). Absorption of m-glucose was decreased by 1 hour, whilst L-glucose was decreased by 3 hours, post-administration into the jejunal lumen. Observations suggest that Fus-X causes specific damage in the active transport system for monosaccharides, which may be linked to an impaired function of the absorption of nutrients.

[14] Bursa of Fabricius; is the site of haematopoiesis and is a specialised organ that is necessary for B cell development (part of the immune system) in birds.

62. However, Awad et al., (2008) studied the effect of Fus-X on nutrient absorption in isolated jejunal epithelial cells of broiler chickens and observed no apparent effect at 10 µg Fus-X/mL (incubation time 30 minutes).

63. Food refusal following oral and ip exposures to Fus-X, persisted from 48 to 96 hours in mouse (n=8 per dose). The no observed adverse effect level was 0.025 mg/kg bw and the lowest observed adverse effect level was 0.25 mg/kg bw (Wu et al., 2012).

64. Fus-X was observed to elicit greater emetic potency than other type B trichothecenes (DON, 15-ADON, 3-DON, and NIV) in a comparative study in female mink (n=60) by Wu et al., (2013), following ip injection at 70 µg Fus-X/kg bw and oral administration at 30 µg Fus-X/kg bw.

65. Yang et al., (2017) assessed the individual or combined toxicological effects of multiple deoxynivalenol-family mycotoxins including; DON, 3-DON, 15-DON, Fus-X and NIV on human gastric epithelial cells (hGES). NIV was observed to be the most toxic out of the tested mycotoxins followed by Fus-X, for individual toxic effects at 0 to 3 ppm (cell viability decreased in a dose-dependent manner). For combined toxicological effects, Fus-X + NIV at 2 ppm each decreased cell viability by up to 30%, whilst Fus-X + 15-ADON (2 ppm and 6 ppm, respectively) exhibited the least toxic effect. The former mycotoxin combination resulted in a complete synergistic cytotoxicity and the latter almost entirely antagonistic cytotoxic effects in hGES.

Hypothermia

66. In the general pharmacological studies carried out Matsuoka et al., (1979), as described in paragraphs 20-22, Fus-X was found to induce hypothermia, although appreciable behavioural changes in mice were not observed.

Summary of toxic effects on humans

67. The major toxicity of Fus-X is mediated through the inhibition of protein synthesis, which disrupts the process of DNA synthesis. Furthermore, Fus-X has been shown to induce apoptosis in in vitro and in vivo animal studies. The target organs of Fus-X are those that contain actively proliferating cells e.g. thymus, spleen, small intestine, testes and the bone marrow.

HBGV

68. Due to the data insufficiencies for Fus-X, especially the limited number of oral studies, their limitations, and the lack of carcinogenicity data; RIVM were unable to establish a temporary TDI in their 2002 evaluation.

69. The EFSA Panel on Contaminants in the Food Chain (CONTAM) in 2017 considered the appropriateness of performing a hazard characterisation of Fus-X and

to evaluate whether it should be included in the group HBGV with NIV, however, further assessment on this has not yet been made public (EFSA CONTAM, 2017).

Considerations prior to exposure assessment

70. Poapolathep et al., (2004) investigated the transfer of Fus-X from pregnant to fetal mice and from lactating to suckling mice. 0.5 mg/kg bw of ^{3}H-Fus-X was orally administered to pregnant (n=8)/lactating (n=6) mice. NIV was detected as a large peak on results from high-performance liquid chromatography, while Fus-X was not identified as a clear peak.

71. Nakagawa et al., (2011) has reported the detection of a new Fusarium masked mycotoxin; Fus-X glucoside in infected wheat grain utilising high-resolution LC-Orbitrap™- mass spectroscopy. In general, masked mycotoxins are less potent relatives to the unmodified forms, however, it is assumed that masked mycotoxins can be reactivated during mammalian digestion by cleavage of the polar group and liberation of the native toxin (Berthiller et al., 2013).

