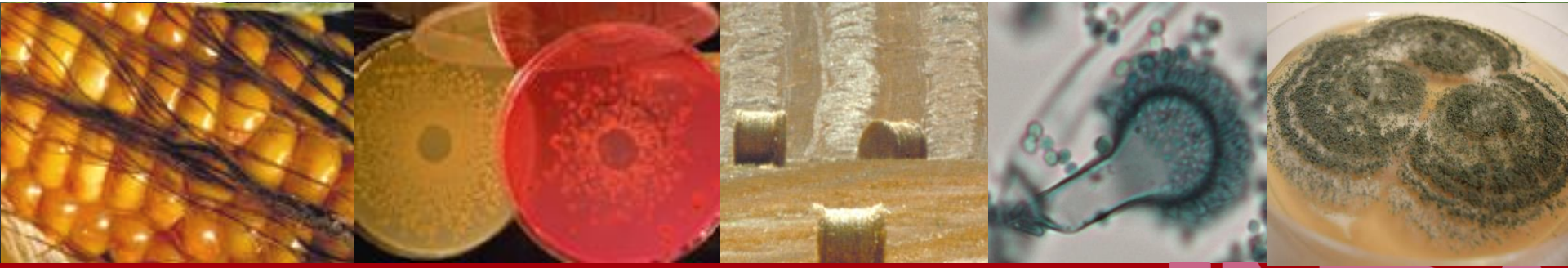
Multiexposition aux mycotoxines : analyse des interactions

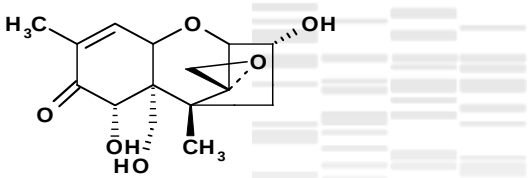
Isabelle P. OSWALD
Imourana ALASSANE-KPEMBI
Philippe PINTON

INRA - ToxAlim -Toulouse, FRANCE
Research Center in Food Toxicology
Institut National de la Recherche Agronomique



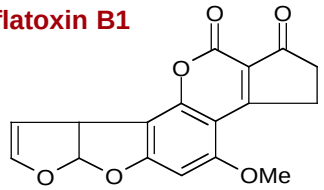
SFT, November 23-24, Paris

ALIMENTATION
AGRICULTURE
ENVIRONNEMENT



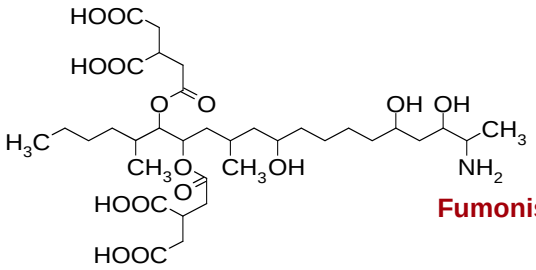
Deoxynivalenol - DON

Mycotoxins

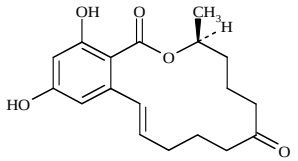


Aflatoxin B1

- Fungal secondary metabolites that exert toxic effects on animals and human
- More than 1000 mycotoxins have been described
- The chemical structure of mycotoxins is very diverse
- Chemical structure and toxic properties of mycotoxins are conserved during both storage and processing/cooking of food or feed



Fumonisin B1



Zearalenone

Mycotoxins, a very old problem

Jesus Christ

500 – 400
Before Christ

Middle Age
850 – 1129

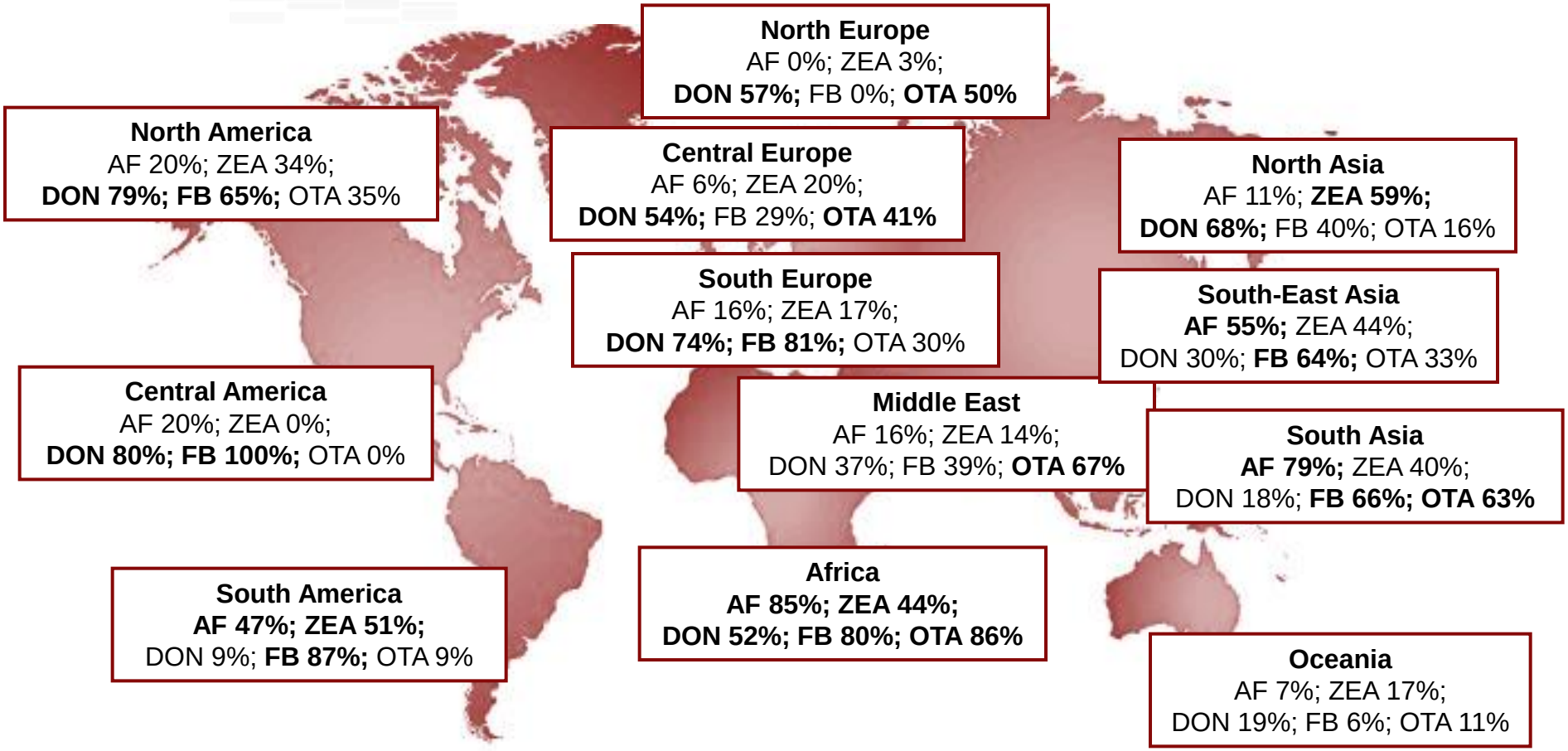
1950 – today

Poisoning with Fusarium toxins
- ❖ **Peloponnese war**
- ❖ **Decline of the etruscan civilization**

