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DEOXYNIVALENOL
(addendum)
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DEOXYNIVALENOL (addendum)

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1. EXPLANATION

Deoxynivalenol (12,13-epoxy-3,4,15-trihydroxy-trichothec-9-en-8-one; DON, also known as vomitoxin; Chemical Abstracts Service [CAS] No. 51481-10-8) is a type B trichothecene mycotoxin produced mainly in cereals by various *Fusarium* species. In addition to DON, 3-acetyl-deoxynivalenol (3-Ac-DON; CAS No. 50722-38-8) and 15-acetyl-deoxynivalenol (15-Ac-DON; CAS No. 88337-96-6) are also naturally occurring fungal secondary metabolites, whereas DON-3β-glucopyranoside (DON-3-glucoside) is a naturally occurring conjugate of DON formed in plants.

DON was previously evaluated by the fifty-sixth meeting of the Committee (Annex 1, reference *152*). The Committee established a provisional maximum tolerable daily intake (PMTDI) of 1 µg/kg body weight (bw) on the basis of the no-observed-effect level (NOEL)[1] of 100 µg/kg bw per day for decreased body weight gain reported in a 2-year feeding study in mice and application of a safety factor of 100. The Committee concluded that intake at this level would not result in effects of DON on the immune system, growth or reproduction. The Committee noted that the available data did not suggest that DON presents a carcinogenic hazard.

DON was on the agenda of the present meeting at the request of the Second Session of the Codex Committee on Contaminants in Food (FAO/WHO, 2008), which asked the Committee to assess exposure on a more global basis, taking new data into account; to review the toxicological data and consider the need for an acute reference dose (ARfD), taking into account data in finished products, but also in raw wheat and other commodities as they are traded internationally, and consideration of processing factors; and to assess the toxicity of 3-Ac-DON and 15-Ac-DON.

The Committee reviewed several new studies on metabolism and toxicokinetics, acute toxicity, genotoxicity, mechanisms of toxicity and developmental toxicity of DON and/or its acetyl derivatives. The Committee also took note of the data previously evaluated at the fifty-sixth meeting. Emphasis was given to studies in which pure DON or acetylated DON was added to defined diets in mammalian species, because naturally contaminated feed commonly contains multiple mycotoxin contaminants. New information on occurrence, processing, prevention and control, and dietary exposure was also considered.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

(a) Mice

The effects of oral (gavage) and intranasal exposure to DON (5 mg/kg bw, purity unknown) on tissue distribution and proinflammatory cytokine induction in

[1] At its sixty-eighth meeting (Annex 1, reference *187*), the Committee decided to differentiate between no-observed-adverse-effect level (NOAEL) and no-observed-effect level (NOEL). This NOEL would now be considered a NOAEL.

adult female B6C3F1 mice (five or more per group) were investigated. Competitive direct enzyme-linked immunosorbent assay (ELISA) revealed that, regardless of exposure route, DON concentrations in plasma, spleen, liver, lung and kidney were maximal within 15–30 min and declined by 75–90% after 120 min. After oral exposure, peak concentrations were approximately 1 µg/ml in plasma, 0.77 µg/g in spleen, 1.1 µg/g in liver, 0.9 µg/g in lung and 1.8 µg/g in kidney. Plasma and tissue DON concentrations were 1.5–3 times higher following intranasal exposure compared with oral exposure. The inductions of interleukin-1 beta (IL-1β), IL-6 and tumour necrosis factor-alpha (TNF-α) messenger ribonucleic acids (mRNAs) were measured in spleen, liver and lung of nasally exposed mice, and these were 2–10, 2–5 and 2–4 times greater, respectively, than the mRNA inductions in the tissues of orally exposed mice (Amuzie, Harkema & Pestka, 2008).

Female B6C3F1 mice (4 weeks old) were fed on a diet containing purified DON (purity unknown) at 20 mg/kg for 2–8 weeks. The capacity of mice to accumulate DON in plasma was measured at weeks 2, 4 and 8 of the feeding period. The body weight of animals fed on a control diet increased from 18 to 26 g over the 8-week period, whereas in DON-treated animals, weight increased from 18 g to only 20 g over the same period. DON was detectable in plasma (48 ng/ml) within 2 weeks of initiating the treatment. The mice maintained a near-steady-state concentration of DON in plasma at weeks 4 (63 ng/ml) and 8 (44 ng/ml). Mice fed control diet (without DON) contained no detectable DON in their plasma. These findings indicate that impaired growth in the mice exposed subchronically to DON was associated with detectable levels of the toxin in circulation (Amuzie & Pestka, 2010).

(b) Rats

The metabolism of [^{14}C]DON (5 mg/kg bw, radiochemical purity 93%) was investigated in male Sprague-Dawley rats. The animals (n = 15) received the radiolabelled compound, dissolved in water containing 15% ethanol, by gavage, and the distribution of DON in body fluids was investigated over 72 h. DON and its metabolites were detectable in the plasma of rats, with the highest levels at 8 h, at which time approximately 9% was bound to plasma protein. After 72 h, a total of 37% of the radiolabel was excreted in the urine, and DON-glucuronide was implicated as the major urinary metabolite based on reversed-phase high-performance liquid chromatographic (HPLC) analysis (Meky et al., 2003).

(c) Pigs

Castrated male pigs (n = 11, body weight 88.1 ± 3.9 kg) received a *Fusarium*-contaminated diet (restricted to 2.2 kg/day) containing DON at 4.2 mg/kg diet over a period of 7 days. The pigs were slaughtered at 1, 2, 3, 4, 5, 6, 8, 15, 18 and 24 h after feeding on day 7, with the exception of one pig, which was slaughtered unfed. DON and de-epoxy-DON were analysed in serum and digesta from consecutive segments of the digestive tract (stomach, small intestine divided into three parts of a similar length, caecum, colon, rectum). DON was rapidly and nearly completely absorbed while passing through the stomach and the proximal small intestine. The maximum serum concentration appeared 4.1 h after the DON-containing meal had been ingested, and half of the systemically absorbed

DON was eliminated after 5.8 h. De-epoxy-DON appeared in increasing proportions in the distal small intestine and reached approximately 80% of the sum of DON plus de-epoxy-DON in faeces collected from the rectum. The study authors concluded that de-epoxidation of DON, which occurs primarily in the hindgut, probably does not contribute much to detoxification in the pig (Dänicke et al., 2004).

The toxicokinetics of DON was investigated in castrated male pigs (5–6 per group, body weight 41.5 ± 2.0 kg). Pigs were fed naturally contaminated wheat containing DON at 5.7 mg/kg for at least 4 weeks (subchronic) or on a single day (acute). In addition, a group of pigs received an intravenous injection of DON ("pure", but percentage unknown) at a dose of 53 µg/kg bw. After intravenous DON application, serum DON concentrations decreased biphasically, with terminal elimination half-lives of between 4.2 and 33.6 h. DON was rapidly absorbed following oral exposure and reached maximal plasma concentrations of 21.79 and 15.21 ng/ml serum after 88.4 and 99.1 min in the subchronically and acutely fed groups, respectively. Thereafter, serum DON levels declined slowly, with elimination half-lives of 6.28 h and 5.32 h for the subchronic and acute groups, respectively. The mean bioavailability of DON was 89% for the subchronic group and 54% for the acute group. DON was highly distributed in all groups, with an apparent volume of distribution higher than the total body water volume. Glucuronide conjugation of DON was found in serum samples after oral exposure, but not after intravenous application. Dietary DON caused a significant increase in DON concentrations in urine and faeces, whereas the metabolite de-epoxy-DON was found only in the trials with 4 or more weeks of treatment. The total recovery was about 66.6% and 54.0% for the control and the subchronic DON groups, respectively, with urine being the main excretory route. Twenty-four hours following oral dosing, DON could not be detected in the serum, except in one subchronically fed pig, in which it was detected at the limit of detection (LOD). The study indicates that in pigs orally administered DON, more than 50% of the DON is quickly absorbed, highly distributed and only poorly metabolized (Goyarts & Danicke, 2006).

A dynamic laboratory model simulating the gastrointestinal tract of healthy pigs (TNO-Intestinal Model of the stomach and small intestine) was used to evaluate the small-intestinal absorption of DON and nivalenol (NIV), another type B trichothecene, and the efficacy of activated carbon in reducing the relevant absorption. The in vitro intestinal absorptions of DON and NIV were 51% and 21%, respectively, following the ingestion of 170 µg DON and 230 µg NIV, respectively, through contaminated (spiked) wheat. Most absorption occurred in the jejunal compartment for both mycotoxins. The inclusion of activated carbon produced a significant reduction in the intestinal mycotoxin absorption. At a 2% inclusion level, the absorption with respect to the intake was lowered from 51% to 28% for DON and from 21% to 12% for NIV. The Committee noted that this mechanistic study was not relevant for the evaluation (Avantaggiato, Havenaar & Visconti, 2004).

2.1.2 Biotransformation

Five castrated male pigs (body weight 29 kg), in which the gastrointestinal microflora lacked the ability to transform 3-Ac-DON and NIV to their corresponding de-epoxidated metabolites, were equipped with post-valve T-caecum cannulas for

collection of ileal digesta and were fed on control diet for 2 weeks, followed by a diet naturally contaminated with DON at 0.8 mg/kg for 3 weeks and subsequently a diet naturally contaminated with DON at 1.2 mg/kg for 4 weeks. The gastrointestinal microorganisms did not acquire the de-epoxidation ability during the 7-week-long exposure period. At the end of the exposure period, faeces from pigs with a known de-epoxidation ability were spread out in the pens and left for 24 h. One week after the faeces had been spread out in the pens, the de-epoxidation ability was found in faecal incubations from four out of five experimental pigs. This change in the intestinal de-epoxidation ability was not accompanied by any detectable changes in the deoxyribonucleic acid (DNA) profiles of the bacterial community. The results show that the intestinal de-epoxidation ability is common at pig farms in the Uppsala area in Sweden and that the ability may be transferred between pigs in a stock (Eriksen et al., 2002).

2.1.3 Absorption, distribution and excretion of 3-Ac-DON

The absorption, metabolism and excretion of 3-Ac-DON (purity >95%) in pigs were studied. Pigs with a faecal microflora known to be able to de-epoxidate trichothecenes were used in the experiment. The pigs were fed a commercial diet with 3-Ac-DON added to provide a concentration of 2.5 mg/kg feed for 2.5 days. No traces of 3-Ac-DON or its de-epoxide metabolite were found in plasma, urine or faeces. DON was detected in plasma as soon as 20 min after the start of feeding. The maximum concentration of DON in plasma was reached after 3 h and decreased rapidly thereafter. Only low concentrations close to the LOD were found in plasma 8 h after the start of feeding. A significant part of the DON in plasma was in a glucuronide-conjugated form (42% ± 7%). No accumulation of DON occurred in plasma during the 60 h of exposure. The excretion of DON was mainly in urine (45% ± 26% of the toxin ingested by the pigs), and only low amounts of metabolites of 3-Ac-DON (2% ± 0.4%) were recovered in faeces. De-epoxy-DON constituted 52% ± 15% of the total amount of 3-Ac-DON metabolites detected in faeces. The remaining part in faeces was DON. DON was still present in the urine and faeces at the end of the sampling period 48 h after the last exposure. The results show that no de-epoxides are found in plasma or urine in pigs after trichothecene exposure, even in pigs having a faecal microflora with a de-epoxidation activity. The acetylated form of the toxin is deacetylated in vivo. Furthermore, the experiment shows that the main part of DON is rapidly excreted and does not accumulate in plasma, but a minor part of the toxin is retained and slowly excreted from the pigs. It has to be noted that about half of the administered dose was not accounted for. This study indicates that there is substantial conversion of 3-Ac-DON to DON in vivo in pigs (Eriksen, Pettersson & Lindberg, 2003).

2.2 Toxicological studies

Since the last evaluation, a large number of toxicity studies of DON have been published. Many of those were excluded from this addendum, based on the following criteria:

- studies using naturally contaminated feed, which, although relevant for dealing with DON in practice, are not useful for derivation of a no-observed-adverse-effect level (NOAEL) for pure DON;
- studies dosing DON in combination with other mycotoxins, as no NOAEL for pure DON can be derived;
- in vitro studies aimed at elucidating mechanistic effects of DON toxicity, as no in vivo NOAEL for DON can be derived;
- studies using chickens, ducks or turkeys as test species, as these species are not considered representative for toxicity in humans.

2.2.1 Acute toxicity

Results of acute studies on lethality (median lethal dose [LD_{50}]) and emesis in animals treated with DON were presented in the previous monograph (Annex 1, reference *153*). Since then, a number of acute studies have been performed, mostly on immunological parameters; these are summarized in section 2.2.6. One acute study on 3-Ac-DON has been summarized in the section on metabolites (section 2.2.7). Those studies on the emetic effects of DON in the diet of pigs from the 2001 monograph that had clear dosing regimes are described in more detail below for purposes of derivation of the ARfD.

(a) Pigs

Groups of four young pigs (average weight 8 kg) received feed containing contaminated corn at a concentration of 0% or 36% for 10 days, 0%, 6%, 12%, 18% or 24% for 4 days, 0%, 1.5%, 3%, 4.5% or 6% for 11 days or 0%, 0.3%, 0.6%, 0.9%, 1.2%, 1.5%, 1.8% or 2.1% for 21 days in four trials. The DON and the *Fusarium* mycotoxin zearalenone (ZEA) contents of the diets were analysed. A pilot study was perfomed, but the starting weights of the pigs were not given, and feed refusal occurred. Trial 2 was terminated after 4 days because the pigs that received the diets containing mouldy corn were consuming very little feed. In the third trial, pigs were vomiting at day 1 from mouldy corn concentrations of 3% (determined analytically to contain DON at a concentration of 19.7 mg/kg diet). In the 3%, 4.5% and 6% groups, there were indications that at least one pig vomited. Feed intake was reduced to 45% of that of controls in the 1.5% group and to 12% and less in higher dose groups. There were no pigs vomiting in trial 4, but the inclusion of mouldy corn in the diet resulted in a linear reduction in rate of body weight gain and a linear and quadratic reduction in feed consumption and body weight gain per kilogram of feed. The DON content of the diet used in trial 4 was not given. For this evaluation, it was assumed that the ZEA content did not contribute to the emetic effect. The lowest dose at which no emesis was seen was 9 mg/kg diet. The authors reported that this was equal to a dose of 0.15 mg/kg bw per day, but data on food intake from the first day were not given (Young et al., 1983).

Pollman et al. (1985) exposed groups of eight starter pigs (average body weight 7.7 kg) in a first trial to DON through contaminated wheat in the diet at DON concentrations of 0, 0.9, 2.0 and 2.8 mg/kg diet (analysed values) for 3 weeks. No emesis was seen in any dose group, but feed intake was reduced at 2.0 mg/kg diet, equal to 0.17 mg/kg bw per day (using starting weights). In a second trial, groups

of four pigs (average body weight 8.3 kg) were exposed to DON at 1.3, 1.4, 2.3 or 2.7 mg/kg feed through contaminated wheat in the diet (analysed concentrations, no control group) for 2 weeks. No emesis was seen; feed intake was reduced at 1.4 mg/kg diet, but not at higher doses. This dose is equal to 0.1 mg/kg bw per day based on measured feed intake. A third trial was done with grower-finishing pigs of average body weight 60.8 kg, which were exposed to 0, 0.9, 2.2, 2.8 or 4.2 mg/kg diet (analysed concentrations) for 6 weeks. Evidence of emesis was seen only once in the 2.2 mg/kg diet group, but not at higher doses. Reduced feed intake was seen at 2.2 mg/kg feed. The two highest dose groups were taken off the feed after 2 weeks because of very poor performance. The lowest dose that did not induce emesis in this study was 2.8 mg/kg feed, the highest dose tested, equal to 0.24 mg/kg bw per day based on starting weight and measured feed intake.

Groups of four nursery pigs of mixed breed (Polish White Large × Polish White Ear-pendent) with an average body weight of 35 kg were given a single dose of DON at 0, 0.2 or 0.4 mg/kg bw in the feed. The animals were euthanized on day 5, and, based on macroscopic examination, segments of duodenum, jejunum, ileum, liver and mesenteric lymph nodes were sampled and assigned for histopathological examination. Histopathological examination indicated that the regressive lesions were expressed more in the experimental group treated with the higher concentration of DON (Zielonka et al., 2009).

2.2.2 Short-term studies of toxicity

(a) Rats

The effects of DON (purity not reported) on blood biochemical parameters in growing Wistar rats were studied. Male rats (10 per group) were treated subcutaneously with DON at 1 mg/kg bw per day for 3 days. After 3 days, significant increases in blood insulin, glucose and free fatty acids were observed in the DON-treated animals in comparison with the control group. DON treatment caused an increment in glycogen depots and a reduction in the triglyceride content of the muscle (Szkudelska, Szkudelski & Nogowski, 2002).

(b) Pigs

Groups of 6–10 pigs (sex unknown, body weights 15–20 kg at the start of the study) were fed on a diet containing DON at 0 or 2.85 mg/kg for 5 weeks (equivalent to 0 or 0.11 mg/kg bw per day). In intestinal tissues of pigs treated with DON, an increased intestinal barrier permeability and a reduction in the expression of claudins (a component of tight junctions) were observed. In vitro studies demonstrated that in intestinal epithelial cell lines from porcine (IPEC-1) or human (Caco-2) origin, DON decreased trans-epithelial electrical resistance and increased, in a time- and dose-dependent manner, the paracellular permeability to 4-kilodalton dextran and to pathogenic *Escherichia coli* across intestinal cell monolayers. The data suggested that porcine epithelial cells were more susceptible than human cells to the effects of DON. As only one dose was tested in vivo, a NOAEL could not be established, but would be below 0.11 mg/kg bw per day (Pinton et al., 2009).

In a study in which pigs (five of each sex per dose, 5 weeks old) were fed corn–soya bean diets containing 0, 0.5 or 1.5 mg DON (purity and source unknown) per kilogram (equivalent to 0, 0.02 and 0.06 mg/kg bw per day; conversion from Bohm & Razzazi, 2003) for 15 days, sera samples were collected at day 35 of treatment for biochemical analysis. DON treatment at 0.5 and 1.5 mg/kg diet increased serum urea by 43% and 51%, respectively. Gamma glutamyl transferase (GGT) activity was increased about 2.5-fold at 1.5 mg/kg diet. DON treatment did not affect serum protein levels or aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. As the source and purity of DON could not be determined, the Committee did not consider this study suitable for the evaluation (Dinischiotu et al., 2007).

Female weaned piglets (nine per group; mean body weight 9.8 kg at the start of the study) were given 0, 0.3, 0.6 or 1.2 mg/kg of isolated, pure DON in the diet (equivalent to 0, 0.012, 0.024 and 0.48 mg/kg bw per day) for 8 weeks. Pigs were fed restrictively to allow a complete feed intake by all animals. Body weight, feed intake, parameters of liver integrity, haematological data and blood concentrations of some selected components of energy and protein metabolism were examined weekly. Body weight gain, feed intake and feed conversion rate were not affected by DON treatment. No toxicologically significant effects of DON treatment on plasma levels of AST, ALT, GGT, glutamate dehydrogenase, sorbitol dehydrogenase, haemoglobin, urea, albumin or glucose were observed (Drochner et al., 2006).

The effects of DON on weaned piglets (average body weight 8 kg) were investigated. Two feeding trials were conducted with wheat naturally contaminated with DON. In the first trial, as a preliminary study, weaned piglets (9–13 per age group) in age groups of 3, 4 or 5 weeks were fed for 1 week with DON at 7.7 mg/kg diet (reported to be equal to 0.35 mg/kg bw per day). The intake of DON-contaminated diets was not associated with any obvious negative health effects. In the main feeding trial, in which piglets (13–15 per group) were treated with control feed or feed containing DON at 3 mg/kg diet, no vomiting or other negative clinical symptoms were observed. At the end of the 8-week treatment period, body weights in the control and treatment groups were 49.8 kg and 45.7 kg, respectively. The weekly feed intake was decreased by 4–19% in the DON-fed group compared with the control group, but the feed conversion rates were slightly improved in the DON-fed group (Bohm & Razzazi, 2003).

2.2.3 Long-term studies of toxicity and carcinogenicity

No new long-term studies of toxicity or carcinogenicity were identified.

2.2.4 Genotoxicity

For the present addendum, no new studies into the genotoxic potential of DON conducted according to Organisation for Economic Co-operation and Development (OECD) guidelines were available. In a review by Ma & Guo (2008), it is reported that DON was positive in an unscheduled DNA synthesis (UDS) test and a replicative DNA synthesis (RDS) test in rat primary hepatocytes and in a number of comet assays. However, these studies were not available for the present

evaluation, as indicated in Table 1. In a study by Sakai et al. (2007), in which the effects of various mycotoxins on initiation and promotion of v-Ha-ras-transfected BALB/3T3 cell transformation was studied, DON (and NIV) was negative (see Table 1). In an in vivo study in which chickens were treated for 17 days with DON at 10 mg/kg bw per day, DON induced a slight, but statistically significant, increase in DNA damage in spleen leukocytes, as measured by the comet assay (Frankic et al., 2006).

Table 1. Results of assays for the genotoxicity of DON

End-point	Test object	Concentration	Results	Reference[a]
In vitro				
UDS	Male SD rat primary hepatocytes	0, 0.003, 0.03, 0.3 µg/ml	Positive	Guo & Xu (1997)*
RDS	Male SD rat primary hepatocytes	0, 0.01, 0.1, 1 µg/ml (incubation time 3 h)	Positive	Li & Guo (2000)*
Comet assay (DNA breaks)	Male SD rat primary hepatocytes	0, 0.01, 0.1, 1 µg/ml (incubation time 2 h)	Positive	Li & Guo (2001)*
Comet assay (DNA breaks)	Vero cells	0, 1, 5, 10 µmol (incubation time 4 and 16 h)	Positive	Li & Sun (2004)*
Comet assay (DNA breaks)	Vero cells	0, 10 µmol (incubation time 4 and 16 h, reincubate for 15, 30, 60 and 120 min) 0, 1, 5, 10 µmol (incubation time 4 and 16 h)	Positive	Li & Sun (2004)*
Comet assay (DNA breaks)	Human Caco-2 cells	0.01–0.5 µmol/l (incubation time 24 and 72 h)	Positive	Bony et al. (2006)*
Cell transformation (tumour initiation and promotion)	BALB/c3T3 cells	0.01–0.2 µg/ml	Negative	Sakai et al. (2007)
In vivo				
Comet assay (DNA breaks)	Chicken spleen leukocytes	10 mg/kg bw per day by gavage for 17 weeks in diet	Positive	Frankic et al. (2006)

[a] An asterisk (*) indicates that the original study was not available; the data were reported in the review by Ma & Guo (2008).

2.2.5 Reproductive and developmental toxicity

(a) Effects on reproductive organs

(i) Rats

Male Sprague-Dawley rats were treated with DON (0, 0.5, 1, 2.5 or 5 mg/kg bw) daily via gastric intubation for 28 days. Epididymal (right and left) and seminal vesicle weights (expressed per gram of body weight and brain weight) were significantly reduced in animals treated with 2.5 and 5 mg/kg bw. Decreased prostate weight (expressed per gram of body weight and brain weight), spermatid numbers, cauda epididymal sperm numbers and cauda epididymal sperm numbers per gram cauda epididymis were observed in the 5 mg/kg bw dose group. Increased sperm tail abnormalities (broken tails) were also observed in the 5 mg/kg bw dose group, whereas sperm swimming speed was increased only in the 2.5 mg/kg bw dose group. Serum concentrations of follicle-stimulating hormone and luteinizing hormone were increased, whereas the testosterone concentration was decreased in a dose-dependent manner. Increases in germ cell degeneration, sperm retention and abnormal nuclear morphology were observed at doses above 2.5 mg/kg bw. A NOAEL of 1 mg/kg bw per day was derived based on reduced epididymal (right and left) and seminal vesicle weights in the next higher dose group (Sprando et al., 2005).

(b) Developmental toxicity

(i) Mice

In a review aimed at determining the relative developmental toxicity potential of DON and benomyl, both present on wheat, Hicks et al. (2000) identified a dietary NOAEL for DON for decreased body weight in mouse pups of 0.375 mg/kg bw per day from Khera et al. (1984). This study was described in the 2001 monograph (Annex 1, reference *153*). The authors also claimed that the toxic actions of DON in pregnant animals are consistent from species to species. The decrease in body weight in dams and pups at lower doses and complete resorptions at higher doses were stated to be consistent with the primary mechanism of action, which was inhibition of protein synthesis.

DON was administered to 3-month-old nulliparous female NMRI mice by intraperitoneal injection (3.3, 4.2, 5 or 10 mg/kg bw [11, 14, 17 or 34 µmol/kg bw] on gestation days 7 and 9 or 1.6, 2.5 or 3.3 mg/kg bw [5.4, 8.4 or 10 µmol/kg bw] daily on gestation days 7–10), and the mice were sacrificed on day 18 of gestation. The total numbers of implants, resorptions and dead and live fetuses were recorded. Resorption was considered as early if fetal structures were resorbed and late if some recognizable fetal tissue remained. Live fetuses were examined for external malformation, weighed and then sacrificed and prepared for histological examination of the skeleton. High maternal deaths were seen at the two highest doses in each set of doses. In embryos, the number of resorptions was dose-dependently increased in treated animals compared with controls. Skeletal abnormalities (mostly in the axial skeleton) were observed. Exencephaly was seen mainly at 2.5 or 3.3 mg/kg bw during the 4-day treatment. Neural arch defects and

fusion occurred more in the 2-day treatment than in the 4-day treatment. In both experiments, vertebral bodies showed various deformities (destruction or division), as well as hemivertebrae (except with 2.5 mg/kg bw given for 4 days) and fused, branched and/or cervical ribs. In the 2-day experiment, the effects were dose dependent, and in the 4-day experiment, the incidences were lower (Debouck et al., 2001).

(ii) Rats

Groups of 24 pregnant female Charles River Sprague-Dawley rats were gavaged once daily with purified DON at a dose of 0, 0.5, 1, 2.5 or 5 mg/kg bw per day on gestation days 6–19. At caesarean section on gestation day 20, reproductive and developmental parameters were measured. All females survived to caesarean section. DON caused a dose-related increase in excessive salivation by the pregnant females in all dose groups, statistically significant at 2.5 mg/kg bw per day, a reaction probably linked to the lack of emetic reflex in rats. At 5 mg/kg bw per day, feed consumption and mean body weight gain were significantly decreased throughout gestation, mean weight gain (carcass weight) and gravid uterine weight were significantly reduced, 52% of litters (12/23) were totally resorbed, the average number of early and late deaths per litter was significantly increased, average fetal body weight and crown–rump length were significantly decreased, the incidence of runts was significantly increased and the ossification of fetal sternebrae, centra, dorsal arches, vertebrae, metatarsals and metacarpals was significantly decreased. At 2.5 mg/kg bw per day, DON significantly decreased average fetal body weight, crown–rump length and vertebral ossification. These effects may be secondary to maternal toxicity and the reduced size of the fetuses. The incidence of misaligned and fused sternebrae was significantly increased at 5 mg/kg bw per day. No adverse developmental effects were observed at 0.5 and 1 mg/kg bw per day. Dose-related increases in maternal liver weight to body weight ratios were observed in all treated groups (significant at 1, 2.5 and 5 mg/kg bw per day). The weight changes were correlated with dose-related cytoplasmic alterations of hepatocytes. The NOAEL for maternal toxicity in this study is 0.5 mg/kg bw per day based on the dose-related increase in liver to body weight ratio at 1 mg/kg bw per day. The NOAEL for fetal toxicity is 1 mg/kg bw per day based on the general reduction in fetal development at 2.5 and 5 mg/kg bw per day. The NOAEL for teratogenicity is 2.5 mg/kg bw per day based on the increase in misaligned and fused sternebrae at 5 mg/kg bw per day (Collins et al., 2006).

(iii) In vitro

In porcine cumulus oocyte complexes, DON (0.94, 1.88, 3.75 or 7.5 µmol/l [0.28, 0.557, 1.11 or 2.2 µg/ml]) dose-dependently decreased maturation (telophase 1 and metaphase 2) rates and increased degeneration rates after 48 h culture in vitro (Alm et al., 2002).

2.2.6 Special studies on immunotoxicity

Details on the special studies on the immunotoxicity of DON are summarized in Table 2. The individual studies are described more fully below.

Table 2. Details and end-points of studies on immunotoxicity

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw)[a]	Route	Effect	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)	Reference
Female BALB/c mice, 5 weeks old	DON (Sigma)	Single dose, reovirus after 2 h, effect assessment after 10 days	6		0, 2, 5, 10 or 25	Gavage	Decreased viral clearance	2	<2	Li et al. (2007)
Male and female BALB/c mice, 6 weeks old	DON (>98%)	14 or 28 days	10 + 10	0, 0.25, 0.5, 1 or 2	Eq 0, 0.038, 0.075, 0.15 or 0.3	Diet	Decreased CD19+ (B cells in peripheral blood lymphocytes)	—	No NOAEL derived	Wu et al. (2009)
Male BALB/c mice	DON (unknown)	14 days	12	0 or 2	Eq 0 or 0.3	Diet	Splenocyte proliferation suppression	0.3	<0.3	Landgren, Hendrich & Kohut (2006)
Male C57BL6 mice, 6 weeks old	DON (pure, but % unknown)	4 weeks, 3 days/week	10		0, 0.014, 0.071, 0.355 or 1.774	Gavage	Increased plasma IgA levels	—	No NOAEL derived	Gouze et al. (2006)
							Increased liver PROD activity	—	No NOAEL derived	

Table 2 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw)[a]	Route	Effect	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)	Reference
Male wild-type (WT; P53N5-W) and p53+/− (TG; P53N5-T) mice, 5–7 weeks old	DON (95%)	26 weeks	20 (10 + 10)	0, 1, 5 or 10	Eq 0, 0.15, 0.75 or 1.5	Diet	Increased plasma IgA, decreased IgM in WT	1.5	0.75	Bondy et al. (2009)
							Increased kidney weight in WT	0.75	0.15	
							Increased kidney weight in TG	1.5	0.75	
							Increased IgA positive glomeruli in kidney	1.5	0.75	
Female B6, 129P2-Ptgs2tm1Smi (002181-W) mice, wild type compared with COX-2 knockout mice	DON (reagent grade or better, purity checked by HPLC)	12 weeks	3–6	0 or 10	Eq 0 or 1.5	Diet	Increased serum IgA	1.5	<1.5	Jia & Pestka (2005)

Table 2 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw)[a]	Route	Effect	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)	Reference
Hybrid commercial castrated male pigs, 8 weeks old	DON (D0156, Sigma)	6 weeks	14		0 or 0.5 (1 week) and 1 (5 weeks)	Oral	No effects	—	1	Ferrari et al. (2009)
Female weaned piglets (mean body weight 9.8 kg)	DON (pure)	8 weeks	9	0, 0.3, 0.6 or 1.2	Eq 0, 0.012, 0.024 or 0.048	Diet	Increased IgA levels in serum	—	0.048	Drochner et al. (2004)
Weanling pigs, 4 weeks old, sex unknown	DON, naturally contaminated, no myocotoxins other than DON detectable	9 weeks in different age periods	6	0, 2.2 or 2.5	0, 0.088 or 0.1	Diet	Increased serum IgA, reduction mesenteric lymph node IFN-α and TGF-α mRNA	0.088	<0.088	Pinton et al. (2008)

Table 2 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw)[a]	Route	Effect	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)	Reference
Male B6C3F1, B6 129-IL6tmi Kopf, L6 knockout and B6129F2l wild-type control mice	DON purified and checked	12 weeks	3–6	0 or 10	Eq 0 or 1.5	Diet	Increased serum IL-6 and IgA and increased mesangial IgA deposition in the kidney	1.5	<1.5	Pestka & Zhou (2000)
							Reduced feed intake and body weight gain	1.5	<1.5	
Female B6C3F1 mice, 8–9 weeks old	DON (purity unknown)	Treatment: 16–24 weeks Withdrawal: 8 weeks followed by control diet for remaining 8–16 weeks	12	0 or 25	Eq 0 or 3.75	Diet	Increased serum IgE from 12 weeks onwards in both treatment and withdrawal groups	3.75	<3.75	Pestka & Dong (1994)

Table 2 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw)[a]	Route	Effect	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)	Reference
Female B6C3F1 mice, 3–4 weeks old	DON (unknown from Sigma labs)	Single dose, assessment each hour for 5 h	≥5		0, 0.1, 0.5, 1, 5 or 12.5	Gavage	Induction of SOCS in liver	0.1	<0.1	Amuzie, Shinozuka & Pestka (2009)
Female B6C3F1 mice, 3–4 weeks old	DON (unknown from Sigma labs)	Single dose, assessment after 2 h	4–5		0, 0.1, 0.5, 1, 5 or 12.5	Gavage	Decreased IGFALS in liver	0.5	0.1	Amuzie & Pestka (2010)
Male B3C3F1 mice, 8–10 weeks old	DON (unknown from Romer labs)	Single dose, effect assessment 2 and 3 h after exposure	3		0, 0.1, 0.5, 1, 5 or 25	Gavage in buffer	Increased cytokine mRNA in spleen and Peyer's patches	5	1	Zhou, Yan & Pestka (1997)
Male B3C3F1 mice, 8–10 weeks old	DON and LPS (from Sigma labs)	Single dose, effect assessment after 12 h	3		0 or 25 DON and/or 0.5 LPS	Gavage and/or ip injection	Enhanced LPS induction, TNF-α, IL-6 and IL-1β	5	<5	Zhou et al. (2000)
							Apoptosis	—	—	
Male B6C3F1 mice, 20 weeks old, 30–34 g	DON, purified and analysed	Single dose, effect assessment after 2 h	5		0 or 25	Gavage	Upregulation spleen cytokine and chemokine expression	25	<25	Kinser et al. (2004)

Table 2 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw)[a]	Route	Effect	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)	Reference
Female B6C3F1 mice, weanling (3–4 weeks) and young adult (8–10 weeks)	DON (Sigma)	Single dose, effect assessment after 15, 30, 60 and 120 min	≥5		0 or 5	Gavage	Expression of mRNAs for TNF-α, IL-1β and IL-6 in spleen, but not in liver or lung, 2-fold higher in weanling than adult	5	<5	Pestka & Amuzie (2008)
Male B6C3F1 mice, 8 weeks old	DON (Sigma)	Single dose	Unknown		0, 12.5 or 25	Gavage	Lymphoid apoptotic depletion	12.5	<12.5	Islam et al. (2002, 2003)
Male B6C3F1 mice, 8–10 weeks old	DON (Sigma)	Single dose, effect assessment after 3 h	3		0, 1, 5 or 25	Gavage	Lymphoid apoptotic depletion	25	<25	Zhou et al. (1999)

Eq, equivalent to; IFN, interferon; IgA, immunoglobulin A; IGFALS, IGF acid-labile subunit; IgM, immunoglobulin M; ip, intraperitoneal; LOAEL, lowest-observed-adverse-effect level; LPS, lipopolysaccharides; PROD, pentoxyresorufin-*O*-deethylase; SOCS, suppressors of cytokine signalling; TG, transgenic; TGF, transforming growth factor; WT, wild type

[a] Where Eq is stated, doses were calculated from the feed using standard conversion factors.

