

Comparison of Emetic Potencies of the 8-Ketotrichothecenes Deoxynivalenol, 15-Acetyldeoxynivalenol, 3-Acetyldeoxynivalenol, Fusarenon X, and Nivalenol

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Although the acute toxic effects of trichothecene mycotoxin deoxynivalenol (DON or vomitoxin), a known cause of human food poisoning, have been well characterized in several animal species, much less is known about closely related 8-ketotrichothecenes that similarly occur in cereal grains colonized by toxigenic fusaria. To address this, we compared potencies of DON, 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), fusarenon X (FX), and nivalenol (NIV) in the mink emesis model following intraperitoneal (ip) and oral administration. All five congeners dose-dependently induced emesis by both administration methods. With increasing doses, there were marked decreases in latency to emesis with corresponding increases in emesis duration and number of emetic events. The effective doses resulting in emetic events in 50% of the animals for ip exposure to DON, 15-ADON, 3-ADON, FX, and NIV were 80, 170, 180, 70, and 60 µg/kg bw, respectively, and for oral exposure, they were 30, 40, 290, 30, and 250 µg/kg bw, respectively. The emetic potency of DON determined here was comparable to that reported in analogous studies conducted in pigs and dogs, suggesting that the mink is a suitable small animal model for investigating acute trichothecene toxicity. The use of a mouse pica model, based on the consumption of kaolin, was also evaluated as a possible surrogate for studying emesis but was found unsuitable. From a public health perspective, comparative emetic potency data derived from small animal models such as the mink should be useful for establishing toxic equivalency factors for DON and other trichothecenes.

Key Words: mycotoxin; trichothecene; emesis; deoxynivalenol; vomitoxin; 15-acetyldeoxynivalenol; 3-acetyldeoxynivalenol; fusarenon X; nivalenol.

Food contamination by trichothecene mycotoxins has long been associated with human and animal toxicoses and remains a worldwide public health concern (Pestka, 2010a,b). The 8-ketotrichothecenes produced by the mold genus

Fusarium are common contaminants of cereal grains. This family is characterized by having a keto group at carbon 8 of the parent epoxytrichothecene nucleus and includes five closely related congeners: (1) deoxynivalenol (DON), (2) 15-acetyldeoxynivalenol (15-ADON), (3) 3-acetyldeoxynivalenol (3-ADON), (4) fusarenon X (FX; 4-acetylnivalenol), and (5) nivalenol (NIV) (Fig. 1).

Adverse effects of trichothecenes that have been reported in experimental animal studies include emesis, nausea, anorexia, growth retardation, neuroendocrine changes, and immunosuppression (Pestka, 2010a). Investigations of human food poisoning outbreaks that have been etiologically linked to trichothecene exposure have identified nausea and vomiting as primary symptoms (Luo, 1994; Ueno, 1987; Yoshizawa, 1983). Given the high relevance of the emetic response to human food poisoning, it is surprising that there have been relatively few studies on this effect in experimental animals with respect to DON and even fewer for the other 8-ketotrichothecenes. Defined as a forceful expulsion of the contents of the gastrointestinal tract through the mouth and nose, emesis serves as a reflex defense mechanism to protect the body from absorption of ingested toxins (Andrews and Hawthorn, 1988). Severe emesis can disrupt normal nutrition, hydration, and electrolyte balance, thereby having serious implications for human and animal health.

DON, the most commonly encountered 8-ketotrichothecene in food, was discovered 40 years ago (Yoshizawa and Morooka, 1973) and is colloquially referred to as “vomitoxin” because of its potent emetic effects in pigs consuming *Fusarium*-contaminated feed (Vesonder *et al.*, 1973). Indeed, DON readily induces vomiting in several animal models following oral, intraperitoneal (ip), intravenous (iv), and subcutaneous (sc) exposure (Friend *et al.*, 1982; Forsyth *et al.*, 1977; Hughes *et al.*, 1999;

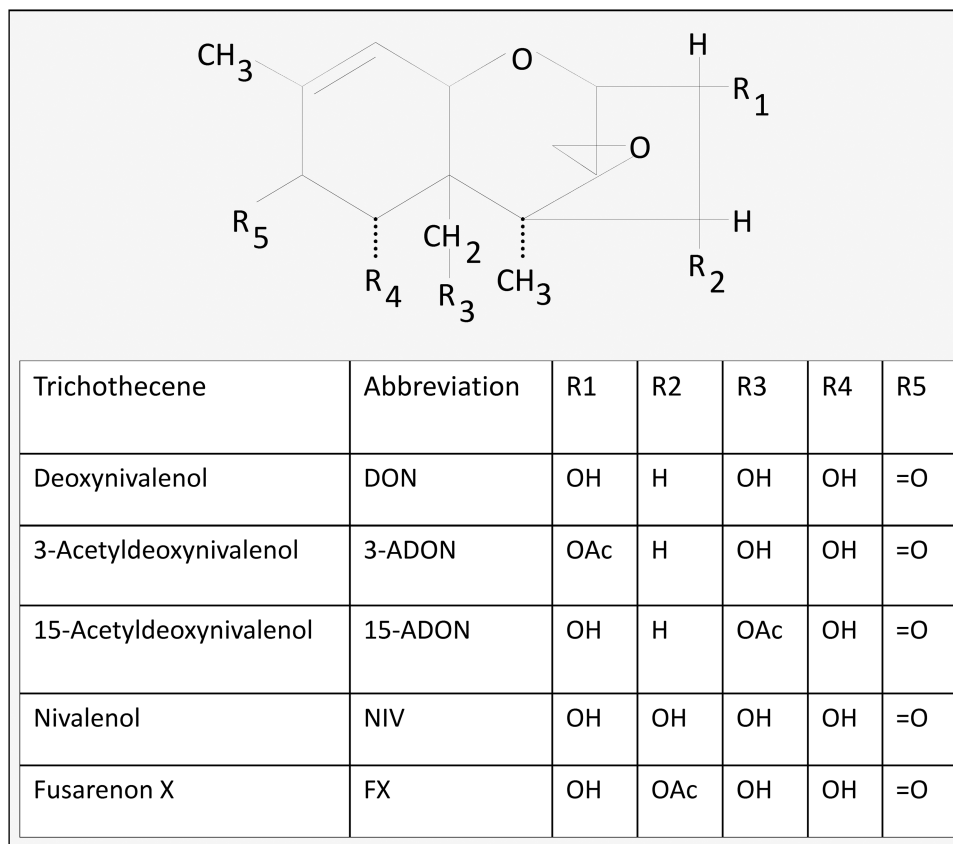


FIG. 1. Structures of 8-ketotrichothecenes.

Pestka *et al.*, 1987; Prelusky and Trenholm, 1993; Ueno *et al.*, 1974; Yoshizawa and Morooka, 1977; Young *et al.*, 1983). The 8-ketotrichothecenes 15-ADON, 3-ADON, FX, and NIV have also been reported to cause vomiting in experimental animals (Hedman *et al.*, 1997; Matsuoka *et al.*, 1979; Pestka *et al.*, 1987; Ueno *et al.*, 1971, 1974; Yoshizawa and Morooka, 1974, 1977). However, to date, the emetic capacities of these latter congeners have not been systematically compared with respect to DON in a common animal model, making it impossible to accurately predict their relative potential to induce nausea and vomiting in humans and animals.

