In vitro and in vivo effects of a mycotoxin, deoxynivalenol, and a trace metal, cadmium, alone or in a mixture on the intestinal barrier

Su Luo, Chloé Terciolo, Ana-Paula Loureiro-Bracarense, Delphine Payros, Philippe Pinton, Isabelle P. Oswald

Environment International 132 (2019) 105082

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

In vitro and *in vivo* effects of a mycotoxin, deoxynivalenol, and a trace metal, cadmium, alone or in a mixture on the intestinal barrier

Su Luo[a], Chloe Terciolo[a], Ana Paula F.L. Bracarense[b], Delphine Payros[a], Philippe Pinton[a,*], Isabelle P. Oswald[a]

[a] Toxalim (Research Center in Food Toxicology), Université de Toulouse, INRA, ENVT, INP-Purpan, UPS, Toulouse, France
[b] Laboratory of Animal Pathology, Universidade Estadual de Londrina, Londrina, Brazil

ARTICLE INFO

Handling Editor: Daniel Doerge

Keywords:
Deoxynivalenol
Cadmium
Intestinal barrier function
Mixture exposure
Junctional proteins
Mixture risk assessment

ABSTRACT

Deoxynivalenol (DON), one of the most widespread mycotoxins in Europe, and cadmium (Cd), a widespread environmental pollutant, are common food contaminants. They exert adverse effects on different organs including kidney, liver, and intestine. The intestine is a common target of DON and Cd when they are ingested. Most studies have focused on their individual effects whereas their combined toxicity has rarely been studied. The aim of this study was thus to evaluate their individual and combined effects on the intestinal barrier function *in vitro* and *in vivo*. *In vitro*, Caco-2 cells were treated with increasing concentrations of DON and Cd (1–30 μM). *In vivo*, Wistar rats were used as controls or exposed to DON contaminated feed (8.2 mg/kg feed), Cd-contaminated water (5 mg/l) or both for four weeks. In Caco-2 cells, DON, Cd and the DON + Cd mixture reduced transepithelial electrical resistance (TEER) and increased paracellular permeability in a dose-dependent manner. Impairment of the barrier function was associated with a decrease in the amount of E-cadherin and occludin after exposure to the two contaminants alone or combined. A decrease in E-cadherin expression was observed in rats exposed to the two contaminants alone or combined, whereas occludin expression only decreased in animals exposed to DON and DON + Cd. Jejunal crypt depth was reduced in rats exposed to DON or Cd, whereas villi height was not affected. *In vitro* and *in vivo* results showed that the effects of exposure to combined DON and Cd on the intestinal barrier function in the jejunum of Wistar rats and in the colorectal cancer cell line (Caco-2) was similar to the effects of each individual contaminant. This suggests that regulations for each individual contaminant are sufficiently protective for consumers.

1. Introduction

Food safety is a major concern worldwide. Food and feedstuffs are frequently contaminated by multiple contaminants (Rather et al., 2017; Silins and Högberg, 2011). The toxic effects of these mixtures cannot be predicted based on the toxicity of individual contaminants alone, as simultaneous exposure to several contaminants can have synergistic, antagonistic or additive effects (Alassane-Kpembi et al., 2017b; More et al., 2019). In recent years, combined toxicity studies have assessed the effects of mixtures of food contaminants such as mycotoxins (Alassane-Kpembi et al., 2017a), trace metal elements (Claus Henn et al., 2014), pesticides (Lukowicz et al., 2018) and carcinogens (Miller et al., 2017). However, the toxicity of mixtures of contaminants from different families remains poorly documented (Le et al., 2018; More et al., 2019; Payros et al., 2017).

Mycotoxins are the most frequently occurring natural food contaminants in human and animal diets. Among mycotoxins, Deoxynivalenol (DON), mainly produced by *Fusarium graminearum* and *F. culmorum*, frequently contaminates cereals and cereal products. Almost half of 26,613 cereal samples collected from 21 European countries were found to be contaminated by DON, with the highest levels observed in wheat, maize and oat grains (EFSA, 2017). Tolerable daily intake (TDI) for DON and its derivative was established at 1 μg/kg b.w./day (EFSA, 2017). Analyses of adult urine samples in the United Kingdom revealed that 98% of them had been exposed to DON (Turner et al., 2008), while national and European surveys indicate that the health based guidance values are exceeded in children (EFSA, 2017; Sirot et al., 2013). DON interacts with the peptidyl transferase region of the 60S ribosomal subunit, inducing “ribotoxic stress,” resulting in the activation of mitogen-activated protein kinases and their downstream pathways (Lucioli et al., 2013; Pestka, 2010). Symptoms of intoxication in animals exposed to DON may include reduced food consumption and

* Corresponding author at: Toxalim, INRA, 180 chemin de tournefeuille, 31300 Toulouse, France.
E-mail address: Philippe.Pinton@inra.fr (P. Pinton).

https://doi.org/10.1016/j.envint.2019.105082
Received 27 March 2019; Received in revised form 31 July 2019; Accepted 1 August 2019
Available online 07 August 2019

weight gain, neuro-endocrine changes as well as alteration of intestinal and immune functions. Following acute exposure, vomiting and bloody diarrhea have been reported (Payros et al., 2016; Pestka, 2010; Pinton and Oswald, 2014).

Cadmium (Cd) is a heavy metal element naturally present in the Earth's crust. It is a common environmental pollutant that occurs both naturally and as a result of industrial and agricultural activities (ATSDR, 2012). Natural sources of Cd include volcanic activity, weathering of rocks, sea aerosols and forest fires. Anthropic origins of Cd include batteries, pigments, plastic stabilizers, pesticides and fertilizers, and photovoltaic devices, as well as rubber processing, galvanization, fossil combustion and waste incineration. Cadmium compounds are soluble in water and can be taken up by plant roots and translocated to edible parts where they accumulate. For example, large quantities of Cd accumulate in tobacco leaves and tobacco smokers are thus exposed by inhalation (Ganguly et al., 2018). Among nonsmokers, ingestion of contaminated food is a major source of Cd exposure. Sixty-six percent of 137,200 food samples analyzed in 20 European countries contained 5% more than the maximum recommended level of Cd (EFSA, 2009). Tolerable weekly intake (TWI) for Cd is set at 2.5 μg/kg b.w. (EFSA, 2009). European adults' mean dietary exposure to Cd was shown to be close to this TWI, and subgroups, including vegetarians, children, and smokers, exceeded the TWI about 2-fold (EFSA, 2009). Grain and cereal products, as well as fish and offal were the major contributors to human Cd exposure (ATSDR, 2012; Filipič et al., 2006). Cd causes inflammation, apoptosis and oxidative stress in liver and kidney (Kim et al., 2015; Liu et al., 2015). Cadmium absorbed by inhalation and ingestion mainly accumulates in the liver and kidney, but the small intestine and lung are other targets (Tinkov et al., 2018; Zhang et al., 2015). Cd increases the risk of cancer through oxidative stress, which damages DNA and inhibits DNA repair systems, and cadmium is classified as a (group 1) human carcinogen (Bishak et al., 2015).

The intestinal epithelium is the first barrier against ingested chemicals and food contaminants. The gut barrier is formed to a large extent by intercellular junctions on the apical side of epithelial cells. These junctions seal the cells together and regulate the passage of ions and water across the epithelium (Suzuki, 2013; Terciolo et al., 2019). Following ingestion of contaminated food, intestinal epithelial cells may be chronically exposed to contaminants including DON and Cd. The effects of DON on the intestine are well described (Maresca, 2013; Pinton and Oswald, 2014). DON alters the intestinal structure, reduces the expression of several junctional proteins, reduces the barrier function, affects nutrient absorption, modulates intestinal microbiota and the local immune responses (Payros et al., 2016; Pestka, 2010; Pinton and Oswald, 2014). The effects of Cd on the intestine are less well documented, but recent studies have shown that it alters the gut microbiota, triggers a local inflammatory response and disrupts tight junctions (Breton et al., 2016; Tinkov et al., 2018).

Human and animals are exposed to both compounds through ingestion of contaminated food and feed, especially cereals and cereal by-products (Arnich et al., 2012; Sirot et al., 2013). To the best of our knowledge, with the exception of one study (Le et al., 2018), the combined effects of these two contaminants have not been documented to date. The aim of the present study was thus to assess the individual and combined effects of DON and Cd on the intestinal barrier using *in vitro* and *in vivo* models. *In vitro*, the individual and combined effects of DON and Cd (0–30 μM) on Caco-2 cells were analyzed by measuring transepithelial electrical resistance (TEER), paracellular permeability and the abundance of junctional proteins. *In vivo* experiments were performed to investigate the effects on intestinal histomorphology, the abundance and localization of junctional proteins in rats exposed to DON (8.2 mg/kg feed) and Cd (5 ml/l of drinking water). Our *in vitro* and *in vivo* data on the parameters cited above demonstrated that the effects of different combinations of DON and Cd tested were comparable to those of the highest dose of each individual contaminant.

2. Materials and methods

2.1. Reagents

DON and $CdCl_2$ were purchased from Sigma (St Quentin Fallavier, France). For the *in vitro* experiments, DON and $CdCl_2$ were dissolved in water and stock solutions (5 mM) were stored at −20 °C before dilution in complete cell culture medium. For the *in vivo* experiments, DON was included in the rats' diet as previously described (Bracarense et al., 2017; Payros et al., 2016) while $CdCl_2$ was added to drinking water at a concentration of 5 mg/l.

2.2. Cells

The Caco-2 cell line (Sigma, 86010202) was originally isolated from a primary colonic tumor in a 72-year-old Caucasian male. The cells were maintained in DMEM-Glutamax (Gibco, Life Technologies, Courtaboeuf, France) supplemented with 10% fetal calf serum (FCS) (Eurobio, Courtaboeuf, France), 1% non-essential amino acid (Sigma) and 0.5% gentamycin (Eurobio). Cells were maintained in a humidified atmosphere at 37 °C with 5% CO_2 and passaged by trypsinization (0.5% trypsin in 0.5 mM EDTA) when they reached 80% confluency. Caco-2 cells were differentiated on 0.3 cm^2 polyethylene terephthalate membrane inserts with 0.4 μm pores (Corning Inc., Corning, NY, USA) for the assessment of transepithelial electrical resistance (TEER) and paracellular permeability, as previously described (Pierron et al., 2016b; Pinton et al., 2009).

2.3. Animals

The experimental protocol was carried out in accordance with European Guidelines for the Care and Use of Animals for Research Purposes and was validated by the Toxcomethique Ethics Committee and the French Ministry of National Education Higher Education and Research (TOXCOM/0142/PP). Three of the authors (DP, PP and IPO) have official authorization from the French Veterinary Services for animal experimentation.

