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Trans-/multi-generational effects of deoxynivalenol on *Caenorhabditis elegans*

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HIGHLIGHTS

- Effects on development, reproduction, and behavior were assessed for DON exposure on *C. elegans*.
- DON exposure produced significant trans-/multi-generational toxic effects on *C. elegans*.
- Significant interactions between concentrations and generations on DON exposure and response were found in *C. elegans* model.

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ABSTRACT

Deoxynivalenol (DON, vomitoxin) is one of the most widely distributed trichothecene mycotoxins commonly found in cereal food and feeds. Significant acute and potential chronic toxic effects of DON have been observed in animals and human populations. However, potential adverse effects associated with DON exposure across multiple generations have not been extensively investigated. In this study, *Caenorhabditis elegans* (*C. elegans*) were used to evaluate the trans-/multi-generational toxicities of DON via 3 physiological endpoints: growth, brood size, and feeding ability. DON concentration at higher than 100 μg/mL significantly inhibited growth, decreased brood size, and reduced food intake in a concentration-dependent manner. Gradual decline in DON-induced impairments was observed in the filial generations when only the parental generation was exposed. However, greater damages in filial generations were found as compared to the parental generation ($p < 0.01$) with all generations continuously exposed to DON. Overall, the endpoints of brood size and food intake were more sensitive for both trans- and multi-generational toxic effects of DON. Additionally, the interactions between concentrations and generations significantly influence the response of *C. elegans* to DON exposure, based on a mixed-effect model with multi-level analysis. Taken together, our results demonstrated that DON exposure produced significant trans-/multi-generational toxic effects on *C. elegans*, which may serve as a model organism to explore molecular mechanisms of long-term adverse health effects of DON.

1. Introduction

Deoxynivalenol (DON, vomitoxin), a type B trichothecene mycotoxin, is a secondary metabolite mainly produced by *Fusarium (F.) graminearum* and *F. culmorum* in the field and/or during storage, and is commonly found in some grain-based food and feeds, such as wheat, barley, and maize (Pestka, 2010b; Voss and Snook, 2010; Rodriguez-Carrasco et al., 2015). In general, DON contamination is more commonly found in many Asian and African countries, where grain-based products are consumed as the staple food (Wang et al., 2010; Srey et al., 2014; Selvaraj et al., 2015). But high concentration of DON was also found in feed samples from Europe and America. A survey conducted by the BIOMIN Company (BIOMIN, 2017) from January to March 2017 on 3715 finished feed and raw commodity samples sourced from 54 countries showed that DON is the most contaminated mycotoxin in East Asia (96%), South America (84%), North America (78%), Europe (northern/79%; central/74%; eastern/73%), Africa (71%), and Middle East (69%) with the maximum concentrations of 1,206, 12,802, 51,374, 28,470 and 4801 μg/kg,

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respectively. Though the levels of DON contamination in processed food were significantly lower than those in raw cereal grains due to the postharvest handling and milling processing, the global average contamination rate of DON in processed food still can reach at 56% with the maximum concentration at 6,178 μg/kg (Lee and Ryu, 2017). International Agency for Research on Cancer (IARC) once noted that DON in processed grains was detected at a concentration as high as 500 mg/kg (IARC, 1993). As a naturally occurring mycotoxin, DON contamination is influenced by various environmental parameters, especially temperature and moisture (Lee and Ryu, 2017). Additionally, DON is quite stable during storage, processing and cooking (Larsen et al., 2004, Canady et al.) which makes elimination or decontamination difficult. DON has been regarded as one of the top dietary mycotoxin contaminants since its discovery (Canady et al.; JECFA, 2001; Schatzmayr and Streit, 2013; Streit et al., 2013).

Owing to DON's extensive contamination and difficulties of removal, a large number of studies have been conducted to investigate DON-induced adverse effects on various *in vitro* models, *in vivo* animals, as well as human observations (Pestka and Smolinski, 2005; Zhou et al., 2017). In general, acute exposures to high concentration of DON induced nausea, vomiting, and diarrhea, moreover, at extremely high concentrations, additional toxic syndromes could be caused including gastrointestinal hemorrhage, leukocytosis, circulatory shock, and ultimately death. Contrarily, chronic exposures to low/moderate-concentrations of DON resulted in anorexia, reduced food intake, growth retardation, reproductive and developmental impairments (Wu et al., 2014). In addition, DON affected immune regulation, disturbed neuroendocrine signaling, and deleterious effects on gut microbiota (Pestka and Smolinski, 2005; Bonnet et al., 2012; Robert et al., 2017). Many cytotoxic studies had been conducted to assess the toxic mechanisms of DON and found the inhibition of protein and nucleic acid synthesis via binding to ribosome, and/or activation of cellular kinases (e.g., Mitogen-activated protein kinase/MAPKs) as the main toxic effects of DON at the cellular level (Zhou et al., 2003; Pestka, 2010a; Mishra et al., 2014). Disruptions of macromolecule synthesis, cell signaling, differentiation, proliferation, and lethal effect were also commonly induced by DON exposure (Pestka, 2010b).

