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Exposure to mixtures of Persistent Organic Pollutants (POPs) can reduce the transactivation activities of Aryl hydrocarbon Receptor (AhR) in Dioxin Responsive (DR)-H4IIE cells

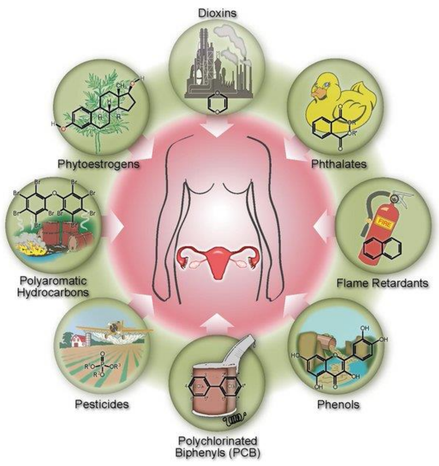
Doan TQ.[1], Muller M.[2], Connolly L.[3], Scippo ML.[1*]

1 Department of Food Science, FARAH, ULiège.
2 GIGA-R, Laboratory for Organogenesis and Regeneration, ULiège.
3 Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast.

Corresponding author: mlscippo@uliege.be

INTRODUCTION

❖ Persistent organic pollutants (POPs) are defined as organic chemicals
- resistant to degradation in the environment
- bioaccumulate and biomagnify in living organisms
- have potential harmful impacts on humans and wildlife



POPs and Early Menopause in U.S. Women http://t.co/ycXekUG2AA"

❖ Humans are exposed to POP mixtures.
❖ However, most available scientific data focus on:
- the effect of single compounds at a time
- do not address the cocktail/mixture effect of mixtures of POPs

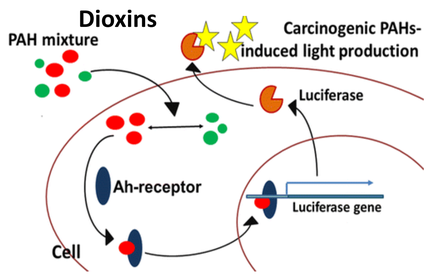
Cocktail effects???
- **Additive**
- **Antagonistic**
- **Synergistic**

➢ This study aims to determine *in vitro* the cocktail effect of a mixture of POPs in reporter cell lines at the level of the Aryl hydrocarbon Receptor (AhR) function.

*AhR is a key receptor regulating the metabolism of xenobiotics including POPs.

MATERIALS AND METHODS

❖ Dioxin Responsive and luciferase gene transformed rat hepatoma DR-H4IIE
➢ Induced light production will be in proportion with the concentration of Ahr ligands.

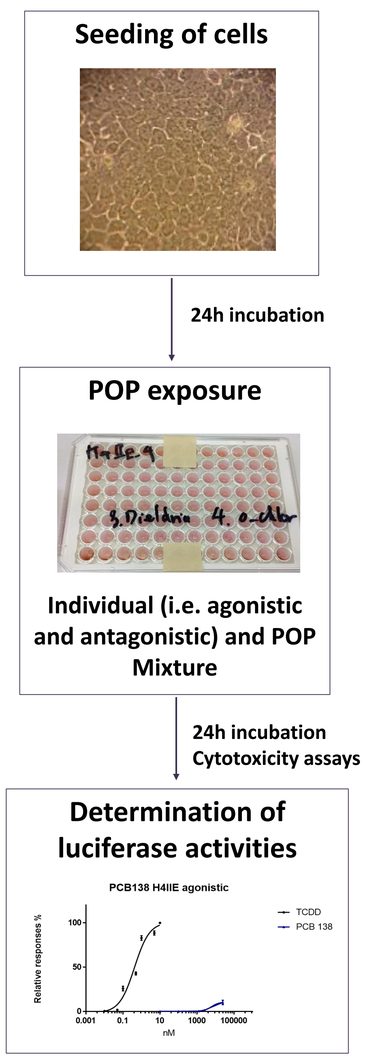


DR-CALUX (Dioxin Responsive Chemical Activated LUciferase gene eXpression) cell-based assays (Pieterse et al., 2013)

❖ POP Mixture = Mixture of 29 tested POPs belonging to 3 groups of perfluorinated, brominated and chlorinated compounds

PFCs	PBDEs	Chlorinated compounds	
PFHxS	PBDE 47	PCB 28	HCB
PFOS	PBDE 99	PCB 52	α-chlordane
PFOA	BDE 100	PCB 101	o-chlordane
PFNA	BDE 153	PCB 118	t-nonachlor
PFDA	BDE 154	PCB 138	α-HCH
PFUnDA	BDE 209	PCB 153	β-HCH
	HBCD	PCB 180	γ-HCH
		p,p'-DDE	Dieldrin

*Concentration = 1000 x human blood levels (Stockholm Convention on Persistent Organic Pollutants) Berntsen et al., 2017



RESULTS

❖ Only 4 out of the 29 compounds showed AhR agonistic activities on DR-H4IIE cells

Table 1: EC50, efficiency and potency values for the 5 AhR agonistic compounds in DR-H4IIE cells.

