PrediTox
Genetic Assays for Safety Use

INRA
SCIENCE & IMPACT

Effet génotoxique combiné des contaminants environnementaux

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MeX

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APRONA
l'observatoire de la nappe d'Alsace

La problématique des mélanges chimiques
Effet « cocktail »

➔**<u>Définition</u>** : mélange chimique « *ensemble de substances, identifiées ou non, indépendamment de leur source ou de leur proximité temporelle ou spatiale, qui peuvent contribuer de façon conjointe à la toxicité dans la population d'étude* » (*US EPA, 2000*).

L'a ues
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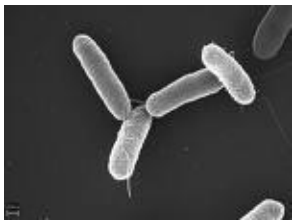
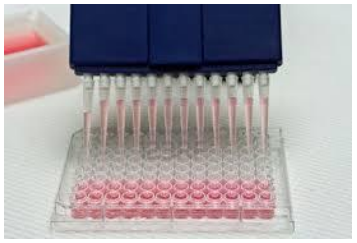
Limitations of the genotoxicity assays

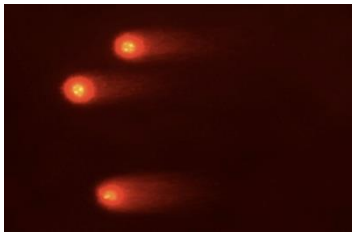
- **Inter and intra-specie differences** (Metabolism, DNA repair,...)



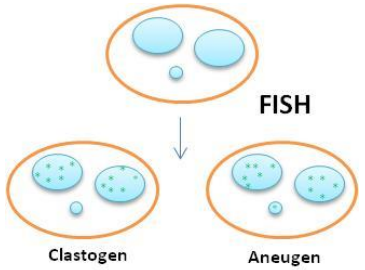
- **High-throughput** screening possibility.



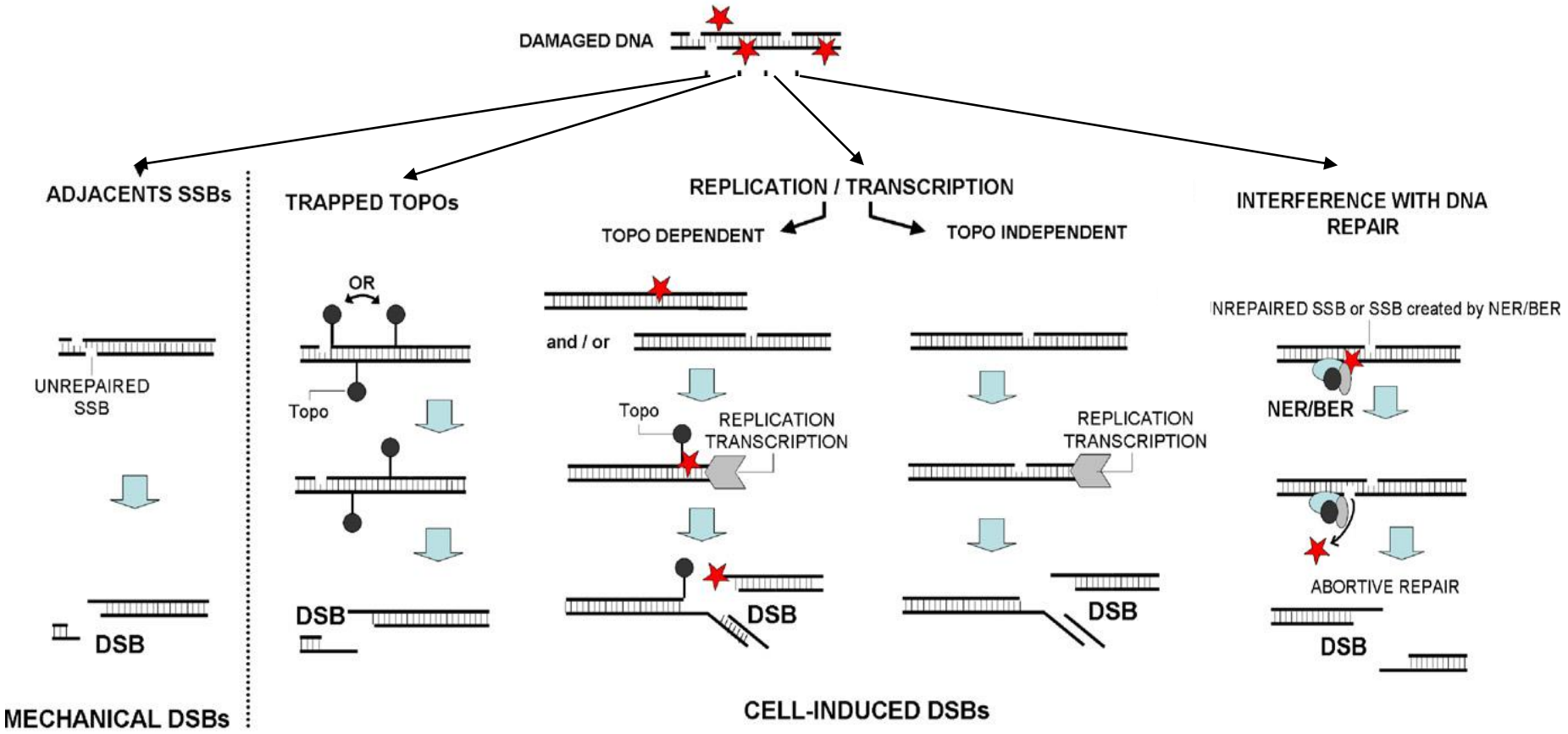
- **Cytotoxic** compounds.



- Determination of the **genotoxic mode of action**.



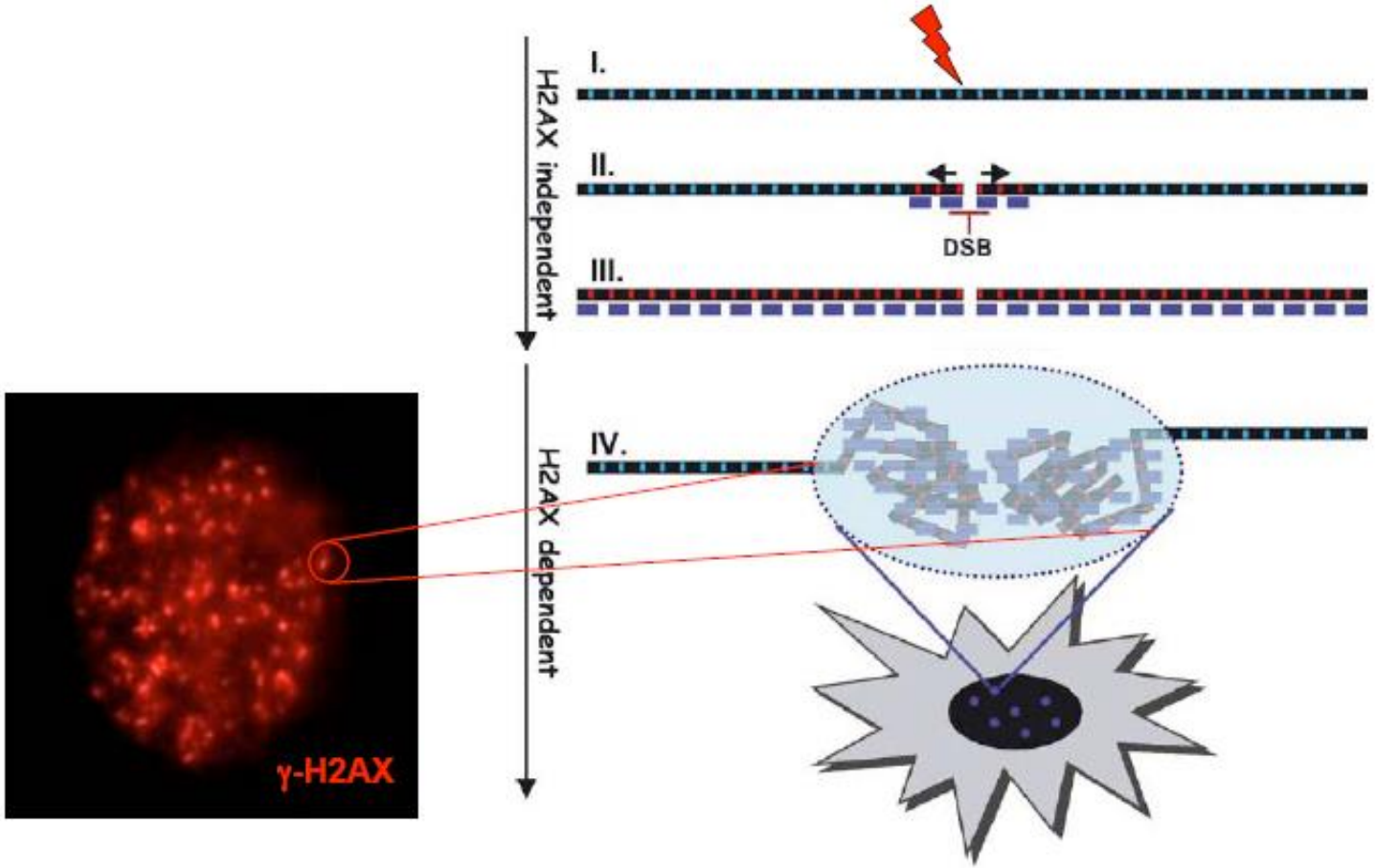
DNA Double strand breaks (DSB) formation



From O.A. Sedelnikova, et al., Mutat. Res.Rev. Mutat. Res. 704 (2010), 152-159.