(a) Altered host resistance and humoral and cell-mediated responses

(i) Mice

Groups of six female 5-week-old BALB/c mice were intubated with a single dose of DON at 10 mg/kg bw or with water vehicle and then intranasally instilled 2 h later with reovirus or saline vehicle. After 10 days, viral titres, virus ribonucleic acid (RNA) (L2) expression and histopathology of lungs in infected mice were determined. For a dose–response study, mice were gavaged with DON at 0, 2, 5, 10 or 25 mg/kg bw, followed by the same treatment as for the single-dose group, but effects were determined 3 days post-dosing. No pulmonary effects were seen in mice exposed to DON alone or in control mice. DON markedly exacerbated bronchopneumonia compared with exposure to reovirus alone. After 10 days, viral titres and viral L2 RNA expression of lung in infected mice were 10 times higher in the DON-treated group than in saline-treated mice (control group to reovirus). In the dose–response groups, viral L2 RNA expression in lung was increased at 2, 5, 10 and 25 mg/kg bw compared with controls. Viral-induced elevations of protein, monocyte chemotactic protein-1 (MCP-1), TNF-α and inflammatory cells in bronchoalveolar lavage fluid (BALF) were markedly enhanced at 3 days post-instillation in 10 mg/kg bw DON-exposed mice. DON exposure also upregulated induction of reovirus-specific immunoglobulin A (IgA) in BALF, faecal pellets and serum, preceded by elevated IL-6 expression and secretion in the lung. As effects on viral clearance were seen in the lowest dose group, a NOAEL could not be derived, but would be lower than 2 mg/kg bw (Li et al., 2007).

BALB/c mice (10 of each sex per dose, 6 weeks of age) were fed on a diet containing DON (purity >98%) at concentrations of 0, 0.25, 0.5, 1 and 2 mg/kg feed, equivalent to 0, 0.038, 0.075, 0.15 and 0.3 mg/kg bw per day, for 14 or 28 days. Food intake and body weights were measured weekly. After 14 or 28 days, the mice were killed, and blood was collected for haematology. Spleens were removed and used to prepare single-cell suspensions. Food intake and body weight gain were not affected by DON treatment. At day 14, but not at day 28, the percentages of CD19(+) leukocytes (in both sexes) in peripheral blood cells were statistically significantly decreased by 11%, 15% and 15% at dietary doses of 0.5, 1 and 2 mg/kg, respectively. Decreases in the percentages of mononuclear cells (up to 10%, in females only) were observed at dietary doses of 1 and 2 mg/kg at day 14 only. The percentages of CD11b(+) monocytes in peripheral blood leukocytes and total CD11b(+) splenic leukocytes were decreased (up to 32%) only in female mice fed DON at 1 and 2 mg/kg after 28 days. (Note that control levels in females had almost doubled between days 14 and 28.) The authors concluded that BALB/c mice adapted to DON exposure, as effects observed after 14 days had largely disappeared after 28 days of treatment. As effects of DON were most prominent in females, the study authors suggested that female sex hormones potentiate one potential marker of DON immunotoxicity in BALB/c mice (Wu et al., 2009). The Committee considered the transient decrease in CD19(+) leukocytes not biologically relevant.

Groups of 12 male BALB/c mice were dosed with DON at 0 or 2 mg/kg diet (equivalent to 0 or 0.3 mg/kg bw per day) for 14 days and then exercised to fatigue

on a treadmill. Mice were euthanized by decapitation, and trunk blood and spleens were collected for analysis of splenocyte proliferation, serum cytokine concentration and antibody response to sheep red blood cells. Only the non-exercised DON-fed mice showed significant suppression of splenocyte proliferation, 32.9% ± 17.9% of that of non-exercised controls (P = 0.021). Exercised controls and DON-fed exercised animals showed splenocyte proliferation of 68–75% of that of non-exercised controls. Antibody response to a T cell–dependent antigen, sheep red blood cells, was significantly less for exercised DON-fed mice than for controls (P = 0.031). Serum corticosterone levels were significantly higher for both exercised groups than for the unexercised groups (P < 0.001). IL-4 secretion from mitogen-stimulated splenocytes was elevated by DON alone (P < 0.05), whereas IL-2 concentration was elevated by DON with exercise stress (P < 0.05). As only one dose was given, a NOAEL could not be derived, but would be lower than 0.3 mg/kg bw per day (Landgren, Hendrich & Kohut, 2006).

Female BALB/c mice (n = 10) were given drinking-water containing DON (purity not reported) at 0.2–6 mg/l for 4 weeks. On day 14, the mice received a gastric inoculation with *Salmonella* Enteritides. The survival rate of mice was decreased at DON concentrations of 2 mg/l and higher. DON reduced the serum levels of TNF-α at 0.2 mg/l and increased the TNF-α levels at 2 and 6 mg/l (Sugita-Konishi, 2003).

(ii) Pigs

Two groups of 14 piglets aged 8 weeks (hybrid commercial, clinically healthy and pathogen-free castrated males) received tested control feed, devoid of mycotoxins, ad libitum or the same feed with pure DON (D0156, Sigma) daily for 6 weeks, at 0.5 mg/kg per pig (0.5 mg/kg bw per day) over the first week and 1 mg/kg per pig (1 mg/kg bw per day) over the following 5 weeks. Clinical assessment, haemochromocytometric examination, and characterization and quantification of CD3CD8+, CD4+CD8, CD4CD8+, CD8high, CD4+CD8+ and TCRγ/δ cells were performed at termination of the exposure. Histological and histochemical analyses were performed on samples of lymphoid organs (thymus, spleen, palatine tonsils, mediastinic and mesenteric lymph nodes), lungs, heart, skeletal muscle, liver, kidney, stomach and segments of the small and large intestine. The treatment of the pigs with DON did not induce alterations due to pathological effects on either clinical or cellular parameters. Although higher mean absolute values of natural killer (NK) cells and cytotoxic T lymphocytes were observed in the control group over the last experimental weeks, the treatment with DON did not significantly influence the levels of leukocyte subsets. The histopathological investigation of lymphoid tissues did not show any particular lesions of the parenchymal morphology and detected, by immunohistochemical assays, a normal composition and distribution of the lymphocyte subsets in the gut-associated lymphoid tissue. As no significant effects could be determined, the NOAEL was 1 mg/kg bw per day, the highest dose tested (Ferrari et al., 2009).

(b) Altered serum IgA levels

(i) Mice

Groups of 10 male C57BL6 mice were treated orally 3 days/week (Monday, Wednesday, Friday) for 4 weeks with "pure" DON (per cent purity not reported) in 0.150 ml 5% gum arabic solution at doses of 0, 0.014, 0.071, 0.355 or 1.774 mg/kg bw. Body weight was measured 3 times per week. After 4 weeks, the animals were killed, blood was collected and livers were weighed and stored for biochemical analysis. In the plasma, the following biochemical parameters were measured: alkaline phosphatase activity, osmolarity and levels of sodium, chlorine, carbon dioxide, phosphate, urea, glucose, IgA, immunoglobulin G (IgG) and immunoglobulin M (IgM). In liver tissue, patterns of P450 expression and activities of P450 (by measuring ethoxyresorufin-*O*-deethylase [EROD], methoxyresorufin-*O*-deethylase [MROD] and pentoxyresorufin-*O*-deethylase [PROD]) and glutathione-*S*-transferase were assessed. Body weight gain and liver weight were not affected by DON treatment. Plasma IgA levels were statistically significantly increased by 66%, 48% and 47% at DON doses of 0.071, 0.355 and 1.774 mg/kg bw, respectively. The other investigated plasma parameters were not affected by treatment. Treatment with DON at 0.014, 0.071 or 0.355 mg/kg bw increased liver microsomal PROD activity by 43%, 53% and 47%, respectively. Protein expression of the cytochrome P450 2b subfamily was increased by approximately 30% and 50% at 0.071 and 0.355 mg/kg bw, respectively. Glutathione-*S*-transferase activity was increased up to 39% and 78% by DON at doses of 0.071 and 0.355 mg/kg bw, respectively. At the highest dose, no effects on liver P450 and glutathione-*S*-transferase activity were observed. A significant competitive inhibition of 1-chloro-2,4-dinitrobenzene conjugation by DON in vitro suggests that DON may be a substrate for glutathione-*S*-transferases. The data suggest that a subchronic exposure to low (but not high) doses of DON causes changes in the normal liver metabolism of xenobiotics. The Committee noted that this specific low-dose effect of DON was not observed in other studies and concluded that these were of questionable biological relevance. As doses were given only 3 days/week, a daily dose could not be set for a NOAEL for elevated IgA in the serum (Gouze et al., 2006).

Transgenic p53+/− and corresponding wild-type mice (5–7 weeks old at the start of the study; starting body weights 23.0 ± 2.0 g for wild-type mice and 24.7 ± 1.3 g for transgenic mice) were exposed to DON (purity 95%) at 0, 1, 5 or 10 mg/kg diet (equivalent to 0, 0.15, 0.75 and 1.5 mg/kg bw per day) for 26 weeks. DON caused a significant dose-dependent reduction in body weight in wild-type and transgenic mice in the middle dose group, accompanied by declining liver fat stores. In wild-type mice, there was a significant trend towards increased plasma total IgA and decreased total IgM levels with increasing DON exposure, which was statistically significant in the highest dose group. In transgenic mice, plasma immunoglobulin levels were not affected. Kidney weights were increased in wild-type mice from the middle dose group and in transgenic mice in the highest dose group. IgA-positive glomeruli in kidney were increased in the highest dose groups in both strains. Real-time polymerase chain reaction (PCR) analyses indicated that

kidney cyclin D and cyclin E expression declined in DON-treated wild-type and transgenic mice. Overall, the effects of 26-week DON exposure on wild-type and transgenic mice were consistent with those previously seen in B6C3F1 mice exposed to DON for 2 years (Iverson et al., 1995). Based on the decreased body weight and increased kidney weight seen in the middle dose group, a NOAEL of 1 mg/kg, equivalent to 0.15 mg/kg bw per day (i.e. a similar order of magnitude as in the long-term mouse study from Iverson et al. [1995]), could be established (Bondy et al., 2009).

In a study on the mechanism of the immunotoxicity of DON, groups of 3–6 IL-6 knockout, wild-type and IL-6 sentinel mice were exposed to purified DON at 10 mg/kg bw in the diet (equivalent to 1.5 mg/kg bw per day) for 12 weeks. This dose induced serum IL-6 and IgA concentrations and increased mesangial IgA deposition in the kidney, but not in the IL-6 knockout mice. All treated groups had statistically significantly reduced feed intake and body weight gain during the study, as measured at weeks 6 and 12, which were similar for all three mouse strains (Pestka & Zhou, 2000). A later study by this group indicated that, in contrast to earlier assumptions, inhibition of cyclooxygenase-2 (COX-2, also induced by DON) expression or function did not prevent the DON-induced IgA increase but rather enhanced DON's capacity to promote IgA elevation after 16 weeks of exposure to the same dose (Jia & Pestka, 2005).

The effects of dietary treatment with purified DON (purity not reported) on serum IgE were assessed in female B6C3F1 mice (12 per group). Ingestion of DON at 25 mg/kg in the diet (equivalent to 3.75 mg/kg bw per day) resulted in 2.7-, 4-, 5- and 2.3-fold increases in serum IgE relative to controls after 12, 16, 20 and 24 weeks, respectively. When mice were fed DON at 25 mg/kg in the diet for 8 weeks and continued on toxin-free diet, serum IgE levels were 2.4-, 4-, 4.9- and 2-fold those of controls at 12, 16, 20 and 24 weeks, respectively. IgE levels were not significantly different between treatment and withdrawal groups at weeks 12–24. As only one dose was tested, a NOAEL for the reversible increase in serum IgA levels could not be determined, but would be below 3.75 mg/kg bw per day (Pestka & Dong, 1994). This study was not included in the 2001 monograph (Annex 1, reference *153*).

(ii) Pigs

Since the previous evaluation of DON, evidence for the induction of IgA concentrations in serum of pigs by DON has become available, as described below.

Groups of nine female weaned piglets (mean body weight 9.8 kg at the start of the study) were given 0, 0.3, 0.6 or 1.2 mg of isolated, pure DON per kilogram in the diet (equivalent to 0, 0.012, 0.024 and 0.048 mg/kg bw per day) for 8 weeks. Pigs were fed restrictively to allow complete feed intake by all animals. Body weight gain and biochemical and haematological values in the blood and serum, including concentrations of IgA, blood glucose, cortisol and insulin-like growth factor 1 (IGF-1), were determined. Body weight gain, food intake and feed conversion rate were not affected by DON treatment. Glucose levels tended to be decreased at the high dose throughout the treatment period (including at the start of the treatment). Cortisol and IGF-1 levels were not significantly affected. Small increases (up to

20%) in IgA levels were found at 0.6 and 1.2 mg/kg diet. As these latter effects in the two highest dose groups were not statistically significant, the NOAEL was 0.048, the highest dose tested (Drochner et al., 2004).

Effects on serum IgA levels were seen in 24 weanling pigs that were fed either control feed or feed naturally contaminated with DON at 2.2–2.5 mg/kg (equivalent to 0.088–0.1 mg/kg bw per day; other mycotoxins under LOD of 10–50 µg/kg feed) for 9 weeks. At days 4 and 15 of the experiment, the animals were subcutaneously immunized with ovalbumin. Total and specific IgA and IgG levels in serum and expression of mRNA encoding for cytokines such as TGF-α, IFN-α, IL-4 and IL-6 were also investigated in mesenteric lymph nodes, ileum and the spleen of piglets. IgA but not IgG upregulation could be observed in the serum of pigs exposed to the naturally contaminated diet. In vaccinated animals, DON also increased the concentration of ovalbumin-specific IgA and IgG. No significant effect of DON was observed in the samples from the ileum and spleen. By contrast, a significant reduction of mRNA expression encoding for both IFN-α and TGF-α was observed in mesenteric lymph nodes from DON-intoxicated animals. As only one dose was given, a NOAEL could not be derived, but would be lower than 2.2 mg/kg feed, equivalent to 0.088 mg/kg bw per day (Pinton et al., 2008).

Male and female pigs were fed DON at 0 or 3.5–5.3 mg/kg diet for 5–11 weeks in unequal group sizes. In total, six pigs were fed a DON-containing diet, but background levels were present in the control diet. Based on measured concentrations and feed intake, the DON exposure ranged from 0.051 to 0.213 mg/kg bw per day. Controls received a background concentration of DON, a maximum of 0.007 mg/kg bw per day. In vitro treatment of porcine monocyte-derived dendritic cells with DON interfered with phenotypic maturation of the dendritic cells, but also with antigen uptake and IL-10 secretion. Chronic dietary exposure of pigs to DON resulted in the generation of dendritic cells that failed to mature in response to TNF-α/lipopolysaccharides (LPS), but acquired a more mature phenotype in response to DON treatment in vitro. The study authors concluded that DON disrupts porcine dendritic cell function in vitro and in vivo. The Committee concluded that the study design is unsuitable for deriving a NOAEL (Bimczok et al., 2007).

(c) IgA-associated nephropathy

Increased mesangial IgA deposition in the kidney was found, together with induced serum IL-6 and IgA concentrations, in wild-type and IL-6 sentinel mice, but not in IL-6 knockout mice, after exposure to purified DON in the diet at 10 mg/kg bw (equivalent to 1.5 mg/kg bw per day) for 12 weeks. All treated groups had statistically significantly reduced feed intake and body weight gain during the study, as measured at weeks 6 and 12, which were similar for all three mouse strains (Pestka & Zhou, 2000).

(d) Cytokine expression

(i) Mice

Groups of 4–5 female B6C3F1 mice (3–4 weeks old) were treated with DON in phosphate-buffered saline by a single gavage at 0, 0.1, 0.5, 1, 5 or 12.5 mg/kg bw. Each hour up to 5 h after dosing, mice were euthanized, and blood, spleen, liver and muscle were sampled. In plasma, concentrations of TNF-α, IL-6, MCP-1, interferon-gamma (IFN-γ), IL-10 and IL-12p70 were determined using a bioassay kit. DON concentrations in serum were determined by ELISA. Suppressors of cytokine signalling (SOCS), some of which impair growth hormone (GH) signalling, are known to be induced by proinflammatory cytokines, which are upregulated by DON. In spleen, liver and muscle, concentrations of mRNA for four well-characterized SOCSs (cytokine-inducible SH2 domain protein [CIS], SOCS1, SOCS2 and SOCS3) were determined. The results showed that TNF-α and IL-6 mRNA and protein expression were rapidly induced (1 h) after exposure in several organs and plasma, respectively. Upregulation of mRNAs for the four SOCSs was either concurrent with (1 h) or subsequent to (2 h) cytokine upregulation. SOCS mRNAs were induced in muscle and spleen from 0.5 mg/kg bw and in liver from 0.1 mg/kg bw, with CIS, SOCS1 and SOCS2 occurring to a lesser extent than SOCS3. SOCS3 protein was detectable in the liver well after the onset of cytokine decline (5 h). Other SOCSs and cytokines were back to control levels after 5 h. DON concentration did not fully return to control levels after 5 h. Furthermore, hepatic SOCS upregulation was associated with about 75% suppression of GH-inducible IGF acid-labile subunit (IGFALS, an IGF-1-binding partner responsible for increasing the half-life of circulating IGF-1). Taken together, DON-induced cytokine upregulation corresponded to increased expression of several SOCSs and was associated with suppression of GH-inducible gene expression in the liver. As the (reversible) effect on SOCS mRNA expression in liver was seen in the lowest dose group, a NOAEL could not be derived, but would be lower than 0.1 mg/kg bw (Amuzie, Shinozuka & Pestka, 2009).

Groups of 4–5 female B6C3F1 mice (3–4 weeks old) were treated with an acute dose of DON at 0, 0.1, 0.5, 1, 5 or 12.5 mg/kg bw in phosphate-buffered saline by gavage, and liver sections were collected 2 h later. Bovine somatotropin (GH) was administered intraperitoneally at a dose of 5 mg/kg bw, at one or more time intervals (0–2 h) after DON gavage. Mice were euthanized at selected time intervals (1–4 h) after GH exposure, and the caudolateral portion of the liver's lateral lobe was collected for real-time PCR analysis of IGF-1, IGFALS, IGF binding protein 3 (IGFBP3) and SOCS3 mRNAs. In groups dosed with DON at 0.5–12.5 mg/kg bw, hepatic IGFALS mRNA levels were suppressed in a dose-dependent fashion, whereas DON at 0.1 mg/kg bw was without effect. In GH-treated mice, DON selectively suppressed hepatic IGFALS mRNA but increased IGF-1 and IGFBP3 mRNAs. The authors suggested that oral DON exposure perturbs the GH axis by suppressing two clinically relevant growth-related proteins, IGFALS and IGF-1, and therefore these effects would be related to the effects of DON on body weight. Based on the suppression of hepatic IGFALS mRNA levels in the second lowest dose group, a NOEL of 0.1 mg/kg bw could be derived (Amuzie & Pestka, 2010).

Groups of three male B3C3F1 mice (8–10 weeks old) were acclimatized for 1 week and given a single oral gavage of DON in 0.5 ml of 0.01 mol/l carbonate/bicarbonate buffer (pH 9.6). For determining dose–response effects on cytokine mRNA expression in spleen and Peyer's patches, groups received DON at 0, 0.1, 0.5, 1, 5 or 25 mg/kg bw and were euthanized 2 h post-dosing for tissue cytokine mRNA determination by reverse transcriptase PCR (RT-PCR) in combination with hybridization analysis. For determination of kinetic effects, mice were given a single dose of DON at 0 or 25 mg/kg bw and euthanized 1, 2, 4, 8 or 24 h after dosing. For serum cytokine determination, animals were given DON at 0 or 25 mg/kg bw, and blood was collected 3 h after exposure. In the two highest dose groups, statistically significantly elevated concentrations of the mRNAs for the proinflammatory cytokines IL-1β, IL-6 and TNF-α, the T helper 1 cytokines IFN-γ and IL-2, and the T helper 2 cytokines IL-4 and IL-10 were found. IL-12p40 mRNA was also induced, but not IL-12p35 mRNA. The effects were more pronounced in spleen than in Peyer's patches. IL-5 and TGF-β mRNAs were expressed constitutively in spleen and Peyer's patches but were not affected by DON. The kinetic study showed that peak levels were reached 2–4 h after exposure, and concentrations returned to control levels after 24 h in spleen and 24 h in Peyer's patches. DON treatment induced serum levels of TNF-α, IL-6 and IFN-γ 3 h after exposure to DON at 25 mg/kg bw. The NOAEL for reversible induction of cytokine mRNA in spleen and Peyer's patches in mice was 1 mg/kg bw (Zhou, Yan & Pestka, 1997). This study was also described in the 2001 monograph (Annex 1, reference *153*), but without the kinetics.

DON exposure enhanced LPS-induced expression of cytokines TNF-α, IL-6 and IL-1β in B6C3F1 mice acutely exposed to DON at 5 mg/kg bw by gavage (Zhou et al., 1999).

A single dose of purified DON at 25 mg/kg bw upregulated spleen cytokine and chemokine mRNA expression in 20-week-old B6C3F1 mice 2 h after acute exposure by gavage (Kinser et al., 2004).

In a study to test possible age differences in toxicokinetics and immune effects of DON, groups of weanling (3–4 weeks) and young adult (8–10 weeks) female mice were given a single dose of DON (Sigma) at 5 mg/kg bw by gavage. Expression of mRNAs for TNF-α, IL-1β and IL-6 in spleen, but not in liver or lung, was 2–3 times greater in weanling than in adult mice. Kinetics showed a higher uptake of DON in plasma, spleen, liver, lung and kidney in weanling mice compared with adult mice, but differences in concentrations were almost entirely diminished after 2 h. These data suggest that at these very high dose levels, young mice are modestly more susceptible than adult mice to the adverse effects of DON and that this might result from a greater toxin tissue burden resulting from differences in uptake (Pestka & Amuzie, 2008).

(e) Apoptosis in lymphoid tissue

DON potentiated LPS-induced lymphoid apoptotic depletion in B6C3F1 mice at acute oral doses of 12.5 mg/kg bw (Islam et al., 2002, 2003) and 25 mg/kg bw (Zhou et al., 2000).

2.2.7 Special studies on metabolites

The details on the special in vivo studies on metabolites of DON are summarized in Table 3 and described more fully below.

Table 3. Summary of special studies on metabolites

Species	Route	Purity	Effect	LOAEL	NOAEL	Reference
Mice, outbred albino [Crl:CDI (ICR) BR], males, weanling	Gavage	Purified 3-Ac-DON (purity not reported)	Clinical signs of toxicity, necrotic lesions in duodenal crypts, thymus and spleen, reduced mitotic activity	5 mg/kg bw per day	—	Schiefer et al. (1985)
Mice CD1 Swiss, male, 18–20 g	Diet	Purified 3-Ac-DON (purity not reported)	Increased T cell–dependent antibody response	10 mg/kg diet, equivalent to 1.5 mg/kg bw per day	5 mg/kg diet, equivalent to 0.75 mg/kg bw per day	Tomar, Blakley & Decoteau (1987)

(a) Mice

Groups of five male weanling outbred albino Crl:CDI (ICR) BR mice were given a single dose of purified 3-Ac-DON (purity not reported) at 0, 5, 10, 20 or 40 mg/kg bw in propylene glycol by intragastric administration and sacrificed 2, 4, 6, 12, 24, 48 or 96 h after dosing. The animals became clinically ill in all dose groups after 12 h, and some animals in the highest dose group died. Histological examination of duodenal crypts, thymus and spleen revealed the presence of necrotic lesions in all dose groups. As soon as 2 h after administration, mitotic activity was significantly reduced in all dose groups. No other tissues were examined. The authors concluded that the intensity of lesions in the 40 mg/kg bw group corresponded to lesions known to be caused by 4 mg/kg bw of T-2 toxin, but data supporting this conclusion were not presented. Together with the results from a rabbit skin bioassay (not summarized), the authors concluded that 3-Ac-DON was considerably less toxic than T-2 toxin, but caused acute effects in the dividing cells of the body in a manner characteristic of trichothecenes. As effects were seen in all dose groups, a NOAEL for 3-Ac-DON could not be derived, but would be lower than 5 mg/kg bw. The Committee concluded that this study could not be used for comparison of toxicity between 3-Ac-DON and DON (Schiefer et al., 1985). This study was not described in the 2001 monograph (Annex 1, reference *153*).

The effects of purified 3-Ac-DON (purity not reported) on mitogen-induced lymphocyte proliferation and antibody production were studied in male CD-I mice exposed to 3-Ac-DON at 0, 2.5, 5 or 10 mg/kg in the diet for 35 days. Concentrations were not checked after preparation of diet. The authors reported no effects on

mitogen-induced lymphocyte proliferation, but a slight (non-significant) effect was seen, and T cell–independent antibody responses to dinitrophenyl-ficoll or *Escherichia coli* were seen. The T cell–dependent antibody response to sheep red blood cells was increased in the group fed 3-Ac-DON at 10 mg/kg. In vitro, 3-Ac-DON inhibited lymphocyte proliferation in a dose-dependent manner. The authors suggested that the in vitro effects of 3-Ac-DON may not reflect its in vivo immunotoxicity. A NOAEL of 5 mg/kg diet could be derived, equivalent to 0.75 mg/kg bw per day (Tomar, Blakley & Decoteau, 1987). This study was not described in the 2001 monograph (Annex 1, reference *153*).

(b) In vitro

The cytotoxicity of the de-epoxy metabolites of trichothecenes NIV and DON was determined by DNA synthesis in 3T3 mouse fibroblasts (5-bromo-2′-deoxyuridine [BrdU] bioassay) and compared with the cytotoxicity of the respective toxin with an intact epoxy group and their acetylated derivatives. The toxicities of NIV and DON expressed as the concentration inhibiting 50% of the DNA synthesis (IC_{50}) occurred at similar micromole per litre concentrations (1.19 ± 0.06 and 1.50 ± 0.34 µmol/l). The toxicity of fusarenon X (4-acetyl-NIV) in the assay was similar to the toxicity of NIV, and the toxicity of 15-Ac-DON was equal to the toxicity of DON. 3-Ac-DON was 9 times less toxic than DON and 15-Ac-DON. The IC_{50} value for de-epoxy-DON was 54 times higher in the assay than the IC_{50} for DON, whereas the IC_{50} of de-epoxy-NIV was 55 times higher than the IC_{50} for NIV (Eriksen, Pettersson & Lundh, 2004).

DON, 3-Ac-DON and ZEA (purities not reported) were examined for their in vitro effect on mitogen-induced lymphocyte blastogenesis using rat or human peripheral blood lymphocytes as measured by incorporated [^{3}H]thymidine. Results were compiled from 20 experiments, and experiments were performed using five replicates. A dose-dependent reduction of lymphocyte proliferation was demonstrated for each mycotoxin. However, the inhibitory effect of DON was significantly higher than that of the acetylated compound. DON concentrations of 90 ng/ml and 220 ng/ml inhibited rat and human lymphocyte blastogenesis by 50%, respectively, whereas 3-Ac-DON concentrations of 450 ng/ml and 1060 ng/ml were required to produce the same effect. The amount of ZEA necessary to inhibit blastogenesis by 50% was 250 times greater than the amount of DON required. In lymphocyte cultures containing 50 ng DON, the addition of 1.5, 2.5 or 5 µg ZEA caused a depression of lymphocyte proliferation that was equal to the sum of that produced by the individual trichothecenes. When 1.5 µg ZEA was added to cultures containing 150 ng DON, a similar additive effect was observed. There was no evidence of cell death, and combinations of DON and ZEA did not alter the expected response. This study shows that 3-Ac-DON is 5 times less potent than DON in inhibiting mitogen-induced lymphocyte blastogenesis in vitro and that rat lymphocytes are approximately 2 times more sensitive to this effect than human lymphocytes. The effects of DON and ZEA were additive in this experiment. The Committee noted that the conversion of 3-Ac-DON to DON as determined in the study of Eriksen, Pettersson & Lindberg (2003) makes this in vitro study of doubtful

relevance for assessing relative potency in vivo (Atkinson & Miller, 1984). This study was not described in the 2001 monograph (Annex 1, reference *153*).

The ability of human gastrointestinal organisms to transform the trichothecenes 3-Ac-DON and NIV was investigated in vitro. Samples of human faeces were incubated under anaerobic conditions for 48 h with 10 µg/l of the toxins. They were then extracted and analysed for trichothecenes and metabolites. The recovery of the toxins in the control sample was 90–96%, and the recovery of the sum of the toxin and de-epoxide metabolite was similar to the recovery in the control sample. 3-Ac-DON was metabolized to DON during the incubation period (78% ± 30%). In contrast to what has been reported for other species such as rats, mice and pigs, no de-epoxidated metabolites were detected in the faecal incubates. The toxicological significance of the difference in the intestinal ability to transform trichothecenes between species is unknown (Eriksen & Pettersson, 2003).

2.2.8 Special studies on species differences

Pestka & Smolinski (2005) concluded from available literature that there are marked species differences in sensitivity to DON, with the pig being most sensitive, followed, in decreasing order, by rodent, dog, cat, poultry and ruminants. They stated that primate or human studies on DON-induced emesis have not been reported to date. However, they concluded that, based on the use of porcine models for human intestinal function (Nejdfors et al., 2000) and drug-induced emesis (Szelenyi, Herold & Gothert, 1994), it was not unreasonable to speculate that humans are as sensitive as pigs to DON.

In a study comparing mycotoxin cytotoxicity in several mammalian cell lines measured by metabolic activity (cleavage of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MTT) assay, sensitivities of cell lines to DON were found in the following decreasing order: Chinese hamster ovary CHO-K1 > Chinese hamster lung fibroblast (V79) > BALB/c mice keratinocyte cell line (C5-O) > human Caco-2 > human hepatocellular carcinoma (HepG2) cells, with IC_{50} values of 0.27, 0.49, 0.54, 1.02 and 8.36 µg/ml, respectively, after 48 h exposure. This could suggest that humans are less sensitive to the cytotoxicity of DON than mice and hamsters (Cetin & Bullerman, 2005). However, the Committee considered that this study did not provide a reliable basis for interspecies comparison of toxicity in vivo.

In a study using in vitro, ex vivo and in vivo approaches to determining the effects of DON on gastrointestinal epithelium, human Caco-2 cells exhibited a lower sensitivity to DON-induced increase in permeability compared with porcine IPEC-1 cells in vitro. The study is described in section 2.2.2 above (Pinton et al., 2009).

2.3 Observations in humans

The potential deleterious effects of DON on humans have been reviewed by Creppy (2002), Sudakin (2003), Pestka & Smolinski (2005), Pronk, Schothorst & van Egmond (2002) and Fokunang et al. (2006).

Since the fifty-sixth meeting of the Committee, risk assessments or reviews on DON have been performed by the Scientific Committee on Food (SCF, 1999,

2002), the European Food Safety Authority (EFSA, 2004), the Health Council of the Netherlands (2001), the Dutch National Institute for Public Health and the Environment (Pieters et al., 2001; Pieters, Bakker & Slob, 2004; Boon et al., 2009) and the French Food Safety Agency (AFFSA, 2006). All but the Health Council of the Netherlands (2001) derived the same health-based guidance value of 1 µg/kg bw per day for long-term intake of DON based on the same critical study used by the fifty-sixth meeting of the Committee (Iverson et al., 1995). The Health Council of the Netherlands (2001) derived a tolerable daily intake (TDI) of 0.5 µg/kg bw per day based on the NOAEL of 0.11 mg/kg bw per day from the study by Iverson et al. (1995), but applied a uncertainty factor of 210, composed of uncertainty factors of 10 for intraspecies differences and 3 for interspecies differences and a scaling factor of 7 for differences in energy use, as an indicator for metabolism differences between humans and mice.

An overview of available data on DON and the research needs has been compiled by the International Life Sciences Institute (Larsen et al., 2004) and the United States National Toxicology Program (NTP, 2009a). Recommendations from the latter were in line with those of the fifty-sixth meeting of the Committee (NTP, 2009b).

2.3.1 Epidemiology

Reddy & Raghavender (2008) reviewed outbreaks of mycotoxicoses in India (Reddy & Raghavender, 2008; Raghavender & Reddy, 2009), but reports relating to the DON outbreak were evaluated by the Committee previously. No new information could therefore be taken from this review.

2.3.2 Development of a urinary biomarker of exposure

Urine samples were collected from 11 female inhabitants of Linxian County, Henan Province, China (studies on occurrence data in these areas were included in the 2001 monograph; Annex 1, reference *153*), a high-risk region for oesophageal cancer and an area of potentially high DON exposure, as the staple diet consists of corn and wheat; and from 4 female inhabitants of Gejiu, Yunnan Province, a low-risk region in China, where the staple diet consists primarily of rice. Participants were selected from eligible non-smoking volunteers between the ages of 19 and 75 years. Each subject was given a sterile container, and up to 100 ml of first-voided morning urine was collected and placed in a light-protected bag. The urine samples were then kept frozen until analysis. DON was detected in all 15 samples following β-glucuronidase treatment and immunoaffinity column enrichment, with the identity of DON being confirmed by mass spectrometry. The mean levels of DON from the suspected high- and low-exposure regions of China were 37 ng/ml (range 14–94 ng/ml) and 12 ng/ml (range 4–18 ng/ml), respectively. Given that approximately 30% of the total DON consumed is excreted during a 24 h period in the animal model and assuming that a 60 kg person produces 1 litre of urine per day and that there is a 40% recovery of DON in human urine samples, the levels detected in the high- and low-risk populations were believed to represent a daily exposure ranging from 1.9 to 13.0 mg/kg bw per day and from 0.6 to 2.5 mg/kg bw per day, respectively (Meky et al., 2003).