Poisoning with Claviceps toxins
- ❖ **Ergotism also called Saint Antony's fire**
  → *more than 50000 death in France*

Other poisoning with mycotoxins
- ❖ **Balkan endemic nephropathy** – ***Ochratoxin A ?***
- ❖ **Acute hepatitis** – ***Aflatoxins***

Mycotoxins: a global threat



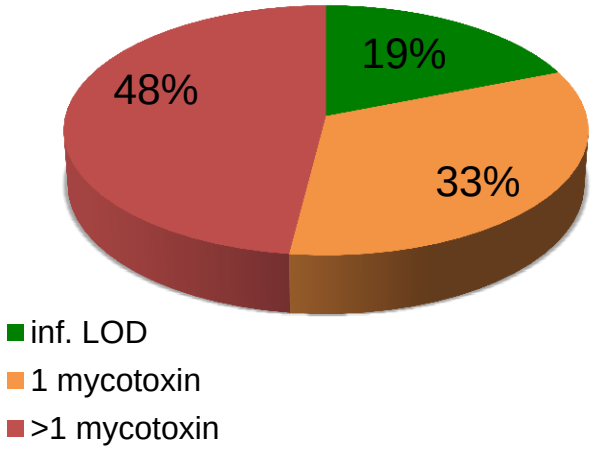
60-70% of raw materials are contaminated

Mycotoxins: a health issue in both developing and developed countries

- Global survey indicate that 60-70% of the world crop production is contaminated by mycotoxins (Schatzmayr & Streit, 2013)
- In France, mycotoxin levels can exceed the health based guidance values (second French total diet study, Sirot *et al.*, 2013)
- In 2014, the high level of mycotoxins observed in French crops lead the authorities to request a temporary derogation from the maximum limit in maize (EFSA J, 2014)
- Climate influences mycotoxin levels. What will be the consequences of the global warming?

The reality of mycotoxin co-contamination

- Fungi produce several mycotoxins simultaneously
- Food may be contaminated by several fungi
- Meals are composed of multiple raw materials



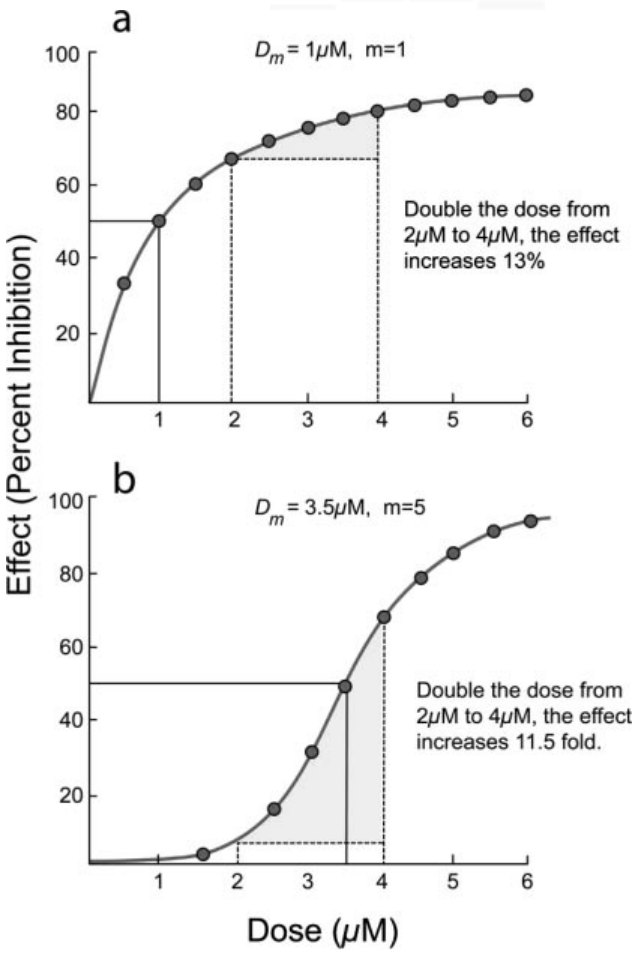
Global co-occurrence of mycotoxins
Rodrigues and Naehrer (2012)
7049 samples (2009-2011)

Co-contamination by several mycotoxins is the RULE, not the exception

Most studies have investigated the effect of mycotoxins when present separately

It is crutial to investigate the effect of mycotoxin mixtures

Experimental designs in mycotoxin mixture studies



- Classically, a two-step approach is recommended for toxicological interaction studies (Suhnel, 1996)
- First the dose-effect relationship analysis of each toxic individually has to be done to allow the prediction of a non-interactive combination effects.
- Then the actual experimental mixture effect data are compared to the predicted ones to draw a conclusion of additive combination (no interaction), synergistic or antagonistic.

Many studies addressing mycotoxins interaction are difficult to interpret, due to the lack of dose-response experiments.

The different models to study the toxicity of mycotoxins mixtures

3 types of approaches to study the interactions

- Experiments lacking of dose-response curve
  (no conclusion about the interaction)
- Experiments with factorial plan
  (conclusion about the interaction but no characterization on the interaction)
- The isobologram and the combination index
  (conclusion about the interaction and characterization of the type of interaction)

Importance of the dose response analysis:
an example

Toxicity		Mycotoxin A	Mycotoxin B	Mixture
Cytotoxic effect (versus control)	Measured value	30 ± 4	30 ± 4	40 ± 5
	Theorical value	-	-	60 ± 8

Additivity model

Toxic effect (mixture) = Toxic effect (mycotoxin 1) +Toxic effect (mycotoxin 2)

Measured value < Theorical value ⟶ Antagonism between Mycotoxin A and B

		FB1 (1µM)	FB1 (1µM)	FB1 (2µM)
Cytotoxic effect (versus control)	Measured value	30 ± 4	30 ± 4	40 ± 5
	Theorical value	-	-	60 ± 8

