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Comparison of anorectic potencies of the trichothecenes T-2 toxin, HT-2 toxin and satratoxin G to the ipecac alkaloid emetine

Wenda Wu[a,b], Hui-Ren Zhou[b], Xiao Pan[c,d], James J. Pestka[b,c,d,*]

[a] College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, PR China
[b] Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824, USA
[c] Center for Integrative Toxicology, Michigan State University, East Lansing, MI 48824, USA
[d] Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA

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ABSTRACT

Trichothecene mycotoxins, potent translational inhibitors that are associated with human food poisonings and damp-building illnesses, are of considerable concern to animal and human health. Food refusal is a hallmark of exposure of experimental animals to deoxynivalenol (DON) and other Type B trichothecenes but less is known about the anorectic effects of foodborne Type A trichothecenes (e.g., T-2 toxin, HT-2 toxin), airborne Type D trichothecenes (e.g., satratoxin G [SG]) or functionally analogous metabolites that impair protein synthesis. Here, we utilized a well-described mouse model of food intake to compare the anorectic potencies of T-2 toxin, HT-2 toxin, and SG to that of emetine, a medicinal alkaloid derived from ipecac that inhibits translation. Intraperitoneal (IP) administration with T-2 toxin, HT-2 toxin, emetine and SG evoked anorectic responses that occurred within 0.5 h that lasted up to 96, 96, 3 and 96 h, respectively, with lowest observed adverse effect levels (LOAELs) being 0.1, 0.1, 2.5 and 0.25 mg/kg BW, respectively. When delivered via natural routes of exposure, T-2 toxin, HT-2 toxin, emetine (oral) and SG (intranasal) induced anorectic responses that lasted up to 48, 48, 3 and 6 h, respectively with LOAELs being 0.1, 0.1, 0.25, and 0.5 mg/kg BW, respectively. All four compounds were generally much more potent than DON which was previously observed to have LOAELs of 1 and 2.5 mg/kg BW after IP and oral dosing, respectively. Taken together, these anorectic potency data will be valuable in discerning the relative risks from trichothecenes and other translational inhibitors of natural origin.

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1. Introduction

Trichothecenes, a family of toxic sesquiterpenoid metabolites produced by *Fusarium*, *Stachybotrys* and other fungi, cause a spectrum of adverse effects in experimental animals that include food refusal, growth suppression, emesis, neuroendocrine changes, and immunotoxicity [1]. Trichothecenes can be encountered both in food and the indoor environment and have been structurally classified into several groups with Types A, B and D being

Abbreviations: NOAELs, no adverse effect levels; AGRP, agouti-related protein; CART, amphetamine-regulated transcript; DON, vomitoxin, deoxynivalenol; DMSO, dimethyl sulfoxide; IL-1β, interleukin-1β; IL-6, interleukin-6; LOAELs, lowest adverse effect levels; MC4R, melanocortin 4 receptor; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; SG, satratoxin G; TNF-α, tumor necrosis factor-α.

* Corresponding author at: Michigan State University, 469 Wilson Road, 234A Trout Food Science Bldg., East Lansing, MI 48824, USA.
Tel.: +1 517 353 1709.
E-mail address: Pestka@msu.edu (J.J. Pestka).

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of greatest significance to public health. The Type B trichothecenes are frequently encountered in wheat, barley, maize and rice that are infected by the fungus *Fusarium graminearum* with deoxynivalenol (DON, vomitoxin) being detected most commonly and at the highest concentrations [2,3]. DON and other Type B trichothecenes are stable during milling and processing and thus can be present in animal and human food [4]. The potential for suppression of food intake and resultant growth retardation by DON and related Type B trichothecenes is of particular concern from the perspective of human and animal health and has been the basis for their regulation by governments around the world [5,6].

DON-induced food refusal in mice has been linked to modulation of anorexigenic (e.g., pro-opiomelanocortin [POMC], melanocortin 4 receptor [MC4R] and amphetamine-regulated transcript [CART]) expression within hypothalamic neurons [7]. Further studies by this group, revealed the presence of NUCB2/nesfatin-1 neurons in various brain regions, and their activation during DON intoxication [8]. Importantly, nesfatin-1 evokes anorectic responses after both peripheral and central exposure [9] as well as activation of NUCB2/nesfatin-1 neurons in the [7–10]. Satiety hormones secreted by enteroendocrine cells located in the intestinal epithelium such as cholecystokinin (CCK) and peptide $YY_{3\text{-}36}$ ($PYY_{3\text{-}36}$) appear to be critical driving factors in aforementioned anorexigenic pathways in the brain [11–13]. These hormones can act in paracrine fashion to stimulate specific receptors located on either vagus nerve or endocrine fashion via the area postrema, eventually evoking anorectic responses [14]. In prior studies, elevated plasma CCK and/or $PYY_{3\text{-}36}$ concentrations were correlated to Type B ketotrichothecene-induced anorexia in mouse [15,16]. Furthermore, inhibition of NPY2 receptor or CCK1 and CCK2 receptor attenuated DON-induced anorexia Elevated plasma CCK and/or $PYY_{3\text{-}36}$ concentrations correlate to Type B ketotrichothecene-induced anorexia in mouse and furthermore receptor inhibition studies support a role for these hormones in this adverse effect [15,16].

Type A trichothecenes are produced by two *Fusarium* head blight fungi, *Fusarium langsethiae* and *Fusarium sporotrichioides.* Although less commonly encountered in cereal grains than the Type B group, they are much more potent toxins in acute toxicity studies of several animal models and in cell culture assays, with T-2 toxin (Fig. 1A) being considered one of the most potent [17,18]. T-2 toxin toxicosis outbreaks in farm animals have been reported in many countries including Canada, United States and Japan [19–21]. T-2 toxin's capacity to cause human food poisoning is demonstrated by a documented food poisoning outbreak in China that involved 97 persons out of an estimated 165 persons that consumed *Fusarium*-contaminated rice that contained T-2 toxin in the range of 0.18–0.42 mg/kg [22]. T-2 toxin readily induces anorectic effects following oral, sublingual, intravenous (iv), subcutaneous (sc) and intraperitoneal (IP) in several animal species [17,23–27]. HT-2 toxin (Fig. 1B) differs from T-2 toxin by being hydroxylated at the C-4 position but has comparable toxicity to the parent toxin [18,28]. To date there have been no studies concerning the anorectic potential of HT-2 toxin.

Type D or macrocyclic trichothecenes are produced by the black mold *Stachybotrys chartarum* and can be encountered in dust and air of water-damaged buildings [3]. Members of the Type D group have equivalent or greater toxicity to T-2 toxin in cell culture assays [29]. Satratoxin G (SG) (Fig. 1C), is one of the most potent Type D trichothecenes. Intranasal installation has been shown to highly destructive to olfactory epithelium of mice and monkey resulting in the loss of the sense of smell [30–33]. Although the potential exists for intranasally instilled SG and other Type D trichothecenes to enter the gut following the action of ciliated respiratory epithelium, their effects on food intake have not been assessed.

Trichothecene-induced food refusal might be linked to their capacity to inhibit translation. Emetine is a natural alkaloid produced from ipecacuanha that has been pharmacologically used to induce emesis against toxic agent ingestion, as well as for its anti-cough and anti-amoebiasis effects [34,35]. Like trichothecenes, emetine has the potential to block protein synthesis by binding to ribosomal subunits [36]. Thus emetine might be useful in mechanism studies as a functional analog of the otherwise structurally unrelated trichothecenes, however, its anorectic effects are not well-characterized. The objective of this study was to compare the anorectic potencies of T-2 toxin, HT-2 toxin, SG and emetine in a mouse bioassay developed previously for the Type B trichothecenes [37,38]. Both parenteral (intraperitoneal [IP]) and natural (oral gavage for T-2, HT-2, emetine and intranasal for SG) routes of exposure were employed. The results indicate that all four toxic agents: (1) induced anorectic responses, (2) were differentially affected by exposure route and (3) were generally much more potent than DON.

