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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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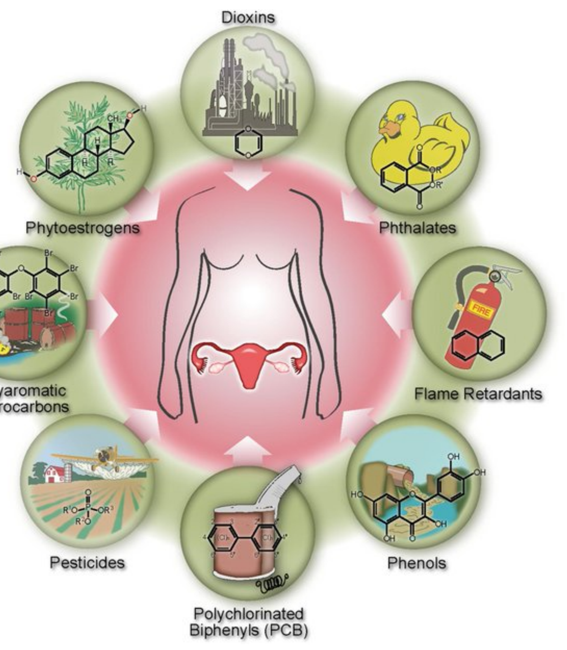
INTRODUCTION

❖ 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**

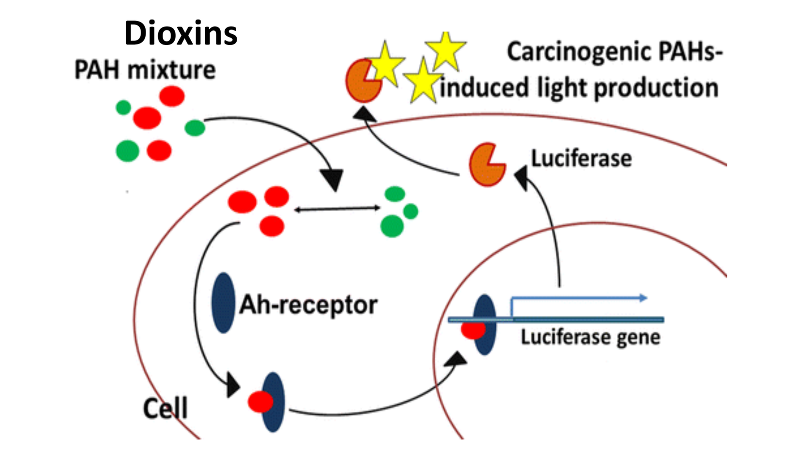
➢ ***Aims to determine, in vitro, how POPs act simultaneously in the mixture to produce an effect at the level of the rat Aryl hydrocarbon Receptor (rAhR) function***

