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# **Analysis of the interactions between environmental and food contaminants, Cadmium and Deoxynivalenol, in different target organs**

*Thanh-Huong Le<sup>1</sup>, Imourana Alassane -Kpembé<sup>1,2</sup>, Isabelle P. Oswald<sup>1\*</sup>, Philippe Pinton<sup>1</sup>*

<sup>1</sup> Toxalim (Research Centre in Food Toxicology), Université de Toulouse, INRA, ENVT, INP-Purpan, UPS, 180 chemin de Tournefeuille, 31027 Toulouse cedex 3, France <sup>2</sup> Hôpital d'Instruction des Armées, Camp Guézo 01BP517 Cotonou, Bénin

\* Correspondence: isabelle.oswald@inra.fr Tel.: +33-582-06-6366

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## **Abstract**

Cadmium (Cd), a common and widespread toxic heavy metal, and mycotoxins such as deoxynivalenol (DON) are frequent contaminants of the food supply. Most of the data on their toxicity concern their effects when present alone. However, consumers can be exposed to a cocktail of DON and Cd. To improve the understanding of their combined toxicity, the effects of DON and Cd alone or in combination were investigated in different human cell lines from the kidney (HEK-293), intestine (Caco-2), blood (HL-60) and liver (HepG2). Cytotoxicity was assessed through ATP measurement and types of interactions determined by the Isobologram-Combination index method. HEK-293 cells were exposed to increasing doses of DON, Cd and their combination at different ratios (DON/Cd of 2/1; 1/1; 1/2 and 1/8). Regardless of the ratio, the type of interaction observed in HEK-293 cells ranged from moderate antagonism to nearly additive with increasing cytotoxicity. In Caco-2 cells, the interactions ranged from nearly additive to antagonism whatever the ratio. At ratio 1/1, in HL-60 and HepG2 cells, interactions ranged from synergy to antagonism depending on the cytotoxicity level. Using human cells lines, this study indicates that the consequences of combined exposure to environmental and food contaminants are specific to the target organ. Further studies are needed to confirm these data *in vivo*.

## Introduction

Exposure to chemical contaminants is an important concern worldwide. Most of the toxicological data concern the effects of chemical contaminants when present alone; however, humans are usually exposed to multiple toxic compounds, which might impact health via food or the environment (Heys et al., 2016). In recent years, combined toxicity studies have been carried out to assess the effects of mixtures of food contaminants such as mycotoxins (Alassane-Kpembi et al., 2017a; Ruiz et al., 2011), endocrine disrupters and carcinogens (Le Magueresse-Battistoni et al., 2017) or a mixture of air pollutants (Coyle et al., 2006).

Deoxynivalenol (DON) is the most prevalent mycotoxin naturally present in grains and other food commodities. It is produced by toxigenic *Fusarium* species that are common pathogens of cereal crops under a temperate climate (Kokkonen et al., 2010). Humans are mainly exposed to DON through cereal-derived food. A European survey showed that 61%, 89% and 47% of wheat, maize, and barley samples, respectively, are contaminated with DON (Schothorst and Van Egmond, 2004). In Europe, a tolerable daily intake (TDI) of 1  $\mu\text{g}/\text{kg}$  body weight per day for DON was established; however, young children can be exposed to DON at levels close to or even higher than the TDI (EFSA, 2013). Intoxication symptoms of DON may include reduced weight gain, a decrease in food consumption, neuro-endocrine changes and alteration of intestinal and immune functions (Payros et al., 2016). Following acute exposure, vomiting and bloody diarrhea have been observed (Pestka, 2010). There is no evidence for carcinogenic properties of DON and it was classified into group 3 by the International Agency for Research on Cancer (IARC) (IARC, 1993; Payros et al., 2017).

Cadmium (Cd) is one of the common and widespread toxic heavy metal found naturally in the Earth's crust and is usually present in the environment as an inorganic salt. Its presence in the environment is a consequence of both natural and anthropic processes. Natural sources of Cd include volcanic activity, weathering consumption of rocks, sea aerosols, forest fires and mobilization of Cd adsorbed in soils and landfills (ATSDR, 2012). The anthropic origin of Cd derives from batteries, pigments, plastic stabilizers, pesticides and fertilizers, and photovoltaic devices, as well as from rubber processing, the galvanization process, fossil combustion and waste incineration (ATSDR, 2012). Cd can be released into soil, water and air, and it bio-accumulates in the organic matter by entering the food chain. Grain and cereal products, fish and offal are the major contributors of Cd exposure in food (IARC, 2012; Kabata-Pendias and Pendias, 1984). A high amount of Cd also accumulates in tobacco leaves, and tobacco smokers are exposed by inhalation. Acute intoxication of Cd may lead to liver, lung, and testis damage, while chronic intoxication may result in pulmonary damage, disturbance of metabolism, dysregulation of blood pressure, obstruction of kidney function, and structural damage of bones (Godt et al., 2006). Cd and Cd compounds have been classified as carcinogenic to humans (group 1) by IARC (IARC, 2012).