72. Fus-X is typically found as a co-contaminant with deoxynivalenol and NIV in cereal based food commodities. Although Fus-X is detected at low levels as a contaminant, its toxicity in experimental animals has been shown to be more potent than that of other regulated trichothecenes in the B subtype family when comparing emetic potencies (Male et al., 2016) and intestinal inflammation (Alassane-Kpembi et al., 2017).

Exposure Assessment

73. Chronic Fus-X exposures were calculated from the DNSIYC and NDNS consumption data and are shown in Tables 1a-c. Levels in majority of food samples were below the Level of Quantification (LOQ; 10 µg Fus-X/kg). Food groups that had values between the Level of Detection (LOD; 5 µg Fus-X/kg) and LOQ for Fus-X were vegetable, oils, herbs, and spices.

74. The mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0 – 0.013 and 0.001 – 0.035 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0.001 – 0.02 - and 0.004 – 0.04 µg/kg bw/day. The calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0.001 – 0.02 and 0.004 – 0.06 µg/kg bw/day.

75. The food groups which contributed most highly to total exposure in infants and young children were “herbs and spices” and “fats and oils”.

Table 1a. Estimated Fus-X chronic exposures from the TDS in infants aged 4 to 12 months (µg/kg bw/day).

Age (months)	Mean	97.5th percentile
4 to <6 (n=116)	0.000-0.002	0.001-0.01
6 to <9 (n=606)	0.000-0.008	0.003-0.03
9 to <12 (n=686)	0.001-0.013	0.003-0.004

Table 1b. Estimated Fus-X chronic exposures from the TDS young children aged 12 to 18 months (µg/kg bw/day).

Age (months)	Mean	97.5th percentile
12 to <15 (n=670)	0.001-0.02	0.005-0.04
15 to 18 (n=605)	0.001-0.02	0.005-0.04

Table 1c. Estimated Fus-X chronic exposures from the TDS young children aged 18 to 60 months (µg/kg bw/day).

Age (months)	Mean	97.5th percentile
18 to 24 (n=118)	0.001-0.02	0.006-0.06
24 to 60 (n=688	0.001-0.002	0.004-0.04

Risk characterisation

76. There is currently no HBGV against which the dietary exposures can be compared, however, from toxicokinetic studies; Fus-X is extensively converted into NIV. NIV is also found to have the same MOA as Fus-X, therefore it is possible to compare exposures to the NIV TDI of 1.2 µg/kg bw by utilising the same $BMDL_{05}$ value of 0.35 mg/kg bw/day.

77. A margin of exposure (MOE) calculation can be carried out, as below:

$$\text{MOE} = \frac{(\text{BMDL05 (mg per kg bw per day) x 1000})}{\text{Exposure value (µg per kg bw per day)}}$$

78. The calculated MOE values for Fus-X are shown in Table 2a-c, for both mean and 97.5th percentile estimated chronic exposure values.

Table 2a. Calculated MOE's for estimated Fus-X chronic exposures from the TDS in infants aged 4 to 12 months (µg/kg bw/day).

Age (months)	**Mean**	**97.5th percentile**
4 to <6	0-180,000	350,000-35,000
6 to <9	0-44,000	120,000-12,000
9 to <12	0-44,000	120,000-88,000

Table 2b. Calculated MOE's for estimated Fus-X chronic exposures from the TDS young children aged 12 to 18 months (µg/kg bw/day).

Age (months)	**Mean**	**97.5th percentile**
12 to <15 (n=670)	350,000-180,000	88,000-8,700
15 to 18 (n=605)	350,000-18,000	70,000-8,800

Table 2c. Calculated MOE's for estimated Fus-X chronic exposures from the TDS young children aged 18 to 60 months (µg/kg bw/day).