Several studies demonstrated that DON exposure caused reproductive impairments on experimental animals. Exposure to DON-contaminated food or feeds affected oocyte developmental competence, by directly interfering with microtubule dynamics during meiosis, and by disturbing oocyte cytoplasmic maturation, which induced teratogenic effects on developing embryos (Schoevers et al., 2010). DON was found to transfer via placenta to fetus and could be detected in the plasma, liver and kidney of fetuses if pregnant sows were treated with the parent mycotoxin (Goyarts et al., 2007; Tiemann et al., 2008). Overt impairments were found after pregnant Swiss-Webster mice were administered DON at concentrations of 0–15 mg/kg body weight (Khera et al., 1982). These studies suggested that maternal exposure to DON may pose adverse effects on offspring. More importantly, animals and humans may be continually exposed to unavoidable toxicants, such as DON, across multiple generations in nature. These findings support the significance for assessment of adverse biological impacts over multiple generations.

Caenorhabditis elegans (*C. elegans*) as a non-parasitic model have been used as model organism for toxicological and biological studies (Brenner, 1974; Anderson et al., 2001; Leung et al., 2008; Boyd et al., 2010a, 2010b; Zhuang et al., 2014). The advantages of using *C. elegans* as an experimental model include their short lifespan at room temperature, rapid reproduction in the laboratory, and large brood size per nematode (Ma, 2009; Megalou and Tavernarakis, 2009; Zhuang et al., 2014) which would be appropriate for multiple generational studies. The translucent body of the worm allows fluorescent markers or dyes to be detected in living conditions (Giles and Rankin, 2009). Further, an ample amount of biological, genetic and genomic data for *C. elegans* have been generated with the completely sequenced genome (García-Sancho, 2012), the complete cell lineage characterized by laser ablation and microscopy (Sulston et al., 1983), and a thorough base of knowledge exists regarding the nervous system (White et al., 1986; Coulson et al., 1991).

In this study, the potential trans-/multi-generational effects of DON were evaluated in the *C. elelgans* model. The aim of the study was to explore potential adverse impacts of DON on the maternal and offspring populations under different exposure scenarios via multiple endpoints, including body length for development, brood size for reproduction, and food intake for feeding ability.

2. Materials and methods

2.1. C. elegans maintenance and DON exposure

C. elegans of wild type Bristol (N2) strain and *E. coli* strain OP50 were purchased from the *Caenorhabditis* Genetics Center (Minneapolis, MN, USA). All nematodes in the experiments were hermaphrodites. Populations of *C. elegans* were grown on nematode growth medium (NGM) plates seeded with *E. coli* strain OP50 as food source at 20 °C based on a standard cultivation protocol (Stiernagle, 2006). NGM was made as previously described by Brenner (1974). DON, the purity (TLC) ≥ 98%, was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The stock solution (10 mg/mL) was freshly prepared weekly with distilled H_2O (dH_2O) produced by Millipore® Synergy Purification System (Millipore, Billerica, MA, USA) and kept in the dark at −20 °C. Based on the reported contamination levels of DON found in food and feeds, the various maximal limits of different countries in diverse foodstuffs (200–1000 μg/kg) (Lee and Ryu, 2017), and the uncertainty factors (10–1000 folds) depending on tested species (Dourson and Stara, 1983), 6 different concentrations of DON were designed for this study: 0 (control), 50, 100, 200, 400 and 800 μg/mL. All treatment solutions were prepared by diluting with K-medium. Synchronized *C. elegans* were treated with the solutions in 12-well sterile tissue culture plates (Fisherbrand, PA, USA). Each well contained 100 μL of *E. coli* OP50 (optical density of 2.0 at 570 nm), 5 μL of worms in K-medium, 5 μL of DON with different concentration or control solution (K-medium), and 890 μL of K-medium to bring total volume to 1 mL (Williams and Dusenbery, 1990). All other chemicals and reagents were purchased commercially at the highest degree of purity available.

2.2. Experimental design

The whole experimental design in this study is illustrated in Fig. 1. Four generations of *C. elegans* in total were tested and all the generations were investigated for endpoints including growth (body length), brood size (egg number) and feeding (food intake). Gravid nematodes and eggs were harvested from the stock NGM plates and then lysed by bleach-sodium hydroxide solution (Stiernagle, 2006) to obtain eggs. These eggs were regarded as the parental generation (P_0) in the experiment. The eggs were cultured in K-medium overnight to obtain synchronized L1/L2-stage N2 larvae. Subsequently, these larvae were exposed to different concentrations of DON for a specific period of time depending on experimental assays. Growth assay and brood size assay were conducted using liquid medium at the concentrations of 0 (control), 50, 100, 200, 400 and 800 (only for growth assay) μg/mL in 12-well tissue culture plate (Fisherbrand, PA, USA), 1 mL/well. Feeding assay