To better assess exposure to DON at the individual level, a urinary assay was developed, incorporating immunoaffinity column enrichment and liquid chromatography–mass spectrometry (LC-MS) detection. Further refinement of this urinary assay, by inclusion of [^{13}C]DON as an internal standard, was then undertaken and tested within the United Kingdom. DON was frequently observed in urine and was associated with cereal intake. A dietary intervention study demonstrated that avoiding wheat in the diet reduced urinary levels of DON (Turner et al., 2008a).

Twenty-five volunteers from the United Kingdom (aged 21–59 years) completed semi-weighed food diaries on days 1 and 2 (normal diet), and a morning urine sample was provided on day 3. On days 3–6 (intervention), individuals restricted major sources of wheat intake following dietary guidance. Diaries were completed on days 5 and 6, and a further morning urine sample was provided on day 7. Urinary DON was measured following immunoaffinity column cleanup and analysis by LC-MS. Wheat-based food intake (mean 322 g/day, range 131–542 g/day) was significantly ($P < 0.001$) reduced during the intervention to 26 g/day (range 0–159 g/day), indicating good compliance. DON was detected in all 25 urine samples taken on day 3 (geometric mean DON concentration of 7.2 ng/mg creatinine; 95% confidence interval [CI] 4.9–10.5 ng/mg), but following the intervention, there was a significant 11-fold reduction ($P < 0.001$) to 0.6 ng/mg (95% CI 0.4–0.9 ng/mg). One individual who increased wheat intake during the intervention instead of lowering it had elevated DON levels in the urine (Turner et al., 2008b).

In another study by the same group, the United Kingdom adult National Diet and Nutrition Survey was used to compare 24 h urinary DON excretion with cereal intake. One hundred subjects were identified for each of the following cereal consumption groups: low (mean 107 g of cereal per day; range 88–125 g/day), medium (mean 179 g/day; range 162–195 g/day) and high (mean 300 g/day; range 276–325 g/day). DON was analysed in 24 h urine samples by LC-MS after purification on immunoaffinity columns. DON was detected in 296 of 300 (98.7%) urine samples. Cereal intake was significantly associated with urinary DON ($P < 0.0005$), with the geometric mean urinary levels being 6.55 µg/day (95% CI 5.71–7.53 µg/day), 9.63 µg/day (95% CI 8.39–11.05 µg/day) and 13.24 µg/day (95% CI 11.54–15.19 µg/day) for low, medium and high exposure groups, respectively. In multivariable analysis, wholemeal bread ($P < 0.0005$), white bread ($P < 0.0005$), "other" bread ($P < 0.0005$), buns/cakes ($P = 0.003$), high-fibre breakfast cereal ($P = 0.016$) and pasta ($P = 0.017$) were significantly associated with urinary DON. Wholemeal bread was associated with the greatest per cent increase in urinary DON per unit of consumption, but white bread contributed approximately twice as much as wholemeal bread to the urinary DON levels, because it was consumed in higher amounts (Turner et al., 2008c). The Committee concluded that this biomarker could be used for systemic DON exposure resulting from dietary exposure to DON and its derivatives, as DON could be metabolized from other precursors.

In a more detailed analysis of the previous study, food diary information ($n = 255$) for the day of urine collection (model I), the previous 24 h period (model II) and the day of urine collection plus the previous 24 h combined (model III) was

further examined to assess whether the recent intake of cereal correlated more strongly with urinary DON, compared with the longer-term assessment of usual cereal intake from 7-day food diaries (model IV). DON was detected in 254/255 (99.6%) urine samples (mean 12.0 µg/day; range not detected to 66 µg/day). For all the models, total cereal intake was positively associated with urinary DON ($P < 0.001$) in each model. The goodness of fit (adjusted R^2 value) was used to assess how well each model explained the variation in urinary DON. Model I provided a better goodness of fit (adjusted R^2 0.22) than did model IV (adjusted R^2 0.19), whereas model III provided the best fit (adjusted R^2 0.27). The authors suggested that the interindividual variation in urinary DON was somewhat better explained by recent cereal intake than by usual cereal intake assessed over 7 days (Turner et al., 2009).

In a study aimed at correlating urinary DON levels with one or more metabolites in the urine, the urinary metabolome of 22 adults from the United Kingdom (7 males, 15 females; age range 21–59 years) for whom urinary DON levels had been previously determined using an established LC-MS assay was analysed using a nuclear magnetic resonance (NMR)-based metabolomics approach coupled with multivariate statistical analysis. The authors suggested that, based on the metabolic profiling, hippurate levels could be used to distinguish between groups with low (3.6 ng/mg creatinine; 95% CI 2.6–5.0 ng/mg) and high (11.1 ng/mg; 95% CI 8.1–15.5 ng/mg) DON exposure, with the concentration of hippurate being significantly ($P = 0.047$) higher (1.5 times) for people with high DON exposure than for those with low DON exposure (Hopton et al., 2010).

2.3.3 Derivation of a lower dose for emetic responses to DON in humans

Analyses of two DON intoxication events in humans reported in the 2001 monograph (Luo, 1988; Guo et al., 1989) provide data that have allowed the Committee to approximate a lower dose in humans that might cause an emetic response. In one event, it was found that foodstuffs contaminated with DON at 1–40 mg/kg did not cause emesis, whereas in the second event, foodstuffs contaminated with DON at concentrations between 3 and 98 mg/kg did cause emesis. If it is assumed that food contaminated at 50 mg/kg could cause an emetic response, the following can be calculated. If a 50 g portion of food were consumed, a dose of 2.5 mg of DON would be delivered (0.05 kg × 50 mg/kg). In a 50 kg individual, this would be a dose of 0.05 mg/kg bw (2.5 mg/50 kg bw).

The use of a small portion of food and a relatively small standard body weight in the estimation above assures that the calculated emetic response level in humans is conservatively low for comparisons with pigs. For illustrative purposes, if a food portion of 200 g was necessary to cause emesis in a 20 kg child, the calculated dose would be 0.5 mg/kg bw, 10-fold higher than the above calculation.

Additional information was available on a lower no-effect dose of DON with respect to emesis. In Henan, China, in 1985, no cases of acute illness were observed among 191 peasant families who ate scabby wheat containing DON at a concentration of 0.016–3.3 mg/kg (mean 0.92 mg/kg) and NIV at a mean concentration of 0.13 mg/kg (both measured by gas chromatography with electron

capture detection [GC-ECD]). Assuming a loss of DON during processing of approximately 30% and consumption of 560 g per person, the authors estimated an intake of DON of 0.380–0.520 mg per adult, which, for a body weight of 50 kg, would give an intake of 0.0075–0.010 mg/kg bw.

3. ANALYTICAL METHODS

3.1 Chemistry

The chemistry of DON, a type B trichothecene, was summarized by the fifty-sixth meeting of the Committee (Annex 1, reference *153*).

3.2 Chemical analysis

Since the monograph for the fifty-sixth meeting of the Committee was written, considerable research has been conducted on analytical methods for the determination of DON, as well as its 3- and 15-acetyl derivatives and DON-3-glucoside. The most important development during this period has been the use of MS or tandem MS (MS/MS) coupled to HPLC (LC-MS/MS) for DON determination in a range of matrices either with or without sample extract cleanup.

The purity and stability of calibrants for mycotoxin analysis are critical issues. DON appears to be stable if kept in acetonitrile solution at 25 °C for 24 months (Widestrand & Pettersson, 2001). The European Commission (EC) funded a project to produce certified calibrants of DON, 3-Ac-DON and 15-Ac-DON in acetonitrile (Krska et al., 2007). These mycotoxins were purified from available *Fusarium* culture material and chemically characterized by ultraviolet (UV) and infrared spectroscopy, HPLC, GC with ECD, flame ionization detection (FID) and MS detection, elemental analysis and NMR. Temperature stability studies confirmed the long-term stability of the standards in acetonitrile. Molar absorptivity coefficients for DON, 3-Ac-DON and 15-Ac-DON were 6805 ± 126 litre/cm per mole, 6983 ± 141 litre/cm per mole and 6935 ± 142 litre/cm per mole, respectively, based on an interlaboratory study. The calibrator for DON produced by the project is commercially available as a sealed ampoule from the Institute for Reference Materials and Measurements in Geel, Belgium.

A number of reviews may be consulted for detailed information on analytical methods. These cover the current state of trichothecene determination in general and DON determination in particular (Krska, Baumgartner & Josephs, 2001; Mateo et al., 2001; Koch, 2004; Lattanzio, Pascale & Visconti, 2009), more general aspects of mycotoxin determinations (Krska et al., 2005, 2008; Sforza, Dall'Asta & Marchelli, 2006; Cigi & Prosen, 2009; Turner, Subrahmanyam & Piletsky, 2009), LC-MS/MS of mycotoxins (Zollner & Mayer-Helm, 2006), immunoaffinity column cleanup techniques for general food analysis, including mycotoxins (Senyuva & Gilbert, 2010), and developments in immunosensors (Ricci et al., 2007).

3.2.1 Screening tests

Various analytical techniques have been adapted and developed to screen for DON. These include thin-layer chromatography (TLC), infrared spectroscopy and a number of assays reliant on immunological principles using anti-DON antibodies, including ELISAs, test strips, surface plasmon resonance and direct fluorescence and fluorescence polarization measurements. A number of these immunological assays are commercially available and have been reviewed (Schneider et al., 2004). They should be used to analyse only matrices for which they are validated and in the test ranges set by the manufacturers. Non-commercial immunochemical assays should be carefully validated in the developing laboratory and are generally limited in use to the research laboratory. A policy on antibody characterization for conducting Association of Official Analytical Chemists (AOAC) collaborative studies for immunochemical methods stipulates that monoclonal and polyclonal antibodies should be described in terms of their purification method, avidity, specificity (cross-reactivity), matrix effects, selectivity (binding in immuno-affinity column formats) and specific capacity in the assay format (Fremy & Usleber, 2003).

ELISA is a well-established analytical format, mostly available from commercial companies with LODs and analytical ranges relevant to legislative requirements. However, useful antibodies, such as one for simultaneously detecting both DON and NIV, are still being described (Maragos, Busman & Sugita-Konishi, 2006). In addition, antibodies described before the discovery of DON-3-glucoside can now be tested for cross-reactivity with this plant metabolite, which can be responsible for overestimation of DON levels in conventional ELISA tests for the mycotoxin (Ruprich & Ostry, 2008). Similarly, commercial companies have continued to develop the concept of fluorometry for rapid (around 15 min) testing in which the cereal extract is cleaned up on proprietary columns and derivatized with fluorogenic reagent before measurement of total fluorescence on a proprietary fluorometer with dedicated software (Malone, 2001; Hafner et al., 2007). Fluorescence polarization is a technique that was first described nearly 50 years ago and has recently, with improved commercial instrumentation, been adapted for mycotoxin determination in wheat, semolina and pasta (Maragos & Plattner, 2002; Maragos, Jolley & Nasir, 2002; Lippolis, Pascale & Visconti, 2006; Maragos, 2006). Fluorescence polarization does not require separation of a bound and free label as in ELISA, but is performed purely as a solution-phase assay. It relies on the measurement of the rate of rotation of fluorescent molecules in which smaller molecules (such as a fluorescent-labelled mycotoxin substrate) rotate faster than larger molecules (such as the same fluorescent-labelled mycotoxin that has competed with unlabelled analyte for binding on the relevant antibody). These assays have been reported to have LODs of 0.1 mg/kg and recoveries above 90%, depending on the matrix and the tracer used (Maragos & Plattner, 2002; Lippolis, Pascale & Visconti, 2006). A disadvantage of fluorescence polarization is the presence of a background or matrix effect from cross-reacting compounds, which made the method unsuitable for maize and required a background correction for wheat and wheat products (Maragos & Plattner, 2002).

A number of other immunological methods have been investigated for the screening of DON. Assays based on surface plasmon resonance have been developed and tested for determination of DON in wheat by comparison with LC-MS/MS determinations (Tudos, Lucas-van den Bos & Stigter, 2003) or by comparison with GC-MS or HPLC (Schnerr, Vogel & Niessen, 2002). One of the simplest and fastest technologies is the lateral flow device, usually in the format of a strip or dipstick, which provides a simple test for contamination above or below a set level (Kolosova et al., 2008; Xu et al., 2010). DON in the sample extract interacts with colloidal gold–conjugated anti-DON antibodies at the base of the stick. Both bound and unbound antibodies are carried along the stick membrane by the extract solvent, passing a test line composed of immobilized mycotoxin, which will bind free antibody to form a visible line indicating a level of DON contamination below the test cut-off value. Typically, commercial kits contain a control line farther along the stick composed of anti-antibodies as a control for complete extract migration along the strip. Issues related to this technology, apart from the matrices for which the test is valid, include the cut-off limit set by the producer and the degree of false negatives during testing. As this is a screening technology, false positives are less serious, as such samples would normally be further tested by a fully quantitative method. This system has been commercialized for semiquantitative results by including two test lines and a proprietary photometric reader (Chrpova et al., 2008).

The desire for multiple analyses has resulted in the development of array biosensors, which can be used for simultaneous analysis of multiple samples or simultaneous analyses of multiple target analytes (Ngundi et al., 2006; Sapsford et al., 2006). The multiple targets for this technology included large pathogenic bacteria (*Campylobacter* spp.), as well as DON and other mycotoxins, such as aflatoxin B_1, ochratoxin A and fumonisin B_1. Silanized microscope slides were patterned with suitable capture species for the sandwich immunoassay used for the bacterial assay and the competitive immunoassay used for the mycotoxin assay. The glass slides acted as a waveguide for the detection system, which involved incident laser light launched into the end of the waveguide and charge coupled device (CCD) camera recording of the fluorescence of surface-bound species resulting from excitation by the evanescent wave.

All the above screening techniques require the extraction of DON from the sample before the analytical step. Considerable interest has been shown in developing a non-destructive instrumental method for detection of DON in ground wheat or maize without sample extraction by using near-infrared (10 000–4000/cm), mid-infrared (4500–650/cm) or Raman (3600–100/cm) spectroscopy combined with chemometric analysis (Pettersson & Aberg, 2003; Kos, Lohninger & Krska, 2003; Kos et al., 2004, 2007; De Girolamo et al., 2009; Liu, Delwiche & Dong, 2009). These approaches have shown potential for discrimination between wheat batches at levels that would be useful in terms of the limits set by the EC for DON, but they require large data sets for calibration.

3.2.2 Quantitative methods

The fifty-sixth meeting of the Committee summarized the basic steps for quantification of DON in cereals and food matrices, reviewing extraction, cleanup,

chromatographic separation, detection and performance characteristics. Apart from separation by TLC, both GC and HPLC have been used, and researchers have investigated two-dimensional GC (Jelen & Wasowicz, 2008). For quantification by GC, flame ionization, electron capture and MS detectors have been used. Of these three methods, FID has had limited use, and most publications have reported either ECD or MS detection (Mateo et al., 2001; Krska, Baumgartner & Josephs, 2001; Koch, 2004; Lattanzio, Pascale & Visconti, 2009). MS or MS/MS has the advantage of sensitivity, as well as providing confirmatory evidence in the form of characteristic fragment ions. DON and other trichothecenes are oxygenated polar compounds and require derivatization to increase volatility before they can be injected into a GC column. However, as the trichothecenes are structurally similar and possess similar chemical properties, GC offers the advantage of being capable of determining a range of trichothecenes simultaneously, including 3-Ac-DON and 15-Ac-DON. Common derivatization reactions at the hydroxyl moieties of DON involve the formation of trimethylsilyl ethers or trifluoroacetyl, pentafluoropropionyl or heptafluorobutyryl ester derivatives. Problems of multiple reaction products can be overcome by using mixtures of derivatization reagents, such as 1-(trimethylsilyl)-imidazole, trimethylchlorosilane and *N,O*-bis(trimethylsilyl)acetamide (Mateo et al., 2001). The silylating reagent, *N,N*-dimethyl-trimethylsilyl-carbamate, has been proposed as a suitable single reagent for silylation of DON, NIV and diacetoxyscirpenol (Eke & Torkos, 2004). The analytical method was applied to the determination of DON and NIV in maize grits and semolina (Eke, Kende & Torkos, 2004). MS detection limits of 0.05–0.35 mg/kg were slightly lower than those of 0.30–0.47 mg/kg achieved for GC-FID. For fluoroacylation of type B trichothecenes, the heptafluorobutyryl esters are preferable to trifluoroacetyl esters in terms of response, but are unsuitable for determination of 15-Ac-DON as a result of stereochemical hindrance during the derivatization reaction (Mateo et al., 2001). Other authors have found pentafluoropropionic anhydride to be preferable to heptafluorobutyric anhydride as a derivatization reagent due to its greater stability against moisture (Valle-Algarra et al., 2005). Quantitative methods have used a number of different internal standards, with mirex being used for ECD (Koch, 2004) and *n*-docosane, neosolaniol or α-chloralose being employed in FID or MS detection (Schothorst & Jekel, 2001; Eke, Kende & Torkos, 2004; Jestoi, Ritieni & Rizzo, 2004). GC-MS has been employed for trichothecene analysis and identification using electron impact ionization, negative chemical ionization and positive chemical ionization (Melchert & Pabel, 2004). Melchert & Pabel (2004) provided a list of key fragment ions of trimethylsilyl derivatives of various trichothecenes for toxin identification using the above three ionization methods in an ion trap system operating in the multiple MS mode. More recently, a fully ^{13}C-labelled DON has been used as an internal standard in GC-MS (Neuhof et al., 2009).

In order to avoid the problems of derivatization for GC, methods have been developed for the determination of DON using HPLC with UV detection at a wavelength of 220 nm. A number of methods for DON in different matrices using HPLC-UV have been validated and their performance characteristics determined by interlaboratory collaborative studies. MacDonald et al. (2005) studied an HPLC method with UV detection (220 nm) for the determination of DON in cereals (oat flour, rice flour and wheat flour) and cereal products (polenta and wheat-based

breakfast cereal). DON was extracted from samples with water by homogenization, and, after filtration, an aliquot was cleaned up on an immunoaffinity column. The column was washed with water and DON eluted with acetonitrile or methanol. Mean recoveries ranged from 78% to 87% at levels between 200 and 2000 µg/kg. Intralaboratory repeatability (relative standard deviation for within-laboratory results) was 3.1–14.1%, and interlaboratory reproducibility (relative standard deviation for between-laboratory results) was 11.3–26.3%. Horwitz ratio values were less than 1.3. The method was validated for determinations above 100 µg/kg. This method was further validated by Neumann et al. (2009) to test its applicability for the analysis of soft wheat. In this second study, repeatabilities ranged from 3.1% to 14.8% and reproducibilities from 21.0% to 32.9%, and Horwitz ratio values were 1.0–1.9. Mean recovery at 500 µg/kg was 84%.

Sugita-Konsihi et al. (2006) also studied an HPLC method with UV detection (220 nm) for the determination of DON in wheat. The method involved extraction of DON with acetonitrile–water (85:15 by volume) by shaking for 30 min. An aliquot was then cleaned up on a multifunctional column, with a portion of the eluate being collected, dried down and reconstituted in HPLC mobile phase. Intralaboratory repeatability was 5.8–11.3%, and interlaboratory reproducibility was 12.0–20.7%. Mean recovery was 100.0% at a level of 1.1 mg/kg, and Horwitz ratio values were less than 1.0. The LOD was 0.10 mg/kg. Another interlaboratory study has determined the performance characteristics of an HPLC method with UV detection for baby food and animal feed (Stroka et al., 2006). The samples were extracted with water by shaking for 1 h. Thereafter, an aliquot was cleaned up on an immunoaffinity column. Repeatability for analysis of baby food was 6.4–14.0% and for animal feed was 6.1–16.5%. Reproducibility was 9.4–19.5% for baby food and 10.5–25.2% for animal feed. Horwitz ratio values were equal to or less than 1.3. The authors recommended the method for DON at levels equal to or above 60 µg/kg for baby food and equal to or above 200 µg/kg for animal feed. Mean recoveries ranged between 89% at 120 µg/kg and 85% at 240 µg/kg for baby food and between 100% at 200 µg/kg and 93% at 400 µg/kg for animal feed.

Although the above validated methods rely on the natural UV absorption of DON, other methods involving derivatization have been developed to improve LODs using fluorescence. Precolumn derivatization with coumarin-3-carbonyl and reversed-phase HPLC with fluorescence detection allowed the determination in wheat of three type A trichothecenes and five type B trichothecenes, including DON, 3-Ac-DON and 5-Ac-DON, with LODs of 0.2–1 µg/kg (Dall'Asta et al., 2004). A post-column derivatization with methyl acetoacetate achieved the determination of DON, 3-Ac-DON and 15-Ac-DON in wheat down to an LOD of 8 µg/kg (Buttinger & Krska, 2003).

Since the fifty-sixth meeting of the Committee, the most significant advance in mycotoxin analysis has been the application of LC-MS(/MS) with atmospheric pressure ionization techniques, such as electrospray ionization, atmospheric pressure chemical ionization or atmospheric pressure photoionization, for the determination and confirmation of mycotoxins. Typically, MS detection is achieved by selected ion monitoring of a pseudo-molecular ion (frequently the protonated molecular ion in positive electrospray ionization) or ion fragment or by multiple

reaction monitoring of a given fragmentation product for quantification and of one or two other specific fragments as confirmation (Zollner & Mayer-Helm, 2006). For mycotoxin determination, triple quadrupole instruments are the most commonly used, although ion trap and time of flight instruments have also found application. LODs achieved in these assays are strongly instrument dependent, but are generally sufficient to meet the strictest legislative requirements. The most important feature (and problem) for method validation of LC-MS/MS methods is the phenomenon of enhancement or suppression of the analytical signal due to the presence of matrix components in the HPLC column eluate entering the MS ionization source. This has the effect of increasing or decreasing the slope of the calibration line (Sulyok, Krska & Schuhmacher, 2007a). The effect is generally overcome by using matrix-matched standard solutions for calibration. Alternatively, some research groups have synthesized stable isotope–labelled (generally ^{13}C) mycotoxins for use as internal standards, and these are now commercially available (Haubl et al., 2006; Asam & Rychlik, 2007). Apart from the above considerations, two distinct trends have emerged in the application of MS in HPLC systems. The MS can be viewed as a sensitive and specific detector for single or chemically similar toxins, such as its use for concurrent analysis of cereals for DON and NIV (Plattner & Maragos, 2003; Tanaka et al., 2009). Alternatively, the power of the modern MS instrument in achieving rapid mass analysis can be utilized to determine multiple toxins in a single HPLC run (Tanaka et al., 2006; Lattanzio et al., 2007; Ren et al., 2007; Sulyok, Krska & Schuhmacher, 2007b; Spanjer, Rensen & Scholten, 2008; Beltrán et al., 2009; Frenich et al., 2009; Monbaliu et al., 2009). This latter use of the MS highlights the second trend in LC-MS/MS analysis of mycotoxins—namely, the use of a "dilute-and-shoot" technique in which the latest instruments are sufficiently sensitive for the sample extract to be merely diluted without a specific cleanup and injected directly into the HPLC system (Sulyok, Krska & Schuhmacher, 2007b; Spanjer, Rensen & Scholten, 2008; Beltrán et al., 2009; Frenich et al., 2009). Where matrix effects are associated with this technique, matrix-matched standards can be used. This approach circumvents the problem of a single cleanup technique for the chemically diverse mycotoxins. Various approaches to cleanup for multitoxin analysis have been tried. Beer samples have been cleaned up by using conventional reversed-phase (C18) solid-phase extraction (Romero-González et al., 2009), sweet peppers have been analysed using multiple types of solid-phase extraction cartridges (Monbaliu et al., 2009), reversed-phase cartridges have been used for wheat, maize, barley, snacks and infant foods (Lattanzio, Solfrizzo & Visconti, 2008) and a multifunctional column has been used for maize feed samples (Ren et al., 2007). An alternative approach is the application of immunoaffinity columns containing antibodies against DON, aflatoxins, fumonisins, ochratoxin A, ZEA and T-2 toxin (Lattanzio et al., 2007). This has been incorporated in a multitoxin analysis for these legislatively important mycotoxins.

The majority of analytical method development has focused on DON and other trichothecenes, and little work has been performed specifically with the acetylated derivatives, 3-Ac-DON and 15-Ac-DON, or with the relatively newly described DON-3-glucoside, a bound form of DON. The acetylated DON mycotoxins can be determined by the GC or MS methods discussed above for DON, although careful selection of GC column that can achieve separation of the

acetylated forms of DON is required (Valle-Algarra et al., 2005). LC-MS/MS is ideal for the identification, analysis and confirmation of DON-3-glucoside, which has been identified, synthesized and determined to occur naturally in cereal samples contaminated by DON (Berthiller et al., 2005; Dall'Asta et al., 2005), as well as in beer at levels comparable to DON itself (Zachariasova et al., 2008). Alternatively, a method has been optimized for the determination of bound DON in barley grain by means of hydrolysis of bound forms in which trifluoroacetic acid is added to the extraction solvent and sample matrix (Zhou et al., 2007). The entire mixture is heated to simultaneously hydrolyse and extract total DON, and the final determination is by GC with ECD.

4. SAMPLING PROTOCOLS

The generation of meaningful analytical data requires the sampling stage to be as representative as possible. Owing to the lack of homogeneity in the distribution of mycotoxin contamination, which results from differences in fungal contamination of individual units of the raw materials, such as cereal kernels and nuts, the sampling stage of the overall mycotoxin analysis can frequently represent the greatest contribution to the overall variance of the result. Previous work on sampling plans for DON in wheat showed that for a batch level of 5.0 mg/kg, the coefficients of variation associated with the three stages of the analytical process —namely, sampling (based on a 0.45 kg sample), sample preparation (based on grinding and subsampling with a Romer mill) and chemical analysis (by Romer fluoroquant method)—were 6.3%, 10% and 6.3%, respectively (Whitaker et al., 2000). Hence, the variance introduced by sampling for the determination of DON in wheat is much less than that associated with other mycotoxin–matrix combinations. This is partly due to the relatively small kernel size of wheat, which implies that a given sample mass will represent a greater number of potentially contaminated units.

For the purpose of official control of the levels of mycotoxins, including DON, in foods, the EC has regulated sampling protocols, which stipulate, for a given batch of a commodity, the number and size of the incremental samples and size of the aggregate sample to be taken for control purposes (EC, 2006). Similarly, the regulations lay down criteria to be met by the analytical methods used in official control laboratories. Based on the EC sampling regulations, the distribution of DON and ochratoxin A within a 26-tonne truckload of wheat kernels was investigated by analysing all the incremental samples (100) taken to form an aggregate sample (Biselli, Persin & Syben, 2008). The results indicated that the variability associated with ochratoxin A (mean level 0.6 µg/kg ± 200% relative standard deviation [RSD]) was much larger than that associated with DON (mean level 1340 µg/kg ± 25% RSD); they also indicated that for multiple mycotoxin testing, the EC-regulated sampling levels could not be reduced. Recently, geostatistical analysis has been applied to the distribution of DON and ochratoxin A in a bulk lot of wheat kernels (Rivas Casado et al., 2009). The results indicated that DON presented spatial structure (possibly as it is formed pre-harvest in the field), whereas ochratoxin A was randomly distributed in the lot (possibly because of its production in "hot spots" during storage). The spatial structure of DON would indicate that the location of

sampling points as well as the number should be considered in designing sampling plans.

5. EFFECTS OF PROCESSING

Knowledge of the fate of mycotoxins during processing is important for both dietary exposure estimation and adoption of measures for its minimization. The fifty-sixth meeting of the Committee (Annex 1, reference *153*) reviewed the use of gravity separators that separate particles on the basis of differences in specific gravity, size, shape and surface texture to reduce DON concentrations. The effectiveness of milling practices, high-temperature and high-pressure cooking, baking and the use of microorganisms in reducing trichothecene concentrations was also reviewed. Additional studies conducted since the review are summarized below.

Studies conducted on the distribution of DON in wheat grains showed that effective removal of all screenings and outer layers of bran from the surface of wheat grains during the cleaning steps reduced the DON content by 50%, 55%, 41% and 47% in four samples, respectively. The highest levels of DON and the sum of 3- and 15-Ac-DON were concentrated in the waste fractions—namely, screenings and outer layers of bran (Lancova et al., 2008a).

Studies involving milling (removal of bran layer by pearling) of barley to produce white flours may lead to reductions in DON levels in the finished products (House, Nyachoti & Abramson, 2003). The distribution of DON in the wheat and processed fractions showed the concentration of the toxin in the outer portions of the kernel (bran), with lowered levels in the flour (Samar et al., 2003).

A single dry milling study to investigate the redistribution of DON and 16 other *Fusarium* toxins in maize resulted in an accumulation of toxins in fractions used mainly for the production of feedstuffs. High concentrations of DON, 3-Ac-DON and 15-Ac-DON were found in screenings, bran, germ or germ meal. 15-Ac-DON, ZEA, HT-2 toxin and T-2 toxin were detected in germ oil as a result of the higher lipophilic properties of these substances compared with the other toxins (Schollenberger et al., 2008). Recent studies conducted by Scudamore & Patel (2009a) showed similar results with mycotoxins concentrated in the feed components, such as the maize germ, meal, bran and broken maize.

The use of high-speed optical sorting for reducing the concentration of DON in *Fusarium*-infected soft red winter wheat has been reported (Delwiche, Pearson & Brabec, 2005). Commercial wheat samples of low (<1 mg/kg) to very high (>20 mg/kg) DON concentrations were sorted by the simultaneous analysis of two wavelengths (675 nm and 1480 nm) at a feed rate of 0.33 kg/(min-channel). On average, with one-pass sorting, the DON concentration of the sorted wheat was 51% of the original concentration, with successive passes further reducing the concentration of DON.

The effectiveness of detoxification procedures for specific mycotoxins depends largely on the structure and reactivity of the toxin molecule. Most chemical methods for DON reduction in cereal grains depend upon wetting with aqueous alkaline solutions, with optimal heat treatment. The fifty-sixth meeting of the

Committee noted the use of sodium carbonate solution for soaking or as a first wash for contaminated barley, maize and wheat in reducing DON levels. Since then, additional studies (Lauren & Smith, 2001; Abramson, House & Nyachoti, 2005; Ragab et al., 2007) have further confirmed the removal of DON from naturally contaminated whole barley and wheat through washing and/or soaking in water or sodium carbonate solutions. The effects of temperature, time and the use of various levels of sodium bicarbonate on naturally contaminated ground corn are also reported (Lauren & Smith, 2001). Subjecting naturally contaminated ground corn to 10% and 20% (volume by weight) 1.19 mol/l sodium bicarbonate solution at 80 °C for 2 days resulted in nearly equal reduction levels. With the same conditions, after 12 days of heating, greater DON reduction was observed at the 20% level. In heating trials with solutions containing DON at 5 µg/ml at 80 °C, 84% of the DON was destroyed with a 1.19 mol/l sodium bicarbonate solution, and 100% of DON was destroyed with a 1 mol/l solution (Lauren & Smith, 2001).

Studies by Abramson, House & Nyachoti (2005) on naturally contaminated barley sealed in polypropylene containers and subjected to heat (80 °C) and varying amounts of water or a 1 mol/l sodium carbonate solution showed reductions in DON down to near-zero values, depending on experimental conditions.

Recent studies on the fate of DON in contaminated wheat grain during the preparation of Egyptian "balila" (soaked and boiled whole wheat kernels with sugar, nuts and milk) showed that boiling contaminated wheat kernels in water reduced the DON content of grain by 70%, most probably through leaching out of DON into the boiling medium, which is subsequently discarded. Combined treatment of soaking in a 0.1 mol/l sodium carbonate solution (pH 11) with subsequent boiling reduced the DON content of the grain by 93% (Ragab et al., 2007). Further studies are required to investigate potentially harmful degradation products as well as consumers' acceptance of the product when sodium carbonate is used.

Visconti et al. (2004) studied the effects of processing and spaghetti cooking on DON levels. Nine samples of durum wheat contaminated with DON under field conditions (three samples naturally contaminated; six samples artificially inoculated with *Fusarium*) at levels ranging from 0.3 to 13.1 µg/g were processed and cooked into spaghetti. Reductions in DON levels occurred during the different steps of processing and spaghetti cooking: 23%, 63%, 67% and 80% in cleaned wheat, semolina, spaghetti and cooked spaghetti, respectively, relative to the uncleaned wheat. A repartition of DON between dry cooked spaghetti and cooking water was observed during cooking, with increasing DON leaching as the water to spaghetti ratios were increased during cooking.

Hot water treatments of *Fusarium*-infected malting barley resulted in significant ($P < 0.05$) reductions in *Fusarium* infection (Kottapalli & Wolf-Hall, 2008). One minute of treatment at 45 °C and 50 °C resulted in 41–66% and 51–69% reductions in *Fusarium* infection, respectively. After 20 min, reductions of 65–92% at 45 °C and 71–98% at 50 °C were reported. Significant reductions in DON (54–71%) were observed in malts prepared from barley treated at 45 °C or 50 °C for 1 min. The largest reductions for DON were observed in malts prepared from barley treated with hot water at 45 °C (79–93%) and 50 °C (84–88%) for 20 min.

DON is stable at 120 °C, moderately stable at 180 °C and partially unstable at 210 °C (Annex 1, reference *153*). Since the last review by the Committee, some studies on the effects of frying, baking and extrusion cooking on DON and, to some extent, 3-Ac-DON have been conducted (Cazzaniga et al., 2001; Samar et al., 2001, 2007; Lancova et al., 2008a; Scudamore et al., 2008a,b, 2009; Valle-Algarra et al., 2009). These studies are briefly reviewed below.