Four-week-old male Wistar rats (140–170 g body weight), (Janvier Labs, Le Genest Saint Isle, France) were kept under the normal conditions with a 12-hour day/night cycle, 19–25 °C temperature, 50–70% humidity, at the Toxalim animal facility (INRA, UMR 1331, Toulouse) with *ad libitum* access to food and water throughout the study. After five days of acclimatization, the rats were divided into four groups of eight animals: a control group, rats exposed to DON contaminated-feed (8.2 mg DON/kg feed), rats exposed to Cd-contaminated water (5 mg $CdCl_2$/l) and rats exposed to both DON and Cd. No other mycotoxin was detected in the feed (Supplementary Table 1). The rats were weighed weekly. After four weeks, the animals were euthanized, and the intestinal tissue was collected and fixed in 10% buffered formalin for histological assessment and immunohistochemical staining.

2.4. Transepithelial electrical resistance assay

Caco-2 cells grown on inserts differentiated and acquired an epithelial phenotype with polarity properties (apical and basolateral sides). They were treated apically with increasing concentrations of DON and Cd (0, 3, 10, 30 μM) alone or combined. Transepithelial electrical resistance (TEER) was measured at 4 hourly intervals for 48 h using a cellZscope device (nanoAnalytics, Münster, Germany). Measurements were made on four replicates of four independent experiments.

2.5. Paracellular tracer flux assay

To assess paracellular flux, the 4-kDa fluorescein isothiocyanate-dextran (FITC-dextran) (Sigma), dissolved in cell culture medium, was

added in the apical compartment (transwell, final concentration 2.2 mg/ml) at 48 h post DON and/or Cd exposure. After 1 h of incubation, fluorescence was measured in the basal compartment (well of plate) with a microplate fluorimeter reader (Tecan, Lyon, France). The excitation and emission wavelengths were 490 and 520 nm, respectively. The background signal resulted from reagent-treated medium without cells. Measurements were made on four replicates of four independent experiments.

2.6. Western blot analysis of junctional proteins

Differentiated Caco-2 cells, cultured in 6-well plates, were exposed for 24 h to DON and Cd individually or combined to analyze junctional proteins. Three independent experiments were performed for each cell culture condition. After cell treatment, proteins were extracted as previously described (Pinton et al., 2009) and separated on SDS-PAGE membranes probed with rabbit polyclonal Abs anti-occludin (#71-1500), -ZO-1 (#61-7300) (Thermo Fisher Scientific, Illkirch, France), -claudin-3 (#34-1700), -claudin-7 (#34-9100) (Invitrogen), rabbit monoclonal Ab anti-E-cadherin (Cell Signaling Technology, Leiden, The Netherlands), or mouse monoclonal Ab anti-claudin-4 (Invitrogen), diluted 1:250. Mouse monoclonal Ab or rabbit polyclonal anti-β-actin (Cell Signaling Technology, #3700 or #4967) was used as control (diluted 1:1000). Membranes were then washed and incubated with secondary antibodies CF680 goat anti rabbit IgG (#20067) or CF680 goat anti mouse IgG (#20065, diluted 1:10000) obtained from Biotium (Hayward, CA). Infrared fluorescence intensity of the specific bands was obtained with Odyssey Infrared Imaging System (LI-COR ScienceTec, Les Ulis, France). Protein expression was estimated after normalization calculated by the ratio of the intensity of the band of interest to that of the β-actin band.

2.7. Immunofluorescence analysis

Differentiated Caco-2 cells cultured on glass cover slips were exposed for 24 h to DON or Cd. The cells were fixed with 4% paraformaldehyde at 4 °C for 30 min and permeabilized with PBS containing 0.1% Triton X-100 for 10 min, then blocked with 10% goat serum at room temperature for 1 h. Occludin and E-cadherin were detected by incubation with rabbit polyclonal Abs anti-occludin (Thermo Fisher Scientific, #71-1500) and monoclonal anti-E-cadherin (Cell Signaling Technology, #3195) respectively. After three washes, the cells were incubated for 1 h with Alexa Fluor 546-conjugated goat immunoglobulin (1:500; Life Technologies, #A11035) raised against rabbit IgGs. Nuclei were stained with DAPI (Vector Laboratories) for 10 min. Cells were washed and mounted in Prolong® Gold antifade reagent (Invitrogen, Oregon, USA). Images were captured using an SP8 Leica confocal microscope and analyzed using FIJI software.

2.8. Histological and morphometric assessment of rat jejunum

The pieces of rat jejunum were fixed in 10% buffered formalin, embedded in paraffin and cut into 5 µm sections. The sections were stained with hematoxylin & eosin for histopathological evaluation. A lesional score, including the morphology of villi and enterocytes, interstitial edema, and lymph vessel dilation was used to compare histological changes in different conditions as previously described (Pierron et al., 2018). Villus height and crypt depth were measured randomly using a MOTIC Image Plus 2.0 ML software (Motic Instruments, Richmond, Canada).

2.9. Immunohistochemical assessment of junctional proteins in the jejunum

After dewaxing and heat-induced antigen retrieval as described previously (Bracarense et al., 2012), paraffin-embedded 5 µm sections of rat jejunum were incubated overnight at 4 °C with the primary antibody anti-E-cadherin (Zymed, San Francisco, CA, USA) and anti-occludin (*Santa Cruz*. Biotechnology Inc., USA). The secondary antibody (SuperPicTure™ Polymer, Zymed) was applied followed by the addition of a chromogen (3,30-diaminobenzidine). Finally, tissue sections were counterstained with hematoxylin. The sections were then examined and the proportion of intestinal section expressing E-cadherin was estimated. Each sample was classified as having either normal or reduced staining.

2.10. Statistical analysis

The results are presented as the means ± standard error of the mean (SEM) of independent experiments. Statistical analysis was performed using GraphPad Software (La Jolla, CA, USA). Significant differences between groups were analyzed by one-way ANOVA (non-parametric) with Bonferroni's multiple comparison test. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Individual effects of DON and Cd on intestinal barrier function

The individual effects of DON and Cd were first assessed on two parameters that reveal the integrity of the intestinal epithelium: the TEER and the paracellular passage of dextran.

As shown in Fig. 1A, both DON and Cd reduced the TEER of Caco-2 monolayers in a dose- and time-dependent manner. The TEER was not affected by 1 and 3 µM of either contaminant but was significantly reduced by 10 µM DON as early as 8 h after exposure (44% decrease, $p < 0.001$) and by 10 µM Cd after 12 h of exposure (40% decrease, $p < 0.001$). Exposure to 30 µM of DON or Cd reduced the TEER as early as 4 h (27%, $p < 0.05$), and the TEER decreased over time: respectively 82% and 87% decrease ($p < 0.001$) at 12 h.

The permeability of Caco-2 monolayers to 4-kDa FITC-dextran was measured at the end of the experiment. As shown in Fig. 1B, this parameter was not affected in cells exposed to 1, 3 µM DON or 10 µM Cd. Higher doses of DON and/or Cd significantly increased the passage of FITC-dextran (18, 67 and 53-fold upon exposure to 10 µM DON, 30 µM DON and 30 µM Cd, respectively).

These results show that both DON and Cd alter the intestinal barrier function as measured by decreased TEER and increased passage of dextran.

3.2. Individual effects of DON and Cd on the abundance of junctional proteins

Adherent and tight junctions belong to apical multiprotein complexes that link adjacent epithelial cells together and play an important role in the formation and the maintenance of the intestinal barrier (Suzuki, 2013). Consequently, the effect of DON and Cd on the abundance of several junctional proteins was examined.

As shown in Fig. 2, DON induced a dose-dependent decrease in the amount of occludin, E-cadherin and claudin-7. In the case of occludin, upon exposure to 10 and 30 µM DON, the decrease reached 54% ($p < 0.05$) and 66% ($p < 0.01$); the reduction in E-cadherin was 50% ($p < 0.05$) and 80% ($p < 0.001$) respectively. For claudin-7, a significant decrease was only observed at 30 µM DON (80%, $p < 0.05$). By contrast, exposure to DON at rates of up to 30 µM, did not affect the amount of claudin-3, -4 and ZO-1.

Cd only caused a significant reduction in the abundance of occludin and E-cadherin upon exposure to 30 µM (58% ($p < 0.05$) and 70% ($p < 0.01$) decrease respectively), whereas claudin-3, -4, -7 and ZO-1 were not affected by the trace metal element.

These results suggest that DON and Cd impair the intestinal barrier function *via* a specific decrease in the abundance of E-cadherin and occludin.

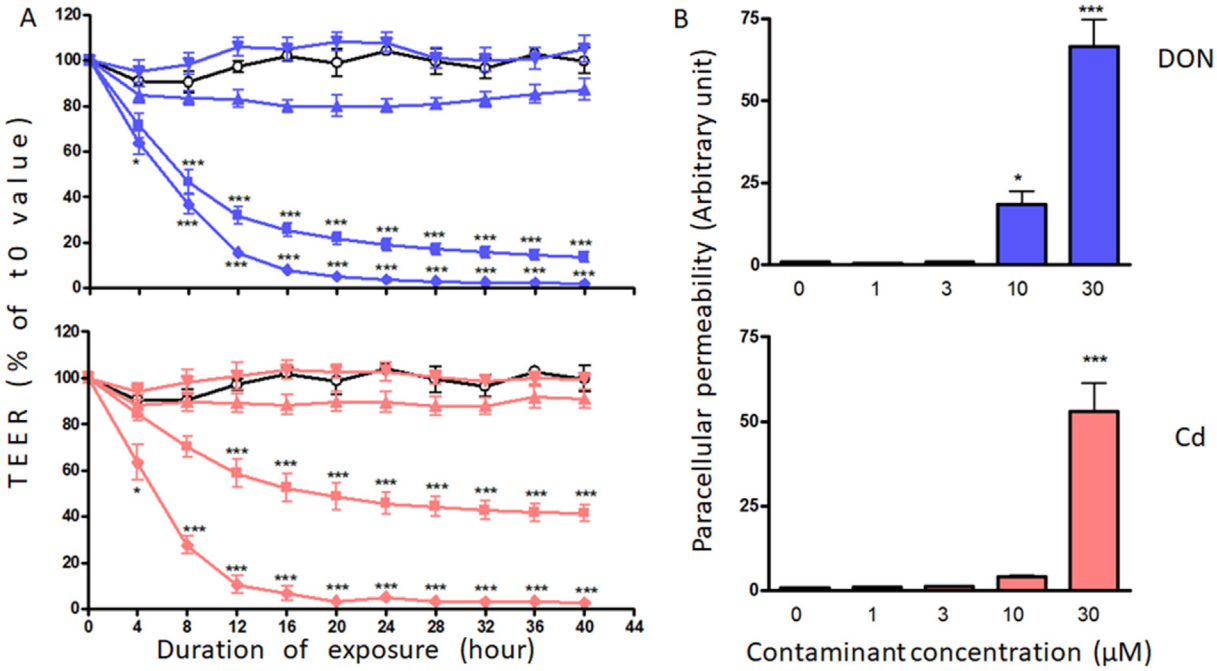

Fig. 1. Effects of DON and Cd on TEER and on the paracellular permeability of differentiated Caco-2 cells.
(A) Effect on the TEER. Caco-2 cells were grown and differentiated on inserts and treated apically with DON (blue upper panel) or Cd (red lower panel) at 1 μM (inverted triangles), 3 μM (triangles), 10 μM (squares) or 30 μM (diamonds); control cells (circles, black line) were untreated. The TEER was recorded over a period of 40 h.
(B) Effect on paracellular permeability. At 48 h, 4-kDa FITC-dextran was added in the apical compartment and fluorescence was assessed in the basal compartment 1 h later. Fluorescent intensity was measured, and background levels subtracted.
Results, normalized to the controls, are expressed as the mean of 4 independent experiments ± SEM (*, $p < 0.05$; ***, $p < 0.001$). Data were analyzed by non-parametric one-way ANOVA.