→ **DSB could be induced by a large class of DNA damage when cells replicated and transcribed normally.**

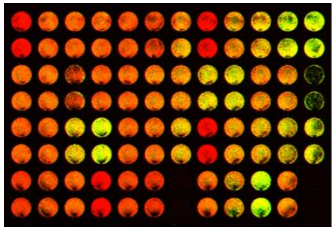
Phosphorylated histone H2AX and DSB



✓**γH2AX** is a rapid and quantitative response to DSB.
✓**γH2AX** have been demonstrated in **precancerous cells *in vivo***.

→ **Detection of DSB could be done by immunohistochemistry.**

γH2AX quantification with the "**I**n **C**ell **W**estern" technique



✓ Determination of **genotoxicity and cytotoxicity** in any cell type.

✓ **High-throughput possibility** for genotoxicity screening**.**

✓ Efficient evaluation of genotoxicity with the γH2AX ICW assay :

- **Pesticides** (Graillot *et Al*., Env Mol Mutagen, 2012; Graillot *et Al*., Mut Res 2012)
- **Bisphenols** (Dolo *et Al*., Arch Tox 2011)
- **PAHs** (Audebert *et Al*., Tox Letter 2010; Audebert *et Al*., Tox And Appl Pharma 2012)
- **HAAs** (Jamin *et Al*., Plos ONE 2013; Chevereau *et Al*., Arch Tox 2017)
- **Pathogenic bacteria** (Martin *et Al*., Plos Pathogen 2013)
- **Oxidized lipids** (Bastide *et Al*., Can. Res. 2016…)
- **Metals** (Kopp et Al. Env Mol Mutagen. 2018)
- **Virus, water…**

Application of the γH2AX assay to screen the genotoxic effect of different food contaminant families, alone or in mixture

HYDROMEL project

Study of the genotoxic potential of Polycyclic aromatic hydrocarbon (PAHs)

Large class of compounds found in our alimentation :

Environnemental contamination

Cooking technics

More than 100 identified chemicals.

Food consomption estimation : 3 µg/day (plus 2-5 µg/day for smoker).

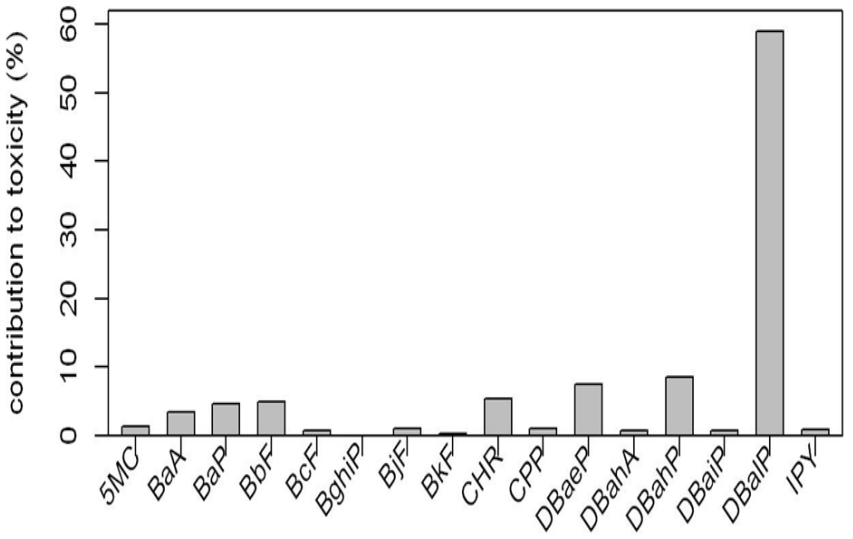
HAPs are genotoxic and carcinogens for Human (BaP classified 1 by IARC).

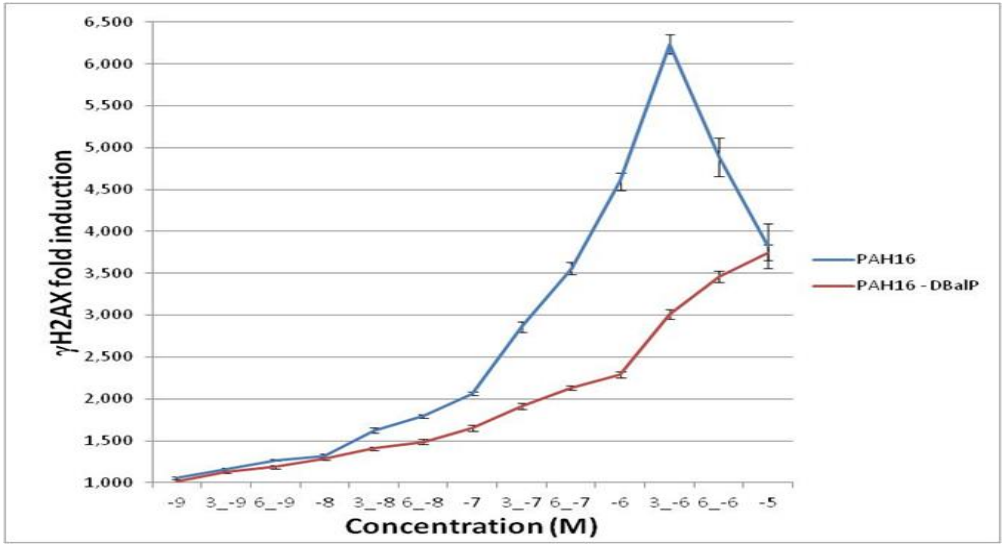
PAHs Classifications

Compound	Cigle
5-Methylchrysene	5MC
7,12-Dimethylbenz [a]antracene	DMBA
Acenaphthene	ACT
Acenaphthylene	ACTY
Anthracene	ANT
Benz[a]anthracene	BaA
Benzo[a]pyrene	**BaP**
Benzo[b]fluoranthene	BbF
Benzo[c]fluorene	BcF
Benzo[e]pyrene	BeP
Benzo[g,h,i]perylene	BghiP
Benzo[j]fluoranthene	BjFa
Benzo[k]fluoranthene	BkF
Chrysene	CHR
Coronene	COR
Cyclopenta[c,d]pyrene	CPP
Dibenz[a,h]anthracene	DBahA
Dibenzo[a,e]pyrene	DBaeP
Dibenzo[a,h]pyrene	DBahP
Dibenzo[a,i]pyrene	DBaiP
Dibenzo[a,l]pyrene	DBalP
Fluoranthene	FLA
Fluorene	FLE
Indeno[1,2,3-cd]pyrene	IPY
Naphtalene	NAP
Phenanthrene	PHE
Pyrene	PYR

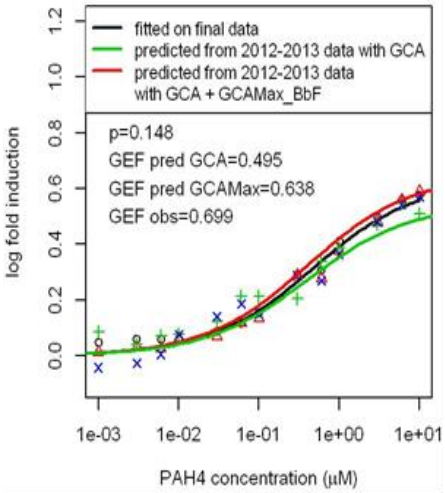
Effect of mixture of PAHs (1)