The toxicity of a mixture cannot always be predicted based on the toxicities of its individual compounds (Alassane-Kpembi et al., 2017a; Sarigiannis and Hansen, 2012). Exposure to toxic mixtures could result in antagonistic, additive or synergistic effects. Various studies have analyzed the interactions between mycotoxins and reported synergistic, additive or antagonistic effects. For example, the combination of DON, nivalenol and their acetyl derivatives resulted in synergistic in vitro cytotoxicity at low doses, while additive and antagonistic interactions were observed at higher doses (Alassane-Kpembi et al., 2013, 2015). Ex vivo, the interaction of DON and NIV in the pro-inflammatory response was synergistic at low doses (Alassane-Kpembi et al., 2017b). In vivo exposure to mixtures of DON and other mycotoxins showed different types of interactions, the most frequent being less than additive (Grenier and Oswald, 2011). A few studies have also investigated the interactions between mycotoxins and other toxins. For example, synergy was observed between DON and

colibactin, a genotoxin produced by some strains of *Escherichia coli* (Payros et al., 2017). Similarly, a synergistic interaction was shown between ochratoxin A, a mycotoxin produced by *Penicillium* and *Aspergillus* and aristolochic acid, a nephrotoxic and genotoxic plant product (Stiborova et al., 2015). As far as Cd is concerned, some in vivo and in vitro studies have focused on the interactions between Cd and pesticides (Xu et al., 2017) or between Cd and other pollutants (Muthusamy et al., 2016).

Humans can be simultaneously exposed to DON and Cd through inhalation or water and food consumption. However, to the best of our knowledge, the combined toxicity of Cd and mycotoxins has never been addressed. The aim of the present study was to assess the combined effects of DON and Cd on different target organs using cell lines from the kidney, intestine, blood and liver.

## Materials and Methods

### 2.1 Cell culture and reagents

Human embryonic kidney 293 cells (HEK-293) and human liver hepatocellular carcinoma (HepG2) cells were maintained in EMEM (Sigma, St Quentin Fallavier, France) supplemented with 10% fetal calf serum (FCS) (Eurobio, Courtaboeuf, France), 1% penicillin/streptomycin (Eurobio) and 0.5% L-glutamine (Eurobio). Human colon carcinoma Caco-2 cells were maintained in DMEM-glutamax (Gibco, Life Technologies, Courtaboeuf, France) supplemented with 10% FCS, 1% amino acid non-essential (Sigma) and 0.5% gentamycin (Eurobio). Human promyelocytic leukemia cells (HL-60) were maintained in IMDM (Sigma) supplemented with 20% FCS, 1% penicillin/streptomycin.

All cell lines were incubated in a humidified atmosphere at 37°C and 5% CO<sub>2</sub> in culture flasks. Adherent cells (Caco-2, HEK-293 and HepG2) were passaged by trypsinization (0.5% trypsin in 0.5 mM EDTA) when they were sub confluent. HL-60 were passaged by dilution when the concentration reached 1 × 10<sup>6</sup> cells/ml.

DON and Cd chloride were purchased from Sigma and were dissolved to 5 mM in water. Stock solutions were stored at -20°C before dilution in complete cell culture medium.

**Table 1.** Experimental design for the different DON/Cd mixtures

Cell type	DON/Cd Ratio	DON and Cd concentration (μM)						
<b>HEK-293</b>	2/1	0/0	0.5/0.25	1.0/0.5	2.0/1.0	4.0/2.0		
	1/1	0/0	0.25/0.25	0.5/0.5	1.0/1.0	2.0/2.0		
	1/2	0/0	0.25/0.5	0.5/1.0	1.0/2.0	2.0/4.0		
	1/8	0/0	0.1/0.75	0.19/1.5	0.38/3.0	0.75/6.0		
<b>Caco-2</b>	2/1	0/0	0.6/0.3	1.25/0.6	2.5/1.25	5/2.5	10/5	20/10
	1/1	0/0	0.3/0.3	0.6/0.6	1.25/1.25	2.5/2.5	5/5	10/10
	1/2	0/0	0.3/0.6	0.6/1.25	1.25/2.5	2.5/5	5/10	10/20
	1/8	0/0	0.19/1.5	0.38/3	0.75/6	1.5/12	3/24	6/48
<b>HL-60</b>	1/1	0/0	0.75/0.75	1.5/1.5	3.0/3.0	6.0/6.0	12/12	24/24
<b>HepG2</b>	1/1	0/0	0.75/0.75	1.5/1.5	3.0/3.0	6.0/6.0		

Adherent cells were seeded in 96-well plates and incubated 24 h in complete media before treatment. HEK-293 and Caco-2 cells were seeded with a density of 5000 cells/well. Because of their slower growth HepG2, with a density of 15000 cells/well (Cervera et al., 2011; Lee et al., 1999; Sambuy et al., 2005). HL-60 suspension cells were treated immediately after being seeded at the density of 25000 cells/well. The stock solutions of DON and Cd chloride were diluted in serum-free medium and all cell types were treated with serial dilutions of each compound or their mixture for 48 h.