Age (months)	**Mean**	**97.5th percentile**
18 to 24 (n=118)	350,000-18,000	58,000-5,800
24 to 60 (n=688)	350,000-180,000	88,000-8,800

Conclusion

79. Fus-X is a type B trichothecene, predominantly found in cereals such as wheat, barley, oats, rye, rice, sorghum, millet and maize.

80. A range of LD_{50} values have been reported for several species, as described in paragraph 19. Fus-X was found to be acutely toxic to rats with a LD_{50} value of 4.4 mg Fus-X/kg bw.

81. Chronic toxicity studies are limited, however, low tumour incidence in rats with long-term feeding of Fus-X at a daily dose of 105 mg (7 ppm in the diet) or 50 mg (3.5 ppm) in male Donryu rats for 1 to 2 years have been reported to have unusual tumours; 1 adenocarcinoma of the stomach, 2 papillary carcinomas of the urinary bladder, 1 adrenocortical adenoma, and 1 leukaemia.

82. Pregnant DDD mice were fed diets mixed with Fus-X at concentrations of approximately 25, 50 and 100 µg/animal/day throughout gestation or during early, mid or late pregnancy. Fus-X inhibited embryonal implantation and induced abortion, foetal absorption or foetal growth retardation when given throughout gestation or during the early stages of gestation. When Fus-X was given during mid-gestation the outcomes were abortion, foetal absorption, and growth retardation in surviving foetuses. Teratogenic effects were not observed.

83. No HBGV has been established by EFSA, JECFA or any EU Member State, however, MOE's for Fus-X were calculated by dividing the $BMDL_{05}$ of the NIV TDI by the estimated UK dietary exposures which showed no concern for adverse toxicological effects since all MOE values are above 1,000. Although, it should be recognised that there are uncertainties involved with extrapolating the $BMDL_{05}$ from NIV.

Questions on which the views of the Committee are sought

84. Members are invited to consider the following questions:

i). Does the current data presented allow the Members of the Committee to derive an appropriate value for a point of departure calculation?

ii). Do the Committee consider the comparison of Fus-X exposure to the NIV TDI, by utilising the same $BMDL_{05}$ value appropriate or should it be considered as a group TDI with NIV?

iii). Do the Committee want a separate statement for Fus-X or can it be included in the overarching statement?

Secretariat

May 2019

Abbreviations

15-ADON	15-acetyldeoxynalenol
3-ADON	3-acetyldeoxynalenol
ADME	Aborsption, Distribution, Metabolism and Excretion
$BMDL_{05}$	Benchmark dose response of 5%
CCL-2	C-C motif chemokine ligand 2
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
COX-2	Cyclooxygenase-2
DDD	Dopamine-deficient dopamine transporter
DNA	Deoxyribonucleic acid
DNSIYC	Diet and Nutrition Survey of Infants and Young Children
DON	Deoxynivalenol
ED_{50}	Effective dose 50
EFSA	European Food Safety Authority
EFSA CONTAM	EFSA Panel on Contaminants in the Food Chain
FSA	Food Standard Agency
Fus-X	Fusarenon-X
HBGV	Health based guidance value
hGES	Human gastric epithelial cell
HPA	Hours post-administration
IARC	International Agency for Research on Cancer
IC_{50}	Inhibiting concentration 50
ip	Intraperitoneal
IPCS	International Programme on Chemical Safety
iv	Intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC_{50}	Lethal concentration 50
LD_{50}	Lethal dose 50
LOD	Limit of detection
LOQ	Limit of quantification
MAPK	Mitogen-activated protein kinases
MOA	Mode of action
MOE	Margin of exposure
mRNA	Messenger ribonucleic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium
NDNS	National Diet and Nutrition Survey
NIV	Nivalenol
RIVM	The Netherlands National Institute for Public Health and the Environment
RNA	Ribonucleic acid
SACN	Scientific Advisory Committee on Nutrition
sc	Subcutaneous
TDI	Tolerable daily intake
TDS	Total diet study
TNF-α	Tumour necrosis factor α

References

Abbas, H. K., Mirocha, C. J. (1988) Production of Fusarenon-X, Nivalenol, and Zearalenone by Gibberella zeae isolates, and their toxicity in fibroblasts and rats. Mycotoxin Research 4, pp. 67-74.