3.3. Individual effects of DON and Cd on the distribution of junctional proteins

We next investigated the effect of DON and Cd on the distribution of junctional proteins in Caco-2 cells. In control cultures, a well-defined band of membrane-associated immunofluorescence staining was observed for E-cadherin and occludin (Fig. 3A and B). Exposure to DON or Cd for 24 h caused significant changes in the E-cadherin pattern analyzed by fluorescence (white arrow) (Fig. 3A). The main modification caused by DON was reduced membranous expression of E-cadherin. At the same time, increased granular fluorescence was observed in the cytoplasm. After exposure to Cd, the redistribution of E-cadherin into the cytoplasm was more visible. The loss of membranous expression with partial redistribution into the cytoplasm was also observed for occludin (white arrow) (Fig. 3B). The effects of DON and Cd on E-cadherin and occludin distribution were dose dependent.

Taken together, these results show that exposure to DON or Cd caused alterations in the cellular localization of E-cadherin and occludin.

3.4. Combined effects of DON and Cd on intestinal barrier function

The data presented above show that individual exposure to DON or Cd has harmful effects on the intestinal barrier; however, in a mixture, their combined effects remain unknown.

The combined effects of DON and Cd on the TEER are summarized in Fig. 4. In this heat-map, the first line and column show the individual effects of DON and Cd while the other cells show the effects of different combinations, with a color code indicating the strength of the effect. A time and dose dependent decrease in the TEER was observed both when the toxins were present alone or in a mixture. Comparison of the combined effect with that of each individual compound revealed that after 8 h of exposure, the impact of Caco-2 cells exposed to 3 μM Cd combined with 10 μM DON was always stronger than that of 3 μM Cd alone ($p < 0.001$) but was similar to that of DON alone. The same effects were observed for 10 μM Cd combined with DON (10 or 30 μM) ($p < 0.001$). Moreover, at 12 h of exposure, 10 μM Cd combined with DON (10 or 30 μM) also had a stronger impact than DON alone ($p > 0.05$). Conversely, 10 μM of Cd combined with 3 μM of DON led to a greater decrease in the TEER than DON alone ($p < 0.001$). These results were also observed when 30 μM Cd was combined with 10 μM DON ($p < 0.001$ from 8 h to 12 h and $p < 0.05$ from 24 h), except for 36 h. At 4 h, 24 h and 36 h, the effect of the combination of DON and Cd 30 μM was stronger than the effect observed with DON or 30 μM Cd alone ($p < 0.01$ at 4 h, 24 h and 36 h). These data show that, as far as the TEER is concerned, in most cases, the combination of DON and Cd had a similar effect to that of the individual contaminants.

Paracellular permeability to 4-kDa FITC-dextran was assessed on cell monolayers exposed to mixtures of DON and Cd for 48 h (Fig. 5). The results showed that, except for the combination 3 μM DON and 10 μM Cd, combinations of low doses of DON and Cd (1 to 3 μM) did not cause any significant changes in paracellular permeability. In this case, the mixture had a stronger effect than the contaminant alone (DON, $p < 0.001$; Cd, $p < 0.05$). Moderate to high doses (10 to 30 μM) resulted in a marked increase in permeability to 4-kDa FITC-dextran. For example, the combination of DON and Cd at 10/10, 30/30, 10/30 and 30/10 μM increased the passage of dextran 46-, 82-, 76- and 81-fold, respectively, compared to untreated cells. Notably, 10 μM DON combined with 10 μM Cd resulted in a greater increase in paracellular permeability than 10 μM of Cd alone ($p < 0.001$). DON (10 μM) combined with 30 μM Cd had a stronger effect than 10 μM DON alone ($p < 0.001$). Similarly, 30 μM DON combined with 10 μM Cd led to a greater increase in paracellular permeability than 10 μM Cd alone ($p < 0.001$). These data show that the combination of DON and Cd has

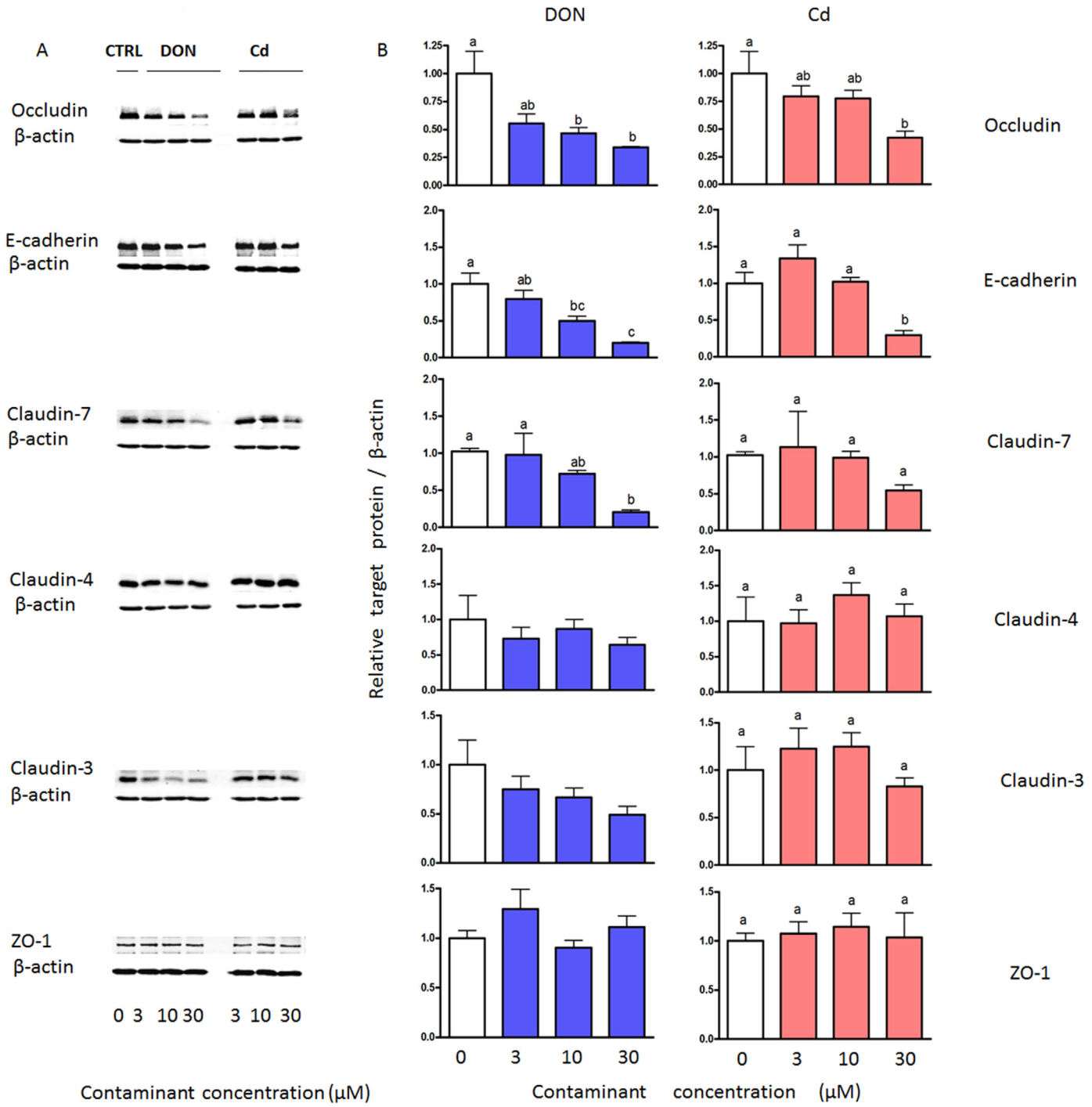

Fig. 2. Effects of DON and Cd on the expression of junctional proteins in Caco-2 cells. Differentiated Caco-2 cells were exposed to different concentrations of DON and Cd for 24 h. (A) After extraction, proteins were analyzed by immunoblotting for claudin-3, 4, 7, occludin, E-cadherin and ZO-1.
(B) Protein expression was analyzed by densitometry and normalized to that of β-actin. Data are presented as means ± SEM of 3 independent experiments. Means with different letters differ ($p < 0.05$). Results were analyzed by one-way ANOVA with Bonferroni's multiple comparison test.

a similar effect on the intestinal permeability to that of the individual contaminant.