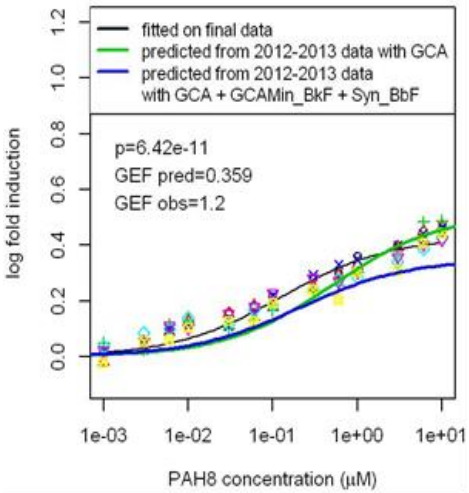
	Mélanges équitoxiques		Mélanges représentatifs de l'exposition		
Substance	PAH4	PAH8	PAH4	PAH8	PAH16
5MC					3.0
BaA	30.4	10.0	20	12.9	8.0
BaP	10.9	3.4	13	8.4	5.2
BbF	14.1	4.4	20.5	13.3	8.2
BcF					7.0
BghiP		12.5		18.1	11.2
BjF					4.2
BkF		29.9		5.8	3.6
CHR	44.6	14.0	46.5	30.1	18.6
CPP					11.4
DBaeP					3.4
DBahA		9.0		3.2	2.0
DBahP					3.0
DBaiP					3.0
DBalP					3.4
IPY		16.9		8.1	5.0

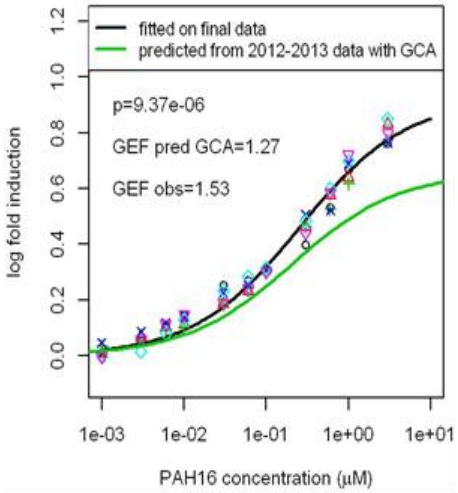


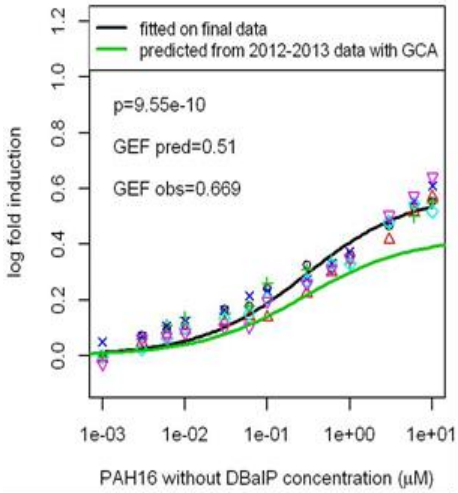


Effect of mixture of PAHs (2)





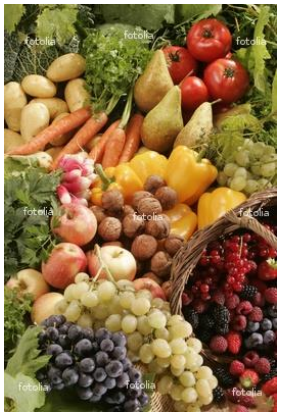




	Predicted GEF	Observed GEF
PAH4 équitoxique	0.47 [0.35 ; 0.56]	0.87 [0.411 ; 1.07]
PAH8 équitoxique	0.26 [0.21 ; 0.33]	0.81 [0.311 ; 1.11]
PAH4 exposition	0.50 [0.35 ; 0.56]	0.77 [0.471 ; 1.03]
PAH8 exposition	0.36 [0.27 ; 0.41]	1.6 [0.992 ; 3.24]
PAH16 exposition	1.27 [0.75 ; 3.0]	2.5 [1.34 ; 4.06]
PAH16 sans DBalP	0.51 [0.44 ; 0.70]	0.95 [0.544 ; 2.25]

PERICLES project

- What are the main mixtures of pesticides that French population is exposed through food?



- Is these mixtures present a genotoxic potential ?

PhD V. Graillot
Graillot *et al.* (2012) Env. Mol. Mut.
Graillot *et al.* (2012) Mutation Research
Crepet *et al*. (2013) Toxicology