2. Materials and methods

2.1. Toxins and drugs

T-2 toxin, HT-2 toxin and emetine were purchased from Enzo Life Sciences with purity ≥98%. SG was isolated and purity was verified by electrospray ionization/collision-induced dissociation tandem mass spectroscopy as previously described [39]. T-2 toxin and HT-2 toxin were dissolved in 1% dimethyl sulfoxide (DMSO) in filter-sterilized phosphate buffered saline (PBS). SG and emetine were dissolved in filter-sterilized PBS.

2.2. Laboratory animals

Animal treatment followed National Institutes of Health guidelines and were approved by the Michigan State University Institutional Animal Care and Use Committee (MSU-IACUC). Female B6C3F1 mice were obtained from Charles River Breeding (Portage, MI) at 10–12 wk of age and housed individually in polycarbonate cages in a room maintained at 21–24 °C and 40–55% relative humidity under a 12 h light (6:00–18:00 h)/dark (18:00–6:00 h) cycle.

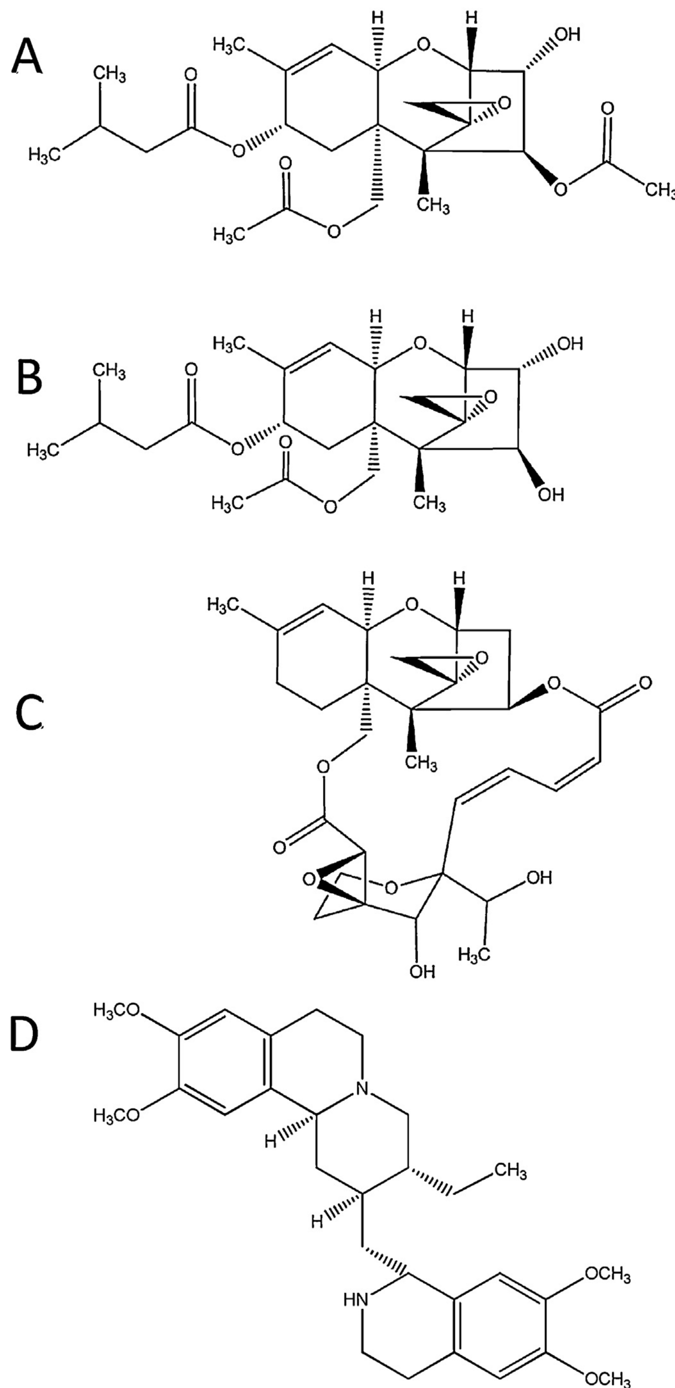

Fig. 1. Structures of T-2 toxin (A), HT-2 toxin (B), SG (C) and emetine (D).

2.3. Experimental design

The general experimental design for assessing anorectic potencies (Fig. 2) was based on previously described protocols [37,38]. Briefly, mice were acclimated for 1 wk after arriving and randomly divided into different groups according to body weight 1 d before experiment. On the day of the experiment, groups of mice ($n = 6$) were fasted but water provided *ad lib* from 10:00 h to 18:00 h. At 18:00 h, toxins were administered to mice in 100 μl volumes by IP injection with a sterile 27 G, 0.5 in. needle, oral gavage using a sterile 22 G, 1.5 in. disposable feeding tube (Instech Solomon; Plymouth Meeting, PA) or by intranasal exposure as previously described [32]. Food was then provided and consumption monitored. Dose selection was based on preliminary range finding studies. Food intake was

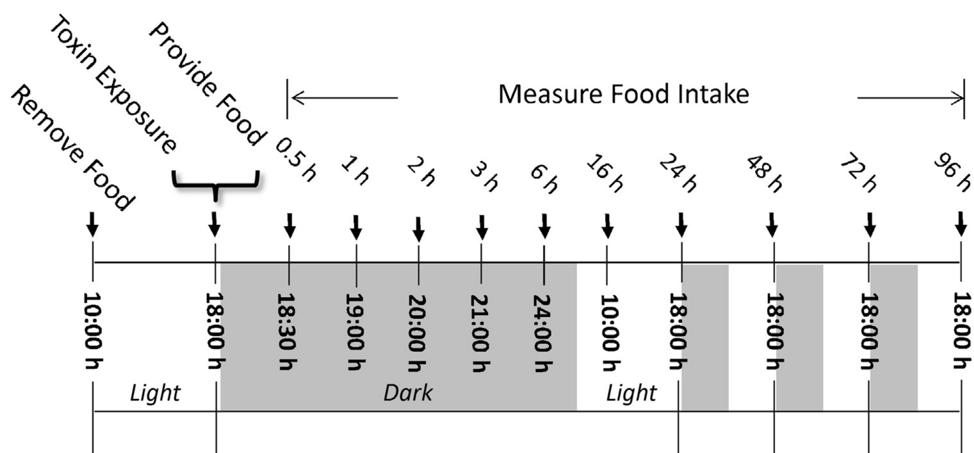

Fig. 2. Experimental design for anorexia bioassay in mice. Mice were fasted from 10:00 to 18:00 h on day 1. At 18:00 h, mice were treated with toxin or vehicle and then immediately provided with pre-weighed food. Food intake was measured up to 0–96 h.

monitored for varying lengths of time up to 96 h depending on the duration of anorexia observed in preliminary experiments.

For T-2 toxin, groups of mice were dosed with 0, 0.01, 0.1, 0.5 and 1 mg/kg BW T-2 toxin in 100 μl 1% DMSO by both IP injection and oral gavage. Mice were then immediately provided six pre-weighed food pellets (≈21 g) and food intake measured at 0.5, 1, 2, 3, 6, 16, 24, 48, 72 and 96 h after IP exposure or 0.5, 1, 2, 3, 6, 16, 24 and 48 h after oral exposure, respectively. Preliminary experiments indicated that feeding responses in mice treated with 1% DMSO in PBS and PBS alone did not differ. The approach for HT-2 toxin was identical to T-2 toxin.