Although mice and rat models are frequently used for toxicology research, they lack an emetic reflex (Horn, 2008). Two surrogate endpoints that have been proposed to be predictive of nausea or an emetic-like sickness response in rodents are feed refusal and pica (Andrews and Horn, 2006; Takeda *et al.*, 1993; Vera *et al.*, 2006). We have developed a mouse anorexia bioassay for DON (Flannery *et al.*, 2011) that has been recently applied to the comparison of the 8-ketotrichothecenes (Wu *et al.*, 2012), however, the direct relevance of these latter findings to emesis has yet to be determined. Pica behavior, defined as ingestion of nonnutritional clay such as kaolin, is also considered as an indicator of nausea in rodents (du Sert *et al.*, 2012). DON recently has been reported to increase kaolin

intake in mice following oral exposure (Girardet *et al.*, 2011), suggesting that pica behavior might be useful for comparing emetic potencies of trichothecenes.

Species with a vomiting reflex used to study trichothecene-induced emesis include pigs, dogs, cats, and ducklings. Pigs are of interest because of their agricultural relevance and the similarity of their gastrointestinal systems to those of humans (Szelenyi *et al.*, 1994). However, this species offers challenges because of its size and expense. Dogs and cats are also sensitive to DON, and both species have been involved in food poisoning outbreaks via contaminated pet food (Hughes *et al.*, 1999). Ethical issues of using companion species and maintenance costs for such studies present complications for their use as emesis models (du Sert *et al.*, 2012). Finally, ducklings are relatively insensitive to trichothecenes (Ueno *et al.*, 1971, 1974; Yoshizawa and Morooka, 1974, 1977) and thus not practical for emesis studies.

Small-animal alternatives to the above-mentioned models include the mink (*Neovison vison*) and ferret (*Mustela putorius furo*), both of which belong to the Mustelidae family. These species display emetic behavior analogous to that reported for humans and thus are applicable to studying emetogenic compounds (du Sert *et al.*, 2011; du Sert *et al.*, 2011; Qian *et al.*, 2009, 2010; Zhang *et al.*, 2006). Notably, we have previously

observed that mink refuse to consume food contaminated with DON (Gibson *et al.*, 1993), suggesting that this species is sensitive to this toxin.

The intent of this study was to compare the potencies of 8-ketotrichothecenes in the mouse pica and mink emesis models. The results suggest that the mouse pica model would not be a satisfactory approach for predicting emesis in susceptible species. In contrast, studies in the mink indicated that (1) this species exhibited similar responses to DON as pigs and dogs, (2) the five major 8-ketotrichothecenes induced vomiting, (3) latency to emesis decreased and frequency of emetic events increased as doses were increased, and (4) the emetic responses to the congeners were differentially affected by ip and oral exposure.

MATERIALS AND METHODS

Toxins. The 8-ketotrichothecenes were isolated, and purity (>98%) was verified by elemental analysis and LC-MS as previously described (Wu *et al.*, 2012). All toxins were dissolved in filter-sterilized phosphate buffered saline (PBS).

Mouse pica study. Male B6C3F1 mice (10-week old) were obtained from Charles River Breeding (Portage, MI) and housed individually in polycarbonate cages in a room with temperature at 21°C–24°C, relative humidity at 40–55%, and lights on a 12-h cycle (6:00–18:00h)/dark (18:00–6:00h). Animal treatment followed National Institutes of Health guidelines and were approved by the Michigan State University Institutional Animal Care and Use Committee (MSU-IACUC). The general experimental design for the pica study (Fig. 2) was based on previously described protocols (Flannery *et al.*, 2011; Girardet *et al.*, 2011). To minimize stress to the animals, all procedures were consistently and rapidly conducted. Briefly, mice were acclimated for 1 week after arriving and randomly divided into different groups according to body weight 1 day prior to the experiment. On the day of the experiment, groups of mice ($n = 5$) were fasted from 10:00h to 18:00h (water provided *ad lib*) and dosed with 0, 2.5, 5, and 10mg/kg bw DON in 100- μ l PBS by oral gavage using a sterile 22 G 1.5" disposable feeding tube (Instech Solomon, Plymouth Meeting, PA). Following DON treatment, mice were then immediately provided two preweighed kaolin pellets (≈ 4 g) and six preweighed food pellets

(≈ 20 g) (Research Diets Inc., New Brunswick, NJ) in two separate 2" high glass jars. Kaolin intake was measured at intervals for 24h after gavage.

Mink emesis studies. Experiments were performed using sixty 1- to 2-year-old, standard dark, female mink bred and housed at the Michigan State University Experimental Fur Farm. Housing of animals was according to guidelines specified in the Standard Guidelines for the Operation of Mink Farms in the United States (Fur Commission USA, 2010) and was approved by MSU-IACUC. Prior to the first experiment, the animals were acclimated for 1 week and fed the MSU Experimental Fur Farm ranch diet, which was formulated to meet the nutrient requirements of mink (Zhang *et al.*, 2009). The mink recovered rapidly after trichothecene exposures and exhibited normal appetite within 24h after challenge. Therefore, to minimize the number of animals used, the mink were rested for a minimum of 1 week between experiments and were randomized and reused in subsequent trials.

For the ip exposure study (Fig. 3A), conducted in March and April, 2011, mink were housed individually in suspended wire cages (76-cm long \times 61-cm wide \times 46-cm high) in an enclosed barn. A wooden nest box (38-cm long \times 28-cm wide \times 27-cm high) bedded with aspen shavings and excelsior (wood wool) was attached to the outside of each cage. Ventilation was provided by two wall fans and ceiling vents. Room temperature was kept above 0°C by two thermostatically controlled heaters. For emesis trials, mink were fasted for 24h (water available *ad lib*) prior to conducting the experiment in order to maintain a constant volume of gastric contents in each animal. On the day of experiment, mink were provided 50-g feed at 12:00h, which was consumed rapidly. After 30 min (12:30h), mink were given either toxin or PBS in a volume of 1 ml/kg bw via ip injection using a sterile 20-G, 2.54-cm needle. After dosing, animals were returned to individual cages, food and water were provided *ad lib*, and emesis was monitored for 6h. During the observation period, the incidence of emesis, latency to emesis, emesis duration, and number of emetic events were recorded. Incidence is defined as the ratio of animals exhibiting emesis to total test animals. Latency to emesis refers to the time from dosing toxins to the first emetic event, whereas emesis duration is the time from the first occurrence of emesis to the end of the last emesis. An emetic event was characterized as either vomiting or retching. Vomiting is defined as rhythmic abdominal contraction with oral expulsion of either solid or liquid material, whereas retching refers to responses that mimicked vomiting but without any material being expelled (Hasegawa *et al.*, 2002).

