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DEOXYNIVALENOL: TOXICOLOGY AND POTENTIAL EFFECTS ON HUMANS

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Deoxynivalenol (DON) is a mycotoxin that commonly contaminates cereal-based foods worldwide. At the molecular level, DON disrupts normal cell function by inhibiting protein synthesis via binding to the ribosome and by activating critical cellular kinases involved in signal transduction related to proliferation, differentiation, and apoptosis. Relative to toxicity, there are marked species differences, with the pig being most sensitive to DON, followed by rodent > dog > cat > poultry > ruminants. The physiologic parameter that is most sensitive to low-level DON exposure is the emetic response, with as little as 0.05 to 0.1 mg/kg body weight (bw) inducing vomiting in swine and dogs. Chinese epidemiological studies suggest that DON may also produce emetic effects in humans. With respect to chronic effects, growth (anorexia and decreased nutritional efficiency), immune function, (enhancement and suppression), and reproduction (reduced litter size) are also adversely affected by DON in animals, whereas incidence of neoplasia is not affected. When hazard evaluations were conducted using existing chronic toxicity data and standard safety factors employed for anthropogenic additives/contaminants in foods, tolerable daily intakes (TDIs) ranging from 1 to 5 μg/kg bw have been generated. Given that critical data gaps still exist regarding the potential health effects of DON, additional research is needed to improve capacity for assessing adverse health effects of this mycotoxin. Critical areas for future DON research include molecular mechanisms underlying toxicity, sensitivity of human cells/tissues relative to other species, emetic effects in primates, epidemiological association with gastroenteritis and chronic disease in humans, and surveillance in cereal crops worldwide.

Strains of *Fusarium*, *Stachybotrys*, and other saprophytic and plant parasitic fungi can elaborate a diverse group of toxic secondary metabolites that are known as trichothecenes (Pestka & Casale, 1990). These compounds are esters of sesquiterpenoid alcohols containing the trichothecene tricyclic ring system. More than 180 trichothecenes have been isolated and characterized (Grove, 1988, 2000). Because all these compounds possess a double bond at C9–C10 and an epoxide at C12–C13, they have been chemically designated as 12,13-epoxy trichothecenes.

Trichothecenes bind readily to eukaryotic ribosomes and are potent inhibitors of translation (Ueno, 1984). These mycotoxins are capable of producing a variety of toxicoses in animals. Acute exposure of experimental animals to high doses of trichothecenes induces radiomimetic effects that include diarrhea, vomiting, leukocytosis, and gastrointestinal hemorrhage. At very high doses, these effects can be accompanied by circulatory shock, reduced cardiac output, and ultimately death. Chronic exposure produces anorexia, reduced weight gain, altered nutritional efficiency, and immunotoxicity.

Given the potential toxicity of trichothecenes and capacity to occur following mold growth in grains, these toxins have been monitored for in human and animal foods. Of the trichothecenes, deoxynivalenol (also known as 12,13-epoxy-3,4,15-trihyroxytrichothec-9-en-8-one; DON; vomitoxin; dehydronivalenol; 4-deoxynivalenol; RD-toxin) is among the most commonly encountered relative to frequency and concentration in wheat, corn, and barley throughout the world (Rotter et al., 1996). DON was first characterized and named following its isolation from *Fusarium*-infected barley in Japan (Morooka et al., 1972; Yoshizawa & Morooka, 1973). Nearly concurrently,

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Vesonder et al. (1973) isolated the same compound from *Fusarium*-infected corn in the United States and named it "vomitoxin" because of its capacity to induce emesis in swine. DON is produced in grains infected with *Fusarium graminearum* and *F. culmorum*. In North America, DON can also co-occur with low concentrations of a related metabolite, 15-acetyl DON, whereas DON-elaborating fusaria in Asian food samples also coproduce the 8-ketotrichothecene 3-acetyl DON and nivalenol (Miller et al., 1991; Yoshizawa, 1983).

The presence of DON in human foods raises serious issues of safety. The purpose of this review is to summarize critical research related to the occurrence, cellular and molecular effects, and toxicity of DON. These data are further related to pertinent human epidemiologic studies and safety assessments. Finally, specific limitations of existing studies and future research needs are delineated.

DON INCIDENCE IN FOOD

Commodities Affected and Economic Consequences

A thorough summary of reported incidence of DON worldwide can be found in Canady et al. (2001).In developed countries where grains are dried to ≤13% moisture content to prevent mold growth, DON is primarily a preharvest problem. However, it can also be produced during storage in areas of the world where moisture content of stored grains is less rigorously controlled. Concurrent infection and DON elaboration in the field are largely dependent on weather and are favored by low temperatures and high humidity (Rotter et al., 1996). Thus, DON levels in wheat, barley, and corn can vary widely from year to year and region to region. DON at low levels (< 1 ppm) is frequently encountered but can sporadically occur at levels as high as 5 to 20 ppm even in human foods such as corn meal and granola (Abouzied et al., 1991). A recent 3-yr evaluation of cereal-based infant foods from the Canadian retail market demonstrated the regular presence of mycotoxins, with DON being the most commonly detected at overall mean levels of 0.032–0.15 ppm (Lombaert et al., 2003). DON is also detectable at very low levels in beer (Scott, 1996)—a result of *F. graminearum* growth in barley in the field or during the germination stage of brewing.

DON is typically associated with the plant diseases corn ear rot and head blight ("scab") in wheat and barley. During the last decade, scab has occurred with greatly increased frequency in the midwestern United States (McMullen et al., 1997) as a result of recurrent cool rainy weather during wheat and barley flowering. Another causative factor is increased no-till farming, which facilitates maintenance of high levels of infective fusaria propagules in the field from one season to the next. To combat and control scab in a coordinated manner, the U.S. Department of Agriculture initiated the National Wheat and Barley Scab National Initiative (http://www.scabusa.org), which involves researchers from major land-grant universities, industry, and government and receives input from wheat- and barley-associated organizations. Analogous activities are underway in Europe and Japan.

Economic losses due to scab from 1991 to 1996 in barley, winter wheat, and spring wheat have been estimated to be over a billion dollars because of DON occurrence as well as decreased grain quality. Computer simulations have suggested that annual costs for DON in the United States are $637 million in crop losses (mainly wheat and corn), $18 million in feed losses, and $2 million in livestock losses (CAST, 2003). Similarly, losses of over $100 million (Canadian dollars) were accrued following a *Fusarium* epidemic in Ontario in 1996 (Schaafsma, 2002). An additional $23 million was lost in 2000.

Effect of Processing on DON

Since DON can be found in many finished foods, it is believed to be generally recalcitrant to standard processes such as milling and baking (Abbas et al., 1985; Hart & Braselton, 1983; Young et al., 1984). Jackson and Bullerman (1999) extensively reviewed the effects of physical, chemical and biological treatments on survival of DON and other trichothecenes. During dry milling, DON concentrates in the bran fraction, with as much as a 40% reduction in the flour. When wheat kernels exhibit shriveling due to scab, sieving or cleaning (via screening, density flotation, washing,

or airflow) can reduce DON concentration by over 60%. During wet milling, DON is predominantly transferred into the steep water. Baking or heating seems to have little effect on DON. Several chemicals such as ammonia, calcium hydroxide, chlorine, hydrochloric acid, ozone, sodium bisulfite, and sodium hydroxide can degrade DON; however, to date it has not been practical to apply these measures because they interfere with standard processing of grains or represent health hazards on their own.

DON Tolerance Limits

The U.S. Food and Drug Administration (FDA) has established an advisory level of 1 ppm DON for bran, flour, and germ targeted for human consumption (Proctor et al., 1995). An advisory level was not established for raw wheat intended for milling because the aforementioned manufacturing practices and additional technology available to millers decrease DON levels in finished wheat products. Other countries have also established limits for DON. For example, Health Canada designated guidelines of 2 ppm DON in uncleaned soft wheat used for nonstaple foods, except for infant foods, where the guideline is 1 ppm (Kuiper-Goodman et al., 1994). Maximum tolerated levels of DON have been set at 0.5 ppm in Austria, Germany, and the Netherlands. China, Hungary, Russia, and Switzerland have established a 1 ppm limit (Eriksen & Alexander, 1998). Currently, DON screening is conducted by mills and processors in the United States and other countries to divert grains exceeding established limits away from entering the human food supply. The level of this surveillance by producers, millers, and the food processing industry has increased sharply in recent years because of the availability of rapid enzyme-linked immunosorbent assay (ELISA) tests and because of increased awareness of DON resulting from *Fusarium* head scab. Thus the possibility of high levels of DON sporadically entering human food is likely to have decreased substantially from 10 yr ago.

CELLULAR AND MOLECULAR EFFECTS

Trichothecene toxicity is partially explained by the capacity of these compounds to bind to eukaryotic ribosomes and inhibit protein synthesis (Ueno, 1983). Marked protein synthesis inhibition occurs throughout most tissues in mice exposed orally to DON at 5 to 25 mg/kg body weight (bw) within 3–9 h (Robbana-Barnat et al., 1987; Azcona et al., 1995a). However, other toxic mechanisms that have been suggested to result from trichothecenes include impaired membrane function (Bunner & Morris, 1988), altered intercellular communication (Khera et al., 1982), and deregulation of calcium homeostasis (Yoshino et al., 1996).

Recent studies suggest that early alterations in cell signaling, particularly at the level of mitogen-activated protein kinases (MAPKs), are critical to trichothecene toxicity (Shifrin & Anderson, 1999; Yang et al., 2000). A “ribotoxic stress response” has been demonstrated for translation inhibitors such as T-2 toxin, anisomycin, ricin, and α-sarcin (Iordanov et al., 2000). In this model, alteration of 28s rRNA by these inhibitors was postulated to be an initiation signal for activation of SAPK/JNK, which is a MAPK. Trichothecene-induced MAPK activity drives activation of transcription factors that promote cytokine production and cyclooxygenase 2 expression as well as induce apoptosis (Moon & Pestka, 2002; Yang et al., 2000; Zhou et al., 2003a). One possible upstream signal transducer for MAPK activation is double-stranded RNA-activated protein kinase (PKR), which has been recently shown to be activated by DON and other translational inhibitors (Zhou et al., 2003b). Inhibition of PKR in DON-exposed macrophages will inhibit MAPK activation as well as transcription factor activation, cytokine expression, and apoptosis (Pestka & Zhou, 2003). A second candidate for upstream signal transduction is hematopoetic cell kinase (Hck), a Src-family tyrosine kinase. Inhibition of this enzyme also inhibits MAPK activation, transcription factor activation, and cytokine expression (Pestka & Zhou, 2003). The transduction mechanisms by which ribosome binding by DON might activate PKR or Hck are unknown at this time. The potential also exists for an as yet unidentified receptor to transduce these signals upon binding DON. A third intermediate signal between trichothecene mycotoxins and MAPK activation might be the generation of reactive oxygen species. In support of this contention, trichothecene mycotoxins produce lipid peroxidation,

and their adverse effects can be inhibited by antioxidants such as vitamin E and *N*-acetylcysteine (Rizzo et al., 1994). Hydrogen peroxide induces activation of MAPKs as well as apoptosis (Wang et al., 1998). The possibility of cross-talk between reactive oxygen species and ribotoxic stress signals cannot be excluded in an explanation of the mechanism of trichothecene-induced MAPK activation.

Studies evaluating the mutagenic potential of DON indicate that it does not pose a risk. Rogers and Heroux-Metcalf (1983) demonstrated that concentrations up to 3 μg/ml DON were not mutagenic to Chinese hamster V79 cells. Similarly, Wehner et al. (1978) showed that DON produced no mutagenic effect on various strains of *Salmonella typhimurium*. However, studies evaluating chromosomal aberrations are positive both in vitro and in vivo, suggesting DON is genotoxic (Knasmuller et al., 1997). DON treatment at 1 μg/ml produced a seven-fold increase in chromosomal aberrations using rat hepatocyte cell.

TOXICOKINETICS OF DON

All animal species tested have been shown to be susceptible to DON. As reported by Prelusky et al. (1994a), experimental animals are sensitive to DON according to the following rank order: swine > mice > rats > poultry ≈ ruminants. Animal species differ with regard to the absorption, distribution, metabolism, and elimination of DON. This may account for the differential sensitivity to the adverse effects of this mycotoxin.