Used strategy for the PERICLES project

Exposure = consomption x food contamination

Enquête INCA 2

EAT2 data

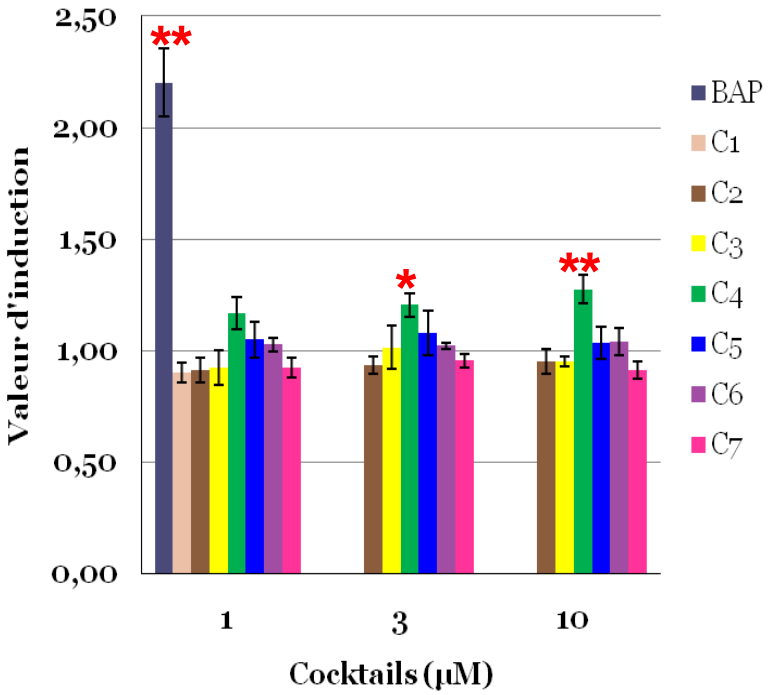
Characterisation of pesticides exposure

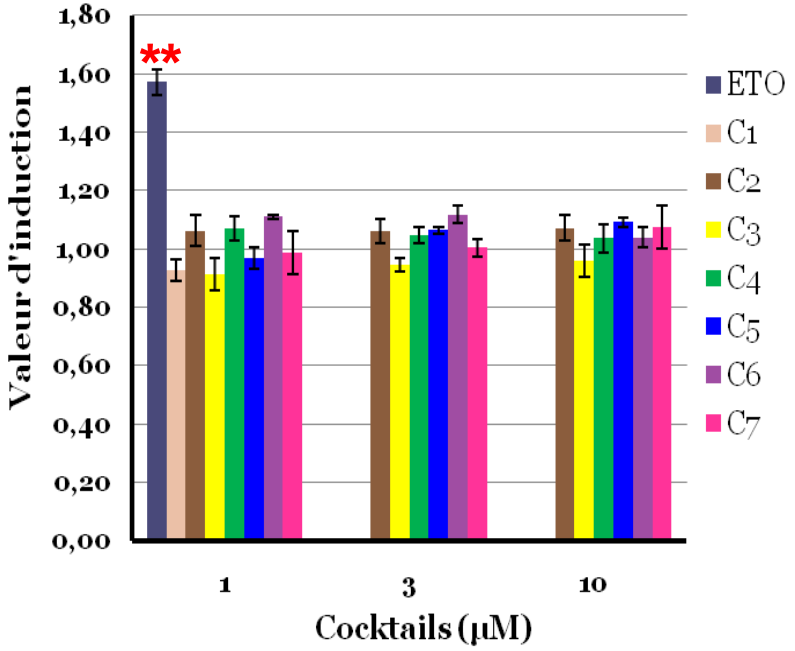
25 pesticides / 7 mixtures

Genotoxicity studies

4 cell lines/ γH2AX ICW test

Genotoxicity of mixtures of pesticides

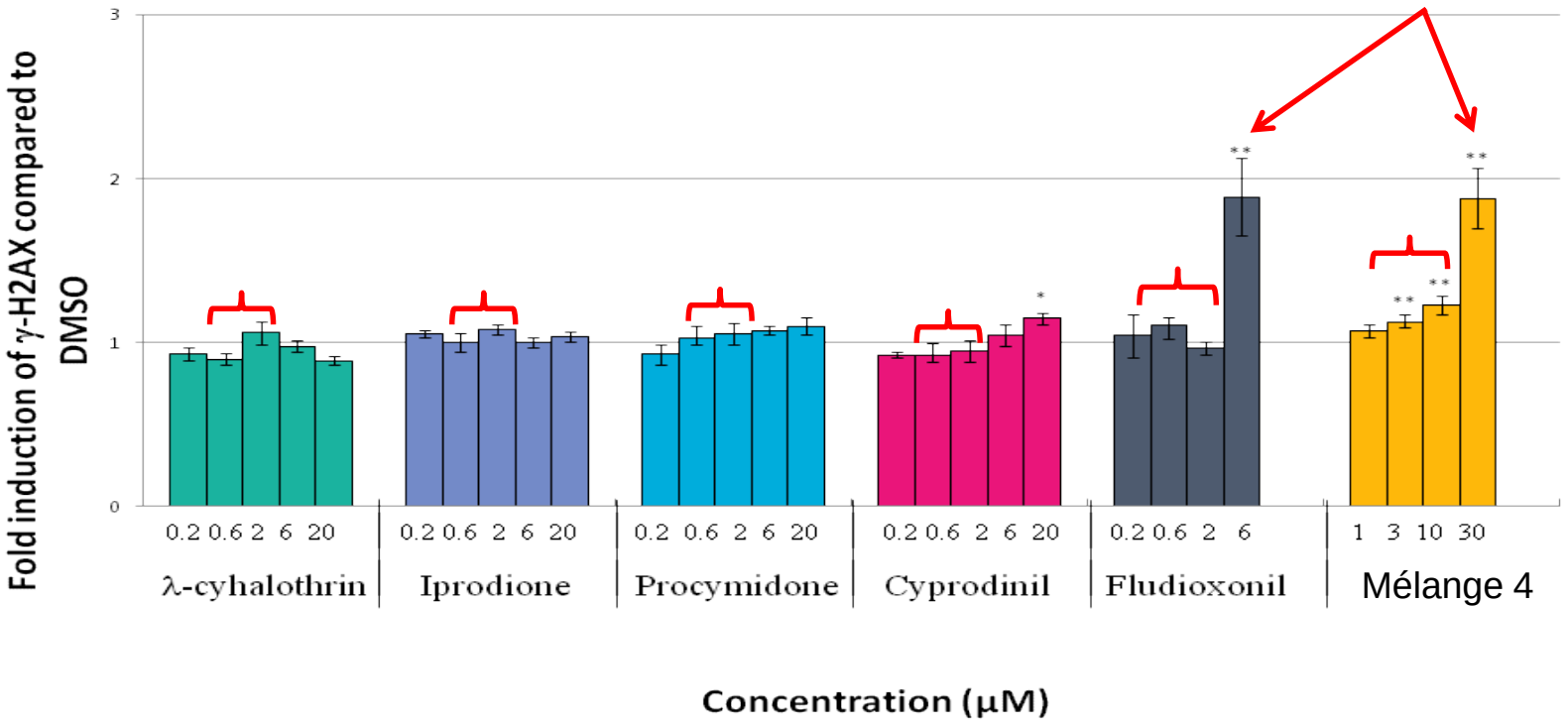




(Moyenne ± SEM, 24 h de traitement, [*p-value<0,05, **p-value< 0,01]).

→ **One compound in mixture 4 is genotoxic.**

« Cocktail » effect of pesticides



Demonstration of a « cocktail » effect : At low concentrations the mixture is genotoxic but chemicals separately did not demonstrated any effect at the concentration present in the mixture.

Graillot V. *et al.* (2012) Env. Mol. Mut.

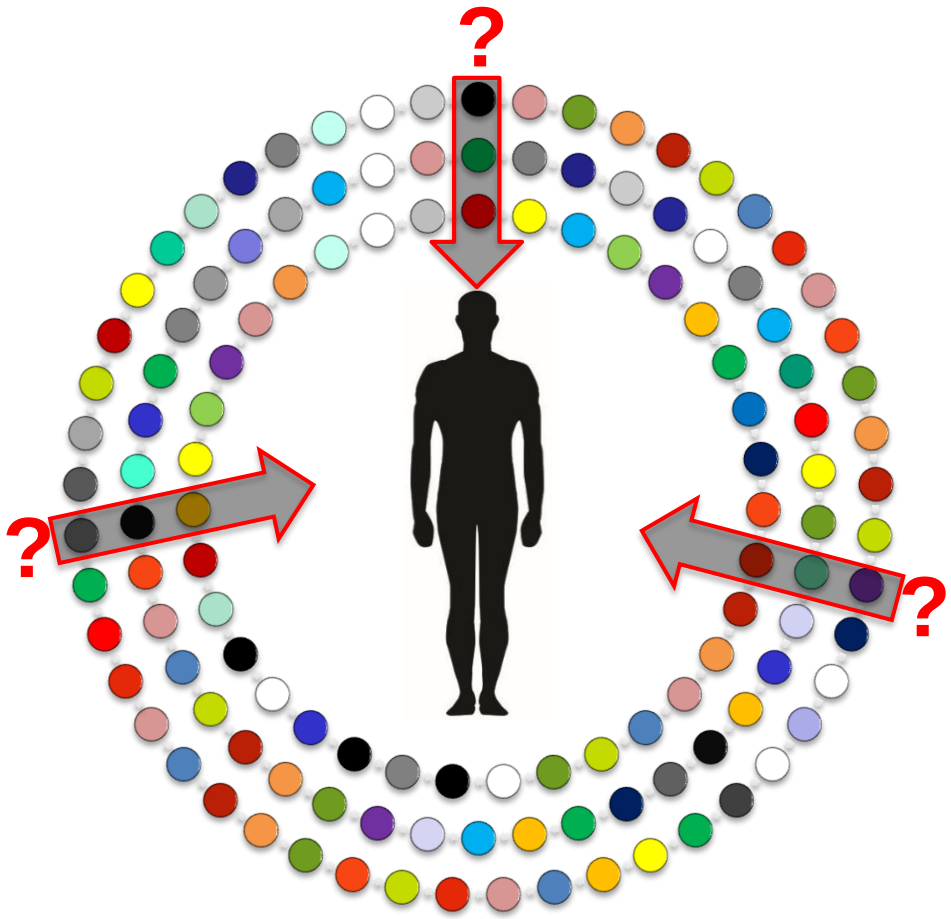
Fludioxonil was demonstrated to be an aneugen (pH3).

Khoury L. *et al.* (2016) Arch. Tox.

EATox project

Effects of simulataneous exposure to different food contaminants

Heavy metals



Pesticides

Mycotoxins

Others

Ph D B. Kopp

Identification of the main mixtures of contaminants

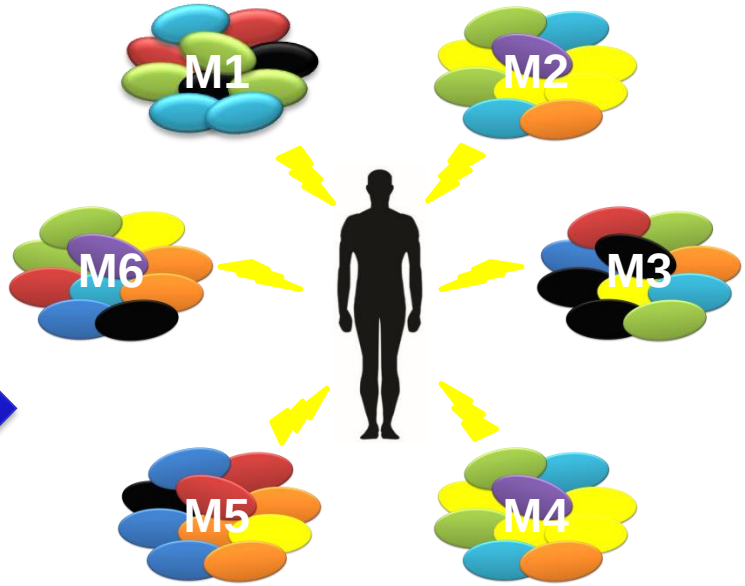
Food consumption data
Definition of food habits (snacking, traditional, vegetarian…)

6 food habits

Food contamination data (pesticides (200), heavy metals (30), mycotoxins (20)…)