For the oral exposure study (Fig. 3B), conducted in June and July, 2011, the same 60 mink were housed singly in wire cages (62-cm long \times 25-cm wide \times 38-cm high) and provided with a nest box (24-cm long \times 24-cm wide \times 29-cm high) bedded with aspen shavings and excelsior within a shaded, open-sided pole barn. Temperature, humidity, and photoperiod were dependent on

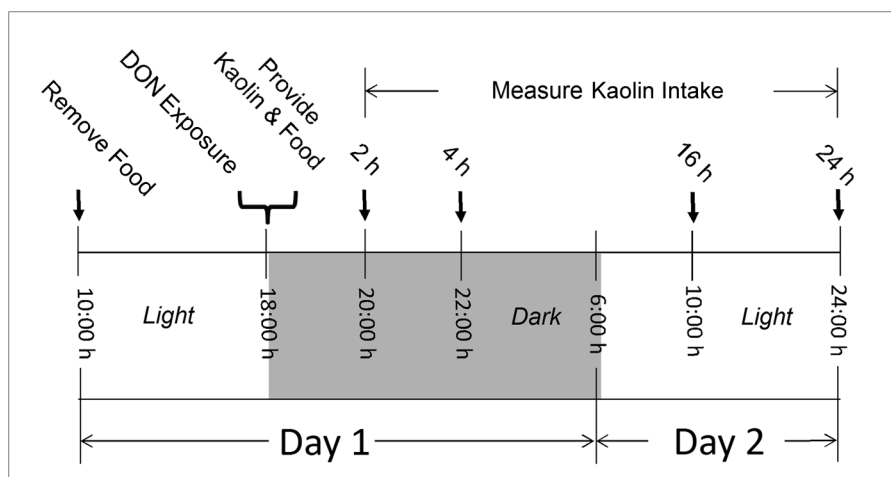


FIG. 2. Experimental design for pica bioassay in mice.

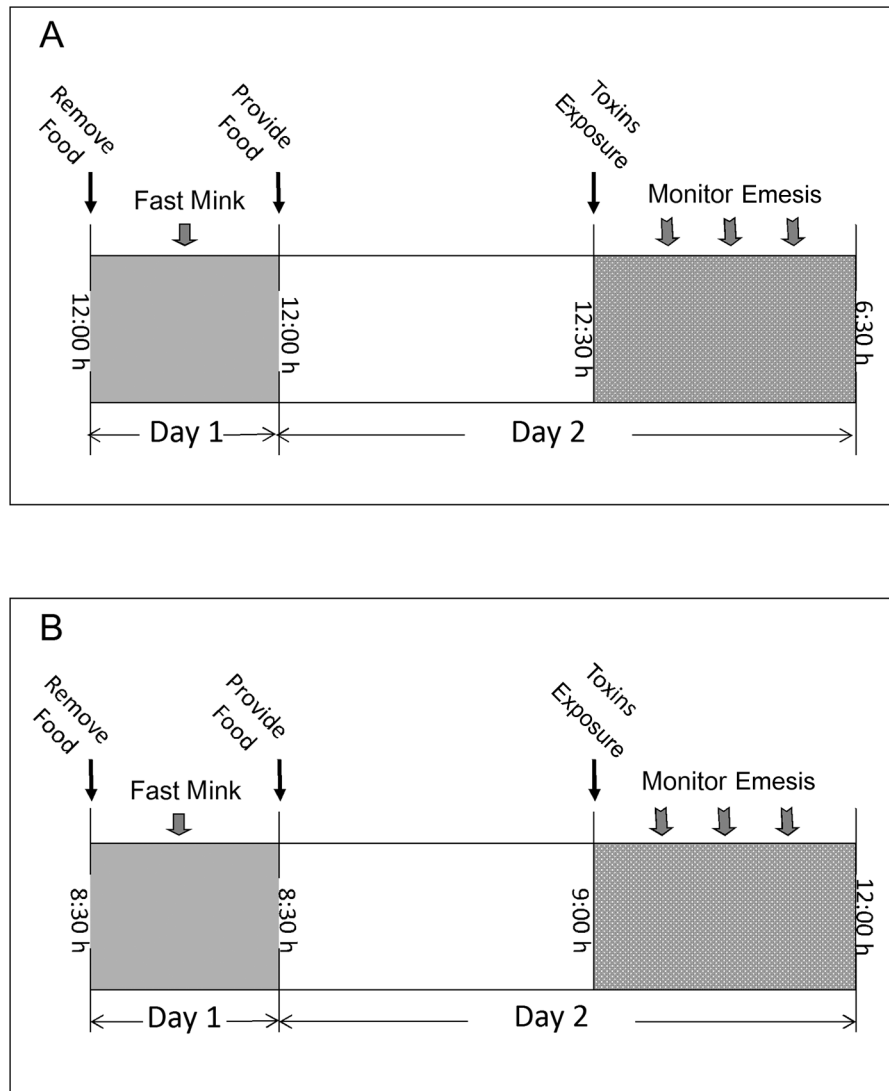


FIG. 3. Experimental design for emesis bioassay following ip (A) and oral (B) exposure.

ambient summer conditions. In an attempt to minimize the effect of higher midday ambient temperatures during the summer, experiments were initiated in the morning (8:30 A.M.). Mink were fasted for 24 h prior to dosing (water available *ad lib*) and provided 50 g of feed at 8:30 h on the day of experiment. After 30 min (9:00 h), mink were administered either toxin or saline (1 ml/kg bw) via oral gavage using a sterile 16-G, 5-cm stainless steel gavage tube. Animals were then monitored for emesis over the subsequent 3 h as described above.

A follow-up study was conducted in June, 2012, to directly compare emetic responses in 30 new female mink (1-year old) following ip and oral exposure to DON as described above to assess seasonal differences.

Data analysis. The emetic dose (ED) was determined with Proc Probit using SAS (Version 9.2, SAS, Cary, NC). All other data were plotted and statistically analyzed using SigmaPlot 11 for Windows (Jandel Scientific, San Rafael, CA). Means were considered significantly different at $p < 0.05$. In the mouse pica model, a two-way repeated ANOVA (dose and time) using Bonferroni *t*-test was used to assess significant differences in kaolin intake. For dose-dependent emesis studies, Fischer's Exact Test was used for incidence, retching, vomiting, and total emetic events. A *t*-test or one-way ANOVA

with Tukey test was used to determine significant differences within doses for latency and duration. A two-way ANOVA using Bonferroni *t*-test was used to assess significant differences in mean cumulative emetic events.

RESULTS

Effects of DON on Kaolin Consumption in the Mouse

Kaolin consumption after 2 and 4 h was not significantly affected by ip exposure to DON at 2.5–10 mg/kg bw ($p < 0.05$) (Fig. 4). Rather than being increased, cumulative kaolin intakes after 16 and 24 h were reduced by 60–80% following exposure to 5 and 10 mg/kg bw DON, respectively. These results were not consistent with the induction of pica for kaolin by DON model. It was therefore concluded that this rodent model was not a suitable substitute for predicting nausea and emesis in other species and therefore was not employed in subsequent studies.

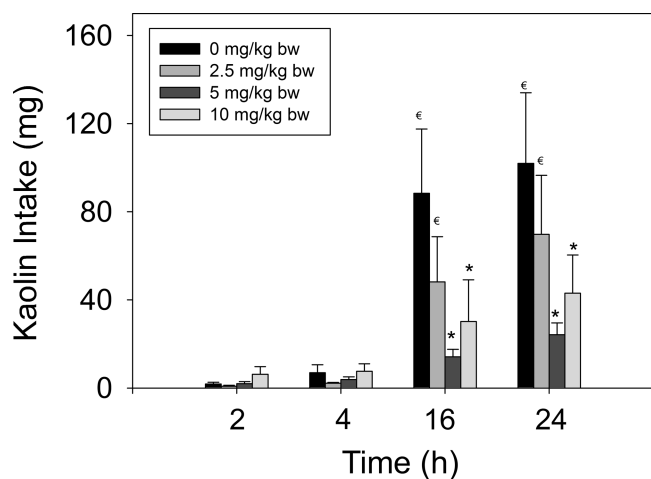


FIG. 4. Effect of DON on kaolin intake. Data represent mean \pm SEM ($n = 8$ per group). Symbols: * indicates statistically significant differences in cumulative emetic episodes compared with the control ($p < 0.05$). € indicates a statistically significant difference relative to the 2-h time point within a given dose ($p < 0.05$).