For SG, groups of mice were given with 0, 0.025, 0.25, 1 and 2.5 mg/kg BW of toxin in 100 μl PBS by IP injection or 0, 0.005, 0.05, 0.25 and 0.5 mg/kg BW of toxin in 100 μl PBS by intranasal exposure. Mice were then immediately provided six pre-weighed food pellets (≈21 g) or two pre-weighed food pellets (≈7 g) and food intake measured at 0.5, 1, 2, 3, 6, 16, 24, 48, 72 and 96 h after IP exposure or 0.5, 1, 2, 3, 6 and 16 h after intranasal exposure, respectively.

For emetine, groups of mice were given with 0, 0.05, 0.5, 2.5 and 5 mg/kg BW of emetine in 100 μl PBS by IP injection or 0, 0.025, 0.25, 1 and 2.5 mg/kg BW of emetine in 100 μl PBS by oral exposure. Mice were then immediately provided two pre-weighed food pellets (≈7 g) and food intake measured at 0.5, 1, 2, 3, 6 and 16 h after both IP and oral exposure.

2.4. Statistics

Data were plotted and statistically analyzed using SigmaPlot 11 for Windows (Jandel Scientific; San Rafael, CA). Means were considered significantly different at $p < 0.05$. Food intake at specific time points was analyzed by one-way ANOVA using Holm–Sidak Method to determine significant differences between treatments and the respective control. Two way repeated ANOVA (one-factor) using Holm–Sidak Method was used to analyze significant differences in food consumption as compared to the control over time.

3. Results

After IP administration with T-2 toxin, marked reductions in food intake were observed in mice at 0.1, 0.5 and 1 mg/kg BW during 0.5–96 h time period whereas 0.01 mg/kg BW had no effect (Fig. 3A and B). The 0.1 mg/kg BW dose evoked 51, 44, 52, 52 and 38% reduction in cumulative food intake at 0.5, 1, 2, 3 and 6 h, respectively, with no differences being observed after 16 h. The 0.5 mg/kg BW dose caused 90, 65, 63, 66, 86 and 70% reduction in cumulative food intake at 0.5, 1, 2, 3, 6 and 16 h, respectively. From 16 to 96 h, there was a trend toward moderately increased food consumption. Dramatic reductions in food intake were observed for the 1 mg/kg BW dose after 0.5 (95%), 1 (83%), 2 (76%), 3 (78%), 6 (90%), 16 (93%) and 24 h (92%), respectively. From 24 to 96 h, there was again a moderate recovery in food intake. However, significant reductions in cumulative food intake were still evident at 96 h for both 0.5 and 1 mg/kg BW doses.

Upon oral dosing with T-2 toxin, food intake was markedly reduced at 0.1, 0.5 and 1 mg/kg BW during 0.5–48 h while 0.01 mg/kg BW was without effect (Fig. 4A and B). The 0.1 mg/kg BW dose caused 58, 50, 46 and 43% reduction in cumulative food intake at 0.5, 1, 2 and 3 h, respectively, with no differences being observed after 6 h. The 0.5 mg/kg BW dose caused 63, 54, 47, 46 and 44% reduction in cumulative food intake at 0.5, 1, 2, 3 and 6 h, respectively. Marked reductions in food intake were observed at 1 mg/kg BW after 0.5 (76%), 1 (59%), 2 (57%), 3 (60%), 6 (59%), 16 (62%) and 24 h (57%), respectively. From 24 to 48 h, there was a trend toward increased food consumption. However, this latter group still did not fully compensate for initial food refusal by 48 h suggesting T-2s effects were long-lasting.

When the anorectic effects of IP exposure to HT-2 toxin was evaluated, significant reductions in food intake were seen at 0.1, 0.5 and 1 mg/kg BW during 0.5–96 h, whereas effects were not observed for 0.01 mg/kg BW (Fig. 5A and B). The 0.1 mg/kg BW dose evoked 68, 53, 45, 53 and 35% reduction in cumulative food intake at 0.5, 1, 2, 3 and 6 h, respectively, with no differences being observed after