Alassane- Kpembi, I., Kolf-Clauw, M., Gauthier, T., Abrami, R., Abiola, F. A., Oswald, I. P., Puel, O. (2013) New insights into mycotoxin mixtures: The toxicity of low doses of Type B trichothecenes on intestinal epithelial cells is synergistic. Toxicology and Applied Pharmacology 272, pp. 191-198.

Alassane-Kpembi, I., Gerez, J. R., Cossalter, A. M., Neves, M., Laffitte, J., Naylies, C., Lippi, Y., Kolf-Clauw, M., Bracarense, A. P. L., Pinton, P., Oswald, I. P. (2017) Intestinal toxicity of the type B trichothecene mycotoxin fusarenon-X: whole transcriptome profiling reveals new signalling pathways. Scientific Reports 8, pp. 7530.

Aoki, I., Toyama, K. (1983) Effects of various antibiotics and natural mycotoxins on hematopoietic stem cells of the bone marrow in adjuvant-treated rats. The Japanese Journal of Antibiotics 36, pp.1638-1644 (abstract only).

Aupanun, S., Poapolathep, S., Imsilp, K., Prapong, T., Poapolathep, A. (2015) Oral exposure of fusarenon-X induced apoptosis in Peyer's patches, thymus, and spleen of mice. Research in Veterinary Science 102, pp. 217-22.

Aupanun, S., Phuektes, P., Poapolathep, S., Sutjarit, S., Giorgi, M., Poapolathep, A. (2016) Apoptosis and gene expression in Jurkat human T cells and lymphoid tissues of Fusarenon-X-treated mice. Toxicon 123, pp. 15-24.

Aupanun, S., Poapolathep, S., Giorgi, M., Imsilp, K., Poapolathep, A. (2017) An overview of the toxicology and toxicokinetics of Fusarenon-X, a type B trichothecene mycotoxin. The Journal of Veterinary Medical Science 79, pp. 6-13.

Awad, W. A., Razzazi-Fazeli, E., Bohm, J., Zentek. (2008) Effects of B-tricothecenes on luminal glucose transport across the isolated jejunal epithelium of broiler chickens. Journal of Animal Physiology and Animal Nutrition 92, pp. 225-230.

Berek, L., Petri, I. B., Mesterházy, Á, Téren, J., Molnár, J. (2001) Effects of mycotoxins on human immune functions in vitro. Toxicology in Vitro 15, pp. 25-30.

Berthiller, F., Crews, C., Dall'Asta, C., Saeger, S. D., Haesaert, G., Karlovsky, P., Oswald, P. I., Seefelder, W., Speijers, G., Stroka, J. (2013) Masked mycotoxins: A review. Molecular Nutrition & Food Research 57, pp. 165-186.

Bony, S., Olivier-Loiseau, L., Carcelen, M., Devaux, A. (2007) Genotoxic potential associated with low levels of the Fusarium mycotoxins nivalenol and fusarenon X in a human intestinal cell line. Toxicology in Vitro 21, pp. 457-465.

Carter, C. J., Cannon, M. (1978) Inhibition of eukaryotic ribosomal function by the sesquiterpenoid antibiotic Fusarenon-X. European Journal of Biochemistry 84, pp. 103-111.

CPDB. (2007) The Carcinogenic Potency Database: Fusarenon-X (CAS 23255-69-3). URL: https://toxnet.nlm.nih.gov/cpdb/chempages/FUSARENON-X.html. Accessed: 25/02/2019.

Ciegler, A. (1978) Trichothecenes: Occurrence and Toxicoses. Journal of Food Protection 41, pp. 399-403.