Emetic Effects of *ip* Exposure of Mink to 8-Ketotrichothecenes

The lowest dose at which DON induced emesis in mink following *ip* exposure was 0.1 mg/kg bw, with 50% of the animals responding (Table 1). After exposure to DON at 0.25 mg/kg bw, all animals vomited. The majority of emetic episodes occurred within 20 min, although new emetic events were detectable up to 80 min at the 0.25-mg/kg bw dose (Fig. 5A).

Following *ip* administration of 15-ADON, the minimum emetic dose was 0.25 mg/kg bw, with 83% of the treated mink experiencing emesis (Table 1). When the dose was increased to 1 mg/kg bw, all mink vomited. Throughout the dose range of 0.25–1.0 mg/kg bw, emetic responses began within 10–15 min after treatment and ended by 60 min (Fig. 5B). When 3-ADON was administered *ip*, doses of 0.2, 0.3, and 0.4 mg/kg bw caused emesis in 50, 83, and 100% of the mink, respectively, whereas 0.1-mg 3-ADON/kg bw had no effect (Table 1). The majority of emetic events occurred within the first 60 min at a dose of 0.4 mg/kg bw and within the first 80 min at doses of 0.2 and 0.3 mg/kg bw (Fig. 5C).

Upon *ip* exposure to 0.1 and 0.25 mg/kg bw, FX induced emesis in 83 and 100% of the mink, respectively, whereas 0.025 and 0.05 mg/kg bw had no effect (Table 1). The two high doses evoked emesis within 20 min with no emetic events recorded after 140 min (Fig. 5D). Mink dosed *ip* with NIV at 0.01 and 0.05 mg/kg bw did not exhibit emesis, whereas exposure to 0.1 and 0.25 mg/kg bw induced emesis in all animals within these groups (Table 1). The dose of 0.1 mg/kg bw induced emesis within 60 min, which continued up to 220 min postdosing (Fig. 5E), whereas 0.25 mg/kg bw caused emesis to occur within 40 min and to persist until 220 min postdosing.

Emetic Effects of Oral Exposure to 8-Ketotrichothecenes

When DON was administered orally, no effects were observed at 0.01 mg/kg bw, whereas the 0.05 mg/kg bw dose caused 83% of the exposed mink to vomit (Table 2). Increasing the dose to 0.25 or 0.5 mg/kg bw evoked emesis in all animals. Most emetic events were observed within 20 min and lasted up to 40 min (Fig. 6A).

The lowest oral dose of 15-ADON that caused emesis was 0.1 mg/kg bw with 83% of the mink responding to the treatment (Table 2). Doses of 0.5 and 1.0 mg/kg bw 15-ADON induced emesis in all mink. Emesis typically occurred within 20 min, and new emetic events were detectable only up to 60 min (Fig. 6B). Oral dosing with 0.25, 0.5, and 1.0 mg/kg bw 3-ADON induced emesis in 17, 83, and 100% of the mink, respectively, whereas 0.05 mg/kg bw had no effect (Table 2). New emetic events were not apparent after 80 min (Fig. 6C).

Following oral dosing with FX at 0.05, 0.25, and 0.5 mg/kg bw, 67, 100, and 100% mink experienced emesis, respectively (Table 2). The 0.05 mg/kg bw dose induced emesis between 40 and 80 min postdosing (Fig. 6D). Doses of 0.25 and 0.5 mg/kg bw both caused emesis within 20 min, and emetic events continued until 140 and 160 min postdosing, respectively. When mink were treated orally with NIV, no emesis occurred at doses of 0.05 and 0.1 mg/kg bw, whereas doses of 0.25 and 0.5 mg/kg bw induced emesis in 67 and 100% of the mink, respectively (Table 2). Dosing with 0.25 mg/kg bw NIV caused emesis between 40 and 80 min postdosing (Fig. 6E). The 0.5-mg/kg bw dose also caused emesis within 40 min, but emesis continued up to 100 min postdosing.

Comparison of Emetic Responses to DON Following Oral and *ip* Exposure

A follow-up study was conducted to compare the emetic responses following concurrent *ip* and oral exposures to DON at 0, 0.025, 0.05, 0.1, and 0.25 mg/kg bw (Table 3). Mink exposed *ip* to DON to 0.05 mg/kg bw had no effect, whereas exposure to 0.10 and 0.25 mg/kg bw caused vomiting in 66 and 100% of the mink, respectively. In comparison, oral exposure up to 0.025 mg/kg bw DON had no effect, whereas 0.05, 0.1, and 0.25 mg/kg bw caused vomiting in 33, 66 mitigating any seasonal effect, and 100% of the animals. Latency, duration, and number of emetic events for the two exposure regimens were thus comparable to those described above (Tables 1 and 2) mitigating any seasonal effect.

DISCUSSION

Although vomiting is a hallmark of human food poisoning by 8-ketotrichothecenes, there have been relatively few investigations of their comparative effects because of the challenges inherent in conducting emesis studies in large animal species such as pigs and dogs. This study is novel because it is the first to employ a small-animal model, the mink, to characterize

TABLE 1
Comparison of Emetic Responses in Mink Following ip Exposure to 8-Ketotrichothecenes

Toxin	Dose (mg/kg bw)	Incidence (responding/tested)	Latency to emesis (min) ^{a,b}	Emesis duration (min) ^{a,b}	Emetic events ^c		
					Retching	Vomiting	Total
Control	0	0/26	-	-	0±0	0±0	0±0
DON	0.025	0/6	-	-	0±0	0±0	0±0
	0.05	0/6	-	-	0±0	0±0	0±0
	0.1	3/6	14±2	6±3	14±8	4±2	17±9
	0.25*	6/6	10±2	38±5	94±13	16±2	110±14
3-ADON	0.1	0/6	-	-	0±0	0±0	0±0
	0.2	3/6	36±14	3±2	7±4	2±1	9±5
	0.3*	5/6	48±9	26±7	28±8	6±2	34±9
	0.4*	6/6	19±4	54±2	81±10	16±2	97±12
15-ADON	0.1	0/6	-	-	0±0	0±0	0±0
	0.25*	5/6	12±2	3±1	26±8	4±1	31±9
	0.5*	5/6	11±1	24±8	38±9	7±2	45±11
	1*	6/6	8±1	32±5	58±5	13±3	71±7
FX	0.025	0/6	-	-	0±0	0±0	0±0
	0.05	0/6	-	-	0±0	0±0	0±0
	0.1*	5/6	63±19	22±4	18±5	5±1	23±6
	0.25*	6/6	20±4	92±22	136±8	26±3	162±10
NIV	0.01	0/6	-	-	0±0	0±0	0±0
	0.05	0/6	-	-	0±0	0±0	0±0
	0.1*	6/6	83±8	56±23	33±7	9±2	42±9
	0.25*	6/6	29±5	158±12	132±20	30±2	162±20

^aAverage of positive responders only.

^bIf animals failed to retch or vomit, the latency and duration of emesis are shown as “-.”

^cAverage of both responders and nonresponders. Data represent the mean ± SEM. Values with an asterisk indicate insignificant differences at $p < 0.05$ relative to the control for incidence, retching, vomits, and total emetic events.

trichothecene-induced emesis. In addition, it is the first to systematically compare the emetogenic potential of the 8-ketotrichothecenes in a common animal model.

