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# EXPOSURE TO DEFINED MIXTURES OF PERSISTENT ORGANIC POLLUTANTS (POPs) LEADS TO MITOCHONDRIAL INJURY IN THE RAT H4IIE HEPATOMA CELLS

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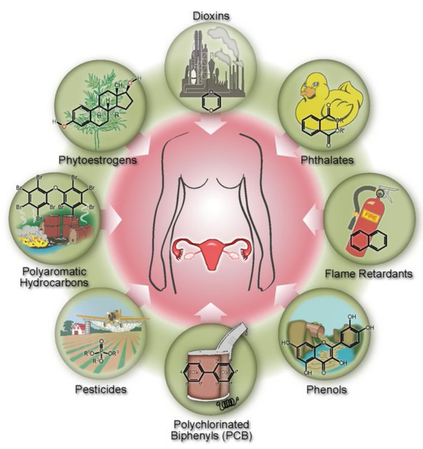
## INTRODUCTION

❖ 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




❖ Humans are exposed to POPs as mixtures and not as a single compound

❖ Few available scientific research on the effect in mixture of POPs

❖ Rat liver transgenic lines (DR)-H4IIE (Biodetection system, Netherlands) are widely applied for screening the Aryl hydrocarbon receptor transactivity of POPs

❖ High Content Analysis (HCA): a high-throughput, quantitative fluorescence technique to study sublethal and subcellular cytotoxicity

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

➢ ***Aims to determine the overall health effects regarding several cellular responses of seven complex mixtures in the rat DR-H4IIE using HCA***

## MATERIALS AND METHODS

### ❖ Test chemicals

❑ **29 POPs** prevalent in Scandinavian human blood (Stockholm Convention 2001)

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

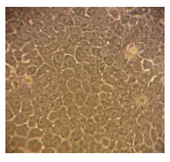
| 6 PFAA: Perfluorinated Compounds | 7 Br: Brominated Compounds | 13 Cl: 7 PCBs + 9 Organochlorine Compounds | |
|---|---|---|---|
| • PFHxS | • BDE 47 | • PCB 28 | • HCB |
| • PFOS | • BDE 99 | • PCB 52 | • α-chlordane |
| • PFOA | • BDE 100 | • PCB 101 | • o-chlordane |
| • PFNA | • BDE 153 | • PCB 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 |

| | |
|---|---|
| • **PFAA Sub-mixture** | • **Cl + Br Sub-mixture** |
| • **Br Sub-mixture** | • **Cl + PFAA Sub-mixture** |
| • **Cl Sub-mixture** | • **Br + PFAA Sub-mixture** |

### ❖ Methods

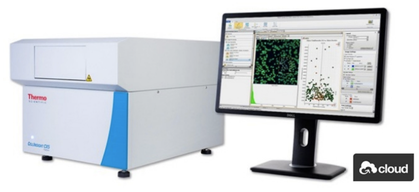
❑ Detect sublethal and subcellular nuclear and mitochondrial cytotoxicity in DR-H4IIE (Biodetection system, Netherlands) exposed to POP (sub-) mixtures by applying High Content Analysis (HCA)

**80% confluence**



**24h seeding ($10^5$ cells/ml)**
**24h exposure to POP (sub-)mixtures**
**Staining with two dyes**

**Read endpoints**
**- Hoechst dye (Ex/Em 350/461 nm): nuclear responses (cell number (CN), nuclear area (NA), and nuclear intensity (NI))**
**- MitoTracker dye (Ex/Em 554/ 576 nm): mitochondrial responses (mitochondrial area (MA) and mitochondrial intensity (MI))**



**Data analyses**


## RESULTS

### ❖ Cell number (CN) and mitochondrial intensity (MI): good markers for sublethal cytotoxicity of the rat DR-H4IIE exposed to the POP (sub)- mixtures

❑ Only Cl containing mixtures (the total POP mixture, Cl, Br+Cl and PFAA+Cl) were significantly decreasing in CN and MI at 1000 folds the blood levels, except the Br+Cl at already 100 blood folds.

❑ PFAA and Br or their combination PFAA+Br sub-mixtures alone did not exhibited significant cytotoxicity effects.