Metabolism

De-epoxy DON (DOM-1) is the primary metabolite found in urine and feces of animals exposed to DON (Yoshizawa et al., 1986). DOM-1 is produced via intestinal or rumen microbe activity (Cote et al., 1986b; He et al., 1992; Swanson et al., 1988; Worrell et al., 1989) rather than by liver or other organs (Cote et al., 1987; Gareis et al., 1987). Human in vitro studies employing nine different cytochrome P-450-expressing cell lines displayed no changes in cytotoxicity upon DON exposure when assessed by MTT assay (Lewis et al., 1999), supporting metabolism routes of other types. DON can be conjugated to glucuronides in liver, and these metabolites can be found in tissue and excreta of animals (Gareis et al., 1987). Sundstol-Eriksen and Pettersson (2003) studied the capacity of human gastrointestinal organisms to transform the trichothecenes 3-acetyldeoxynivalenol and nivalenol. In contrast to what has been reported for other species such as rats, mice and pigs, no de-epoxidated metabolites were detected in the fecal incubation mixtures. The difference in the intestinal ability to transform trichothecenes between species might have toxicological significance.

Toxicokinetics of DON in Different Animal Species

Swine Swine are very sensitive to DON. DON absorption is very rapid and reaches peak plasma concentrations within 15–30 min of dosing (Prelusky et al., 1988). Up to 82% systemic absorption occurred in pigs that had been orally administered DON. Although extensive tissue distribution of DON occurs in swine, the effect is very transient (Rotter et al., 1996). DON has an elimination half-life of 3.9 h and does not accumulate to any significant extent in swine tissues (Prelusky et al., 1988; Prelusky & Trenholm, 1991a, 1991b). It has been suggested that swine do not eliminate DOM-1 (Prelusky et al., 1988). However, the feeding of the closely related 8-ketotrichothecene nivalenol to pigs induces the microflora to adapt and readily metabolize DON or nivalenol (Hedman & Pettersson, 1997).

Rodents Following administration of a single oral 10 mg/kg bw dose of [^{14}C]DON to rats, radioactivity excreted in the urine and feces accounted, respectively, for 25 and 64% of the administered dose within 96 h, with less than 0.15% of the dose being detected in the respired air (Lake et al., 1987). Very little radioactivity appeared to be retained in any of the tissues examined after 96 h.

Azcona-Olivera et al. (1995a) orally dosed mice with ^{14}C-labeled DON and found peak uptake within 30 min, along with extensive distribution in plasma and tissues. Clearance of the DON label followed two-compartment kinetics with an initial rapid disappearance ($t_{1/2}$ = 0.36 h) and a slower terminal elimination ($t_{1/2}$ = 7.62 h).

Yordanova et al. (2003) monitored tissue disposition and clearance of DON, measured by ELISA in mice that were orally administered 25 mg/kg bw of the toxin. Maximal DON was detected at 30 min in all tissues tested, with a rapid clearance following over a 24-h period. At 30 min, DON concentrations (ng/g) were 5680 ± 1480 in kidney, 4530 ± 1140 in heart, 4430 ± 440 in plasma, 3900 ± 206 in liver, 3640 ± 105 in thymus, 2990 ± 110 in spleen, and 763 ± 61 in the brain. DON concentrations were significantly higher in all the organs tested from 30 min to 8 h compared to untreated mice. At 24 h, DON concentrations dropped to levels that were not significantly higher except in kidney. Taken together, the results showed that, in the mouse, DON rapidly distributed in all organs within a short time after exposure according to the rank order kidney > heart > plasma > liver > thymus > spleen > brain.

Meky et al. (2003) administered 5 mg/kg bw [^{14}C]DON by gavage to male Sprague-Dawley rats and measured the distribution of DON in body fluids over a 72-h period. Plasma levels of DON and its metabolites peaked at 8 h, where binding of [^{14}C]DON to plasma proteins was approximately 9% of total plasma DON. Levels decreased rapidly by 24 h, and decreased further at the 72-h time point. The majority of DON was cleared from plasma within 20–30 h. Urinary concentrations of DON/DON metabolites, based on scintillation counts from obtained urine samples, peaked from 0 to 24 h (37% of dose) and decreased rapidly thereafter. At all collection time points, high-performance liquid chromatography (HPLC) analysis of rat urine showed two main peaks; a major peak of approximately 80% of total reactivity, consistent with the presence of polar metabolites, and a minor peak coeluted with [^{14}C]DON standard, accounting for 8% of total radioactivity.

Poultry Tolerance in chickens may reflect the low degree of absorption into plasma and tissues (~1%) and rapid clearance (Prelusky et al., 1986a). Similarly, limited oral absorption (0.96%) and rapid plasma clearance ($t_{1/2}$ = 44 min) occur in turkeys (Gauvreau, 2000). Only a small fraction of the parent toxin can be recovered from chickens fed DON (Lun et al., 1986, 1989). Intestinal microflora have been shown to effectively convert DON to DOM-1 in poultry (He et al., 1992; Lun et al., 1986, 1988). The toxicokinetic characteristics of DON in poultry are consistent with the findings that DON does not accumulate in tissues and eggs to any significant extent (El Banna et al., 1983; Kubena et al., 1985, 1987; Prelusky et al., 1986a).

Ruminants Ruminant species are the animals most resistant to DON. When dairy cows were dosed with 1.9 mg/kg bw DON, less than 1% of the parent toxin was systemically absorbed (Prelusky et al., 1984). Serum DON levels reached 90–200 ng/ml, of which 24–46% existed as beta-glucuronide conjugate; serum half-life was approximately 4 h. Free DON and conjugated DON were also detectable in cow's milk, but only extremely low amounts (less than 4 ng/ml) were found.

When 66 ppm DON is fed to dairy cows, 20% of the consumed toxin is recoverable in urine and feces as unconjugated metabolites, primarily DOM-1 (Cote et al., 1986a). Enzymatic treatment of the excreta releases more DON and DOM-1. In sheep, approximately 6–10% of orally administered DON is absorbed systemically (Prelusky et al., 1986b). About two-thirds of the toxin in serum exists in conjugated form, suggesting that metabolic conjugation is an important step in elimination of DON in sheep.

Rotter et al. (1996) reviewed additional evidence supporting the scenario that DON undergoes significant biotransformation to DOM-1 by rumen microflora. Overall, the rumen affords an exquisite level of protection against DON toxicity.

Conclusions on DON Toxicokinetics

Observations that ruminants and poultry tolerate as much as 20 ppm DON in feed whereas 1–2 ppm of the toxin produces toxicity in swine can be explained by the differences in the toxicokinetics among the different species. Metabolism by gastrointestinal (GI) and rumen microflora plays a major role in DON detoxification. A key finding from these studies is that DON is metabolized in all species and does not bioaccumulate. Thus, residues of this toxin in the milk, meat, and eggs of food-producing animals do not appear to be of public health concern.

ACUTE/SUBACUTE TOXICITY

DON toxicity in animals has been addressed in numerous studies (reviewed in Rotter et al., 1996). Although most of these target a specific toxicologic outcome or mechanism, some provide no observable adverse effect levels (NOAELs) that have value in hazard assessment.

LD50s

Although less toxic than other trichothecenes such as T-2 toxin, acute exposure to extremely high DON doses (i.e., unlikely to be encountered in food) can produce shocklike death. LD50s for mice ranging from 49 to 70 mg/kg bw ip and from 46 to 78 mg/kg bw oral have been reported (Forsell et al., 1987; Yoshizawa, 1983). The LD50 for DON is 27 mg/kg bw in the 10-d-old duckling when administered sc (Yoshizawa & Morooka, 1974) and is 140 mg/kg bw when administered orally in day-old broiler chicks (Huff et al., 1981).

Histopathology

Consistent with other trichothecenes, acute ip exposure of mice to lethal levels of DON results in histopathologic effects ranging from hemorrhage/necrosis of the intestinal tract, to necrosis in bone marrow and lymphoid tissues, to kidney and heart lesions (Forsell et al., 1987; Yoshizawa & Morooka, 1977).

GI Tract Toxicity and Emetic Effects

The symptoms of acute DON toxicity in sensitive species include abdominal distress, increased salivation, malaise, diarrhea, and emesis and anorexia (Forsyth et al., 1977; Friend et al., 1982; Pestka et al., 1987a; Prelusky & Trenholm, 1993; Young et al., 1983). Although DON is considered to be one of the least lethal trichothecenes, its emetic and anorexic potencies are equal to or greater than those reported for the more acutely toxic trichothecenes (Rotter et al., 1996).

Forsyth et al. (1977) found that the ip minimum emetic dose MED for DON in swine is 50 μg/kg bw with a no-observed-adverse-effect level (NOAEL) of 25 μg/kg bw ip; orally, the MED is 100 μg/kg bw with a NOAEL of 75 μg/kg bw. Pestka et al. (1987a) reported that the MED for DON in swine is 50 μg/kg bw ip and oral, with the NOAEL being 25 μg/kg bw. Consistent with these findings, Prelusky and Trenholm (1993) evaluated emesis induction in fasted pigs and determined approximate ED50 (dose producing emesis in 50% of animals) for DON of 75 μg/kg bw following intragastric dosing.

Young et al. (1983) fed groups of 3–4 pigs a wide range of levels of DON-contaminated corn over several trials and found that vomiting occurs within minutes after ingesting diets containing 19.7 ppm DON, which was calculated, using feed intake data, to be approximately 0.15 mg/kg bw/d of the toxin. Vomiting was not detected in pigs fed 11.9 ppm, which suggests a NOAEL of 0.12 mg/kg bw/d based on average daily intake data.

The MEDs for the dog and the 10-d-old duckling are 100 and 10,000 μg/kg bw DON sc, respectively (Yoshizawa & Morooka, 1974). Hughes et al. (1999) prepared extruded foods with naturally contaminated wheat to generate DON concentrations of 0, 1, 2, 4, 6, 8, and 10 ppm and fed these diets to dogs and cats. Ingestion of DON at concentrations of 8 ppm induced vomiting in Brittany and beagle dogs, whereas ≤6 ppm did not (NOAEL = 0.3 mg/kg bw/d). In American Shorthair cats, DON at 10 ppm induced vomiting whereas ≤8 ppm (NOAEL = 0.4 mg/kg bw/d) did not. These latter authors observed tremendous variability in emetic patterns among individual dogs and cats, with some being highly susceptible and others being very resistant. These data suggest not only that species differ in sensitivity but also that individuals within a species may exhibit a wide sensitivity range to DON.

Metabolic Disturbances

Szkudelska et al. (2002) treated male Wistar rats subcutaneously with 1 mg/kg bw DON for 3 d, each as a single morning dose. Blood insulin, glucose, and free fatty acids were significantly increased compared to control animals, while other parameters including blood glucagon, leptin,

TABLE 1. Summary of DON Dose Levels Required for Acute Toxic Effects

Species	Study	Effect	Parameter	Dose[a]	References
Mouse	Acute—oral	Mortality	LD50	46	Yoshizawa and Morooka (1974)
	Acute—ip			70	
Mouse	Acute—oral	Mortality	LD50	78	Forsell et al. (1987)
	Acute—ip			49	
Duck	Acute—sc	Mortality	LD50	27	Yoshizawa and Morooka (1974)
Hen	Acute—oral	Mortality	LD50	140	Huff et al. (1981)
Swine	Acute—oral	Vomiting	LOAEL	0.050	Pestka et al. (1987)
			NOAEL	0.025	
Swine	Acute—oral	Vomiting	LOAEL	0.100	Forsyth et al. (1977)
			NOAEL	0.075	
Swine	Acute—oral	Vomiting	Emetic dose$_{50}$	0.075	Prelusky and Trenholm (1993)
Swine	Diet	Vomiting	LOAEL	0.15[c] (20 ppm feed)	Young et al. (1983)
			NOAEL	0.12[c] (11.9 ppm feed)	
Cat	Diet	Vomiting	LOAEL	0.30[b] (10 ppm feed)	Hughes et al. (1999)
			NOAEL	0.4[b] (8 ppm feed)	
Dog	Acute—sc	Vomiting	MED	0.1	Yoshizawa and Morooka (1974)
Dog	Diet	Vomiting	LOAEL	0.3[b] (8 ppm feed)	Hughes et al. (1999)
			NOAEL	0.40[b] (6 ppm feed)	

[a] All dosages in mg/kg bw/d, unless indicated otherwise.
[b] Calculated based on daily intake.
[c] Calculated from data in paper.

triglycerides, and cholesterol were not significantly affected. Significant increases in muscle glycogen and decreases in triglycerides were observed. No significant effects were noted in liver parameters evaluated.

Conclusions

Acute toxicity studies are summarized in Table 1. Acute exposure to extremely high DON (≥27 mg/kg bw) levels are required to produce mortality or marked tissue injury. In contrast, acute exposure to relatively low doses (≥50 μg/kg bw) can induce emesis in swine, the most sensitive species. For this effect to occur via a contaminated food, it appears likely that the DON dose must be presented as a single bolus rather than as increments over a day.