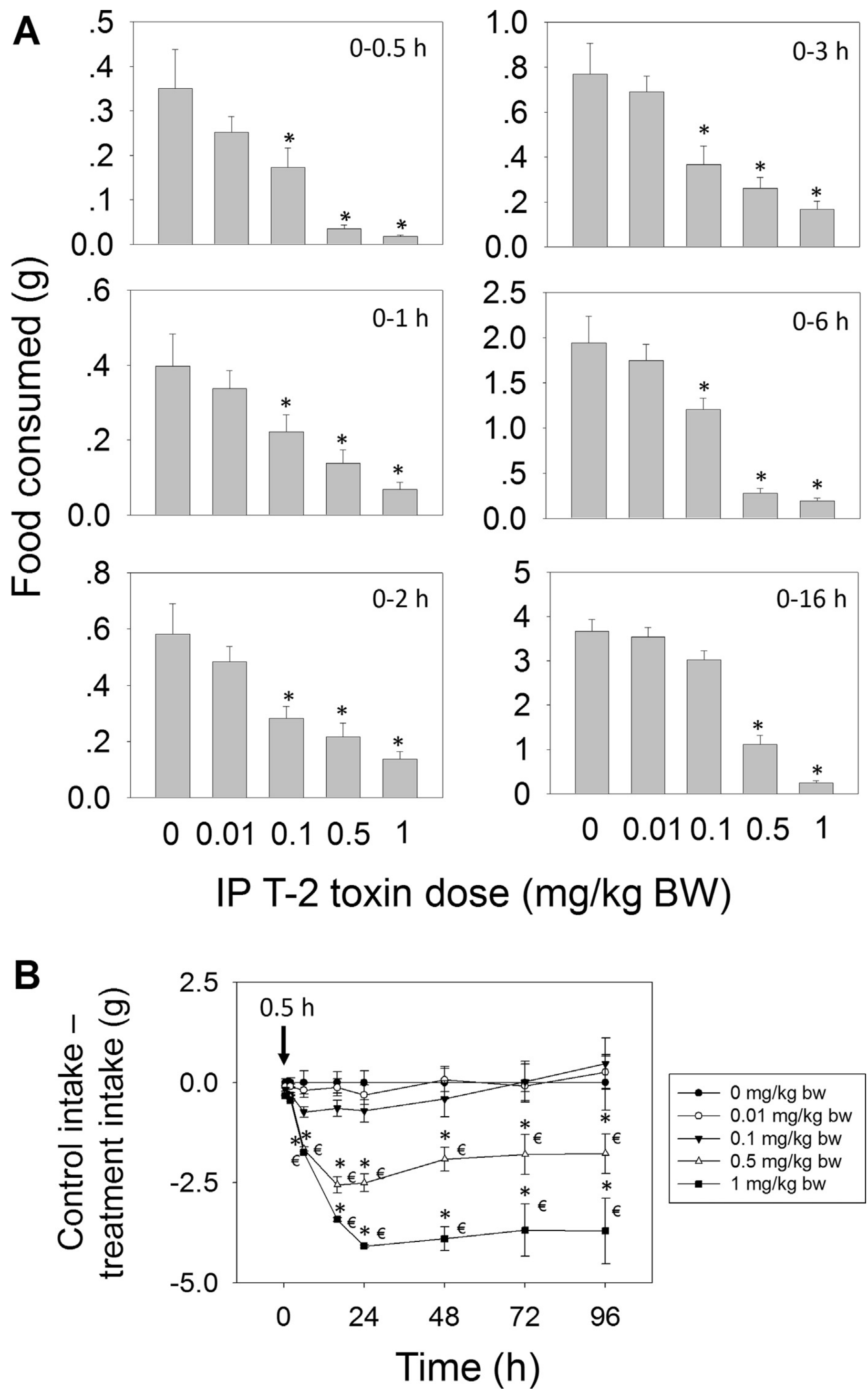

Fig. 3. IP exposure to T-2 toxin impairs food intake. (A) Short term (0–16 h) food refusal induced by IP T-2 toxin exposure. Data are mean ± SEM ($n = 6$/gp). Food intake at specific time points was analyzed by one-way ANOVA using Holm–Sidak Method to determine significant differences between an individual treatment and the vehicle control. The symbol * indicates difference in food consumption as compared to the control ($p < 0.05$) for a given time period. (B) Long term (0–96 h) food refusal induced by IP T-2 toxin exposure. Data are mean ± SEM ($n = 6$/gp). Two way repeated ANOVA (one-factor) using Holm–Sidak Method was used to analyze significant differences in food consumption as compared to the control over time. Symbols: * indicates difference in cumulative food consumption relative to the control at specific time point ($p < 0.05$) and € indicates difference in cumulative food consumption relative to the 0.5 h time point within a given dose ($p < 0.05$).

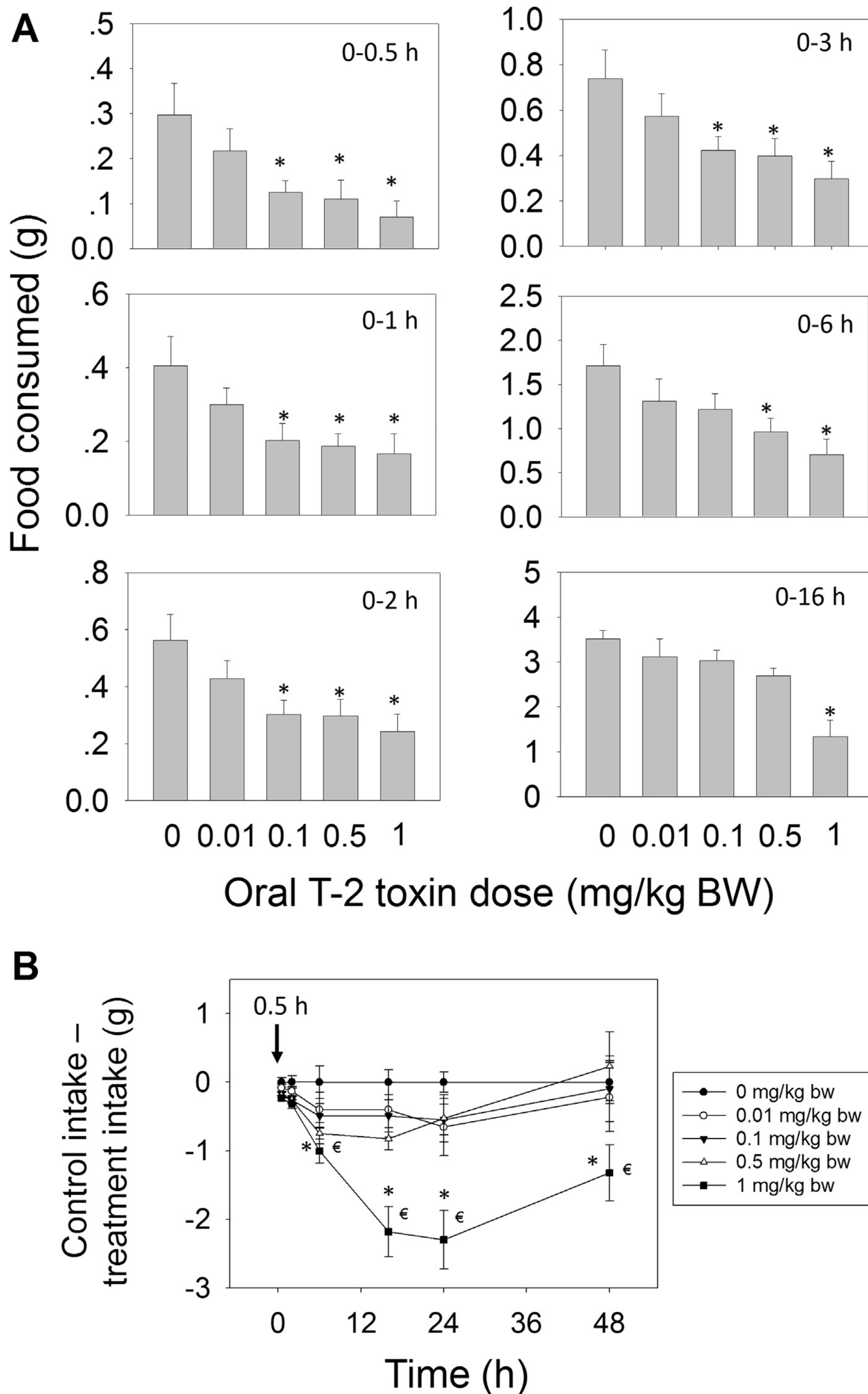

Fig. 4. Oral exposure to T-2 toxin impairs cumulative food intake for up to 48 h. (A) Short term (0–16 h) food refusal induced by oral T-2 toxin exposure. (B) Long term (0–96 h) food refusal induced by oral T-2 toxin exposure. Experiment was conducted and data analyzed as described in Fig. 3 legend.

16 h. The 0.5 mg/kg BW dose caused 83, 67, 61, 66, 77 and 43% reduction in cumulative food intake at 0.5, 1, 2, 3, 6 and 16 h, respectively. A trend toward increased food consumption was observed after 16 h, and the 0.5 mg/kg BW group compensated for initial food refusal by 48 h (Fig. 5B). Marked reductions in food intake were observed at 1 mg/kg BW after 0.5 (86%), 1 (67%), 2 (60%), 3 (67%), 6 (84%), 16 (69%) and 24 h (65%), respectively. From 24 to 96 h, there was a trend toward increased food consumption. However, as observed for T-2 toxin, by 96 h