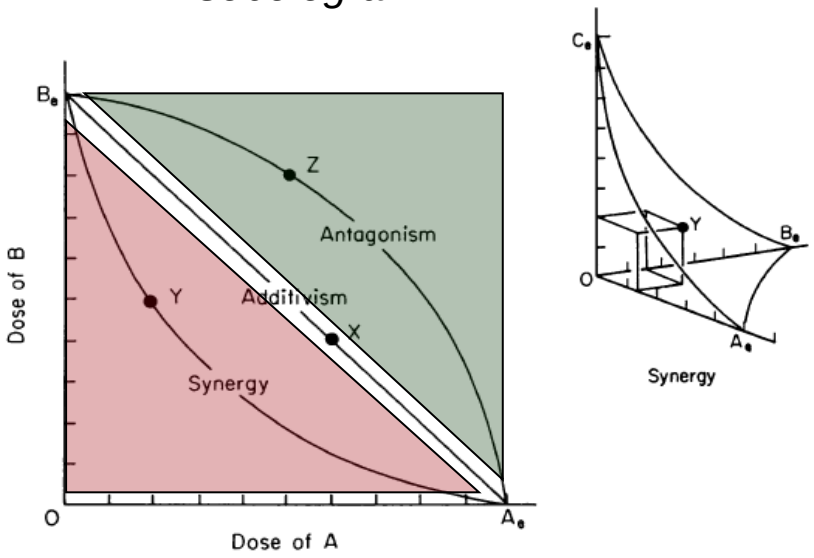
Measured value < Theorical value ⟶ Antagonism between FB1 and FB1!!!

Just a simple dose effect

The isobologram and the combination index

Principle: (i) determine the concentrations of toxins, alone and in combination, required to obtain a given toxic effect and (ii) compare theses concentration with the one that would give a theoretical additive effect.

Graphical approach:
Isobologram



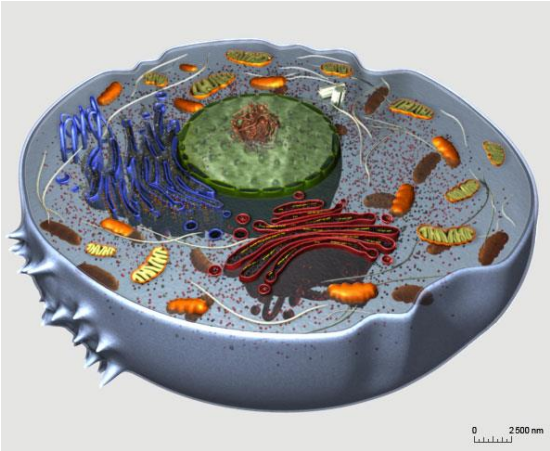
Mathematical approach:
combination index

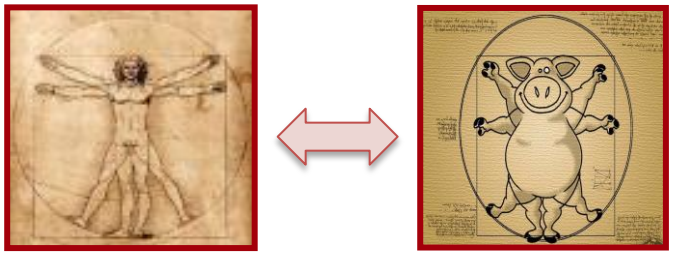
$$^{n}(\mathrm{CI})_x = \sum_{j=1}^{n} (\mathrm{D})_j/(\mathrm{D}_x)_j = \frac{(\mathrm{D}_x)_{1-n}\{[\mathrm{D}]_j \sum_{j-1}^{n}[\mathrm{D}]\}}{(\mathrm{D}_m)_j \{(fax)_j/[1-(fax)_j\}^{1/mj}}$$

Combination index	Type of interaction
Below 0.9	Synergism
0.90 – 1.10	Additive
Above 1.10	Antagonism

Determination of the type of interaction & its amplitude

Interaction between deoxynivalenol and other type B trichothecenes: analysis on intestinal cells





Proliferating intestinal epithelial cells

48 hours-exposure to graded levels of toxin

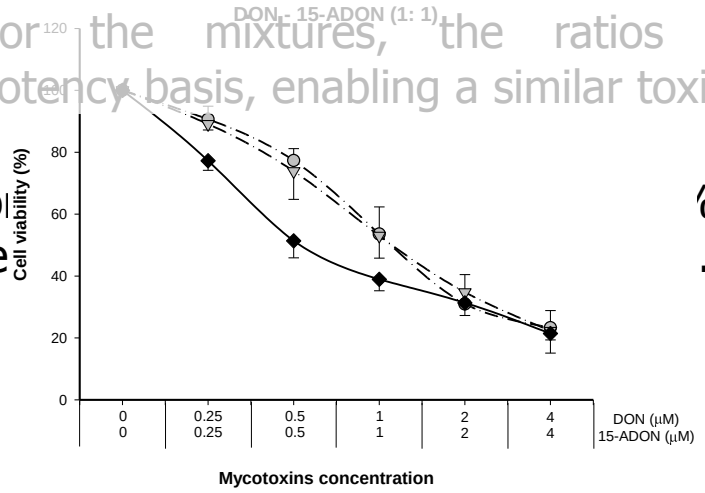
- DON, 3-ADON, 15-ADON, NIV : 0 – 7µM
- FX 0 – 0.12µM

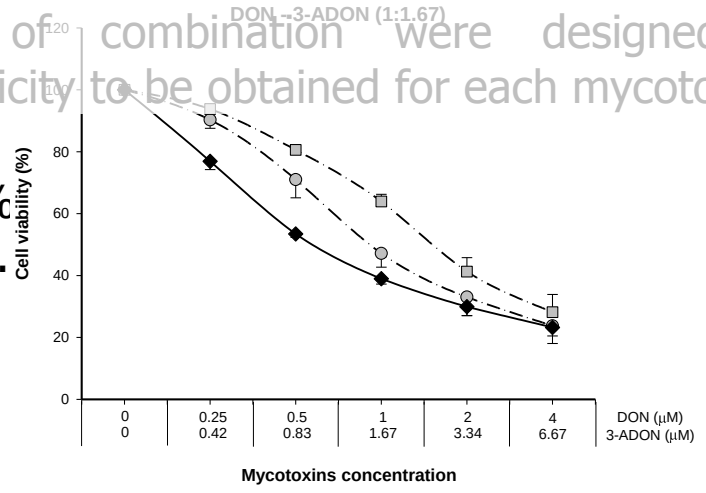
Cytotoxicity assays

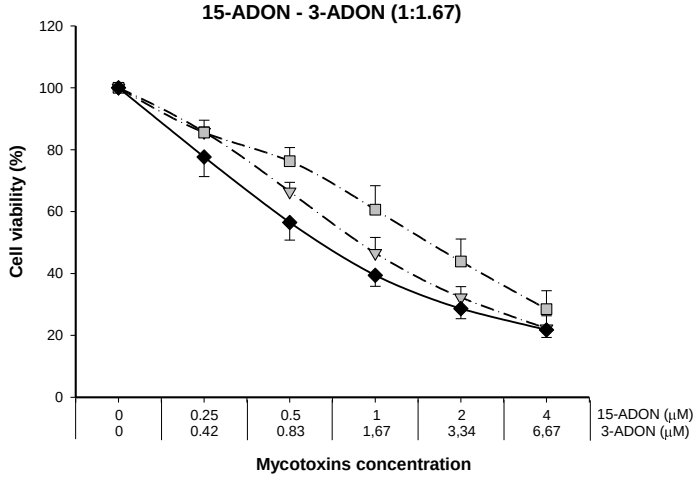
(MTT test, mitochondrial activity)