SUBCHRONIC/CHRONIC TOXICITY

The most common effects of prolonged dietary exposure of experimental animals to DON are decreased weight gain, anorexia, and altered nutritional efficiency (Table 2). As illustrated in the following studies, species differences are apparent.

Swine

Early studies (Abbas et al., 1986; Forsyth et al., 1977; Rotter et al., 1994; Trenholm et al., 1984; Young et al., 1983) have shown that in pigs ingesting feedstuffs naturally contaminated with DON, levels of 1 to 2 ppm typically produced partial feed refusal, whereas 12 ppm produced complete refusal.

Norwegian workers conducted feeding trials on groups of 8 Norwegian Landrace pigs (equal numbers of females and castrated males) to evaluate the effects of ingesting contaminated oats containing approximately 0.5, 1, 2, and 4 ppm DON (Bergsjo et al., 1992). Restricted feeding was compared to ad libitum feeding. The groups fed diets containing 2 and 4 ppm of DON exhibited a dose-related decrease in weight gain during the first 8 wk on experimental diets. The 4 ppm diet group had decreased feed intake, weight gain, and efficiency of feed utilization throughout the experiment. The NOAEL for this study is estimated to be 0.04 mg/kg bw/d.

TABLE 2. Examples of DON Dose Levels Required for Body Weight, Feed Intake, and Other Chronic Effects

Species	Study	Effect	Parameter	Dose[a]	References
Mouse	8 wk, Purified DON diet	Decreased growth	LOAEL	0.30[b] (2 ppm feed)	Forsell et al. (1986)
		Decreased feed intake	NOAEL	0.075[b] (0.5 ppm feed)	
Mouse	5 wk, Purified toxin by gavage	Decreased growth, lesions in lymphoid and GI tract	LOAEL	0.75	Arnold et al. (1986)
			NOAEL	Not determined	
Mouse	18 wk, Naturally contaminated DON diet	Decreased growth	LOAEL	0.93[c] (6.25 ppm feed)	Arnold et al. (1986)
			NOAEL	Not determined	
Mouse	1 wk, Purified DON diet	Decreased growth	LOAEL	0.36[b] (2.5 ppm)	Robbana-Barnat et al. (1987)
		Decreased food uptake	NOAEL	Not determined	
Mouse	5–6 wk, Purified DON diet	Decreased growth	LOAEL	0.6[b] (4 ppm feed)	Rotter et al. (1992)
			NOAEL	Not determined	
Mouse	9 wk, Purified DON diet	Decreased growth	LOAEL	1.5[c] (10 ppm)	Hunder et al. (1991)
			NOAEL	0.15[c] (1 ppm)	
Mouse	2 yr, Purified DON diet	Decreased growth	LOAEL	0.75[b] (5 ppm feed)	Iverson et al. (1995)
			NOAEL	0.15[b] (1 ppm feed)	
Rat	9 wk, Purified DON diet	Decreased growth	LOAEL	0.25	Arnold et al. (1986a)
		Decreased food uptake	NOAEL	0.50	
Rat	18 wk, Naturally contaminated DON diet	Decreased growth	LOAEL	0.63[c] (6.25 ppm feed)	Arnold et al. (1986b)
			NOAEL	Not determined	
Rat	90 d, Purified DON diet	Decreased growth	NOAEL	2.0[b] (20ppm)	Morrissey et al. (1985)
Cat	14 d, Naturally contaminated DON diet	Decreased food intake	LOAEL	>0.4[b] (6 ppm feed)	Hughes et al. (1999)
			NOAEL	0.3[b] (8 ppm feed)	
Dog	14 d, Naturally contaminated DON diet	Decreased food intake	LOAEL	>0.44[b] (10 ppm feed)	Hughes et al. (1999)
			NOAEL	0.3[b] (8 ppm feed)	
Swine	90 d, Naturally contaminated DON diet	Decreased growth	LOAEL	0.08[b] (2 ppm)	Bergsjo et al. (1992)
		Decreased food uptake	NOAEL	0.04[b] (1 ppm feed)	
Swine	95 d, Naturally contaminated DON diet	Decreased growth	LOAEL	0.07[b] (1.7 ppm feed)	Bergsjo et al. (1993b)
		Decreased food uptake	NOAEL	0.03[b] (0.7 ppm feed)	
		Increased liver weight			
		Decreased serum albumin			
Swine	28 d, Naturally contaminated DON diet	Decreased food uptake	LOAEL	0.07[c] (0.95 ppm feed)	Rotter et al. (1994)
		Decreased thyroid weight and α-globulin	NOAEL	Not determined	
		Increased T4, serum-albumin and A/G ratio			
Swine	32 d, Naturally contaminated DON diet 32 d, Purified DON	Decreased growth	LOAEL	0.12[b] (3 ppm feed)	Prelusky et al. (1994b)
		Decreased food intake	NOAEL	0.04[b] (1 ppm feed)	
			LOAEL	Not determined	
			NOAEL	0.12[c] (3 ppm feed)	
Swine	42 d, Naturally contaminated DON diet	Decreased growth	LOAEL	0.16[b] (4 ppm feed)	Rotter et al. (1994)
		Decreased food uptake	NOAEL	Not determined	
		Stomach corrugation			

[a]All dosages in mg/kg bw/day, unless indicated otherwise.
[b]Estimated based on daily intake (Association of Food & Drug Officials, 1975).
[c]Calculated from feed intake and body weight data found in paper.

In a subsequent feeding trial by this group (Bergsjo et al., 1993b), contaminated oats were added to achieve 0, 0.7, 1.7, and 3.5 ppm DON, and this was provided ad libitum to groups of 8 Norwegian Landrace pigs (equal numbers of males and females). Significantly decreased body weight gain, decreased slaughter weight, and reduced feed utilization efficiency were observed for the group fed a diet containing 3.5 ppm DON. At the same DON concentration, there were increased liver weights, decreased concentrations of serum protein and albumin, and a temporary fall in packed blood cell volume, serum calcium, and serum phosphorus. For the groups fed diets containing 1.7 and 3.5 ppm DON, a statistically significant, dose-related decrease in daily feed consumption was observed. No other effects on hematological, biochemical, or immunological parameters were recorded. The NOAEL for this study was estimated to be 0.03 mg/kg bw/d.

Groups of 8 Norwegian-Landrace pigs were fed diets amended with naturally contaminated oats to contain 0.6, 1.8, and 4.7 ppm DON (Øvernes et al., 1997). Effects on weight gain were not observed. Significantly reduced feed intake with increased levels of DON was observed in groups fed restricted rations according to weight, but not in animals fed ad libitum. Efficiency of feed utilization was significantly higher in 1.8- and 4.7-ppm groups. Since in this study the control diet contained 0.6 ppm DON, a NOAEL could not be determined.

The effects of low dietary concentrations of DON on growth and hematological parameters were determined in groups of 6–8 castrated male Yorkshire pigs during a 28-d feeding experiment (Rotter et al., 1994). Clean corn and naturally contaminated corn were incorporated into basal diets formulated to contain 0.00, 0.75, 1.5, and 3 DON ppm and chemically analyzed to contain 0.00, 0.95, 1.78, and 2.85 ppm, respectively. Feed intake was significantly suppressed at all DON levels, with estimated daily intakes of the toxin being 0.07, 0.12, and 0.17 mg/kg bw/d based on the chemical analysis and feed intake data. Several other linear effects were observed: reduction in thyroid size (absolute/relative), increased serum T4 (thyroxine) levels, and changes in the appearance of the esophageal region of the stomach (thicker and higher degree of folding with increasing toxin concentration). In addition, serum albumin levels increased, alpha-globulin levels decreased, and an overall albumin/globulin ratio increased as the level of contamination increased. When animals consuming the highest level of contamination were compared to pair-fed controls, better feed efficiency, more corrugated stomachs, and reduced alpha-globulin levels were observed. These latter two findings suggested that DON could alter stomach epithelial cell layer as well as the protein profile of blood, respectively; however the physiological significance of these data is unclear. Since the lowest dose produced an effect, a NOAEL could not be determined.

Groups of 5 castrated male Yorkshire pigs ingesting diets spiked with 3 ppm purified DON or DON in naturally contaminated corn exhibited decreased feed intake and weight gain during the first week of exposure (Prelusky et al., 1994b). However, during a 32-d feeding period, these effects were detectable only for the naturally contaminated diet. No effects were observed at the 1-ppm level, which provides an estimated NOAEL of 0.04 mg/kg bw/d. It was suggested that differences between naturally contaminated feed ingredients versus feed spiked with purified DON could be attributable to the presence of additional compounds in naturally contaminated grains that contribute additively or synergistically to DON toxicity.

Dogs and Cats

Food intake by groups of 7 Brittany and beagle dogs (1–7 yr) and groups of 2–3 American Shorthair cats (1–9 yr) was significantly reduced during a 14-d feeding period by dietary DON concentrations of greater than 4.5 ± 1.7 ppm (0.34 mg/kg bw/d) or 7.7 ± 1.1 ppm (0.39 mg/kg bw/d), respectively (Hughes et al., 1999).

Rodents

Groups of 8 weanling female B6C3F1 mice were fed semipurified diets containing purified DON at concentrations of 0, 0.5, 2, 5, 10, and 25 ppm for 8 wk (Forsell et al., 1986). This resulted in estimated mean daily intakes of 0, 0.15, 0.3, 0.75, 1.5, and 3.75 mg/kg bw/d, respectively, based on feed intake measurements. Weight gain was depressed by DON, with the lowest-observable-adverse-effect level (LOAEL) and NOAEL being 0.3 and 0.15 mg/kg bw/d, respectively. Feed intake was decreased, with the LOAEL and NOAEL being 3.75 and 1.5 mg/kg bw/d, respectively. Gross examination and histopathological studies showed that the spleen, liver, kidney, uterus, small intestine, colon, heart, brain, lungs, and bone marrow were normal in appearance and histological architecture.

Ingestion of diets containing 6.25 ppm for 18 wk impaired weight gain in both groups of 80–90 weanling Swiss-Webster mice and groups of 50 Sprague-Dawley rats (Arnold et al., 1986b). No histopathological findings were observable for brain, lungs, lymphoid tissues, pancreas, intestinal tract, kidneys, or liver in these animals. A 5-wk gavage study was also performed in groups of 24 weanling Swiss-Webster mice that were fed 0.75, 2.5, or 7.5 mg DON/kg bw/d. Effects on body weight were detectable at all levels tested. Most of the animals given 2.5 or 7.5 mg/kg bw in

the gavage study died during the test period and showed histopathological lesions in primary lymphoid tissues and gastrointestinal tract. Animals given 0.75 mg/kg bw/d exhibited no histopathological alterations.

In a subsequent study by the same laboratory, groups of 6–10 male and female Sprague-Dawley rats were fed diets containing 0, 0.25, 0.5, or 1.0 mg of DON/kg bw for 9 wk (Arnold et al., 1986a). A statistically significant, dose-related decrease in body weight gain was observed for all treated females, but only the males dosed at 1.0 mg/kg bw/d were found to have a treatment-related weight-gain suppression. The histopathological evaluations revealed no lesions attributable to DON in brain, lungs, heart, lymphoid tissues, pancreas, intestinal tract, kidneys, or liver.

Morrissey et al. (1985) fed DON at a level of 20 ppm to male Sprague-Dawley rats ad libitum for 90 d. In contrast to other rodent studies, few clinical signs of toxicity were observed. Rats in the DON treatment group consumed more diet, and terminal body weight was not significantly reduced in the DON treatment group. There were no statistically significant effects on serum enzyme levels, hematological parameters, or tissue lesions, or on liver detoxification systems, as reflected in levels of microsomal cytochrome P-450 or in glutathione *S*-transferase activity.

Robbana-Barnat et al. (1987) fed groups of 12 BALB/*c* mice, 4–6 wk old, with diets containing 0, 2.5, 5, 10, 20, and 50 ppm, which were equated by these authors to 0, 0.375, 0.75, 1.5, 3.0, and 7.5 mg/kg bw/d, respectively. Feed intakes and body weights after 1 wk were significantly reduced at 0.375 and 0.75 mg/kg bw/d, respectively. These effects were also demonstrable in a follow-up study where feeding was extended for 1 mo. Notably, cardiac lesions appearing as white flecks on the ventral surface of the heart were observed in mice given 1.5 to 3 mg/kg bw/d DON for 2–3 wk. This finding was presented in an observational fashion and no statistics were provided. Furthermore, it was not clear from the paper whether these effects occurred at lower doses. Observation of cardiac lesions contrasts sharply with the lack of histopathological findings associated to heart in other DON feeding studies.

When groups of 12–15 weanling ICR mice were fed 0, 4, 8, 12, 16, and 20 ppm DON for 5 to 6 wk, the lowest level that produced significant growth effects was 4 ppm (Rotter et al., 1992). The estimated LOAEL for this study was 0.6 mg/kg bw/d.