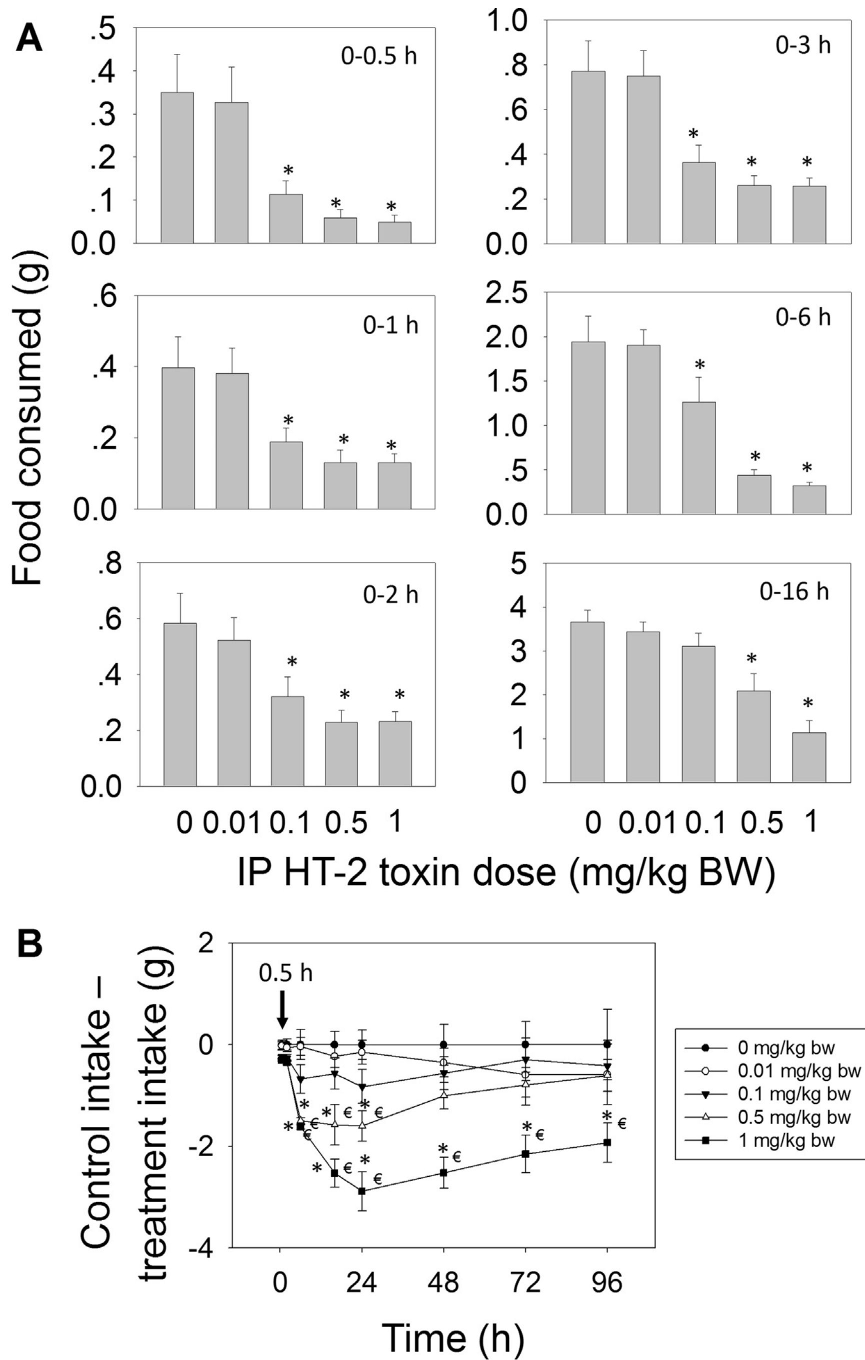

Fig. 5. IP exposure to HT-2 toxin impairs cumulative food intake for up to 96 h. (A) Short term (0–16 h) food refusal induced by IP HT-2 toxin exposure. (B) Long term (0–96 h) food refusal induced by IP HT-2 toxin exposure. Experiment was conducted and data analyzed as described in Fig. 3 legend.

this group still did not fully compensate for initial food refusal.

Significant reductions in food intake were also observed after oral exposure to HT-2 toxin at 0.1, 0.5 and 1 mg/kg BW (Fig. 6A) but, 0.01 mg/kg BW dose had no effect. The 0.1 mg/kg BW dose reduced cumulative food intake 60 and 49% at 0.5 and 1 h, respectively, with no differences being observed after 2 h. Reduced food intake was observed at 0.5 mg/kg BW after 0.5 (86%), 1 (72%), 2 (69%), 3 (65%) and 6 h (48%), respectively, with no differences being observed after 16 h. 1 mg/kg BW dose evoked 90, 66, 68, 64, 60 and 33% reduction in cumulative food intake at 0.5, 1, 2, 3, 6 and 16 h, respectively. An increased rate of food consumption was observed for HT-2 toxin-treated groups during 16–48 h thus compensating for initial food refusal by 48 h (Fig. 6B).

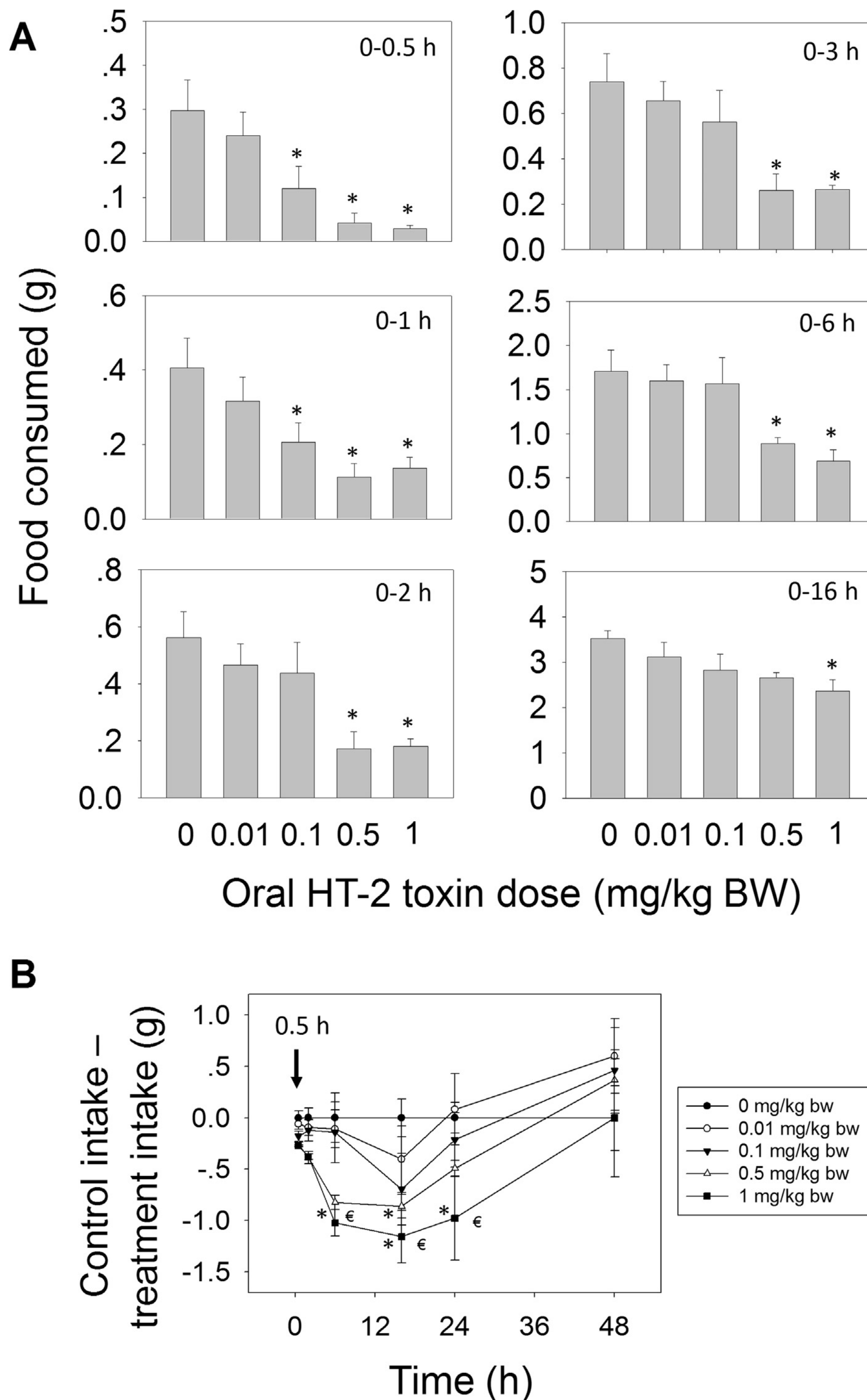

Fig. 6. Oral exposure to HT-2 toxin impairs cumulative food intake for up to 48 h. (A) Short term (0–16 h) food refusal induced by oral HT-2 toxin exposure. (B) Long term (0–96 h) food refusal induced by oral HT-2 toxin exposure. Experiment was conducted and data analyzed as described in Fig. 3 legend.

