Aryl hydrocarbon receptor transactivation assays to study health impacts of exposure to mixtures of endocrine disrupting chemicals.

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

[1]*Departement 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.*

Introduction

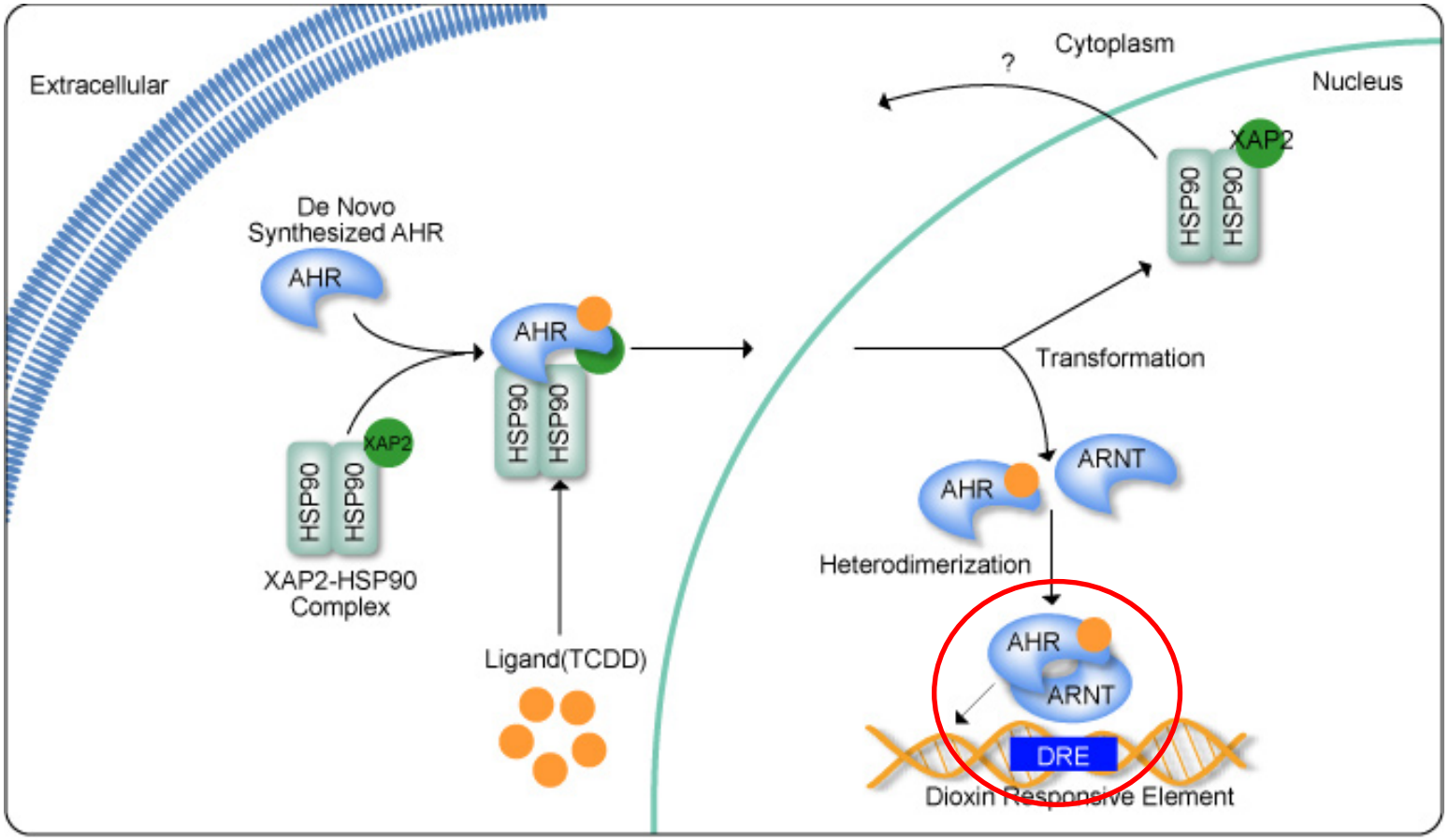
Endocrine Disrupting Chemicals

Cocktail effects???