Groups of 20 male NMRI mice were fed 0, 0.1, 1, and 10 ppm DON (Hunder et al., 1991). Weight gain was significantly reduced in the 10-ppm group but was not affected by the lower DON concentrations. The 10-ppm dose also impaired intestinal transfer of nutrients such as glucose and 5-methyltetrahydrofolic acid. Food intake was not affected. The NOAEL was estimated to be 0.15 mg/kg bw/d.

In the most extensive DON feeding study reported to date, Iverson et al. (1995) examined the effects of feeding 22- to 28-d-old male and female B6C3F1 mice 0, 1, 5, and 10 ppm DON for 2 yr and found significant body weight reduction in mice fed 5 and 10 ppm DON. Food consumption was not affected in females but was decreased for male test animals at the two highest doses. Full histopathologic analysis revealed that DON produced a dose-related decrease in liver preneoplastic and neoplastic lesions that were attributed to reduced caloric intake. No other remarkable lesions were noted in the report. The estimated NOAEL in this study was 0.15 mg/kg bw/d.

Poultry and Ruminants

Poultry tolerate DON, as concentrations up to 8 ppm do not affect productivity (Hamilton et al., 1985a, 1985b). Rapidly growing broilers, however, are more sensitive to feed refusal than laying hens (Huff et al., 1986). DON fed to dairy cows at 66 ppm for 5 d (Cote et al., 1986a) or at 6.4 ppm for 6 wk (Trenholm et al., 1985) does not produce performance reduction or signs of illness.

Conclusions on Subchronic/Chronic Toxicity Studies

Monogastric animals are extremely sensitive to growth and weight-gain suppression upon subchronic and chronic DON exposure. This can be accompanied by anorexia at higher DON concentrations in diet. The NOAELs for rodents were 0.1–0.15 mg/kg bw/d, whereas for swine, NOAELs were 0.03–0.12 mg/kg bw/d. These results suggest swine are more sensitive to DON than mice or rats. Where tissue lesions were detected, the GI tract and lymphoid tissues are common

targets. The finding of cardiac lesions in one mouse study was not supported by other studies where histopathological analyses were conducted.

IMMUNE FUNCTION

Based on in vitro and in vivo mechanistic studies, DON and other trichothecenes are theoretically capable of modulating immune function in multiple fashions (reviewed in Bondy & Pestka, 2000). At low concentrations, they can potentiate or attenuate expression of cytokines, which can disrupt normal regulation of a wide variety of immune functions in positive or negative fashions. At high concentrations, trichothecenes can induce leukocyte apoptosis and produce pronounced immunosuppression. In the case of DON, these effects may dovetail with immunological effects induced by reduced feed intake. Pertinent studies are summarized in Table 3.

Mice

Most immunotoxicology studies for DON and other chemicals have been conducted in mice because (1) their immune system is the most thoroughly characterized of any species, (2) they are small in size, and (3) studies employing mice require much smaller amounts of purified DON.

Altered Host Resistance, Humoral, and Cell-Mediated Responses Groups of 12 weanling Swiss-Webster male mice were gavaged with 0.75 and 2.5 mg/kg bw DON daily for 5 wk and it was found that the antibody response to sheep red blood cells was suppressed (Tryphonas et al., 1984). In addition, 0.75 mg/kg bw/d decreased splenic and thymic weights. In a follow-up study by the

TABLE 3. Examples of DON Dose Levels Required for Immunotoxic Effects

Species	Study	Effect	Parameter	Dose[a]	References
Mouse	5 wk, Gavage, purified DON	Antibody response	LOAEL NOAEL	0.75 Not determined	Tryphonas et al. (1984)
Mouse	5 wk, Purified DON, diet	Host resistance, lymphocyte proliferation	LOAEL NOAEL	0.5 0.25	Tryphonas et al. (1986)
Mouse	2–4 wk, Purified DON, diet	Host resistance, DTH, antibody response	LOAEL NOAEL	3.75[b] (25 ppm diet) 0.75[b] (5 ppm diet)	Pestka et al. (1987b)
Mouse	1–2 wk, Purified DON, diet	Antibody response, lymphocyte proliferation	LOAEL NOAEL	1.5[c] (10 ppm diet) 0.75[c] (5 ppm diet)	Robbana-Barnat et al. (1988)
Mouse	4 wk, Purified DON, drinking water	Host resistance	LOAEL NOAEL	0.12 Not determined	Sugita-Kinoshi et al. (1998)
Mouse	1 wk, Gavage, purified DON	Host resistance	LOAEL NOAEL	6.25 Not determined	Atroshi et al. (1994)
Mouse	6 wk, Purified DON, diet	Serum IgA	LOAEL NOAEL	0.3[b] (2 ppm diet) 0.08[b] (0.5 ppm diet)	Forsell et al. (1986)
Mouse	4–12 wk, Purified DON, diet	Serum IgA	LOAEL NOAEL	1.5[b] (10 ppm diet) 0.3[b] (2 ppm diet)	Greene et al. (1994)
Mouse	12 wk, Purified DON, diet	Kidney IgA deposition, IgA nephropathy	LOAEL NOAEL	0.30[b] (2 ppm diet) Not determined	Greene et al. (1994)
Mouse	Single exposure, purified DON, gavage	Cytokine expression	LOAEL NOAEL	5.0 1.0	Zhou et al. (1997)
Mouse	2–7 d, Purified DON, gavage	Cytokine expression	LOAEL NOAEL	2.0 0.5	Zhou et al. (1998)
Swine	42 d, Naturally contaminated DON diet	Antibody response	LOAEL NOAEL	0.07[b] (1.78 ppm diet) 0.04[b] (0.95 ppm diet)	Rotter et al. (1994)

[a]All dosages in mg/kg bw/d, unless indicated otherwise.
[b]Calculated from feed intake and body weight data found in paper.
[c]Calculated based on daily intake.

same group, immune function was studied in groups of 6–10 male weanling Swiss-Webster mice fed 0, 0.25, 0.5, and 1 mg/kg bw/day of DON for 5 wk (Tryphonas et al., 1986). Spleen plaque-forming colonies (PFC) and serum antibody responses to sheep red blood cells were unaffected by all DON doses. Both 0.5 and 1 mg/kg bw/d of DON produced reduced, dose-related time-to-death interval following a challenge with *Listeria monocytogenes*, and an increased proliferative capacity of splenic lymphocyte cultures stimulated with phytohemagglutinin (PHA). The 0.25 mg/kg bw dose had no adverse effect. The authors estimated that a NOAEL for immunotoxicity in mice would be between 0.25 and 0.5 mg/kg bw/d.

Pestka et al. (1987b) assessed the immune effects of 2–3 wk of feeding 5 and 25 ppm DON (equivalent to 3.75 mg/kg bw/d) to groups of 5 female B6C3F1. As compared to pair-fed controls, 25 ppm DON depressed (1) the plaque-forming response to sheep red blood cells, (2) the delayed hypersensitivity response to keyhole limpet hemocyanin (KLH) and (3) the ability to clear *L. monocytogenes*, whereas diets containing 5 ppm DON had no marked effect on these parameters. The effects on resistance to *Listeria* and delayed hypersensitivity were not detectable in mice ingesting the mycotoxins for 8 wk. In contrast, the plaque-forming response was suppressed after an 8-wk period of DON ingestion. The NOAEL for DON in this study was 0.75 mg/kg bw/d.

When groups of 8 female B6C3F1 mice were fed with AIN 76A semipurified diets containing purified DON at 0, 0.5, 2, 5, 10, and 25 ppm (equivalent to 0, 0.1, 0.4, 1, 2, and 5 mg/kg bw/d) for 6 wk, serum white blood cell counts were depressed at dose levels >10 ppm (Forsell et al., 1986). The NOAEL for this parameter is estimated to be 1.0 mg/kg bw/d.

Robanna-Barnat et al. (1988) observed that groups of 4–17 male BALB/*c* mice fed 10, 20, and 50 ppm DON for 1 and 2 wk exhibited reductions in (1) antibody responses to sheep red blood cell, (2) splenic responses to PHA and lipopolysaccharide (LPS), and (3) thymic responses to PHA and thymus weight, whereas 5 ppm had no marked effect. The estimated NOAEL for DON was 0.75 mg/kg bw/d.

Sugita-Konishi et al. (1998) treated groups of ten 7-wk-old male BALB/*c* mice by adding the toxin to drinking water at 0, 0.2, 1, and 3 ppm for 4 wk and evaluating the resistance to *Salmonella enteritidis*. These DON levels were insufficient to produce refusal to drink or eat. Increased mortality due to *S. enteritidis* exposure was observed at 1 and 3 ppm, but not at the 0.2-ppm dose. However, these data were presented in a descriptive fashion and a statistical analysis was not reported. When mice were provided 2 ppm DON in drinking water, both the immunoglobulin (Ig) M antibody and delayed-type hypersensitivity responses to *S. enteritidis* treatment were significantly suppressed. From these latter data and water intake measurements, the authors calculated that the lowest effect level based on water intake was 0.12 mg/kg bw/d.

Groups of 5 lactating, inbred Han:NMR1 mice were given DON at 12.5 mg/kg bw for 1 d or 6.25 mg/kg bw for 7 consecutive days by gavage and their resistance to the mastitic pathogens *Staphylococcus hyicus* and *Mycobacterium avium* was examined (Atroshi et al., 1994). No suppression of immune response was observed. Rather, both treatments were found to enhance resistance to *S. hyicus*but not to *M. avium*. The 1-d treatment in *S. hyicus*-treated mice was found to increase total serum IgA, whereas the 7-d treatment increased serum IgA, IgM, and IgG. Enhanced host resistance is frequently seen when trichothecenes are administered just prior to challenge with a model pathogen (Bondy & Pestka, 2000).

Altered Serum IgA Levels After feeding groups of 8 weanling female B6C3F1 mice with AIN 76A semipurified diets containing purified DON (0, 0.5, 2,10 and 25 ppm) for 6 wk, it was found that serum IgA levels were increased at levels of DON ≥2 ppm with no effect being observed at 0.5 ppm (Forsell et al., 1986). Serum IgM was decreased by 25 ppm DON. Using serum IgA dysregulation as an endpoint, the estimated LOAEL and NOAEL for DON from this study were 0.3 and 0.075 mg/kg bw/d, respectively.

The same group subsequently reported that the IgA elevation could be maximally induced in female B6C3F1 mice by feeding DON at 25 ppm (Pestka et al., 1989). These effects were detectable at 4 wk and rose to over 17 times control levels after 24 wk. Concurrent decreases in serum IgM and IgG also occurred. Comparison to restricted controls verified that these Ig isotype effects were not solely due to reduced food intake.

In a later study, the same lab compared the sensitivity of male and female B6C3F1 mice (7–9 per group) to IgA hyperelevation and found that a dietary level of 10 ppm DON was necessary to observe consistent, significant increases in serum IgA for both males and females at 4, 8, and 12 wk whereas 2 ppm had no effect (Greene et al., 1994). This would indicate an estimated NOAEL for DON of 0.3 mg/kg bw/d.

In a 2-yr feeding study using B6C3F1 mice conducted by Health Canada at dose levels of 0, 1, 5, and 10 ppm purified DON, a linear dose-related increase in serum IgA and IgG was observed in female but not male mice (Iverson et al., 1995). The increase of about 1.5-fold was much less than that found in the studies of shorter duration from Pestka's lab. The authors suggested that 2-yr feeding with DON may allow for adaptation thus masking earlier effects. Another explanation may relate to differences in diets used—Purina certified feed for the Canadian study versus AIN-76A semipurified diet for the other studies.

IgA Nephropathy Elevation in serum IgA of female B6C3F1 mice following ingestion of 25 ppm DON in diet corresponds with marked deposition of IgA in the kidney mesangium, which mimics the common human glomerulonephritis IgA nephropathy (Dong et al., 1991; Pestka et al., 1989). This effect occurs in multiple strains of mice.

Greene et al. (1994) observed that 12 wk of dietary exposure to DON produced significant increases in kidney mesangial IgA in males and females fed 2 and 10 ppm DON, respectively. Other effects include hematuria and increased IgA–immune complexes. These effects can persist with the kidney IgA deposits for at least 16 wk after withdrawal of 25 ppm DON (after feeding for 8 wk) from the mouse diet (Dong & Pestka, 1993).

Another common feature of human IgA nephropathy and the DON mouse model is the involvement of polyvalent "natural" IgA, which may be associated with immune complex formation and subsequent glomerulonephritis (Rasooly et al., 1994; Rasooly & Pestka, 1992, 1994; Yan et al., 1998a).