The CellTiter-glo® luminescent cell viability assay (Promega, Charbonnières-les-Bains, France) was used as previously described (Tannous et al., 2017). This test is based on the measure of ATP, a widely accepted marker of viable cells (Riss et al., 2013). The luminescent signal produced by the luciferase reaction, reflecting the presence of metabolically active cells, was read using a multiplate reader (TECAN, Lyon, France). The results were obtained by calculating the percentage of viable cells (ratio luminescence in treated samples / luminescence in non-treated samples).

### 2.3 Data analysis

The dose-effect relationships of the individual and combined mycotoxins were biometrically modeled using the Median-Effect Equation of the Mass Action Law as already described (Alassane-Kpembé et al., 2013, 2015).

$$f_a/f_u = (D/D_m)^m$$

D	dose of the toxin
$f_a$	fraction affected by D
$f_u$	Fraction unaffected (i.e., $f_u = 1 - f_a$ )
$D_m$	median-effect dose (e.g., EC50)
$m$	coefficient signifying the shape of the dose-effect relationship ( $m = 1$ , $m >$ and $m < 1$ indicate hyperbolic, sigmoidal, and flat sigmoidal dose-effect curves, respectively)

We verified that the linear regression correlation coefficients of the median effect plots were greater than 0.95 (Chou, 2006). Toxin interactions were analyzed by the isobologram and combination-index (CI) methods. For all toxic combinations, CI values were generated over a range of fractions of cell viability affected ( $f_a$ ) from 0.05–0.97 (5–95% toxicity).  $CI = 0.9$ – $1.1$ ,  $CI < 0.9$ , and  $CI > 1.1$  indicate additive, synergistic, and antagonistic effects, respectively (Chou, 2006).

### 2.4 Statistical analysis

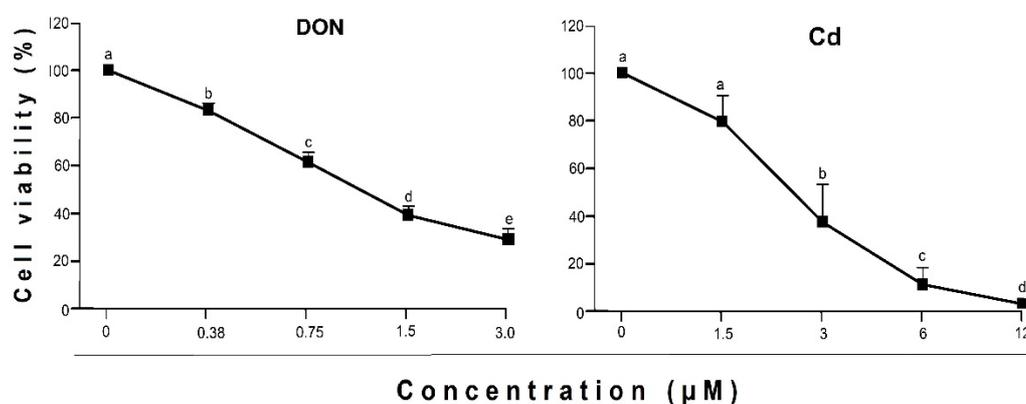
The reported values were expressed as means  $\pm$  standard deviation (SD) of at least three independent experiments, each with triplicate wells per dose level. Statistical analyses were performed using GraphPad Software (La Jolla, CA, USA). Significant differences between groups were analyzed using the Bonferroni's multiple comparison test. The level of  $p < 0.05$  was considered statistically significant. The dose-effect relationship analysis for individual compound cytotoxicities, combination index (CI) and their 95% confidence intervals, the dose reduction index calculation, isobologram plots and  $f_a$ -CI plots for combined effects were all performed using Compusyn software version 3.0.1 (ComboSyn Inc., Paramus, NJ, USA).

## Results

### 3.1. Individual cytotoxicities of DON and Cd in HEK-293 cells

The individual cytotoxicities of DON and Cd were first evaluated using the renal HEK-293 cell line. In humans, kidney is the target organ for Cd where it induces tubular dysfunction and stone formation (Järup and Åkesson, 2009; Johri et al., 2010). Similarly, kidney damage is a common feature of exposure to DON (Pestka, 2003). DON increases serum creatinine and blood urea nitrogen, reflecting damage in kidney glomerular filtration. In addition, DON induced kidney cell apoptosis and oxidative stress (Liang et al., 2015) and exhibited dysregulation of IgA production and IgA nephropathy (Pestka, 2003).