- **Additive**
- **Antagonistic**
- **Synergistic**

http://www.makeupandmedicine.dk/2016/11/16/what-are-endocrine-disrupting-chemicals-part-two/

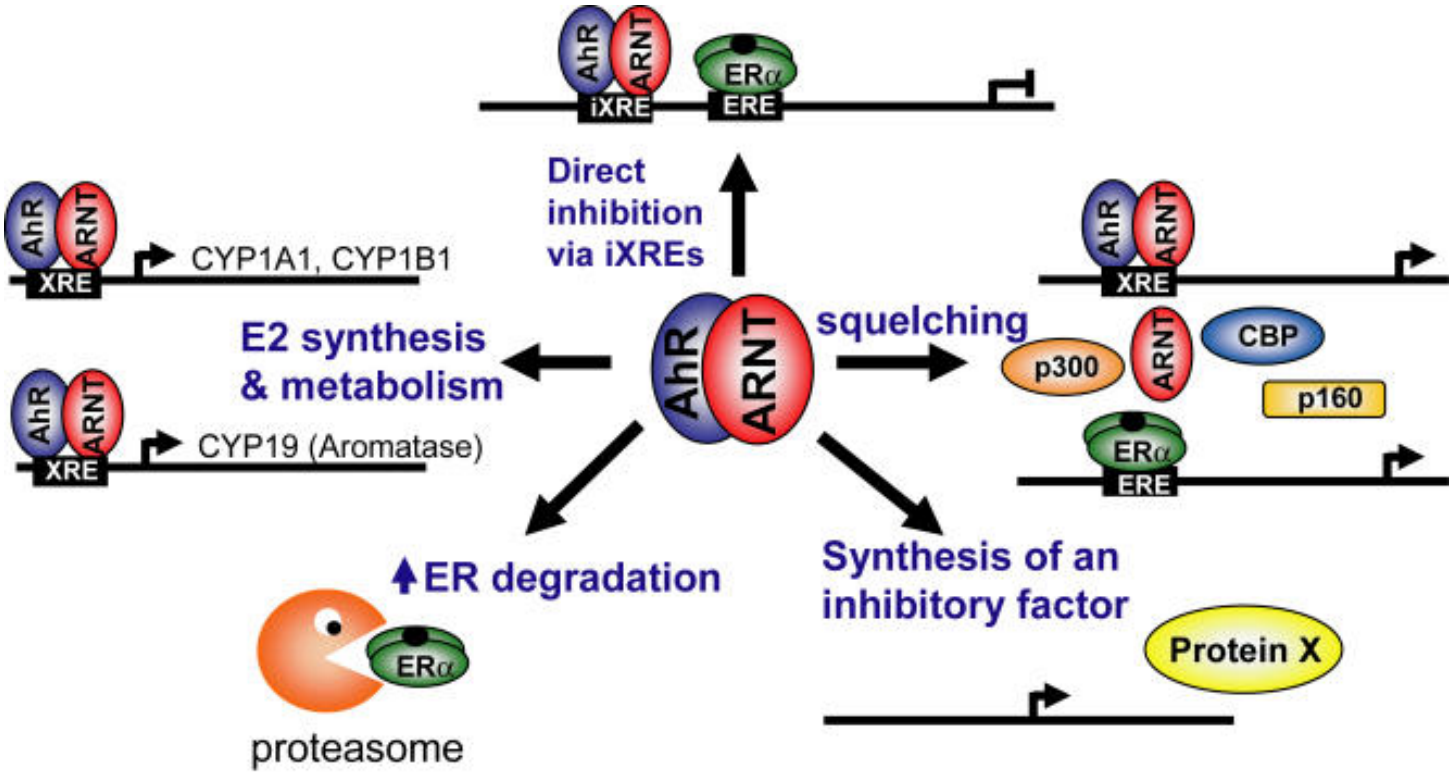
Aryl hydrocarbon receptor (AhR) signal pathway