Intermittent dietary DON exposure is less effective at inducing IgA nephropathy than is continuous exposure (Banotai et al., 1999b). This appears to relate to the capacity of mice to suspend eating until contaminated diet is replaced with clean diet.Dietary DON at 5 and 10 ppm can induce IgA nephropathy in murine models of systemic lupus erythematosus but does not exacerbate the manifestations of lupus (Banotai et al., 1999a).

Mechanisms for DON-Induced IgA Elevation The mechanistic basis for DON-induced IgA elevation has been examined in detail, typically in B6C3F1 mice using toxin concentrations (10–25 ppm) to achieve the maximal response. Peyer's patch lymphocytes and, to a lesser extent, splenic lymphocytes isolated from female B6C3F1 mice fed 25 ppm DON produce significantly more IgA than cultures derived from mice receiving ad libitum or restricted control diets (Bondy & Pestka, 1991; Pestka et al., 1989, 1990a, 1990b). These results suggest that DON enhances differentiation to IgA-secreting cells at the Peyer's patch level and that this impacts the systemic immune compartment.

Using an ex vivo approach and neutralizing antibodies, it was found that potential for enhanced IgA production exists in lymphocytes as early as 2 h and as late as 24 h after a single oral exposure to 5, and 25 mg/kg DON. This observation may be related to the increased capacity to secrete the helper cytokines IL-2, IL-5, and IL-6 (Dong et al., 1994; Warner et al., 1994; Wong et al., 1998). Both $CD4^+$ and macrophage cells appear to be involved in this process (Yan et al., 1998b). Thus, increased cytokine expression may, in part, be responsible for upregulation of IgA secretion in mice exposed orally to DON.

Cytokine Expression The ability of DON to transiently alter expression of cytokines is important because such effects can disrupt normal regulation of a wide variety of immune functions in a positive or negative fashion. DON can upregulate cytokine production in both in vitro and in vivo murine models (Azcona et al., 1995a, 1995b; Dong et al., 1994; Heller et al., 1990; Ji et al., 1998; Ouyang et al., 1995, 1996a; Warner et al., 1994; Wong et al., 1998). The levels required for in vitro effects (50 to 1000 ng/ml) are readily attained within minutes in plasma, lymphoid, and other tissues of mice gavaged with 5 and 25 mg/kg of DON, and can last for several hours (Azcona et al., 1995a; Yordanova et al., 2003). Thus, in vitro approaches should be suitable for exploring in vivo DON mechanisms.

Superinduction of cytokine gene expression by DON is mediated via both transcriptional and/or posttranscriptional mechanisms. For example, DON at 250 ng/ml increases binding of the transcription factors NF-κB and AP-1 in T-cell (Li et al., 2000; Ouyang et al., 1996b) and macrophage cultures (Wong et al., 2002). Using transcriptional inhibitors, increased cytokine mRNA expression by DON was also found to be due, in part, to markedly enhanced mRNA stabilities in the T-cell (Li et al., 1997), and interleukin-6(IL-6)/tumor necrosis factor (TNF)α mRNA stability in the macrophage (Wong et al., 2001).

The effects of a single oral exposure of groups of 3 male B6C3F1 mice to 0, 0.1, 0.5, 1, 5, and 25 mg/kg bw DON on cytokine mRNA abundance in spleen and Peyer's patches (indicators of systemic and mucosal immune compartments, respectively) were assessed at 2 h postexposure using reverse-transcription polymerase chain reaction (RT-PCR) in combination with hybridization analysis (Zhou et al., 1997). Both 5 and 25 mg/kg DON significantly induced the mRNAs for proinflammatory cytokines (IL-1beta, IL-6, and TNF-alpha), T helper 1 cytokines (interferon [IFN]-gamma and IL-2) and T helper 2 cytokines (IL-4 and IL-10), whereas lower doses had no marked effect. IL-12p40 mRNA was also induced, but not IL-12p35 mRNA. The effects were more pronounced in spleen than in the Peyer's patches. IL-5 and TGF-beta mRNAs were expressed constitutively in spleen and Peyer's patches, but were not affected by DON. The NOAEL from this study for DON was 1 mg/kg bw/d.

The same authors subsequently examined the effects of repeated DON dosing on cytokine expression. Male B6C3F1 mice were orally dosed with DON at 0, 0.5, 2, or 5 mg/kg bw consecutively for 2, 4, or 7 d and cytokine mRNAs assessed 2 h after the last treatment in spleen and Peyer's patches (Zhou et al., 1998). Upon exposure to 2 and 5 mg/kg bw DON, the relative abundance of IL-1beta, IL-6, TNF-alpha, IL-12 p35, IL-12 p40, IL-2, and IL-10 mRNAs increased with dose frequency, whereas IFN-gamma and IL-4 mRNAs were unaffected. This suggested that the ability of DON to dysregulate cytokine expression was cumulative. The NOAEL from this study for DON was 0.5 mg/kg bw/d.

Apoptosis in Lymphoid Tissue High doses of trichothecenes promote rapid onset of leukocyte apoptosis, and this will undoubtedly be manifested as immunosuppression. Using flow cytometric analysis, it was observed that DON either inhibits or enhances apoptosis (programmed cell death) in a concentration-dependent manner in T cells, B cells, and IgA^+ cells isolated from spleen, Peyer's patches, and thymus (Pestka et al., 1994). Induction of apoptosis was dependent on lymphocyte subset, tissue source, and glucocorticoid induction.

DON-induced apoptosis occurs in mouse macrophage cells (Yang et al., 2000). These in vitro results are relevant to the whole-animal model since in vivo administration of T-2 toxin and other trichothecenes to rodents results in apoptosis in thymus, spleen, bone marrow, and liver (Ihara et al., 1997, 1998; Islam et al., 1998; Miura et al., 1998; Shinozuka et al., 1997, 1998).

Zhou et al. (2000) demonstrated that oral gavage with 25 mg/kg bw DON could significantly induce apoptosis in B6C3F1 mice. This was markedly potentiated by bacterial lipopolysaccharide (LPS). LPS potentiation of DON-induced lymphocyte apoptosis in thymus, Peyer's patches, spleen, and bone marrow has been linked to elevated corticosterone (Islam et al., 2002, 2003), which is driven by superinduction of IL-1β (Islam & Pestka, 2003).

Chickens

Groups of 10 female white Leghorn chicks were fed 0 and 18 ppm DON for 18 wk (Harvey et al., 1991). The diet suppressed antibody responses to Newcastle disease vaccine. Groups of 3 broilers fed 50 ppm showed a suppressed lymphocyte blastogenesis response.

Swine

There is only limited information on immune effects of DON in swine immune function. In two studies, young Norwegian Landrace female and castrated male pigs were fed 0–4 ppm DON (equivalent to 0–0.16 mg/kg bw/d) for 90–95 d and significant differences in serum IgA levels were not observed (Bergsjo et al., 1992, 1993b).

The effects of feeding diets amended with naturally contaminated corn containing 0, 0.95, 1.78, and 2.85 ppm DON (equivalent to 0, 0.04, 0.07, and 0.1 mg/kg bw/d) was evaluated on

several immune parameters in groups of 6–8 male castrated Yorkshire pigs treated for 28 d (Rotter et al., 1994). Antibody responses to sheep red blood cells were delayed in animals exposed to the two highest concentrations; pair-fed controls indicated that this was not solely a nutritional effect. The treatments had no effect on peripheral blood mononuclear cell proliferative responses to the mitogens concanavalin A (Con A), phytohemagglutinin (PHA), or pokeweed mitogen. At the end of the experiment, the total leukocyte count rose with increasing DON concentrations. This was apparently due to increases in segmented and band neutrophils. No marked alterations were seen in monocyte and eosinophil counts. These data suggest the NOAEL for DON relative to the most sensitive immune parameter was 0.04 mg/kg bw/d.

The effects of diets amended with naturally contaminated wheat containing 0.6, 1.8, and 4.7 ppm DON on the immune response of growing male and female Norwegian Landrace pigs were evaluated (Øvernes et al., 1997). Immune response parameters recorded included primary and secondary antibody titers after injections of five different antigens: human serum albumin (HSA), sheep red blood cells (SRBC), paratuberculosis vaccine (MPT), tetanus toxoid (TT), and diphteria toxoid (DT). Delayed hypersensitivity and lymphocyte stimulation tests were also performed. A significant DON dose-dependent reduction in secondary antibody response to tetanus toxoid was observed. A quantitative higher mitogen response following PHA stimulation in lymphocytes from the medium and high DON groups compared to the low DON group after 9 wk was considered inconclusive. No other indication of dose-dependent immune response inhibition or stimulation was found. Further, there was no apparent evidence of IgA nephropathy. This study was limited by absence of a toxin-free control diet among the experimental groups.

Swamy et al. (2002) fed Yorkshire pigs ($n = 12$) control and a treatment diet that contained 4.6 and 0.5 ppm DON and 15-ADON, respectively, for 21 d. The treatment pigs exhibited significantly increased serum IgA and IgM.

Conclusions on Immune Function Effects

In total, the results suggest that some components of the immune response are very sensitive to DON whereas others are less so. Several points are worth noting. First, mouse studies suggest that DON can suppress host resistance to *Listeria* (Tryphonas et al., 1986; Pestka et al., 1987b) and *Salmonella* (Sugita-Kinoshi et al., 1998), with NOAELs of 0.25 and 0.12 mg/kg bw/d, respectively. Supportive data were provided as to possible mechanisms for these effects.

Second, antibody responses are also affected by DON in mice, with NOAELs of 0.75 mg/kg bw/d being reported (Pestka et al., 1987b; Robbana-Barnat et al., 1988). The pig is potentially more sensitive, with an estimated NOAEL of 0.04 mg/kg bw/d (Rotter et al., 1994).

Third, NOAELs of 0.08 to 0.3 mg/kg bw/d have been reported for serum IgA elevation (Forsell et al., 1986; Greene et al., 1994). The proposed underlying mechanism for this effect, cytokine upregulation, is detectable in mice exposed subchronically to DON with a NOAEL of 0.5 mg/kg bw/d. In contrast, it should be noted that Iverson et al. (1995) did not observe marked IgA elevation or glomerulonephritis in mice exposed to up to 10 ppm DON for 2 yr, suggesting that the effect may be dependent on the length of the study or different diets employed by the two groups conducting these studies. Swamy et al. (2002) has similarly observed increased serum IgA in pigs fed 5 ppm DON. However, several studies in swine suggested that dietary DON up to 4.7 ppm does not elevate IgA or produce IgA nephropathy. This difference between pig and mouse studies may relate to species differences or the use of semipurified diets where IgA nephropathy has been observed in mice fed DON. Until the cause of these differential sensitivities is better understood, caution should be used when considering IgA dysregulation as a possible human hazard.

NEUROENDOCRINE EFFECTS

Neuroendocrine effects of DON have been reviewed in detail by Rotter et al. (1996). The anorectic and emetic responses discussed earlier are believed to be mediated by the serotoninergic system based on increased levels of serotonin or its metabolites in DON-treated animals (Prelusky

et al., 1992; Prelusky, 1996), as well as the capacity of serotonin receptor antagonists to prevent DON-induced emesis (Prelusky and Trenholm, 1993).

Fioramonti et al. (1993) found that oral dosing with 0.05–1 mg/kg DON to rats could delay gastric emptying in a dose-related fashion. Gastric propulsion was attenuated in mice treated with 1 mg/kg of DON. The authors provided further evidence suggesting that DON inhibits gastric emptying via the induction of intestinal migration motor complexes by peripheral action at serotonin-3 receptors.

An interesting feature of DON is its capacity to produce taste aversion to other constituents that may be present in food. Clark et al. (1987) examined the effects of DON on the feeding and development of conditioned taste aversion in rats. It was found that conditioned taste aversion to saccharin could be established by adulteration of the food with 4–8 ppm DON and concurrent presentation of a novel saccharin drinking solution.

The area postrema is a circumventricular organ located on the fourth ventricle of the brain that has been functionally associated in a range of physiological and behavioral processes and can function as a sensor for bloodborne toxins (Borison, 1989). The area postrema has a reduced blood–brain barrier, making it accessible to drugs that do not penetrate other regions of the brain. This organ has been closely associated with conditioned taste aversion. Ossenkopp et al. (1994) studied DON-conditioned aversion to a novel saccharin taste to assess a possible mediating role of the area postrema. Groups of 7 adult male rats drank a novel 0.15% saccharin solution followed by ip injection of DON (0.125 mg/kg) or vehicle. In subsequent two-bottle preference tests, the rats conditioned with DON displayed significantly lower absolute and relative saccharin intake levels in comparison to control rats, which exhibited a strong preference for saccharin solution. In a follow-up experiment, groups of six adult male rats received area postrema ablations or sham lesions. On 2 conditioning days all rats drank a novel 0.15% saccharin solution followed by injections of DON (0.125 mg/kg, ip). In subsequent two-bottle preference tests, the sham-lesioned rats displayed a significant aversion to the saccharin stimulus, relative to the area postrema-ablated rats, which exhibited a preference for the saccharin solution. The results suggested that administration of DON, following a novel taste, induces conditioned taste aversions that were mediated by the area postrema.