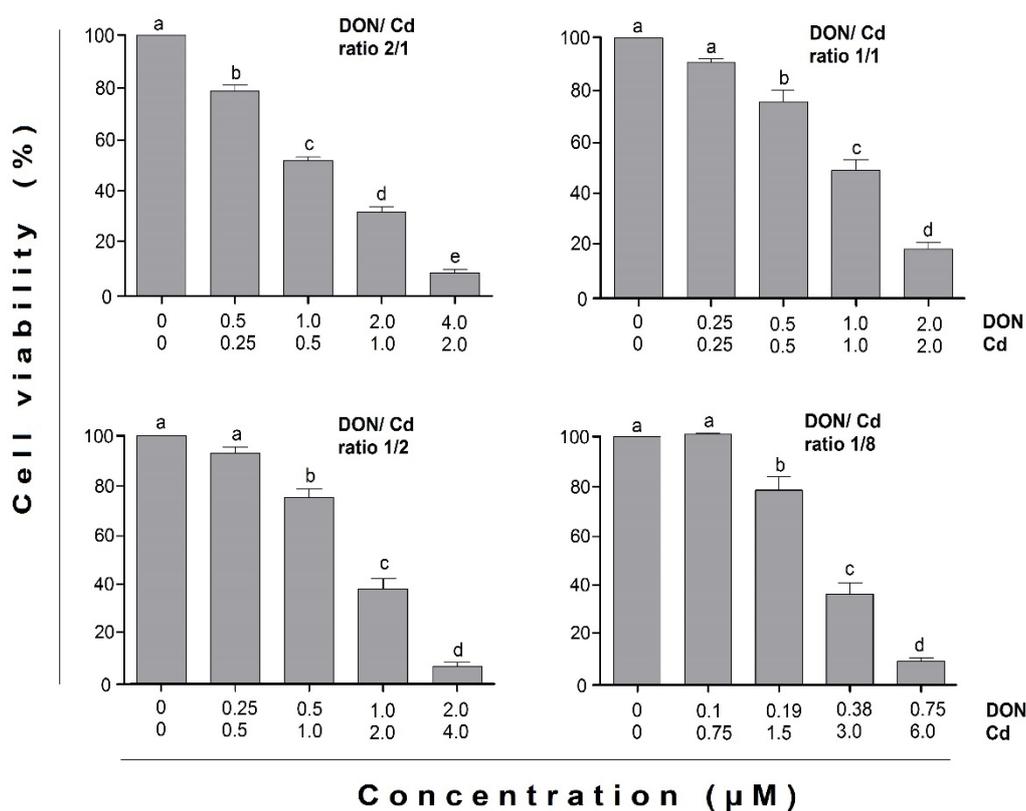
Cells were exposed for 48 h to graded concentrations up to 3  $\mu\text{M}$  for DON or up to 12  $\mu\text{M}$  Cd, and cytotoxicity was assessed through ATP measurement. Both contaminants showed a dose-dependent toxicity toward HEK-293 cells, and this cell line appeared to be more sensitive to DON than to Cd as indicated by the EC<sub>50</sub> (1.24  $\mu\text{M}$  for DON versus 2.53  $\mu\text{M}$  for Cd) (Fig. 1).



**Figure 1.** Toxicities of DON and Cd on HEK-293 cells. Kidney cells were exposed for 48 h to serial dilutions of both contaminants, and cytotoxicity was assessed through ATP measurement. Data values are expressed as the means  $\pm$  SD of 3 experiments. Means not sharing a common superscript are significantly different ( $p < 0.05$ ).

### 3.2. Combined cytotoxicity of DON and Cd on HEK-293 cells

The next step was to determine the cytotoxicity of the mixtures of DON and Cd and to characterize the type of interaction (additivity, synergy or antagonism) between these contaminants. Several combination ratios were tested. These ratios ranged from a high concentration of DON and low concentration of Cd (ratio DON/Cd of 2/1) to a high concentration of Cd and low concentration of DON (ratio DON/Cd of 1/8). Cytotoxicity of the mixtures was assessed through ATP measurement. As shown in Figure 2, the four DON/Cd tested ratios (2/1, 1/1, 1/2 and 1/8) induced a dose-dependent toxicity in kidney cells. The highest concentrations decreased the cell viability by almost 90% when compared to untreated control. The parameters  $D_m$ ,  $m$ , and  $r$  for the dose-effect relationships of DON and Cd and their mixtures are provided in Table 2. All the dose-effect experiments demonstrated good linear correlation coefficients ( $r > 0.95$ ), which are required for the interaction analysis using the Median-Effect principle (Chou, 2006).