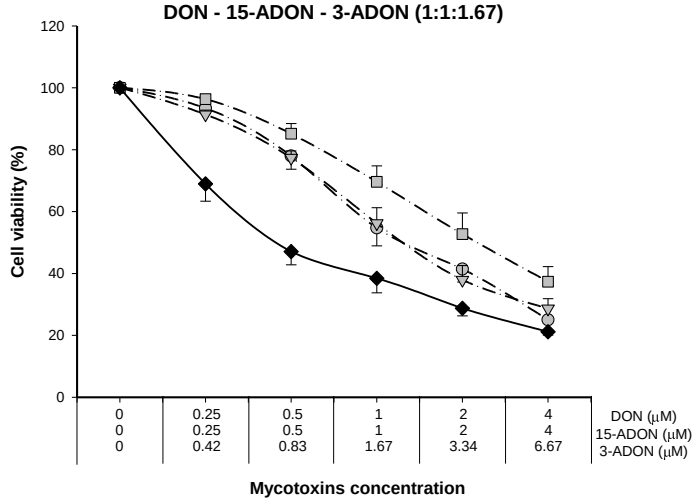
Interaction between DON and other type B trichothecenes

1. We performed dose-response experiments for single mycotoxins and their binary or ternary mixtures simultaneously.
2. For the mixtures, the ratios of combination were designed on an equipotency basis, enabling a similar toxicity to be obtained for each mycotoxin.
3. Fo[illegible] ram and de[illegible].



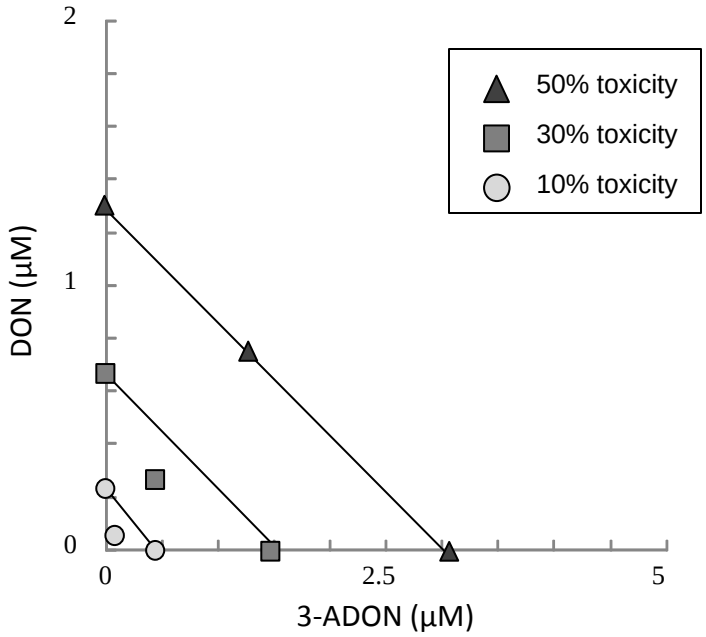




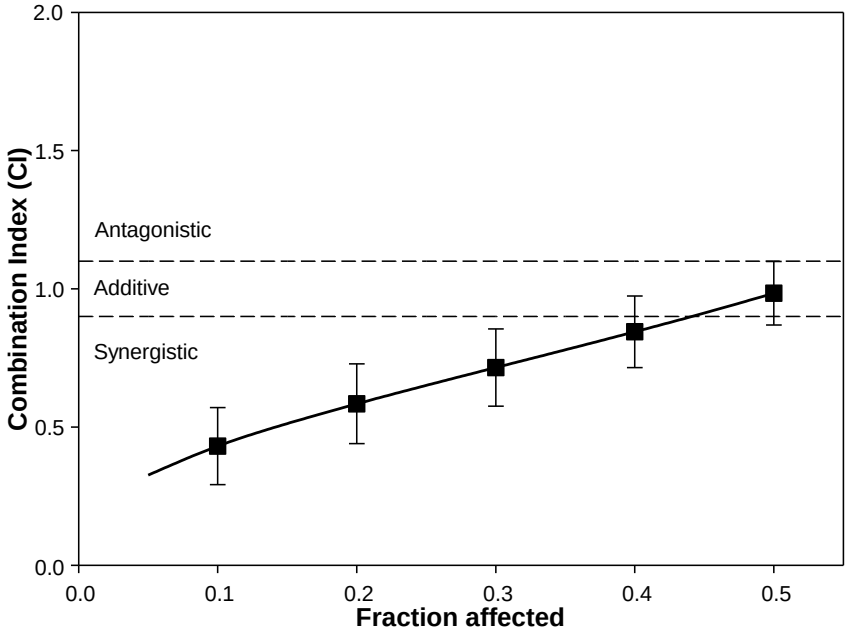


Combined effect of DON and 3-acetyl DON

Graphical approach: Isobologram

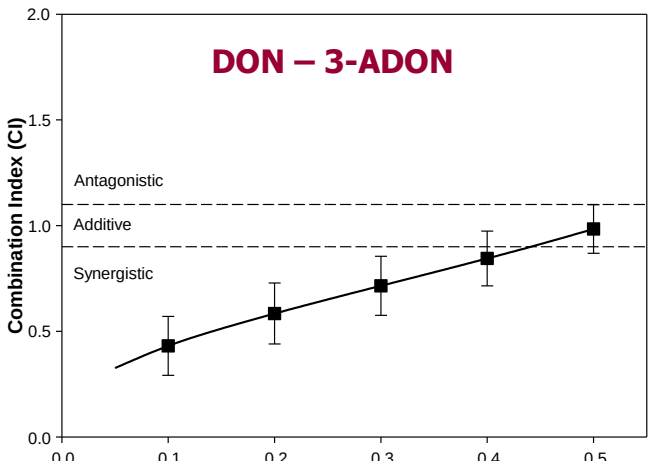


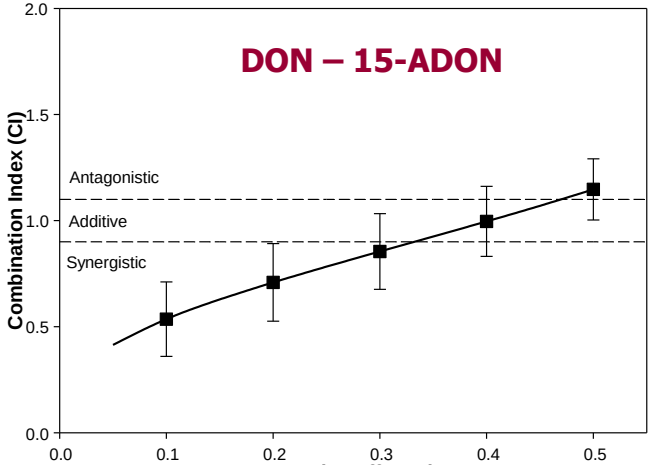
Mathematical approach: Combination index