REPRODUCTIVE AND TERATOGENIC EFFECTS

Mice

The reproductive effects of DON on groups of 15–19 Swiss-Webster mice after daily gavage with 0, 0.5, 1, 2.5, 5, 10, and 25 mg/kg bw DON on d 8–11 of gestation were evaluated (Khera et al., 1982). Significant resorption of embryos was observed at ≥2.5 mg/kg bw, with doses of 10 and 15 mg/kg bw producing complete resorption of fetuses. DON levels of ≥5 mg/kg produced a significant decrease in live fetuses per dam. Skeletal malformations such as absent or fused ribs were observed in a dose-responsive fashion for the 1-, 2.5-, and 5-mg/kg bw doses. This study concluded that the NOAEL for embryo toxicity was 0.5 mg/kg bw/d.

In a second study, Khera et al. (1984) fed groups of 7 to 20 weanling F_0 Swiss-Webster mice, 0 and 2 mg/kg bw/d DON (Experiment 1) or 0, 0.375, 0.75, and 1.5 mg/kg bw/d DON (Experiment 2). After 30 d, mice were mated twice and progeny were evaluated for 19–21 d. At 2 mg/kg bw/d DON, decreased feed consumption, maternal body weight, and postnatal mortality were observed. Fertility was not affected and deformities in offspring were not seen. However, in the second set of progeny (F_{1b}), there were significant decreases in implants per litter, percent fetal resorptions, number of live fetuses, and total weight in toxin-treated mice. Furthermore there was an increased frequency of skeletal malformations. In the second experiment, the 1.5-mg/kg bw/d dose produced significant increases in postnatal mortality. No effects on fetal development or fetal malformations were noted at ≤1.5 mg/kg bw/d DON. The NOAEL for fetal toxicity was 0.75 mg/kg bw/d.

Debouck et al. (2001) treated female NMRI mice by ip administration with DON at 3, 4, 5, and 10 mg/kg bw on gestation d 7 and 9 (Experiment 1) or with 1.5, 2.5, and 3 mg/kg bw on d 7 to

10 (Experiment 2). The experimental design intended to expose embryos to DON during the early organogenetic period. Following treatments, maternal mortalities were increased with higher doses of DON but had a lower impact if given for a longer period, that is, 4 versus 2 d. Implant resorption rates were dose-dependently higher in treatment animals compared to controls, regardless of treatment period. The most frequent deformities were in the axial skeleton, with costo-vertebral segmentation abnormalities most commonly observed. However, statistical analysis of results was limited throughout the study, and the authors noted that the use of ip administration of DON may not follow the same pattern as physiological absorption by means of an oral form of administration.

Rats

Fischer 344 rats were fed DON at 0.0, 0.5, 2, or 5 ppm (equivalent to 0, 0.05, 0.2, and 0.5 mg/kg bw/d) during the entire course of pregnancy to assess possible teratogenicity (Morrissey, 1984). There were no overt signs of toxicity in the dams, and no statistically significant differences in feed consumption at any level compared to the control group. Dams in the two groups receiving the highest levels of DON tended to weigh less at term than other females. After removal of the pups, the uterus and carcass weights were significantly lower than those in the control group. Male and female pup weights were unaffected by the maternal treatment. DON had no statistically significant adverse effects on the incidence of gross, skeletal, or visceral abnormalities. Neither dams nor pups showed any significant histopathological changes. The NOAEL for this study was estimated to be 0.5 mg/kg bw/d.

In a follow-up study, this lab group fed 20 ppm DON to Sprague-Dawley rats (20 male, 25 female, 60 d) before and during pregnancy and observed a significant reduction in fertility (Morrissey & Vesonder, 1985). There were no treatment-related histological abnormalities in testes or ovaries.

When groups of 15 male and 15 female Sprague-Dawley rats were fed 0, 0.25, 0.5, and 1 mg/kg bw/d DON for 6 wk before and throughout pregnancy, fertility was not impaired (Khera et al., 1984). Except for dilation of renal pelvis and urinary bladder at all dose levels, the fetal viscera were normal.

Rabbits

Groups of 13–15 New Zealand white rabbits were fed 0, 0.3, 0.6, 1, 1.6, 1.8, and 2 mg/kg bw/d DON in diet for d 0–30 of gestation (Khera et al., 1986). The DON dose of 1–2 mg/kg bw was maternotoxic as indicated by significant reductions in body weight and feed intake. The 1 and 1.6 mg/kg bw DON levels produced losses in fetus body weight, whereas 1.8 and 2 mg/kg bw produced complete resorption of fetuses. There was no evidence for teratogenic effects. A DON level of 0.6 mg/kg bw/d is the NOAEL for this study.

Swine

Effects of ingesting 0.1 to 4.8 ppm DON exposure during gestation have been examined in 2 studies, one to d 50–54 of gestation and the other until 3 wk of lactation (reviewed by Eriksen & Alexander, 1998). There were no adverse effects on number of offspring or survival, nor were any deformities observed.

Poultry

Dietary administration of DON at 0.2 to 4.9 ppm to White Leghorn hens for 24 wk had no adverse effects on egg numbers, incidence of dead or abnormal embryos, or weight and survival of chicks at hatching (Hamilton et al., 1985a).

Bergsjo et al. (1993a) examined the effects of feeding 0.12, 2.5, 3.1, and 4.9 ppm DON to White Leghorns and found no marked difference in reproduction performance parameters including fertility, hatchability, perinatal mortality, chick body weight, or survival. Several anomalies were detected at the three highest DON concentrations. These included minor malformations, unwithdrawn yolk sac, and incomplete skeletal ossification, indicative of delayed fetal maturation. Major malformations, like cloacal atresia and cardiac anomalies, occurred in numbers too low to allow statistical analyses. The presence of low quantities of other mycotoxins (3-acetyl-DON, zearalenone, and ochratoxin A) complicates interpretation of these results.

Conclusions on Reproductive and Teratogenic Effects

Reproductive studies are summarized in Table 4. DON is capable of inducing reproductive/teratogenic effects similarly in mice and rabbits with NOAELs of 0.5 and 0.6 mg/kg bw/d, respectively. However, there was little distinction between toxin doses that produce maternal toxicity (feed refusal or reduced weight gain) and those that produce adverse reproductive effects. Rats did not seem to be sensitive to these effects, suggesting that species differences exist.

POTENTIAL EFFECTS ON HUMANS

Historical

From acute toxicity studies in animals it seems plausible that DON might produce similar effects in humans. Trichothecenes found in moldy grains have been suspected to produce a human illness known as "taumalgetriede" (staggering grains) that was first observed in Siberia in the 1890s. Symptoms included vomiting, headache, and vertigo (reviewed in Pestka & Casale, 1990). A related disease, "alimentary toxic aleukia" (ATA), was first reported in eastern Siberia in 1913, and subsequent outbreaks occurred in an ever-widening area. ATA was associated with overwintered wheat, barley, and millet and had as its symptoms vomiting, diarrhea, leukopenia, hemorrhage, shock, and sometimes death. Major ATA epidemics occurred in the Orenburg region of the USSR from the 1930s to the late 1940s, with mortality reaching 60% in the affected population in some years. Retrospective studies of fusaria isolated from moldy grains obtained during ATA outbreaks demonstrated that T-2 toxin and related trichothecenes may have been the etiologic agents of this disease (Joffe, 1978).

Epidemiological Studies Related to Gastroenteritis

Yoshizawa (1983) documented outbreaks of human food poisoning with nausea, diarrhea, and vomiting as primary symptoms that were associated with *Fusarium*-infested food between 1946 and 1963 in Japan and Korea. The studies predated the discovery of the possible causative mycotoxin, DON.

Potentially useful information on human DON toxicity comes from China (Luo, 1994). There were 32 outbreaks of food poisoning associated with ingestion of scabby wheat, scabby barley, or moldy corn in China from 1961 to 1981. Of 9382 persons who consumed the moldy cereals, 5998 persons were affected, representing a 63.9% attack rate. Characteristic symptoms in these outbreaks included nausea, vomiting, abdominal pain, diarrhea, headache, dizziness, and fever, all of

TABLE 4. Examples of DON Dose Levels Required for Reproductive/Teratogenic Effects

Species	Study	Effect	Parameter	Dose[a]	References
Mouse	Fed DON d 8–11 gestation	Fetal skeleton abnormalities	LOAEL NOAEL	1.0 0.5	Khera et al. (1982)
Mouse	Continuous feeding of DON to parents from weaning and to progeny	Mortality of pups	LOAEL NOAEL	1.5 0.75	Khera et al. (1984)
Rat	Continuous feeding of DON to parents from weaning and to progeny	Maternal and/or embryo toxicity	LOAEL NOAEL	Not determined 1.0	Khera et al. (1984)
Rat	Continuous DON feeding before breeding, throughout pregnancy and lactation	Mortality, decreased weight of pups	LOAEL NOAEL	Not determined 0.5[b] (5 ppm diet)	Morrissey (1984)
Rat	Continuous DON feeding before breeding throughout pregnancy	Decreased fertility	LOAEL NOAEL	Not determined 2.0[b] (20 ppm)	Morrissey and Vesonder (1985)
Rabbit	Fed DON d 0–30 gestation	Fetal death/resorption	LOAEL NOAEL	1.0 0.6	Khera et al. (1986)

[a]All dosages in mg/kg bw/day, unless indicated otherwise.
[b]Calculated based on daily intake.

which mimic food poisoning by *Staphylococcus* enterotoxin. Onset time was approximately 30 min. DON or other trichothecenes were not sought in food samples from these outbreaks because of the absence of suitable analytical methods. Other causative toxicants were not identified in these outbreaks.

During 1984–1991, 21 more gastroenteritis outbreaks occurred in China that were associated with scabby/moldy cereals. Using newly available analytical methods, investigators in these outbreaks found that associated food samples contained DON and/or other trichothecenes. The most serious outbreak occurred in Anhui province during 1991, when 130,141 people were affected. Analysis of 10 wheat samples collected in this outbreak indicated the presence of DON at 2 to 50 ppm. Tables 5–7 summarize clinical manifestations and analytical data from various scabby/moldy cereal poisoning outbreaks in China.

Li et al. (2002) examined wheat samples from the 1998 and 1999 crops from Puyang, an area in Henan Province, PR China, following a human red mold intoxication episode; the samples were analyzed for trichothecenes and zearalenone. For the 1998 Puyang crop, DON was the predominant toxin detected abundantly and frequently at a level of up to 14 ppm (mean 2.85 ppm) in 30 of 31 (97%) wheat samples. Among these were 21 (70%) with a DON level that exceeded the Chinese regulation limit of 1 ppm. Nivalenol (NIV) and 15-acetyl-DON (15-ADON) were also found at

TABLE 5. Food Poisoning Outbreaks Caused by Scabby/Moldy Cereals in China During 1960–1991 (from Luo, 1994)

Region	Date	Number of outbreaks (% affected)	Number of cases
Zhejiang, Hubei, Anhui, Jiangsu, etc.	1960–1981	32 (63.9)	5998
Xingtai Hebei	March 1984	1 (85.1)	362
Lingtao Gansu	June 1985	1 (87.6)	1357
Puyang Henan	July 1985	1 (46.5)	101
Yulin Guangxi	October 1988	1 (25.0)	40
Pingshan Hebei	November 1988	1 (52.5)	270
Taiyuan Shanxi	December 1988	1 (67.9)	142
Hongxian Guangxi	April 1989	1 (100.0)	10
Zigong Sichuan	May 1989	1 (100.0)	17
Baihe Shanxi	June 1989	1 (14.0)	701
Anhui	1991	8 (?)	130,141
Jiangsu	1991	4 (?)	6560

TABLE 6. Clinical Manifestations of Scabby/Moldy Cereals Poisoning in China (from Luo, 1994)

	Number of people affected								
Outbreak designation	1	2	3	4	5	6	7	8	9
Total cases	257	122	51	59	362	1357	40	263	47
Nausea	199	45	41	50	325	847	40	148	32
Vomiting	78	33	21	29	232		34	59	29
Stomach upset	257			48					
Dizziness	25	51	42	45	283	847	38	179	33
Headache			1	17	466				
Abdominal pain	30	33	10	12	22	466	12	139	37
Abdominal distension			6	6		466	40		35
Diarrhea	29	24	8	7	12		2	94	41
Slobber			13						
Fever	7	1	3	3	20				5
Generalized malaise			3	23		466	34	160	24
Palpitation				15	3				
Convulsions	17			14					
Lethargy									4

TABLE 7. Determination of *Fusarium* Toxins in Scabby/Moldy Cereals Associated with Food Poisoning in China (from Luo, 1994)

Region	Date	Poisonous foods	Number of samples	Assay	Trichothecenes (ppm)		
					DON	T-2	NIV
Xingtai	1984	Moldy corn	2	GC-MS	0.34–3.75	NT	ND
Hebei			3	RIA	5.1–92.8	NT	ND
Puyang, Henan	1985	Scabby wheat	14	TLC	2.0–40.0	NT	NT
Yuling, Guangxi	1988	Wheat flour	3	TLC	1.5–2.2	NT	NT
Pingshan, Hebei	1988	Corn flour	3	TLC	20.0–50.0	NT	NT
			6	GC	2.1–57.9	ND	ND
Taiyuan, Shanxi	1988	Corn flour	1	TLC	3	ND	ND
Hengxian,	1989	Corn flour	5	TLC	4.0–36.0	NT	NT
Guangxi			2	GC	59.3–66.8	ND	ND
Anhui	1991	Moldy wheat	10	TLC	2.0–50.0	NT	NT
Henan	1991	Moldy wheat	35	TLC	1.0–3.5	NT	NT
Anhui	1991	Moldy wheat	61	ELISA	NT	.0032-0.604	NT
		Rice	6	ELISA	NT	.2296-1.002	NT
Henan	1991	Wheat	35	ELISA	NT	.0119-1.004	NT

Note. NT, not tested; ND, not detected; GC-MS, gas chromatography–mass spectrometry; RIA, radioimmunoassay; TLC, thin-layer chromatography; ELISA, enzyme-linked immunosorbent assay.