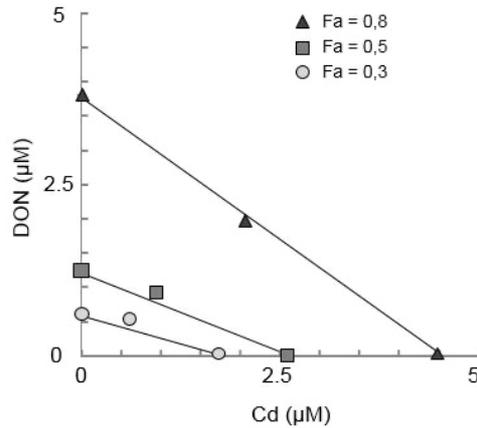
**Figure 2.** Combined toxicity of DON and Cd on proliferating HEK-293 cells. Kidney cells were exposed for 48 h to 4 different ratios of DON and Cd (2/1, 1/1, 1/2 and 1/8). Data values were expressed as the means  $\pm$  SD of 3 independent experiments. Means not sharing a common superscript are significantly different ( $p < 0.05$ ).

**Table 2.** Dose-effect relationship parameters for viability inhibition by DON, Cd, and their combination in HEK-293 cells

Dose-effect Parameters	Contaminants					
	DON	Cd	DON + Cd			
			Ratio 2/1	Ratio 1/1	Ratio 1/2	Ratio 1/8
$D_m$ ( $\mu\text{M}$ )	1.24	2.53	1.64	1.84	2.26	2.77
$m$	1.22	2.46	1.67	1.75	2.47	2.61
$r$	0.98	0.99	0.99	0.99	0.99	0.99

$D_m$ ,  $m$ , and  $r$  are the median-effect dose (e.g., the EC50), slope, and coefficient of linear correlation of the experimental data to the mass-action law, respectively

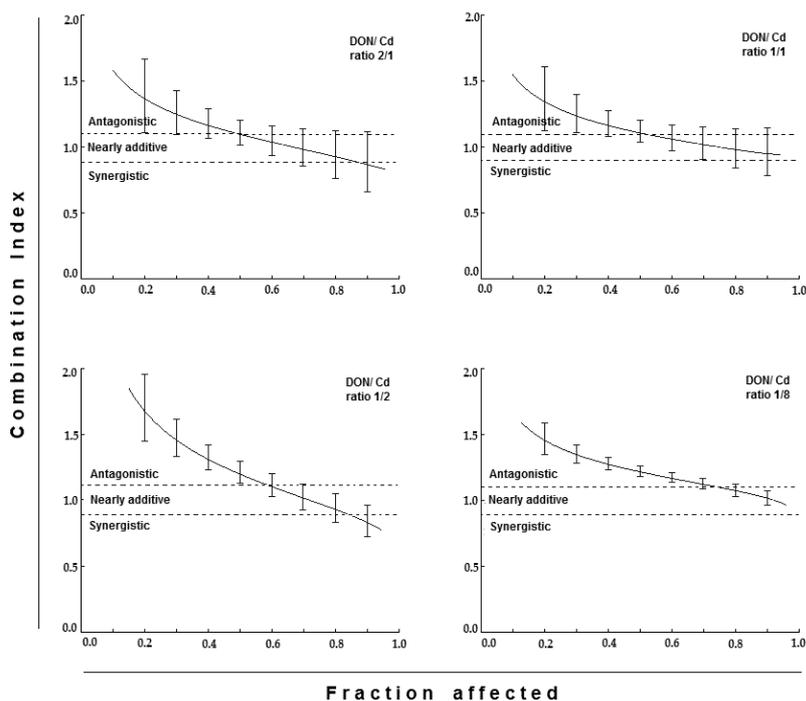
The types of interactions were analyzed by drawing an isobologram and calculating CI values. The isobologram for the DON/Cd combination at ratio 1/1 is presented in Figure 3 for three different cytotoxicity levels (30%, 50% and 80%).



**Figure 3.** Isobologram illustrating the iso-effective doses of DON and Cd and their combined cytotoxicity at concentrations eliciting 30% (filled circle), 50% (filled square) or 80% (filled triangle) toxicity. The points represent the mean concentrations obtained from 3 cytotoxicity dose-response experiments ( $n=3$ ) for DON, Cd and their combination (Compusyn software analysis). The linear correlation coefficients of the median-effect plots ( $r$  values) were higher than 0.95, indicating the goodness of fit of the mean experimental data with the median-effect equation (Chou 2006).

In this type of graph, the additive combined effect follows the diagonal line between the effective concentrations of each single contaminant. If the measured combined effect of two contaminants is above or below the diagonal line, it indicates an antagonistic or a synergistic effect of the combination, respectively. For the combination of DON and Cd (ratio 1/1), this isobologram indicates an additive effect or slight antagonism between the two contaminants at the three cytotoxicity levels presented.