Dellafiora, L., Galaverna, G., Dall'Asta, C. (2017) In silico analysis sheds light on the structural basis underlying the ribotoxicity of trichothecenes – A tool for supporting the hazard identification process. Toxicology Letters 270, pp. 80-87.

ECHA. (2018) CLP Fusarenon-X Infocard. URL: https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/notification-details/155143/1102116. Accessed: 06/02/2019.

EFSA. (2013) Scientific Opinion on risks for animal and public health related to the presence of nivalenol in food and feed. URL: https://www.efsa.europa.eu/en/efsajournal/pub/3262. Accessed: 08/02/2019.

EFSA CONTAM. (2017) Appropriateness to set a group health-based guidance value for nivalenol and its modified forms. URL: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.4751. Accessed: 28/03/2019.

Eriksen, G. S., Pettersson, H., Lundh, T. (2004) Comparative cytotoxicity of deoxynivalenol, nivalenol, their acetylated derivatives and de-epoxy metabolites. Food and Chemical Toxicology 42, pp. 619-624.

Forsell, J.H., Pestka, J.J. (1985) Relation of 8-ketotrichothecene and zearalenone analog structure to inhibitions of mitogen-induced human lymphocyte blastogenesis. Applied Environmental Microbiology 50, pp. 1304-1307.

IARC. (1993) Monographs Volume 56. URL: https://monographs.iarc.fr/wp-content/uploads/2018/06/mono56-15.pdf. Accessed: 05/02/2019.

IPCS. (1990) Environmental Health Criteria 105. Selected Mycotoxins: Ochratoxins, Trichothecenes, Ergot. URL: http://apps.who.int/iris/bitstream/handle/10665/39552/9241571055_eng.pdf?sequence=1&isAllowed=y. Accessed: 05/02/2019.

Ito, Y., Ohtsubo, K., Saito, M. (1980) Effects of fusarenon-X, a trichothecene produced by Fusarium nivale, on pregnant mice and their fetuses. The Japanese Journal of Experimental Medicine 50, pp. 167-172.

Ito, H., Watanabe, K., Koyama, J. (1982) The immunosuppressive effects of trichothecenes and cyclochlorotine on the antibody responses in guinea pigs. Journal of Pharmacobio-dynamics 5, pp. 403-409.

Kumagai, S., Shimuzu, T. (1988) Effects of Fusarenon-X and T-2 toxin on intestinal absorption of monosaccharide in rats. Archives of Toxicology 61, pp. 489-495.

Li, J., Shimizu, T., Miyoshi, N., Yasuda, N. (1997) Rapid apoptotic changes in the gastric glandular epithelium of rats administered intraperitoneally with fusarenon-X. The Journal of Veterinary Medical Science 59, pp. 17–22.

Li, J., Shimizu, T. (1997) Course of apoptotic changes in the rat gastric mucosa caused by oral administration of fusarenon-X. The Journal of Veterinary Medical Science 59, pp.191–199.

Male, D., Wu, W., Mitchell, N. J., Bursian, S., Petska, J., J., Wu, F. (2016) Modelling the emetic potencies of food-borne trichothecenes by benchmark dose methodology. Food and Chemical Toxicology 94, pp. 178-185.

Masuda, E., Takemoto, T., Tatsuno, T., Obara, T. (1982) Immunosuppressive effects of a trichothecene mycotoxin, Fusarenon-X in mice. Immunology 45, pp. 743-749.

Matsuoka, Y., Kubota, K., Ueno, Y. (1979) General pharmacological studies of fusarenon-X, a trichothecene mycotoxin from Fusarium spp. Toxicology and Applied Pharmacology 50, pp. 87-94.

Matsuoka, Y., Kubota, K. (1981) Studies on mechanisms of diarrhea induced by fusarenone-X, a trichothecene mycotoxin from Fusarium species. Toxicology and Applied Pharmacology 57, pp. 293-301.