http://www.genecopoeia.com/product/search/pathway/h_acrPathway.php

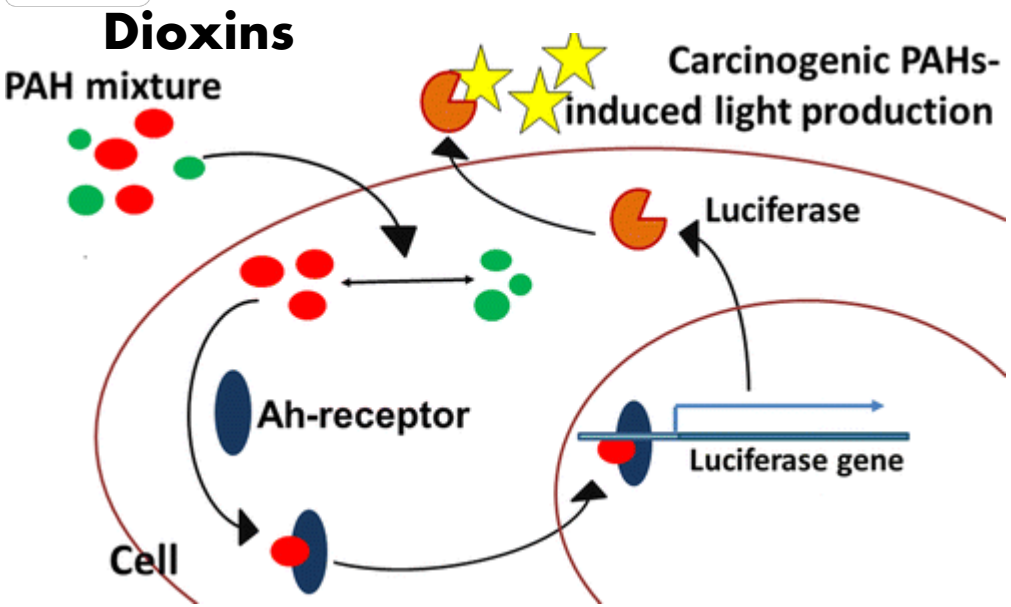
*Aryl Hydrocarbon Receptor Nuclear Translocator (ARNT)

AhR-ER cross-talk: AhR ↑ ➔ ER ↓



Proposed mechanisms of crosstalk between AhR (Aryl hydrocarbon receptor) and ER (Estrogen receptor) signaling pathways
Matthews and Gustafsson, 2006

Materials - Methods



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

H4IIE	T47D	Hep G2
• Rat hepatoma • Commercial, BDS • **EC50~15pM**	• Human breast • Home-made, ULg • **EC50~150pM**	• Human hepatoma • Home-made, ULg • **EC50~650pM**

➢ **Species, tissue-specific effects?**

Materials - Methods

Mixture stock concentration (1,000,000 x human blood Levels)
(Stockholm Convention on Persistent Organic Pollutants)
Berntsen et al., In Press

Perfluorinated compounds		Polybrominated diphenyl ethers		Chlorinated compounds			
PFHxS	PFNA	PBDE 47	BDE 153	PCB 28	PCB 118	p,p'-DDE	α-HCH
PFOS	PFDA	PBDE 99	BDE 154	PCB 52	PCB 138	HCB	β-HCH
PFOA	PFUnDA	BDE 100	BDE 209	PCB 101	PCB 153	α – chlordane	γ-HCH
		HBCD			PCB 180	oxy – chlordane	Dieldrin
						trans-nonachlor	

- **Individual (i.e. agonistic and antagonistic) vs cocktail effects**
- **No effects seen for perfluorinated compounds!!!**

Agonistic effects

Compounds	PBDE 47	PBDE 99	PBDE 100	PBDE 153	PBDE 154	PBDE 209	HBCD
H4IIE	-	+	-	-	-	-	-
T47D	-	+	-				-
Hep G2	-	-	-	-			-

Compounds	PCB 28	PCB 52	PCB 101	PCB 118	PCB 138	PCB 153	PCB 180
H4IIE	-	-	-	+	+	-	-
T47D	-	-	-	-	-	-	-
Hep G2	-	-	-	-	-	-	-

Compounds	p,p'-DDE	HCB	α_ chlordane	Oxy-chlordane	trans-nonachlor	α-HCH	β-HCH	γ-HCH	Dieldrin
H4IIE	-	-	-	-	-	-	-	-	-
T47D	-	-	-	-		-	-	+	-
Hep G2	-	-	-	-	-	-	-	-	-

