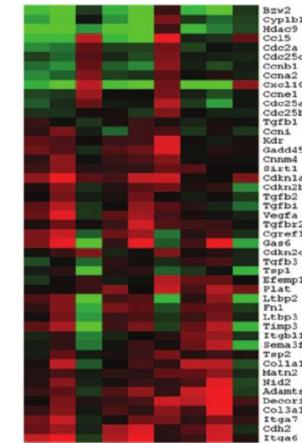
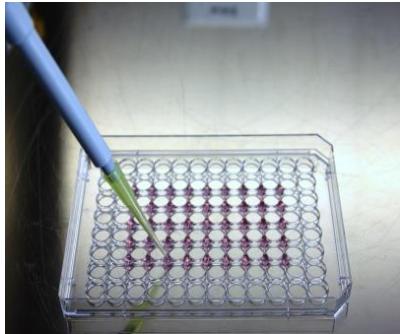


# A Battery of In Vitro Assays for Prenatal Developmental Toxicity Testing of Petroleum Substances

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12<sup>th</sup> Concawe Symposium  
March 21<sup>st</sup>, 2017  
Antwerp, Belgium

# Petroleum substances

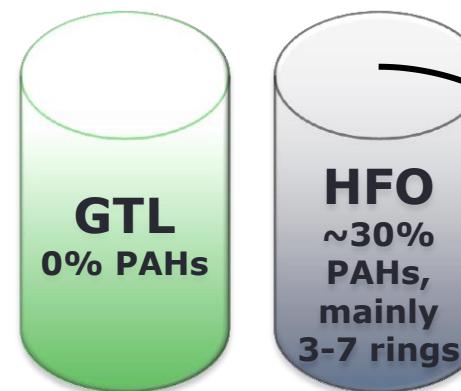
are UVCBs\* comprising a wide variety and amount of hydrocarbons, including **polycyclic aromatic hydrocarbons (PAHs)**.



\*Substances of Unknown or Variable composition, Complex reaction products or Biological materials.

## Negative:

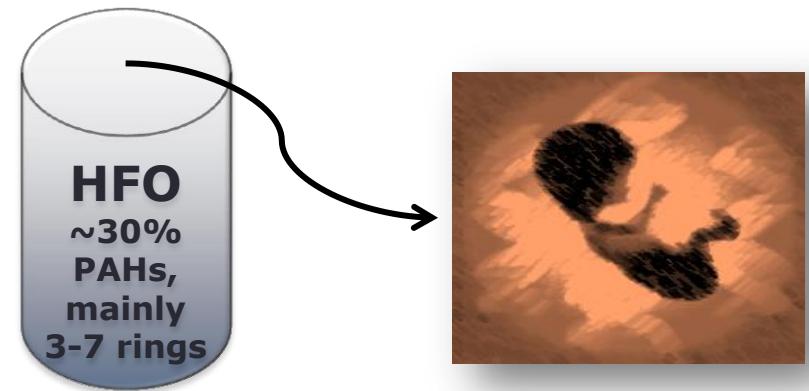
- prenatal developmental toxicity
    - two generation reproductive toxicity studies
- (both GTL gas oil and base oil)



prenatal developmental toxicity  
(PNDT)

HFO: heavy fuel oil  
GTL: gas-to-liquid

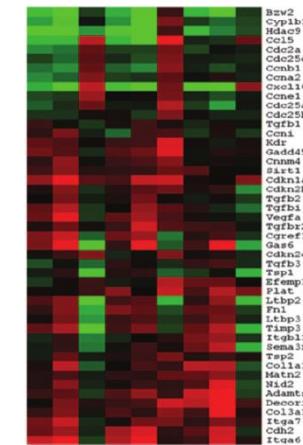
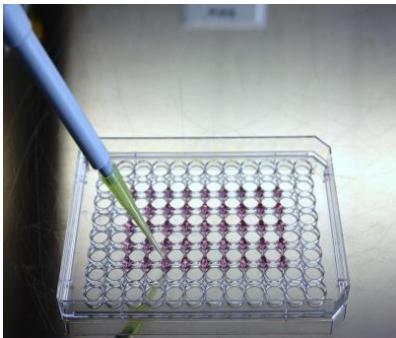
**Hypothesis:** PAHs are the sole inducer of prenatal developmental toxicity (PNDT) in petroleum products.