The effectiveness of traditional home frying of turnover pie dough cover of "empanadas" in reducing DON concentrations was studied at three ordinary frying temperatures (Samar et al., 2007). Frying flour artificially contaminated with DON at 260 µg/kg at 169, 205 and 243 °C resulted in reductions of 66%, 43% and 38%, respectively. Flour naturally contaminated at a DON level of 1200 µg/kg was also fried at similar temperatures (169, 205 and 243 °C). DON concentrations were reduced by 28%, 21%, and 20%, respectively.

The processes used for baking bread and non-yeasted products (cakes/biscuits) vary considerably in fermentation, baking conditions, time, temperatures and the inclusion of additives in the dough mixture. Available data on the effects of baking are therefore conflicting. Some studies report an increase, whereas other work observed a reduction by over 40% during dough fermentation. These have been reviewed by Pacin et al. (2010).

Recent studies have shown that baking bread at 210 °C for 14 min had no significant effect on DON levels (Lancova et al., 2008a).

A study to evaluate the stability of naturally occurring DON (150 mg/kg) during the fermentation stage of the bread-making process was conducted by Samar et al. (2001). Controlled experimental conditions were employed, and dough was fermented at 30, 40 and 50 °C according to standard procedures used in Argentinean low-technology bakeries. Fermenting dough at 50 °C resulted in a maximum reduction in DON levels by 56% for Vienna bread and 41% for French bread.

Scudamore et al. (2009) studied the effect of baking bread, cake and biscuits on DON concentrations. Baking of both white and wholemeal bread at 210 °C for 21 min from flour naturally contaminated with DON at 284 µg/kg reduced DON concentrations by 35–40%. These results are based on an "as is" basis; if moisture content and the presence of other ingredients are taken into account, the loss of DON was less than 5% or 11%, respectively, confirming the stability of DON during the processing. Reductions in concentrations of DON during baking of biscuits and cakes when compared with the concentrations in the flour were due to dilutions with other ingredients and not to processing.

Valle-Algarra et al. (2009) monitored changes in DON, 3-Ac-DON, NIV and ochratoxin A levels in wheat flour during the bread-making process. Wheat flour used was spiked at three levels (200, 750 and 1500 µg/kg) for both DON and 3-Ac-DON. Dough was fermented with *Saccharomyces cerevisiae*. Baking was at different combinations of temperature (190, 207, 223 and 240 °C) and time (50, 40, 35 and 30 min). Fermentation did not affect the levels of DON, 3-Ac-DON or NIV, but ochratoxin A levels were significantly ($P < 0.05$) reduced by between 29.8% and

33.5%, depending on the initial concentration of toxin in the flour. Reductions in all four toxin levels were reported during baking. There were significant differences ($P < 0.05$) among the different mycotoxins. The average reduction percentages for DON, 3-Ac-DON, ochratoxin and NIV were 47.9%, 65.6%, 32.9% and 76.9%, respectively. No significant differences ($P < 0.05$) in the reduction percentages of each toxin in relation to the temperature–time combination used were reported.

The effect of extrusion cooking on the stability of DON in maize flour in the presence and absence of additives has been studied by Cazzaniga et al. (2001). Detoxification levels higher than 95% were obtained using moisture contents of 15% and 30%, temperatures of 150 and 180 °C and metabisulfite concentrations of 0% and 1%.

In a study by Scudamore et al. (2008a), concentrations of NIV and ZEA were minimally changed by extrusion of wholemeal wheat grain. The amount of DON was decreased by 18.9–23.4% at the lowest moisture content of 15%. This effect was not temperature dependent and may be due to either binding or inability to extract the toxin from the extruded product (Scudamore et al., 2008a). Further extrusion studies with naturally contaminated maize grits (143 µg/kg) by Scudamore et al. (2008b) showed DON to be relatively stable. Temperature had little or no effect on DON concentration, although minor losses were reported under all conditions, probably for the same reasons noted above (binding to cereal components and/or reduced extractability). Addition of 2% sucrose (by weight) had no effect on DON levels. The presence of 2% sodium chloride resulted in slightly higher DON levels, which may be due to the fact that sodium chloride assists in extraction and is used in extraction procedures for several mycotoxins (Scudamore et al., 2008b).

The effects of superheated steam as a processing medium on *Fusarium*-infected wheat kernels with a DON concentration of 15.8 mg/kg have been studied. Reductions in DON concentrations of up to 52% were achieved at 185 °C with superheated steam and 6 min processing time. Thermal degradation was found to be the dominant factor in the destruction of DON (Cenkowski et al., 2007).

Treatment of wheat containing DON at 7.6 mg/kg with sodium metabisulfite at 10 g/kg for 15 min at 100 °C, at a moisture content of 22% and a permanently saturated steam supply under permanent mixing, reduced the DON concentration to 0.28 mg/kg (Dänicke et al., 2005).

The fate of DON during malting, mashing and fermentation has been reviewed (Hazel & Patel, 2004). Steeping lowers DON levels due to the water solubility of the toxin. Germination tends to increase DON levels because of conducive conditions created for *Fusarium* growth and toxin formation. Mashing results in increases in DON levels due to enzymatic release of the toxin from protein conjugates. During the first 20 h of fermentation, DON levels are reported to increase due to the conversion of metabolic precursors to DON. The subsequent decrease during the fermentation process has been attributed to yeast absorption or metabolism of the toxin (Hazel & Patel, 2004).

DON-3-glucoside was detected in malt and beer made from barley naturally contaminated with *Fusarium* (Lancova et al., 2008b; Kostelanska et al., 2009). Although DON conjugates with higher masses (presumably diglucosides and triglucosides) have been found in beer, significant increases in DON-3-glucoside

levels in malt over the DON plus DON-3-glucoside in the grain used have also been observed (Berthiller et al., 2009a). The possibilities are that additional mycotoxin that is conjugated is produced by the fungus during the initial steps of the malting process of *Fusarium*-infected barley or that bound mycotoxin originally present in the cell wall polymer fraction might be enzymatically released during malting.

The fate of DON and the sum of 15-Ac-DON and 3-Ac-DON during the malting and brewing processes using naturally infected barley and barley artificially inoculated with *Fusarium* spp. during the time of flowering as raw material was studied (Lancova et al., 2008b). Steeping reduced DON to levels below the limit of quantification (LOQ) of 5–10 µg/kg. There was accumulation of DON during germination to levels higher than in the original barley, but no significant change occurred during the final malting stage (kilning). Overall, DON levels were 2.1 times higher in malt than in barley. Additionally, the occurrence of DON-3-glucoside was monitored during the beer production process. DON-3-glucoside levels were 8.6 times higher in malt than in barley, with further significant increases in levels occurring during the brewing process. Concentrations of the acetylated DON derivatives increased by 1.1 times during the malting process (Lancova et al., 2008b).

The fifty-sixth meeting of the Committee reviewed the microbiological transformation of DON to less toxic metabolites using microorganisms isolated from rumen fluid and soil under anaerobic and aerobic conditions in liquid culture (Annex 1, reference *153*).

Selected strains of *Lactobacillus* (*Lactobacillus rhamnosus* strains GG and LC-705) and *Propionibacterium* (*Propionibacterium freudenreichii* ssp. *shermanii* JS) were able to remove DON and some other trichothecenes from liquid media, although their abilities varied significantly. Both viable and non-viable forms of the bacteria removed DON, whereas 3-Ac-DON was not affected. GC-MS chromatographic peaks suggesting possible degradation of the toxin were absent, implying binding rather than metabolism as the explanation for removal (El-Nezami et al., 2002).

In another study, glucomannans extracted from the external part of the cell wall of *Saccharomyces cerevisiae* showed a binding capacity of 12.6% for DON (Yiannikouris & Jouany, 2002).

In vitro screening tests to ascertain the capacity of non-nutritive adsorbent materials to bind DON at various concentrations in phosphate-buffered solutions showed binding levels generally higher than 50% with activated carbon (Avantaggiato, Solfrizzo & Visconti, 2005).

6. PREVENTION AND CONTROL

6.1 Pre-harvest control

The fifty-sixth meeting of the Committee reviewed measures to prevent and control *Fusarium* infection and DON contamination. The review covered culture techniques such as suitable crop rotation, appropriate use of fertilizers, irrigation and weed control, as well as growing resistant cultivars and the use of fungicides

or biological antagonists and decontamination procedures to reduce infection and DON formation (Annex 1, reference *153*).

Since the monograph for the fifty-sixth meeting of the Committee was prepared, several studies have concentrated on the use of fungicidal/biological antagonists to control *Fusarium* head blight (FHB) and reduce DON formation. Fewer studies are available on the use of culture techniques, use of resistant cultivars and decontamination procedures. Strategies to prevent mycotoxin contamination of food and animal feed have been reviewed (Kabak, Dobson & Var, 2006).

The use of suitable crop rotation is important and focuses on breaking the chain of production of infectious material, such as using wheat/legume rotations. The use of maize in a rotation is, however, to be avoided, as maize is also susceptible to *Fusarium* infection and can lead to carry-over onto wheat via stubble/crop residues. It is accepted that wheat that follows an alternative host for *Fusarium* pathogens is at greater risk of FHB and subsequent DON contamination of grain. Evidence is, however, conflicting that wheat following wheat is more at risk than wheat following a non-cereal crop (Edwards, 2004). It has also been observed that FHB disease severity and DON contamination of grain were significantly different when the previous crop was maize, wheat or soya bean, with the highest levels following maize and the lowest levels following soya bean (Dill-Macky & Jones, 2000). The Codex Alimentarius Commission has recommended that crops such as potato, other vegetables, clover and alfalfa that are not hosts to *Fusarium* species should be used in rotation to reduce the inoculums in the field (FAO/WHO, 2002). Applications of nitrolime to wheat plots reduced the incidence of FHB by 59% when compared with plots treated with calcium ammonium nitrate. There was, however, no significant effect on DON concentrations in harvested grain (Yi et al., 2001).

The Codex Alimentarius Commission (FAO/WHO, 2002) has further recommended that the soil must be tested to determine if there is a need to apply fertilizer and/or soil conditioners to ensure adequate soil pH and plant nutrition to avoid stress, especially during seed development. Fertilizer regimes may affect FHB incidence and severity by altering the rate of residue decomposition, by creating a physiological stress on the host plant or by altering the crop canopy structure. Lemmens et al. (2004) concluded that FHB cannot be sufficiently controlled by manipulating only the nitrogen input. Their work showed a significant increase in FHB intensity and DON contamination in the grain when the application of a mineral nitrogen fertilizer was increased from 0 to 80 kg/ha.

Irrigation is a valuable method of reducing plant stress in some growing situations. It is necessary that all plants in the field have an adequate supply of water if irrigation is used. Excess precipitation during anthesis creates conditions favourable for dissemination and infection by *Fusarium* spp., so irrigation during anthesis and during ripening of crops, specifically wheat, barley and rye, should be avoided (FAO/WHO, 2002).

There are inherent differences in the susceptibility of various cereal species to FHB, which are reflected in differences in the degree of mycotoxin contamination to which each species is susceptible. The differences in susceptibility between crop species appear to vary by country, probably due to differences in the genetic pool

within each country's breeding programme as well as the different environmental and agronomic conditions in which crops are cultivated (Edwards, 2004). Obtaining high levels of native genetic resistance in various crop types to toxigenic fungi has proven difficult. Problems in this regard have centred primarily on the lack of resistant control genotypes and the lack of involvement of single major genes (Munkvold, 2003). Kolb et al. (2001) and Ruckenbauer, Buerstmayr & Lemmens (2001) reviewed information on molecular markers associated with quantitative trait loci for resistance to FHB in wheat and barley and breeding strategies in resistance breeding against FHB, respectively.

Another factor known to increase the susceptibility of agricultural commodities to toxigenic mould invasion is injury due to insect, bird or rodent damage. These must be controlled in the vicinity of the crop by proper use of registered insecticides, fungicides and other appropriate practices within an integrated pest management control programme (FAO/WHO, 2002). The use and effects of some fungicides were reviewed by the fifty-sixth meeting of the Committee (Annex 1, reference *153*), and there was evidence that under certain conditions, fungicide use may actually stimulate toxin production. Studies on fungicides in common use have shown differential effects against toxin-forming *Fusarium* species and related non-toxin-forming pathogens, such as *Microdochium nivale* on ears (Simpson et al., 2001). The reliability of the use of fungicides seems to depend on the fungal species present, the effect the particular fungicide has on the species, the dose rate used, the time of application and even perhaps the method of application.

Recent in vitro studies on a range of *Fusarium culmorum* strains showed stimulation in DON production in the presence of epoxiconazole and propiconazole (Magan et al., 2002). Additional studies are available on the efficacy of the fungicides azoxystrobin, metconazole and tebuconazole at anthesis against *Fusarium* spp. and *Microdochium nivale* and for years on naturally infected fields of soft wheat, durum wheat and barley (Ioos et al., 2005). Infection levels of *F. graminearum, F. culmorum* and *M. nivale* were significantly reduced by the application. Tebuconazole and metconazole effectively controlled *Fusarium* spp. but had little effect on *M. nivale*. The control was, however, seasonal: tebuconazole controlled fungi in 2001 but had little effect in 2000 and 2002; metconazole significantly reduced levels in 2000 and 2001, but not in 2002. Although a few countries have recently allowed the use of several fungicides, including tebuconazole and metconazole, for the control of FHB at or about anthesis, in the European Union, fungicides must be shown to be safe to both the environment and humans before being authorized for use (Kabak, Dobson & Var, 2006).

The effects of prochloraz, tebuconazole, benomyl, carbendazim, guazatine and iminoctadine on mycelial growth of *F. graminearum* and 3-Ac-DON have been reported (Matthies, Walker & Buchenauer, 1999). Prochloraz inhibited mycelial growth and reduced 3-Ac-DON production. Tebuconazole inhibited fungal growth at 0.1, 0.5 and 1.0 µg/ml. At 0.5 µg/ml, however, 3-Ac-DON production was increased 4-fold compared with control experiments. Benomyl increased mycelial growth of *F. graminearum* by 22% and reduced 3-Ac-DON production by 22% compared with the untreated control. Carbendazim showed a dose-related inhibition

of mycelial growth and mycotoxin production when added to media at 0.5, 0.7, 1, 1.5 and 2 µg/ml. Guazatine and iminoctadine significantly reduced mycelial growth of *F. graminearum* in vitro, but increased 3-Ac-DON production by up to 200%.

The use of microorganisms is one of the most recent approaches currently employed to reduce mycotoxin contamination. Antagonistic microorganisms can reduce growth in *Fusarium* species, reduce severity of disease symptoms and reduce the levels of DON production. These microorganisms have been reviewed (Kabak & Dobson, 2009). Important factors for the successful application of FHB antagonists in the field have been listed as the potential deleterious effect of UV light, variable and sporadic arrival of pathogen inoculums on wheat heads over extended periods of head susceptibility and the phylloplane environment, with marked fluctuations in temperature, moisture and available nutrients (Schisler et al., 2002).

At the time of the fifty-sixth meeting of the Committee, only a few reports were available on biological control of FHB. Additional studies conducted since then are briefly described below.

Bacillus subtilis Ehrenberg strains NRRL B-30210 and B-30211 reduced FHB disease severity by 66% and 92% and reduced disease incidence by 35% and 78%, respectively (Schisler et al., 2002). A strain of *Fusarium equiseti* (G9) was found to be effective in controlling FHB and reducing DON formation by more than 70% on wheat (Dawson et al., 2004). Yeasts of the genus *Cryptococcus* are also reported to be effective against FHB. *Cryptococcus* sp. OH 71.4, OH 181.1 and OH 182.9 reduced FHB by up to 59% on durum wheat in the field (Khan et al., 2001). Treatment of heads of FHB-susceptible wheat with a *Streptomyces* sp. reduced both FHB disease severity and associated loss in grain weight by approximately 50% under glasshouse conditions (Nourozian, Etebarian & Khodakaramian, 2006). *Pseudomonas fluorescens* strains MKB 158 and MKB 249 and *Pseudomonas frederiksbergensis* strain 202 significantly reduced the severity of FHB disease symptoms caused by *F. culmorum* in wheat and barley grown under both glasshouse and field conditions. Treatment with either of the two strains in addition resulted in a 74–78% reduction in DON levels in wheat and barley grains in the *F. culmorum*-inoculated field trials (Khan & Doohan, 2009a). Additional studies showed that chitosan (the deacetylated derivative of chitin) was effective in reducing DON contamination of grain caused by *F. culmorum* and also reduced the severity of FHB symptom development on wheat and barley by over 74% (Khan & Doohan, 2009b). *Pseudomonas* sp. AS 64.4 isolated from wheat anthers was as effective as the fungicide tebuconazole for controlling FHB disease severity under field conditions (Kabak & Dobson, 2009). In another study, 22 bacterial strains isolated from wheat anthers in Argentina reduced the growth of *F. graminearum* and reduced the production of DON on irradiated wheat grains by 60–100% (Palazzini et al., 2007). In vitro studies on wheat and maize residues (straw/stalk and grain) showed that inoculating residues with a *Microsphaerosis* species (isolate P130A) significantly reduced *G. zeae* ascospore production by 73%. When applied to crop residues in the field, the *Microsphaerosis* species had no effect on the pattern of perithecial formation, but significantly reduced perithecial production (Bujold, Paulitz & Carisse, 2001).

Discrepancies between the performance of biocontrol agents under environmentally controlled and field conditions are an issue that is commonly observed and are a major obstacle to the development of commercial biocontrol products.

Specific tools, such as DONcast®, have been developed to assist in ameliorating mycotoxin contamination. DONcast® is a weather prediction–based tool to assist Canadian wheat farmers in deciding whether or not to apply appropriate fungicide treatments at anthesis to reduce the risk of eventual DON contamination (Weather Innovations Incorporated, 2008).

6.2 Decontamination

Numerous chemicals that have been tested for their ability to decontaminate trichothecene-contaminated grain/feed were reviewed by the fifty-sixth meeting of the Committee (Annex 1, reference *153*). These chemicals included sodium bisulfite, hypochlorite bleach and natural and modified clays, as well as treatment with moist ozone, ammonia and microwave radiation. Since the last review, no new information has been made available for review. This may be due to the fact that while some chemical treatments may destroy mycotoxins present in many foods and feeds, in many cases, they significantly decrease the nutritional value of the foods or produce toxic products or other products with undesirable effects, thus limiting their widespread use (Kabak, Dobson & Var, 2006).

7. LEVELS AND PATTERNS OF CONTAMINATION IN FOOD COMMODITIES

Information on the natural occurrence of DON was drawn from data received from a number of countries (Austria, Belgium, Brazil, China, Finland, France, Hungary, Japan, the Netherlands, Norway, Singapore and the United Kingdom), as well as surveys published in the open literature. The period of publication for incorporation of data was 2001–2009. Results of the EC's Scientific Cooperation on Questions relating to Food (SCOOP) report on mycotoxins (Schothorst & van Egmond, 2004) have been incorporated. Data gathered have been tabulated by region and country in the occurrence tables provided in Appendix 1. Data collected in the tables include information on LOD (or LOQ or both) and number of positive samples. For individual reports of surveys, the mean values reported are generally the mean of all samples, with concentrations in samples below the LOD being taken as zero. In some instances, particularly with the SCOOP data, means have been calculated based on a value of LOD/2 (or LOQ/6) for the samples without detected contamination; this is noted in the occurrence table footnote. Although the maximum level analysed in a given set of samples is recorded, information on distribution and 90th-percentile levels are mostly lacking.

It is noted that DON was a common contaminant in cereals (wheat, maize, oats, rye, barley, rice) and their products. Highest reported mean levels for raw cereals were as follows: wheat, 9900 µg/kg; maize, 4772 µg/kg; rice, 183 µg/kg; barley, 6349 µg/kg; oats, 537 µg/kg; and rye, 190 µg/kg. Contamination levels vary

widely between and within regions. Relatively lower levels were detected in processed products, such as baby food, beer, bread, biscuits, pasta, muesli, noodles, cereal-based snacks, pizza, polenta, couscous, flours and fermented soya bean, most likely due to the decrease in contamination resulting from cereal milling and processing. Mean levels of DON in samples of processed products did not exceed 1250 µg/kg. As noted by the fifty-sixth meeting of the Committee (Annex 1, reference *153*), carry-over of DON into animal products is negligible due to feed refusal, rapid metabolism and elimination in livestock species. A few reports have dealt with DON in hens' eggs and have concluded that transmission rates from feed to egg are between 15 000:1 and 29 000:1, implying that, compared with other routes of exposure, this is insignificant (Sypecka, Kelly & Brereton, 2004).

As was observed by the fifty-sixth meeting of the Committee (Annex 1, reference *153*), a range of analytical methods have been used for DON analysis. LODs and LOQs can vary with different methods and with different instrument sensitivities on the same method. This has a clear influence on the number of positive samples found in a batch. Some of these methods are applicable to DON itself, whereas certain chromatographic methods, such as HPLC-MS(/MS) or GC, are capable of determining DON derivatives, such as 3-Ac-DON, 15-Ac-Don and DON-3-glucoside. The occurrence data for the DON derivatives 3-Ac-DON and 15-Ac-DON in wheat, maize, barley, oats, rye and their products were considered by the Committee for the first time at the present meeting. In addition to data submitted by China, France, Japan and the United Kingdom, published data from studies conducted in Austria, Finland, France, Germany, the Netherlands, Norway, Sweden, the United Kingdom and the USA were also assessed. Data were available on 3-Ac-DON from 6980 samples (92% from Europe and 8% from Asia) and on 15-Ac-DON from 4300 samples (81% from Europe, 16% from Asia and 3% from the USA). Generally, these derivatives are infrequently detected, and levels were typically less than 10% of those reported for DON. Highest reported mean levels in wheat, maize and barley for 3-Ac-DON were 193 µg/kg, 27 µg/kg and 19 µg/kg, respectively; for 15-Ac-DON, the corresponding highest reported mean levels were 365 µg/kg, 236 µg/kg and 0.3 µg/kg. The Committee was aware of reports on DON-3-glucoside in cereals and beer (data on 500 samples were assessed, with 79% from China, 15% from Europe and 6% from the USA), but considered that the data were too limited for dietary exposure assessment.

For comparison of concentrations of DON and its derivatives, a separate table was compiled containing data of samples in which DON as well as (all or some of) its derivatives have been determined (see Table A9 in Appendix 1). The potential exposure to DON and its derivatives via beer consumption has been highlighted (Kostelanska et al., 2009). Levels of DON-3-glucoside have been reported to rise during brewing, and its level in beer can exceed that of DON itself (Lancova et al., 2008b).

8. FOOD CONSUMPTION AND DIETARY EXPOSURE ESTIMATES

Dietary exposure to DON was assessed according to the recommendations of a Food and Agriculture Organization of the United Nations/World Health

Organization (FAO/WHO) workshop on methods for assessing exposure to contaminants and toxins, which was held in Geneva in June 2000 (FAO/WHO, 2000). The workshop recommended that the median concentration should be given when data on individual samples are available, whereas a mean should be given when only pooled or aggregated data are available. In the case of commodities that contribute significantly to exposure, distribution curves should be generated to allow risk managers to determine the effects on dietary exposure of different maximum levels.

The workshop further recommended that international estimates of dietary exposure should be calculated by multiplying the mean or median concentration by the values for consumption of the commodity in the five Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) regional diets (WHO, 1998). In the interim, the number of regional diets has been expanded to 13 consumption cluster diets to better represent dietary tendencies around the world. They were established on the basis of information on food balance sheets compiled by FAO. As such information is available for most countries, the data are comparable across countries and regions of the world. The consumption cluster diets represent the average availability of food commodities per capita rather than actual food consumption.

The report of the workshop (FAO/WHO, 2000) noted that national exposure estimates should also be reported when available, as they may provide information about exposure by specific population subgroups or consumers with extreme exposure, which cannot be derived from GEMS/Food regional diets.

8.1 Methods

For this assessment, concentrations of DON in food commodities and in some processed foods were reported to FAO/WHO or were obtained from the literature. The quality and reporting of the data are discussed in the previous section. As the dietary exposures were based on the GEMS/Food consumption cluster diets, which include information on consumption of raw or minimally processed foods, concentrations of DON in processed foods were not used to estimate dietary exposure.

Information was available on the concentrations of six commodities: barley, maize, oats, rice, rye and wheat. Additionally, information on beer, the majority of which is produced from barley, was included. Data originating in 40 countries were analysed, representing 10 of the 13 GEMS/Food consumption cluster diets; no data were reported for the A, H and J clusters. These are primarily Central and East African countries (clusters A and J) and Central American and Caribbean countries (cluster H). The majority of the data were from European countries—more specifically, France. Of the six commodities for which data were available for the exposure assessment, data on barley, maize and wheat predominated, with limited reports on oats, rice and rye.

Most of the data available for this evaluation were pooled; that is, each data point represented the mean concentration in a number of individual samples. In calculating the mean values, samples in which the concentration was below the

LOQ or LOD were assumed to have a value of zero. The maximum analytical value was also reported for each data point. In total, 401 data points (mean values) representing 16 569 individual samples were included in the exposure assessment. Of those 401 data points, 207 were reported from cluster diet E countries, primarily Europe and the United Kingdom countries. The remaining 194 data points represented exposure to DON in the six commodities for the remaining nine reporting cluster diets.

For each commodity, the data were sorted according to the country groupings of the GEMS/Food consumption cluster diets. The number of data points reported, the number of individual samples represented, the highest maximum analytical value reported and the weighted average of all mean values are summarized in Table 4.

8.2 Concentrations in foods

The concentrations of DON used in estimating dietary exposures, summarized by commodity and region in Table 4, are described briefly below:

- *Barley:* Data on the concentrations of DON in barley were received from 10 countries. For the 1353 samples analysed, 433 (32%) had concentrations below the LOD. The weighted mean for all samples combined was 442 µg/kg, and the maximum analytical value reported was 10 000 µg/kg.
- *Beer:* Twenty-two countries reported data on a total of 727 samples of beer. Of these, 297 (41%) contained concentrations below the LOD. The weighted mean of all samples combined was 7 µg/kg, and the maximum analytical value reported was 57 µg/kg.
- *Maize:* Fourteen countries reported data on a total of 2643 samples of maize. Of these, 210 (8%) contained concentrations below the LOD. The weighted mean of all samples combined was 625 µg/kg, and the maximum analytical value reported was 13 000 µg/kg.
- *Oats:* Eight countries representing only three cluster diets submitted data on a total of 478 samples of oats. Of these, 238 (50%) contained concentrations below the LOD. The weighted mean of all samples combined was 79 µg/kg, and the maximum analytical value reported was 5004 µg/kg.
- *Rice:* Five countries representing four cluster diets submitted data on rice, including a total of 462 samples. Of these, 121 (26%) contained concentrations below the LOD. The weighted mean of all samples combined was 12 µg/kg, and the maximum analytical value reported was 34 µg/kg.
- *Rye:* Six countries reported data on a total of 909 samples of rye. Of these, 633 (70%) contained concentrations below the LOD. The weighted mean of all samples combined was 63 µg/kg, and the maximum analytical value reported was 1095 µg/kg.
- *Wheat:* Wheat was the only commodity with data reported from each of the 10 cluster diets that reported data. Twenty-nine countries reported data on 9997 samples of wheat. Of these, 2690 (27%) contained concentrations below the LOD. The weighted mean of all samples combined was 367 µg/kg, and the maximum analytical value reported was 14 000 µg/kg.

Table 4. Summary of data on concentrations of DON in commodities and beer from the GEMS/Food consumption cluster diets[a]

Commodity	Global total	B	C	D	E	F	G	I	K	L	M
Barley											
No. of data points	38			4	13	3	2			8	8
No. of individual samples	1 353			146	443	52	12			400	300
Weighted mean concentration (µg/kg)	442			570	27	60	8			142	1 475
Maximum value (µg/kg)				4 000	550	619	51			4 700	10 000
Beer											
No. of data points	32	7			18	3		2		2	
No. of individual samples	727	146			442	18		75		46	
Weighted mean concentration (µg/kg)	7	3			9	13		3		3	
Maximum value (µg/kg)		29			57	33		6		50	
Maize											
No. of data points	71	3		4	29	2	7	5		1	20
No. of individual samples	2 643	72		216	605	60	240	470		82	898
Weighted mean concentration (µg/kg)	625	3 729		209	551	143	292	843		103	582
Maximum value (µg/kg)		11 000		2 460	3 680	1 022	4 374	13 000		807	2 150
Oats											
No. of data points	18				8	9	1				
No. of individual samples	478				262	204	12				
Weighted mean concentration (µg/kg)	79				23	156	0				

Table 4 (contd)

Commodity	Global total	B	C	D	E	F	G	I	K	L	M
Maximum value (µg/kg)					530	5 004	0				
Rice											
No. of data points	17				8	1	2		6		
No. of individual samples	462				115	16	71		260		
Weighted mean concentration (µg/kg)	12				5	10	0		18		
Maximum value (µg/kg)					12	10	0		34		
Rye											
No. of data points	35			2	28	3	2				
No. of individual samples	909			187	691	28	3				
Weighted mean concentration (µg/kg)	63			0	82	15	0				
Maximum value (µg/kg)				0	1 095	178	0				
Wheat											
No. of data points	190	5	1	9	103	11	11	6	7	12	25
No. of individual samples	9 997	72	17	799	6 170	297	487	202	63	1 377	513
Weighted mean concentration (µg/kg)	367	802	27	46	304	192	399	777	303	65	2 339
Maximum value (µg/kg)		3 600	128	1 840	10 400	2 033	14 000	2 700	1 500	2 100	11 400
Total no. of data points	401										
Total no. of individual samples	16 569										

[a] Clusters A, H and J reported no data and are excluded.

8.3 Estimates of dietary exposure at the international level

The average dietary exposures to DON were calculated by multiplying the weighted mean concentration of each commodity by the corresponding amount of each commodity consumed in each of the 10 GEMS/Food consumption cluster diets that reported data (Table 5).

The total exposure to DON was estimated to range from 0.2 µg/kg bw per day (cluster C) to 14.5 µg/kg bw per day (cluster B). The main source of exposure in clusters C, D, E, F, G, K and M was wheat (56–100% of total exposure), whereas the main source in clusters B and I was maize; exposure in cluster L was evenly spread among maize, wheat and barley. The estimates of average exposure were based on the assumption that consumers choose foods randomly with respect to the distribution of concentrations of contaminants and will therefore be exposed to an approximation of the mean of that distribution over time. The Committee noted that the high estimates of DON exposure in clusters B and M were due to unusually high reported DON levels in maize and wheat in single countries for each cluster and that these data may not be representative of normal dietary exposures. The range of estimates in the remaining clusters is in agreement with those prepared at the fifty-sixth meeting.

It should be noted that any reduction in the concentration of DON as a result of processing has not been taken into consideration in this assessment.

8.4 National estimates of dietary exposure

Since the evaluation of DON at the fifty-sixth meeting of the Committee in 2001, a number of national evaluations of dietary exposure have been published. The Committee considered evaluations by the European Union (collectively) and for foods and cereal products in Argentina, Belgium, Czech Republic, Denmark, Ethiopia, France, Germany, Ireland, Lebanon, Morocco, the Netherlands, Nigeria, Republic of Korea and Thailand. Some of these reports contained overall dietary exposure assessments, whereas others assessed single commodities (or their products) considered to be the potential primary source of DON dietary exposure.

8.4.1 European Union

Following a number of years of high contamination levels of mycotoxins in grains in the 1990s, the European Union established a task for SCOOP to conduct a survey of levels of and resultant dietary exposures to mycotoxins, including DON (Schothorst & van Egmond, 2004). Austria, Belgium, Denmark, Finland, France, Germany, the Netherlands, Norway, Portugal, Sweden and the United Kingdom submitted data on DON, with appropriate food consumption data. France and the Netherlands submitted the majority of the analytical data, with wheat and wheat products having the most data points. For all of the reporting countries, mean dietary exposures were less than 1 µg/kg bw per day for all age groups considered. For France (all age groups) and Germany (young children), high-level exposure exceeded 1 µg/kg bw per day. Wheat flour and bread were the major contributors to dietary exposure.

Table 5. Commodity consumption (g/person per day) and the resulting dietary exposures (µg/day and µg/kg bw per day) for DON in the GEMS/Food consumption cluster diets[a]

	B	C	D	E	F	G	I	K	L	M
Food consumption (g/day)										
Barley	16.8	93.9	13.2	48.6	36.1	5.9	5.9	20.2	16.8	43.8
Beer	84.1	4.1	66.0	243.1	161.3	21.9	29.5	100.9	82.2	218.8
Maize	148.4	135.9	31.8	33.3	7.5	35.2	248.1	63.1	58.6	85.5
Oats	0.6	0.2	4.2	5.7	8.9	0.2	0.8	3.5	0.7	7.6
Rice	31.6	94.6	33.2	12.7	12.7	376.9	38.0	238.4	381.3	34.6
Rye	3.7	0.3	24.3	25.8	45.8	0.4	0.2	0.1	0.9	0.8
Wheat	396.3	426.5	390.2	236.3	216.0	172.9	68.1	114.1	103.4	234.2
Weighted DON concentration (µg/kg)										
Barley	N/A	N/A	570	27	60	8	N/A	N/A	142	1475
Beer	3	N/A	N/A	9	13	N/A	3	N/A	3	N/A
Maize	3729	N/A	209	551	143	292	843	N/A	103	582
Oats	N/A	N/A	N/A	23	156	0	N/A	N/A	N/A	N/A
Rice	N/A	N/A	N/A	5	10	0	N/A	18	N/A	N/A
Rye	N/A	N/A	0	82	15	0	N/A	N/A	N/A	N/A
Wheat	802	27	46	304	192	399	777	303	65	2339

Table 5 (contd)

	B	C	D	E	F	G	I	K	L	M
DON exposure (µg/day)										
Barley			7.5	1.3	2.2	0.0			2.4	64.6
Beer	0.3			2.2	2.1		0.1		0.2	
Maize	553.4		6.6	18.3	1.1	10.3	209.1		6.0	49.8
Oats				0.1	1.4	0.0				
Rice				0.1	0.1	0.0		4.3		
Rye			0.0	2.1	0.7	0.0				
Wheat	317.8	11.5	17.9	71.8	41.5	69.0	52.9	34.6	6.7	547.8
DON exposure (µg/kg bw per day)										
Barley			0.130	0.020	0.040	0.000			0.040	1.080
Beer	0.000			0.040	0.030				0.000	
Maize	9.220		0.110	0.310	0.020	0.170	3.490		0.100	0.830
Oats				0.000	0.020	0.000				
Rice				0.000	0.000	0.000		0.070		
Rye			0.000	0.040	0.010	0.000				
Wheat	5.300	0.190	0.300	1.200	0.690	1.150	0.880	0.580	0.110	9.130
Total DON exposure (µg/kg bw per day)	**14.520**	**0.190**	**0.540**	**1.610**	**0.810**	**1.320**	**4.370**	**0.650**	**0.250**	**11.040**

N/A, no data reported for this cluster

[a] Clusters A, H and J reported no data and are excluded.