0.58 ppm and in 20 samples. ZEA co-occurred in 21 samples at 0.01–1.4 ppm (mean 0.21 ppm). Twenty-five (89%) wheat samples from Zhumadian, a region in the same province without a history of human red mold intoxication, contained low levels of DON (0.5–1.24 ppm; mean, 0.22 ppm). All were free from 15-ADON and NIV. Mean DON in similar samples in 1999 in Henan were <1 ppm. The environmental conditions for *Fusarium* species surviving winter combined with unusually high precipitation during wheat flowering were believed responsible for a high concentration of DON mycotoxins in the 1998 Puyang wheat and possibly contributed to the outbreak of gastroenteritis.

An outbreak of gastroenteritis was reported that affected several thousand individuals consuming products made from rain-damaged moldy wheat in the Kashmir Valley of India (Bhat et al., 1989). In this study, 11 of 24 samples taken from the affected area contained DON in the range of 0.34 to 8.4 ppm. The authors used the lowest level of DON found in wheat (0.34 ppm), an average intake of 67 g of wheat products, and a mean body weight of 52 kg to generate a no-observed-effect level (NOEL) of 0.438 μg/kg bw/d. Kuiper-Goodman et al. (1994) questioned this NOEL because samples were not collected until 4 mo after the outbreak. Thus, a direct association between specific samples and the illness could not be established.

There have been no recorded outbreaks of gastroenteritis in the United States that have been etiologically linked to DON. However, the U.S. Centers for Disease Control and Prevention (CDC) reported 16 gastroenteritis outbreaks in school children from 6 states who consumed burritos from 2 manufacturing plants (Anonymous, 1999). Symptoms included abdominal cramps, vomiting, diarrhea, headache, and dizziness. In a Georgia outbreak, the median onset and duration times were 15 min and 4.5 h, respectively. The rapid onset times suggested that a toxin was producing the illness. *Bacillus cereus* and *Staphylococcus aureus* toxins were not detectable in associated samples. Other tests for putative toxic agents such as metals, alkaloids, biogenic amines, and pesticides were negative. Interestingly, DON was detectable in some of these samples; however, the levels were below the FDA advisory guideline of 1 ppm. Although the investigators concluded that etiology of these outbreaks remains unknown, the results suggest that the possibility of a link between DON and human gastroenteritis outbreaks requires further scrutiny.

Other Related Epidemiological Studies

Luo et al. (1990) conducted a comparative study on the natural occurrence of *Fusarium* toxins in 47 corn and 30 wheat samples collected in 1989 from Linxian and Shanqui counties in Henan province, a high-risk (death rate = 132 per 100,000) and a low-risk area (death rate = 15.7 per

100,000), respectively, for esophageal cancer in China. Samples from Linxian were obtained from esophageal cancer patients' families. Mean DON levels in corn and wheat from Linxian were 574 and 59 ppb, respectively. Linxian DON concentrations were significantly higher (2.4–5.8 times for corn and 3.3 times for wheat) than in Shanqui. A subsequent study by Yoshizawa et al. (1994) also found higher levels of the suspected carcinogenic mycotoxin fumonisin B1 in Linxian as compared to Shanqui. Thus it is difficult to define, at this time, a clear role for DON in human esophageal cancer, particularly in light of the many studies that deem DON noncarcinogenic.

Kristensen et al. (1997) studied gestational age, birth weight, and perinatal death among 192,417 births to a cohort of Norwegian farmers from 1967 to 1991 and found a relationship between grain farming and risk to perinatal health. Furthermore, the authors reported that the risk was highest after harvest in seasons with a poor quality harvest. It was stated that these results support the hypothesis that occupational exposure to mycotoxins, notably *Fusarium* toxins, in grain induces labor at an early stage of pregnancy. No specific data were provided on actual levels or types of mycotoxins that might have been present during the poor quality harvests.

The recent development of a urinary biomarker to DON will aid epidemiological studies involving suspected human exposure to DON (Meky et al., 2003). This new method will allow measurement of urinary concentrations of DON and its metabolites in humans with the use of an immunoaffinity column–high-performance liquid chromatography technique.

Human In Vitro Studies

A number of studies have assessed the effect of DON exposure on human cells in culture. The majority of the effects have evaluated the functional characteristics of cells of the immune system.

Effects on Leukocyte Function The effect of DON on human lymphocyte proliferation and cytokine production was evaluated (Meky et al., 2001). DON, at concentrations greater than 100 ng/ml (ED50 216 ng/ml), decreased proliferation of PHA-stimulated lymphocytes derived from the blood of healthy volunteers, as assessed by the MTT assay, following a 5-d incubation. No effect of DON was observed in unstimulated lymphocytes. DON (100–400 ng/ml) concentration- and time-dependently increased IL-2 production by PHA-stimulated lymphocytes, whereas no significant effects were observed for IL-4 and IL-6 production up to 72 h. Evaluation of cytokine production of PHA-stimulated lymphocytes following extended incubation periods with 400 ng/ml DON was determined. Significant increases in IL-2 and IL-4 were observed from 24 to 144 h and from 72 to 113 h, respectively, whereas a significant reduction in IL-6 was observed at 24 h. In this study, it was not clear how the influence of DON on proliferation of cells may have affected the observed cytokine response.

The effects of DON on induction of the proinflammatory cytokines IL-6 and TNF-α and the chemokine IL-8 were evaluated in a clonal human macrophage model (Sugita-Konishi & Pestka, 2001). PMA-differentiated U-937 cells, a human monocytelike histocytic lymphoma, were incubated with or without LPS and supernatants were analyzed. In the absence of LPS, DON at 500 and 1000 ng/ml upregulated TNF-α production as early as 3 h and up to 6 h, whereas 100 to 1000 ng/ml DON significantly increased production of IL-6 from 3 to 24 h and IL-8 from 6 to 48 h. Cells costimulated with LPS (200 ng/ml) and DON at 500 or 1000 ng/ml markedly superinduced TNF-α and IL-8 production. IL-6 production was potentiated by coexposure with LPS and 100 ng/ml DON; however, 500 or 1000 ng/ml of the toxin suppressed the LPS-induced IL-6 response.

To study the acute effects of DON on human cytokine production in peripheral mononuclear blood leukocytes, Penner et al. (2003) developed a culture approach using a 20% dilution of whole blood in medium and a 6-h exposure. Cultures were exposed to DON at concentrations of 0, 10, 50, 100, 250, and 500 ng/ml. IL-6mRNA was significantly induced by DON at 250 ng/ml (~9.5 fold) and 500 ng/ml (~14 fold). IL-8 mRNA was significantly induced by DON at 250 ng/ml (~8.5 fold) and 500 ng/ml (~3 fold). TNF-alpha mRNA was significantly induced by DON at 10 ng/ml (~1-fold), 250 ng/ml (~1-fold), 250 ng/ml (~1-fold), and 500 ng/ml (~4-fold). Taken together, the capacity of DON to induce IL-8, IL-6, and TNF-alpha gene expression and the threshold doses to achieve these effects were consistent with previous findings in cloned human and mouse macrophage cultures. Interestingly, a high degree of variability was observed in blood cultures from different

donors, thus raising the possibility that some individuals may have greater sensitivity to DON than others.

The immunomodulating effects of DON were investigated in human peripheral blood mononuclear (PBM) cells obtained from the blood of healthy volunteers (Berek et al., 2001). Effects were evaluated through assessment of lymphocyte blast transformation activity, antibody-dependent cell-mediated cytotoxicity (ADCC), and natural killer (NK) cell activity. DON (100–5000 ng/ml) significantly and concentration-dependently inhibited concanvalin A (Con A)- and PHA-induced lymphocyte blast transformation (T lymphocyte proliferation). DON (50–1000 ng/ml) also significantly inhibited the antibody-dependent cell-mediated cytotoxicity of monocyte-free PBM cells. Additionally, DON concentration-dependently inhibited natural killer cell activity.

Apoptosis in Lymphocyte Populations Apoptosis in human peripheral blood lymphocytes (HPBLs) was monitored following exposure to DON (Sun et al., 2002). Time- and concentration-dependent effects of DON exposure on HPBL apoptosis were monitored by flow cytometric cellular DNA analysis, and additional time-course effects were evaluated by DNA agarose gel electrophoresis. Flow cytometry data showed that DON (1000 ng/ml) treatment significantly increased apoptosis in HPBLs compared to control groups, and that the rate of apoptosis increased with time DON (2–72 h). Apoptosis rates also rose with increasing concentrations of DON (50–2000 ng/ml). The observation of characteristic laddering on DNA gels confirmed flow cytometric data.

An additional study assessing the effect of DON on apoptosis in a human monocyte line showed no significant effects between control and treated cells (Yang et al., 2000). However, DON levels tested were well below those employed in the Sun et al. (2002) study, 5–10 ng/ml versus 50–2000 ng/ml, and may account for the observed differences.

Intestinal Metabolism of DON Sundstol-Eriksen and Pettersson (2003) studied the ability of human gastrointestinal organisms to transform the trichothecenes 3-acetyldeoxynivalenol and nivalenol. Samples of human feces were incubated under anaerobic conditions for 48 h with the toxins. 3-Acetyldeoxynivalenol was metabolized to deoxynivalenol during the incubation period; however, in contrast to what has been reported for other species such as rats, mice, and pigs, no de-epoxidated metabolites were detected in the fecal incubates. This suggests that humans in this study may have lacked the microflora for a key detoxification step for DON.

Conclusions on Human In Vitro Studies Exposure of various human cells to DON resulted in both stimulation (cytokines) and an impairment (apoptosis) of immune function. Decreases in cell proliferation were observed, as well impairment of cellular defense activities, that is, alteration of cytokine production and natural killer cell activity. These results suggest that exposure to DON through food may negatively impact the human immune system. Although it is not clear how these in vitro studies translate to an intact human system, modulation of immune function through consumption of DON-contaminated food commodities has the potential to increase susceptibility to disease.