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

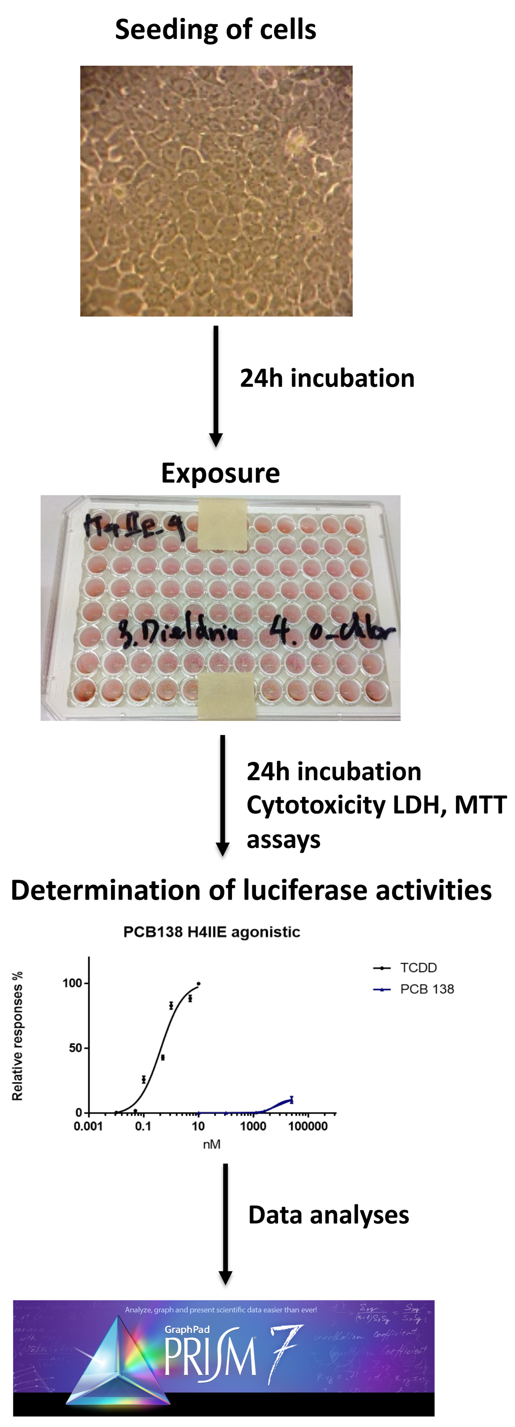
MATERIALS AND METHODS

❖ 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**

❑ **<u>29 POPs</u>** (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

❑ **<u>POP mixture</u>** = Mixture of 29 tested POPs

❑ **<u>6 Sub-mixture</u>** (Berntsen et al., 2017)

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

REFERENCES

1. Berntsen, H.F, Berg, V. Thomsen, C., Ropstad E., & Zimmer, K.E. (2017) The design of an environmentally relevant mixture of persistent organic pollutants for use in in vivo and in vitro studies, Journal of Toxicology and Environmental Health, Part A, 80:16-18, 1002-1016
2. Payne, J., Nissanka, R., Megan, W., & Andreas, K. 2000. "Prediction and Assessment of the Effects of Mixtures of Four Xenoestrogens." Environmental Health Perspectives 108(10):983–87.

RESULTS

❑ **<u>rAhR mediated-activities for 29 POPs</u>**

▪ 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*

❑ **<u>rAhR mediated-activities POP Mixture and 6 sub-mixtures</u> : Antagonism**

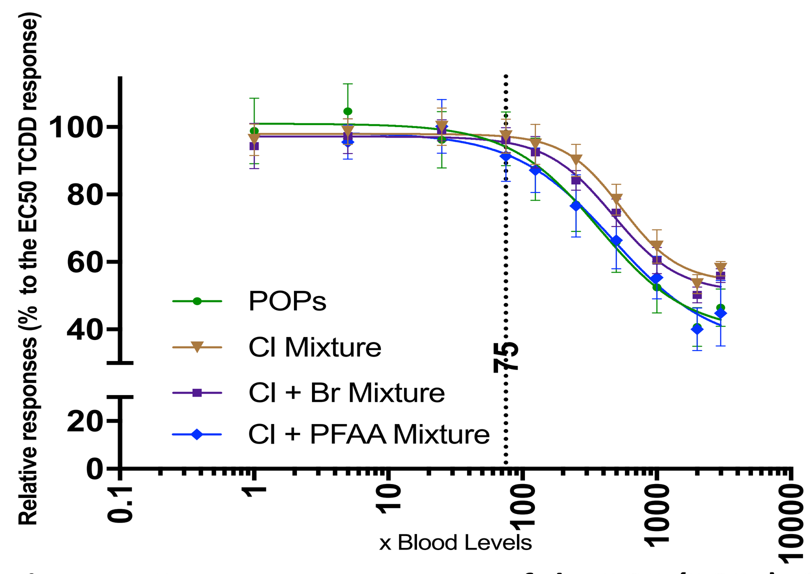


Figure 1: Dose-response curves of the POP (POPs), Cl, Cl + Br and Cl + PFAA Mixture co-exposed with 15 pM TCDD