# Objectives

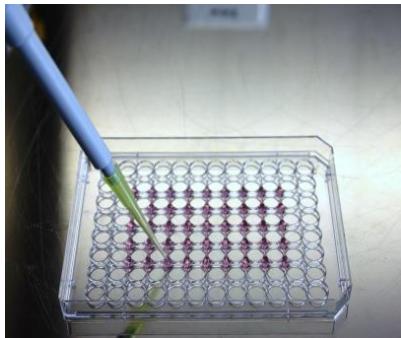
**PNDT studies are animal intensive**

applying a battery of in vitro test systems  
in combination with -omics technologies



to study the PNDT potencies of petroleum substances

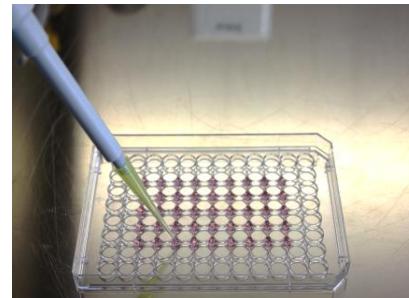
# Objectives



## Embryonic stem cell test (EST)

- **ES-D3 cell viability assay**
- **ES-D3 cell differentiation assay**

- DMSO-extract of:
  - ✓ 9 pet. substances:
    - 2 GO
    - 1 VTO
    - 2 RAE
    - 3 DAE
    - 1 HFO
  - ✓ 2 GTL products:
    - GTL base oil
    - GTL gas oil