The interaction between DON and Cd regarding the cytotoxicity toward HEK-293 cells was also analyzed using the CI calculation. Figure 4 presents the CI values calculated for a wide range of cytotoxicity levels (from 20% to 90%). As shown in this figure, the main type of interaction between those two contaminants ranged from moderate antagonism to nearly additive.



**Figure 4.** Combination index-fraction affected curves for the combination of DON and Cd in HEK-293 at 4 different ratios. CI values were calculated from the data obtained from 6 independent experiments. The vertical bars indicate 95% confidence intervals for CI values based on sequential deletion analysis. Horizontal dashed lines correspond to lower and upper limits of the additivity zone

### 3.3. Individual and combined cytotoxicities of DON and Cd on other cell types (Caco-2; HL-60 and HepG2 cells)

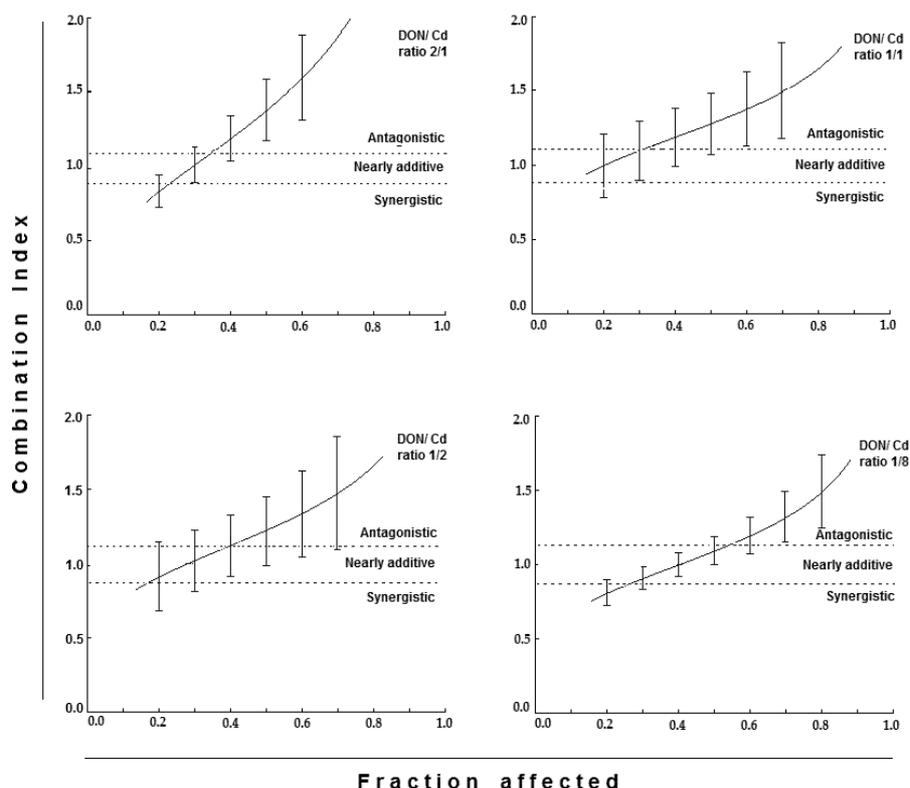
Next, we investigated the interaction between DON and Cd in human cell lines from the intestines (Caco-2), blood (HL-60) and liver (HepG2). First, the individual toxicities of DON and Cd were assessed (Table 3). As already observed for HEK-293, Caco-2 cells were more resistant to Cd ( $EC_{50} = 5.1 \mu M$ ) than to DON ( $EC_{50} = 2.88 \mu M$ ). A similar finding was observed for HL-60 cells, which were 93 times more resistant to Cd than to DON with an extremely high  $EC_{50}$  value ( $EC_{50} = 118.6 \mu M$  for Cd). By contrast, HepG2 cells showed higher sensitivity to Cd ( $EC_{50} = 1.64 \mu M$ ) than to DON ( $EC_{50} = 18.4 \mu M$ ).

**Table 3.** Dose-effect relationship parameters for viability inhibition by DON, Cd, and their combination in Caco-2, HL-60 and HepG2 cells

Dose-effect Parameters	Cell line								
	Caco-2			HL-60			HepG2		
	DON	Cd	DON+Cd	DON	Cd	DON+Cd	DON	Cd	DON+Cd
$D_m$ ( $\mu M$ )	2.88	5.10	4.19	1.27	118.6	4.19	18.4	1.64	2.92
$m$	1.01	1.36	0.95	1.67	7.33	0.79	0.37	1.98	3.34
$r$	0.96	0.99	0.98	0.99	0.99	0.99	0.97	0.99	0.99

$D_m$ ,  $m$ , and  $r$  are the median-effect dose (e.g., the  $EC_{50}$ ), slope, and coefficient of linear correlation of the experimental data to the mass-action law, respectively.

