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Exposure to mixtures of Persistent Organic Pollutants (POPs) can inhibit the transactivation activities of the rat Aryl hydrocarbon Receptor (rAhR) *in vitro*

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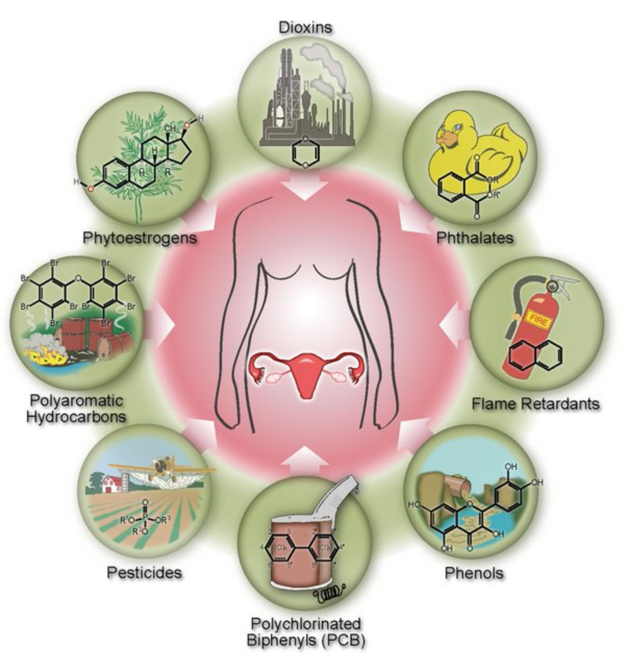
INTRODUCTION

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



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

❖ Humans are exposed to POP mixtures not as a simple compound, but few available scientific data have addressed the effect of POPs in mixture.

Mixture effects???
- **Additive**
- **Antagonistic**
- **Synergistic**

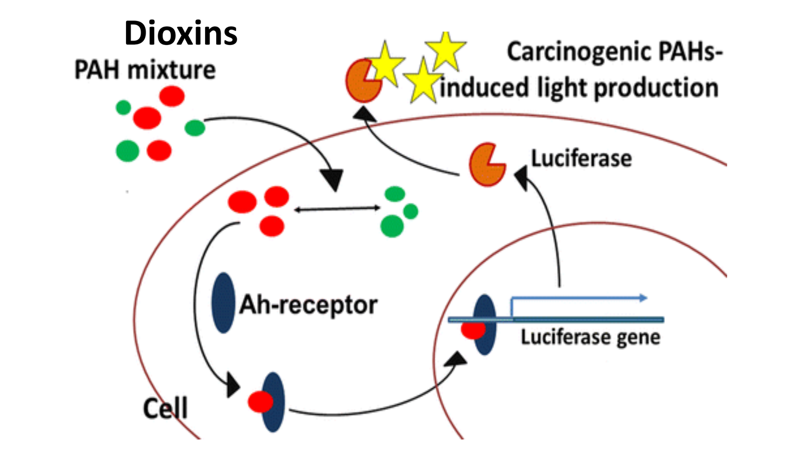
➢ ***Aims to determine, in vitro, how POPs act simultaneously in the mixture to produce an effect at the level of the rat Aryl hydrocarbon Receptor (rAhR) function***