Incidence, latency, duration, and intensity of emetic events are the key considerations when characterizing potencies of emesis-inducing chemicals (Kris *et al.*, 2006). Using incidence data, we determined each toxin's no-observed adverse effect level (NOAEL) and lowest observed effect level (LOAEL) as well as its ED₅₀ value (Fig. 7 and Table 4). The NOAEL and LOAEL of DON (0.05 and 0.1 mg/kg bw for ip exposure, respectively, and 0.01 and 0.05 mg/kg bw for oral exposure, respectively) were similar to those reported previously in studies with other species (Table 5). For example, it was reported that a NOAEL and LOAEL for DON-induced emesis following ip exposure in pigs were 0.025 and 0.05 mg/kg bw, respectively, and 0.075 and 0.1 mg/kg bw after oral exposure, respectively (Forsyth *et al.*, 1977). We similarly reported a NOAEL and LOAEL of 0.025 and 0.05 mg/kg bw for both ip and oral exposure of pigs to DON (Pestka *et al.*, 1987). The ED₅₀ observed here for DON (0.08 mg/kg bw, ip; 0.03 mg/kg bw, oral) were also generally consistent with those reported for pigs (0.02, mg/kg bw, iv; 0.075 mg/kg bw, oral) (Prelusky and Trenholm, 1993; Young *et al.*, 1987).

ED₅₀ values have not been reported previously for 15-ADON. The NOAELs and LOAELs for this congener were reported to be 0.05 mg/kg bw and 0.075 mg/kg bw, respectively, following

both oral and ip exposure (Pestka *et al.*, 1987). These values are consistent with the oral data reported here (0.01 and 0.1 mg/kg bw, respectively), but different than the ip NOAEL and LOAEL in the present study (0.1 and 0.25 mg/kg bw, respectively). Species differences in absorption, distribution, metabolism, and excretion of 15-ADON following ip exposure might explain the differences in these values. ED₅₀, NOAELs, and LOAELs for emesis induced by 3-ADON, FX, and NIV following ip and oral exposure presented here are the first published values for any species.

The latency to first emesis reflects a toxicant's capacity to activate the emetic mechanism, whereas the duration of emesis is indicative of the rate and extent of excretion or elimination of the emetic agent. In general, DON evoked emesis more rapidly than that of the other 8-ketotrichothecenes tested. Furthermore, the emetic response was transient, suggesting that DON is absorbed and metabolized rapidly. In pigs and mice, absorption of DON is rapid, reaching a peak plasma concentration of 15–30 min following oral administration and declining rapidly thereafter with 75–90% cleared after 3 h (Amuzie *et al.*, 2008; Azcona-Olivera *et al.*, 1995; Pestka *et al.*, 2008; Prelusky *et al.*, 1988).

Both ADONs are identical in structure to DON with the exception of being acetylated at the 15- or 3- positions. Although no data exist yet on the toxicokinetics of 15-ADON, it is generally assumed that the rate and extent of absorption and elimination of 15-ADON are similar to those of DON because the latency

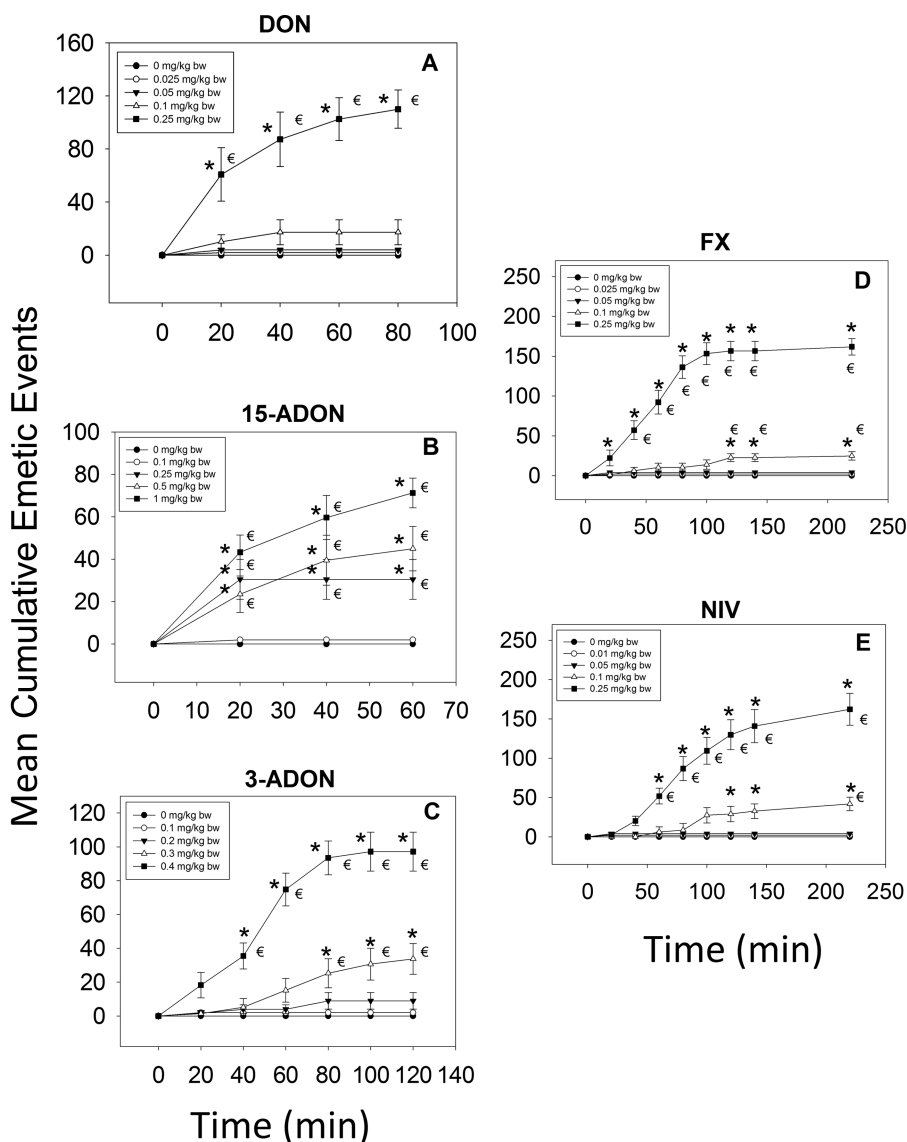


FIG. 5. Mean cumulative emetic events in mink following ip exposure to 8-ketotrichothecenes. Data are averages for both responders and nonresponders and represent mean \pm SEM ($n = 6$ per group). Symbols: * indicates statistically significant differences in cumulative emetic episodes compared with the control ($p < 0.05$). € indicates a statistically significant difference relative to the 0-min time point within a given dose ($p < 0.05$).

to and duration of emesis were similar for the two chemicals after oral exposure (Pestka *et al.*, 1987). The duration of emesis after ip exposure was less for 15-ADON compared with DON, suggesting that the former might be metabolized at a faster rate than the latter.

3-ADON is generally considered to be equivalent to DON in terms of causing anorexia, growth retardation, and other similar effects (Yoshizawa and Morooka, 1977). However, the emetic response induced by 3-ADON here was very different compared with DON in that oral exposure to 3-ADON evoked an emetic effect with a longer latency as well as duration. Because 3-ADON must be deacetylated to DON before appreciable absorption occurs in the gut following consumption (Eriksen *et al.*, 2003), emesis induction might

require more time due to the need for metabolic conversion of 3-ADON to DON.