The Committee noted that this report sparked a number of national evaluations by member countries to consider dietary exposure to mycotoxins in more detail. These European national DON evaluations (published) are explored individually throughout the remainder of this section.

8.4.2 Argentina

Dietary exposure to DON from bread consumption in Argentina was reported in 2010 (Pacin et al., 2010). Bread was considered to be the likely primary source of DON exposure in the Argentinean diet. It was noted that baking of French and Vienna breads resulted in 33% and 58% reductions, respectively, of DON from the levels in the wheat flour. The resulting estimations of dietary exposure were 0.065 µg/kg bw per day for French bread and 0.019 µg/kg bw per day for Vienna bread. The authors noted that consumption of other wheat-containing products, such as pizza, noodles, pasta, cookies and beer, could result in a higher total dietary exposure.

8.4.3 Belgium

The dietary exposures to DON from the consumption of beer and home-produced eggs in Belgium have been reported (Harcz et al., 2007; Tangni et al., 2009). DON exposure from consumption of conventional beer, at the 97.5th percentile, was 0.23 µg/kg bw per day. Mean exposure was less than 0.07 µg/kg bw per day for all beer types reported. DON exposure from the consumption of home-produced eggs had a maximum estimated level of 0.05 µg/kg bw per day.

8.4.4 Czech Republic

As part of a risk–risk analysis of the trade-off of use of fungicides versus mycotoxin contamination of grains, a margin of exposure (MOE) for DON in the Czech Republic was reported (Muri et al., 2009). Although dietary exposure was not reported, the lowest MOE (at the 1st percentile for the age group 4–19 years) was 11; a calculation using an effect dose of 30 µg/kg bw per day suggests that dietary exposure at the 99th percentile would be less than 3 µg/kg bw per day. The mean concentration of DON in foods was found to be 96 µg/kg.

8.4.5 Denmark

In the paper reporting the risk–risk analysis noted above for the Czech Republic, an MOE for DON in Denmark was also included (Muri et al., 2009). As for the Czech Republic, MOEs were reported. The lowest MOE (at the 1st percentile for the age group 4–19 years) was 14; a calculation using an effect dose of 30 µg/kg bw per day suggests that dietary exposure at the 99th percentile would be less than 2 µg/kg bw per day. The mean concentration of DON in foods was found to be 118 µg/kg.

Rasmussen, Petersen & Ghorbani (2007) reported on DON exposure from consumption of wheat and rye flour produced in Denmark between 1998 and 2003. The authors noted the great variability from year to year, with DON levels in wheat

flour ranging from 32 to 255 µg/kg. Although they noted reports that production of breads from contaminated wheat resulted in lower DON levels in the final products than in the raw material, they did not assume any reduction for the purposes of the dietary exposure assessments. They found mean exposure for the total population to be 0.17 µg/kg bw per day, with an exposure of 0.32 µg/kg bw per day for children using a deterministic approach. Use of the DON level in wheat flour from the year with the highest level of contamination resulted in a population mean of 0.31 µg/kg bw per day. A probabilistic evaluation of exposure to DON from consumption of wheat and rye flour found that at the 99.9th percentile for children, exposure was 0.9 µg/kg bw per day.

8.4.6 Ethiopia

A report concerning DON levels in barley, sorghum, teff and wheat from the 1999 crop year in Ethiopia stated that DON was found in only 4 of 23 wheat samples, with a mean contamination level of less than 90 µg/kg (Ayalew et al., 2006). Sorghum was found to have the highest percentage of contaminated samples, at 48% positives (mean level 360 µg/kg), but the authors noted that these were "suspect" samples, visibly damaged by *Fusarium* species. No dietary exposures were reported. The authors concluded that the levels of DON found in the grains were too low to be of concern to farmers.

8.4.7 France

Following the publication of the SCOOP task report noted above for the European Union (Schothorst & van Egmond, 2004), France undertook a total diet study including DON dietary exposure (Leblanc et al., 2005). Total diet studies examine the dietary exposure to substances (nutrients, additives or contaminants) by measuring the level of the substance of interest in all food products in a representative diet, prepared for normal consumption. These studies often give the best measure of dietary exposure to a substance because they explicitly account for any changes in the level of the substance during food production and preparation.

The French study considered the total population as well as those consuming three types of vegetarian diets (vegetarians comprised 138 of the total surveyed population of 3003). The mean dietary exposure for the adult non-vegetarian population was found to be 0.281 µg/kg bw per day, whereas that for children was 0.451 µg/kg bw per day. At the 95th percentile, the corresponding exposures were 0.571 and 0.929 µg/kg bw per day, respectively. The authors noted that all of these estimates are below the PMTDI of 1 µg/kg bw and "significantly lower" than the estimates reported in the SCOOP task report (0.46 and 0.73 µg/kg bw per day at the mean for adults and children, respectively). They suggested that this was due to reduction of DON levels in food on cooking. The range of mean DON exposures for the vegetarian diets was 0.32–0.41 µg/kg bw per day at the mean and 0.72–0.96 µg/kg bw per day at the 95th percentile. The analysis showed that 0.4% of the adult population could be above the PMTDI for DON.

The Committee noted that this analysis was the best available for this evaluation of DON dietary exposure.

8.4.8 Germany

Schollenberger, Müller & Drochner (2005) reported on consumption of trichothecene toxins for the population in south-west Germany in 1998–1999. They considered consumption of bread and pasta by infants, children and adults. DON levels in 1999 were twice those in 1998. For 1999, the DON exposure for adults was 0.45 µg/kg bw per day at the mean and 0.90 µg/kg bw per day for "high" consumption. Exposure for infants in 1998 was as high as 0.35 µg/kg bw per day. No evaluation for infants in 1999 was undertaken. The authors reported that children's exposure to DON in 1999 was 1.59 µg/kg bw per day at the mean and 3.17 for "high" consumption. The Committee believes that these estimates are reported in error. In the discussion section outlining the process used to prepare the estimates, it is stated that a 20 kg child would consume 20 g of bread and pasta per day, with a high consumption of 40 g/day. In the reported estimates, 170 g of food was used to calculate the mean, the same as for adults. The high estimates used 340 g for both populations also. If the lower food consumptions are correct, the exposures would be approximately one eighth of the reported figures, or 0.19 µg/kg bw per day at the mean and 0.38 µg/kg bw per day for "high" consumption.

8.4.9 Ireland

DON exposure from the consumption of milk in Ireland was analysed using a probabilistic assessment method employing Monte Carlo modelling (Coffey, Cummins & Ward, 2009). The authors reported DON exposure to be less than 0.001 µg/kg bw per day at the mean and 0.019 µg/kg bw per day at the 95th percentile.

8.4.10 Lebanon

The dietary exposure to DON for children and teenagers in Beirut, Lebanon, from consumption of cereal products (bread, cakes, pizza, etc.) was reported in 2009 (Soubra et al., 2009). Approximately 45% of samples had non-detectable levels, with the highest DON levels found in bread (176 µg/kg). For 8- to 13-year-olds, the mean exposure was estimated to be less than 0.55 µg/kg bw per day, with a 95th-percentile exposure of less than 1.0 µg/kg bw per day. The exposures for 14- to 18-year-olds were lower (mean 0.41 µg/kg bw per day; 95th percentile 0.66 µg/kg bw per day).

8.4.11 Morocco

DON contamination of wheat grains in Morocco was examined by Hajjaji et al. (2006). Seven of 17 samples were found to contain measurable DON levels, with a mean concentration of 27 µg/kg. Although dietary exposure was not estimated, the authors concluded that the presence of DON in wheat, even at levels below proposed regulatory limits (500 µg/kg), may constitute a risk to human health.

8.4.12 Netherlands

Three published reports have contained information concerning dietary exposure to DON in the Netherlands. The Muri et al. (2009) risk–risk analysis described above found MOEs for DON in the Netherlands to be as low as 29 (at the 1st percentile for the age group 4–19 years); a calculation using an effect dose of 30 µg/kg bw per day suggests that dietary exposure at the 99th percentile would be less than 1 µg/kg bw per day (Muri et al., 2009).

Pieters, Bakker & Slob (2004) explored the effect that government risk management efforts put in place following the high mycotoxin contamination years of the late 1990s had on dietary exposure to DON. They noted that mean DON levels in samples taken from February 2000 to December 2002 were reduced by 50% when compared with samples from the period 1998–2000. The levels were highest in maize and wheat. Using a probabilistic exposure assessment method, they estimated 95th-percentile exposure for 1-year-olds to be approximately 1.0 µg/kg bw per day (0.46 µg/kg bw per day at the mean); exposures for 5% of 1-year-olds would exceed 1.0 µg/kg bw per day. For all age groups above 10 years, the mean exposures were below 0.2 µg/kg bw per day, with 95th-percentile exposures below 0.4 µg/kg bw per day.

Schothorst et al. (2005) explored dietary DON exposures in children using a duplicate diet study, in which all portions of food prepared for normal consumption are divided in two, with half being analysed for DON; exposure is then estimated using the actual measured consumption of the food. This method is extremely effective for accurately measuring the dietary exposure to a substance. Seventy-four children were included in the study. The mean dietary exposure to DON was 0.66 µg/kg bw per day, with a maximum of 1.98 µg/kg bw per day. Nine of the 74 participants had exposures above 1.0 µg/kg bw per day.

8.4.13 Nigeria

Maize for human consumption (180 samples) was analysed for the presence of DON, among other mycotoxins (Adejumo, Hettwer & Karlovsky, 2007). Forty samples contained DON (22%) at a mean concentration of 226 µg/kg. Although no dietary exposure analysis was performed, the authors stated that since the DON levels were below the United States Food and Drug Administration's (USFDA) advisory level of 1000 µg/kg, these samples do not present a health risk for consumers. The Committee noted, however, that chronic consumption of 265 g of maize per day would result in an exposure to DON from maize at the PMTDI of 1 µg/kg bw. Nigeria is in GEMS/Food cluster diet J, with a per capita exposure to maize of 57.4 g/day. Only cluster diet H, primarily Central America and the Caribbean nations, exceeds 265 g/day (298 g/day).

8.4.14 Republic of Korea

DON exposure from numerous commodities collected from 2005 to 2008 was evaluated for the Republic of Korea (Ok et al., 2009b). DON was below the LOD in more than 60% of the samples, with dried maize having the highest levels, at a mean of 128 µg/kg. Using a probabilistic Monte Carlo model, mean exposures

for all age groups over 7 years were found to be less than 0.1 µg/kg bw per day. The 95th-percentile exposures for these groups were all below 0.2 µg/kg bw per day. For children aged 3–6 years, the mean and 95th-percentile exposures were 0.14 and 0.30 µg/kg bw per day, respectively. Polished rice consumption provided 73–91% of the DON exposure. Breads, biscuits and beer contributed less than 10% of the total for all groups.

8.4.15 Thailand

Wheat products in Thailand were examined for DON contamination (Poapolathep et al., 2008). Ninety samples were examined, 30 each of noodles, pasta and cereals. DON was detected in 18.9% of the samples (LOD 100 µg/kg), with 1 above 1 mg/kg. The mean contamination levels in quantifiable samples were 0.26, 0.37 and 0.24 mg/kg for noodles, pasta and cereals, respectively. A dietary exposure analysis was undertaken for a number of age groups. The highest exposure reported was for the 3- to 6-year-olds, with an "upper estimated exposure" of 0.0038 µg/kg bw per day. The dietary exposures to DON from bread and noodles are reported as ranging from 0.33 to 2.05 g/person per day. The Committee questions these values and could not conclude that the reported dietary exposures are valid.

8.4.16 Summary

The data used for the preparation or analyses of national estimates of dietary exposure to DON are summarized in Table 6.

The Committee concluded that all of the mean estimates of national exposure to DON were below the PMTDI of 1 µg/kg bw. In only a few cases, and typically for children only at upper percentiles, national reports showed dietary exposures that were above 1 µg/kg bw per day.

9. DOSE–RESPONSE ANALYSIS

9.1 BMD modelling for chronic effects

Since the previous evaluation, a derivation of a benchmark dose (BMD) for humans has been performed from the 2-year feeding study in mice (Iverson et al., 1995), the study on which the PMTDI for DON was based. Based on a 5% reduction in body weight, a value of 8.6 µg/kg bw per day was derived, with a lower confidence limit of 0.6 µg/kg bw per day (Slob & Pieters, 1998; Pieters et al., 2001). As the current PMTDI was not under re-evaluation at the present meeting, the Committee did not use this derived BMD.

9.2 BMD modelling for acute reference dose

The Committee considered emesis the critical end-point for acute effects, as this effect was observed consistently following DON intoxication in experimental animals and humans. Because the emetic effect was considered to be dependent on the maximum plasma concentration (C_{max}), the Committee concluded that for the

Table 6. National dietary exposures to DON[a]

Country/region	Mean exposure (µg/kg bw per day)	Upper-percentile exposure (µg/kg bw per day)
Argentina	0.02–0.06 (breads)	Not reported
Belgium	<0.07 (beer)	0.23 (97.5th, beer) 0.05 (eggs)
Czech Republic	Not reported	3 (4–19 years, 99th)
Denmark	0.02–0.03 (adults) 0.32 (children)	2 (4–19 years, 99th) 0.9 (children, 99.9th)
France	0.28 (adults) 0.45 (children) 0.32–0.45 (vegetarians)	0.57 (adults, 95th) 0.93 (children, 95th) 0.72–0.96 (vegetarians, 95th)
Germany	0.45 (adults) 0.19 (children)	0.90 (adults) 0.38 (children)
Ireland	0.001 (milk)	0.02 (milk)
Japan	Not reported	0.69 (1–6 years, 95th) 0.49 (7–14+ years, 95th) 0.24 (>19 years, 95th)
Lebanon	0.55 (8–13 years) 0.41 (14–18 years)	<1.0 (8–13 years, 95th) 0.66 (14–18 years, 95th)
Netherlands	0.46 (1 year) 0.66 (children) 0.2 (10+ years)	1 (4–19 years, 99th) 1 (1 year, 95th) 0.4 (10 years, 95th) 1.98 (children, 100th)
Republic of Korea	0.1 (7+ years) 0.14 (3–6 years)	0.2 (7+ years) 0.30 (3–6 years)
European Union	<1.0 (all age groups)	>1.0 (France, all ages, Germany, young children)
GEMS/Food clusters	0.19 (cluster C)—14.5 (cluster B)	

[a] Where no age group is specified, the dietary exposure is for the total population; when a food is specified, the dietary exposure included consumption of that food only.

purpose of establishing an ARfD, studies in which DON was administered via the diet were more appropriate than studies that used gavage dosing.

Two studies on emesis in piglets and pigs following exposure to DON via the diet (Young et al., 1983; Pollman et al., 1985) were combined for BMD modelling. Doses were calculated from the measured DON concentrations in the feed and the observed feed intake. In the first study, dietary concentrations above 3 mg/kg feed resulted in drastically reduced average feed intakes (reduced by 88–94% compared with controls) and decreases in body weights during the test period; for these

groups, it was assumed that the total feed intake over 4 or 11 days was actually all consumed on day 1. This assumption was made because it has often been observed that pigs stop eating after DON-induced vomiting on day 1. For the three dose groups in which it was reported that at least one pig vomited, it was assumed that the incidence was one. In the second study, the average feed intake was taken from the first week of exposure, although intake was decreased in the dose groups given 1.4 mg/kg feed or more, compared with controls. The initial body weights were used for the calculations, because the emesis was observed on day 1 of exposure.

The dose–response analysis was performed using the PROAST software (version 23.2). The benchmark response (BMR) was set at 10% extra risk. As Table 7 shows, the lower limit on the benchmark dose for a 10% extra risk ($BMDL_{10}$) among the accepted models ranged between 0.21 and 0.74 mg/kg bw per day. The lowest value in this range was used as a point of departure for establishing an ARfD. Figure 1 shows the dose–response data, with the fitted log-logistic model.

Table 7. Outcome of dose–response models on emesis in pigs[a]

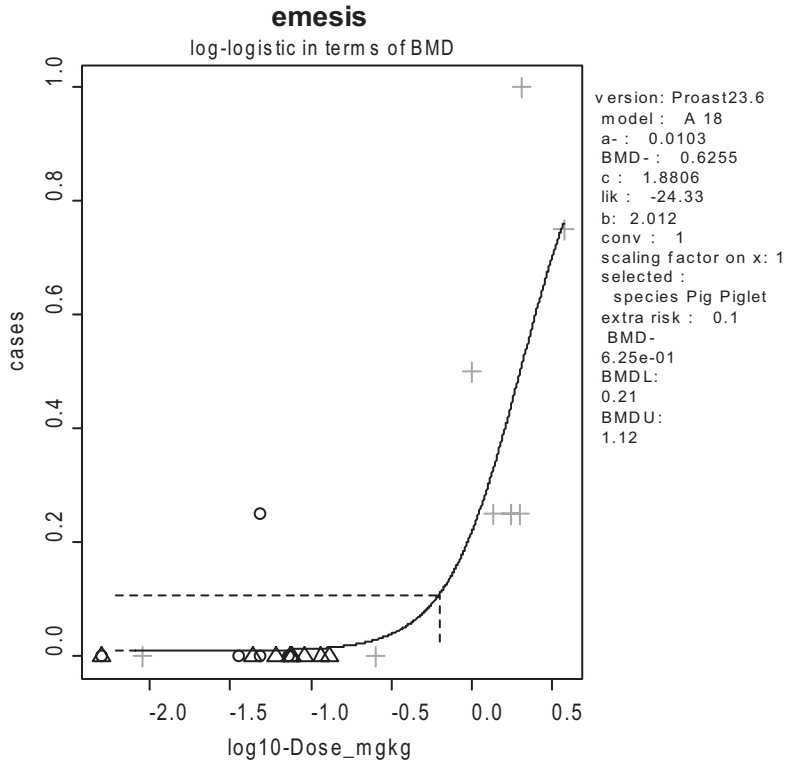
Model	npar	Log-likelihood	Accepted	BMD_{10} (mg/kg bw per day)	$BMDL_{10}$ (mg/kg bw per day)	$BMDU_{10}$ (mg/kg bw per day)
Null	1	−43.23	—	—	—	—
Full	22	−16.27	—	—	—	—
One-stage	2	−24.78	Yes	0.34	0.22	0.55
Two-stage	3	−24.54[b]	No	0.49	—	—
Log-logistic	3	−24.33	Yes	0.63	0.21	1.12
Weibull	3	−24.43	Yes	0.57	0.21	1.06
Log-probit	3	−24.21	Yes	0.61	0.21	1.09
Gamma	3	−24.36	Yes	0.62	0.21	1.10
Logistic	2	−26.14	Yes	0.99	0.74	1.29
Probit	2	−25.75	Yes	0.93	0.69	1.22

$BMDU_{10}$, upper limit on the benchmark dose for a 10% extra risk; npar = number of parameters in dose–response model

[a] No constraint; *P*-value goodness-of-fit test: 0.05.

[b] Not accepted for not being significantly better than the one-stage model.

Figure 1. Dose–response data on emesis in piglets and pigs after exposure to DON via the diet, with fitted log-logistic model



Note: Circles: Pollman et al. (1985) (pigs); triangles: Pollman et al. (1985) (piglets); plus signs: Young et al. (1983) (piglets). Note that the outlying circle (at response fraction 0.25) reflects one out of four animals.

10. COMMENTS

10.1 Absorption, distribution, metabolism and excretion (ADME)

The additional studies on metabolism in mice, rats and pigs confirmed that DON and its acetyl derivatives are rapidly and extensively absorbed from the upper gastrointestinal tract and cleared with a short plasma half-life. After absorption of 3-Ac-DON, DON was the principal metabolite observed in plasma, and acetylated DON was not detected, indicating that deacetylation is an extensive and rapid metabolic process. De-epoxidation of DON is a microbial pathway that occurs in the lower gut and does not appear to be a significant route of detoxification in the pig and other monogastric animals.

The Committee noted that the new ADME studies addressed the request made at the fifty-sixth meeting for data from comparative studies on toxicokinetics.

10.2 Toxicological data

As concluded at the previous meeting, emesis is the most sensitive functional manifestation of acute toxicity in the pig, dog and cat after either oral or parenteral administration. This is a systemic effect and is believed to arise from increased central serotonergic activity. The lowest doses that did not induce emesis in the pig were 0.025 mg/kg bw by gavage and 0.25 mg/kg bw by exposure via the diet. The Committee took note of the fact that much higher doses were tolerated when DON was given in the diet than by gavage.

New toxicological studies in mice, rats and pigs have provided insights into the mode of action of DON in causing reduced weight gain, which was the basis for the PMTDI established at the fifth-sixth meeting, and into its immunological and related effects in single-dose and repeated-dose studies. These studies indicated that the effects were largely due to the induction of suppressors of cytokine signalling and to effects on the pituitary GH axis. Changes in these parameters are observable very soon after acute dosing in vivo and are rapidly reversible in parallel with the decline in DON concentrations in plasma. At the levels likely to be encountered in the diet (described below), sustained exposure would be necessary to cause functional effects on growth or the immune system.

At its previous evaluation, the Committee concluded that DON is not mutagenic in bacteria but gave rise to chromosomal aberrations both in vitro and in vivo, but their overall significance remained equivocal. The limited new information regarding the potential genotoxicity of DON did not alter the Committee's previous conclusion.

Despite the request from the fifty-sixth meeting of the Committee, no new long-term study in a species other than mouse has become available, and the support for the lack of carcinogenic potential in humans remains dependent on a single mouse study.

One study on reproductive toxicity in rats became available, from which a NOAEL of 1 mg/kg bw per day was derived for reduced epididymal and seminal vesicle weights in rats, as well as increased sperm swimming speed. An additional developmental toxicity study in rats was available in which the NOAELs were 0.5 mg/kg bw per day for maternal toxicity, 1 mg/kg bw per day for fetal toxicity and 2.5 mg/kg bw per day for teratogenicity.

Results from studies on immunotoxicity in mice and pigs showed that low doses of DON increase IgA levels in the blood. The Committee noted, however, that there were insufficient data with which to establish a threshold for IgA nephropathy. Most mechanistic studies on immunological end-points in mice and pigs were unsuitable for deriving a NOAEL, but in one study, an acute NOEL of 0.1 mg/kg bw was derived based on suppression of hepatic mRNA for IGFAL. However, the toxicological significance of this finding is unknown.

The Committee considered the toxicity data on derivatives of DON. A few new studies have been published on the toxicity of acetylated DON, and these were considered together with the derivative studies in the previous evaluation. Given the results from the ADME studies, the toxicity of the acetylated DON compounds is likely to arise from conversion to DON. In vitro cytotoxicity and immunotoxicity studies of the relative potencies of DON and its acetylated derivatives are not considered to provide a reliable indication of relative potency in vivo, as they generally do not take account of this conversion. LD_{50} studies indicated their toxicity in mouse to be similar to that of DON. The acetylated DON compounds were therefore considered to be as toxic as DON.

No toxicological studies were found on DON-3-glucoside, a fungal metabolite recently detected in wheat and beer. The Committee considered it possible that this compound would be hydrolysed in the body and the DON would become bioavailable, but noted that ADME studies would be necessary to confirm this.

10.3 Observations in humans

No new epidemiological studies were found. With respect to possibilities for derivation of a NOAEL from outbreaks of mycotoxicosis in humans, recent studies indicate that urinary biomarkers may be used for assessing human exposure to DON. As DON can be formed from its acetylated derivatives, the Committee considered that these biomarkers could provide an indication of total dietary exposure to DON and its derivatives. Using the limited information on outbreaks from epidemiological studies summarized for the previous evaluation, the Committee noted that the calculated level that was not likely to elicit acute intoxication in humans was around 50 µg/kg bw.

10.4 Analytical methods

Since the fifty-sixth meeting, when the Committee reviewed the range of screening and quantitative methods available for the determination of DON in various foods, a number of advances have been made in the analysis of both DON and its derivatives, and certified standard solutions for DON, 3-Ac-DON and 15-Ac-DON have been made available.

Immunoassays for screening purposes for DON have been further developed and, in some instances, commercialized. These methods include lateral flow devices, fluorescence polarization and direct fluorometry after extract cleanup and derivatization. New antibodies continue to be developed. Possible cross-reactivity between DON and its derivatives in ELISA has been demonstrated in comparative studies and possibly accounts for the previously noted higher levels of naturally occurring DON determined by ELISA as opposed to chromatographic methods. Commercialized screening methods are usually developed with LODs targeted to meet legislative or other requirements.

Major advances have been made in DON determination by HPLC in which analytical methods using UV detection for DON in cereals (oat flour, wheat flour and rice flour), cereal products (polenta and wheat-based breakfast cereals), soft wheat

and baby food have been validated by international collaborative studies. These methods, using either immunoaffinity column or multifunctional column cleanup, have been validated down to 60 µg/kg for baby foods and to 100 µg/kg for all other products. The application of HPLC coupled to MS has enabled multi-mycotoxin analysis to be undertaken. The major problem of LC-MS and LC-MS/MS—namely, matrix effects in which signal enhancement or suppression occurs—is generally overcome by the use of isotope-labelled internal standards or matrix-matched standard solutions. These methods can be used for a limited range of mycotoxins for which a common cleanup, such as by multi-mycotoxin immunoaffinity column, is available; alternatively, a more diverse analysis can be performed by an injection of an aliquot of diluted sample extract without prior cleanup.

Based on current knowledge, the main derivatives of DON that might contribute to exposure are 3-Ac-DON, 15-Ac-DON and DON-3-glucoside. The analysis of these compounds requires chromatographic separation. They can be determined simultaneously with DON by LC-MS/MS. Alternatively, the acetyl derivatives have been determined by GC after suitable derivatization.

10.5 Sampling protocols

Owing to the lack of homogeneity in the distribution of mycotoxins, the sampling stage of the overall mycotoxin analysis can frequently represent the greatest contribution to the overall variance of the result. This was noted by the fifty-sixth meeting of the Committee. Specific sampling protocols for DON should be followed, such as the one provided by the EC, which regulates the number and size of incremental samples as well as the size of the aggregate sample to be taken for control purposes.

10.6 Effects of processing

The fifty-sixth meeting of the Committee reviewed the effects of gravity separation, milling, washing, soaking in water or sodium carbonate solutions, baking, extrusion cooking, fermentation and the use of microorganisms on DON levels. These are documented in the monograph of the fifty-sixth meeting of the Committee (Annex 1, reference *153*). Milling redistributes DON, with the highest amounts appearing in the bran, which is sometimes used in human food and most often in animal feed. Additional studies conducted since then have shown that removal of screenings and bran from wheat grains reduced DON levels by 41–50%. Current data have also confirmed the efficacy of washing or soaking in water or sodium carbonate solutions in reducing DON levels in barley and wheat. Although results of frying and baking studies have been conflicting, the use of extrusion cooking indicated a reduction of DON levels by between 18% and 95%, depending on the moisture content and temperature. It is, however, suggested that apparent reductions may be due to binding or the inability to extract the toxin from the extruded matrix using current analytical techniques. Few studies exist on the effects of malting and brewing processes on DON levels. Steeping lowered DON levels as a result of the water solubility of the toxin. During germination, DON levels increased 2-fold because of the conditions conducive for *Fusarium* growth and toxin formation. A subsequent decrease in DON levels during fermentation was observed, which

was attributed to yeast absorption. Additional studies are required to confirm these changes as well as the effects of processing on the acetyl derivatives of DON.

10.7 Prevention and control

Prevention and control practices include the use of suitable crop rotation, appropriate use of fertilizers, irrigation and weed control, and the use of resistant cultivars and decontamination procedures. The use of microorganisms is a recent approach employed to reduce growth of *Fusarium* species, severity of disease symptoms and DON levels. Strains of *Bacillus subtilis*, *Fusarium equiseti* and *Cryptococcus* sp. have given encouraging results (controlling FHB and reducing DON formation) in field studies with wheat. Experimental studies under glasshouse conditions with *Streptomyces* sp., *Pseudomonas fluorescens* and *Pseudomonas frederiksbergensis* strains similarly reduced both the severity of FHB symptoms caused by *Fusarium culmorum* in wheat and barley and DON levels under both glasshouse and field conditions. The use of chitosan (deacetylated derivative of chitin) for reducing DON levels as well as the severity of FHB symptom development in wheat and barley has been studied, but additional data are required to confirm the effects.

No new data are available on the use of chemicals such as sodium bisulfite, hypochlorite bleach, ammonia, moist ozone, and natural and modified clays to decontaminate grain.

10.8 Levels and patterns of contamination in food commodities

Information on the occurrence of DON was drawn from data received from a number of countries (Austria, Belgium, Brazil, China, Finland, France, Hungary, Japan, the Netherlands, Norway, Singapore and the United Kingdom), surveys published in the open literature from 42 countries, as well as the EC's SCOOP report on mycotoxins. Only DON data published since the previous evaluation were included in this assessment. In total, data on 23 980 samples analysed for DON were collected (68% from Europe, 17% from Asia, 6% from North America, 5% from South America and 3% from Africa). It was noted that DON remains a common contaminant in cereals (wheat, maize, oats, rye, barley, rice) and their products. Highest reported mean levels for raw cereals were as follows: wheat, 9900 µg/kg; maize, 4772 µg/kg; rice, 183 µg/kg; barley, 6349 µg/kg; oats, 537 µg/kg; and rye, 190 µg/kg. Contamination levels vary widely between and within regions. Relatively lower levels were detected in processed products, such as baby food, beer, bread, biscuits, pasta, muesli, noodles, cereal-based snacks, pizza, polenta, couscous, flours and fermented soya bean, most likely due to the decrease in contamination resulting from cereal milling and processing. Mean levels of DON in samples of processed products did not exceed 1250 µg/kg. As noted by the fifty-sixth meeting of the Committee, carry-over of DON into animal products is negligible due to feed refusal, rapid metabolism and elimination in livestock species.

The occurrence data for the DON derivatives 3-Ac-DON and 15-Ac-DON in wheat, maize, barley, oats, rye and their products were considered by the Committee for the first time at the present meeting. In addition to data submitted by

China, France, Japan and the United Kingdom, published data from studies conducted in nine countries were also assessed. Data were available on 3-Ac-DON from 6980 samples (92% from Europe and 8% from Asia) and on 15-Ac-DON from 4300 samples (81% from Europe, 16% from Asia and 3% from the USA). Generally, these derivatives are infrequently detected, and levels were typically less than 10% of those reported for DON. Highest reported mean levels in wheat, maize and barley for 3-Ac-DON were 193 µg/kg, 27 µg/kg and 19 µg/kg, respectively; for 15-Ac-DON, the corresponding highest reported mean levels were 365 µg/kg, 236 µg/kg and 0.3 µg/kg. The Committee was aware of reports on DON-3-glucoside in cereals and beer (data on 500 samples were assessed, with 79% from China, 15% from Europe and 6% from the USA), but considered that the data were too limited for dietary exposure assessment.

10.9 Food consumption and dietary exposure assessment

Dietary exposure to DON was evaluated at the fifty-sixth meeting of the Committee. Using the then-available five regional diets from GEMS/Food, the total dietary exposure to DON was estimated to range from 0.77 µg/kg bw per day in the African diet to 2.4 µg/kg bw per day in the Middle Eastern diet. The major source of dietary exposure in three of the five regional diets (European, Latin American and Middle Eastern) was wheat (64–88% of total exposure), whereas the sources in the other two regional diets were more varied (wheat, rice and maize in the African diet and wheat and rice in the Far Eastern diet).

At the current meeting, the Committee prepared updated international estimates using the consumption cluster diets from GEMS/Food and occurrence data reported in the literature or supplied to the Committee by Member States. Information was available on the concentrations of DON in six commodities: barley, maize, oats, rice, rye and wheat. Additionally, information on beer, the majority of which is produced from barley, was included. Data originating in 42 countries were analysed, representing 10 of the 13 GEMS/Food consumption cluster diets; no data were reported for the A, H and J clusters. Of the six commodities for which information was available for the exposure assessment, data on DON concentrations in barley, maize and wheat predominated, with limited reports on concentrations in oats, rice and rye. In total, 401 data points (mean values) representing 16 569 individual samples sorted by specific cluster diet were included in the exposure assessment. As the acetylated derivatives of DON are, in general, found at levels less than 10% of those for DON, they were not included in the dietary exposure estimates. Their inclusion would not be expected to change the estimates significantly.

The average dietary exposures to DON were calculated by multiplying the weighted mean concentration of each commodity by the corresponding amount of each commodity consumed in each of the 10 GEMS/Food consumption cluster diets for which occurrence data were available. The total dietary exposure to DON was estimated to range from 0.2 µg/kg bw per day (cluster C) to 14.5 µg/kg bw per day (cluster B). The main source of exposure in clusters C, D, E, F, G, K, L and M was wheat (56–100% of total exposure), whereas the main source in clusters B and I was maize. Three of the clusters had dietary exposure estimates above the PMTDI

of 1 µg/kg bw established previously. The Committee noted that the high estimates of dietary exposure to DON in clusters B and M were due to unusually high reported DON levels in maize and wheat in single countries for each cluster and that these data may not be representative of chronic dietary exposures. The range of estimates in the remaining clusters is in agreement with those prepared at the fifty-sixth meeting. It should be noted that any reduction in the concentration of DON as a result of processing has not been taken into consideration in this assessment.

Since the evaluation of DON at the fifty-sixth meeting of the Committee, a number of national evaluations of dietary exposure to DON have been published. The Committee considered evaluations of dietary exposure to DON from Argentina, Belgium, Czech Republic, Denmark, Ethiopia, France, Germany, Ireland, Japan, Lebanon, Morocco, the Netherlands, Nigeria, Republic of Korea and Thailand. Some of these reports contained overall dietary exposure assessments, whereas others assessed single commodities (or their products) considered to be the potential primary source of dietary exposure to DON in the population assessed. The evaluations that contained numerical estimates are summarized in Table 6 in section 8.4.16.