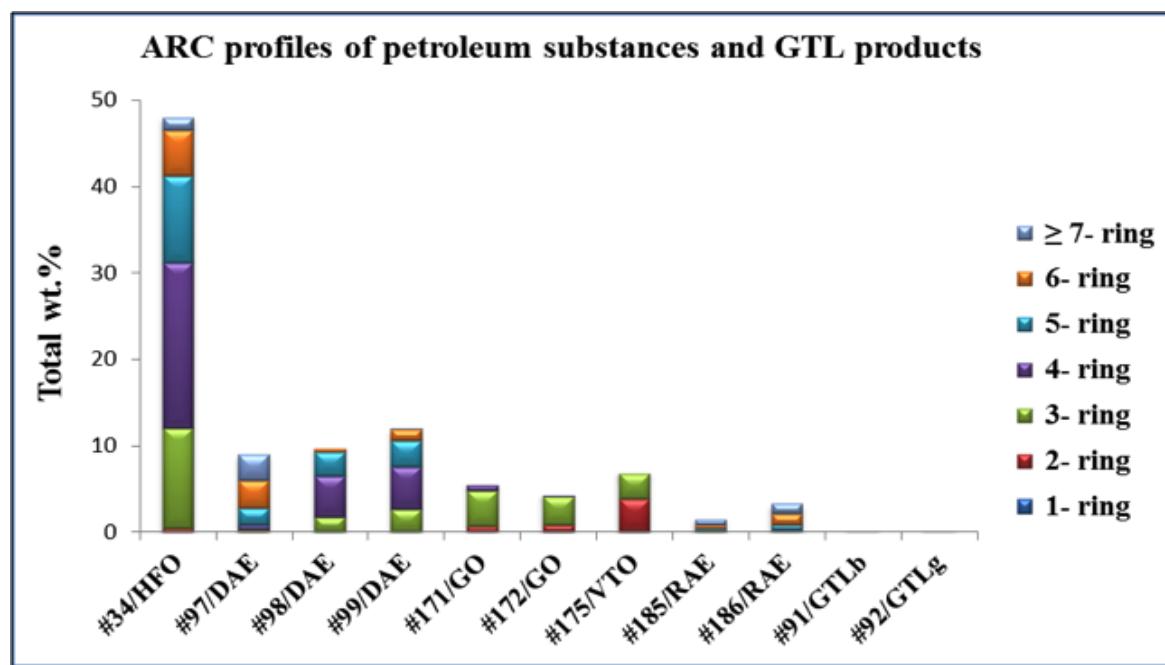


**ES-D3 cell viability  
&  
ES-D3 cell differentiation  
assays of the EST**

**In vitro prenatal  
developmental  
toxicity potencies**



**in vitro  
in vivo  
comparison**



Note:

HFO: heavy fuel oil

DAE: distillate aromatic extract

GO: gas oil

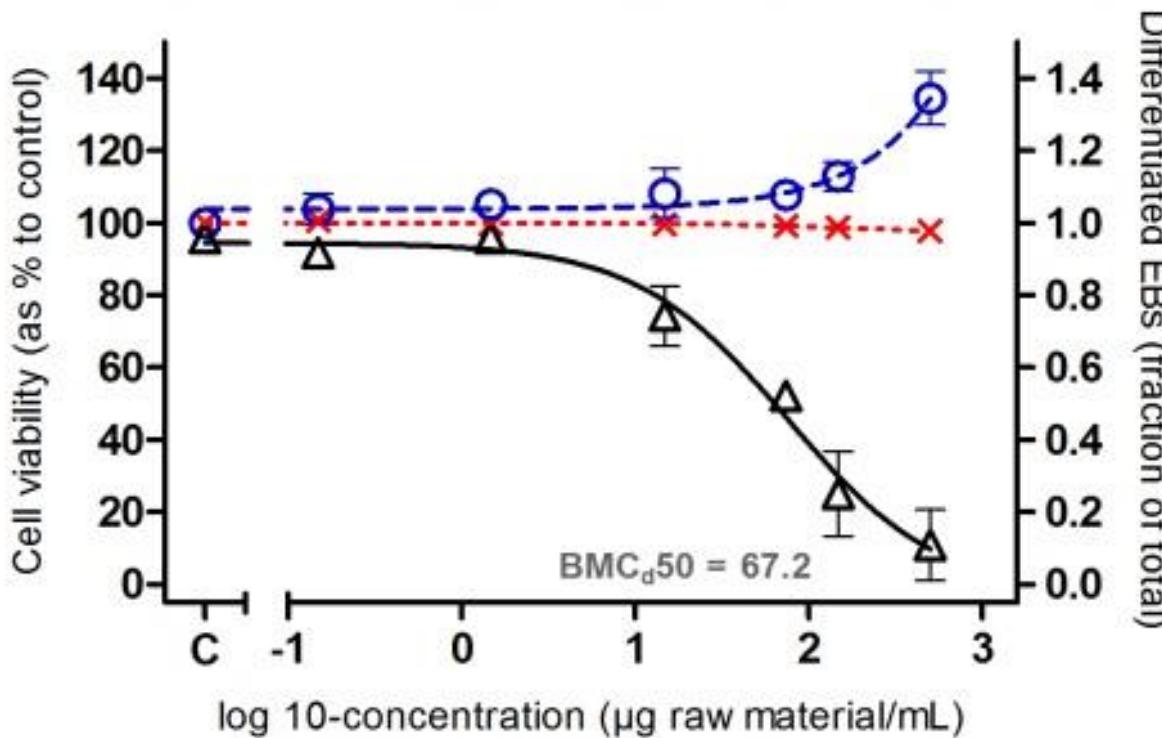
VTO: vacuum tower overhead

RAE: residual aromatic extract

GTL: gas-to-liquid

**ARC profile:** the weight percent of the DMSO-soluble 1- to ≥7 aromatic-ring compounds present in each sample, from the starting material of 4.0 gram sample.