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

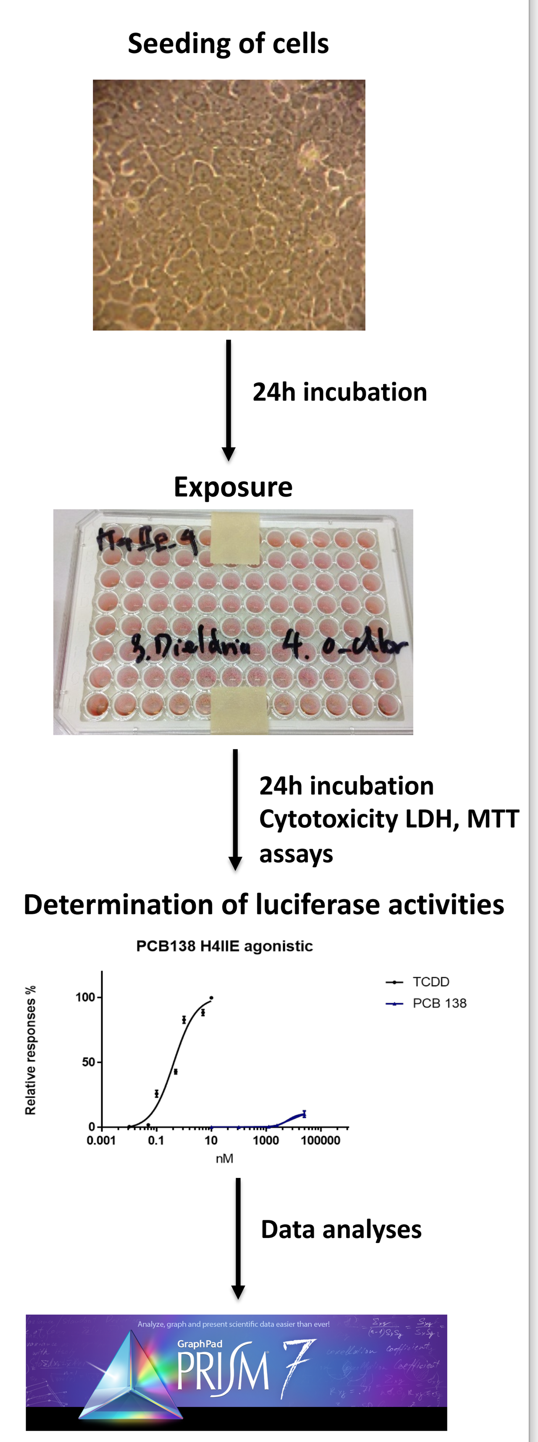
MATERIALS AND METHODS

❖ Dioxin Responsive luciferase gene transformed rat hepatoma DR-H4IIE cells

➢ Induced light production will be in proportion with the concentration of rAhR ligands



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



❖ **Test chemicals**

❑ **29 POPs** (Stockholm Convention 2001)

6 Perfluorinated (PFAA) Compounds	7 Brominated (Br) Compounds	7 PCBs + 9 Organochlorine (Cl) Compounds	
PFHxS	BDE 47	PCB 28	HCB
PFOS	BDE 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
			Dieldrin
			p,p'-DDE

❑ **POP mixture** = Mixture of 29 tested POPs and **6 Sub-mixture** (Berntsen et al., 2017) at concentration found in Scandinavian human blood (Berntsen et al., 2017)

PFAA Mixture	Cl + Br Mixture
Br Mixture	Cl + PFAA Mixture
Cl Mixture	Br + PFAA Mixture

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RESULTS

❑ **rAhR mediated-activities for 29 POPs**

- 5 out of the 29 compounds: rAhR agonistic activities

Table 1: : EC_{50}, efficiency and potency values for the 5 AhR agonistic compounds in DR-H4IIE cells.

Compounds	BDE 99	BDE 153	BDE 154	PCB 118	PCB 138
EC_{50} (µM)	4 ± 0.78	No full curve	No full curve	25 ± 13	28 ± 6.4
Efficiency	8.6%	-	-	43%	106%
Potency	3.8E-06	-	-	6E-07	5.4E-07

** EC_{50} = concentration giving half-maximal response*
**Efficiency = maximum response expressed in % of the maximum response of TCDD*
**Potency = EC_{50} TCDD / EC_{50} substance, with EC_{50} TCDD (DR-H4IIE) = 15 pM*

- In contrast, 16 out of 29 compounds: rAhR antagonistic activities

Table 2: : IC_{50} and efficiency values of 16 rAhR antagonistic compounds.