For risk characterization, the Committee chose a dietary exposure of 0.5 µg/kg bw per day for an average exposure and 1.0 µg/kg bw per day for a high exposure.

The Committee was asked to consider the need for an ARfD for DON. In this regard, the Committee prepared an estimate of acute dietary exposure to DON. The Committee chose to use a high-percentile daily consumption (97.5th, taken from the WHO GEMS/Food database) with a high concentration of DON (and its acetyl derivatives) in food (the highest mean value taken from the review of occurrence data at the present meeting). The consumptions for the foods most likely to be contaminated with DON were as follows: maize, 4.06 g/kg bw per day; wheat flour, 9.17 g/kg bw per day; white bread, 9.08 g/kg bw per day; and wheat, 13.46 g/kg bw per day. Considering that breads were the mostly likely foods to be regularly consumed, the Committee used a figure of 9 g/kg bw per day in making the estimate. Combining this with a DON contamination level of 10 mg/kg of wheat gives an acute dietary exposure estimate of 90 µg/kg bw per day. The Committee noted that regulatory limits for DON in foods in various countries range up to 1 mg/kg food. Using this limit with the high consumption figure would result in an acute dietary exposure of 9 µg/kg bw per day.

10.10 Dose–response analysis

The Committee was aware that acute exposure to high doses of DON and its derivatives has resulted in emesis in humans and considered it appropriate to establish an ARfD. Although developmental toxicity might be considered a potential effect of acute intoxication during critical periods of embryogenesis, the NOAEL for teratogenicity in the rat was 1 order of magnitude greater than the level found not to induce emesis in the pig; therefore, emesis in pigs was chosen to derive an acute health-based guidance value. Because the emetic effect was considered to be dependent on C_{max}, the Committee concluded that for the purpose of establishing

an ARfD, studies in which DON was administered via the diet were more appropriate than studies that used gavage dosing.

Data on DON-induced emesis in pigs, cats and dogs were available; although the effect was noted at similar concentrations in the three species, the dog and cat data were deemed not suitable for dose–response modelling. Two studies on emesis in piglets and pigs following exposure to DON via the diet (Young et al., 1983; Pollman et al., 1985) were combined for BMD modelling. Doses were calculated from the measured DON concentrations in the feed and the observed feed intake. In the first study, dietary concentrations above 3 mg/kg of feed resulted in drastically reduced average feed intakes (reduced by 88–94% compared with controls) and decreases in body weights during the test period; for these groups, it was assumed that the total feed intake over 4 or 11 days was actually all consumed on day 1. This assumption was made because it has often been observed that pigs stop eating after DON-induced vomiting on day 1. For the three dose groups in which it was reported that at least one pig vomited, it was assumed that the incidence was one. In the second study, the average feed intake was taken from the first week of exposure, although intake was decreased in the dose groups given 1.4 mg/kg of feed or more compared with controls. The initial body weights were used for the calculations, because the emesis was observed on day 1 of exposure.

The dose–response analysis was performed using the PROAST software (version 23.2). The BMR was set at 10% extra risk. The $BMDL_{10}$s among the accepted models ranged between 0.21 and 0.74 mg/kg bw per day. The lowest value in this range was used as a point of departure for establishing an ARfD.

11. EVALUATION

At its fifty-sixth meeting, the Committee established a PMTDI of 1 µg/kg bw for DON on the basis of the NOEL[2] of 100 µg/kg bw per day based on decreased body weight gain from a 2-year feeding study in mice and application of a safety factor of 100. Repeated-dose short-term studies considered in the present evaluation indicated that this NO(A)EL remains appropriate.

Since 3-Ac-DON is converted to DON and therefore contributes to the total DON-induced toxicity, the Committee decided to convert the PMTDI for DON to a group PTMDI of 1 µg/kg bw for DON and its acetylated derivatives (3-Ac-DON and 15-Ac-DON). In this regard, the Committee considered the toxicity of the acetylated derivatives equal to that of DON. The Committee concluded that, at this time, there was insufficient information to include DON-3-glucoside in the group PMTDI.

The Committee derived a group ARfD for DON and its acetylated derivatives using the lowest $BMDL_{10}$ of 0.21 mg/kg bw per day for emesis in pigs. The Committee considered that because DON-induced emesis is a systemic effect and more dependent on C_{max} than on area under the plasma concentration–time curve (AUC), it would be appropriate to apply an uncertainty factor of 25, which is the value

[2] At its sixty-eighth meeting (Annex 1, reference *187*), the Committee decided to differentiate between NOAEL and NOEL. This NOEL would now be considered a NOAEL.

used by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) for acute C_{max}-dependent effects. The Committee established a group ARfD for DON and its acetylated derivatives of 8 µg/kg bw. Limited data from human case reports indicated that dietary exposures to DON up to 50 µg/kg bw per day are not likely to induce emesis.

Estimation of dietary exposure was made using data from 42 countries, representing 10 of the 13 GEMS/Food consumption cluster diets, and was therefore considered to be more globally representative than the previous evaluation. The Committee concluded that all of the mean estimates of national exposure to DON were below the group PMTDI of 1 µg/kg bw. National reports showed dietary exposures that were above 1 µg/kg bw per day in only a few cases, only for children at upper percentiles. For acute dietary exposure, the estimate of 9 µg/kg bw per day, based on high consumption of bread and a regulatory limit for DON of 1 mg/kg food, was close to the group ARfD.

The acetylated derivatives have not been included in the estimates of dietary exposure to DON prepared at this meeting. The Committee noted that in general they are found at levels less than 10% of those for DON, and inclusion would not be expected to significantly change the estimates of dietary exposure to DON. Data are limited on the occurrence of DON-3-glucoside, which might be an important contributor to dietary exposure; this derivative was also not included in the dietary exposure estimates.

11.1 Recommendations

- As DON-3-glucoside has been detected in cereals and beers and might therefore contribute to systemic exposure to DON, the Committee recommended that ADME studies be conducted on this substance.
- Additional data on the occurrence of and the effects of processing on 3-Ac-DON, 15-Ac-DON and DON-3-glucoside are needed, as well as their co-occurrence with DON.

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Appendix 1:

DON occurrence tables

Table A1. Occurrence data for DON (Africa)

Country/ region	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Kenya	Beer (Tusker)		39	1.045				3.29/6.40			Mbugua & Gathumbi (2004)
Kenya	Beer (Pilsner)		36	1.045				3.57/4.35			Mbugua & Gathumbi (2004)
Kenya (Nakuru)	Wheat	2004	48			12		132.7/303			Muthomi et al. (2008)
Kenya (Nyandura)	Wheat	2004	34			20		113/289			Muthomi et al. (2008)
Morocco	Wheat		17	50	85		10	27.1/128			Hajjaji et al. (2006)
South Africa	Maize	2003–2004	90	500				200/13 000			Southern African Grain Laboratory (http://www.sagl.co.za/)
	Maize	2004–2005	100	500				600/3260			Southern African Grain Laboratory (http://www.sagl.co.za/)
	Maize	2005–2006	90	500				2740/6200			Southern African Grain Laboratory (http://www.sagl.co.za/)
	Maize	2006–2007	90	500				530/3100			Southern African Grain Laboratory (http://www.sagl.co.za/)
	Maize	2007–2008	100	250				240/1700			Southern African Grain Laboratory (http://www.sagl.co.za/)
	Wheat	2004–2005	30	500				1060/1800			Southern African Grain Laboratory (http://www.sagl.co.za/)

Table A1 (contd)

Country/ region	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat	2005–2006	30	500				1010/1500			Southern African Grain Laboratory (http://www.sagl.co.za/)
	Wheat	2006–2007	30	500				1460/2400			Southern African Grain Laboratory (http://www.sagl.co.za/)
	Wheat	2007–2008	30	500				1360/2700			Southern African Grain Laboratory (http://www.sagl.co.za/)
Tunisia	Durum wheat	2007	65	10	30		11	7200/54 000			Bensassi et al. (2010)

Table A2. Occurrence data for DON (Americas)

Country	Commodity	Year/season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Argentina	Maize	2003	12	9	12		8	118.5/834.4			Broggi et al. (2007)
	Maize	2004	9	9	12		9	nd			Broggi et al. (2007)
	Maize, Bt & non-Bt	2002–2003		50		0.44		1700			Barros et al. (2009)
	Maize, Bt & non-Bt	2003–2004		50		0.28		2150			Barros et al. (2009)
- Miramar	Wheat	?	10	50		0		1300/3040			Lori et al. (2003)
- La Dulce	Wheat	?	10	50		1		900/2400			Lori et al. (2003)
- Barrow	Wheat	?	10	50		7		100/570			Lori et al. (2003)
- Bordenave	Wheat	?	10	50		10		nd			Lori et al. (2003)
- Balcarce	Wheat	Consecutive	12	50		0		2600/8440			Lori et al. (2003)
- Miramar	Wheat	Consecutive	12	50		0		2000/4220			Lori et al. (2003)
- La Dulce	Wheat	Consecutive	12	50		0		1600/3200			Lori et al. (2003)
- Barrow	Wheat	Consecutive	12	50		1		2100/5330			Lori et al. (2003)
- Bordenave	Wheat	Consecutive	12	50		12		nd			Lori et al. (2003)

Table A2 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Brazil	Wheat	2005	5			0		1321			Miranda et al. (2006)
	Maize-based foods	November 2001–January 2002	78	20–60	70–300	77	78	nd/167			Milanez, Valente-Soares & Baptista (2006)
	Whole rice	2003–2008	16	10			16	nd			Nunes, Garda & Furlong (2001)
	Parboiled rice	2003–2008	16	10			16	nd			Nunes, Garda & Furlong (2001)
	Polished rice	2003–2008	24	10			22	190/300			Nunes, Garda & Furlong (2001)
	Rice by-products	2006	24	10			21	183/250			Nunes, Garda & Furlong (2001)
	Wheat grain	2002–2003	14	80	100		9	?/1500			Lamardo, Navas & Sabino (2006)
	Wheat flour	2002–2003	28	80	100		14	296/600			Lamardo, Navas & Sabino (2006)
	Sandwich cookie	2008	5	<80	80		0	461/594			Unpublished
	Wheat flour	2008	2	<80	80		0	834/1450			Unpublished

Table A2 (contd)

Country	Commodity	Year/season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat bran	2008	6	<80	80		0	386/863			Unpublished
	Rice	November 2007	101	30	100		88	108/192			Unpublished
	Rice	January–December 2008	65	30	100		52	172/244			Unpublished
	Rice bran	June 2008	11	30	100		11	nd			Unpublished
	Wheat grain	July 2008	7	30	100		5	293/378			Unpublished
	Wheat flour	March 2009	1	30	100		1	nd			Unpublished
	Filled biscuit wafer	October 2009	5				0	1246/1348			Unpublished
Canada	Maize, Bt	1996	17	200		7		450			Schaafsma et al. (2002)
	Maize, non-Bt	1996	17	200		1		1250			Schaafsma et al. (2002)
	Maize, Bt	1997	27	200		16		360			Schaafsma et al. (2002)
	Maize, non-Bt	1997	27	200		9		510			Schaafsma et al. (2002)

Table A2 (contd)

Country	Commodity	Year/season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Maize, Bt	1998	31	200		8		690			Schaafsma et al. (2002)
	Maize, non-Bt	1998	31	200		3		1150			Schaafsma et al. (2002)
	Maize, Bt	1999	27	200		4		1060			Schaafsma et al. (2002)
	Maize, non-Bt	1999	27	200		4		1190			Schaafsma et al. (2002)
	Infant cereal, oat-based	1997–1999	53		20		20	32/90			Lombaert et al. (2003)
	Infant cereal, barley-based	1997–1999	50		20		21	150/980			Lombaert et al. (2003)
	Infant cereal, soya-based	1997–1999	8		20		0	116/240			Lombaert et al. (2003)
	Infant cereal, rice-based	1997–1999	9		20		9	nd			Lombaert et al. (2003)
	Infant cereal, multigrain	1997–1999	86		20		24	83/400			Lombaert et al. (2003)
	Infant teething biscuits	1997–1999	24		20		6	45/120			Lombaert et al. (2003)

Table A2 (contd)

Country	Commodity	Year/season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Infant soya formula	1997–1999	1		20		1	nd			Lombaert et al. (2003)
	Infant creamed corn	1997–1999	6		20		6	nd			Lombaert et al. (2003)
	Breakfast cereal, maize-based	1999–2001	34	10	20		22	30/420			Roscoe et al. (2008)
	Breakfast cereal, multigrain	1999–2001	36	10	20		15	80/770			Roscoe et al. (2008)
	Breakfast cereal, oat-based	1999–2001	27	10	20		10	20/80			Roscoe et al. (2008)
	Breakfast cereal, rice-based	1999–2001	29	10	20		28	1.4/40			Roscoe et al. (2008)
	Breakfast cereal, wheat-based	1999–2001	29	10	20		8	110/940			Roscoe et al. (2008)
	Breakfast cereal, buckwheat	1999–2001	1	10	20		1	nd			Roscoe et al. (2008)
Canada, eastern	Maize	1991	54		10		25	370/1070			Campbell et al. (2002)
	Maize	1992	145		10		25	500/17 500			Campbell et al. (2002)

Table A2 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Maize	1993	93		10		5	550/2360			Campbell et al. (2002)
	Maize	1994	109		10		6	490/2090			Campbell et al. (2002)
	Maize	1995	89		10		10	400/2640			Campbell et al. (2002)
	Maize	1996	69		10		0	640/2550			Campbell et al. (2002)
	Maize	1997	74		10		0	750/5880			Campbell et al. (2002)
	Maize	1998	40		10		2	470/1710			Campbell et al. (2002)
	Wheat	1991	4		10		2	170/200			Campbell et al. (2002)
	Wheat	1992	12		10		3	1370/6990			Campbell et al. (2002)
	Wheat	1993	12		10		4	520/1080			Campbell et al. (2002)
	Wheat	1994	21		10		4	1870/9160			Campbell et al. (2002)
	Wheat	1995	11		10		1	730/1320			Campbell et al. (2002)
	Wheat	1996	10		10		0	3210/9070			Campbell et al. (2002)
	Wheat	1997	20		10		5	1150/3370			Campbell et al. (2002)
	Wheat	1998	9		10		1	1680/5440			Campbell et al. (2002)
Uruguay	Barley	1996	100		500		70	326/2100			Pan et al. (2007)
	Barley	1997	59		500		19	794/3000			Pan et al. (2007)
	Barley	1998	45		500		33	294/1900			Pan et al. (2007)

Table A2 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Barley	1999	26		500		19	411/2600			Pan et al. (2007)
	Barley	2000	8		500		1	3592/5000			Pan et al. (2007)
	Barley	2001	25		500		0	6349/10 000			Pan et al. (2007)
	Barley	2002	37		500		0	4098/10 000			Pan et al. (2007)
	Wheat	1997	13		500		5	1539/3400			Pan, Graneri & Bettucci (2009)
	Wheat	1998	10		500		10	nd			Pan, Graneri & Bettucci (2009)
	Wheat	1999	10		500		10	nd			Pan, Graneri & Bettucci (2009)
	Wheat	2000	10		500		2	3233/5000			Pan, Graneri & Bettucci (2009)
	Wheat	2001	81		500		0	6593/11 400			Pan, Graneri & Bettucci (2009)
	Wheat	2002	20		500		0	5880/8800			Pan, Graneri & Bettucci (2009)
	Wheat	2003	142		500		59	1360/7500			Pan, Graneri & Bettucci (2009)

Table A2 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
USA	Durum wheat, Montana	2001	23		50			nd			Manthey et al. (2004)
	Durum wheat, NW North Dakota	2001	27		50			2700/13 000			Manthey et al. (2004)
	Durum wheat, NC North Dakota	2001	24		50			9100/23 000			Manthey et al. (2004)
	Durum wheat, NE North Dakota	2001	15		50			9900/22 000			Manthey et al. (2004)
	Durum wheat, SW North Dakota	2001	17		50			400/4100			Manthey et al. (2004)
	Durum wheat, SE North Dakota	2001	17		50			2900/9100			Manthey et al. (2004)
	Wheat, hard red spring	2005	28	1	0.5		10	1400/10 000			Sasanya, Hall & Wolf-Hall (2008)

Bt, *Bacillus thuringiensis*; NC, north-central; nd, not detected; NE, north-east; NW, north-west; SE, south-east; SW, south-west

Table A3. Occurrence data for DON (Asia)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
China	Maize	2008	203	0.1	0.3		103	144/4374			GEMS/Food database
	Wheat	2008	162	0.1	0.3		23	63/591			GEMS/Food database
	Wheat flour	2008	30	0.1	0.3		0	52/3425			GEMS/Food database
China, Henan Province											
- Puyang	Wheat	1998	31	10		1		2850/14 000			Li et al. (2002)
- Zhumadian	Wheat	1998	28	10		3		223/1240			Li et al. (2002)
- Puyang	Wheat	1999	34	10		5		294/941			Li et al. (2002)
Japan	Wheat	2002	199		50		118	180/2100		550	GEMS/Food database
	Wheat	2003–2004	213		50		136	67/580		260	GEMS/Food database
	Wheat	2004	226	20	50		145	44/930		130	GEMS/Food database
	Wheat	2005	200	4	10		108	18/230		42	GEMS/Food database
	Wheat	2006	100	3	10		13	130/880	42	410	GEMS/Food database
	Wheat	2007–2008	100	3	9		43	23/290	10	55	GEMS/Food database
	Wheat	2008	120	5	13		39	33/460	14	53	GEMS/Food database
	Barley	2002	50		50		28	280/4800		720	GEMS/Food database
	Barley	2003–2004	54		50		34	290/3700		910	GEMS/Food database

Table A3 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Barley	2004	56	2	50		23	250/1800	90	670	GEMS/Food database
	Barley	2005	50	4	10		20	60/460	11	190	GEMS/Food database
	Barley	2006	10	3	10		0	550/2500	370	1000	GEMS/Food database
	Barley	2007	10	3	7		3	64/320	16	190	GEMS/Food database
	Barley	2008	100	3	7		22	32/560	15	61	GEMS/Food database
	Bread	2007	50	20	40		49	1/40			GEMS/Food database
	Uncooked noodles	2007	20	20	40		19	2/40			GEMS/Food database
	Dried noodles	2007	20	20	40		20	nd			GEMS/Food database
	Boiled noodles	2007	20	20	40		20	nd			GEMS/Food database
	Uncooked noodles	2007	10	20	40		10	nd			GEMS/Food database
	Steamed noodles	2007	10	20	40		10	nd			GEMS/Food database
	Boiled noodles	2007	10	20	40		10	nd			GEMS/Food database
	Instant noodles	2007	20	20	40		19	2/40			GEMS/Food database
	Macaroni	2007	20	20	40		17	9/100			GEMS/Food database
	Baked goods	2007	10	20	40		10	nd			GEMS/Food database
	Wheat flour	2007	79	2	5		8	60/630		170	GEMS/Food database

Table A3 (contd)

Country	Commodity	Year/season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat flour	2008	50	20	40		48	2/40			GEMS/Food database
	Bean paste	2009	20	30	60		20	nd			GEMS/Food database
	Soya sauce	2009	20	20	50		20	nd			GEMS/Food database
	Beer	2009	20	20	50		19	3/50			GEMS/Food database
	Barley tea	2009	20	20	50		20	nd			GEMS/Food database
	Bread	2007	35		1		0	8.6/25.2			Sugiyama et al. (2009)
	Wheat flour	2007	12		5		1	31.3/113			Sugiyama et al. (2009)
	Biscuits (wheat)	2004–2006	201	0.9	3.0	5		23/791			Tanaka et al. (2010)
Lebanon	Biscuits	2005	20	30		10		31/70			Soubra et al. (2009)
	Bread	2005	40	30		22		176/700			Soubra et al. (2009)
	Cakes	2005	20	30		15		60/100			Soubra et al. (2009)
	Cornflakes	2005	20	30		14		58/100			Soubra et al. (2009)
	Croissant	2005	20	30		10		50/120			Soubra et al. (2009)
	Doughnuts	2005	20	30		12		60/130			Soubra et al. (2009)
	Kaak assrounieh	2005	20	30		10		50/130			Soubra et al. (2009)
	Kaak tea	2005	20	30		10		70/220			Soubra et al. (2009)
	Lahm bi ajin	2005	20	30		11		88/240			Soubra et al. (2009)

Table A3 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Manakeesh	2005	20	30		10		88/300			Soubra et al. (2009)
	Pizza	2005	20	30		10		85/200			Soubra et al. (2009)
	Toast	2005	20	30		10		52/120			Soubra et al. (2009)
Malaysia	Wheat-based noodles	?									Moazami & Jinap (2009)
	- Instant, domestic	?	42	0.63		18		<1.0			Moazami & Jinap (2009)
	- Instant, imported	?	48	0.63		24		<1.0			Moazami & Jinap (2009)
	- Yellow alkaline, domestic	?	21	0.63		9		<1.0			Moazami & Jinap (2009)
	- Yellow alkaline, imported	?	9	0.63		9		nd			Moazami & Jinap (2009)
	- White salted	?	15	0.63		15		nd			Moazami & Jinap (2009)
Nepal											
- Bagmati	Maize	2004	12	100		8		663/4070			Desjardins et al. (2008)
- Bheri	Maize	2004	5	100		4		4150			Desjardins et al. (2008)
- Dhaulagiri	Maize	2004	6	100		3		440/1040			Desjardins et al. (2008)
- Gandaki	Maize	2004	3	100		2		3130			Desjardins et al. (2008)

Table A3 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Republic of Korea	Corn breakfast cereal	2007–2008	18	2.2	5.6	12		8.0/36.5			Ok et al. (2009a)
	Dried corn	2007–2008	82	2.2	5.6	18		103.2/807.3			Ok et al. (2009a)
	Canned corn	2007–2008	25	2.2	5.6	25		nd			Ok et al. (2009a)
	Barley	2007–2008	70	2.2	5.6	32		9.4/36.8			Ok et al. (2009a)
	Barley beer	2007–2008	26	2.2	5.6	23		2.4/28.6			Ok et al. (2009a)
	Biscuits	2007–2008	8	2.2	5.6	5		9.4/35.2			Ok et al. (2009a)
	Bread	2007–2008	8	2.2	5.6	5		19.6/78.1			Ok et al. (2009a)
	Wheat	2007–2008	41	2.2	5.6	17		32.7/353.6			Ok et al. (2009a)
	Wheat flour	2007–2008	37	2.2	5.6	21		18.8/172.9			Ok et al. (2009a)
	Glutinous rice	2007–2008	43	2.2	5.6	41		1.4/37.4			Ok et al. (2009a)
	Rice-based mixed cereal	2007–2008	50	2.2	5.6	31		5.9/57.5			Ok et al. (2009a)
	Polished rice	2007–2008	62	2.2	5.6	57		0.8/16.0			Ok et al. (2009a)
	Unpolished rice	2007–2008	44	2.2	5.6	38		6.0/127.9			Ok et al. (2009a)

Table A3 (contd)

Country	Commodity	Year/season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Singapore	Buckwheat	2005	1		13		1	nd			GEMS/Food database
	Buckwheat	2008	3	4	13		3	nd			GEMS/Food database
	Wheat	2002, 2005	2		13		2	nd			GEMS/Food database
	Cereal grains	2005	21		13		18	17/259			GEMS/Food database
	Cereal grains	2008	16	4	13		12	16/137			GEMS/Food database
	Spices	2005	2		13		1	81			GEMS/Food database
	Rye	2002, 2005	2		13		2	nd			GEMS/Food database
	Rye	2008	1	4	13		1	nd			GEMS/Food database
	Popcorn	2002, 2005	2		13		2	nd			GEMS/Food database
	Sweet corn kernels	2002	9		13		9	nd			GEMS/Food database
	Tapioca	2005	1		13		1	nd			GEMS/Food database
	Vegetable spaghetti	2005	23		13		19	8.8/102			GEMS/Food database
	Corn flour	2008	2		13		2	nd			GEMS/Food database
	Noodles	2008	26	4	13		23	7.9/89			GEMS/Food database
	Wheat, whole meal	2008	1	4	13		1	nd			GEMS/Food database

Table A3 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat, bulgur	2008	1	4	13		1	nd			GEMS/Food database
Thailand	Noodles	2007	30	100		28		4.3/350			Poapolathep et al. (2008)
	Breads	2007	30	100		25		62/1130			Poapolathep et al. (2008)
	Cereals	2007	30	100		20		80/390			Poapolathep et al. (2008)
Turkey	Beer	2002–2003	50	125 µg/l		50		nd			Omurtag & Beyoğlu (2007)
	Wheat	?	27	100		27		nd			Omurtag & Beyoğlu (2003)
	Corn starch	?	3	100		3		nd			Omurtag & Beyoğlu (2003)
	Corn flour	?	7	100		3		517/2670			Omurtag & Beyoğlu (2003)
	Dried corn	?	11	100		10		650			Omurtag & Beyoğlu (2003)
	Home-made macaroni	?	3	100		2		470			Omurtag & Beyoğlu (2003)
	Other cereals / processed food	?	17	100		17		nd			Omurtag & Beyoğlu (2003)
	Processed pulses	?	15	100		15		nd			Omurtag & Beyoğlu (2003)

nd, not detected

Table A4. Occurrence data for DON (Europe)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Austria, Germany, Slovakia	Wheat	2005	23	8	20			1500/4130			Berthiller et al. (2009b)
Austria	Maize	2006	54	16	40			753/3680			Berthiller et al. (2009b)
	Beer	2000–2002	33	3.7[a]		8		10.2/29.5[a]	8.7[a]		Papadopoulou-Bouraoui et al. (2004)
	Oat	2000	96		100	83		43.5/530[b]	2000		Schothorst & van Egmond (2004)
	Oat	2001	40		100	36		29.3/200[b]	15		Schothorst & van Egmond (2004)
	Wheat	2000	62		100	24		744/6090[b]	2000		Schothorst & van Egmond (2004)
	Wheat	2001	36		100	18		175.6/1230[b]	72.5		Schothorst & van Egmond (2004)
Belgium	Wheat	2001	33		250	28		87/504[b]	<250		Food Inspection Service and Institute for Public Health
	Wheat	2002	14		50	14		8/<50[b]	<50		Food Inspection Service and Institute for Public Health

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Biscuits	2000	5		250	5		42/<250[b]	<250		Food Inspection Service and Institute for Public Health
	Baby food	2000	5		250	5		42/<250[b]	<250		Food Inspection Service and Institute for Public Health
	Baby food	2002	5		50	5		8/<50[b]	<50		Food Inspection Service and Institute for Public Health
	Bread	2000	10		250	6		187/560[b]	<250		Food Inspection Service and Institute for Public Health
	Bread	2001	5		200	4		97/350[b]	<200		Food Inspection Service and Institute for Public Health
	Bread	2001	5		175	5		29/<175[b]	<175		Food Inspection Service and Institute for Public Health
	Bread	2002	18		50	18		8/<50[b]	<50		Food Inspection Service and Institute for Public Health
	Pasta	2000	10		250	7		159/559[b]	<250		Food Inspection Service and Institute for Public Health
	Pasta	2001	10		250	7		193/716[b]	<250		Food Inspection Service and Institute for Public Health
	Pasta	2002	9		50	8		16/74[b]	<50		Food Inspection Service and Institute for Public Health

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat bran	2000	5		250	4		128/475[b]	<250		Food Inspection Service and Institute for Public Health
	Muesli bars	2000	5		250	5		42/<250[b]	<250		Food Inspection Service and Institute for Public Health
	Beer	2003–2004	80	2	6	21	36	5/22	4		Anselme et al. (2006)
	Beer	2000–2002	47	3.7[a]		0		18.1/56.7[a]	15.1[a]		Papadopoulou-Bouraoui et al. (2004)
	Eggs	2006	10	0.6	2	2	6	2.3/7.5	2		Tangni et al. (2009)
	Eggs	2007	10	0.6	2	1	4	4.5/17.9	3		Tangni et al. (2009)
Cyprus	Beer	2000–2003	6	3.7[a]		1		8/12.2[a]	7.8		Papadopoulou-Bouraoui et al. (2004)
Czech Republic	Beer	2000–2003	17	3.7[a]		0		21.5/55.3	18.8[a]		Papadopoulou-Bouraoui et al. (2004)
	Winter wheat	2003	42		250	0		330/3500			Ostry et al. (2005)
Denmark	Wheat	2000	28	20		2		59/330	33		Rasmussen, Ghorbani & Berg (2003)
	Wheat	2001	30	20		11		32/204	10		Rasmussen, Ghorbani & Berg (2003)
	Wheat flour	2000	28	20	20	2		59/33[b]	33		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat flour	2001	30	20	20	11		32/204[b]	10		Schothorst & van Egmond (2004)
	Rye flour	2000	17	20	20	9		23/84[b]	10		Schothorst & van Egmond (2004)
	Rye flour	2001	20	20	20	9		17/55[b]	10		Schothorst & van Egmond (2004)
	Durum wheat flour	2000	23	20	20	0		1157/2591[b]	1242		Schothorst & van Egmond (2004)
	Durum wheat flour	2001	10	20	20	0		1153/1619[b]	1224		Schothorst & van Egmond (2004)
	Rye	2000	17	20		9		23/84	10		Rasmussen, Ghorbani & Berg (2003)
	Rye	2001	20	20		9		17/55	10		Rasmussen, Ghorbani & Berg (2003)
	Beer	2000–2002	9	3.7[a]		0		19.9/47.1	19.9[a]		Papadopoulou-Bouraoui et al. (2004)
Finland	Wheat	2000	35		50	10		169.9/1026[b]	111		Schothorst & van Egmond (2004)
	Wheat	2001	39		25/40	25		36.3/376[b]	4.17		Schothorst & van Egmond (2004)
	Barley	2000	20		50	12		57/293[b]	8.33		Schothorst & van Egmond (2004)
	Barley	2001	20		25/40	10		78.8/619[b]	17.4		Schothorst & van Egmond (2004)
	Barley malt	2000	25		50	18		45.7/394[b]	8.33		Schothorst & van Egmond (2004)
	Barley malt	2001	25		25/40	15		28.4/144[b]	4.17		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Oats	2000	25		50	10		537.2/5004[b]	97.6		Schothorst & van Egmond (2004)
	Oats	2001	30		25/40	4		236.1/1560[b]	89.9		Schothorst & van Egmond (2004)
	Rye	2000	15		50	14		19.6/178[b]	8.33		Schothorst & van Egmond (2004)
	Rye	2001	12		25/40	10		9.18/37.4[b]	4.17		Schothorst & van Egmond (2004)
	Rye organic	2001	1		25/40	1		6.67[b]			Schothorst & van Egmond (2004)
	Beer	2000–2002	4	3.7[a]		1		7.4/10.6	6.3[a]		Papadopoulou-Bouraoui et al. (2004)
France	Wheat	2001	30		20			132.15/2125[b]	3.3		Schothorst & van Egmond (2004)
	Wheat	2001	22		20			10.88/170[b]	3.3		Schothorst & van Egmond (2004)
	Wheat	2000	1	100		1		50/0[b]	50		Schothorst & van Egmond (2004)
	Wheat	2001	1	50		0		105/85[b]	40		Schothorst & van Egmond (2004)
	Wheat	2002	3	100		1		100.33/120[b]	50		Schothorst & van Egmond (2004)
	Corn	2001	29		20			494.12/8850[b]	50		Schothorst & van Egmond (2004)
	Corn	2000	25		125	0		895/2000[b]			Schothorst & van Egmond (2004)
	Corn	2001	25	30		1		1056/4800[b]			Schothorst & van Egmond (2004)
	Corn	2000	59		60–100	0		475/3390[b]	300		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Corn	2001	107		50–60	0		903/5400[b]	650		Schothorst & van Egmond (2004)
	Corn	2001	9	25	50	5		19/36[b]	12		Schothorst & van Egmond (2004)
	Barley	2001	9		20			6.47/35[b]	3.3		Schothorst & van Egmond (2004)
	Wheat bran	2001	1		100	1		16.67/[b]	16.67		Schothorst & van Egmond (2004)
	Wheat bran	2002	6		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Wheat bran	2001	3		200	1		33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Wheat bran	2000	9	50		0		525.56/2000[b]	240		Schothorst & van Egmond (2004)
	Wheat bran	2001	8	20		1		59.38/170[b]	13		Schothorst & van Egmond (2004)
	Wheat bran	2002	13	20		4		222.23/915[b]	140		Schothorst & van Egmond (2004)
	White wheat flour	2000	15	60	200	6		155.62/500[b]	125		Schothorst & van Egmond (2004)
	White wheat flour	2000	1		100			125/[b]	125		Schothorst & van Egmond (2004)
	White wheat flour	2001	170		200	14		143.14/1213[b]	33.3		Schothorst & van Egmond (2004)
	White wheat flour	2002	11		200			45.5/136[b]	33.3		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	White wheat flour	2002	46		100	13		63.08/595[b]	16.7		Schothorst & van Egmond (2004)
	White wheat flour	2000	55	51		9		992.89/50 000[b]	45		Schothorst & van Egmond (2004)
	White wheat flour	2001	38	50		16		105/400[b]	40		Schothorst & van Egmond (2004)
	White wheat flour	2002	33	50		16		100.33/330[b]	50		Schothorst & van Egmond (2004)
	White wheat flour	2000	10	50		3		153/300[b]	45		Schothorst & van Egmond (2004)
	White wheat flour	2001	4	50		2		105/280[b]	40		Schothorst & van Egmond (2004)
	White wheat flour	2002	3	100		3		50/0[b]	50		Schothorst & van Egmond (2004)
	White wheat flour	2000	37		50	0		304/2100[b]	220		Schothorst & van Egmond (2004)
	White wheat flour	2001	101		20–50	0		53/328[b]	25		Schothorst & van Egmond (2004)
	Corn fractions	2000	29	60	200	11		30/[b]	30		Schothorst & van Egmond (2004)
	Corn fractions	2001	1					340/340[b]	340		Schothorst & van Egmond (2004)
	Corn fractions	2001	1					620/620[b]	620		Schothorst & van Egmond (2004)
	Corn fractions	2000	1	100		1		50/[b]	50		Schothorst & van Egmond (2004)
	Corn fractions	2001	17	100		3		105/1400[b]	40		Schothorst & van Egmond (2004)
	Corn fractions	2002	7	20		0		100.33/825[b]	50		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Corn meal	2001	1		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Corn meal	2001	2					435/450[b]	435		Schothorst & van Egmond (2004)
	Corn meal	2001	3		200			331.1/1400[b]	340		Schothorst & van Egmond (2004)
	Corn meal	2002	1					245/245[b]	245		Schothorst & van Egmond (2004)
	Wheat products	2000	3	60		3		30/[b]	30		Schothorst & van Egmond (2004)
	Wheat products	2001	5		200	1		33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Wheat products	2001	75		200	8		286.4/1826[b]	235		Schothorst & van Egmond (2004)
	Wheat products	2002	2		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Wheat products	2002	8		200	2		348.28/502[b]	33.3		Schothorst & van Egmond (2004)
	Wheat products	2000	1	50		0		289/289[b]	289		Schothorst & van Egmond (2004)
	Wheat products	2001	15	50		13		105/250[b]	40		Schothorst & van Egmond (2004)
	Wheat products	2002	7	100		6		100.33/220[b]	50		Schothorst & van Egmond (2004)
	Wheat products	2000	12	50		1		992.89/1000[b]	45		Schothorst & van Egmond (2004)
	Wheat products	2001	15	50		3		105/410[b]	40		Schothorst & van Egmond (2004)
	Wheat products	2002	3	100		2		100.33/220[b]	50		Schothorst & van Egmond (2004)
	Pasta	2001	1		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Pizza	2000	1					150/150[b]	150		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Pizza	2001	1					216/216[b]	216		Schothorst & van Egmond (2004)
	Pizza	2001	2					33.3/611[b]	33.3		Schothorst & van Egmond (2004)
	Corn products	2001	1					33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Corn products	2001	8			2		237.59/320[b]	225.95		Schothorst & van Egmond (2004)
	Polenta	2001	1					33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Polenta	2002	3		100			63.9/[b]	25		Schothorst & van Egmond (2004)
	Sweet corn	2002	4			1		33.3/222[b]	33.3		Schothorst & van Egmond (2004)
	Sweet corn	2002	9		200			57.49/224[b]	33.3		Schothorst & van Egmond (2004)
	Soft wheat	2001	31		20			22 068/230[b]	3.3		Schothorst & van Egmond (2004)
	Soft wheat	2000	82	30	60	7		270/1500[b]	190		Schothorst & van Egmond (2004)
	Soft wheat	2001	72	30	60	48		62/700[b]	15		Schothorst & van Egmond (2004)
	Soft wheat	2002	71	30	60	15		216/1900[b]	100		Schothorst & van Egmond (2004)
	Soft wheat	2000	276	30–250	40–100	47		283/1520[b]	190		Schothorst & van Egmond (2004)
	Soft wheat	2001	252	25–30	10–100	67		95/1038[b]	25		Schothorst & van Egmond (2004)
	Soft wheat	2001	8	25	50	5					Schothorst & van Egmond (2004)
	Durum wheat	2000	16	30	60	7		372/1600[b]	175		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Durum wheat	2001	13	30	60	4		169/730[b]	110		Schothorst & van Egmond (2004)
	Durum wheat	2002	52	30	60	9		689/3600[b]	470		Schothorst & van Egmond (2004)
	Grain fractions	2000–2001	145	200	200	50					Schothorst & van Egmond (2004)
	Grain fractions	2002	69	200	200	24					Schothorst & van Egmond (2004)
	Rye flour	2000	1					120/120[b]	120		Schothorst & van Egmond (2004)
	Rye flour	2001	2		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Rye flour	2002	1		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Rye flour	2002	3		200	1		174/595[b]	33		Schothorst & van Egmond (2004)
	Rice flour	2001	1		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Rice flour	2002	1		100	1		16.7/[b]	16.7		Schothorst & van Egmond (2004)
	Buckwheat	2000	1	60		1		30/[b]	30		Schothorst & van Egmond (2004)
	Buckwheat	2000	5	60		5		30/[b]	30		Schothorst & van Egmond (2004)
	Buckwheat	2001	3		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Buckwheat	2001	15		200	6		33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Buckwheat	2002	1		100			16.07/[b]	16.07		Schothorst & van Egmond (2004)
	Buckwheat	2002	3		100	2		34.5/70[b]	16.07		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Oat products	2000	1	60		1		30/[b]	30		Schothorst & van Egmond (2004)
	Oat products	2001	11		200	1		33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Oat products	2001	3		200	1		33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Oat products	2002	5		50	4		8.3/[b]	8.3		Schothorst & van Egmond (2004)
	Barley products	2000	1	60		1		30/[b]	30		Schothorst & van Egmond (2004)
	Barley products	2001	9		200	3		33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Rice products	2000	1	60		1		30/[b]	30		Schothorst & van Egmond (2004)
	Rice products	2001	7		200	1		33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Rice products	2002	1		100	1		16.7/[b]	16.7		Schothorst & van Egmond (2004)
	Rice products	2002	3		200	1		33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Rye flour	2001	11		200	1		103.76/350[b]	33.3		Schothorst & van Egmond (2004)
	Breakfast cereals	2001	14		200			63/235[b]	33		Schothorst & van Egmond (2004)
	Breakfast cereals	2002	1		200			33.3/[b]	33.3		Schothorst & van Egmond (2004)
	Breakfast cereals	2000	9	50		3		993/250[b]	45		Schothorst & van Egmond (2004)
	Breakfast cereals	2001	1	50		0		105/0[b]	40		Schothorst & van Egmond (2004)
	Breakfast cereals	2002	4	50		3		100/80[b]	50		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Malting barley	2001	30	30		30		15/[b]	15		Schothorst & van Egmond (2004)
	Malting barley	2001	52	30		50		9.76/200[b]	5		Schothorst & van Egmond (2004)
	Malting barley	2002	44	30		32		5/500[b]	5		Schothorst & van Egmond (2004)
	Malting barley	2002	68	30		55		21.1/310[b]	5		Schothorst & van Egmond (2004)
	Malting barley	2000	50		20			46/550[b]	10		Schothorst & van Egmond (2004)
	Malting barley	2000	64		20			65/350[b]	37		Schothorst & van Egmond (2004)
	Conventional wheat		11		10		1	215 (maximum)	55		Malmauret et al. (2002)
	Organic wheat		11		10		5	494 (maximum)	106		Malmauret et al. (2002)
	Conventional barley		5		10		1	73 (maximum)	41		Malmauret et al. (2002)
	Organic barley		5		10		2	209 (maximum)	69		Malmauret et al. (2002)
	Beer	2000–2002	27	3.7[a]		3		11/30.2	8.4[a]		Papadopoulou-Bouraoui et al. (2004)
	Baby food	2001	2		200		2				National monitoring programmes
	Baby food	2004	13		60–200		9	291.8/507			National monitoring programmes
	Baby food	2006	12		100		10	242.5/360			National monitoring programmes
	Baby food	2006	9	30		8	1				National monitoring programmes