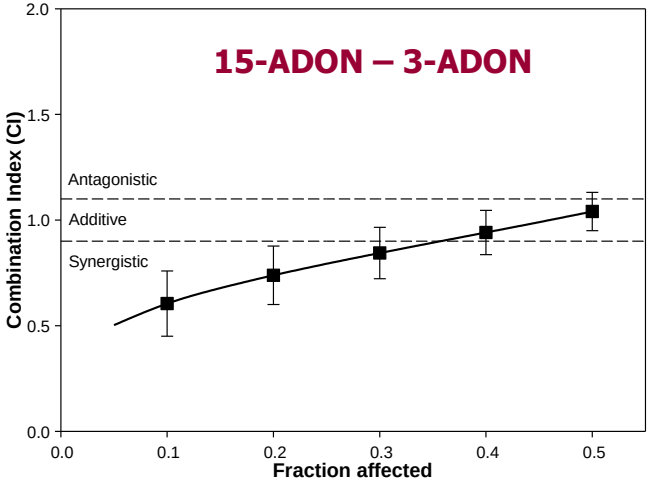


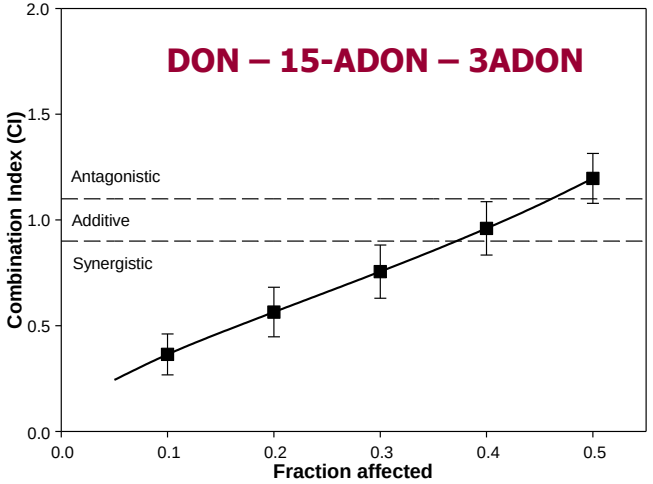
The type of interaction depends on the dose.
At low doses synergy is observed.

Combined cytotoxicity of Type B trichothecenes









- The type of interaction changes with the concentration
- At low concentrations, a synergy is observed

Magnitude of the synergy

DRI (dose reduction index): ratio between the concentration of mycotoxins when used alone or in combination to achieve the same toxicity level.

Magnitude of the synergy

Mycotoxin	Ratio	10% cytotoxicity		30% cytotoxicity	
		CI	DRI	CI	DRI
DON	1:1	0.54	4.2	0.85	2.5
15-ADON			3.4		2.2

Compared to single mycotoxins, the toxicity of the TCT mixture could be obtained with 10-fold less toxin

Combined cytotoxicity of TCT

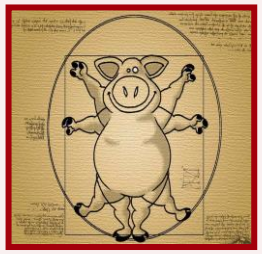
Mycotoxin association	Type of interaction at low doses
	Human Caco-2 cells Alassane-Kpembi *et al.*, 2013
DON & 3-ADON	Synergy
DON & 15-ADON	Synergy
15-ADON & 3-ADON	Synergy
DON & NIV	Synergy
DON & FX	Synergy
NIV & FX	Additivity

Combined cytotoxicity of TCT
Comparison between different cell lines

Mycotoxin association	Type of interaction at low doses	
	Human Caco-2 cells Alassane-Kpembi *et al.*, 2013	**Porcine Ipec-1 cells** Alassane-Kpembi *et al.*, 2015
DON & 3-ADON	Synergy	Antagonism
DON & 15-ADON	Synergy	Synergy
15-ADON & 3-ADON	Synergy	Synergy
DON & NIV	Synergy	Synergy
DON & FX	Synergy	Antagonism
NIV & FX	Additivity	Additivity

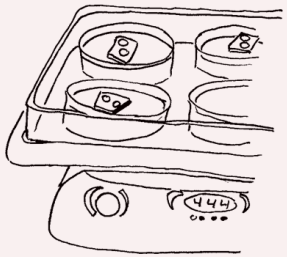
- In different cell line the type of interaction is similar
- At low concentrations, synergy is the main type of interaction observed

Interaction between deoxynivalenol and other type B trichothecenes: analysis on intestinal explants



Pig jejunum

Explants



Porcine intestinal explants

4 hours-exposure to graded levels of DON & NIV

Analysis of the expression of cytokines (IL-1α, IL-1β, IL-8, IL-17a, IL-22) **by qPCR**

Combined cytotoxicity of trichothecenes

Inflammatory response of DON & NIV

Cytokine	Interaction at low doses	
	Type	Magnitude (DRI)
IL-1α	Synergy	3.58
IL-1β	Synergy	15.06
IL-8	Synergy	22.6
IL-17A	Synergy	7.75
IL-22	Synergy	15.27

At low concentrations, synergy is the main type of interaction observed between DON and NIV

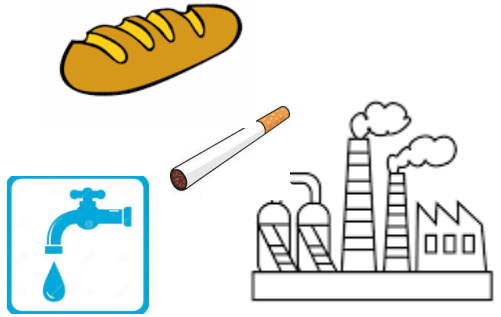
Alassane-Kpembi *et al.*, 2017

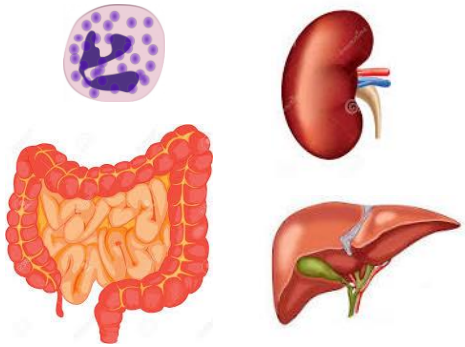
Interaction between mycotoxins & others food contaminants