3.5. Combined effects of DON and Cd on junctional protein abundance

To investigate the combined effect of DON and Cd on the intestinal barrier function in more detail, the abundance of two junctional proteins, occludin and E-cadherin, was analyzed. Fig. 6 is a heatmap of the individual and combined effects of DON and Cd. The levels of the two proteins decreased continuously with increasing concentrations of combined compounds. The combination of DON and Cd at a rate of 3 μM led to a similar decrease in occludin and E-cadherin as 3 μM of DON or 3 μM of Cd alone. Only 10 μM Cd combined with 10 μM DON led to a bigger decrease in occludin and E-cadherin abundance than 10 μM of Cd alone ($p < 0.001$). When the cells were exposed to a combination of 30 μM DON and 30 μM Cd, the levels of occludin and E-cadherin were lower than the level observed in cells only exposed to DON or Cd alone (DON, $p < 0.001$; Cd, $p < 0.05$). The data show that except for very high concentrations, the effect of a combination of DON and Cd on the abundance of occludin and E-cadherin was similar to the

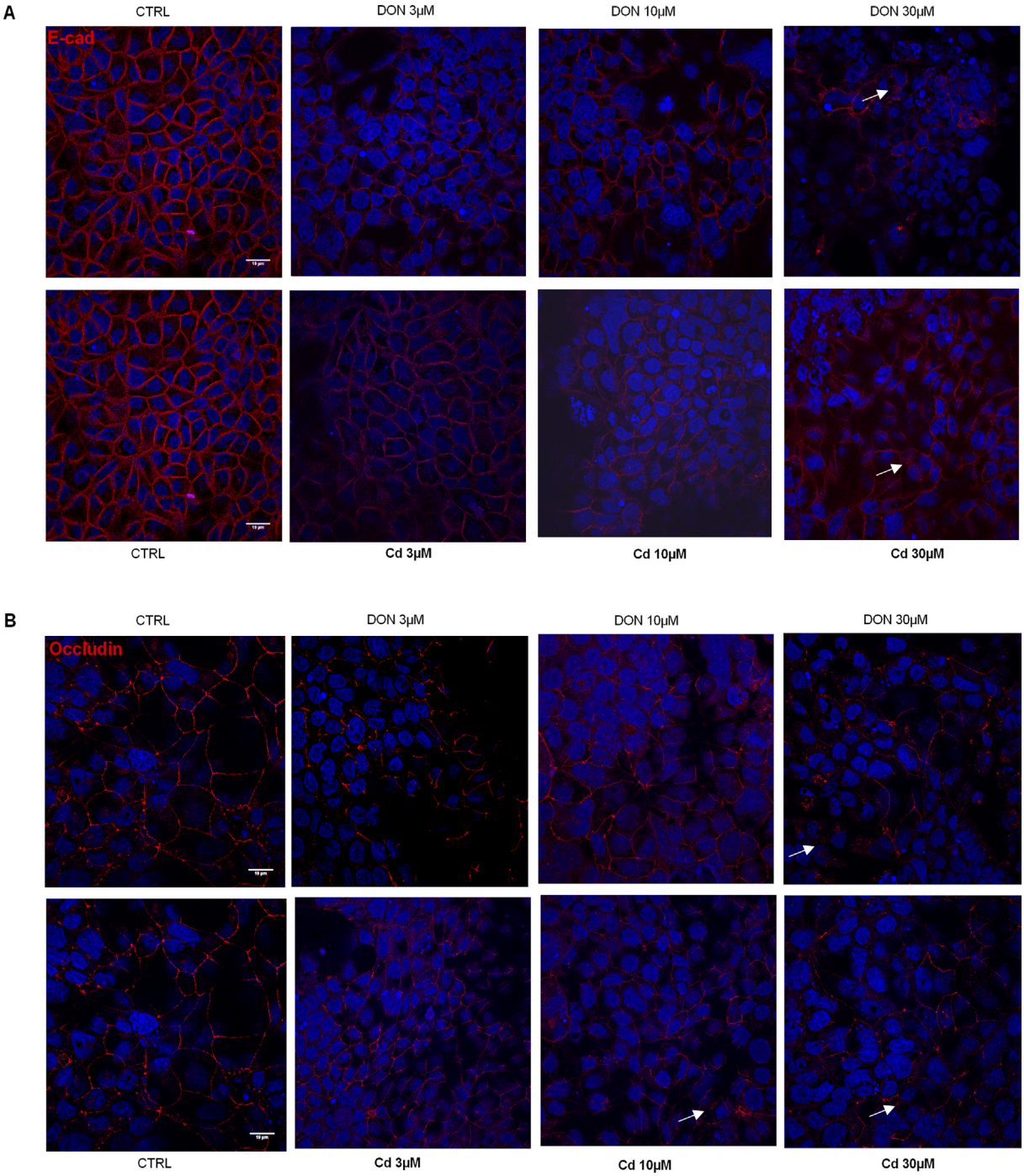

Fig. 3. Effect of DON and Cd on E-cadherin and occludin distribution in differentiated Caco-2 cells.
Differentiated Caco-2 cells were exposed to different concentrations of DON or Cd for 24 h. E-cadherin (A) and occludin (B) distribution (white arrow) was analyzed after immunostaining with specific antibody.

effect of the individual compounds.

3.6. In vivo effects of DON and Cd alone or in combination on the histomorphometry of the jejunum

Given the impact of DON and Cd on intestinal barrier function on Caco-2 cells, experiments were also performed on animals. Rats were exposed to DON-contaminated feed (8.2 mg/kg), Cd-contaminated water (5 mg/l) or both. The individual and combined effects of these contaminants were assessed in the intestine after four weeks of exposure.

The animals' body weight gain was not affected in any of the conditions (Fig. 7). Histomorphometrical changes were analyzed in the jejunum of rats fed DON and Cd alone or in combination. These results showed moderate but significant lesions compared to the intestine of control rats. The main histological changes were atrophy and fusion of the villi. Interstitial edema and enterocyte apical flattening were also observed (Fig. 8A, B, C, D). Lesional scores were about 4-fold higher in rats exposed to DON, Cd or both than that in control rats (Fig. 8E). These results show that intestinal damage caused by the combination of DON and Cd were as severe as the damage caused by each contaminant individually.

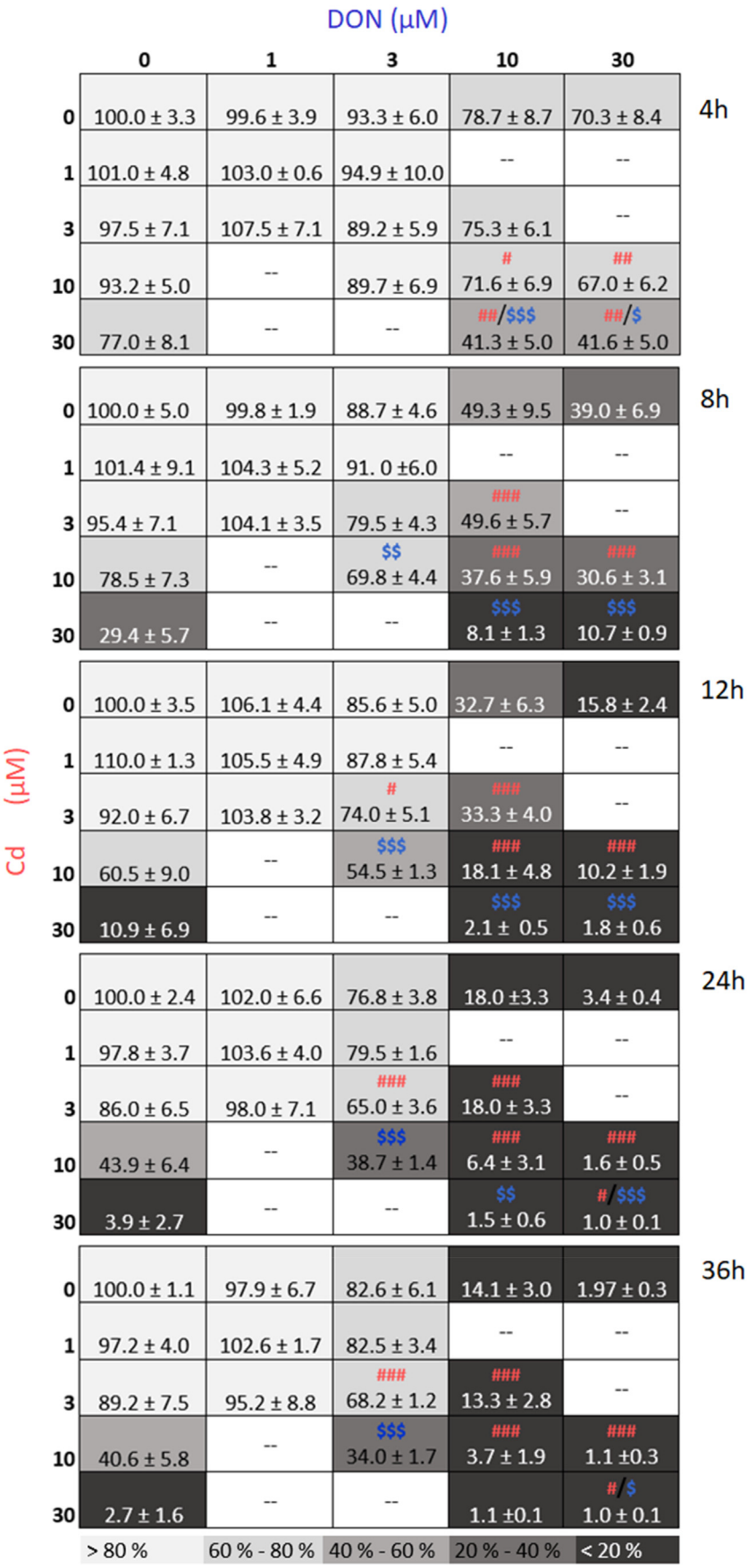

Fig. 4. Heatmap showing the effects of single and binary combinations of DON and Cd on the TEER of Caco-2 monolayers after 4 h, 8 h, 12 h, 24 h and 36 h of exposure. At each time-point, the TEER of control untreated cells was considered to be 100%. Results are expressed as the mean of four independent experiments ± SEM (#, combined effects *vs* effects of Cd alone, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$. $, combined effects *vs* effects of DON alone, $p < 0.05$; $$, $p < 0.01$; $$$, $p < 0.001$). Data were analyzed by non-parametric one-way ANOVA with Bonferroni's multiple comparison test. The intensity of the color reflects the strength of the effect on the TEER.

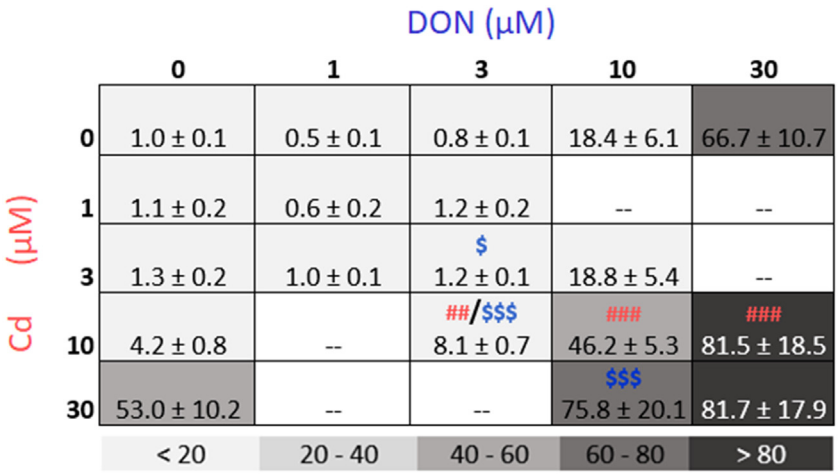

Fig. 5. Heatmap showing the effects of single compound and binary combinations of DON and Cd on the paracellular permeability of Caco-2 monolayers after 48 h of exposure. After 1 h of incubation with FITC-Dextran, the intensity of fluorescent was measured in the basal compartment. Results are expressed as fold increase in fluorescent intensity relative to controls. Results are expressed as the mean of four independent experiments ± SEM. (#, combined effects *versus* effects of Cd alone, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$. $, combined effects *VS* effects of DON alone, $p < 0.05$; $$, $p < 0.01$; $$$, $p < 0.001$). The depth of color reflects the strength of the effect on permeability. Data were analyzed by non-parametric one-way ANOVA with Bonferroni's multiple comparison test.