445 contaminants

153 contaminants



Six complex mixtures (identification et proportion)
Traoré et al., 2016

Traoré et al. Chem. Toxicol. ***98,*** *179–188 (2016)*

Composition of the Mixture 1

	Chemicals	Family	Proportion (%)
Mixture 1	Baryum	HM	**50,98**
« ***classic*** »	Nickel	HM	**40,25**
18 % population	Cobalt	HM	3,62
	Cadmium	HM	1,67
	Déoxynivalénol	MY	1,65
	Plomb	HM	0,97
	Tellure	HM	0,29
	Bisphénol A	XE	0,26
	Nivalénol	MY	0,10
	Phénanthrène	HAP	0,05
	Pyrène	HAP	0,04
	Mycotoxin HT-2	MY	0,02
	Zéaralénone	MY	0,02
	Fluoranthène	HAP	0,01

Composition of the Mixture 3

	Chemicals	Family	Proportion (%)
Mixture 1 « ***vegetarian*** » 8 % population	Nickel	HM	**60,84**
	Vanadium	HM	**26,52**
	Arsenic inorganique	HM	5,44
	Cadmium	HM	2,00
	Iprodione	PES	1,53
	Plomb	HM	1,29
	Germanium	HM	1,16
	Cyprodinyl	PES	0,31
	Bisphénol A	XE	0,31
	Procymidone	PES	0,21
	Chlorpyrifos-éthyl	PES	0,19
	Diéthofencarb	PES	0,06
	Phosmet	PES	0,05
	λ-Cyhalothrin	PES	0,05
	Chlorothalonil	PES	0,02
	Acrinathrin	PES	0,01
	Pyriproxyfen	PES	0,003

Genotoxic effect of each chemical

Concentration-Response of chemicals alone (49), HepG2 cells, 24h treatment, γH2AX ICW

Métaux lourds

Chemical	Results	LOEC (µM)
Cadmium	**+**	**25**
Arsenic inorganique	**+**	**25**
Antimoine	**+**	**100**
Tellure	**+**	**500**
Aluminium	-	
Baryum	-	
Cobalt	-	
Germanium	-	
Nickel	-	
Plomb	-	
Vanadium	-	

Mycotoxines

Chemical	Results	LOEC (µM)
Mycotoxin HT-2	**+**	**0,1**
Nivalénol	**+**	**10**
Déoxynivalénol	**+**	**10**
Zéaralénone	-	

Pesticides

Chemical	Results	LOEC (µM)
Chlorothalonil	**+**	**10**
Acrinathrine	-	-
Chlorpyrifos-éthyl	-	-
Cyprodinyl	-	-
Diéthofencarb	-	-
Ethion	-	-
Etofenprox	-	-
Fluoranthène	-	-
Flutriafol	-	-
Iprodione	-	-
Lindane	-	-
Métalaxyl Méfenoxam	-	-
Phosmet	-	-
Piperonyl butoxide	-	-
Pirimiphos-méthyl	-	-
Procymidone	-	-
Pyriméthanil	-	-
Pyrimicarb	-	-
Pyriproxyfen	-	-
Triadimenol	-	-
λ-Cyhalothrin	-	-

HAP

Chemical	Results	LOEC (µM)
DiBenz[ah]anthracène	**+**	**+ (0.1)**
Benzo[c]fluorène	**+**	**1**
Dibenzo[ae]pyrène	**+**	**1**
Dibenzo[ai]pyrène	**+**	**1**
Indéno[1,2,3cd]pyrène	**+**	**1**
Benzo[ghi]pérylène	-	
Cyclopenta(cd)pyrène	-	
Phénanthrène	-	

Autres xénobiotiques

Chemical	Results	LOEC (µM)
Acrylamide	**+**	**2500**
Acide perfluorooctanoïque	-	
Bisphénol A	-	
Sodium métabisulfite	-	

2. Kopp et al. Environ. Mol. Mutagen (2018); 1. Kopp et al. Environ. Mol. Mutagen 59(3):202-210 (2018)

Predicted genotoxic effect of each mixture

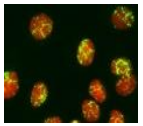
Chemicals	LOEC (*µM*)	Concentration in the mixture (*µM*)	
Mycotoxine HT-2	0.1	0,02	5-fold
Pyrène		0,04	
Cadmium	25	1,67	15-fold
Tellure	500	0,30	1666-fold
Bisphénol A		0,30	
Cobalt		3,63	
Déoxynivalénol	10	1.65	6-fold
Zéaralénone		<0,01	
Plomb		0,01	
Barium		50,99	
Nivalénol	10	0,11	90-fold
Fluoranthène		0,02	
Nickel		40,25	
Phénanthrène		<0,01	
Mixture 1		**100 µM**	

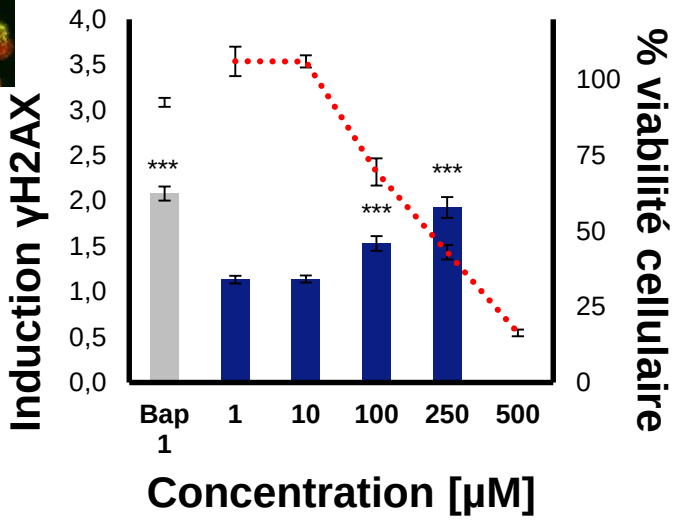
➔**Based on concentrations tested for each mixture and LOEC of each compound, no mixture is expected to be genotoxic nor cytotoxic**

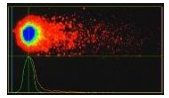
2. Kopp et al. Environ. Mol. Mutagen (2018)

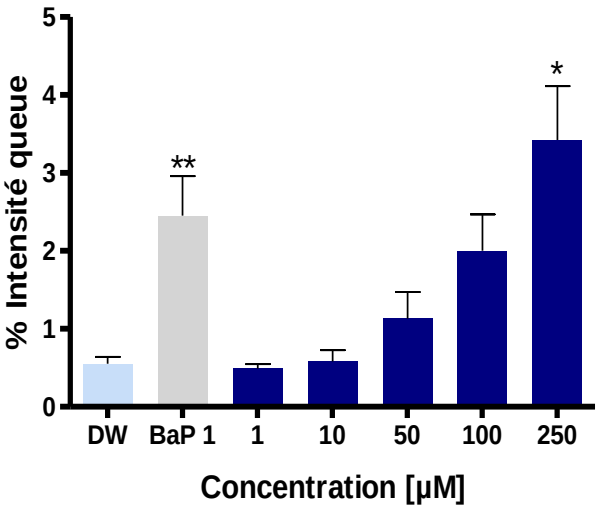
Observed genotoxic effect of each mixture