We next studied the interaction between DON and Cd in Caco-2, HL-60 and HepG2 cells. The results obtained in Caco-2 cells are presented Figure 5 and, as already observed for HEK-293, no effect of the ratio was observed on the nature of interaction between DON and Cd. Because we observed that, for HEK-293 and Caco-2 cells the nature of interactions between DON and Cd did not vary regardless of the ratio, the experiments for the two other cells types were performed at the DON/Cd ratio of 1/1.



**Figure 5.** Combination index-fraction affected curves for the combination of DON and Cd in Caco-2 at 4 different ratios. CI values were calculated from the data obtained from 3 independent experiments. The vertical bars indicate 95% confidence intervals for CI values based on sequential deletion analysis. Horizontal dashed lines correspond to lower and upper limits of the additivity zone.

Table 4 presents, for the different cell lines, the CI for DON and Cd at different cytotoxicity levels. As shown, the type of interaction between DON and Cd varied according to the cell type. In Caco-2 cells, for the fraction affected above 20%, the type of interaction between DON and Cd ranged from nearly additive to antagonism.

In the blood cell line, HL-60, for the lowest fractions affected (below 30%), synergy was observed between DON and Cd. With higher concentrations of DON and Cd (60 to 70% cytotoxicity), their interaction became antagonism and strong antagonism (80% cytotoxicity).

In the liver cell line, HepG2, synergy (moderate to normal) was observed between DON and Cd for the high cytotoxicity level (60 to 80%). For 40 and 50% cytotoxicity, the interaction between the two contaminants was slightly antagonistic to nearly additive. For the doses inducing low cytotoxicity levels (20 to 30%), antagonism (normal to strong) was observed.

**Table 4.** Nature of the interaction between DON and Cd in four different cell types (HEK-293, Caco-2, HL-60 and HepG2)

Fa	Cell type				
	HEK-293	Caco-2	HL-60	HepG2	
0.2	1.51 ± 0.04	0.98 ± 0.28	0.43 ± 0.04	3.80 ± 1.07	Strong antagonism
0.3	1.27 ± 0.11	1.13 ± 0.22	0.65 ± 0.04	1.73 ± 0.02	Antagonism
0.4	1.18 ± 0.08	1.28 ± 0.18	0.94 ± 0.03	1.17 ± 0.06	Moderate antagonism
0.5	1.12 ± 0.07	1.45 ± 0.15	1.33 ± 0.04	0.95 ± 0.04	Slight antagonism
0.6	1.07 ± 0.06	1.64 ± 0.19	1.87 ± 0.08	0.82 ± 0.05	Nearly additive
0.7	1.02 ± 0.06	1.90 ± 0.33	2.73 ± 0.17	0.72 ± 0.05	Moderate synergism
0.8	0.98 ± 0.04	2.30 ± 0.61	4.32 ± 0.39	0.64 ± 0.05	Synergism

HEK-293, Caco-2, HL-60 and HepG2 cells were exposed to graded concentrations of DON/Cd mixture (ratio 1/1). CI values were calculated for seven different level of cytotoxicity (Fa) using the Compusyn program. Data were expressed as the mean of the combination index ±SD calculated from 3 to 6 independent experiments. The color code characterizes the nature of the interaction.

## Discussion

Health can be compromised by agrochemicals, environmental contaminants and natural toxins frequently present as toxic mixtures in the environment and in foods (Clarke et al., 2015). Traditionally, the study of the toxic effects has focused on exposure to single compounds without estimation of the risk associated with the toxic action of mixtures. This is of importance because the toxicity of contaminants present together cannot always be predicted based on their individual toxicities (Alassane-Kpembé et al., 2017a; Sarigiannis and Hansen, 2012). In this context, the present study was designed as an *in vitro* assessment of the combined toxicity of a mycotoxin, DON, and a heavy metal, Cd, on different organs. Indeed, humans are exposed simultaneously to DON and/or Cd through the consumption of contaminated food, water or through inhalation.

The different DON/Cd ratio tested were chosen to encompass the plausible scenario of human exposure to these contaminants. They ranged from a high concentration of DON and low concentration of Cd (ratio DON/Cd of 2/1) to a high concentration of Cd and low concentration of DON (ratio DON/Cd of 1/8). The DON/Cd ratio 1/1 was based on the mean exposure to both contaminants in food (EFSA CONTAM Panel, 2017; EFSA, 2009). The DON/Cd ratio 1/2 corresponded to the toxicity ratio (EC50) of DON and Cd observed for HEK-293 and Caco-2 cell lines. The DON/Cd ratio 2/1, simulated an acute exposure to both contaminants taking into account the difference in absorption (50 to 80 % for DON versus 1 to 10% for Cd) and in excretion (rapid for DON and low for Cd). The last DON/Cd ratio 1/8 mimicked a chronic exposure to DON and Cd. It was based on carry-over factors observed in different organs from pigs experimentally exposed for 12 weeks to feed contaminated with either DON or Cd (Goyarts et al., 2007; Hoogenboom et al., 2015).