The *in vivo* effects of the mycotoxin and the heavy metal on intestinal cell proliferation were also assessed by measuring villus height and crypt depth. A significant reduction in crypt depth was observed in the jejunum of rats exposed to the contaminants compared with control animals (Fig. 8F). The reduction in crypt depth in animals exposed to both DON and Cd was similar to the reduction observed in animals exposed to Cd alone but slightly greater than the reduction observed in the animals only exposed to DON (−16%, $p < 0.01$). Villus heights did not differ significantly whatever the group of animals considered (Fig. 8G).

Taken together, these results indicate that ingestion of a combination of DON and Cd induces similar or slightly bigger lesions and has greater histomorphological effects on the intestine than ingestion of the individual contaminants.

3.7. *In vivo effects of DON and Cd alone or in combination on the abundance of E-cadherin and occludin in the jejunum*

As DON and Cd significantly reduced the amount of E-cadherin and occludin in Caco-2 cells, their abundance was also assessed in the jejunum of rats exposed to the same contaminants. Immunohistochemical staining revealed a significant reduction in E-cadherin in all exposed animals (Fig. 9). In control rats, strong homogeneous immunostaining was observed at the intercellular borders of epithelial cells (Fig. 9A). The intensity of E-cadherin staining was significantly reduced in rats exposed to either DON or Cd (Fig. 9B, C). Weak heterogeneous staining was also observed in rats exposed to the mixture (Fig. 9D). The

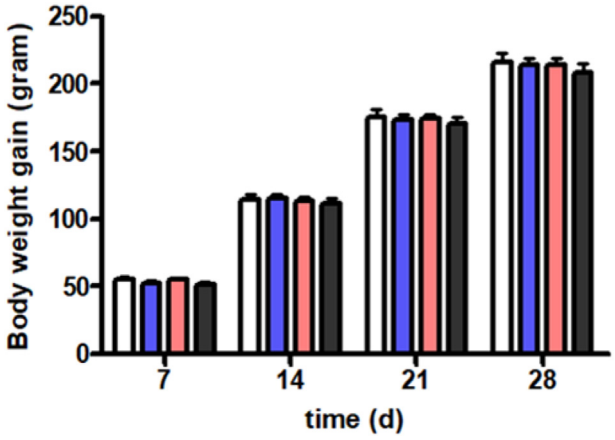

Fig. 7. Individual and combined effects of DON and Cd on body weight gain. Male Wistar rats were divided into four groups: control (white column), exposed to 10 mg/kg DON-contaminated feed (blue column), exposed to 5 mg/l Cd-contaminated feed (red column) or exposed to both DON and Cd (black column). Values are mean ± SEM ($n = 8$ animals). Data were analyzed by non-parametric one-way ANOVA.

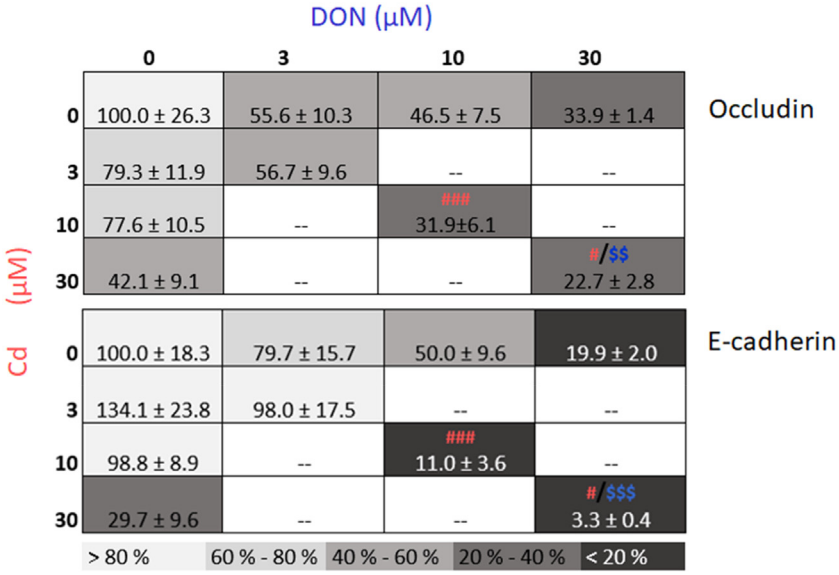

Fig. 6. Heatmap showing the effects of individual compounds and binary combinations of DON and Cd on the expression of junctional proteins after 24 h of exposure. Results are expressed as means of three independent experiments ± SEM. (# combined effects *versus* effects of Cd alone, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$. $, combined effects *versus* effects of DON alone, $p < 0.05$; $$, $p < 0.01$; $$$, $p < 0.001$). The intensity of the color reflects the extent of the decrease in the junctional proteins. Data were analyzed by non-parametric one-way ANOVA with Bonferroni's multiple comparison test.

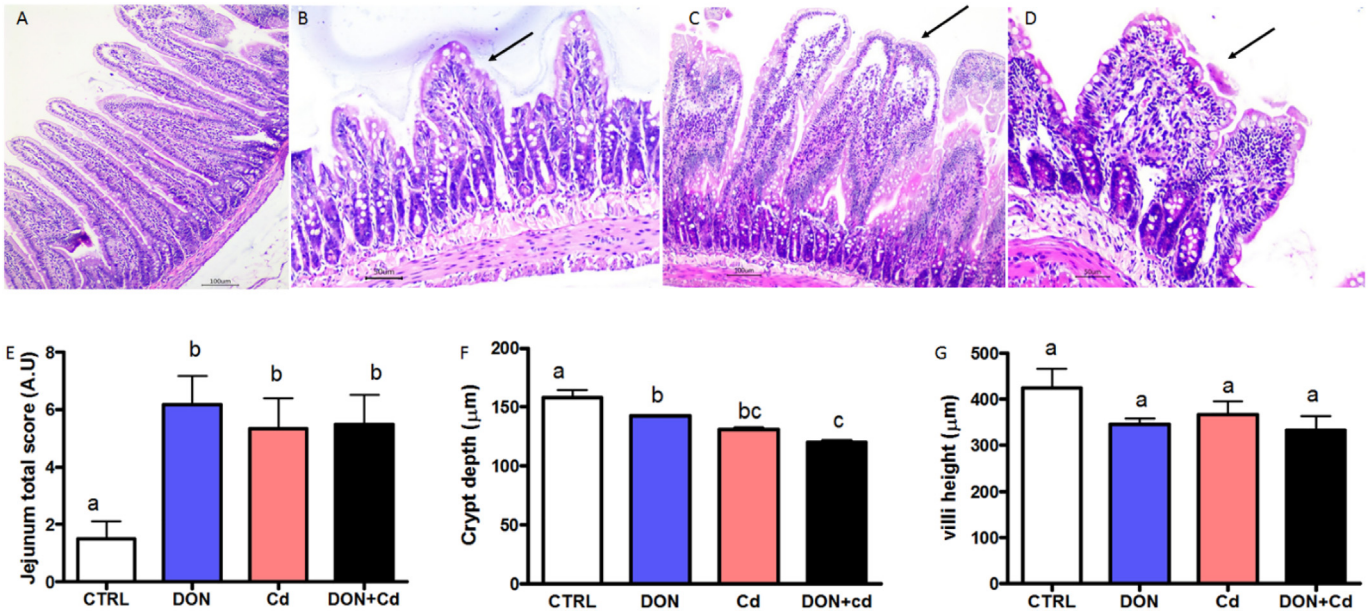

Fig. 8. Individual and combined effects of DON and Cd on jejunum histology. Histology of the jejunum after hematoxylin–eosin staining: control rat (A), DON treated rat (B, villus atrophy and fusion, arrow; 20×), Cd treated rat (C, moderate interstitial edema and enterocyte flattening, arrow; 10×) and DON + Cd treated rat (D, villus atrophy and fusion, arrow; 20×). Lesional score (E); crypt depth (F) and villus height (G). Values are means ± SEM (n = 8). Mean values with different letters differ ($p < 0.05$). Data were analyzed by non-parametric one-way ANOVA with Bonferroni's multiple comparison test.

abundance of E-cadherin decreased by 57%, 49% and 71% in animals treated with DON, Cd, and DON + Cd respectively (Fig. 9E).

For occludin, strong homogeneous immunostaining at the cell membrane was observed in control rats as well as in rats exposed to Cd (Fig. 10A, C). By contrast, weak heterogeneous staining was observed in the jejunum of animals exposed to DON and to the combination of DON and Cd, (Fig. 10B, D) compared to that in control rats, the abundance of occludin decreased by 84% and 62% ($p < 0.001$), respectively (Fig. 10E).

These results show that exposure to either DON or Cd reduces the expression of the E-cadherin and occludin at the cell membrane. Exposure to the mixture led to the same reduction in junctional protein as ingestion of DON alone.