Mycotoxins and heavy metals, two important classes of contaminants

example of the interaction of DON and Cd

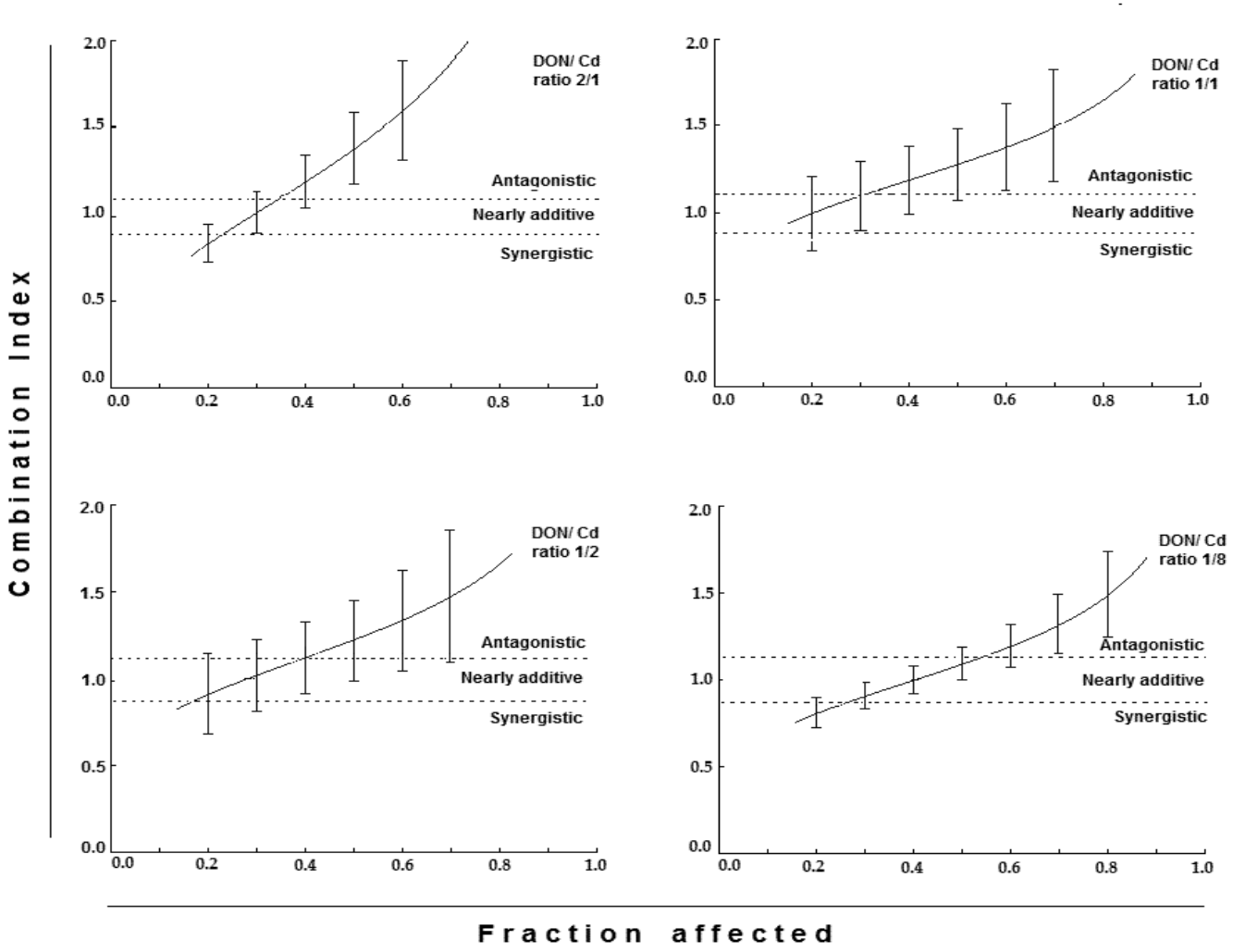
- Mycotoxins such as deoxynivalenol (DON) are frequent food contaminants
- Cadmium (Cd), a common and widespread toxic heavy metal,





How do these contaminants interact in different organs

Interaction between deoxynivalenol & Cadmium in Caco-2 cells



Interaction between deoxynivalenol and Cadmium: analysis on different cells lines

Le *et al.*, submited

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

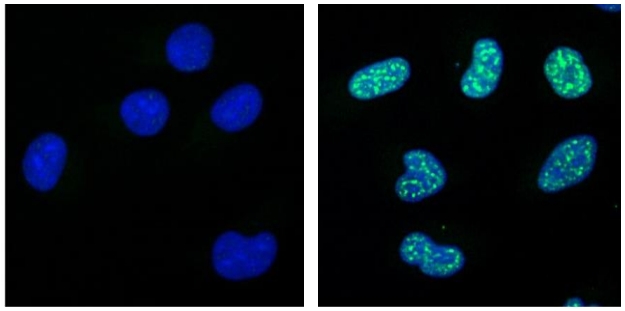
The interaction between DON and Cd is organ specific

Interaction between mycotoxins & bacterial toxins

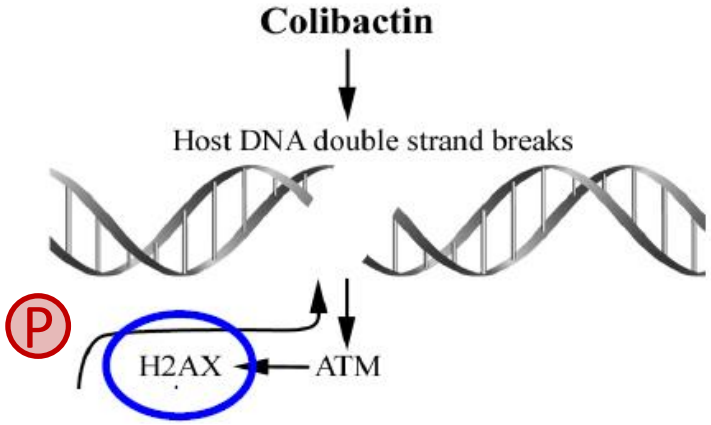
Colibactin, a genotoxin present in commensal and pathogenic *Escherichia coli*