Mixture 1

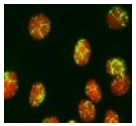


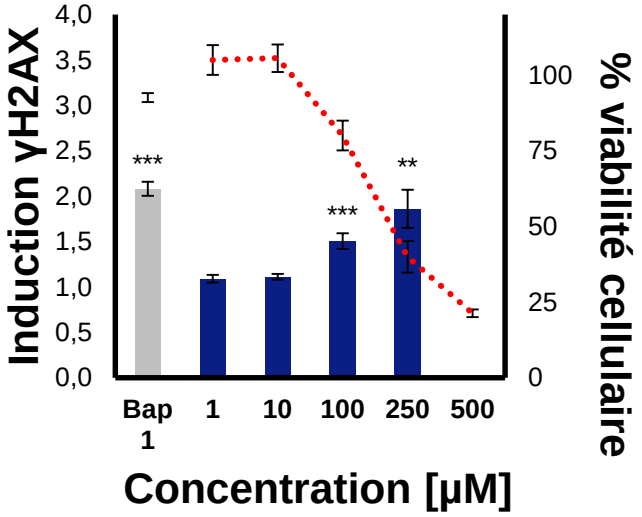


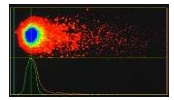


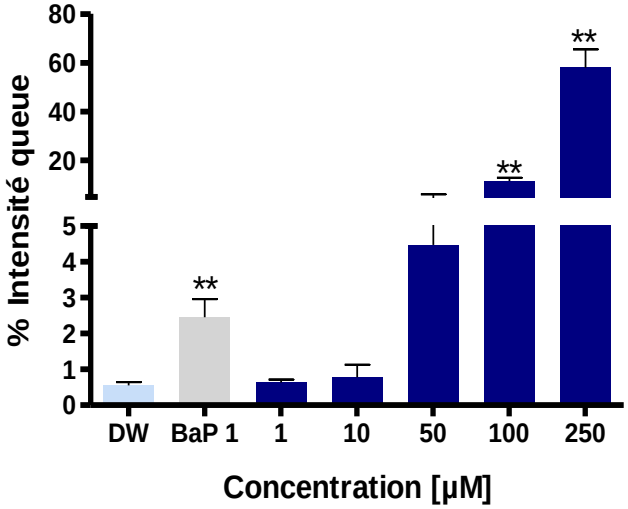


Mixture 3



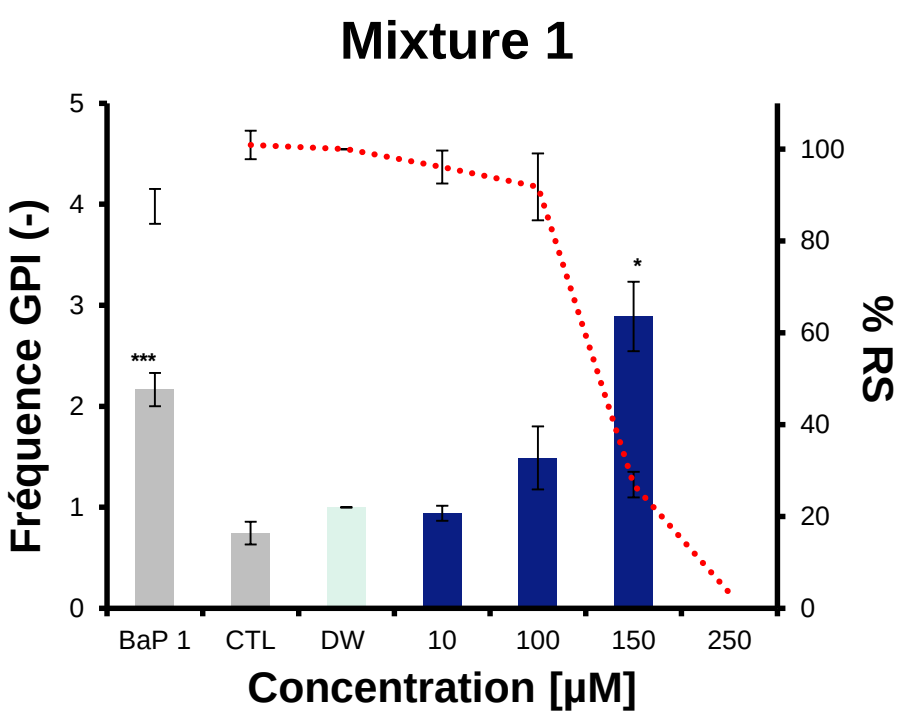


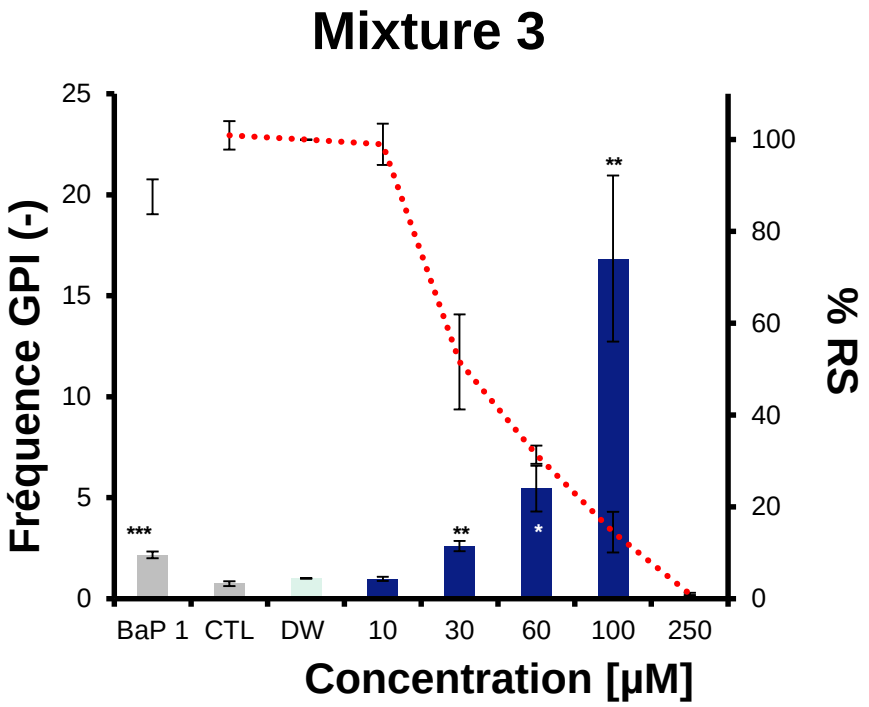




Mutagenic effect of each mixture

PIG-A *in vitro*



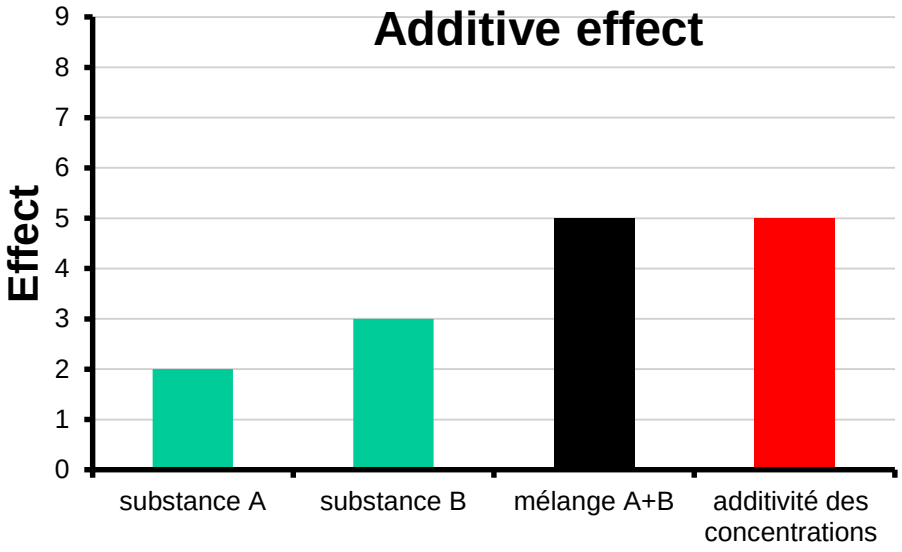


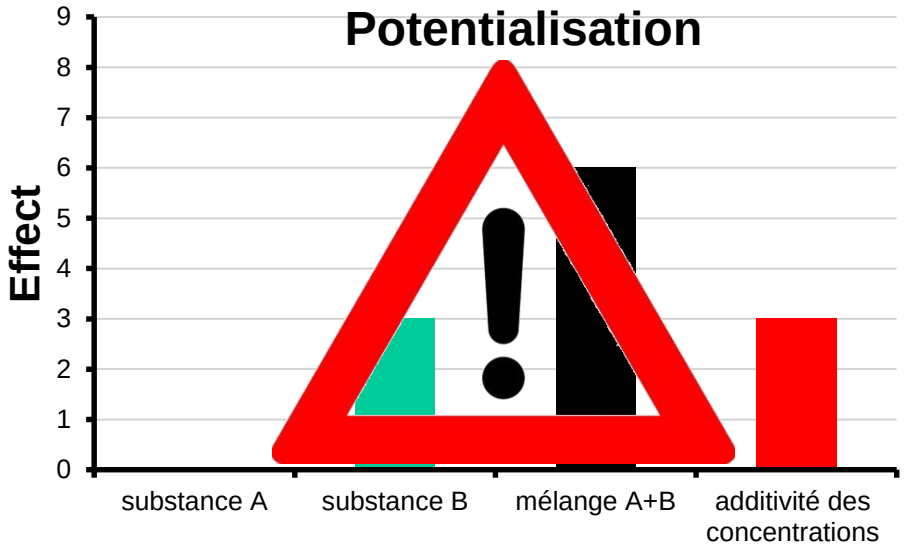
These results demonstrated that the DNA damage observed with the γH2AX assay or COMET induced mutagenesis