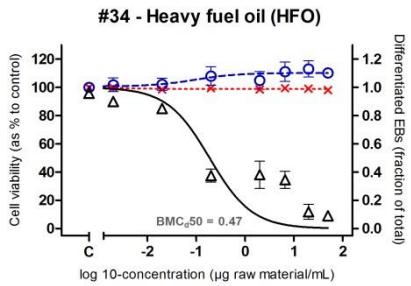
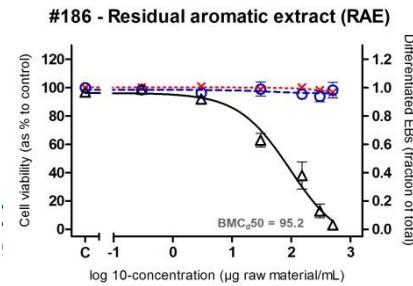
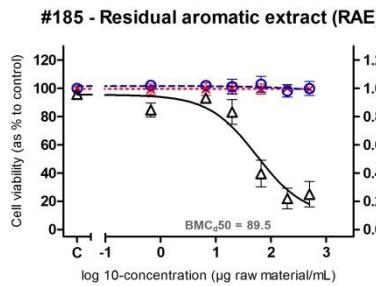
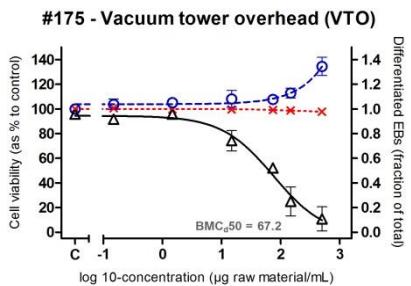
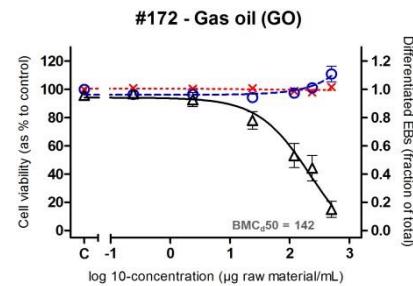
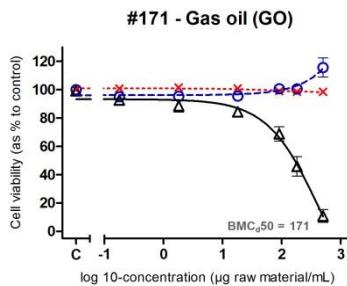
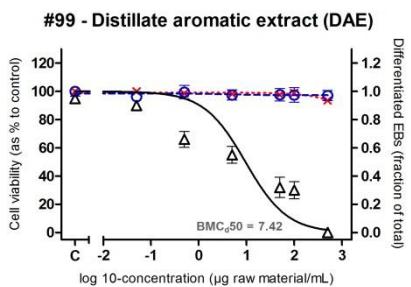
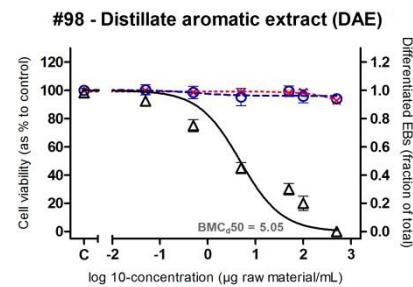
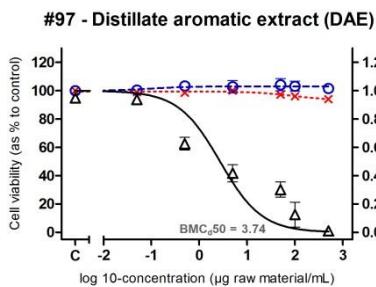
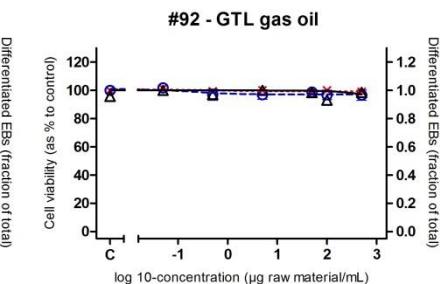
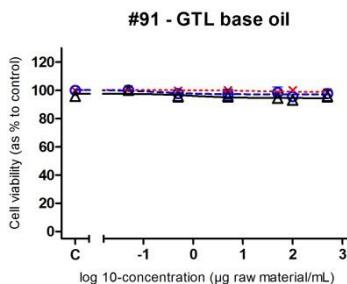
## #175 - Vacuum tower overhead (VTO)