Control

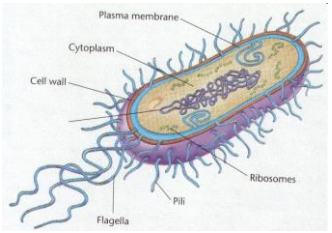
E. coli WT (colibactin +)



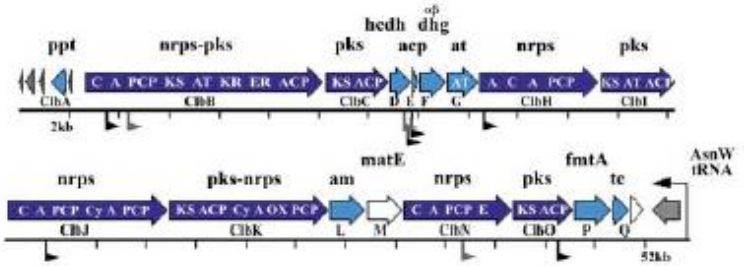
DNA
Phospho-H2AX



Escherichia coli genome



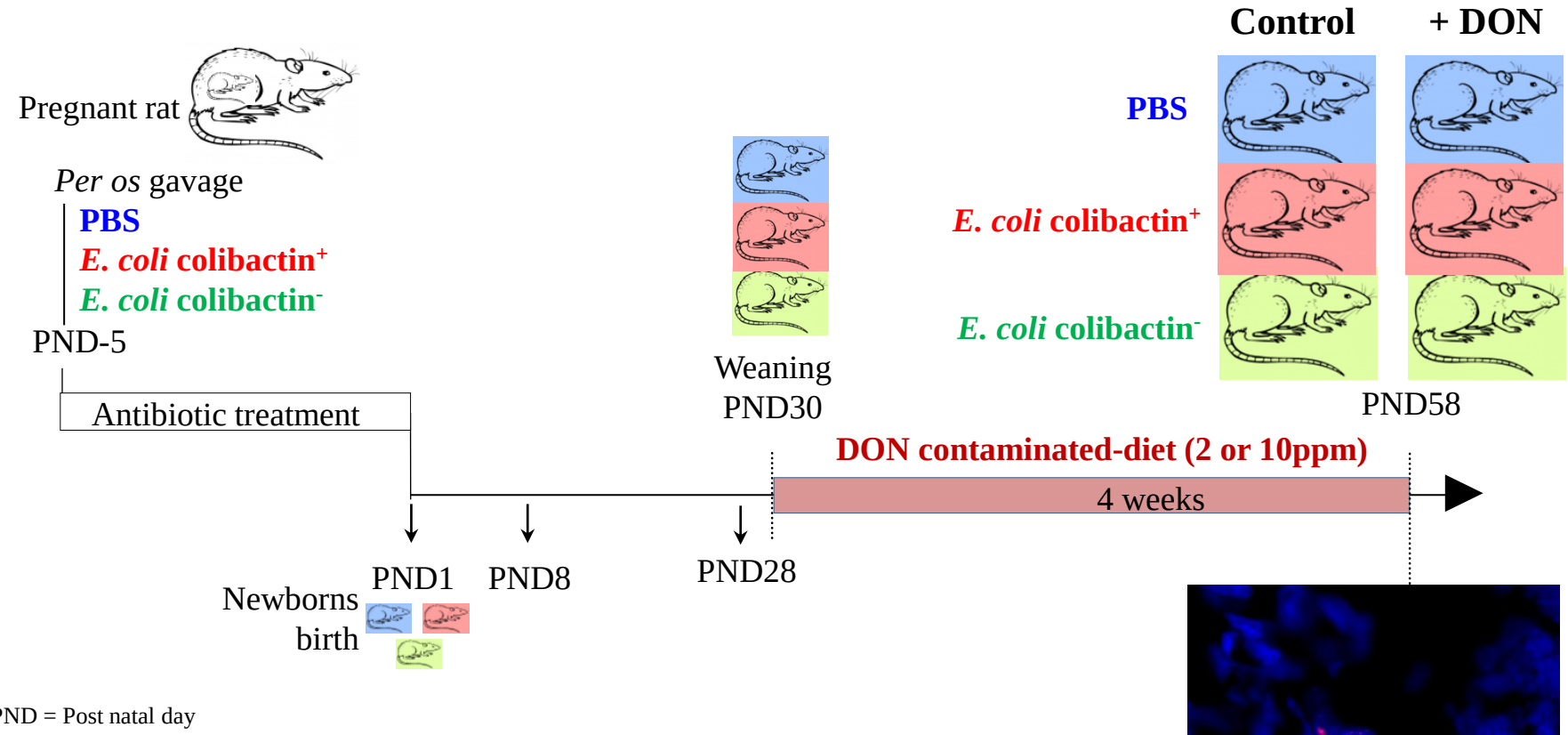
Pathogenicity island = *pks* island



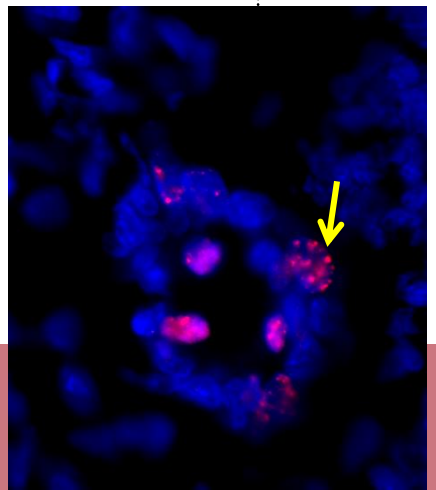
*Up to **18%** of infants and up to **25%** of adults carried E. coli strains colibactin⁺.*

Nougayrède et al., Science, 2006

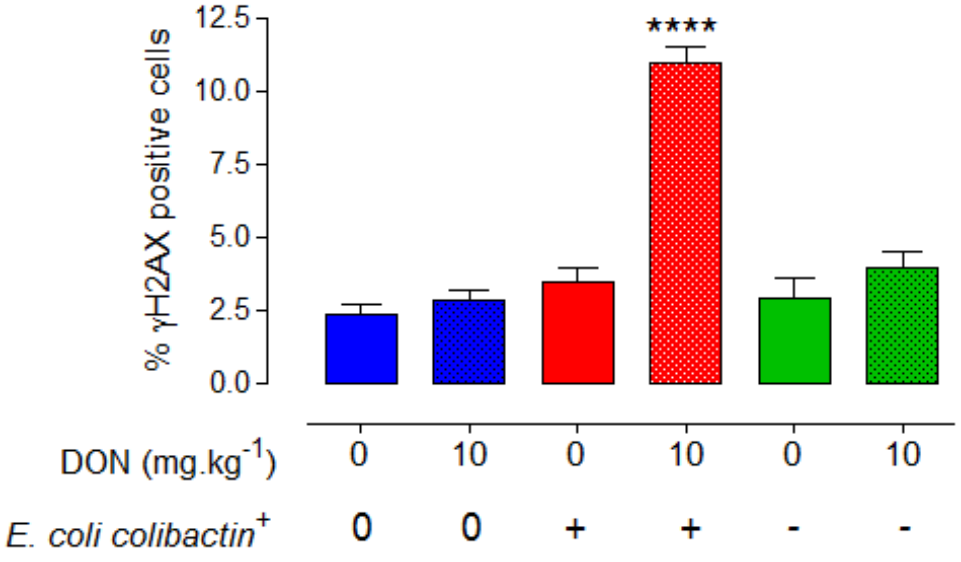
In vivo interaction between DON and a colibactin, a bacterial genotoxin: protocol