IP exposure to SG at 0.25, 1 and 2.5 mg/kg BW caused decreased food consumption in mice during 0.5–96 h while the 0.025 mg/kg BW dose had no effect (Fig. 7A and B). Marked reductions in food intake were observed after 0.5 (67, 60 and 72%), 1 (50, 54 and 57%), 2 (45, 51 and 56%), 3 (42, 51 and 59%), 6 (42, 60 and 81%) and 16 h (25, 69 and 89%) at 0.25, 1 and 2.5 mg/kg BW, respectively. From 24 to 96 h, there is a trend toward increased food consumption at

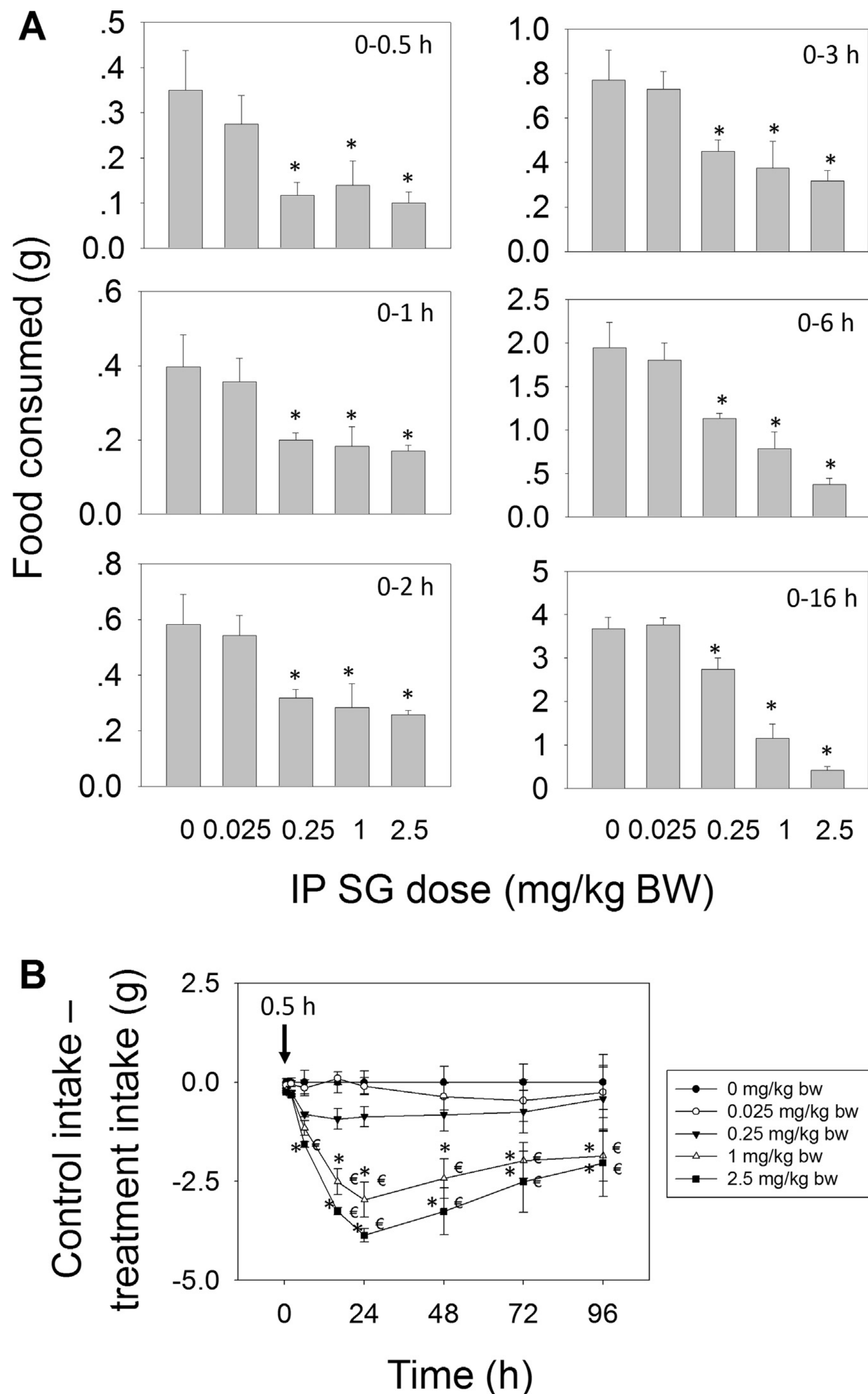

Fig. 7. IP exposure to SG impairs cumulative food intake for up to 96 h. (A) Short term (0–16 h) food refusal induced by IP SG exposure. (B) Long term (0–96 h) food refusal induced by IP SG exposure. Experiment was conducted and data analyzed as described in Fig. 3 legend.

1 and 2.5 mg/kg BW doses (Fig. 7B). However, by 96 h these groups still did not fully compensate for initial food refusal.

Intranasal exposure to SG at 0.5 mg/kg BW caused 70, 56 and 52% reduction in cumulative food intake at 0.5, 1 and 2 h, respectively, with no differences being observed after 3 h (Fig. 8). Comparatively, the 0.005, 0.05 and 0.25 mg/kg BW doses had no effect.

Food intake was reduced in mice exposed IP to emetine at 2.5 and 5 mg/kg BW, but 0.05 and 0.5 mg/kg BW had no effect (Fig. 9). The 2.5 mg/kg BW evoked 50% reduction in cumulative food intake at 0.5 h with no differences being observed after 1 h. Marked reductions in food intake were observed at 5 mg/kg BW after 0.5 (71%), 1 (58%), 2 (57%) and 3 h (54%), respectively. Beginning at 6 h,