(x) : 1-day exposure ES-D3 cell viability assay (n=3)  
(o) : 5-days exposure ES-D3 cell viability assay (n=3)  
(Δ) : ES-D3 cell differentiation assay (n=5)

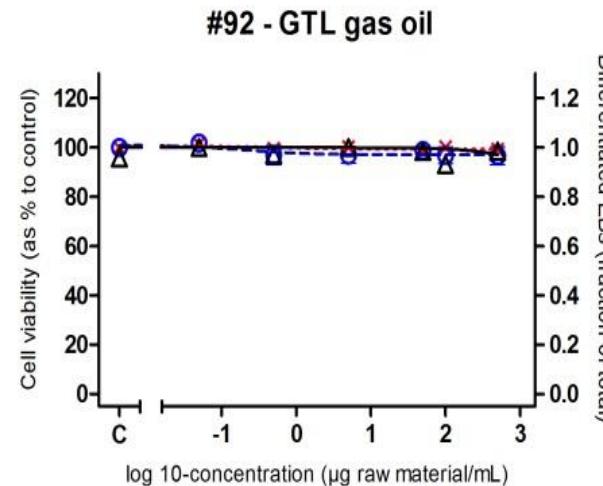
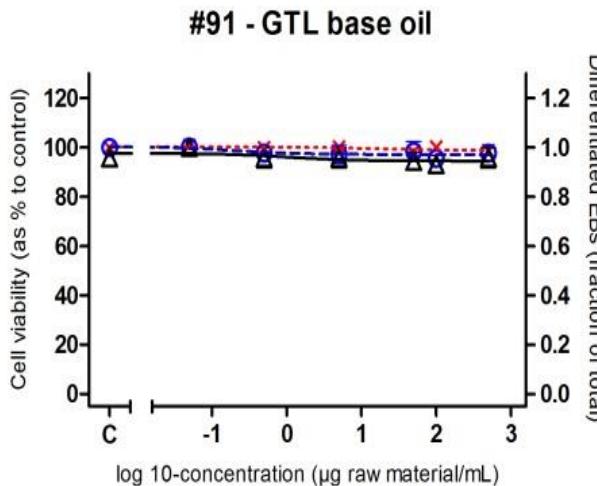
\* $BMC_d50$ : the benchmark concentration for 50% inhibition of cell differentiation

## ES-D3 cell viability assay of the EST



→ There is no decrease in cell viability after 1-day & 5-days of exposure.

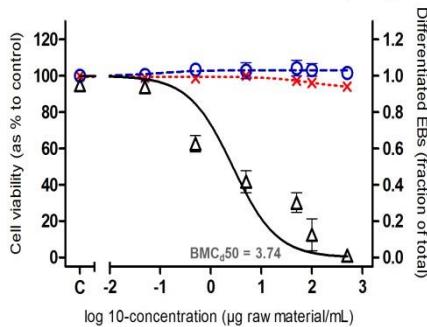
## ES-D3 cell differentiation assay of the EST – GTL products



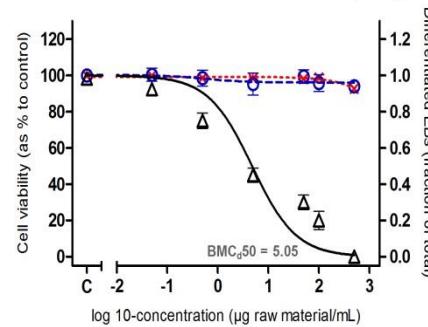
→ **GTL products:**  
no inhibition of ES-D3 cell differentiation.