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Baby food	2007	13	30	100	13					National monitoring programmes
	Baby food	2008	8	30	100	7	1				National monitoring programmes
	Biscuit	2003	4					131.7/151.3			National monitoring programmes
	Biscuit	2004	2	30		1		437/			National monitoring programmes
	Biscuit	2006	9		100			116.7/139			National monitoring programmes
	Biscuit	2006	5	30		5	6				National monitoring programmes
	Biscuit	2007	5	30	100	3	1	167/			National monitoring programmes
	Biscuit	2008	5	30	100	1	3	129/			National monitoring programmes
	Biscuit	2008	8	10–20	20	2	1	168/263.4			National monitoring programmes
	Breakfast cereal	2001	9		200		7	242/250			National monitoring programmes
	Breakfast cereal	2003	10		50		9	65.1/			National monitoring programmes
	Breakfast cereal	2003	3	30		3					National monitoring programmes
	Breakfast cereal	2003	3		100		3				National monitoring programmes
	Breakfast cereal	2004	4	30		2	1	99/			National monitoring programmes
	Breakfast cereal	2004	3		100–200		3				National monitoring programmes
	Breakfast cereal	2006	7	30	100	2	3	167/244			National monitoring programmes
	Breakfast cereal	2007	2	30	100	2					National monitoring programmes

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Breakfast cereal	2008	9	30	100	5	2	360/615			National monitoring programmes
	Breakfast cereal (maize)	2002	11		200		8	168.7/224.3			National monitoring programmes
	Breakfast cereal (maize)	2003	6		50–100		4	174.5/191			National monitoring programmes
	Breakfast cereal (maize)	2004	14	30	60–200	3	11				National monitoring programmes
	Breakfast cereal (maize)	2006	2		100		1	160/			National monitoring programmes
	Breakfast cereal (maize)	2007	3	30	90–100		2	160/			National monitoring programmes
	Breakfast cereal (maize)	2008	4	30	100	3	1				National monitoring programmes
	Breakfast cereal (wheat)	2003	6	30	50–100	2	2	191.9/255.8			National monitoring programmes
	Breakfast cereal (wheat)	2006	3	30	100	1	1	218.8/			National monitoring programmes
	Breakfast cereal (wheat)	2007	1	30	100			240/			National monitoring programmes
	Breakfast cereal (wheat)	2008	3	30	100	1		286.5/341			National monitoring programmes
	Breakfast cereal (oats)	2001	5		200		5				National monitoring programmes
	Breakfast cereal (oats)	2002	5	30		4	1				National monitoring programmes
	Breakfast cereal (oats)	2003	3		50		3				National monitoring programmes

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Buckwheat flour	2001	9		200		9				National monitoring programmes
	Buckwheat flour	2003	4	30		4					National monitoring programmes
	Buckwheat flour	2005	1	30		1					National monitoring programmes
	Buckwheat flour	2008	5	30	100	5					National monitoring programmes
	Cereal bar	2008	3	30	100	2					National monitoring programmes
	Couscous	2001	7		200	1	2	304/371			National monitoring programmes
	Couscous	2002	2		200	1	1	234/			National monitoring programmes
	Couscous	2003	1		50	1	1				National monitoring programmes
	Couscous	2004	1		200	2	1				National monitoring programmes
	Cupcake	2998	3	30	100	10	2	140/			National monitoring programmes
	Durum wheat flour	2001	26	30	200		10	785/1780			National monitoring programmes
	Durum wheat flour	2002	6					461/632			National monitoring programmes
	Durum wheat flour	2003	9			4		334/653			National monitoring programmes
	Durum wheat flour	2005	12	30	50–70		4	127/184			National monitoring programmes
	Durum wheat flour	2006	1	30		1					National monitoring programmes
	Durum wheat flour	2007	5	30	100	1	1	427/443			National monitoring programmes
	Durum wheat flour	2008	13	30	100		1	330/631			National monitoring programmes

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Muesli	2001	1		200		1				National monitoring programmes
	Muesli	2003	3		50		3				National monitoring programmes
	Muesli	2004	7	30	200	2	4	116/			National monitoring programmes
	Muesli	2006	6		100		4	227/330			National monitoring programmes
	Muesli	2007–2008	2	30	100	2					National monitoring programmes
	Pasta	2003	9		50–100		5	166/238			National monitoring programmes
	Wheat bread	2002	1		200		1				National monitoring programmes
	Wheat bread	2003	8	30	50–200	2	4	96/110			National monitoring programmes
	Wheat bread	2005	2	30	50		2				National monitoring programmes
	Wheat bread	2006	14	30	100	3	10				National monitoring programmes
	Wheat bread	2007	6	30	90–100	2	2	146/154			National monitoring programmes
	Wheat bread	2008	5		100			328/540			National monitoring programmes
	Malt	2001	7		200		7				National monitoring programmes
	Malt	2003	1	30	100		1				National monitoring programmes
	Malt	2007	4	30	100	1		457/500			National monitoring programmes
	Maize fecula	2002	1	30		1					National monitoring programmes
	Maize starch	2004	3		200	2	1				National monitoring programmes

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Maize bread	2006	4		100		4				National monitoring programmes
	Dough	2006	2		100		2				National monitoring programmes
	Almond cake	2006	1		100		1				National monitoring programmes
	Coconut balls cookie	2008	1								National monitoring programmes
	Rice flour	2005	1	30		1					National monitoring programmes
	Crepe	2005–2006	3	30		2		67.8/			National monitoring programmes
	Chocolate cake	2006	2	30		3					National monitoring programmes
	Chocolate biscuit	2006	1	30		1					National monitoring programmes
France (imported)	Pasta	2006	6	30	200	3	2	50/			National monitoring programmes
	Pasta	2007–2008	6	30	100	4	1	131/			National monitoring programmes
	Rye bread	2006	1		200		1				National monitoring programmes
	Rye biscuit	2006	1		200		1				National monitoring programmes
	Fermented soya bean	2006	1					372/			National monitoring programmes
	Panettone	2008	1					164.9/			National monitoring programmes
	Rice pancake	2002	1	30		1					National monitoring programmes

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Germany	Beer	2000–2002	46	3.7[a]		1		4.7/40.5	4.0[a]		Papadopoulou-Bouraoui et al. (2004)
	Soft wheat	2006	78			10		217 (maximum)	28		Curtui et al. (2006)
	Durum wheat semolina	2006	6			10		203 (maximum)	38		Curtui et al. (2006)
	Durum wheat pasta	2006	49			10		119 (maximum)	24		Curtui et al. (2006)
	Wheat and wheat products	2005–2006	130	0.038	0.11	0		57/1160	23		Gottschalk et al. (2009)
	Rye and rye products	2005–2006	61	0.038	0.11	0		28/288	15		Gottschalk et al. (2009)
	Oat and oat products	2005–2006	98	0.038	0.11				0.53		Gottschalk et al. (2009)
	Cereal food products	2001	333	20–100				251/	142		Majerus et al. (2002)
	Wheat (integrated cultivation)	2000	47	50		44		140/280	80		Meister (2005)
	Wheat (integrated cultivation)	2001	47	50		35		310/1350	90		Meister (2005)
	Wheat (integrated cultivation)	2002	46	50		15		470/4870	240		Meister (2005)
	Wheat (integrated cultivation)	2003	46	50		37		140/540	90		Meister (2005)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat (integrated cultivation)	2004	41	50		26		195/1120	70		Meister (2005)
	Wheat (integrated cultivation)	2005	42	50		22		212/1730	98		Meister (2009)
	Wheat (integrated cultivation)	2006	43	50		23		200/1020	88		Meister (2009)
	Wheat (integrated cultivation)	2007	43	50		3		1211/10 400	428		Meister (2009)
	Wheat (ecological cultivation)	2000	16	50		16		n/a	n/a		Meister (2005)
	Wheat (ecological cultivation)	2001	12	50		11		70/70	70		Meister (2005)
	Wheat (ecological cultivation)	2002	14	50		8		100/200	90		Meister (2005)
	Wheat (ecological cultivation)	2003	10	50		10		n/a	n/a		Meister (2005)
	Wheat (ecological cultivation)	2004	16	50		10		100/220	80		Meister (2005)
	Wheat (organic cultivation)	2005	13	50		12		87 (value for lone sample)	n/a		Meister (2009)
	Wheat (organic cultivation)	2006	14	50		13		147 (value for lone sample)	n/a		Meister (2009)
	Wheat (organic cultivation)	2007	15	50		5		262/782	133		Meister (2009)
	Rye (integrated cultivation)	2000	43	50		33		90/200	80		Meister (2005)
	Rye (integrated cultivation)	2001	44	50		35		150/420	60		Meister (2005)
	Rye (integrated cultivation)	2002	48	50		32		190/750	120		Meister (2005)
	Rye (integrated cultivation)	2003	45	50		40		60/80	50		Meister (2005)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Rye (integrated cultivation)	2004	39	50		23		175/1095	90		Meister (2005)
	Rye (integrated cultivation)	2005	39	50		21		89/261	72		Meister (2009)
	Rye (integrated cultivation)	2006	41	50		17		84/271	67		Meister (2009)
	Rye (integrated cultivation)	2007	38	50		13		156/677	74		Meister (2009)
	Rye (ecological cultivation)	2000	18	50		18		n/a	n/a		Meister (2005)
	Rye (ecological cultivation)	2001	18	50		17		50 (value for lone sample)	50		Meister (2005)
	Rye (ecological cultivation)	2002	20	50		20		n/a	n/a		Meister (2005)
	Rye (ecological cultivation)	2003	20	50		19		50 (value for lone sample)	50		Meister (2005)
	Rye (ecological cultivation)	2004	27	50		21		105/290	70		Meister (2005)
	Rye (organic cultivation)	2005	24	50		22		85/119	85		Meister (2009)
	Rye (organic cultivation)	2006	23	50		9		61/86	60		Meister (2009)
	Rye (organic cultivation)	2007	23	50		22		80 (value for lone sample)	n/a		Meister (2009)
	Wheat flour	2000	29	111	111	11		198/690[b]	166		Schothorst & van Egmond (2004)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat flour	2001	108	25	25	34		120/640[b]	86		Schothorst & van Egmond (2004)
	Wheat	2000	27	20	40	9		112/402[b]	115		Schothorst & van Egmond (2004)
	Pasta	2000	8	20	40	1		145/370[b]	123		Schothorst & van Egmond (2004)
	Pasta	2001–2002	102	30	30	6		292/3200[b]	177		Schothorst & van Egmond (2004)
	Baby food	2000	32	20	40	10		134/1075[b]	102		Schothorst & van Egmond (2004)
	Baby food	2001–2002	132	30	30	37		70/220[b]	46		Schothorst & van Egmond (2004)
Greece	Beer	2000–2002	4	3.7[a]		0		17.0/16.8	16.5		Papadopoulou-Bouraoui et al. (2004)
Hungary	Beer	2000–2002	2	3.7[a]		0		10.8/11.1	10.8		Papadopoulou-Bouraoui et al. (2004)
	Wheat	2001	10		100		1	182.2/340	182.2		GEMS/Food database
	Cereal grains	2001	16		220		16	/220			GEMS/Food database
	Wheat flour	2001	16		100		11	/455			GEMS/Food database
Ireland	Beer	2000–2002	2	3.7[a]		0		8.7/9.6	8.7		Papadopoulou-Bouraoui et al. (2004)
Italy	Cereals and related foods	2001–2002	202	7		32		7–930 (range)	65		Cirillo et al. (2003)
	Ground wheat	2005–2006	57	2.8				7.7/17.3			Lattanzio, Solfrizzo & Visconti (2008)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Infant semolina	2005–2006	1	5.3				7.1			Lattanzio, Solfrizzo & Visconti (2008)
	Infant biscuits	2005–2006	1	4.6				nd			Lattanzio, Solfrizzo & Visconti (2008)
	Bacon biscuits	2005–2006	1	3				191			Lattanzio, Solfrizzo & Visconti (2008)
	Cocoa wafers	2005–2006	1	4				38.4			Lattanzio, Solfrizzo & Visconti (2008)
	Coconut snack	2005–2006	1	3.8				9.5			Lattanzio, Solfrizzo & Visconti (2008)
	Beer	2000–2002	16	3.7[a]		0		10.5/29.4	8.4		Papadopoulou-Bouraoui et al. (2004)
Lithuania	Winter wheat	1999	23	10				29/142			Garaleviciene, Pettersson & Agnedal (2002)
	Barley	1999	12	10				34/66			Garaleviciene, Pettersson & Agnedal (2002)
	Oats	1999	5	10				20/40			Garaleviciene, Pettersson & Agnedal (2002)
Netherlands	Beer	2000–2002	4	3.7[a]		1		8.0/9.7	8.5		Papadopoulou-Bouraoui et al. (2004)
	Beer	2000–2001	32	8[a]	25[a]	24	24	41 (value for lone sample)	n/a		Schothorst & Jekel (2003)
	Beer (imported from Germany)	2000–2001	9	8[a]	25[a]	8	8	n/a			Schothorst & Jekel (2003)
	Beer (imported from Belgium)	2000–2001	9	8[a]	25[a]	7	7	31/36			Schothorst & Jekel (2003)

Table A4 (contd)

Country	Commodity	Year/season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Beer (imported from Ireland)	2000–2001	1	8[a]	25[a]	1	1	n/a			Schothorst & Jekel (2003)
	Infant food	2000	5	10	20	1		131/270[b]	130		Inspectorate for Health Protection and Veterinary Public Health (unpublished results)
	Barley	2000–2001	4	10	20	4		5/0[b]	<220		Inspectorate for Health Protection and Veterinary Public Health (unpublished results)
	Barley	2000	6	110		3		113/230[b]	<50		Anonymous
	Barley	2001	12	220		10		153/510[b]	<50		Anonymous
	Oat	2000–2001	8	10	20	8		5/0[b]			Inspectorate for Health Protection and Veterinary Public Health (unpublished results)
	Corn	2000	23	110		2		379/1300[b]	293		Anonymous
	Corn	2001	84	220		10		761/3920[b]	640		Anonymous
	Rye	2000	8	110		1		150/220[b]	142.5		Anonymous
	Rye	2001	20	220		20		110/[b]	<220		Anonymous

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat	2000	175	50	100	107		145/3280[b]	<10		Inspectorate for Health Protection and Veterinary Public Health (unpublished results)
	Wheat	2001	98	10	20	43		206/1200[b]	110		Inspectorate for Health Protection and Veterinary Public Health (unpublished results)
	Wheat	2000	939	110		150		315/5000[b]	220		Anonymous
	Wheat	2001	765	220		583		206/2300[b]	<220		Anonymous
	Beer	2000	51	12.5	25	48		7.9/41[b]	<12.5		National Institute for Public Health and the Environment
	Puffed wheat	2001	12	220		11		121/240[b]	240		Anonymous
	Wheat bio	2001	14	220		12		144/430[b]	345		Anonymous
	Wheat durum	2000	22	110	110	2		370/1700[b]	230		Anonymous
	Wheat durum	2001	19	220		14		204/680[b]	460		Anonymous
	Wheat durum bio	2000	4	110	110	1		183/235[b]	220		Anonymous
	Wheat durum bio	2001	8	220		8		110/[b]			Anonymous
	Couscous/ semolina	2003	2	20	60			690/700			Food and Consumer Product Safety Authority - Survey

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Couscous/ semolina	2003	3	20	60			473/510			Food and Consumer Product Safety Authority - Monitoring
	Wheat	2003	5	20	60			324/480			Food and Consumer Product Safety Authority - Survey
	Wheat	2003	51	20	60			119/610			Food and Consumer Product Safety Authority - Monitoring
	Wheat flour brown	2003	13	20	60			104/242			Food and Consumer Product Safety Authority - Monitoring
	Wheat loaves/ rolls, wholemeal	2003	1	20	60			70/70			Food and Consumer Product Safety Authority - Monitoring
	Pasta, egg-free	2003	5	20	60			28.2/87			Food and Consumer Product Safety Authority - Monitoring
	Rye	2003	2	20	60	2		0/0			Food and Consumer Product Safety Authority - Monitoring
	Barley	2003	5	20	60			51.6/138			Food and Consumer Product Safety Authority - Monitoring
	Maize	2003	2	20	60			117.5/170			Food and Consumer Product Safety Authority - Monitoring
	Maize milled	2003	2	20	60			260/300			Food and Consumer Product Safety Authority - Monitoring

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Popcorn	2003	2	20	60			110/110			Food and Consumer Product Safety Authority - Survey
	Rice	2003	2	20	60	2		0/0			Food and Consumer Product Safety Authority - Monitoring
	Peanut	2003	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Survey
	Baking flour	2004	2	20	60			55/110			Food and Consumer Product Safety Authority - Monitoring
	Couscous/ semolina	2004	7	20	60			212.8/400			Food and Consumer Product Safety Authority - Monitoring
	Wheat	2004	20	20	60			107.2/340			Food and Consumer Product Safety Authority - Survey
	Wheat	2004	42	20	60			147.7/580			Food and Consumer Product Safety Authority - Monitoring
	Wheat flour, brown	2004	2	20	60			86/107			Food and Consumer Product Safety Authority - Survey
	Wheat flour, brown	2004	36	20	60			120.9/234			Food and Consumer Product Safety Authority - Monitoring
	Wheat loaves/rolls, brown	2004	1	20	60			110/110			Food and Consumer Product Safety Authority - Monitoring

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Pasta, egg-free	2004	2	20	60			57.5/60			Food and Consumer Product Safety Authority - Survey
	Pasta, egg-free	2004	32	20	60			157.5/630			Food and Consumer Product Safety Authority - Monitoring
	Rye	2004	2	20	60			110/110			Food and Consumer Product Safety Authority - Monitoring
	Barley	2004	1	20	60			325/325			Food and Consumer Product Safety Authority - Monitoring
	Maize	2004	1	20	60			210/210			Food and Consumer Product Safety Authority - Monitoring
	Maize milled	2004	4	20	60			53.7/90			Food and Consumer Product Safety Authority - Survey
	Maize milled	2004	10	20	60			69.2/150			Food and Consumer Product Safety Authority - Monitoring
	Maize semolina	2004	4	20	60			175/360			Food and Consumer Product Safety Authority - Monitoring
	Popcorn	2004	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Cereals, mixed grains	2004	1	20	60			63/63			Food and Consumer Product Safety Authority - Monitoring

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Cashew nut	2004	1	20	60			140/140			Food and Consumer Product Safety Authority - Monitoring
	Hazelnut	2004	1	20	60			2.5/2.5			Food and Consumer Product Safety Authority - Monitoring
	Honey	2004	1	20	60	1		0/0			Food and Consumer Product Safety Authority - National Plan Animal Products
	Toast	2004	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Baking flour	2005	3	20	60			106/110			Food and Consumer Product Safety Authority - Monitoring
	Breadcrumbs	2005	3	20	60			93/110			Food and Consumer Product Safety Authority - Monitoring
	Couscous/semolina	2005	5	20	60			112/350			Food and Consumer Product Safety Authority - Monitoring
	Gluten	2005	1	20	60			164/164			Food and Consumer Product Safety Authority - Monitoring
	Wheat	2005	16	20	60			186.2/451			Food and Consumer Product Safety Authority - Survey
	Wheat	2005	22	20	60			164.4/420			Food and Consumer Product Safety Authority - Monitoring

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat flour, brown	2005	26	20	60			92.9/460			Food and Consumer Product Safety Authority - Monitoring
	Wheat flour, white	2005	2	20	60			40/80			Food and Consumer Product Safety Authority - Monitoring
	Wheat germ	2005	1	20	60			100/100			Food and Consumer Product Safety Authority - Monitoring
	Wheat loaves/rolls, mixed flour	2005	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Wheat starch	2005	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Biscuits	2005	6	20	60			54.1/140			Food and Consumer Product Safety Authority - Monitoring
	Crackers	2005	2	20	60			85/110			Food and Consumer Product Safety Authority - Monitoring
	Pasta, egg-free	2005	4	20	60			86.2/150			Food and Consumer Product Safety Authority - Survey
	Pasta, egg-free	2005	52	20	60			88.6/550			Food and Consumer Product Safety Authority - Monitoring
	Rye	2005	4	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Barley	2005	5	20	60			915/3184			Food and Consumer Product Safety Authority - Monitoring
	Maize	2005	1	20	60			140/140			Food and Consumer Product Safety Authority - Monitoring
	Maize milled	2005	12	20	60			50/100			Food and Consumer Product Safety Authority - Survey
	Maize milled	2005	18	20	60			173/550			Food and Consumer Product Safety Authority - Monitoring
	Maize semolina	2005	12	20	60			70/140			Food and Consumer Product Safety Authority - Survey
	Maize semolina	2005	1	20	60			210/210			Food and Consumer Product Safety Authority - Monitoring
	Popcorn	2005	2	20	60			102.5/110			Food and Consumer Product Safety Authority - Survey
	Popcorn	2005	2	20	60			80/160			Food and Consumer Product Safety Authority - Monitoring
	Rice	2005	2	20	60	2		0/0			Food and Consumer Product Safety Authority - Monitoring
	Unripe spelt flour	2005	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Cereals, mixed grains	2005	1	20	60			106/106			Food and Consumer Product Safety Authority - Monitoring
	Almond	2005	1	20	60			50/50			Food and Consumer Product Safety Authority - Monitoring
	Fig	2005	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Survey
	Wine, alcohol > 9%	2005	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Grape juice	2005	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Toast	2005	1	20	60			70/70			Food and Consumer Product Safety Authority - Monitoring
	Wheat	2006	2	20	60			29/58			Food and Consumer Product Safety Authority - Survey
	Wheat	2006	10	20	60	10		0/0			Food and Consumer Product Safety Authority - Monitoring
	Wheat flour, brown	2006	4	20	60	4		0/0			Food and Consumer Product Safety Authority - Monitoring
	Pasta, egg-free	2006	11	20	60			5.8/64			Food and Consumer Product Safety Authority - Monitoring

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Maize	2006	2	20	60	2		0/0			Food and Consumer Product Safety Authority - Monitoring
	Maize milled	2006	2	20	60	2		0/0			Food and Consumer Product Safety Authority - Survey
	Maize milled	2006	4	20	60	4		0/0			Food and Consumer Product Safety Authority - Monitoring
	Maize semolina	2006	3	20	60	3		0/0			Food and Consumer Product Safety Authority - Survey
	Maize semolina	2006	3	20	60	3		0/0			Food and Consumer Product Safety Authority - Monitoring
	Spelt	2006	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Cereals, mixed grains	2006	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Paranut	2006	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Walnuts	2006	1	20	60	1		0/0			Food and Consumer Product Safety Authority - Monitoring
	Honey	2006	1	20	60	1		0/0			Food and Consumer Product Safety Authority - National Plan Animal Products

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Beer, alcohol > 5%	2006	8	20	60	8		0/0			Food and Consumer Product Safety Authority - Monitoring
	Wheat	2007	25	20	60			972/3400			Food and Consumer Product Safety Authority - Survey
	Pasta, egg-free	2007	9	20	60			126.4/300			Food and Consumer Product Safety Authority - Survey
	Rye	2007	1	20	60			79/79			Food and Consumer Product Safety Authority - Survey
	Barley	2007	3	20	60			388/630			Food and Consumer Product Safety Authority - Survey
	Maize	2007	18	20	60			287.6/1000			Food and Consumer Product Safety Authority - Survey
	Buckwheat	2007	1	20	60			250/250			Food and Consumer Product Safety Authority - Survey
	Cereals, mixed grains	2007	11	20	60			160.6/380			Food and Consumer Product Safety Authority - Survey
	Pistachio nuts	2007	2	20	60			85.5/88			Food and Consumer Product Safety Authority - Survey
	Honey	2007	1	20	60	1		0/0			Food and Consumer Product Safety Authority - National Plan Animal Products

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Beer, alcohol > 5%	2007	2	20	60			34.9/36.5			Food and Consumer Product Safety Authority - Survey
Norway	Wheat (imported)	2001	64	20	60	30		87.1/464[b]	35		Schothorst & van Egmond (2004)
	Wheat	2001	44	20	60	40		12.1/51[b]	10		Schothorst & van Egmond (2004)
	Wheat	2001	39	20	60	26		27.8/183[b]	10		Schothorst & van Egmond (2004)
	Oats	2001	24	20	60	24		10/[b]	10		Schothorst & van Egmond (2004)
	Oats	2001	58	20	60	38		26.2/220[b]	10		Schothorst & van Egmond (2004)
	Oats	2001	28	20	60	28		10/[b]	10		Schothorst & van Egmond (2004)
	Maize	2001	19	20	60	1		450.9/1022[b]	609		Schothorst & van Egmond (2004)
	Rice	2001	16	20	60	16		10/[b]	10		Schothorst & van Egmond (2004)
	Cereal fraction	2001	19	20	60	9		25.4/86[b]	21		Schothorst & van Egmond (2004)
	Beer	2000–2002	4	3.7[a]		1		7.7/9.9	7.3		Papadopoulou-Bouraoui et al. (2004)
	Wheat flour (organic)	2008	2	5	15	1		352/			GEMS/Food database
	Wheat flour (spelt)	2008	2	5	15			23.5/33			GEMS/Food database
	Wheat flour	2008	60	5	15			210.8/639			GEMS/Food database

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat flour (wholemeal)	2008	52	5	15	7		162.9/430			GEMS/Food database
	Wheat (wholemeal, spelt)	2008	1	5	15			49/			GEMS/Food database
	Wheatbran (processed)	2008	23	5	15			382.6/1093			GEMS/Food database
	Maize (baby porridge, organic)	1999–2002	10	20	60			819/1022			GEMS/Food database
	Maize (baby porridge)	1999–2002	12	20	60	4		47.4/78			GEMS/Food database
	Maize (baby porridge)	2008	2	5	15			46/46			GEMS/Food database
	Wheat (baby porridge)	2008	5	5	15	2		17/23			GEMS/Food database
	Oats (baby porridge)	2008	4	5	15	1		33.3/70			GEMS/Food database
	Oats	2008	30	5	15			282/520			GEMS/Food database
	Oatbran	2008	1	5	15			372/			GEMS/Food database
	Rice (baby porridge)	1999–2002	4	20	60	4					GEMS/Food database
Norway (imported)	Cereal grain (baby porridge)	2008	4	5	15	1		15.3/31			GEMS/Food database
	Cereal grain (baby porridge with fruits)	2008	2	5	15			6.5/8			GEMS/Food database
	Barley (baby porridge)	2008	2	5	15	1		15/			GEMS/Food database