In the present study, we observed that DON and Cd alone or in combination were cytotoxic for kidney cells in a dose-dependent manner. However, as already described, Cd was two times less cytotoxic than DON (Dinu et al., 2011; Lawal and Ellis, 2010). Beside kidney

cells, interactions between DON and Cd were also investigated in intestinal cells (Caco-2 cells), which represent the first barrier after the ingestion of food contaminants, in blood cells (HL-60) as the contaminant circulate through this fluid into the body after absorption and in liver cells (HepG2) to mimic the effects observed in this detoxification organ. The dose-dependent toxicity of DON and Cd confirms previous studies performed in intestinal, liver and blood cells (Juan-García et al., 2016; Kondoh et al., 2002; Lawal and Ellis, 2010; Sergent et al., 2006).

The interactions between DON and Cd cells were analyzed using the Chou-Talalay method (Chou, 2006), which has already been used for toxin combinations, including combinations of mycotoxins (Alassane-Kpembé et al., 2013, 2015; Juan-García et al., 2016). This method allows a qualitative assessment of interactions via an isobolographic analysis and quantification of synergy or antagonism by calculating a combination index. Our analysis revealed that, in HEK-293 cells, the types of combined effects of DON and Cd are from antagonism to additivity and depends on the dose of contaminants but not on their ratio. Regarding the other cell types, at moderate cytotoxicity levels (fraction affected from 40 to 50%), interactions between DON and Cd were also mostly antagonistic. The antagonism observed for DON and Cd corresponds to an overall lower toxicity than would have been predicted for the combined effect. It might involve interfering actions with cellular transport processes (Yang and Shu, 2015) and could be explained by a higher affinity of the most toxic compound for certain common transporters as metallothioneins (Sobrova et al., 2012), which could result in the accumulation of the less toxic one. Finally, the variation in the magnitude and/or type of the interactions with the concentration may be explained by the graded levels of the saturation of efflux systems from low to high toxin concentrations. In addition, DON and Cd can activate the mitogen-activated protein kinase (MAPK) pathway via the induction of reactive oxygen species and free radicals (Alassane-Kpembé et al., 2017c; Chen et al., 2008; Luciola et al., 2013). They can also enhance the synthesis of metallothioneins, playing an important role in the detoxification of DON and Cd (Li et al., 2005; Sabolic et al., 2010). The demonstration of the involvement of these pathways deserves to be further investigated.

For the low and high cytotoxicity levels, differences appeared between the four-tested cell types. At low concentrations of DON and Cd (fraction affected below 40%), the interactions varied from synergy (HL-60)-slight antagonism to moderate synergy (Caco-2)-antagonism (HEK-293) to strong antagonism (HepG2). When the cytotoxicity increased (fraction affected above 50%), interactions between DON and Cd were reversed and varied from antagonism (HL-60) -moderate antagonism (Caco-2) -additivity (HEK-293) to synergy (HepG2). Among the four cell types, HL-60 and HepG2 demonstrated the more contrasted response depending on the dose of the mixture. That could be related to the particular resistance of HepG2 to DON and of HL-60 to Cd as already observed by others (Juan-García et al., 2016; Li et al., 2017).

When exposed to the same mixture, cell type-dependent and dose-specific interactions have already been observed. For example, when human intestinal Caco-2 cells are exposed to a mixture of NIV-fusarenon X (FX), synergistic interaction at low concentrations and additivity at higher concentrations are observed (Cervera et al., 2011). When exposed to the same mixture NIV-FX, additivity was observed in IPEC-1 (intestinal porcine epithelial cells) (Alassane-Kpembé et al., 2015) and in intestinal explants (Alassane-Kpembé et al., 2017b), whereas synergy was observed for human gastric epithelial (GES-1) cells (Yang et al., 2017). Conversely, for the mixture DON-FX, antagonism in IPEC-1 cells (Alassane-Kpembé et al., 2015) and synergy in GES-1 cells (Yang et al., 2017) were observed.

In conclusion, this study characterized the nature of the interactions between two contaminants DON and Cd, in different cell types. Overall, for the doses inducing low cytotoxic effects, the interactions ranged from nearly additive to slight antagonism. The

mechanism leading to these interactions was not established in this study. The hypothesis of competition between DON and Cd in common targets needs further investigation to be confirmed. Moreover, the nature of the interactions between DON and Cd should be investigated in whole animals to confirm these data.

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