Matsuoka, Y., Kubota, K. (1987) Characteristics of inflammation induced by Fusarenon-X, a trichothecene mycotoxin from Fusarium species. Toxicology and Applied Pharmacology 91, pp. 333-340.

Miura, K., Nakajima, Y., Yamanaka, K., Terao, K., Shibato, T., Ishino, S. (1998) Induction of apoptosis with fusarenon-X in mouse thymocytes. Toxicology 127, pp. 195-206.

Miura, K., Aminova, L., Murayama, Y. (2002) Fusarenon-X induced apoptosis in HL-60 cells depends on caspase activation and cytochrome c release. Toxicology 172, pp. 103-112.

Mizuno, S. (1975) Mechanism of inhibition of protein synthesis initiation by diacetoxyscirpenol and fusarenon-x in the reticulocyte lysate system. Biochimica et Biophysica Acta 383, pp. 207-214.

Moon, Y., Uzarski, R., Petska, J. J. (2003) Journal of Toxicology and Environmental Health, Part A 66, pp. 1967-1983.

Nakagawa, H., Ohmichi, K., Sakamoto, S., Sago, Y., Kushiro, M., Nagashima, H., Yoshida, M., Nakajima, T. (2011) Detection of a new Fusarium masked mycotoxin in wheat grain by high-resolution LC-Orbitrap™ MS. Food Additives & Contaminants: Part A 28, pp 1447-1456 (abstract only).

Ohtsubo, K., Saito, M. (1970) Cytotoxic effects of scirpene compounds, fusarenon-X produced by Fusarium nivale, dihyronivalenol and dihydyrofusarenon-x, on Hela cells. Japanese Journal of Medical Science and Biology 23, pp. 217-225.

Ohtsubo, K., Kaden, P., Mittermayer, C. (1972) Polyribosomal breakdown in mouse fibroblasts (L-cells) by Fusarenon-X, a toxic principle isolated from Fusarium nivale. Biochimica et Biophysica Acta 287, pp. 520–525.

Petska, J. J., Uzarski, R. L., Islam, Z. (2005) Induction of apoptosis and cytokine production in the Jurkat human T cells by deoxynivalenol: role of mitogen-activated protein kinases and comparison to other 8-ketotrichothecenes. Toxicology 206, pp. 207-219.

Poapolathep, A., Sugita-Konishi, Y., Doi, K., Kumagai, S. (2003) The fates of trichothecene mycotoxins, nivalenol and fusarenon-X, in mice. Toxicon 41, pp.1047-1054.

Poapolathep, A., Sugita-Konishi, Y., Phitsanu, T., Doi, K., Kumagai, S. (2004) Placental and milk transmission of trichothecene mycotoxins, nivalenol and fusarenon-X in mice. Toxicon 44, pp. 111-113.

Poapolathep, A., Poapolathep, S., Sugita-Konishi, Y., Imsilp, K., Tassanawat, T., Sinthusing, C., Itoh, Y., Kumagai, S. (2008) Fate of Fusarenon-X in broilers and ducks. Poultry Science 87, pp. 1510-1515.

Phruksawan, W., Poapolathep, S., Giorgi, M., Imsilp, K., Sakulthaew, C., Owen, H., Poapolathep, A. (2018) Toxicokinetic profile of fusarenon-X and its metabolite nivalenol in the goat (Capra hircus). Toxicon 153, pp. 78-84.

Pronk, M. E. J., Schothorst, R. C., van Egmond, H. P. (2002) RIVM Report 388802024/2002. Toxicology and occurrence of nivalenol, fusarenoN X, diacetoxyscirpenol, neosolaniol and 3- and 15-acetyldeoxynivalenol: a review of six trichothecenes. URL: https://www.rivm.nl/bibliotheek/rapporten/388802024.pdf. Accessed: 05/02/2019.

PubChem. (2018) Fusarenone X 2D Structure. URL: https://pubchem.ncbi.nlm.nih.gov/image/imgsrv.fcgi?cid=304599&t=l. Accessed: 25/02/2019.