	PBDE 99	PBDE 153	PBDE 154	PCB 118	PCB 138
EC50 (µM)	0.15±0.07	Not full curve	0.26±0.7	9.5±3.7	6.1±10.7
Efficiency	10%	15%	8%	39%	11%
Potency	1.0E-7	-	1.8E-8	6.3E-07	4.07E-07

** Efficiency = Rmax (maximum response expressed in % of the maximum response of TCDD)*
** Potency = EC50 TCDD / EC50 substance, with EC50 TCDD (DR-H4IIE) = 15 pM, EC50 TCDD (DR-T47D) = 150 pM*

❖ In contrast, 19 out of 29 compounds showed AhR antagonistic activities on DR-H4IIE cells

Table 2: IC 50 and efficiency values of the POP Mixture and 19 AhR antagonistic compounds in DR-H4IIE cells.

	POP mixtures		PBDE 47	PBDE 99	HBCD	PFOA	PFNA
IC50 (µM)	5.07 ± 2.02		0.051 ± 0.033	0.047 ± 0.024	0.32 ± 0.49	71.3 ± 802.5	22.2 ± 89.1
Efficiency	36%		18.3%	38.3%	58.3%	69%	67.5%
	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	PCB 118
IC50 (µM)	5.9 ± 8.6	3.6 ± 1.3	~ 3.6 ± 9438	1.002 ± 0.2	7.2 ± 12.9	7.8 ± 12.8	~ 4.3
Efficiency	27.6%	22.6%	58%	21%	21%	20.3%	62%
	HCB	α-chlordane	o-chlordrane	t-nonachlor	αHCH	γHCH	Dieldrin
IC50 (µM)	28.4 ± 83.2	~ 350 ± 2611	~ 55885 ± 31E7	59.67 ± 299.5	18.5 ± 17.6	20.6 ± 14.1	17 ± 5.3
Efficiency	64.6%	19%	26.7%	45.7%	68.7%	45.7%	69.3%

**Efficiency = Response obtained for the higher tested concentration, expressed in % of the response of 15 pM TCDD*

❖ POP Mixture effect: antagonistic

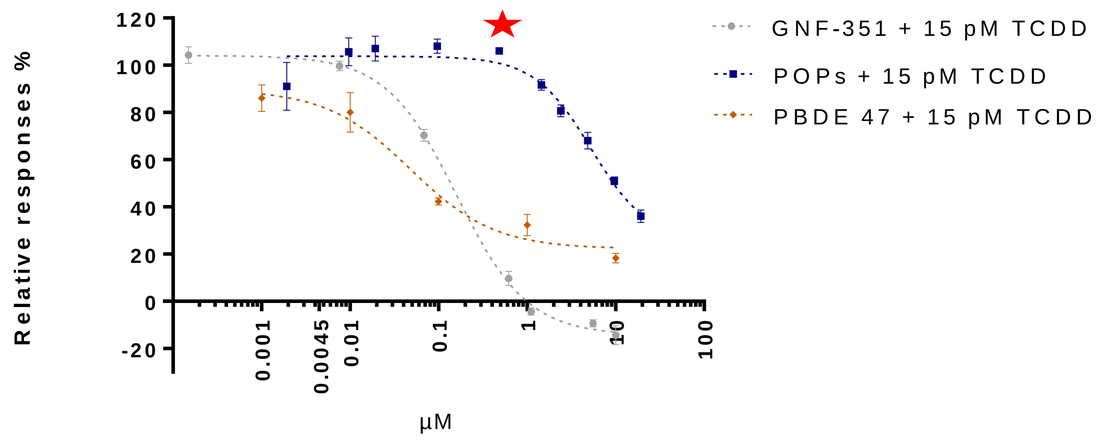


Figure 1: Dose-response curves of the POP Mixture (POPs), GNF-351 (a typical AhR antagonistic) and PBED 47 (the most efficiency AhR antagonistic in the mixture) on DR-H4IIE cells co-exposed with 15 pM TCDD.

➢ The effect starts increasing significantly when the mixture concentration is 75 times higher than their levels in the human blood.

❖ According to the addition concentration model (Payne et al., 2000):
Calculated IC50 = 16.8 µM < Measured IC50 = 5.07 ± 2.02 µM

DISCUSSIONS AND CONCLUSIONS

❖ The POP Mixture is an AhR antagonistic in DR-H4IIE cells.

❖ The compounds in the POP Mixture could act additively or even synergically as AhR antagonists in DR-H4IIE cells.

❖ Extrapolating from *in vitro* to *in vivo*, we could say that a contamination incident leading to an increase of the POP mixture blood concentration up to 75 times the background level could result in an inhibition of the AhR transactivation activities.

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