Latency to emesis induced by FX was similar to that induced by DON. When radiolabeled FX was administered to mice by gavage, like DON, plasma radioactivity reached a maximum concentration at 30 min (Poapolathep *et al.*, 2003). NIV had a longer latency to emesis than DON. After oral exposure of mice to radiolabeled NIV, peak plasma radioactivity was detected after 60 min. Slower absorption of NIV might be a reason for a greater latency to emesis compared with DON.

The duration of emesis induced by both FX and NIV was longer compared with DON, which might relate to differences in their respective elimination rates. Clearance of radiolabeled DON in the mouse following administration follows

TABLE 2
Comparison of Emetic Responses in Mink Following Oral Exposure to 8-Ketotrichothecenes

Toxin	Dose (mg/kg bw)	Incidence (responding/tested)	Latency to emesis (min) ^{a,b}	Emesis duration (min) ^{a,b}	Emetic events ^c		
					Retching	Vomiting	Total
Control	0	0/30	-	-	0±0	0±0	0±0
DON	0.01	0/6	-	-	0±0	0±0	0±0
	0.05*	5/6	15±2	1±1	12±4	3±1	15±5
	0.25*	6/6	17±2	6±1	44±13	8±1	52±15
	0.5*	6/6	11±3	14±3	62±9	9±1	71±10
3-ADON	0.05	0/6	-	-	0±0	0±0	0±0
	0.25	1/6	62±0	1±0	2±2	1±1	3±3
	0.5*	5/6	53±9	12±9	16±8	4±1	20±9
	1*	6/6	19±5	44±9	65±11	14±2	79±13
15-ADON	0.01	0/6	-	-	0±0	0±0	0±0
	0.1*	5/6	20±4	1±1	9±3	2±1	11±4
	0.5*	6/6	18±3	3±1	24±5	6±1	30±5
	1*	6/6	17±4	14±7	26±5	6±1	32±6
FX	0.01	0/6	-	-	0±0	0±0	0±0
	0.05*	4/6	23±2	13±10	8±3	2±1	10±4
	0.25*	6/6	36±10	34±11	38±5	11±2	49±7
	0.5*	6/6	15±3	63±21	45±4	13±2	58±4
NIV	0.05	0/6	-	-	0±0	0±0	0±0
	0.1	0/6	-	-	0±0	0±0	0±0
	0.25*	4/6	31±4	15±10	21±14	4±2	25±16
	0.5*	6/6	29±4	32±10	55±14	9±2	64±15

Average of positive responders only.

If animals failed to retch or vomit, the latency and duration of emesis are shown as “-.”

Average of both responders and nonresponders. Data represent the mean ± SEM. Values with an asterisk indicate insignificant differences at $p < 0.05$ relative to the control for incidence, retching, vomits, and total emetic events.

two-compartment kinetics with an initial rapid phase of elimination ($t_{1/2\alpha} = 0.36$ h) and a slower terminal elimination phase ($t_{1/2\beta} = 7.62$ h) (Azcona-Olivera *et al.*, 1995). A study employing ELISA similarly reported $t_{1/2\alpha}$ and $t_{1/2\beta}$ of 0.29 h and 11.8 h, respectively. (Pestka *et al.*, 2008). In contrast, although following two-compartment kinetics clearance in mice, longer half lives were observed for FX ($t_{1/2\alpha} = 0.878$ h, $t_{1/2\beta} = 37.63$ h) and NIV ($t_{1/2\alpha} = 2.5$ h, $t_{1/2\beta} = 14.34$ h) (Poapolathep *et al.*, 2003). Longer elimination half-life may lead to greater durations of emetic events compared with DON. The duration of emesis induced by NIV was longer compared with FX following ip exposure, but shorter compared with FX via oral administration. Absorption of FX is more rapid and efficient compared with NIV, as a large proportion of NIV may have passed through the gastrointestinal lumen without being absorbed following oral exposure (Poapolathep *et al.*, 2003). This implies that limited absorption of NIV after oral exposure might have diminished its emetic effect.

Oral exposure to DON, 15-ADON, and FX evoked stronger emetic effects than ip exposure based on NOAEL, LOAEL, and ED₅₀ values (Tables 1–3). This observation is not consistent with previous reports in pigs for DON and 15-ADON (Forsyth *et al.*, 1977; Pestka *et al.*, 1987), which was suggestive of less absorption of these toxins by the oral route. Similarly, ED₅₀ values for DON based on emesis were 0.020 and 0.085 µg/kg bw following intravenous and oral exposure in pigs, respectively

(Prelusky and Trenholm, 1993), again suggesting that oral exposure was less effective in emesis induction. It should be noted that, unlike previous studies in pigs that did not control food intake prior to toxin exposure, we fasted mink for 24 h in this study and provided a measured amount (50 g) of food, which might have facilitated efficient uptake. Other possible reasons might relate to species differences in absorption, metabolism, distribution, and bioavailability of 8-ketotrichothecenes for the different exposure routes (ip vs. oral). In addition, it is plausible that DON and its congeners bind to one or more unidentified chemoreceptors with varying affinities located in the gastrointestinal tract. Differences in the distribution of such receptors throughout the GI tract or systemically as well as differences in receptor–ligand affinity may be responsible for the observed differences in potency of the emetic response following oral and ip exposure to DON, 15-ADON, and FX. Our lab is currently conducting research to identify receptors through which DON and its congeners may act to elicit an emetic response.

It is further important to note that there were differences in environmental conditions under which the initial ip (Table 1) and oral (Table 2) studies were carried out. Specifically, the ip study was conducted in March and April, 2011, which had average temperatures of 1.1°C and 8.1°C, respectively, whereas the oral study was carried out in June and July, 2011, with average temperatures being 19.4°C and 21.4°C, respectively. Behavior of animals can be affected by heat stress, which can