	BDE 47	BDE 99	HBCD				
IC_{50} (µM)	3.028 ± 0.34	5.11 ± 0.39	15.91 ± 6.86				
Efficiency	0.3%	35%	40%				
	PCB 28	**PCB 52**	**PCB 101**	**PCB 118**	**PCB 138**	**PCB 153**	**PCB 180**
IC_{50} (µM)	6.25 ± 0.92	3.90 ± 0.20	26.87 ± 8.42	0.304 ± 0.051	0.707 ± 0.057	5.3 ± 1.103	3.06 ± 0.072
Efficiency	15%	28%	40%	67%	40%	34%	33%
	HCB	**α-chlordane**	**o-chlordrane**	**t-nonachlor**	**γHCH**	**Dieldrin**	
IC_{50} (µM)	12.85 ± 4.57	18.31 ± 8.24	26.47 ± 19.35	30.71 ± 1.26	34.47 ± 6.68	18.16 ± 7.12	
Efficiency	27%	25%	0%	38%	4%	51%	

** IC_{50} = concentration able to reduce by half the response of 15 pM TCDD*
**Efficiency = maximum activities expressed in % of the response of 15 pM TCDD*

❑ **rAhR mediated-activities POP Mixture and 6 sub-mixtures : Antagonism**

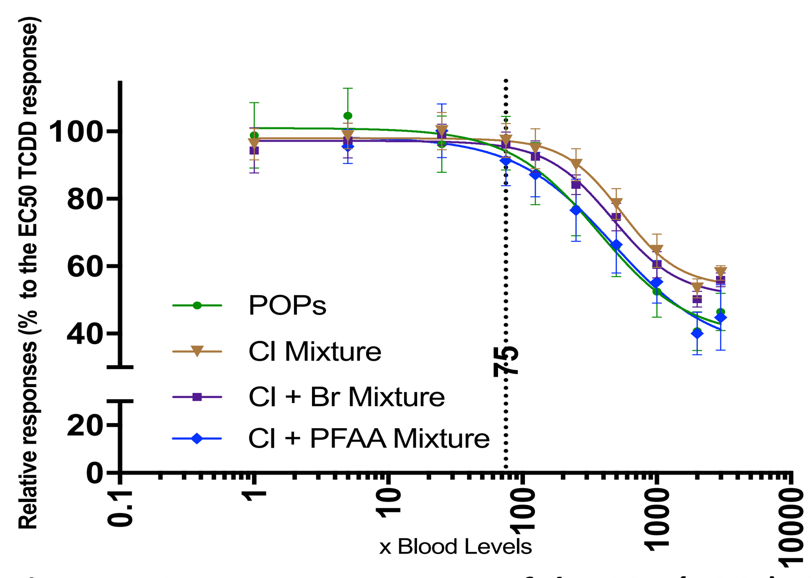

Figure 1: Dose-response curves of the POP (POPs), Cl, Cl + Br and Cl + PFAA Mixture co-exposed with 15 pM TCDD

Table 3: IC_{50} (x blood levels, µM) and efficiencies of POP and 6 sub-mixtures

Mixtures	IC_{50} (xblood levels)	IC_{50} (µM)	Efficiency
POP	**371 ± 52**	**21.77 ± 3.1**	**39%**
PFAA	No	No	No
Br	No	No	No
Cl	**547 ± 44**	**1.9 ± 0.15**	**54%**
Cl + Br	468 ± 38	1.5 ± 0.12	51%
Cl + PFAA	**472 ± 87**	**27 ± 5**	**35%**
PFAA + Br	No	No	No

➢ Lowest effective concentration already at 75 times the blood level (dash line in Figure 1)
➢ Cl mixture is responsible for 80% of the POP response, no effects seen for PFAA and Br mixtures
➢ But only Cl + PFAA mixture induced the same response as the POP mixture
  ➔ Perfluorinated compounds are probably non-specific rAhR antagonists

❑ **Measued vs Predicted IC_{50} of POP and Cl mixtures**

➢ Cl mixture: calculated IC_{50} (2.3 µM) = measured IC_{50} (1.9 µM)
  ➔ 7 PCBs + 9 Organochlorine compounds act additively in the Cl mixture
➢ POP mixture: calculated IC_{50} (43.25 µM) > measured IC_{50} (21.77 µM), along with non-specific rAhR antagonism of PFAA mixture ➔ **possible synergistic effect**