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 corresponding to 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

❑ **<u>Measued vs Predicted IC_{50} of POP and Cl mixtures</u>**

➢ 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 ➔ **<u>possible synergistic effect</u>**

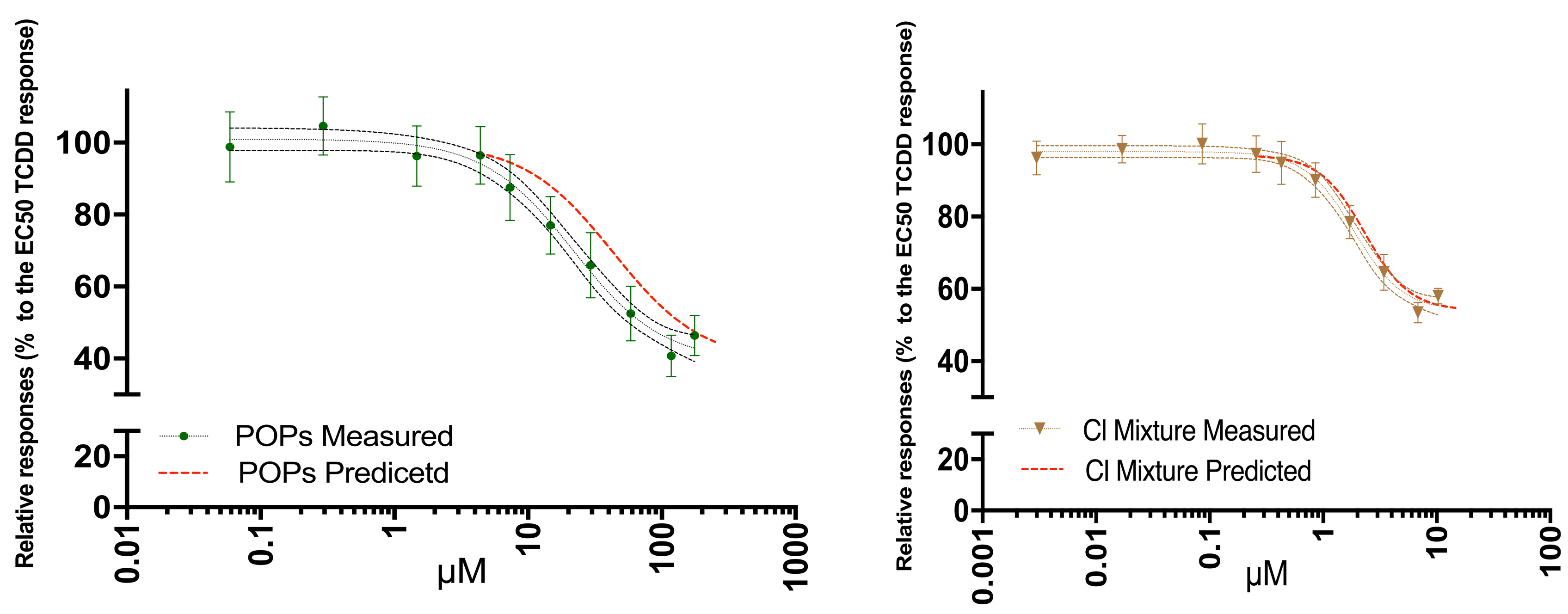


Figure 2: Dose-response curves of the POP (right) and Cl (left) mixtures measured and predicted according to an addition concentration model (Payne et al., 2000) co-exposed with 15 pM TCDD

DISCUSSIONS AND CONCLUSIONS

❖ POP mixture acts as rAhR antagonist, not agonist

❖ Lower POP mixture effective concentration of 75 times the blood level
➔ plausibly reached in humans after a food contamination incident or even in 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

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