4. Discussion

The combined toxicity of pollutants is hard to predict based on the toxic effect of a single compound (Alassane-Kpembi et al., 2017b; More et al., 2019). In the last few years, the number of studies of the combined toxicity of pollutants has increased but knowledge of the impact on animal and human health of exposure to mixtures of pollutants is nevertheless still very poor (Silins and Högberg, 2011). The main objective of the present study was to investigate the effects of a mycotoxin, DON, and a heavy metal, Cadmium, alone and in combination, on the intestinal epithelial barrier function. Two experimental approaches were used: *in vitro* exposure of human intestinal epithelial cells (Caco-2) to DON and Cd alone or in combination, and the *in vivo* dietary exposure of rats to these contaminants. The range of concentrations tested *in vitro* (1 to 30 μM) was chosen to screen a large panel of plausible exposure and adverse effects. In human, assuming that DON consumption in one meal is diluted in 1 l of gastrointestinal fluid and is totally bioaccessible (Sergent et al., 2006). The doses used in this study correspond to 0.296, 0.89, 2.96 and 8.89 μg/ml, which lay in the range of the plausible intestinal concentrations of DON (0.25–10 μg/ml) (De

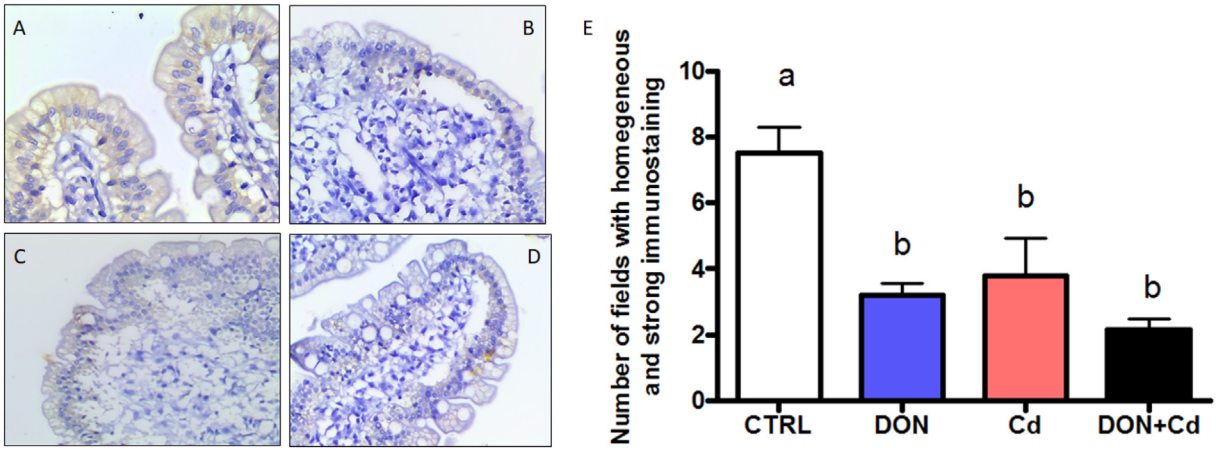

Fig. 9. Individual and combined effects of DON and Cd on E-cadherin expression in jejunum. (A, 60×) Jejunum of rats in the control group showed strong homogeneous staining on E-cadherin; less intense immunostaining on E-cadherin occurred in the jejunum of rats in the groups treated with DON (B, 40×), Cd (C, 40×) and DON + Cd (D, 40×). (E) The number of fields with strong homogeneous immunostaining. Values are mean ± SEM (n = 8). Means with a different letter differ ($p < 0.05$). Data were analyzed by non-parametric one-way ANOVA with Bonferroni's multiple comparison test.

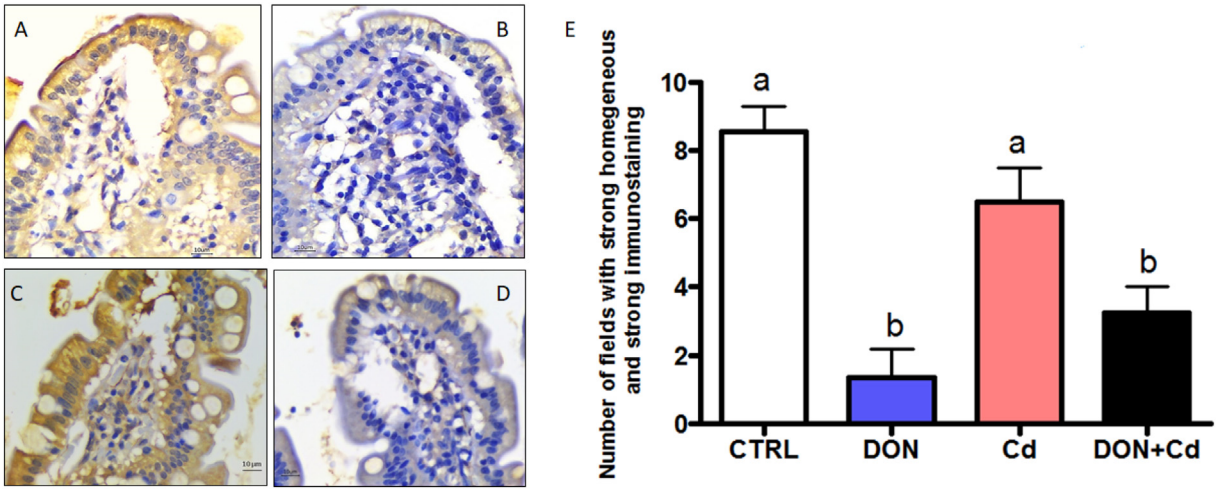

Fig. 10. Individual and combined effect of DON and Cd on occludin expression in jejunum. (A, 60×) The jejunum of rats in the control group showed strong homogeneous staining on occludin; less intense immunostaining on occludin occurred in the jejunum of rats in the groups treated with DON (B, 40×), Cd (C, 40×) and the combination of DON and Cd (D, 40×). (E) The number of fields with strong homogeneous immunostaining. Values are mean ± SEM (n = 8). Means with a different letter differ ($p < 0.05$). Data were analyzed by non-parametric one-way ANOVA with Bonferroni's multiple comparison test.

Walle et al., 2008). Furthermore, as previous report indicates that the molar ratio of mean exposure to DON and Cd in food in Europe is 1:1 (Le et al., 2018), the doses chosen for Cd were in the same range of doses as DON. The recommendation for DON in feed intended for farm animals is 5 mg/kg feed (European Union, 2006). In our experiment, the concentration of DON in feed was 8.2 mg/kg. Indeed, the concentrations of 2–10 mg/kg feed of DON are the most frequently used in toxicological studies of farm and experimental animal models and demonstrate adverse effects in specific target organs (intestine, liver, kidney) (Bracarense et al., 2012; Pinton et al., 2009, 2008). The concentration of 5 mg/l CdCl2 in drinking water is an environmentally realistic low dose (Benoff et al., 2008; Brzóska et al., 2003). It led only to modification of the phenotype of innate immune cells in the mesenteric lymph nodes in a rat model (Ninkov et al., 2015). Our proposal was to investigate the potential increase of the adverse effects when cadmium was in combination with another toxic compound. We took into account that the molar ratio of mean exposure of European population to DON and Cd in food is estimated to be 1:1 (Le et al., 2018). Considering the daily ingestion of food and water by the rat and the content of DON in food, the concentration of 5 mg of Cd/l of drinking was in accordance to reach this realistic ratio.

To evaluate the effects of DON and Cd alone or in combination on the intestine *in vivo*, rats were exposed to the contaminants for four weeks. The body weight gain of animals was not affected in any of the conditions tested. However, alterations in the histology and the morphology of the intestine were observed after exposure to DON and Cd alone or in combination. A significant decrease was observed in crypt depth in the jejunum of rats exposed to DON, Cd and DON + Cd. The effect of DON on crypt depth has already been reported in pigs (Gerez et al., 2015) and might be due to the DON-induced ribotoxic stress that compromised protein synthesis and triggered apoptosis (Pestka, 2010; Pierron et al., 2016a; Pinton et al., 2010). In piglets exposed to DON, a decrease in the number of proliferating cells in the intestinal crypt in response to DON has been already observed (Bracarense et al., 2012; Gerez et al., 2015). A study on broiler chickens showed that Cd ingestion caused histological alterations such as a decrease in leaf-like villi and a reduction in crypt depth (Teshfam et al., 2006). Moreover, inhibition of proliferation and induction of apoptosis has been observed in human renal mesangial cells and subventricular neural stem cells exposed to Cd. These effects were mediated by activation of the JNK or p-38 pathway (Wang et al., 2017). It is thus tempting to hypothesize that Cd inhibits the proliferation of intestinal epithelial cells *via* the same MAPK-dependent mechanism. By contrast, villus height was not affected irrespective of the contaminant to which the rats were exposed. This suggests proliferating cells are more sensitive to DON and/or Cd than differentiated ones, as already described *in vitro* for DON (Bony et al., 2006; Pierron et al., 2016a).

The histological and morphological alterations induced by the two contaminants tested here suggest that the intestinal barrier function may also be impaired. A dose-dependent reduction in the TEER and an increase in paracellular permeability were observed in DON and in Cd treated cells. The reduction in the barrier function caused by DON is mediated by MAPKs/ERK activation, as inhibition of this signaling pathway restored TEER in porcine and human intestinal epithelial cells (Du et al., 2018; Pinton et al., 2010; Springler et al., 2016). A MAPKs/ERK activation in the differentiated Caco-2 cells treated with Cd has also been reported (Mantha and Jumarie, 2010), and may be involved in the disruption of the intestinal barrier by Cd.

The intestinal barrier function is closely linked to the junctional protein network and several studies have shown that DON or Cd alter some junctional proteins including occludin or E-cadherin (De Walle et al., 2010; Pinton et al., 2009; Templeton and Liu, 2013). However, the effects of combinations of DON and Cd on the junctional proteins have not previously been investigated. Here, we observed a decrease in E-cadherin expression in the three groups of exposed rats (DON, CD and DON + Cd) while the decrease in occludin was only observed in animals exposed to DON (with or without Cd). By contrast, in Caco-2 cells, the amount of E-cadherin and occludin decreased after exposure to the two contaminants (DON and Cd) alone or in combination. This was associated with redistribution of the junctional proteins from the membrane to the cytoplasm. A similar decrease in the expression of E-cadherin has already been observed in pigs fed a DON-contaminated diet (Bracarense et al., 2012; Pierron et al., 2018). As far as Cd is concerned, several studies have shown that, in intestinal cells, intercellular junctions, mainly E-cadherin and actin cytoskeleton, are sensitive to this contaminant (Duizer et al., 1999; Rusanov et al., 2015). In MCF-7 cells, Cd has been shown to disturb calcium homeostasis and cause a depletion of Ca^{2+} (Zhou et al., 2015); this induces the activation of Cdc42, a member of Rho family GTPases, and leads to the ubiquitination and degradation of E-cadherin *via* Src-mediated pathway (Shen et al., 2008). In a human airway tissue model, Cd induced redistribution of occludin by tyrosine phosphorylation through c-Src and PKC activation, resulting in TJ disruption (Cao et al., 2015). Whether these modes of action of Cd also occur in the intestine remains to be determined. Disruption of junctional adhesion associated with increased paracellular permeability can facilitate the passage of pathogens and/or

harmful substances into the body. Therefore, animals or humans exposed to these toxins are likely to be more sensitive to intestinal pathogens. In addition, increased intestinal permeability may also favor permeability to other contaminants (Payros et al., 2017).