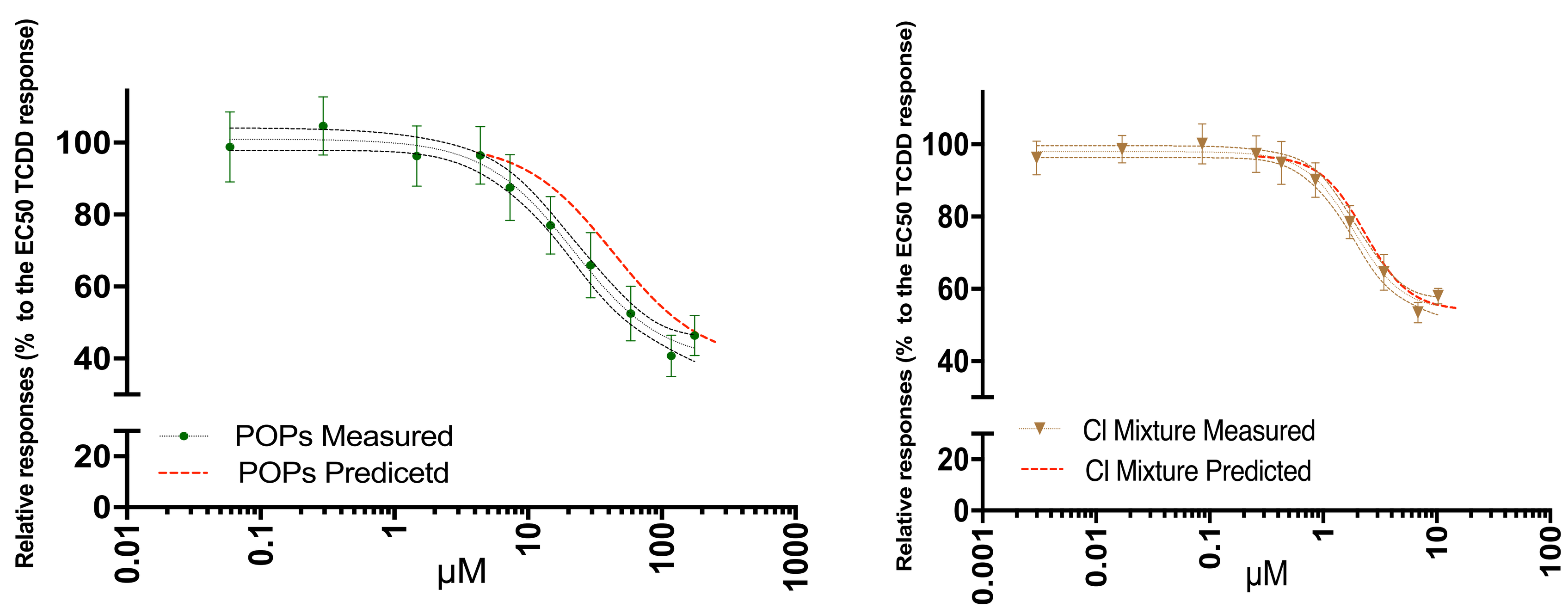

Figure 2: Dose-response curves of the POP (right) and Cl (left) mixtures measured and predicted according to an addition concentration model (Payne et al., 2000) co-exposed with 15 pM TCDD

DISCUSSIONS AND CONCLUSIONS

❖ POP mixture acts as rAhR antagonist, illustrating the principle of "something from nothing" (Thrupp et al. 2018)
❖ Lower POP mixture effective concentration of 75 times the blood level ➔ plausibly reached in humans after a food contamination incident/ highly exposed sub-populations
❖ Perfluorinated compounds are probably non-specific rAhR antagonists
❖ Additive effect seen for the sub Cl mixture but a possible synergistic effect seen for the POP mixture