Extrapolation of Experimental Studies to Human Risk Assessment

The capacity for DON to produce chronic effects such as anorexia, reduced weight gain, or immunotoxicity in humans is of obvious concern. Epidemiological studies have not yet targeted these possibilities. However, animal studies using the most sensitive endpoints (i.e., food refusal, reduced weight gain) have been used to establish tolerable daily intakes (TDIs) for DON. In 1983, the Canadian government established provisional TDIs of 1.5 and 3 μg/kg bw for children and adults, respectively (Kuiper-Goodman et al., 1994). Ehling et al. (1997) suggested that, using these TDIs and worst-case estimates of DON intake via wheat, international estimated daily intakes exceeded the TDI by up to five-fold. In 1998, a Nordic Working group used more recent animal studies to generate a TDI of 1 μg/kg bw (Eriksen and Alexander, 1998). It was reported that, based on estimated daily intakes, DON intake may exceed that TDI in consumers who eat large amounts of cereal grains

In 1999, the National Institute of Public Health and the Environment in the Netherlands conducted a risk assessment of DON and proposed concentration limits for this mycotoxin in wheat and wheat-containing food products (Pieters et al., 1998). Taking into account quality of the studies

evaluated and the relevance of toxicological endpoints, NOAELs were derived from mouse chronic (0.11 mg/kg bw/d; Iverson et al., 1995), immunotoxicity (0.25 mg/kg bw/d; Tryphonas et al., 1984), teratogenicity (0.5 mg/kg bw/d; Khera et al., 1982), and reproduction (0.375 mg/kg bw/d; Khera et al., 1984) studies as well as subchronic swine studies (0.04–0.06 mg/kg bw/d; Bergsjo et al., 1992, 1993b). Uncertainty factors for species (mouse = 10; swine = 2) and interindividual (mouse, swine = 10) differences were employed to generate estimated TDIs of 1.1, 1.5, 2.5, 3.75, and 2–3 μg/kg bw, respectively, for these studies. The authors selected 1.1 μg/kg bw TDI as optimal based on the thorough nature of the chronic mouse study and because swine studies had not employed purified DON.

Using data obtained from the Dutch National Food Consumption Survey, an estimate of wheat consumption in the Dutch population was calculated (Pieters et al., 1998). Based on consumption data and an evaluation of DON in food products, it was suggested that the provisional TDI was exceeded, especially in children, where 80% of 1-yr-olds exceeded the level, 20% of which was greater than twice the provisional TDI. Children (1–4 yr), considered to be at most risk to DON exposure, were estimated to ingest 8.5 g wheat per day based on the 95th percentile consumption. Dividing 1.1 μg/kg bw by 0.0085 kg/kg bw yielded a provisional concentration limit of 129 μg/kg or ppm wheat. Based on the wheat content of various food products, general concentration limits were proposed for two food categories: (1) bread, 60 ppb, and (2) wheat-containing food products, 120 ppb. To facilitate government surveillance, a concentration limit of 120 ppb was selected for cleaned wheat. The Dutch authors noted that because of the ubiquitous nature of DON, the aforementioned concentration limits may be difficult to enforce. Therefore, an alternative was suggested whereby the average consumption (50th percentile), 4.5 g wheat/kg bw, is employed. This would result in a doubling of the proposed concentration limits as follows: (1) bread, 120 ppb, (2) wheat-containing food products, 240 ppb, and (3) cleaned wheat, 240 ppb.

A risk assessment of DON, and other *Fusarium* mycotoxins, was also performed in 1999 by the European Commission Scientific Committee on Food (Anonymous, 2002). Critical effects were considered in the establishment of a temporary TDI for DON related to general toxicity and immunotoxicity. Temporary TDIs were established by the committee pending a group evaluation of several *Fusarium* toxins possessing a common basic chemical structure. The initial consideration of a "group TDI" for several trichothecenes including DON, nivalenol, T-2 toxin, and HT-2 toxin was dismissed when it was determined the data did not support the concept, or that of toxic equivalency factors. The committee subsequently adopted the full TDI for DON at 1 μg/kg bw in 2002.

An additional risk assessment was conducted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2001 (Canady et al., 2001). JECFA recognized DON as a potential cause of acute human illness, but did not believe the data permitted setting an acute reference dose. A provisional maximum tolerable daily intake (PMTDI) of 1 μg/kg bw was determined based on potential effects of DON on the immune system, growth, or reproduction. JECFA's analysis of toxicological data evaluated through the risk assessment process concluded DON does not present a carcinogenic hazard. Similarly, a committee of the International Agency for Research on Cancer (IARC) placed DON in Group 3, not classifiable as to carcinogenicity for human beings, following a 1993 review (IARC, 1993). An additional study of carcinogenicity in mice has since been conducted that demonstrates a lower incidence of liver tumors in DON-treated males when compared to controls; no differences were noted in female mice. The committee attributed the lower tumor incidence to reduced body weight of treated animals.

JECFA assessed human dietary intake of DON using food consumption data and dietary intake estimates (Canady et al., 2001). The mean or median concentration of DON in specific food commodities was multiplied by the amount of each corresponding commodity consumed, using data obtained from the five Global Environmental Monitoring Systems (GEMS) food regional diets: African, European (Australia, Canada, Europe, New Zealand, United States) Far Eastern, Latin American, and Middle Eastern. Intake estimates per person per day were made and converted to intake per kilogram body weight, based on an average body weight of 60 kg. The computed values provide an estimate of the average dietary intake of DON in total, and for specific commodity groups within the five regions (Table 8). Overall levels of DON consumed were highest in the Middle Eastern

TABLE 8. Comparison of Intakes of DON in the GEMS/Food Regional Diets

	African		European		Far Eastern		Latin American		Middle Eastern	
Commodity	μg/kg bw	Percent total intake	μg/kg bw	Percent total intake	μg/kg bw	Percent total intake	μg/kg bw	Percent total intake	μg/kg bw	Percent total intake
Barley	0.022	3	0.24	16	0.042	3	0.078	7	0.012	1
Maize	0.31	40	0.026	2	0.091	6	0.12	10	0.14	5.9
Oats	<0.001	<1	0	<1	0	0	0	<1	0	0
Popcorn	0	<1	0	<1	0	<1	0	<1	0	0
Rice	0.26	33	0.03	2	0.7	44	0.22	18	0.12	5
Rye	0	0	0	<1	0	<1	0	0	0	0
Sorghum	0	0	0	0	0	0	0	0	0	0
Triticale	0	0	0	0	0	<1	0	0	0	0
Wheat	0.18	24	1.2	79	0.74	47	0.76	64	2.1	88
Other cereals	0	0	0	<1	0	<1	0	0	0	<1
Total intake	0.78	100	1.4	100	1.6	100	1.2	100	2.4	100

Note. Reproduced from the "Safety evaluation of certain mycotoxins in food," Canady et al., 2001.

region (2.4 μg/kg bw/d), followed by Far Eastern (1.6 μg/kg bw/d), European (1.4 μg/kg bw/d), Latin American (1.2 μg/kg bw/d), and African (0.78 μg/kg bw/d). In general, levels of DON were highest in wheat, rice, and maize samples.

SUMMARY OF DON TOXICITY AND IMPLICATIONS TO HUMAN HEALTH

General Effects

DON is biologically active and can disrupt cell signaling, differentiation, growth, and macromolecular synthesis. This undoubtedly contributes to its broad spectrum of effects, which impact gastrointestinal homeostasis, growth, neuroendocrine function, and immunity. There are marked species differences, with the pig being most sensitive, followed by rodent > dog > cat > poultry > ruminants. This spectrum of sensitivities relates to differences in toxicokinetics, metabolism, and feeding habits of different species. DON does not accumulate in tissues or appear at high concentrations as residues in animal foods.

The parameter that is most sensitive to low level DON is the emetic response. Swine are susceptible to extremely low doses of DON (LOAELs = 0.05–0.1 mg/kg bw and NOAELs = 0.028–0.075 mg/kg bw) (Table 1). Evoking this response in an individual animal depends not only on the DON concentration in a food, but also on the size of portion ingested. DON can also induce emesis in cats and dogs. Since mice and rats do not have an emetic response, no insight can be drawn on this endpoint from rodent studies. Primate or human studies on DON-induced emesis have not been reported to date. However, based on the use of porcine models for human intestine function (Nejdfors et al., 2000) and drug-induced emesis (Szelenyi et al., 1994), it is not unreasonable to speculate that humans are as sensitive to DON as pigs. The limited data from Chinese epidemiological studies indeed suggest that DON might induce emetic effects in humans.

Growth is a second parameter that is particularly sensitive to DON, with LOAELs and NOAELs for swine ranging from 0.06 to 0.12 and 0.03 to 0.12 mg/kg bw/d, respectively, whereas LOAELs and NOAELs for other monogastric species ranged from 0.25 to 1.5 and from 0.1 to 0.39 mg/kg bw/d, respectively (Table 2). Reduction in weight appears to result from reduced feed intake (anorexia) and is reversible once DON is removed from the diet. The anorectic response could likely derive from disturbances in the serotonergic system, as well as upregulation of proinflammatory cytokines such as TNF-α that are known to produce cachexia.

A third parameter of potential significance is immunotoxicity. DON can be immunostimulatory or immunosuppressive with LOAELs and NOAELs ranging from 0.4 to 6.25 and 0.4 to 1 mg/kg bw/d in mice, respectively, and a LOAEL of 0.12 mg/kg bw/d reported in a single swine study (Table 3).

A fourth parameter where notable effects by DON have been observed is reproduction. LOAELs for reproductive effects range from 1 to 1.5 mg/kg bw/d (Table 4) and appear to result from maternal toxicity associated with feed refusal and weight loss.

Risk Assessments and Their Limitations

Evaluation of DON toxicity leads to the obvious consideration of the appropriateness of current and proposed regulatory guidelines for DON in foods. The aforementioned Dutch risk assessment (Pieters et al., 1998) employed an approximately 200-fold safety factor to achieve a proposed regulatory limit of 120 ppb in wheat. This was based on the standard 100-fold uncertainty factor and a 2-fold modifying factor by using the 95th percentile intake value. By comparison, the current U.S. advisory level of 1 ppm would employ only a 24-fold safety factor if the Dutch consumption data for children were used. This might raise the question as to whether a 1-ppm guideline adequately ensures human safety.

The strategy just described may have limitations for several reasons. It should be reemphasized that DON is metabolized rapidly, it does not accumulate in tissue, and its effects are typically transitory. DON's effects are most pronounced when the toxin is administered in single or multiple bolus doses as compared to presentation via diet. This is dramatically exemplified by studies of DON-induced emesis in pigs. When pigs are orally gavaged with DON, the LOAELs and NOAELs are 0.05–0.1 mg/kg bw/d and 0.025–0.075 mg/kg bw/d, respectively (Table 1). In contrast, when DON is administered via diet, the LOAEL (0.15 mg/kg bw/d) and NOAEL (0.12 mg/kg bw/d) are higher. An analogous finding was made in mice when evaluating the effects of DON on a sensitive molecular endpoint, IL-6 mRNA expression (Zhou et al., 1998). It should be further recognized that because DON is a naturally occurring toxicant that occurs sporadically depending on weather, the compound would not be uniformly present in different wheat-derived foods within an individual's diet.

Another concern is that risk assessments for DON are based on its effects on growth, immunity, and reproduction. For humans who consume a more diverse diet at multiple times throughout a day, the greater health concern would be the development of gastroenteritis with attendant vomiting after a single meal containing a high concentration of DON. In other words, DON may function similarly to preformed bacterial toxins such as *Staphylococcus aureus* and *Bacillus cereus* enterotoxins. In samples from Chinese scabby/moldy cereal outbreaks, minimum detectable levels of DON typically exceed 1 ppm and have ranged as high as 93 ppm. To date, there is no definitive evidence that DON has produced cases of human gastroenteritis in the United States or Canada where 1–2 ppm regulatory levels are in place. The obvious limitation of this observation is that the toxin has not been systematically sought out in outbreaks of gastroenteritis as has been done for foodborne bacterial toxins.

Conclusions and Research Needs

The primary human safety concern for DON should be its potential for inducing acute gastroenteritis with vomiting. The mechanisms for this effect may be related to dysregulation of immune and/or neuroendocrine function. In addition, there is potential for chronic effects on growth, immune function and reproduction exist based on animal studies. Currently, the regulatory guidelines of 1–2 ppm appear to have been effective in preventing obvious cases/outbreaks of gastroenteritis in the U.S. and Canadian populations. Given the potential for loss of valuable food from food security and nutritional standpoints and the economic impact to cereal grain producers and processors that would occur should this limit be reduced, it might be difficult to justify, at this time, use of the 100- to 200-fold safety factor that has been applied conventionally to anthropogenic compounds encountered in foods. Clearly, efforts are needed to fill critical data gaps regarding the potential health effects of DON. Specifically, it would be useful to have more definitive data in the following interrelated areas:

1. DON's emetic effects in primates.
2. Occurrence of DON in foods and human clinical samples associated with outbreaks of gastroenteritis.

3. Worldwide occurrence of DON and other 8-ketotrichothecenes in raw and finished foods over multiple years.
4. Comparative incidence of acute (e.g., gastroenteritis) and chronic disease (e.g., esophageal cancer, IgA nephropathy, cardiac effects) in geographical areas of high and low DON exposure.
5. Mechanistic basis for DON toxicity in human cells and relationship to toxicity in animal cells.
6. Relationship between early molecular endpoints and acute/chronic effects in animal models.
7. Capacity of human tissue and gut microflora to detoxify DON.

Data resulting from these aforementioned studies will enhance our capacity to conduct DON risk assessments for validation or modification of current tolerance limits/guidelines.

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