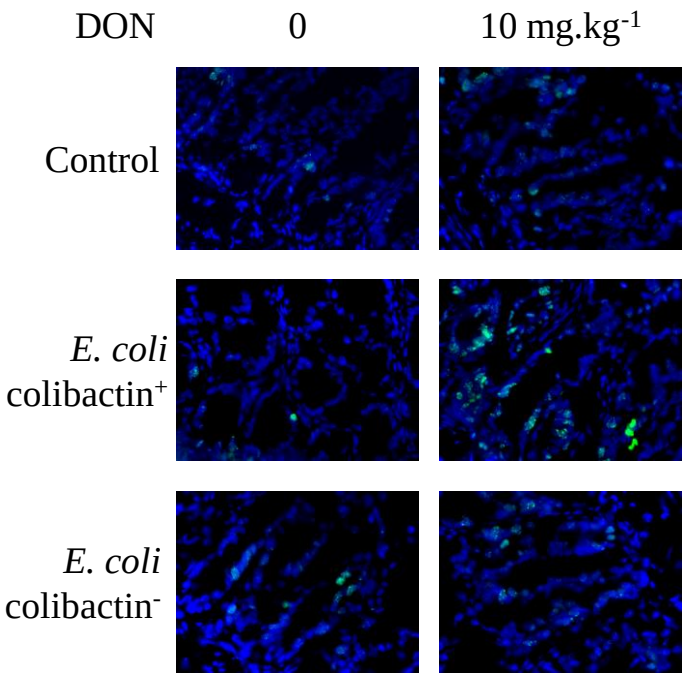


PND = Post natal day



In vivo interaction between DON and a colibactin, a bacterial genotoxin: results





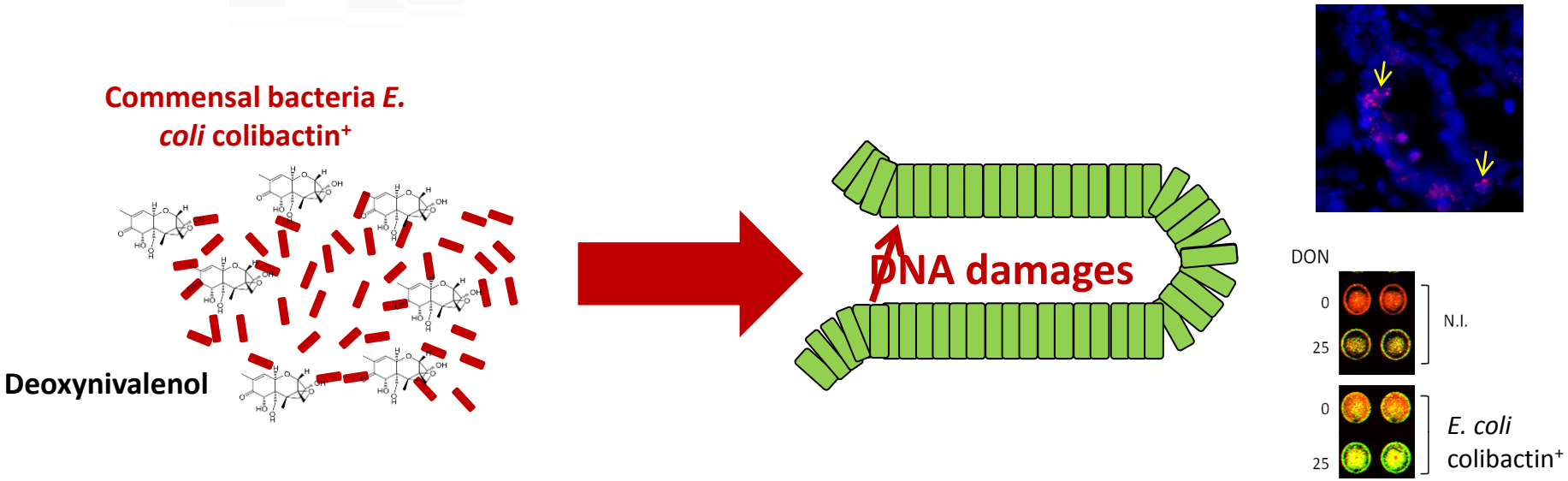
PBS

E. coli colibactin$^+$

E. coli colibactin$^-$

Exacerbation of DNA double strand breaks observed in jejunal epithelial cells of adult animals and exposed to DON-contaminated diet is dependent of the colibactin production

Interaction between DON and a colibactin, a bacterial genotoxin: conclusion



DON exacerbates colibactin-induced DNA damages in intestinal epithelial cells

The microbiota modulates the genotoxic risk of animals exposed to DON-contaminated diet.

Take-home message

- Analysis of interactions requires a dose-effect response for the individual compounds and the mixtures.
- For Trichothecenes the type of interaction depends on
  - the toxins
  - their ratio
  - their concentration
- At low concentrations the main type of interaction observed is a synergy.
- The synergy may pose a significant threat to public health, as they occur in a range of doses considered as realistic in human gut.

Take-home message

- Mycotoxins interact with other contaminants.

  Analysis of the interaction between the mycotoxin DON and Cadmium indicate that the type of interaction is organ dependent

- Mycotoxins interact with the microbiota.

  The microbiota modulates the genotoxic risk of animals exposed to mycotoxin. DON exacerbates colibactin-induced DNA damages in intestinal epithelial cells

- The toxicity of mycotoxin should be considered in a global context taking into account the host, especially its microbiota, and other contaminants we are exposed to.

FUTURE CHALLENGES

- Need to extrapolate from *in vitro* to *in vivo* data (the explant model is an alternative)
- Need to include in the combined toxicity of all mycotoxins (regulated, emerging, masked/modified….)
- Need to consider not only mycotoxins but also other contaminants and the microbiota
- The toxicity of mycotoxin mixture remains a complex problem
- Regulation should evolve and take into account the co-contamination

The team

biosynthesis and toxicity of mycotoxins

Memorial Sloan-Kettering Cancer Center New-York: Dr. T.C. CHOU

Merci pour votre attention