Humans and animals are exposed to multi-component mixtures in the environment and food, whereas chemical risk assessment generally concerns the toxicity of single compounds (More et al., 2019). The effects of combinations of several food contaminants are very poorly documented. In the case of DON and Cd, apart from a previous study by our team (Le et al., 2018), no study has investigated their combined effect. The *in vitro* and *in vivo* data presented in this paper indicate that, whatever the parameter analyzed (TEER, paracellular permeability or the abundance of junction protein), the toxicity resulting from exposure to a combination of DON and Cd is similar to that caused by each individual contaminant. It is well known that chemical compounds have multiple modes of action, a range of targets and different degrees of affinity for these targets, and, conversely, that each mode of action can be used by a variety of chemical compounds (Thrupp et al., 2018). As described above, the mechanisms of action of DON and Cd share some pathways, one of which is the MAPkinase pathway. Indeed, DON and Cd induce the activation of ERK1/2 and p38 respectively, leading to disruption in tight junctions (Pinton et al., 2010; Rather et al., 2017). We hypothesize that, when combined, DON and Cd play competitive roles in the pathway, which would explain why the effect of a mixture of DON and Cd is no greater than the effect of each individual contaminant.

From the point of view of human health, our results suggest that existing regulation for DON and Cd are sufficient to protect consumers exposed to a mixture of these contaminants. Indeed, their combined effects on the intestine were identical to the effect of the most toxic compound alone, whatever the parameter analyzed. These results point to a less than additive effect. More data are needed to determine if the results concerning the effect of DON and Cd on the intestine can be extended to other organs and/or to other mixtures.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105082.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

We are grateful to the staff of the team EZOP for animal care. We thank the UPAE (*Unité de Preparation des Aliments Expérimentaux*, UE03000, Jouy-en-Josas, France) for the preparation of the diets.

Funding

The work was supported, in part, by the French National Research Agency (project CaDON 15-CE21-0001-02) and by the CAPES Cofecub SV 947 19.

Su Luo was supported by the China Scholarship Council.

References

Alassane-Kpembi, I., Puel, O., Pinton, P., Cossalter, A.-M., Chou, T.-C., Oswald, I.P., 2017a. Co-exposure to low doses of the food contaminants deoxynivalenol and nivalenol has a synergistic inflammatory effect on intestinal explants. Arch. Toxicol. 91, 2677–2687. https://doi.org/10.1007/s00204-016-1902-9.

Alassane-Kpembi, I., Schatzmayr, G., Taranu, I., Marin, D., Puel, O., Oswald, I.P., 2017b. Mycotoxins co-contamination: methodological aspects and biological relevance of combined toxicity studies. Crit. Rev. Food Sci. Nutr. 57, 3489–3507. https://doi.org/10.1080/10408398.2016.1140632.

Arnich, N., Sirot, V., Rivière, G., Jean, J., Noël, L., Guérin, T., Leblanc, J.-C., 2012. Dietary exposure to trace elements and health risk assessment in the 2nd French Total Diet Study. Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc. 50, 2432–2449. https://doi.org/10.1016/j.fct.2012.04.016.

ATSDR, 2012. Toxicological Profile for Cadmium. Available on line.. https://www.atsdr.cdc.gov/toxprofiles/tp5.pdf, Accessed date: 7 December 2018.

Benoff, S., Auborn, K., Marmar, J.L., Hurley, I.R., 2008. Link between low-dose environmentally relevant cadmium exposures and asthenozoospermia in a rat model. Fertil. Steril. 89, e73–e79. https://doi.org/10.1016/j.fertnstert.2007.12.035.

Bishak, Y.K., Payahoo, L., Osatdrahimi, A., Nourazarian, A., 2015. Mechanisms of cadmium carcinogenicity in the gastrointestinal tract. Asian Pac. J. Cancer Prev. 16, 9–21.

Bony, S., Carcelen, M., Olivier, L., Devaux, A., 2006. Genotoxicity assessment of deoxynivalenol in the Caco-2 cell line model using the Comet assay. Toxicol. Lett. 166, 67–76. https://doi.org/10.1016/j.toxlet.2006.04.010.

Bracarense, A.-P.F.L., Lucioli, J., Grenier, B., Drociunas Pacheco, G., Moll, W.-D., Schatzmayr, G., Oswald, I.P., 2012. Chronic ingestion of deoxynivalenol and fumonisin, alone or in interaction, induces morphological and immunological changes in the intestine of piglets. Br. J. Nutr. 107, 1776–1786. https://doi.org/10.1017/S0007114511004946.

Bracarense, A.P.F.L., Basso, K.M., Da Silva, E.O., Payros, D., Oswald, I.P., 2017. Deoxynivalenol in the liver and lymphoid organs of rats: effects of dose and duration on immunohistological changes. World Mycotoxin J. 10, 89–96. https://doi.org/10.3920/WMJ2016.2094.

Breton, J., Daniel, C., Vignal, C., Body-Malapel, M., Garat, A., Plé, C., Foligné, B., 2016. Does oral exposure to cadmium and lead mediate susceptibility to colitis? The dark-and-bright sides of heavy metals in gut ecology. Sci. Rep. 6, 19200. https://doi.org/10.1038/srep19200.

Brzóska, M.M., Kamiński, M., Supernak-Bobko, D., Zwierz, K., Moniuszko-Jakoniuk, J., 2003. Changes in the structure and function of the kidney of rats chronically exposed to cadmium. I. Biochemical and histopathological studies. Arch. Toxicol. 77, 344–352. https://doi.org/10.1007/s00204-003-0451-1.

Cao, X., Lin, H., Muskhelishvili, L., Latendresse, J., Richter, P., Heflich, R.H., 2015. Tight junction disruption by cadmium in an in vitro human airway tissue model. Respir. Res. 16, 30. https://doi.org/10.1186/s12931-015-0191-9.

Claus Henn, B., Coull, B.A., Wright, R.O., 2014. Chemical mixtures and children's health. Curr. Opin. Pediatr. 26, 223–229. https://doi.org/10.1097/MOP.0000000000000067.

De Walle, J.V., Romier, B., Larondelle, Y., Schneider, Y.-J., 2008. Influence of deoxynivalenol on NF-kappa B activation and IL-8 secretion in human intestinal Caco-2 cells. Toxicol. Lett. 177, 205–214. https://doi.org/10.1016/j.toxlet.2008.01.018.

De Walle, J. Van, Sergent, T., Piront, N., Toussaint, O., Schneider, Y.-J., Larondelle, Y., 2010. Deoxynivalenol affects in vitro intestinal epithelial cell barrier integrity through inhibition of protein synthesis. Toxicol. Appl. Pharmacol. 245, 291–298. https://doi.org/10.1016/j.taap.2010.03.012.

Du, Z., Kim, K.H., Kim, J., Moon, Y., 2018. Fungal deoxynivalenol-induced enterocyte distress is attenuated by adulterated adlay: in vitro evidences for mucoactive counteraction. Front. Immunol. 9, 186. https://doi.org/10.3389/fimmu.2018.00186.

Duizer, E., Gilde, A.J., Versantvoort, C.H., Groten, J.P., 1999. Effects of cadmium chloride on the paracellular barrier function of intestinal epithelial cell lines. Toxicol. Appl. Pharmacol. 155, 117–126. https://doi.org/10.1006/taap.1998.8589.

EFSA, 2009. Cadmium in food - scientific opinion of the panel on contaminants in the food chain. EFSA J. 7. https://doi.org/10.2903/j.efsa.2009.980.

EFSA, 2017. Risks to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed. EFSA J. 15. https://doi.org/10.2903/j.efsa.2017.4718.

European Union, 2006. Commission Recommendation of 17 August 2006 on the Presence of Deoxynivalenol, Zearalenone, Ochratoxin A, T-2 and HT-2 and Fumonisins in Products Intended for Animal Feeding [WWW Document]. URL. https://eur-lex.europa.eu/eli/reco/2006/576/oj, Accessed date: 7 March 2019.

Filipič, M., Fatur, T., Vudrag, M., 2006. Molecular mechanisms of cadmium induced mutagenicity. Hum. Exp. Toxicol. 25, 67–77. https://doi.org/10.1191/0960327106ht590oa.

Ganguly, K., Levänen, B., Palmberg, L., Åkesson, A., Lindén, A., 2018. Cadmium in tobacco smokers: a neglected link to lung disease? Eur. Respir. Rev. 27, 170122. https://doi.org/10.1183/16000617.0122-2017.

Gerez, J.R., Pinton, P., Callu, P., Grosjean, F., Oswald, I.P., Bracarense, A.P.F.L., 2015. Deoxynivalenol alone or in combination with nivalenol and zearalenone induce systemic histological changes in pigs. Exp. Toxicol. Pathol. 67, 89–98. https://doi.org/10.1016/j.etp.2014.10.001.

Kim, H.S., Kim, Y.J., Seo, Y.R., 2015. An overview of carcinogenic heavy metal: molecular toxicity mechanism and prevention. J. Cancer Prev. 20, 232–240. https://doi.org/10.15430/JCP.2015.20.4.232.

Le, T.-H., Alassane-Kpembi, I., Oswald, I.P., Pinton, P., 2018. Analysis of the interactions between environmental and food contaminants, cadmium and deoxynivalenol, in different target organs. Sci. Total Environ. 622–623, 841–848. https://doi.org/10.1016/j.scitotenv.2017.12.014.

Liu, L., Tao, R., Huang, J., He, X., Qu, L., Jin, Y., Zhang, S., Fu, Z., 2015. Hepatic oxidative stress and inflammatory responses with cadmium exposure in male mice. Environ. Toxicol. Pharmacol. 39, 229–236. https://doi.org/10.1016/j.etap.2014.11.029.

Lucioli, J., Pinton, P., Callu, P., Laffitte, J., Grosjean, F., Kolf-Clauw, M., Oswald, I.P., Bracarense, A.P.F.R.L., 2013. The food contaminant deoxynivalenol activates the mitogen activated protein kinases in the intestine: interest of ex vivo models as an alternative to in vivo experiments. Toxicon 66, 31–36. https://doi.org/10.1016/j.toxicon.2013.01.024.