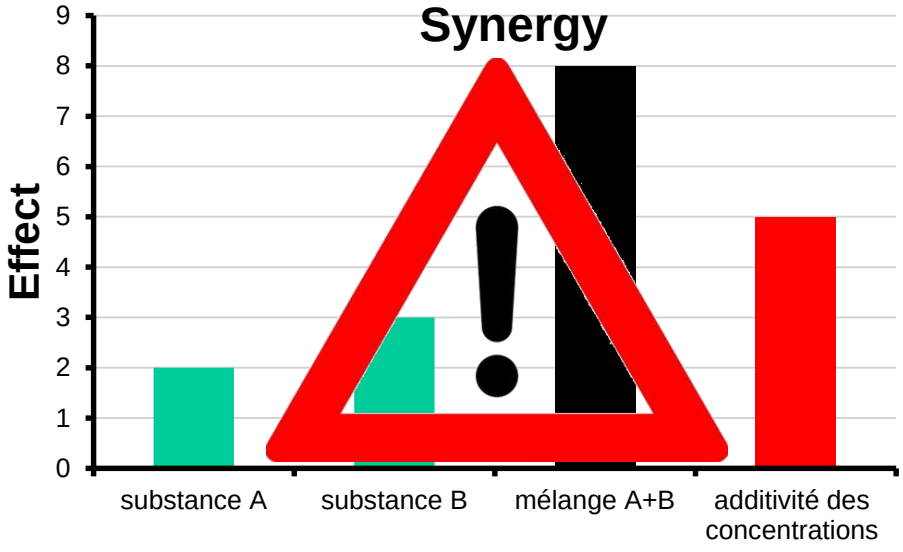
RS (relative survival) after 10 days is used as a cytotoxic measure after treatment

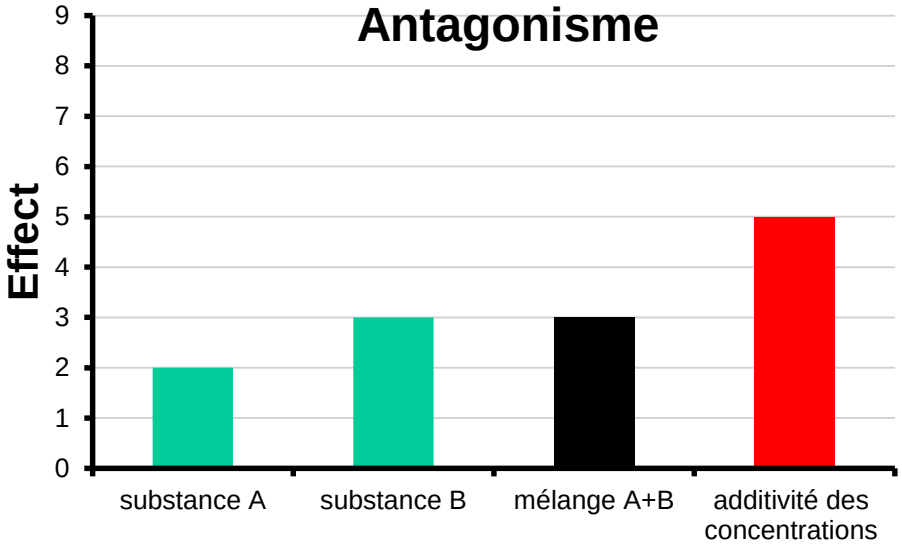
2. Kopp et al. Environ. Mol. Mutagen (2018)