Antagonistic effects

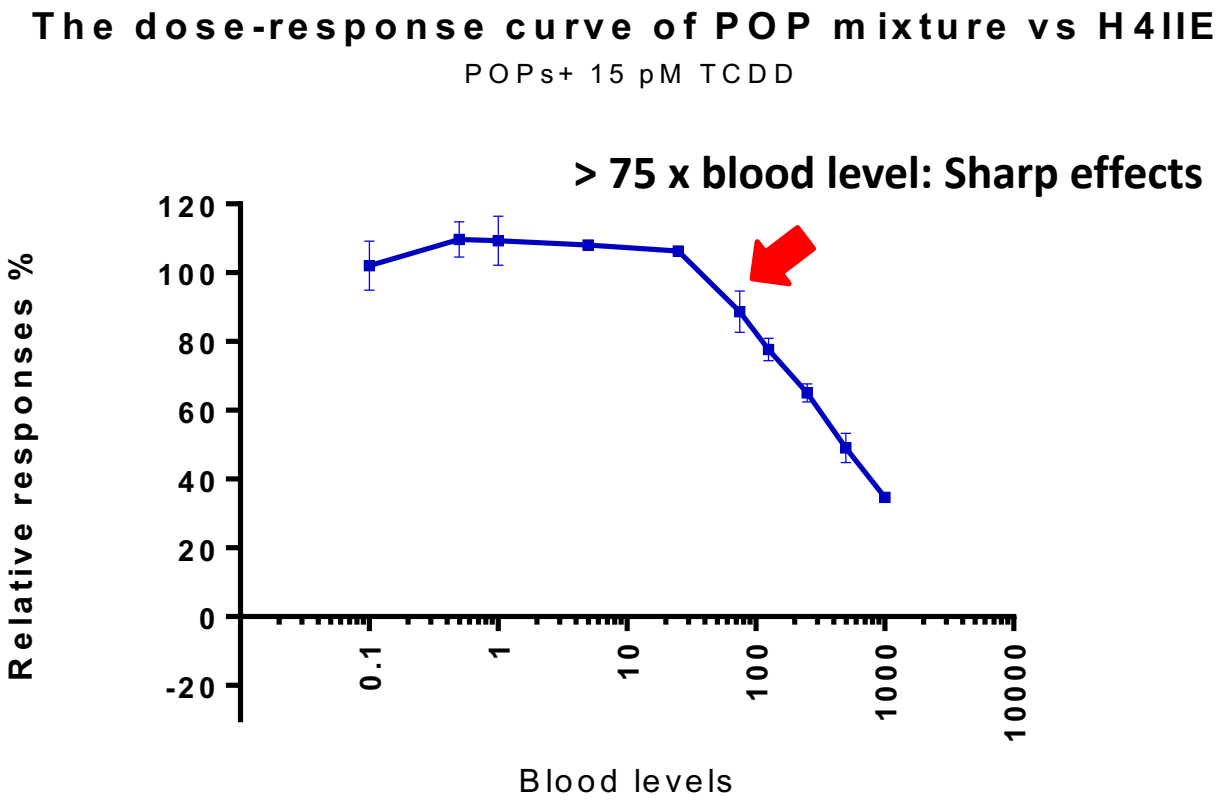
Compounds	PBDE 47	PBDE 99	PBDE 100	PBDE 153	PBDE 154	PBDE 209	HBCD
H4IIE	+	+	-	-	-	-	+
T47D	-	-	-				+
Hep G2	-	-	-	-	-	-	-

Compounds	PCB 28	PCB 52	PCB 101	PCB 118	PCB 138	PCB 153	PCB 180
H4IIE	+	+	+	+	+	+	+
T47D	+	-	-	+	+	+	-
Hep G2	+	-	-	+	+	+	-

Compounds	p,p'-DDE	HCB	α_ chlordane	Oxy-chlordane	trans-nonachlor	α-HCH	β-HCH	γ-HCH	Dieldrin
H4IIE	-	+	+	+	+	+	-	+	+
T47D	-	+	+	+	+	-	-	-	+
Hep G2	-	+	-	-	-	-	-	+	-

- Species, tissue-specific effects!