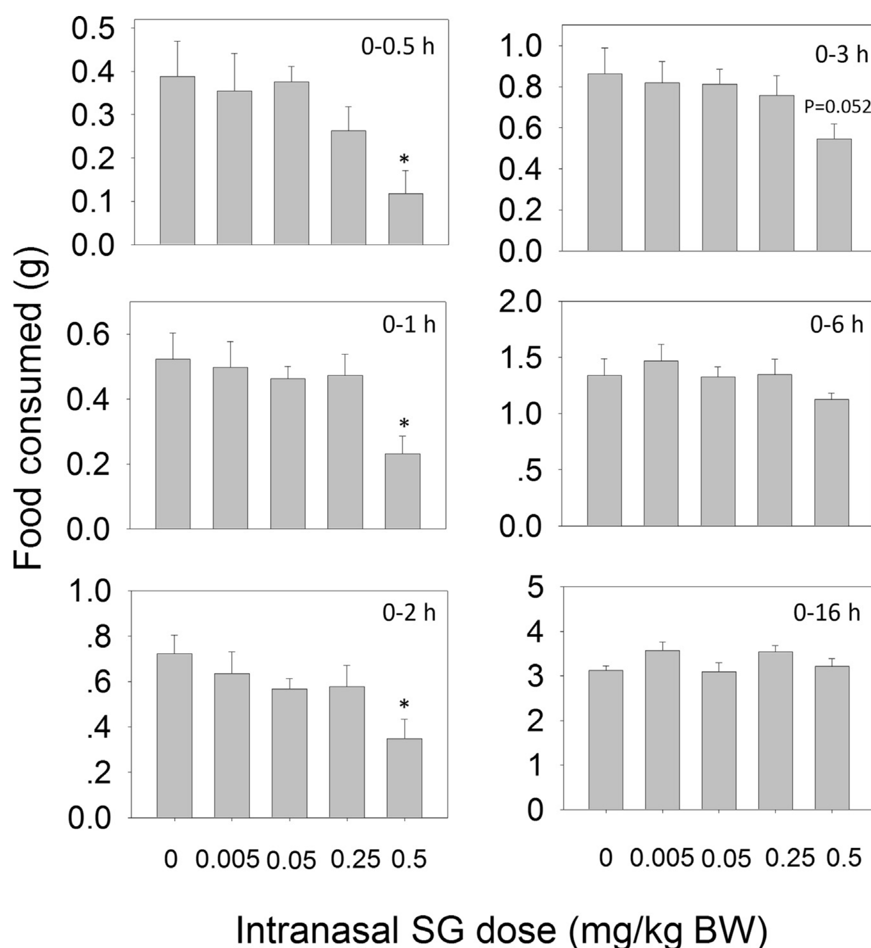

Fig. 8. Intranasal exposure to SG impairs cumulative food intake for up to 3 h. Experiment was conducted and data analyzed as described in Fig. 3 legend.

no differences between control and emetine group were observed.

Following oral exposure to emetine, significant food reductions were observed at 0.25, 1 and 2.5 mg/kg BW during 0.5–6 h, while 0.025 mg/kg BW dose had no effect (Fig. 10). Emetine at 0.25 mg/kg BW evoked 54 and 49% reduction in cumulative food intake at 0.5 and 1 h, respective, with no differences being observed after 2 h. Marked reductions in food intake were observed after 0.5 (65 and 76%), 1 (51 and 66%), 2 (57 and 56%) and 3 h (48 and 56%) at 1 and 2.5 mg/kg BW, respectively, with no differences being observed beginning at 6 h.

4. Discussion

Reduced food intake resulting from trichothecene exposure with consequential impairment of growth is a major concern from the perspective of animal and human health. This study is novel because it is the first to systematically compare the anorectic potentials of T-2 toxin, HT-2 toxin and SG and in a defined animal model and relate these to a structurally unrelated translational inhibitor, emetine, that has a long history of medicinal use. The results revealed that T-2 toxin, HT-2 toxin, SG and emetine caused anorectic effects in the mouse with different response kinetics and durations. Furthermore, the anorectic responses to these toxins were differentially affected relative to dose and/or duration when delivered by parenteral or natural exposure routes. The no adverse effect levels (NOAELs) and lowest adverse effect levels (LOAELs) for T-2 toxin, HT-2 toxin, SG and emetine following IP and oral exposure are summarized in Table 1. These data suggest that anorectic potencies followed approximate rank orders of T-2 toxin = HT-2 toxin > SG > emetine for IP exposure and T-2 toxin = HT-2 toxin > emetine > SG for oral or intranasal exposure. Importantly, these compounds were generally much more potent than DON which was previously found to have a LOAELS of 1 and 2.5 mg/kg BW after IP and oral dosing, respectively [38].

Anorectic potency data that have been previously reported for the studied compounds are summarized in Table 2. The anorectic NOAEL and LOAEL of T-2 toxin were previously reported to be 1 and 3 mg/kg BW in rat following oral exposure [23]. Recently, the anorectic LOAEL for oral exposure to this toxin in the mouse was reported to be 0.5 mg/kg BW [25]. The prolonged duration observed here (48 h) following oral exposure is very consistent with this aforementioned study. Some of these values were higher than the data presented here for T-2 toxin (0.01 and

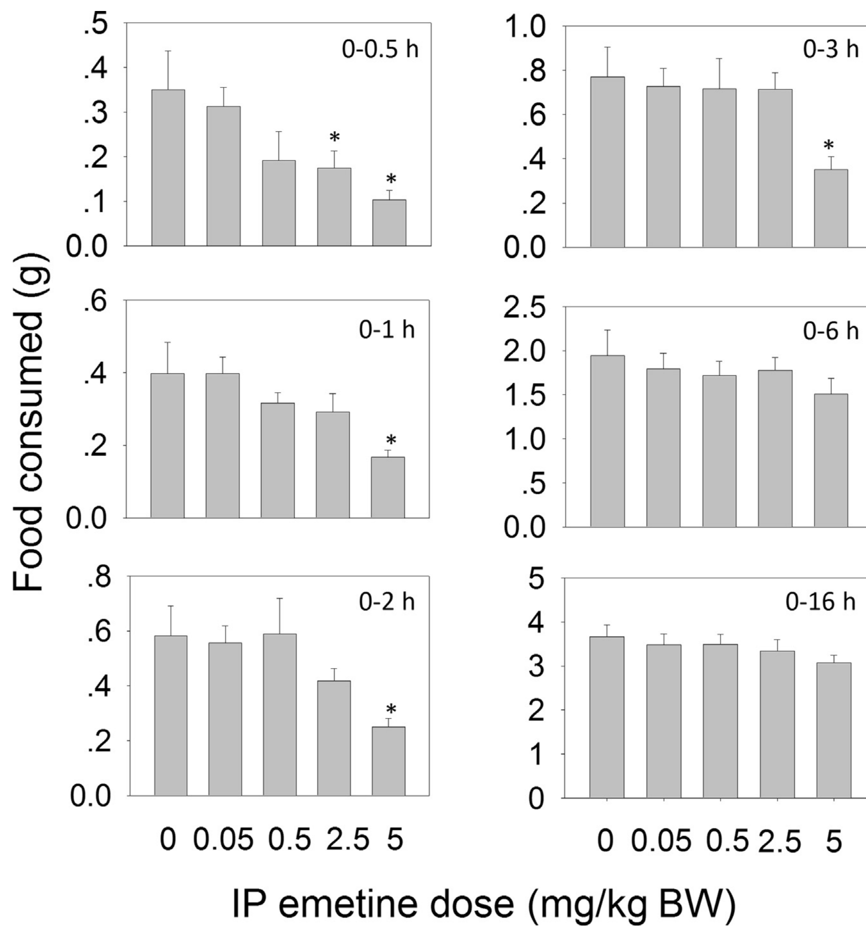

Fig. 9. IP exposure to emetine impairs cumulative food intake for up to 6 h. Experiment was conducted and data analyzed as described in Fig. 3 legend.

Table 1
Comparison of anorectic effects of T-2 toxin, HT-2 toxin, satratoxin G and emetine in mouse.

Toxin (mg/kg BW)	IP		Oral		Intranasal	
	NOAEL[a]	LOAEL[b]	NOAEL[a]	LOAEL[b]	NOAEL[a]	LOAEL[b]
T-2 toxin	0.01	0.1	0.01	0.1	–	–
HT-2 toxin	0.01	0.1	0.01	0.1	–	–
SG	0.025	0.25	–	–	0.25	0.5
Emetine	0.5	2.5	0.025	0.25	–	–

[a] NOAEL, no observed adverse effect level.
[b] LOAEL, lowest observed adverse effect level.

0.1 mg/kg BW, respectively). For emetine, the LOAEL for anorectic responses were reported to be 3 and 0.1 mg/kg BW in dog following iv and interventricular exposure, respectively [34]. Differences in experimental design and animal species in absorption, distribution, metabolism, and excretion of T-2 toxin and emetine could explain the differences observed between these prior studies and the current investigation.

Table 2
Previously reported anorectic effects of T-2 toxin and emetine.