The different mixture effects



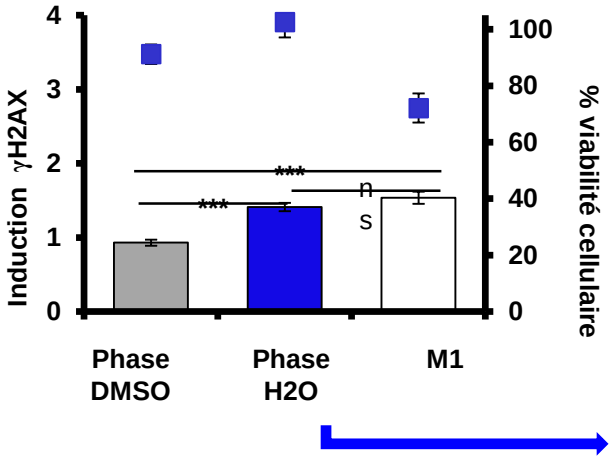


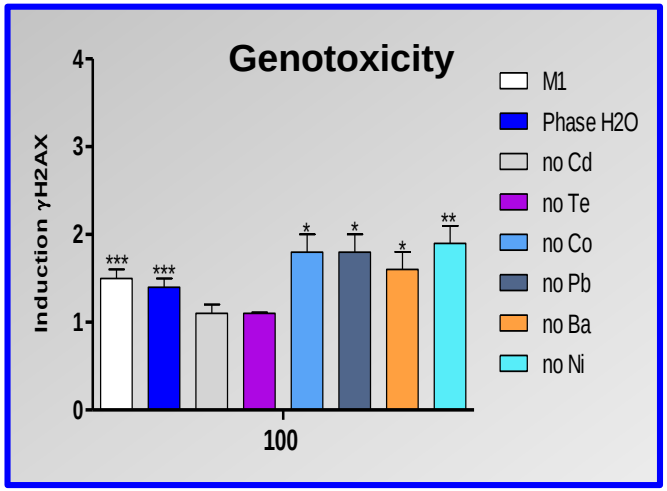


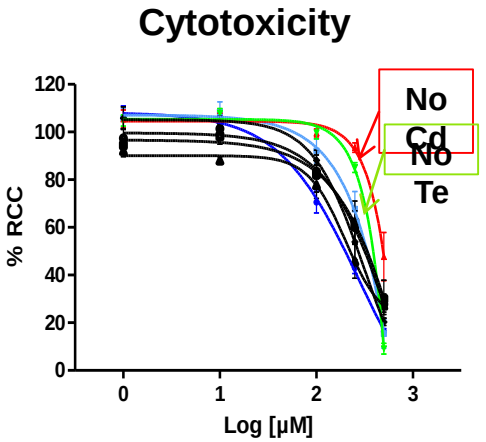


Observed combined effect of each mixture

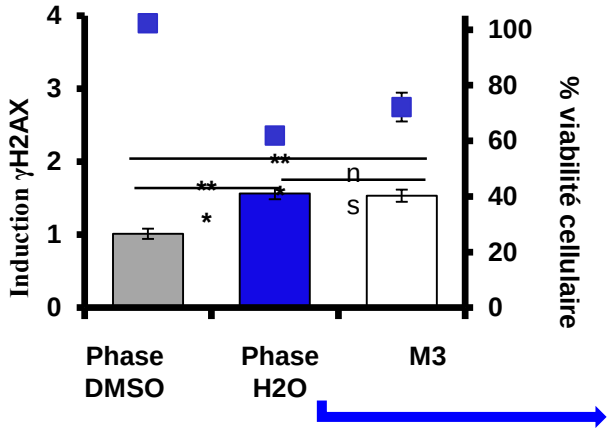
Mixture 1

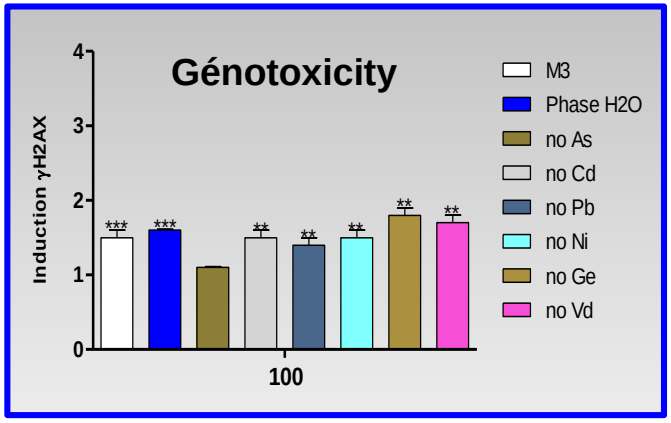


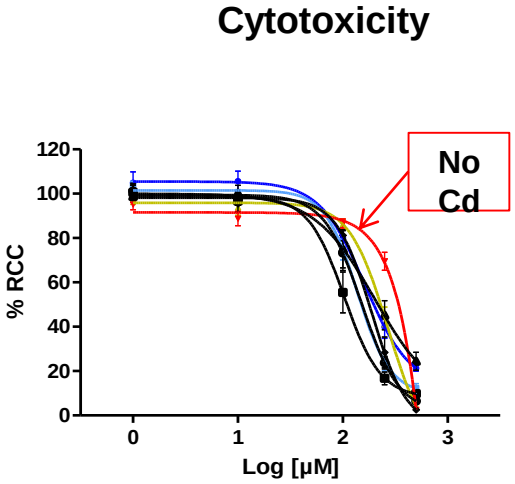




Mixture 3





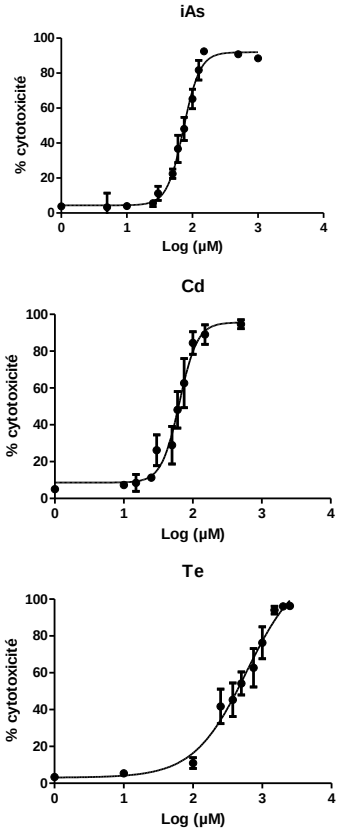


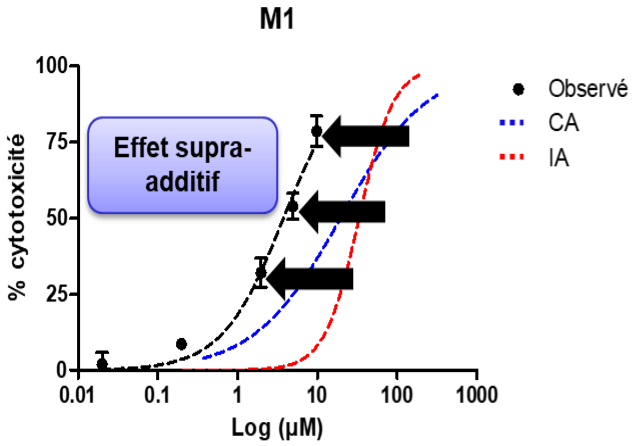
3. Kopp et al. [submitted]

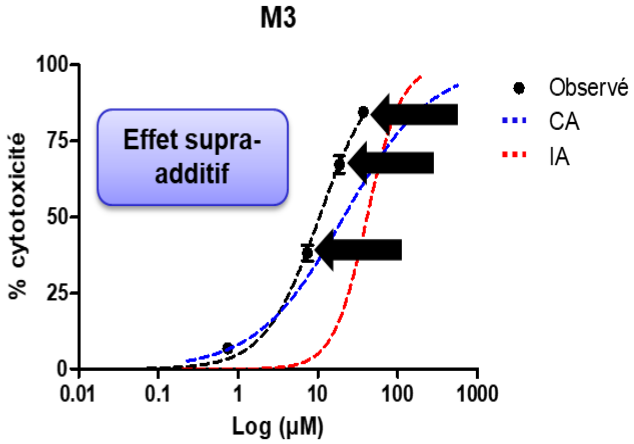
Modelization of the combined effect

Dose-reponse of cytotoxicity of As, Cd and Te alone

Additivity models





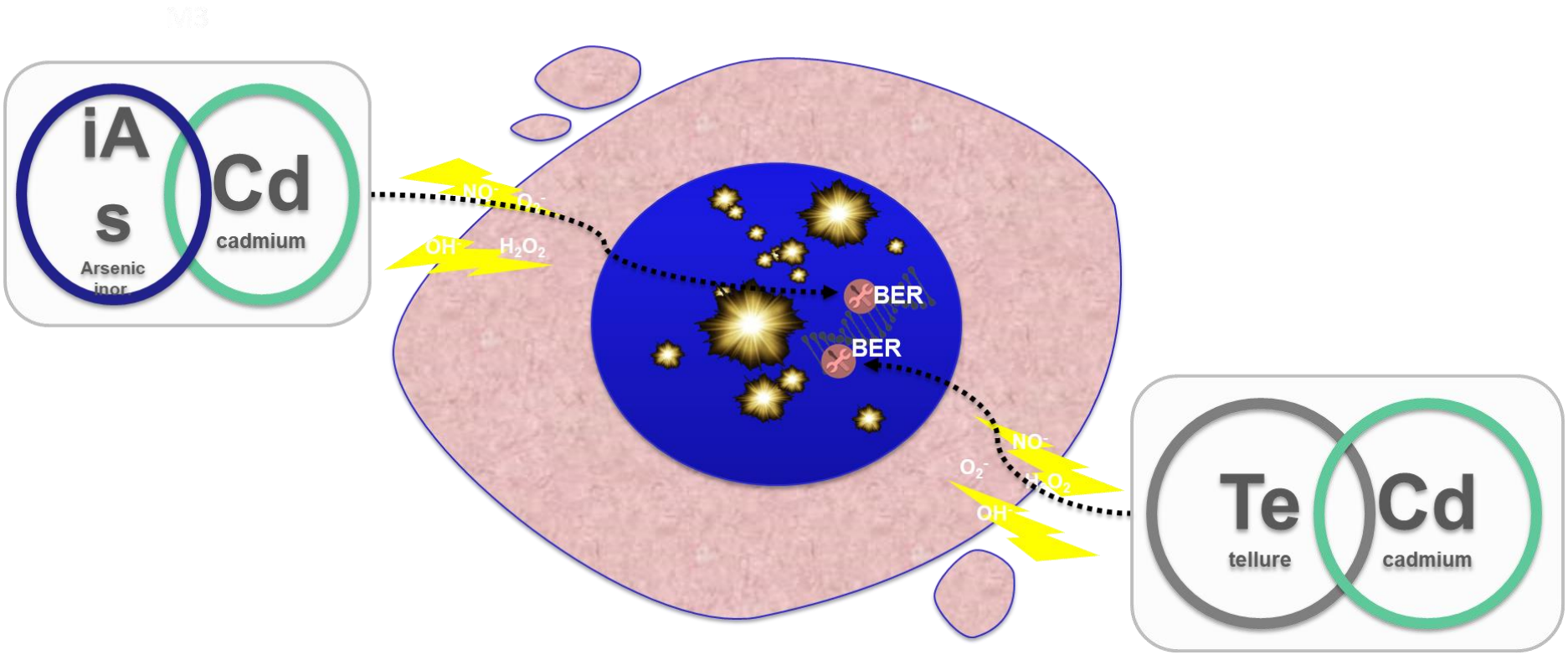


- Modèle d'addivité des concentrations (CA) *Loewe and Muischnek, 1926*
- Modèle d'additivité des réponses (IA) *Bliss, 1939*

The predicted data for M1 et M3 did not overlapped the observed data ➔ the effect are synergic

***3. Kopp et al. [submitted]**, Loewe and Muischnek. Pathol. Pharmakol. 114, 313–326 (1926); Bliss. Appl. Biol. 26, 585–615 (1939).*

Synergistic mode of action



M1 and M3

OXIDATIVE STRESS + DNA REPAIR INHIBITION (BER)

Thank you for your attention

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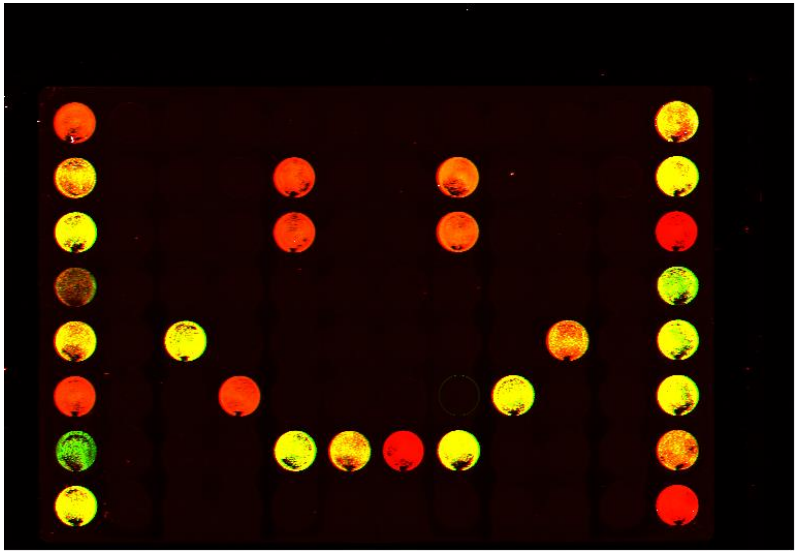

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…

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