➔ Mixture effects also!!! Antagonistic

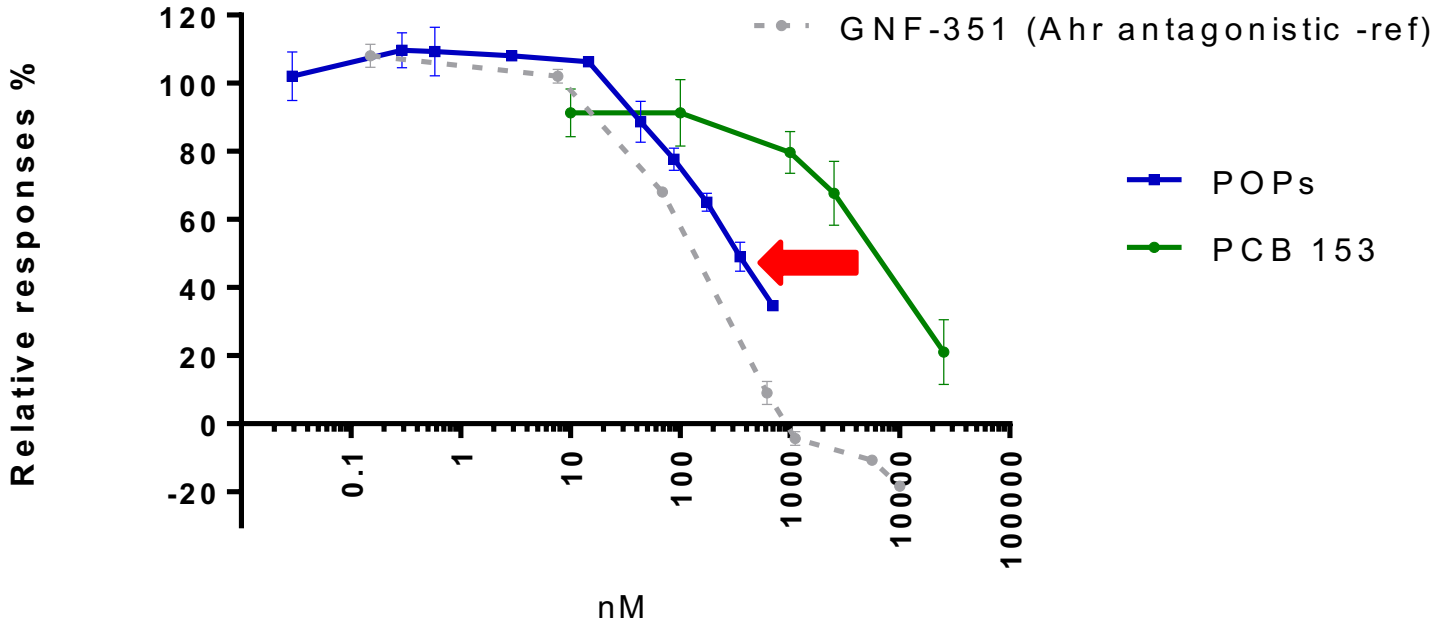


➔Inhibition of AhR transactivation activities *in vitro*

➔ Possible adverse effect ER ↑?

Results

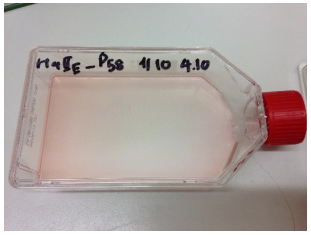
➢ **Scenario : C mixture = highest C of one compound (PCB 153)**

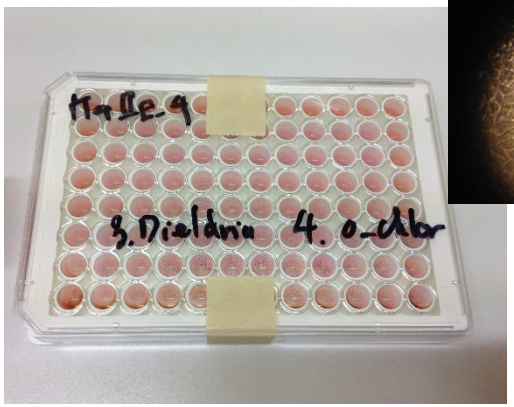


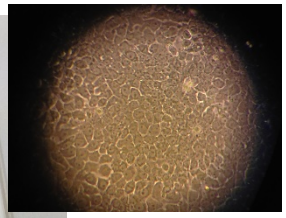
➢ **The dose-response curve is shifted 20 times less.**

➔ **The mixture effect is indeed greater than one single compounds in the mixture.**

Acknowledgements







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QUEEN'S UNIVERSITY BELFAST
DR LISA CONNOLLY
COORDINATOR

INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE
DR ISABELLE OSWALD

UNIVERSITY OF ABERDEEN
PROF PAUL FOWLER

BIODETECTION SYSTEMS
DR BART VAN DER BURG

PROTOQSAR SL
DR RAFAEL GOZALBES

LIÈGE université

UNIVERSIDAD AUSTRAL DE CHILE
DR GUDRUN KAUSEL

NORWEGIAN VETERINARY INSTITUTE
DR GUNNAR ERIKSEN

JOHN THOMPSON & SONS LTD
MR DECLAN BILLINGTON

NORWEGIAN INSTITUTE OF PUBLIC HEALTH

NORWEGIAN UNIVERSITY OF LIFE SCIENCES
PROF ERIK ROPSTAD

PUBLIC HEALTH FOUNDATION OF INDIA
DR SIDDHARTHA MANDAL

LA SOCIETE WALLONE DES EAUX