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Modeling the emetic potencies of food-borne trichothecenes by benchmark dose methodology

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ABSTRACT

Trichothecene mycotoxins commonly co-contaminate cereal products. They cause immunosuppression, anorexia, and emesis in multiple species. Dietary exposure to such toxins often occurs in mixtures. Hence, if it were possible to determine their relative toxicities and assign toxic equivalency factors (TEFs) to each trichothecene, risk management and regulation of these mycotoxins could become more comprehensive and simple. We used a mink emesis model to compare the toxicities of deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol, fusarenon-X, HT-2 toxin, and T-2 toxin. These toxins were administered to mink via gavage and intraperitoneal injection. The United States Environmental Protection Agency (EPA) benchmark dose software was used to determine benchmark doses for each trichothecene. The relative potencies of each of these toxins were calculated as the ratios of their benchmark doses to that of DON. Our results showed that mink were more sensitive to orally administered toxins than to toxins administered by IP. T-2 and HT-2 toxins caused the greatest emetic responses, followed by FX, and then by DON, its acetylated derivatives, and NIV. Although these results provide key information on comparative toxicities, there is still a need for more animal based studies focusing on various endpoints and combined effects of trichothecenes before TEFs can be established.

1. Introduction

Trichothecene mycotoxins, produced most commonly by the fungi *Fusarium graminearum, F. culmorum*, and *F. sporotrichioides*, often contaminate common cereal grains such as wheat, barley, rye, and oats (Foroud and Eudes, 2009; Stanciu et al., 2015). The most commonly occurring and regulated trichothecene is deoxynivalenol (DON), also called "vomitoxin," as it induces emesis in multiple species. The United States Food and Drug Administration (FDA) has set an industry guideline for the maximum allowable concentration of DON at 1 mg/kg (FDA, 2010). Other regulatory bodies such as the European Commission have set similar or more stringent DON standards in food (European Commission, 2006).

DON is one of the trichothecenes that make up the sub-class type B trichothecenes. The type B trichothecenes are characterized by a keto group at carbon-8 of the parent epoxy-trichothecene nucleus. This group includes five associated congeners: DON, its acetylated derivatives 15-acetyldeoxynivalenol (15-ADON) and 3-acetyldeoxynivalenol (3-ADON), nivalenol (NIV) and its acetylated derivative fusarenon X (FX) (Fig. 1). In addition to the type B trichothecenes, there are those trichothecenes classified as type A, of which T-2 and HT-2 toxins are the most toxic. Type A trichothecenes are characterized by the presence of a hydroxyl group, ester function, or no oxygen substitution at carbon-8 (McCormick et al., 2011). DON and its related trichothecene mycotoxins cause a variety of adverse effects in multiple species. These adverse effects have been reviewed by Pestka et al. (2010a, 2010b) and include emesis, nausea, anorexia, diarrhea, growth retardation, neuroendocrine effects, and disruption of the immune system. The primary clinical signs associated with exposure to DON and related trichothecenes in human populations are nausea and vomiting. Therefore, these specific effects should be the endpoints utilized in risk assessment for human consumption of trichothecenes (Luo, 1994; Yoshizawa, 1983).

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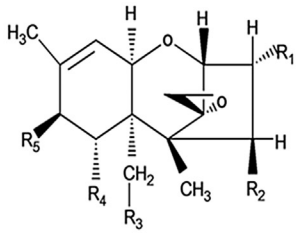
Structure	Trichothecene	Abbreviation	R1	R2	R3	R4	R5
	deoxynivalenol	DON	OH	H	OH	OH	=O
	3-acetyldeoxynivalenol	3-ADON	OAc	H	OH	OH	=O
	15-acetyldeoxynivalenol	15-ADON	OH	H	OAc	OH	=O
	nivalenol	NIV	OH	OH	OH	OH	=O
	fusarenon X	FX	OH	OAc	OH	OH	=O
	HT-2 toxin	HT-2	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
	T-2 toxin	T-2	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$

Fig. 1. The general structure of trichothecenes and the various functional groups.