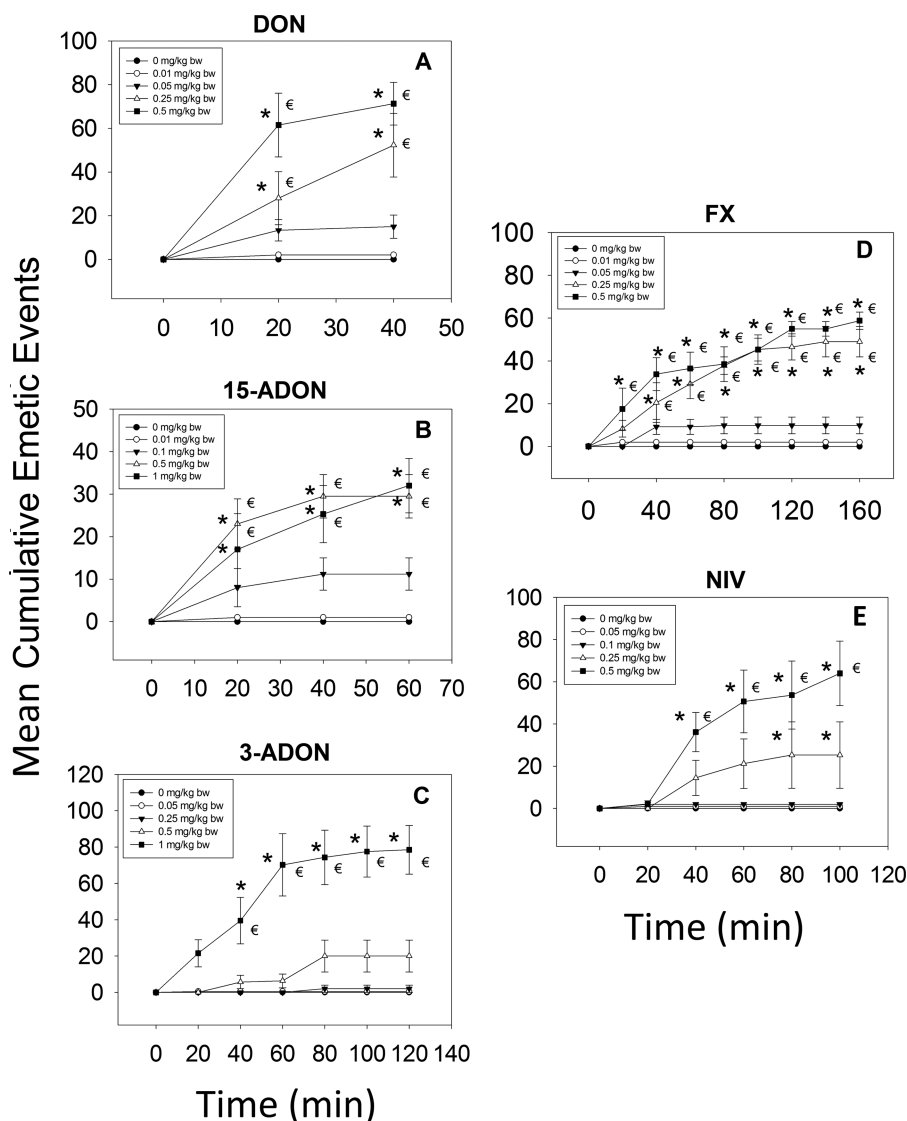


FIG. 6. Mean cumulative emetic events in mink following oral exposure to 8-ketotrichothecenes. Data are averages for both responders and nonresponders and represent mean \pm SEM ($n = 6$ per group). Symbols: * indicates statistically significant differences in cumulative emetic episodes compared with the control ($p < 0.05$). € indicates a statistically significant difference relative to the 0-min time point within a given dose ($p < 0.05$).

cause effects such as feed refusal, emesis, nausea, and low blood pressure (Lugo-Amador *et al.*, 2004). As thermoregulation by mink at high ambient temperatures is difficult (Hansen and Jeppesen, 2003), conducting the oral study in the summer with mink being housed in an open-sided shed could possibly have enhanced the emetic response. To address this concern, we conducted a follow-up study in a new cohort of mink in June 2012 (19.5°C, average ambient temperature) in the latter environment and verified that emesis incidence was indeed greater for oral exposure than for ip exposure (Table 3). Thus differences in sensitivity to ip and oral challenges were not attributable to the temperature or housing conditions.

Conversely to the three aforementioned compounds, 3-ADON and NIV were more effective in inducing emesis

following ip exposure compared with oral exposure. It is likely that 3-ADON and NIV were more completely absorbed following ip exposure. Although no data on absorption, distribution, excretion, and metabolism following oral administration of 3-ADON are available, a large proportion of NIV passes through the gastrointestinal tract without being absorbed after oral exposure in mice (Poapolathep *et al.*, 2003). This would likely reduce the emetic effect of NIV when administered by this route. Moreover, intestinal microorganisms further attenuate the emetic response by de-epoxidation of 8-ketotrichothecenes after oral exposure (Eriksen *et al.*, 2003; Onji *et al.*, 1989). These possibilities will require further study.

Interestingly, the average duration and intensity of emesis after oral exposure was generally less than that for ip exposure.

TABLE 3
Comparison of Emetic Responses in Mink Following ip and Oral Exposure

Toxin DON	Dose (mg/kg bw)	Incidence (responding/tested)	Latency to emesis (min) ^{a,b}	Emesis duration (min) ^{a,b}	Emetic events ^c		
					Retching	Vomiting	Total
IP	0	0/3	-	-	0±0 ^d	0±0 ^d	0±0 ^d
	0.025	0/3	-	-	0±0 ^d	0±0 ^d	0±0 ^d
	0.05	0/3	-	-	0±0 ^d	0±0 ^d	0±0 ^d
	0.1	2/3	13±2	9±4	37±29 ^e	7±5 ^e	44±35 ^e
	0.25	3/3	11±1	48±4	84±4 ^e	20±1 ^e	105±3 ^e
Oral	0	0/3	-	-	0±0 ^d	0±0 ^d	0±0 ^d
	0.025	0/3	-	-	0±0 ^d	0±0 ^d	0±0 ^d
	0.05	1/3	8±0	7.5±0	24±24 ^e	4±4 ^e	28±28 ^e
	0.1	2/3	14±6	5±0.3	23±14 ^e	7±4 ^e	30±16 ^e
	0.25	3/3	11±3	5±2	44±23 ^e	10±1 ^e	55±24 ^e

^aAverage of positive responders only.

^bIf animals failed to retch or vomit, the latency and duration of emesis is shown as “-.”

^cAverage of both responders and nonresponders. Data represent the mean ± SEM. Values without the same superscript within a column differ ($p < 0.05$).

^{d,e}Different letters indicate a statistically significant difference between dose groups.

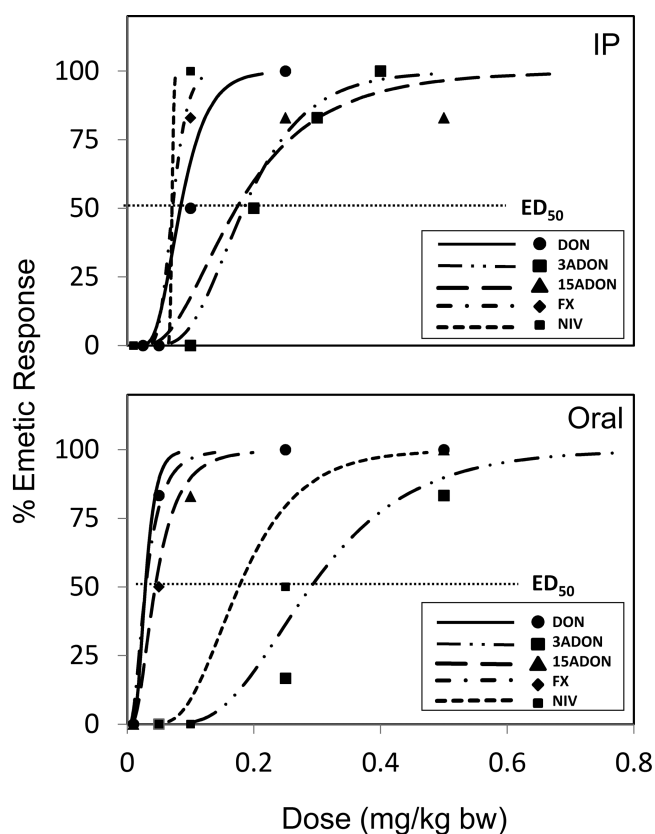


FIG. 7. Emetic dose curves for 8-ketotrichothecenes following ip and oral exposure.

Salivation and vomiting induced by 8-ketotrichothecenes at relatively high doses probably reduced the amount of toxin available for absorption, leading to an overall decrease in the average duration of emesis, particularly because absorption of orally administered doses may start in the stomach or from the upper part of the duodenum (Eriksen *et al.*, 2003; Hedman

et al., 1997). The average duration of emesis directly affected the average number of emetic events because the duration of the emetic response induced by oral exposure was less compared with ip exposure.