Saengtienchai, T., Poapolathep, S., Isariyodom, S., Ikenaka, Y., Ishizuka, M., Poapolathep, A. (2014) Toxicokinetics and tissue depletion of Fusarenon-X and its metabolite nivalenol in piglets. Food and Chemical Toxicology 66, pp. 307-312.

Sakai, A., Suzuki, C., Masui, Kuramashi, A., Takatori, K., Tanaka, N. (2007) The activities of mycotoxins derived from Fusarium and related substances in a short-

term transformation assay using v-Ha-ras-transfected BALB/3T3 cells (Bhas 42 cells). Mutation Research 630, pp. 103-111.

Shimizu, T., Nakano, N., Matsui, T., Aibara, K. (1979) Hypoglycaemia in mice administered with fusarenon-X. The Japanese Journal of Medical Science and Biology 32, pp. 189–198.

Sutjarit, S., Nakayama, S. M. M., Ikenaka, Y., Ishizuka, M., Banlunara, W., Rerkamnuaychoke, Kumagai, S. (2014) Apoptosis and gene expression in the developing mouse brain of fusarenon-X-treated pregnant mice. Toxicology Letters 229, pp. 292-302.

Sutjarit, S., Poapolathep, A. (2016) Fusarenon-X induced apoptosis in the liver, kidney, and spleen of mice. Journal of Toxicologic Pathology 29, pp. 207-211.

Terao, K., Kera, K., Yazima, T. (1978) The effects of trichothecene toxins on the Bursa of Fabricius in day-old chicks. Virchows Archiv B Cell Pathology 27, pp. 359-370 (abstract only).

Ueno, Y., Ueno, I., Iitoi, Y., Tsunoda, H., Enomoto, M., & Ohtsubo, K. (1971) Toxicological approaches to the metabolites of Fusaria. Part 3. Acute toxicity of fusarenon-X. The Japanese Journal of Experimental Medicine 41, pp. 512-539.

Ueno, Y., Nakajima, M., Sakai, K., Ishii, K., Sato, N., Shimada, N. (1973) Comparative toxicology of trichothec mycotoxins: inhibition of protein synthesis in animal cells. Journal of Biochemistry 74, pp. 285–296.

Ueno, Y. (1984) Toxicological features of T-2 toxin related trichothecenes. Fundamental and Applied Toxicology 4, pp. S124-S132.

Wu, W., Flannery, B. M., Sugita-Konishi, Y., Watanabe, M., Zhang, H., Petska, J. J. (2012) Comparison of murine anorectic responses to the 8-ketotrichothecenes 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, fusarenon X and nivalenol. Food and Chemical Toxicology 50, pp. 2056-2061.

Wu, W., Bates, M. A., Bursian, S. J., Link, J. E., Flannery, B. M., Sugita-Konishi, Y., Watanabe, M., Zhang, H., Petska, J. J. (2013) Comparison of Emetic Potencies of the 8-Ketotrichothecenes Deoxynivalenol, 15-Acetyldeoxynivalenol, 3-Acetyldeoxynivalenol, Fusarenon X, and Nivalenol. Toxicological Sciences 131, pp. 279-291.

Wu, W., He, K., Zhou, H., Berthiller, F., Adam, G., Sugita-Konishi, Y., Watanabe, M., Krantis, A., Durst, T., Zhang, H., Petska, J. J. (2014) Effects of oral exposure to naturally-occuring and synthethic deoxynivalenol cogners on proinflammatory cytokine and chemokine mRNA expression in the mouse. Toxicology and Applied Pharmacology 278, pp. 107-115.

Yang, Y., Yu, S., Tan, Y., Liu, N., Wu. A. (2017) Individual and combined cytotoxic effects of co-occuring deoxynivalenol family mycotoxins on human gastric epithelial cells. Toxins 9, pp. 96-105.