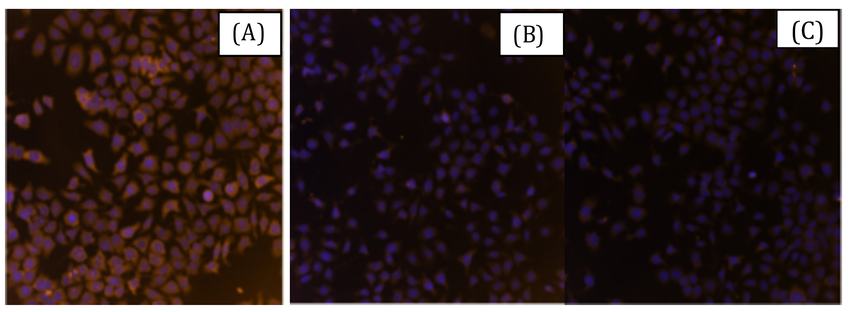
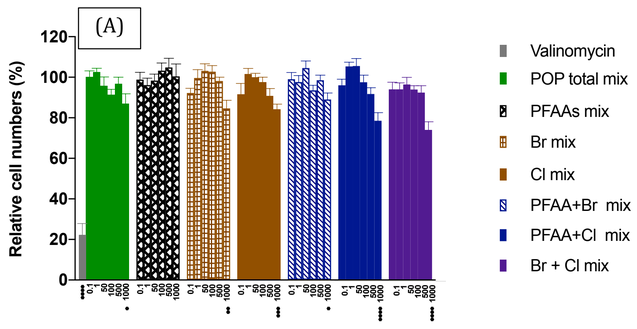



**Figure 1:** HCA images for (A) negative control (DMSO), (B) positive control (100 nM Valinomycin), (C) an example of DR-H4IIE exposed to the total POP mixture at 1000 folds blood levels after 24h exposure. The image was acquired at 10× objective magnification using Hoechst dye (blue; nuclear staining) and MitoTracker dye (orange; mitochondrial staining).



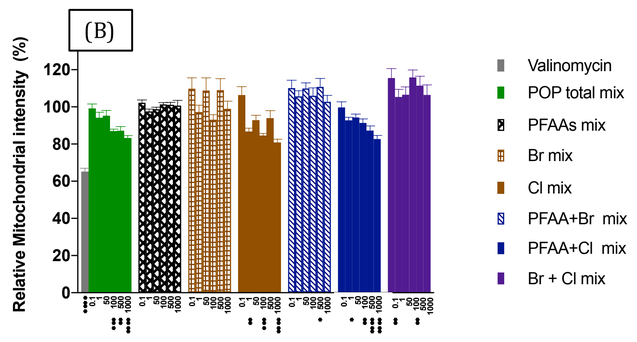




**Figure 2:** Cell viability in cell number (CN, Figure 1A), and mitochondrial intensity (MI, Figure 1B) of the total POP mixture (green, solid), PFAAs (black, triangle), Br (brown, square), Cl (brown, solid), PFAA+Br (blue, parallel), PFAA+Cl (blue, solid), and Br+Cl mixtures (purple, solid) in DR-H4IIE cells exposed to six different concentrations (1/10, 1, 50, 100, 500 and 1000 times serum levels) for 24h. Data expressed relative to the control DMSO treatment, Mean ± SE, n=3, p≤0.05 (*), p≤0.01 (**), p≤0.001 (***), p≤0.0001 (****) expressing statistical significance.

❖Expose to the Cl containing mixtures decreased the cell number by about 20%
➔ PFAA+Cl and Br+Cl mixtures by 21% and 26%
➔ Total POP and Cl mixture by only 13 % and 14%

❖However, ***MI is the most sensitive parameter for detection of cytotoxicity***
➔ Significant decreases at only 100 folds the blood levels in three out of the four Cl containing mixtures (the total POP mixture, the PFAA+Cl and Cl mixtures by around 20%), while no MI reduction in the Br+Cl mixture
➔ MI decrease reflects cells experiencing the loss of their mitochondrial potential, but no changes were observed for MA

## DISCUSSIONS AND CONCLUSIONS

❖MI reduction: an early marker for an apoptosis-induction pathway (Gottlieb et al 2003)
➔ At a concentration of 100 folds the blood levels, DR-H4IIE cells are entering apoptosis, but the other markers namely, MA or NI and NA, have not occurred yet due to insufficient exposure time and/or concentrations.

❖ Potential associated health effects
➔ Exposure to POPs *in vitro* (AhR agonists) leads to mitochondrial impairment of generating ATP, which could lead to weight gain, glucose intolerance and metabolic syndrome (Park et al. 2013). But no relationship was seen for BDE-47, OCPs or PFAAs.
➔ our group also showed an antagonistic effect on the aryl hydrocarbon receptor transactivity of the total POP mixture as well as the other three Cl containing mixtures (Cl, PFAA+Cl, and Br+Cl)

❖ In conclusion, the MI was observed as the earliest marker for apoptosis in the rat DR-H4IIE exposed to the total POP and two other Cl containing sub-mixtures (Cl and PFAA+Cl), at a concentration of already 100 blood folds, followed by the CN at 1000 folds the blood levels for all seven mixtures, except the PFAA mixture. The MA or NI and NA showed no changed within the tested concentration range.

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