The neurologic and physiologic basis for emesis involves a complex reflex pathway involving neurotransmitters, hormones, and afferent fibers and coordination of neural, respiratory, and digestive systems (Carpenter, 1990). The mechanisms for trichothecene-induced vomiting are not fully understood but appear to involve activation of the chemoresponsive area postrema of the brain, as suggested by studies of T-2 toxin-induced emesis in the cat (Borison and Goodheart, 1989) and DON-induced conditioned taste aversion in the rat (Ossenkopp *et al.*, 1994). Furthermore, DON's emetic action is likely to be at least partially due to release of the neurotransmitter 5-hydroxytryptamine (5-HT, serotonin) (Prelusky and Trenholm, 1993). 5-HT is synthesized and secreted by enterochromaffin cells and plays an important role in emesis caused by cytotoxic chemicals (Endo *et al.*, 2000). Increased 5-HT concentration will trigger the 5-HT₃ receptors on the vagal afferent nerves, activating the emesis reflex via vagal afferent nerve depolarization (Andrews *et al.*, 1990; Hillsley and Grundy, 1998; Hillsley *et al.*, 1998).

Although the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recognized that DON potentially causes human acute illness, this group concluded that there were insufficient data to determine an acute reference dose for this effect (Canady *et al.*, 2001). An even greater paucity of data on other 8-ketotrichothecenes makes such estimates for these congeners even more problematic (Pestka, 2010a). The findings of this study provide new insight into the relative potential of DON and its congeners to contribute to human food poisoning. The estimated mean and 95th percentile per capita grain intakes in the United States for 1- to 6-year olds are 6.3 and 12 g/kg bw/d and for 16- to 21-year olds are 2.5 and 5.1 g/kg bw/d, respectively (EPA, 2011). If it is assumed that humans have a similar threshold for oral DON-induced emesis (0.05 mg/kg

TABLE 4
Summary of NOAELs, LOAELs, and ED₅₀ for Emetic Effects of 8-Ketotrichothecenes

Toxin	Intraperitoneal			Oral		
	NOAEL ^a	LOAEL ^b	ED ₅₀ ^c	NOAEL ^a	LOAEL ^b	ED ₅₀ ^c
DON	0.05	0.1	0.08 (0.05–0.18)	0.01	0.05	0.03 (0–0.05)
15-ADON	0.1	0.25	0.17 (0.07–0.29)	0.01	0.1	0.04 (0–0.11)
3-ADON	0.1	0.2	0.18 (0.1–0.27)	0.05	0.25	0.29 (0.01–0.47)
FX	0.05	0.1	0.07 (0.04–0.1)	0.01	0.05	0.03 (0.01–0.08)
NIV	0.05	0.1	0.06 (0–0)	0.1	0.25	0.18 (0.1–0.29)

^aNOAEL = no observed adverse effect level.

^bLOAEL = lowest observed adverse effect level.

^cED₅₀ = Dose causing emesis in 50% of the animals tested. ED₅₀ values were determined using a Proc Probit model.

TABLE 5
Previously Reported Emetic Effects of 8-Ketotrichothecenes

Toxin	Species	Route	NOAEL (mg/kg bw)	LOAEL (mg/kg bw)	References
DON	Pig	ip	n.d.	0.05	Vesonder <i>et al.</i> , 1973
DON	Pig	oral	0.075	0.1	Forsyth <i>et al.</i> , 1977
		ip	0.025	0.05	
DON	Pig	ip	0.025	0.05	Pestka <i>et al.</i> , 1987
DON	Pig	oral	0.025	0.05	Prelusky <i>et al.</i> , 1993
		iv	0.015	0.02	
DON	Dog	sc	n.d.	0.1	Yoshizawa <i>et al.</i> , 1974
	Dog	iv	n.d.	0.1	Yoshizawa <i>et al.</i> , 1977
			0.42		
15-ADON	Pig	oral	0.05	0.075	Pestka <i>et al.</i> , 1987
		ip	0.05	0.075	
15-ADON	Dog	sc	n.d.	0.2	Yoshizawa <i>et al.</i> , 1974
3-ADON	Dog	sc	n.d.	0.2	Yoshizawa <i>et al.</i> , 1977
3-ADON	Dog	iv	n.d.	0.2	Yoshizawa <i>et al.</i> , 1977
FX	Dog	iv	n.d.	0.1	Matsuoka <i>et al.</i> , 1979
FX	Cat	sc	n.d.	1	Ueno <i>et al.</i> , 1971
NIV	Dog	iv	n.d.	0.3	Matsuoka <i>et al.</i> , 1979

bw) and a worst case scenario it is further assumed in which an entire day's grain intake is eaten at a single meal (e.g., breakfast) by those in the 95th percentile groups, it could be speculated that contamination levels of 4 and 10 ppm could cause vomiting episodes in the 1- to 6-year and the 16- to 21-year-old groups, respectively. Consistent with these estimates, a very large Chinese outbreak of gastrointestinal illness that included vomiting in 1984 was associated with consumption of maize contaminated with DON at levels between 3.8 and 93 mg/kg (Luo, 1994). Assuming consumption of 560 g of grain and 50 kg bw, as estimated in another Chinese study (Gao and Yoshizawa, 1997), the LOAEL could be expected to fall between 0.04 and 1.04 mg/kg bw. Similar calculations for high grain consumers can be extrapolated from the mink data for 15-ADON (8 and 20 ppm), 3-ADON (20 and 50 ppm), FX (4 and 10 ppm), and NIV (20 and 50 ppm). It must be cautioned that these estimates are preliminary at best because they do not take into account potential differences in absorption and metabolism between species or possible differences among humans in sensitivities

to these toxins. Nevertheless, this research represents a first step in identifying threshold doses for DON and its congeners for induction of vomiting and noninfectious gastroenteritis.

Because emesis is stressful, it would be desirable to have an alternative rodent model based on anorexia or pica. In previous studies, the NOAELs and LOAELs for 8-ketotrichothecene-induced feed refusal in mice were approximately 10-fold greater than corresponding values based here on emesis (Flannery *et al.*, 2011; Wu *et al.*, 2012). Furthermore, the anorectic potencies of 8-ketotrichothecenes followed rank orders of NIV > FX > DON ≈ 3-ADON ≈ 15-ADON for ip exposure and FX > NIV > DON ≈ 3-ADON ≈ 15-ADON for oral exposure. Possible reasons for the observed species differences in sensitivity based on anorexia and emesis might be due to differences in sensitivity to and toxicokinetics of these toxins between mouse and mink, but also differences in the mechanisms involved in anorexia and emesis. Nevertheless, anorectic studies in the mouse cannot be fully relied on for prediction of emetic potencies of 8-ketotrichothecenes. It is further

notable that DON caused significant reduction of kaolin consumption, instead of increased consumption associated with pica behavior as observed in a recent study (Girardet *et al.*, 2011). Although mice displayed a trend toward pica behavior early after oral exposure to DON in that study, we found that it was rather difficult to measure kaolin as it was consumed in very small amounts. Other researchers have found difficulty in determining exact kaolin consumption in mice after exposure to emetic stimuli, suggesting that it is not useful to use pica to predict nausea in this species (Yamamoto *et al.*, 2002).

In conclusion, the mink emesis model described herein was robust and should be applicable to further investigation of the mechanisms of 8-ketotrichothecene-induced emesis. Such studies will improve our ability to predict specific thresholds for the emetic response during trichothecene food poisoning as well as the persistence and reversibility of the emetic effect in the human population. From a public health perspective, comparative emetic potency data derived from this model should be useful for establishing toxic equivalency factors for DON and other trichothecenes.

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