Gastroenteritis outbreaks in the U.S. and abroad have been associated, inconclusively, with DON contamination of foods. From October 1997 to October 1998 there were 16 outbreaks of gastrointestinal illness affecting more than 1900 school children following the ingestion of burritos from two unrelated companies (Steinberg et al., 2006). The children who consumed the burritos suffered nausea, headache, abdominal cramps, vomiting, and diarrhea. Laboratory analysis did not find contamination with common bacterial strains associated with gastroenteritis, suggesting that the symptoms observed were due to a toxin contamination. Some burrito samples had detectable levels of DON, although they were below the FDA regulatory guideline of 1 mg/kg. Outbreaks in China from 1984 to 1991 were linked to moldy cereal grains. DON, as well as other trichothecenes, were verified in samples taken during this time period, and found at concentrations ranging from 2 to 50 mg/kg (Pestka and Smolinski, 2005). Several thousand individuals from the Kashmir Valley of India were affected with gastroenteritis from consumption of foods made with moldy wheat. Samples were found to contain DON at 0.34–8.4 mg/kg (Bhat et al., 1989). It is important to note, that although food samples may contain trichothecene levels below the FDA guideline, the threshold for human emesis is still unknown and that this threshold might also vary greatly due to differences in age, sex, diet, health status, genetic differences, etc. making it difficult to assess this value (Stadler et al., 2003).

The Joint Expert Committee on Food Additives of the Food and Agriculture Organization and World Health Organization (JECFA) has determined a recommended acute reference dose (ARfD) for DON and its acetylated derivatives of 8 μg/kg bw/day. Although JECFA has not set a similar ARfD for NIV and FX, the European Food Safety Authority (EFSA) has made a recommendation for a joint tolerable daily intake of 1.2 μg/kg bw/day (EFSA, 2013). JECFA has established a provisional maximum tolerable daily intake (PMTDI) of 60 ng/kg bw/day for either T-2 toxin alone or a mixture of T-2 and HT-2 (JECFA, 2001).

Although trichothecenes co-occur in numerous food commodities, a lack of robust toxicology and epidemiology studies makes it difficult to determine appropriate regulatory levels for each individual trichothecene. Additionally, risk assessment of foods with potential co-contamination is extremely difficult when there are limited studies on the toxicity of mixtures. Since there is a lack of consensus among governmental agencies on regulation of trichothecenes, assigning each trichothecene a toxic equivalency factor (TEF) would be desirable for risk assessment and regulatory purposes. Using such an approach, a single regulatory standard could be set for the sum total of all co-occurring trichothecenes in cereals and their products.

TEF values were first introduced in the practice of regulatory risk assessment for the polychlorinated dibenzodioxins (PCDDs), the polychlorinated biphenyls (PCBs), and the polychlorinated dibenzofurans (PCDFs) (Van den Berg et al., 1998; Van den Berg et al., 2006). The theory behind associated TEF values is that, because compounds in a particular group (e.g., dioxins) usually co-occur, are structurally similar, have similar modes of toxicity, and have additive impacts, they are each assigned an equivalency factor in comparison to the toxicity value of a reference compound. Although there is limited evidence for the additive effect of trichothecenes when consumed as mixtures, the initiation of the process to develop a similar regulatory mechanism to that of the TEF values of PCDDs, PCBs, and PCDF is warranted and essential to future risk assessment practices for these similar, co-occurring food mycotoxins.

Previously, the relative anorectic potencies of the above-mentioned trichothecenes were calculated by Male et al. (2015), using raw data collected from a mouse anorexia study (Wu et al., 2012a,b). In that analysis, two methods were used to rank the potency of each trichothecene relative to DON based on feed refusal: 1) benchmark dose (BMD) analysis and 2) incremental area under the curve (IAUC). The mouse model cannot be used to study emetic responses to trichothecenes, because mice are incapable of vomiting. Therefore, the present study applied the same principles, with a new set of data from a mink model to utilize emesis as the outcome variable. Currently, the BMD is used by regulatory bodies worldwide as the "point of departure" from which to calculate tolerable daily intakes or reference doses for humans. The BMD methodology allows for use of animal data with limited number of dose groups and small n values. Here, DON, 3-ADON, 15-ADON, NIV, FX, HT-2, and T-2 toxins were ranked by potency to induce emesis, based on their individual BMD values following oral gavage and intraperitoneal (IP) dosing in mink. Relative potencies were assigned to each toxin in relation to DON. This work is an important initial step in developing a uniform risk assessment strategy for complex mixtures of trichothecenes.

2. Materials and methods

2.1. Mink feeding and emesis trials

The raw emesis data used in this analysis were collected from prior mink experiments. The experimental design and procedures,