Lukowicz, C., Ellero-Simatos, S., Régnier, M., Polizzi, A., Lasserre, F., Montagner, A., Lippi, Y., Jamin, E.L., Martin, J.-F., Naylies, C., Canlet, C., Debrauwer, L., Bertrand-Michel, J., Al Saati, T., Théodorou, V., Loiseau, N., Mselli-Lakhal, L., Guillou, H., Gamet-Payrastre, L., 2018. Metabolic effects of a chronic dietary exposure to a low-dose pesticide cocktail in mice: sexual dimorphism and role of the constitutive

androstane receptor. Environ. Health Perspect. 126, 067007. https://doi.org/10.1289/EHP2877.
Mantha, M., Jumarie, C., 2010. Cadmium-induced hormetic effect in differentiated Caco-2 cells: ERK and p38 activation without cell proliferation stimulation. J. Cell. Physiol. 224, 250–261. https://doi.org/10.1002/jcp.22128.
Maresca, M., 2013. From the gut to the brain: journey and pathophysiological effects of the food-associated trichothecene mycotoxin deoxynivalenol. Toxins Basel 5, 784–820. https://doi.org/10.3390/toxins5040784.
Miller, M.F., Goodson, W.H., Manjili, M.H., Kleinstreuer, N., Bisson, W.H., Lowe, L., 2017. Low-dose mixture hypothesis of carcinogenesis workshop: scientific underpinnings and research recommendations. Environ. Health Perspect. 125, 163–169. https://doi.org/10.1289/EHP411.
More, S.J., Hardy, A., Bampidis, V., Benford, D., Hougaard Bennekou, S., Bragard, C., Boesten, J., Halldorsson, T.I., Hernández-Jerez, A.F., Jeger, M.J., Knutsen, H.K., Koutsoumanis, K.P., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Schlatter, J.R., Silano, V., Nielsen, S.S., Schrenk, D., Solecki, R., Turck, D., Younes, M., Benfenati, E., Castle, L., Cedergreen, N., Laskowski, R., Leblanc, J.C., Kortenkamp, A., Ragas, A., Posthuma, L., Svendsen, C., Testai, E., Dujardin, B., Kass, G.E., Manini, P., Zare Jeddi, M., Dorne, J.C., Hogstrand, C., Hogstrand, C., 2019. Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA J. 17. https://doi.org/10.2903/j.efsa.2019.5634.
Ninkov, M., Popov Aleksandrov, A., Demenesku, J., Mirkov, I., Mileusnic, D., Petrovic, A., Grigorov, I., Zolotarevski, L., Tolinacki, M., Kataranovski, D., Brceski, I., Kataranovski, M., 2015. Toxicity of oral cadmium intake: impact on gut immunity. Toxicol. Lett. 237, 89–99. https://doi.org/10.1016/j.toxlet.2015.06.002.
Payros, D., Alassane-Kpembi, I., Pierron, A., Loiseau, N., Pinton, P., Oswald, I.P., 2016. Toxicology of deoxynivalenol and its acetylated and modified forms. Arch. Toxicol. 90, 2931–2957. https://doi.org/10.1007/s00204-016-1826-4.
Payros, D., Dobrindt, U., Martin, P., Secher, T., Bracarense, A.P.F.L., Boury, M., Laffitte, J., Pinton, P., Oswald, E., Oswald, I.P., 2017. The food contaminant deoxynivalenol exacerbates the genotoxicity of gut microbiota. mBio 8. https://doi.org/10.1128/mBio.00007-17.
Pestka, J.J., 2010. Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. Arch. Toxicol. 84, 663–679. https://doi.org/10.1007/s00204-010-0579-8.
Pierron, A., Mimoun, S., Murate, L.S., Loiseau, N., Lippi, Y., Bracarense, A.P., Liaubet, L., Schatzmayr, G., Berthiller, F., Moll, W.D., Oswald, I.P., 2016a. Intestinal toxicity of the masked mycotoxin deoxynivalenol-3-β-D-glucoside. Arch. Toxicol. 90, 2037–2046. https://doi.org/10.1007/s00204-015-1592-8.
Pierron, A., Mimoun, S., Murate, L.S., Loiseau, N., Lippi, Y., Bracarense, A.P., Schatzmayr, G., He, J.W., Zhou, T., Moll, W.D., Oswald, I.P., 2016b. Microbial biotransformation of DON: molecular basis for reduced toxicity. Sci. Rep. 6, 29105. https://doi.org/10.1038/srep29105.
Pierron, A., Bracarense, A.P.F.L., Cossalter, A.-M., Laffitte, J., Schwartz-Zimmermann, H.E., Schatzmayr, G., Pinton, P., Moll, W.-D., Oswald, I.P., 2018. Deepoxy-deoxynivalenol retains some immune-modulatory properties of the parent molecule deoxynivalenol in piglets. Arch. Toxicol. 92, 3381–3389. https://doi.org/10.1007/s00204-018-2293-x.
Pinton, P., Oswald, I.P., 2014. Effect of deoxynivalenol and other type B trichothecenes on the intestine: a review. Toxins Basel 6, 1615–1643. https://doi.org/10.3390/toxins6051615.
Pinton, P., Accensi, F., Beauchamp, E., Cossalter, A.-M., Callu, P., Grosjean, F., Oswald, I.P., 2008. Ingestion of deoxynivalenol (DON) contaminated feed alters the pig vaccinal immune responses. Toxicol. Lett. 177, 215–222. https://doi.org/10.1016/j.toxlet.2008.01.015.
Pinton, P., Nougayrède, J.-P., Del Rio, J.-C., Moreno, C., Marin, D.E., Ferrier, L., Bracarense, A.-P., Kolf-Clauw, M., Oswald, I.P., 2009. The food contaminant deoxynivalenol, decreases intestinal barrier permeability and reduces claudin expression. Toxicol. Appl. Pharmacol. 237, 41–48. https://doi.org/10.1016/j.taap.2009.03.003.
Pinton, P., Braicu, C., Nougayrede, J.-P., Laffitte, J., Taranu, I., Oswald, I.P., 2010. Deoxynivalenol impairs porcine intestinal barrier function and decreases the protein expression of Claudin-4 through a mitogen-activated protein kinase-dependent mechanism. J. Nutr. 140, 1956–1962. https://doi.org/10.3945/jn.110.123919.
Rather, I.A., Koh, W.Y., Paek, W.K., Lim, J., 2017. The sources of chemical contaminants in food and their health implications. Front. Pharmacol. 8, 830. https://doi.org/10.3389/fphar.2017.00830.
Rusanov, A.L., Smirnova, A.V., Poromov, A.A., Fomicheva, K.A., Luzgina, N.G., Majouga, A.G., 2015. Effects of cadmium chloride on the functional state of human intestinal cells. Toxicol. Vitro Int. J. Publ. Assoc. BIBRA 29, 1006–1011. https://doi.org/10.1016/j.tiv.2015.03.018.
Sergent, T., Parys, M., Garsou, S., Pussemier, L., Schneider, Y.-J., Larondelle, Y., 2006. Deoxynivalenol transport across human intestinal Caco-2 cells and its effects on cellular metabolism at realistic intestinal concentrations. Toxicol. Lett. 164, 167–176. https://doi.org/10.1016/j.toxlet.2005.12.006.
Shen, Y., Hirsch, D.S., Sasiela, C.A., Wu, W.J., 2008. Cdc42 regulates E-cadherin ubiquitination and degradation through an epidermal growth factor receptor to Src-mediated pathway. J. Biol. Chem. 283, 5127–5137. https://doi.org/10.1074/jbc.M703300200.
Silins, I., Högberg, J., 2011. Combined toxic exposures and human health: biomarkers of exposure and effect. Int. J. Environ. Res. Public Health 8, 629–647. https://doi.org/10.3390/ijerph8030629.
Sirot, V., Fremy, J.-M., Leblanc, J.-C., 2013. Dietary exposure to mycotoxins and health risk assessment in the second French total diet study. Food Chem. Toxicol. 52, 1–11. https://doi.org/10.1016/j.fct.2012.10.036.
Springler, A., Hessenberger, S., Schatzmayr, G., Mayer, E., 2016. Early activation of MAPK p44/42 is partially involved in DON-induced disruption of the intestinal barrier function and tight junction network. Toxins 8, 264. https://doi.org/10.3390/toxins8090264.
Suzuki, T., 2013. Regulation of intestinal epithelial permeability by tight junctions. Cell. Mol. Life Sci. 70, 631–659. https://doi.org/10.1007/s00018-012-1070-x.
Templeton, D.M., Liu, Y., 2013. Effects of cadmium on the actin cytoskeleton in renal mesangial cells. Can. J. Physiol. Pharmacol. 91, 1–7. https://doi.org/10.1139/cjpp-2012-0229.
Terciolo, C., Dapoigny, M., Andre, F., 2019. Beneficial effects of Saccharomyces boulardii CNCM I-745 on clinical disorders associated with intestinal barrier disruption. Clin. Exp. Gastroenterol. 12, 67–82. https://doi.org/10.2147/CEG.S181590.
Teshfam, M., Gharagozlou, M.J., Salaramoli, J., Hassanpour, H., 2006. Morphological alterations of the small intestine mucosa following oral administration of cadmium in broiler chickens. J. Appl. Anim. Res. 29, 65–68. https://doi.org/10.1080/09712119.2006.9706573.
Thrupp, T.J., Runnalls, T.J., Scholze, M., Kugathas, S., Kortenkamp, A., Sumpter, J.P., 2018. The consequences of exposure to mixtures of chemicals: something from 'nothing' and 'a lot from a little' when fish are exposed to steroid hormones. Sci. Total Environ. 619–620, 1482–1492. https://doi.org/10.1016/J.SCITOTENV.2017.11.081.
Tinkov, A.A., Gritsenko, V.A., Skalnaya, M.G., Cherkasov, S.V., Aaseth, J., Skalny, A.V., 2018. Gut as a target for cadmium toxicity. Environ. Pollut. 235, 429–434. https://doi.org/10.1016/j.envpol.2017.12.114.
Turner, P.C., Rothwell, J.A., White, K.L.M., Gong, Y., Cade, J.E., Wild, C.P., 2008. Urinary deoxynivalenol is correlated with cereal intake in individuals from the United Kingdom. Environ. Health Perspect. 116, 21–25. https://doi.org/10.1289/ehp.10663.
Wang, H., Engstrom, A.K., Xia, Z., 2017. Cadmium impairs the survival and proliferation of cultured adult subventricular neural stem cells through activation of the JNK and p38 MAP kinases. Toxicology 380, 30–37. https://doi.org/10.1016/j.tox.2017.01.013.
Zhang, S., Jin, Y., Zeng, Z., Liu, Z., Fu, Z., 2015. Subchronic exposure of mice to cadmium perturbs their hepatic energy metabolism and gut microbiome. Chem. Res. Toxicol. 28, 2000–2009. https://doi.org/10.1021/acs.chemrestox.5b00237.
Zhou, X., Hao, W., Shi, H., Hou, Y., Xu, Q., 2015. Calcium homeostasis disruption - a bridge connecting cadmium-induced apoptosis, autophagy and tumorigenesis. Oncol. Res. Treat. 38, 311–315. https://doi.org/10.1159/000431032.