Toxin	Species	Route	NOAEL (mg/kg BW)	LOAEL (mg/kg BW)	Study
T-2 toxin	Mouse	Oral	n.d.	0.5	[25]
T-2 toxin	Rat	Oral	1	3	[23]
T-2 toxin	Rabbit	Oral	n.d.	2	[26]
T-2 toxin	Cat	sc	n.d.	0.05	[27]
T-2 toxin	Cat	Oral	n.d.	0.08	[24]
Emetine	Dog	iv	n.d	3	[34]
	Dog	intraventricular	n.d	0.1	

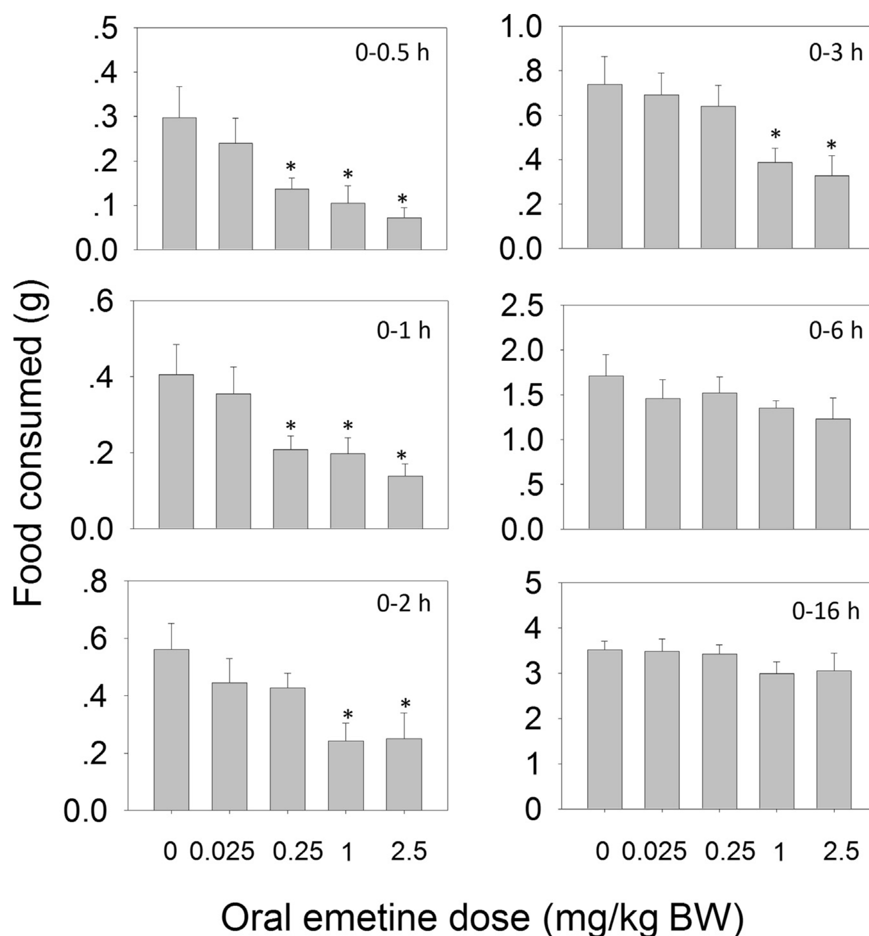

Fig. 10. Oral exposure to emetine impairs cumulative food intake for up to 6 h. Experiment was conducted and data analyzed as described in Fig. 3 legend.

The anorectic NOAEL and LOAEL of HT-2 toxin and SG following IP and oral or intranasal exposure presented here are the first published values for any species. These data indicate that the kinetics and dose-dependency of the anorectic responses to T-2 toxin and HT-2 toxin were similar. Anorexia occurred at both 0.5 h but lasted 96 h and 48 h following IP and oral exposure, respectively. T-2 toxin is rapidly and almost completely biotransformed to HT-2 toxin after exposure in dogs [40] and rats [41] and similar metabolic capabilities can be found in mouse microsomes [42]. Therefore, the equivalent anorectic potencies of these two compounds might be due to efficient biotransformation of T-2 toxin. In contrast to the Type A trichothecenes, the kinetics of the anorectic responses to SG following IP and intranasal exposure were quite different lasting 96 h vs 2 h, respectively. While no toxicokinetic data are available relative to IP exposure to SG, in a prior study [43], it was observed that following intranasal exposure of the mouse to SG 0.5 mg/kg BW SG, the highest plasma toxin levels were detected within 5 min and returned to basal levels by 2 h, which was consistent with transient anorexia induction by SG observed here.

Using the mouse model employed herein, our laboratory demonstrated in prior work that upstream mechanisms for central anorexigenic signaling by DON and other Type B trichothecenes is likely to involve secretion of two satiety hormones by gut enteroendocrine cells, CCK and $PYY_{3\text{-}36}$ [15,16]. T-2 toxin has been shown to induce c-Fos in key brainstem and hypothalamic nuclei indicating that, like DON, this toxin targets the central nervous system and evoke anorexia [25]. T-2 toxin also activates central anorectic pathways by stimulating NUCB2/nesfatin-1 expressing neurons in the hypothalamic nuclei and brainstem. Thus, as observed for DON, activation in the hypothalamus likely participates in T-2 toxin-induced anorectic responses. It might be speculated that CCK and $PYY_{3\text{-}36}$ are similarly involved in anorexia induction by the panel of compounds tested here. Trichothecenes are also known to induce gene expression and release of proinflammatory cytokines including interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) [1], these cytokines can also contribute to anorexia induction by both peripheral and central administration [44–47]. T-2 toxin and SG were reported to cause mRNA levels of IL-1β, IL-6 and TNF-α upregulation in rodents [25,39,48,49]. It is therefore possible that anorexia induction by T-2 toxin, HT-2 toxin and SG might also be mediated in part by sickness responses to these cytokines.

It was notable that emetine also had anorectic effects and was much more potent at reducing food intake when delivered orally than parenterally. Emetine is the principal alkaloid in ipecac syrup derived from ipecacuana plants and has medicinal properties that include gastrointestinal decontamination by induction of emesis as well as treatment of amoebiasis and malaria [50]. Ipecac has, however, been used as a drug of abuse by individuals with eating disorders such as anorexia nervosa and bulimia and therefore, its use has been discouraged and it is no longer available over the counter in the U.S. The greater efficacy of emetine following oral delivery as compared to parenteral administration suggests contact with gut enteroendocrine cells is required for the anorectic effects. It is further notable that emetine inhibits translation by interacting with the E-site of the ribosomal subunit thereby blocking mRNA/tRNA translocation [51]. Interestingly, this compound can block ribosomal binding of T-2 toxin in eukaryotic cells [52,53]. Thus, it might be speculated that emetine and trichothecenes evoke food refusal by causing metabolic dysfunction through protein synthesis inhibition in enteroendocrine cells which are known to rapidly turn over, however, this will require further study.

Taken together, the results presented here indicate that T-2 toxin, HT-2 toxin, SG and emetine dose-dependently evoked anorectic responses in the mouse. Measurement of the anorectic potencies of these toxins will be valuable in predicting their potentials to cause food refusal and resultant growth suppression in animals and humans. It is also important to note that all of the toxins tested here were more effective at reducing food intake than DON. We recognize that the latter interpretation must be made cautiously given the inherent limitations of comparing the results of two different studies. Future studies should focus on the underlying mechanisms for toxin-induced anorexia, particularly in regard to neuroendocrine hormones and cytokines. From animal and public health perspectives, the comparative anorectic potency data presented here could be useful for establishing toxic equivalency factors for trichothecenes as well as other natural translation inhibitors. Furthermore, studies such as this will improve our understanding of how these compounds adversely affect humans and animals.

Transparency document

The Transparency document associated with this article can be found in the online version.

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