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Maize (baby porridge)	2008	1	5	15			34/			GEMS/Food database
Poland	Beer	2000–2002	10	3.7[a]		0		17.2/32.9	18		Papadopoulou-Bouraoui et al. (2004)
Portugal	Corn flour		41	100		41					Martins et al. (2008)
	Popcorn		49	100		49					Martins et al. (2008)
	Cornflakes		15	100		15					Martins et al. (2008)
	Wheat	2002	3	25	50	2		256.3/744[b]			Schothorst & van Egmond (2004)
	White wheat flour	2002	3	25	50	2		119.3/333[b]			Schothorst & van Egmond (2004)
	Wheat bran	2002	4	25	50	2		761/1821[b]			Schothorst & van Egmond (2004)
	Cereal breakfast	2002	10	25	50			161.6/426[b]	161		Schothorst & van Egmond (2004)
Romania	Maize	2002–2004	54	25				4772/11 000			Tabuc et al. (2009)
	Wheat	2002–2004	35	25				1531/3600			Tabuc et al. (2009)
	Barley	2002–2004	21	25				3923/4000			Tabuc et al. (2009)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Russian Federation	Stored wheat grain	2000	40	50		30		40/420	0	170	Tutelyan (2004)
	Stored wheat grain	2001	167	50		118		80/1590	0	0	Tutelyan (2004)
	Stored wheat grain	2002	59	50		56		10/390	0	0	Tutelyan (2004)
	Wheat grain	2000	222	50		216		10/770	0	0	Tutelyan (2004)
	Wheat grain	2001	252	50		240		10/620	0	0	Tutelyan (2004)
	Barley	2000	57	50		56		5/280	0	0	Tutelyan (2004)
	Barley	2001	64	50		64		0/0	0	0	Tutelyan (2004)
	Rye grain	2000	99	50		99		0/0	0	0	Tutelyan (2004)
	Rye grain	2001	88	50		88		0/0	0	0	Tutelyan (2004)
Serbia	Soya bean and soya bean meal	2004	13		40			110/110	110		Jajić et al. (2008)
	Soya bean and soya bean meal	2005	11		40			100/100	100		Jajić et al. (2008)
	Sunflower and sunflower meal	2004	9		40			155/304	138		Jajić et al. (2008)
	Sunflower and sunflower meal	2005	10		40			447/788	467		Jajić et al. (2008)
	Barley	2005	4		40			140/140	140		Jajić et al. (2008)

Table A4 (contd)

Country	Commodity	Year/season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Maize	2004	10		40			536/2460	50		Jajić et al. (2008)
	Maize	2005	66		40			363/2210	213		Jajić, Jurić & Abramović (2008)
	Maize	2006	21		40			426/1340	260		Jajić et al. (2008)
	Maize	2007	119	25				58/172	40		Jajić, Jurić & Abramović (2008)
	Wheat	2004	4		40			1235/1840	1235		Jajić, Jurić & Abramović (2008)
	Wheat	2005	12		40			182/423	124		Jajić, Jurić & Abramović (2008)
	Wheat	2006	34		40			223/410	223		Jajić, Jurić & Abramović (2008)
	Wheat	2007	9		40			177/208	182		Jajić, Jurić & Abramović (2008)
Slovakia	Beer	2000–2002	12	3.7[a]		3		13.5/36.9	10.4		Papadopoulou-Bouraoui et al. (2004)
	Wheat	2004	139					560/1500			Šliková, Šudyová & Gregová (2008)
	Wheat	2005	139					230/300			Šliková, Šudyová & Gregová (2008)
	Wheat	2006	139					730/1300			Šliková, Šudyová & Gregová (2008)
	Wheat	2004	139					910/7200			Šliková, Šudyová & Gregová (2008)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat	2005	139					390/900			Šliková, Šudyová & Gregová (2008)
	Wheat	2006	139					650/1100			Šliková, Šudyová & Gregová (2008)
	Wheat	2004	139					1000/7300			Šliková, Šudyová & Gregová (2008)
	Wheat	2005	139					490/2200			Šliková, Šudyová & Gregová (2008)
	Wheat	2006	139					1100/1700			Šliková, Šudyová & Gregová (2008)
Spain	Corn-based breakfast cereals	2005	55	14.4	25.4	33		30.1–121.1 (range)	44.5		Castillo et al. (2008)
	Baked corn snacks	2005	57	14.4	25.4	44		36.4–131.7 (range)	62.5		Castillo et al. (2008)
	Fried corn snacks	2005	63	14.4	25.4	51		26.1–80.4 (range)	55.5		Castillo et al. (2008)
	Fried corn snacks	2002–2005	446		10			19/1416	11	35	Castillo et al. (2008)
	Beer	2000–2002	13	3.7[a]		7		7.3/12.2	6.3		Papadopoulou-Bouraoui et al. (2004)
	Beer	2000–2002	7	3.7[a]		3		5.1/14.6	3.6		Papadopoulou-Bouraoui et al. (2004)
Sweden	Durum wheat flour	2000	23	20		0		1157/2591	1242		Rasmussen, Ghorbani & Berg (2003)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Durum wheat flour	2001	10	20		0		1153/1619	1224		Rasmussen, Ghorbani & Berg (2003)
	Processed cereals	?	68	100		62		2670 (maximum)			Omurtag & Beyoğlu (2003)
	Wheat	2001	27	2	10	2		61/333[b]	26		Schothorst & van Egmond (2004)
	Wheat	2001	17	2	10			1427/2033[b]	1367		Schothorst & van Egmond (2004)
	Oat	2001	3	2	10			87/174[b]	66		Schothorst & van Egmond (2004)
United Kingdom	Barley grain	2001–2005	1624		10			230/20 333	42	368	Edwards (2009)
	Beer	2000–2002	33	3.7[a]		8		10.9/30.8	10.2		Papadopoulou-Bouraoui et al. (2004)
	Maize (imported from France)	2004–2007	25	5	10			139/444	63		Scudamore & Patel (2009b)
	Maize (imported from France)	2004–2007	24	5	10			271/932	254		Scudamore & Patel (2009b)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Maize (imported from Argentina)	2004–2007	15	5	10			89/220	74		Scudamore & Patel (2009b)
	Rice	2000	100		10	99		1.8/12[b]	<10		Food Standards Agency (2002a)
	Cereal fractions	2000	16		10	15		27.6/61[b]	<10		Food Standards Agency (2002b)
	Cereal fractions	2000	11		10	1		106/531[b]	50		Food Standards Agency (2002b)
	Barley	2000	50		5	46		1.5/20[b]	<5		Schothorst & van Egmond (2004)
	Barley	2001	49		5	29		5.9/53[b]	<5		Schothorst & van Egmond (2004)
	Beer	2000	28		5	28		0.8/<5[b]	<5		Schothorst & van Egmond (2004)
	Corn products	2000	15		10	0		132.7/683[b]	46		Food Standards Agency (2002b)
	Corn products	2000	30		10	4		257.3/879[b]	88		Food Standards Agency (2002b)
	Corn products	2000	1		10	0		16/16[b]	16		Food Standards Agency (2002b)
	Corn products	2001	24		10	3		109.5/275[b]	127		Food Standards Agency (2002b)
	Wheat products	2000	14		10	3		3.5/31[b]	24		Food Standards Agency (2002b)
	Wheat products	2000	14		10	0		235.6/2261[b]	54		Food Standards Agency (2002b)
	Wheat products	2000	40		10	21		12.4/67[b]	<10		Food Standards Agency (2002b)
	Wheat products	2000	6		10	0		75.7/156[b]	67		Food Standards Agency (2002b)
	Wheat products	2001	13		10	0		47.5/199[b]	25		Food Standards Agency (2002b)

Table A4 (contd)

Country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
	Wheat products	2000	8		10	1		53.5/177[b]	36		Food Standards Agency (2002b)
	Wheat flour	2000	29		10	3		35.1/99[b]	32		Food Standards Agency (2002b)
	Flour	2000	8		10	8		1.7/<10[b]	<10		Food Standards Agency (2002b)
	Polenta	2000	8		10	1		153.3/466[b]	140		Food Standards Agency (2002b)
	Biscuits	2000	54		10	8		29.1/315[b]	18.5		Food Standards Agency (2002b)
	Bread	2000	40		10	2		47.8/366[b]	25.5		Food Standards Agency (2002b)
	Bread	2000	16		10	0		79.9/198[b]	82		Food Standards Agency (2002b)

n/a, not applicable; nd, not detected

[a] µg/l.

[b] Arithmetic mean value of all samples (both positive and negative samples). Concentration in samples less than LOD is considered as LOD/2. Where only LOQ is available, then values less than LOQ are considered as LOQ/6.

Table A5. Occurrence data for DON-3-glucoside (Americas, Asia and Europe)

Region/country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median	90th percentile (µg/kg)	References
Americas											
USA	Wheat, hard red spring	2005	28	1	0.5		22	200/5400			Sasanya, Hall & Wolf-Hall (2008)
Asia											
China	Maize	2008	203	1	3		134	22/499			GEMS/Food database
China	Wheat	2008	162	1	3		82	26/238			GEMS/Food database
China	Wheat flour	2008	30	1	3		9	7.3/39			GEMS/Food database
Europe											
Austria, Germany, Slovakia	Wheat	2005	23	4	10			393/1070			Berthiller et al. (2009b)
Austria	Maize	2006	54	4	10			141/763			Berthiller et al. (2009b)
Belgium (De-epoxy-DON)	Eggs	2006	10	0.6	2	7	7	1.2/4.8	2		Tangni et al. (2009)
	Eggs	2007	10	0.6	2	9	9	2.4/23.7	3		Tangni et al. (2009)

Table A6. Occurrence data for 15-Ac-DON (Americas)

Region/ country	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
USA	Durum wheat, Montana	2001	23	50				nd			Manthey et al. (2004)
	Durum wheat, NW North Dakota	2001	27	50				0.0/0.5 mg/kg			Manthey et al. (2004)
	Durum wheat, NC North Dakota	2001	24	50				100/800			Manthey et al. (2004)
	Durum wheat, NE North Dakota	2001	15	50				0.0/0.5			Manthey et al. (2004)
	Durum wheat, SW North Dakota	2001	17	50				nd			Manthey et al. (2004)
	Durum wheat, SE North Dakota	2001	17	50				nd			Manthey et al. (2004)

NC, north-central; nd, not detected; NE, north-east; NW, north-west; SE, south-east; SW, south-west

Table A7. Occurrence data for 3-Ac-DON and 15-Ac-DON (Asia)

Country/compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
3-Ac-DON											
China	Maize	2008	203	0.1	0.3		131	6.6/368			GEMS/Food database
China	Wheat	2008	162	0.1	0.3		120	1.8/35			GEMS/Food database
Japan	Wheat	2008	120	6	16		114	0.5/18			GEMS/Food database
Japan	Barley	2008	100	3	8		81	2.9/53			GEMS/Food database
15-Ac-DON											
China	Maize	2008	203	0.1	0.3		106	75/1734			GEMS/Food database
China	Wheat	2008	162	0.1	0.3		118	1.7/71			GEMS/Food database
China	Wheat flour	2008	30	0.1	0.3		15	1.5/5			GEMS/Food database
China, Henan Province											
- Puyang	Wheat	1998	31	10		11		365/1800			Li et al. (2002)
- Zhumadian	Wheat	1998	28	10		28		nd			Li et al. (2002)
- Pujang	Wheat	1999	34	10		34		nd			Li et al. (2002)
Japan	Wheat	2008	120	3	8		120	nd			GEMS/Food database
Japan	Barley	2008	100	2	7		92	0.3/8.8			GEMS/Food database

nd, not detected

Table A8. Occurrence data for 3-Ac-DON and 15-Ac-DON (Europe)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
Austria (3-Ac-DON)	Corn	1996	46		50	39		20.9/125[a]	<LOQ		Schothorst & van Egmond (2004)
	Corn	1997	58		50	58		8.3/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Corn	1998	48		50	48		8.3/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Oat	1999	96		100	96		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Oat	2000	96		100	96		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Oat	2001	40		100	40		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Wheat	1999	68		100	68		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Wheat	2000	62		100	62		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Wheat	2001	36		100	36		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
Austria (15-Ac-DON)	Corn	1996	46		50	17		170.3/975[a]	97.5		Schothorst & van Egmond (2004)
	Corn	1997	58		50	48		23.2/190[a]	<LOQ		Schothorst & van Egmond (2004)
	Corn	1998	48		50	22		122.8/830[a]	57.5		Schothorst & van Egmond (2004)
	Oat	1999	96		100	96		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Oat	2000	96		100	96		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Oat	2001	40		100	40		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Wheat	1999	68		100	68		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
	Wheat	2000	62		100	60		19.5/110[a]	<LOQ		Schothorst & van Egmond (2004)
	Wheat	2001	36		100	36		16.7/<LOQ[a]	<LOQ		Schothorst & van Egmond (2004)
Finland (3-Ac-DON)	Wheat	1998	27	5		27		2.5/[a]	2.5		Schothorst & van Egmond (2004)
	Wheat	1999	37	25		37		12.5/[a]	12.5		Schothorst & van Egmond (2004)
	Wheat	2000	35		50	35		8.33/[a]	8.33		Schothorst & van Egmond (2004)
	Wheat	2001	35		25/40	35		4.17/[a]	4.17		Schothorst & van Egmond (2004)
	Barley	1998	7	5		7		2.5/[a]	2.5		Schothorst & van Egmond (2004)
	Barley	1999	30	25		25		19/70.8[a]	12.5		Schothorst & van Egmond (2004)
	Barley	2000	20		50	20		8.33/[a]	8.33		Schothorst & van Egmond (2004)
	Barley	2001	20		25/40	19		4.17/101[a]	4.17		Schothorst & van Egmond (2004)
	Barley malt	1999	18	25		18		12.5/[a]	12.5		Schothorst & van Egmond (2004)
	Barley malt	2000	25		50	25		8.33/[a]	8.33		Schothorst & van Egmond (2004)
	Barley malt	2001	25		25/40	25		4.17/[a]	4.17		Schothorst & van Egmond (2004)
	Oats	1998	7	5		6		8.4/79[a]	8.4		Schothorst & van Egmond (2004)
	Oats	1999	10	25		10		12.5/[a]	12.5		Schothorst & van Egmond (2004)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Oats	2000	25		50	19		53.7/438[a]	197.4		Schothorst & van Egmond (2004)
	Oats	2001	30		25/40	22		25.5/183[a]	84.3		Schothorst & van Egmond (2004)
	Rye	1998	6	5		6		2.5/[a]	2.5		Schothorst & van Egmond (2004)
	Rye	1999	2	25		2		12.5/[a]	12.5		Schothorst & van Egmond (2004)
	Rye	2000	15		50	15		8.33/[a]	8.33		Schothorst & van Egmond (2004)
	Rye	2001	10		25/40	10		4.17/[a]	4.17		Schothorst & van Egmond (2004)
	Rye organic	2001			25/40						Schothorst & van Egmond (2004)
France (3-Ac-DON)	Organic wheat		11		10		10	/17	10		Malmauret et al. (2002)
	Wheat	2001	30		20			4.69/45[a]	3.3		Schothorst & van Egmond (2004)
	Wheat	2001	22		20			3.33/[a]	3.33		Schothorst & van Egmond (2004)
	Corn	2001	29		20			26.75/520[a]	3.3		Schothorst & van Egmond (2004)
	Corn	2001	25	30		25		15/15[a]			Schothorst & van Egmond (2004)
	Corn	2000	10		10–30	0		11/18[a]	15		Schothorst & van Egmond (2004)
	Corn	2001	55	20–25		28		21/172[a]	12		Schothorst & van Egmond (2004)
	Barley	2001	9		20			3.33/[a]	3.33		Schothorst & van Egmond (2004)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Soft wheat	2001	31		20			3.33/[a]	3.33		Schothorst & van Egmond (2004)
	Soft wheat	2000	82	30	60	82		15/[a]	15		Schothorst & van Egmond (2004)
	Soft wheat	2001	72	30	60	72		15/[a]	15		Schothorst & van Egmond (2004)
	Soft wheat	2002	71	30	60	71		15/[a]	15		Schothorst & van Egmond (2004)
	Soft wheat	2000	204	30	10–60	185		15/30[a]	15		Schothorst & van Egmond (2004)
	Soft wheat	2001	112	25–30	10–60	85		19/50[a]	15		Schothorst & van Egmond (2004)
	Durum wheat	1999	16	30	60	16		15/[a]	15		Schothorst & van Egmond (2004)
	Durum wheat	2000	16	30	60	16		15/[a]	15		Schothorst & van Egmond (2004)
	Durum wheat	2001	13	30	60	13		15/[a]	15		Schothorst & van Egmond (2004)
	Durum wheat	2002	52	30	60	52		15/[a]	15		Schothorst & van Egmond (2004)
	Malting barley	2001	30	30		30		15/[a]	15		Schothorst & van Egmond (2004)
	Malting barley	2001	52	30		52		15/[a]	15		Schothorst & van Egmond (2004)
	Malting barley	2002	44	30		44		15/[a]	15		Schothorst & van Egmond (2004)
	Malting barley	2002	68	30		68		15/[a]	15		Schothorst & van Egmond (2004)
	Muesli		3	30		3		nd			National monitoring programmes
	Wheat bread		5	30		5		nd			National monitoring programmes

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Breakfast cereal (wheat)		1	30		1		nd			National monitoring programmes
	Breakfast cereal (wheat)		4	30		4		nd			National monitoring programmes
	Pasta		2	30		2		nd			National monitoring programmes
	Wheat bread		1	30		1		nd			National monitoring programmes
	Viennese pastry		5	30		5		nd			National monitoring programmes
	Brioche		1	30		1		nd			National monitoring programmes
	Brownie		1	30		1		nd			National monitoring programmes
	Fruit cake		1	30		1		nd			National monitoring programmes
	Shell-shaped cookie		1	30		1		nd			National monitoring programmes
	Chocolate biscuit		1	30		1		nd			National monitoring programmes
	Boiled beef		1	30		1		nd			National monitoring programmes
	Stuffed crepe		1	30		1		nd			National monitoring programmes
	Pastry		1	30		1		nd			National monitoring programmes
	Fruit tart		2	30		2		nd			National monitoring programmes
	Doughnut		1	30		1		nd			National monitoring programmes

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Cream cake		1	30		1		nd			National monitoring programmes
	Omelette		3	30		3		nd			National monitoring programmes
	Hazel-based sweet spread		1	30		1		nd			National monitoring programmes
	Bean curd		1	30		1		nd			National monitoring programmes
	Beer		2	30		2		nd			National monitoring programmes
	Alcoholic beverage		1	30		1		nd			National monitoring programmes
	Pizza		1	30		1		nd			National monitoring programmes
	Quiche Lorraine		1	30		1		nd			National monitoring programmes
	Vegetable tart		1	30		1		nd			National monitoring programmes
	Burger		3	30		3		nd			National monitoring programmes
	Sandwich		3	30		3		nd			National monitoring programmes
	Couscous		3	30		3		nd			National monitoring programmes
	Paella		3	30		3		nd			National monitoring programmes

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Lasagna		3	30		3		nd			National monitoring programmes
	Pasta		1	30		1		nd			National monitoring programmes
	Toasted ham and cheese sandwich		1	30		1		nd			National monitoring programmes
	Stuffed crepe		1	30		1		nd			National monitoring programmes
	Spring roll		1	30		1		nd			National monitoring programmes
	Tabbouleh		1	30		1		nd			National monitoring programmes
	Soya dessert		1	30		1		nd			National monitoring programmes
	Rice or semolina pudding		2	30		2		nd			National monitoring programmes
	Biscuit		2	30		2		nd			National monitoring programmes
	Wheat	2001	30		20			4.86/50[a]	3.33		Schothorst & van Egmond (2004)
	Wheat	2001	22		20			3.33/[a]	3.33		Schothorst & van Egmond (2004)
	Corn	2001	29		20			76.88/1320[a]	3.33		Schothorst & van Egmond (2004)
	Corn	2001	25	30		3		236/700[a]			Schothorst & van Egmond (2004)
	Corn	2000	10		30	0		52/152[a]	28		Schothorst & van Egmond (2004)
	Corn	2001	54	20–25	30	4		197/883[a]	160		Schothorst & van Egmond (2004)
	Barley	2001	9		20			3.33/[a]	3.33		Schothorst & van Egmond (2004)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Soft wheat	2001	31		20			3.33/[a]	3.33		Schothorst & van Egmond (2004)
	Soft wheat	2001	31		20			3.33/[a]	3.33		Schothorst & van Egmond (2004)
	Soft wheat	2000	82	30	60	82		15/[a]	15		Schothorst & van Egmond (2004)
	Soft wheat	2001	72	30	60	72		15/[a]	15		Schothorst & van Egmond (2004)
	Soft wheat	2002	71	30	60	71		15/[a]	15		Schothorst & van Egmond (2004)
	Soft wheat	2000	203	30	10–60	185		19/50[a]	15		Schothorst & van Egmond (2004)
	Soft wheat	2001	112	25–30	10	84		15/[a]	15		Schothorst & van Egmond (2004)
	Durum wheat	1999	16	30	60	16		15/[a]	15		Schothorst & van Egmond (2004)
	Durum wheat	2000	16	30	60	16		15/[a]	15		Schothorst & van Egmond (2004)
	Durum wheat	2001	13	30	60	13		15/[a]	15		Schothorst & van Egmond (2004)
	Durum wheat	2002	52	30	60	52		15/[a]	15		Schothorst & van Egmond (2004)
France (15-Ac-DON)	Muesli		3	30		3		nd			National monitoring programmes
	Wheat bread		5	30		5		nd			National monitoring programmes
	Breakfast cereal (wheat)		1	30		1		nd			National monitoring programmes
	Breakfast cereal (wheat)		4	30		4		nd			National monitoring programmes

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Pasta		2	30		2		nd			National monitoring programmes
	Wheat bread		1	30		1		nd			National monitoring programmes
	Viennese pastry		5	30		5		nd			National monitoring programmes
	Brioche		1	30		1		nd			National monitoring programmes
	Brownie		1	30		1		nd			National monitoring programmes
	Fruit cake		1	30		1		nd			National monitoring programmes
	Shell-shaped cookie		1	30		1		nd			National monitoring programmes
	Chocolate biscuit		1	30		1		nd			National monitoring programmes
	Boiled beef		1	30		1		nd			National monitoring programmes
	Stuffed crepe		1	30		1		nd			National monitoring programmes
	Pastry		1	30		1		nd			National monitoring programmes
	Fruit tart		2	30		2		nd			National monitoring programmes
	Doughnut		1	30		1		nd			National monitoring programmes
	Cream cake		1	30		1		nd			National monitoring programmes
	Omelette		3	30		3		nd			National monitoring programmes
	Hazel-based sweet spread		1	30		1		nd			National monitoring programmes

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Bean curd		1	30		1		nd			National monitoring programmes
	Beer		2	30		2		nd			National monitoring programmes
	Alcoholic beverage		1	30		1		nd			National monitoring programmes
	Pizza		1	30		1		nd			National monitoring programmes
	Quiche Lorraine		1	30		1		nd			National monitoring programmes
	Vegetable tart		1	30		1		nd			National monitoring programmes
	Burger		3	30		3		nd			National monitoring programmes
	Sandwich		3	30		3		nd			National monitoring programmes
	Couscous		3	30		3		nd			National monitoring programmes
	Paella		3	30		3		nd			National monitoring programmes
	Lasagna		3	30		3		nd			National monitoring programmes
	Pasta		1	30		1		nd			National monitoring programmes
	Toasted ham and cheese sandwich		1	30		1		nd			National monitoring programmes
	Stuffed crepe		1	30		1		nd			National monitoring programmes

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Spring roll		1	30		1		nd			National monitoring programmes
	Tabbouleh		1	30		1		nd			National monitoring programmes
	Soya dessert		1	30		1		nd			National monitoring programmes
	Rice or semolina pudding		2	30		2		nd			National monitoring programmes
	Biscuit		2	30		2		nd			National monitoring programmes
Germany (3-Ac-DON)	Wheat and wheat products	2005–2006	130	0.14	0.42			0.57/15	0.21		Gottschalk et al. (2009)
	Oat and oat products	2005–2006	98	0.14	0.42			0.43/8.2	<LOD		Gottschalk et al. (2009)
	Rye and rye products	2005–2006	61	0.14	0.42			0.39/5	0.21		Gottschalk et al. (2009)
	Wheat and wheat products	2005–2006	130	0.033	0.1			0.9/26	0.17		Gottschalk et al. (2009)
	Oat and oat products	2005–2006	98	0.033	0.1			0.11/1.4	<LOD		Gottschalk et al. (2009)
	Rye and rye products	2005–2006	61	0.033	0.1			0.73/8.6	0.35		Gottschalk et al. (2009)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
Netherlands (3-Ac-DON)	Beer	2000–2001	32	8[a]	25[a]	32	32	n/a			Schothorst & Jekel (2003)
	Beer (imported from Germany)	2000–2001	9	8[a]	25[a]	9	9	n/a			Schothorst & Jekel (2003)
	Beer (imported from Belgium)	2000–2001	9	8[a]	25[a]	9	9	n/a			Schothorst & Jekel (2003)
	Beer (imported from Ireland)	2000–2001	1	8[a]	25[a]	1	1	n/a			Schothorst & Jekel (2003)
Norway (3-Ac-DON)	Wheat	1990	138	30	90	138		15/[a]	15		Schothorst & van Egmond (2004)
	Wheat (imported)	1990	27	30	90	27		15/[a]	15		Schothorst & van Egmond (2004)
	Wheat	1991	107	30	90	106		16/120[a]	15		Schothorst & van Egmond (2004)
	Wheat	1992	112	30	90	109		16.5/96[a]	15		Schothorst & van Egmond (2004)
	Wheat (imported)	1992	16	30	90	16		15/[a]	15		Schothorst & van Egmond (2004)
	Wheat	1993	101	30	90	101		15/[a]	15		Schothorst & van Egmond (2004)
	Wheat (imported)	1993	29	30	90	28		15.9/40[a]	15		Schothorst & van Egmond (2004)
	Wheat	1994	112	30	90	112		15/[a]	15		Schothorst & van Egmond (2004)
	Wheat (imported)	1994	30	30	90	30		15/[a]	15		Schothorst & van Egmond (2004)
	Wheat	1995	26	30	90	26		15/[a]	15		Schothorst & van Egmond (2004)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Wheat (imported)	1995	13	30	90	13		15/[a]	15		Schothorst & van Egmond (2004)
	Barley	1990	20	30	90	20		15/[a]	15		Schothorst & van Egmond (2004)
	Rye	1990	2	30	90	2		15/[a]	15		Schothorst & van Egmond (2004)
	Rye (imported)	1990	18	30	90	18		15/[a]	15		Schothorst & van Egmond (2004)
	Rye (imported)	1993	11	30	90	11		15/[a]	15		Schothorst & van Egmond (2004)
	Rye (imported)	1994	12	30	90	12		15/[a]	15		Schothorst & van Egmond (2004)
	Rye (imported)	1995	11	30	90	11		15/[a]	15		Schothorst & van Egmond (2004)
	Oat	1990	40	30	90	34		19.1/60[a]	15		Schothorst & van Egmond (2004)
	Oat	1993	3	30	90	1		35.3/52[a]	15		Schothorst & van Egmond (2004)
	Oat	1994	3	30	90	2		34/72[a]	15		Schothorst & van Egmond (2004)
	Oat	1995	26	30	90	26		15/[a]	15		Schothorst & van Egmond (2004)
Sweden (3-Ac-DON)	Wheat	1996–1997	57	2	10	53		3/86[a]	1		Schothorst & van Egmond (2004)
	Wheat	1999	75	2	10	74		1/10[a]	1		Schothorst & van Egmond (2004)
	Wheat	2001	17	2	10			193/239[a]	191		Schothorst & van Egmond (2004)
	Oat	1996–1997	23	2	10	23		0/0	0		Schothorst & van Egmond (2004)
	Oat	1999	10	2	10	10		0/0	0		Schothorst & van Egmond (2004)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Rye	1996–1997	28	2	10	27		2/19[a]	1		Schothorst & van Egmond (2004)
	Rye	1999	19	2	10	19		0/0	0		Schothorst & van Egmond (2004)
United Kingdom (3-Ac-DON)	Barley grain	2002–2005	446		10			<10/15	<10	<10	Edwards (2009)
	Wheat	2001–2005	1292		10			<10/44	<10	<10	Edwards (2009)
	Rice	2000	100		10	100		1.67/<10[a]	<10		Food Standards Agency (2002a)
	Cereal fractions	2000	16		10	16		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Cereal fractions	2000	11		10	11		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Barley	1999	54		5	51		1.18/8[a]	<5		Schothorst & van Egmond (2004)
	Barley	2000	50		5	50		0.83/<5[a]	<5		Schothorst & van Egmond (2004)
	Barley	2001	49		5	44		2.07/37[a]	<5		Schothorst & van Egmond (2004)
	Beer	2000	28		5	25		1.64/10.2[a]	<5		Schothorst & van Egmond (2004)
	Corn products	2000	15		10	14		2.29/11[a]	<10		Food Standards Agency (2002b)
	Corn products	2000	30		10	26		3.04/15[a]	<10		Food Standards Agency (2002b)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Corn products	2001	24		10	24		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Corn products	2000	1		10	1		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	14		10	14		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	14		10	13		4.12/36[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	40		10	40		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	6		10	6		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2001	13		10	13		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	8		10	8		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat flour	2000	29		10	29		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Flour	2000	8		10	8		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Polenta	2000	8		10	8		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Biscuits	2000	54		10	54		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Bread	2000	40		10	40		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Bread	2000	16		10	16		1.67/<10[a]	<10		Food Standards Agency (2002b)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
United Kingdom (15-Ac-DON)	Barley grain	2002–2005	446		10			<10/35	<10	<10	Edwards (2009)
	Wheat	2001–2005	1292		10			<10/217	<10	<10	Edwards (2009)
	Rice	2000	100		10	100		1.67/<10	<10		Food Standards Agency (2002a)
	Cereal fractions	2000	16		10	16		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Cereal fractions	2000	11		10	11		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Barley	2001	49		10	48		0.94/6[a]	<5		Schothorst & van Egmond (2004)
	Corn products	2000	15		10	9		20.6/157[a]	17		Food Standards Agency (2002b)
	Corn products	2000	30		10	14		51.48/214[a]	17		Food Standards Agency (2002b)
	Corn products	2001	24		10	10		22.15/59[a]	<10		Food Standards Agency (2002b)
	Corn products	2000	1		10	1		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	14		10	14		1.67/<10[a]			Food Standards Agency (2002b)
	Wheat products	2000	14		10	13		62.21/806[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	40		10	40		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	6		10	6		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2000	13		10	13		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Wheat products	2001	8		10	8		1.67/<10[a]	<10		Food Standards Agency (2002b)

Table A8 (contd)

Country/ compound	Commodity	Year/ season	No. of samples	LOD (µg/kg)	LOQ (µg/kg)	*n* < LOD	*n* < LOQ	Mean/ maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)	References
	Wheat products	2000	29		10	29		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Flour	2000	8		10	8		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Polenta	2000	8		10	4		16.96/46[a]	<10		Food Standards Agency (2002b)
	Biscuits	2000	54		10	54		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Bread	2000	40		10	40		1.67/<10[a]	<10		Food Standards Agency (2002b)
	Bread	2000	16		10	16		1.67/<10[a]	<10		Food Standards Agency (2002b)

n/a, not applicable; nd, not detected

[a] Arithmetic mean value of all samples (both positive and negative samples). Concentration in samples less than LOD is considered as LOD/2. Where only LOQ is available, then values less than LOQ are considered as LOQ/6.

[b] µg/l.

Table A9. Comparative data for DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside

Sample	LOR (µg/kg)	*n* < LOR	Mean (µg/kg)	Maximum (µg/kg)
Austria, maize, 2006, *n* = 54				
DON	40		753	3 680
DON-3-glucoside	10		141	763
Austria, Germany, Slovakia, wheat, 2005, *n* = 23				
DON	20		1 500	4 130
DON-3-glucoside	10		393	1 070
China, maize, 2008, *n* = 203				
DON	0.3	103	144	4 374
3-Ac-DON	0.3	131	6.6	368
15-Ac-DON	0.3	106	75	1 734
DON-3-glucoside	3	134	22	499
China, wheat, 2008, *n* = 162				
DON	0.3	23	63	591
3-Ac-DON	0.3	120	1.8	35
15-Ac-DON	0.3	118	1.7	71
DON-3-glucoside	3	82	26	238
China, wheat, flour, 2008, *n* = 30				
DON	0.3	0	52	3 425
15-Ac-DON	0.3	15	1.5	5
DON-3-glucoside	3	9	7.3	39
China, Henan Province, Puyang, wheat, 1998, *n* = 31				
DON	10	1	2 850	14 000
15-Ac-DON	10	11	365	1 800
China, Henan Province, Zhumedian, wheat, 1998, *n* = 28				
DON	10	3	223	1 240
15-Ac-DON	10	28	nd	
China, Henan Province, Puyang, wheat, 1999, *n* = 34				
DON	10	5	294	941
15-Ac-DON	10	34	nd	nd
Japan, wheat, 2008, *n* = 120				
DON	13	39	33	460
3-Ac-DON	16	114	0.5	18
15-Ac-DON	8	120	nd	nd

Table A9 (contd)

Sample	LOR (µg/kg)	*n* < LOR	Mean (µg/kg)	Maximum (µg/kg)
Japan, barley, 2008, *n* = 100				
DON	7	22	32	560
3-Ac-DON	8	81	2.9	53
15-Ac-DON	7	92	0.3	8.8
USA, Hard Red Spring Wheat, 2005, *n* = 28				
DON	0.5	10	1 400	10 000
DON-3-glucoside	0.5	22	200	5 400
USA, durum wheat, North Dakota, 2001, *n* = 24				
DON	50		9 100	23 000
15-Ac-DON	50		100	800

The following cereals had no or very few samples with detectable Ac-DON:

Austria, oats, *n* = 136	
DON	
No 3-Ac-DON or 15-Ac-DON detected	530
Austria, wheat, *n* = 98	
DON	6 090
No 3-Ac-DON or 15-Ac-DON detected	
Finland, wheat, *n* = 35	
DON	1 026
No 3-Ac-DON detected	
Finland, barley, *n* = 20	
DON	619
One sample: 3-Ac-DON at 101 µg/kg	
Finland, oats, *n* = 55	
DON	5 004
14 samples: 3-Ac-DON maximum 438 µg/kg	
Finland, rye, *n* = 15	
DON	37
No 3-Ac-DON detected	
France, maize, *n* = 25	
DON	4 800
No 3-Ac-DON detected	
France, barley, *n* = 9	
DON	35
No 3-Ac-DON detected	

Table A9 (contd)

Sample	LOR (µg/kg)	*n* < LOR	Mean (µg/kg)	Maximum (µg/kg)
France, soft wheat, *n* = 225				
DON				1 500
No 3-Ac-DON detected				
France, durum wheat, *n* = 81				
DON				3 600
No 3-Ac-DON detected				
United Kingdom, rice, *n* = 100				
DON				12
No 3-Ac-DON detected				
United Kingdom, barley, *n* = 99				
DON				53
Five samples: 3-Ac-DON maximum 37 µg/kg				