## ES-D3 cell differentiation assay of the EST – petroleum substances

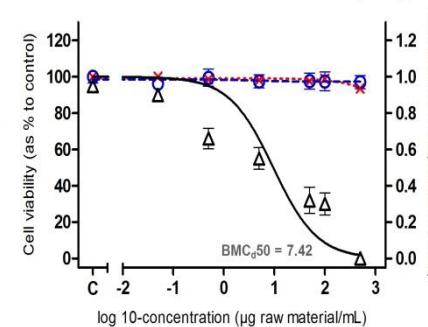
#97 - Distillate aromatic extract (DAE)



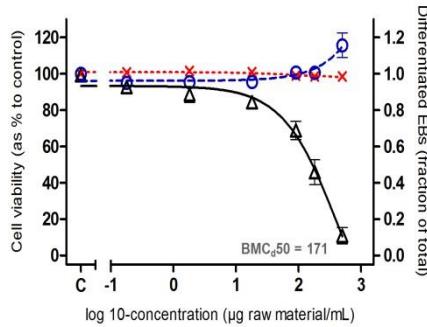
#98 - Distillate aromatic extract (DAE)



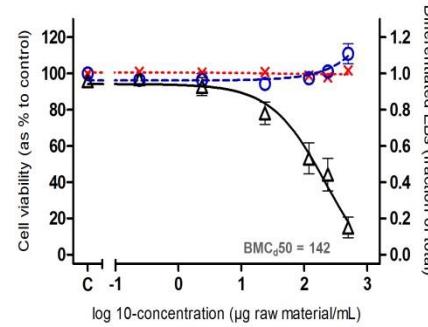
#99 - Distillate aromatic extract (DAE)



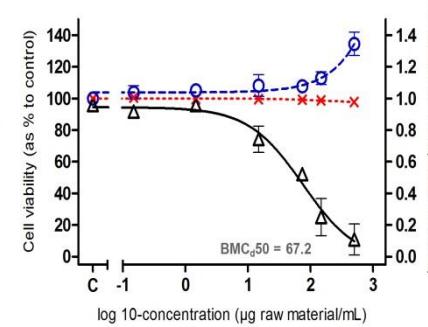
#171 - Gas oil (GO)



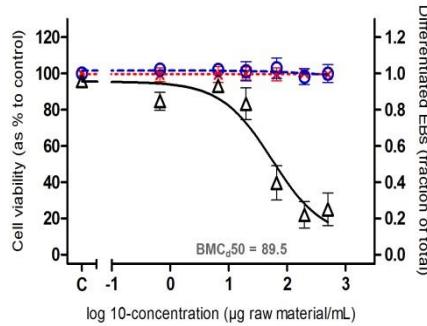
#172 - Gas oil (GO)



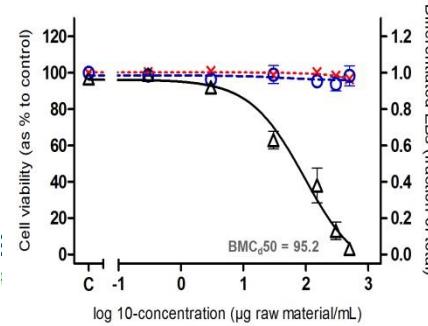
#175 - Vacuum tower overhead (VTO)



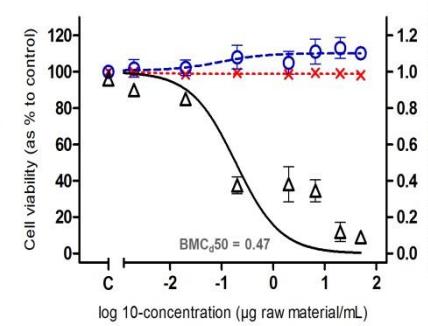
#185 - Residual aromatic extract (RAE)



#186 - Residual aromatic extract (RAE)

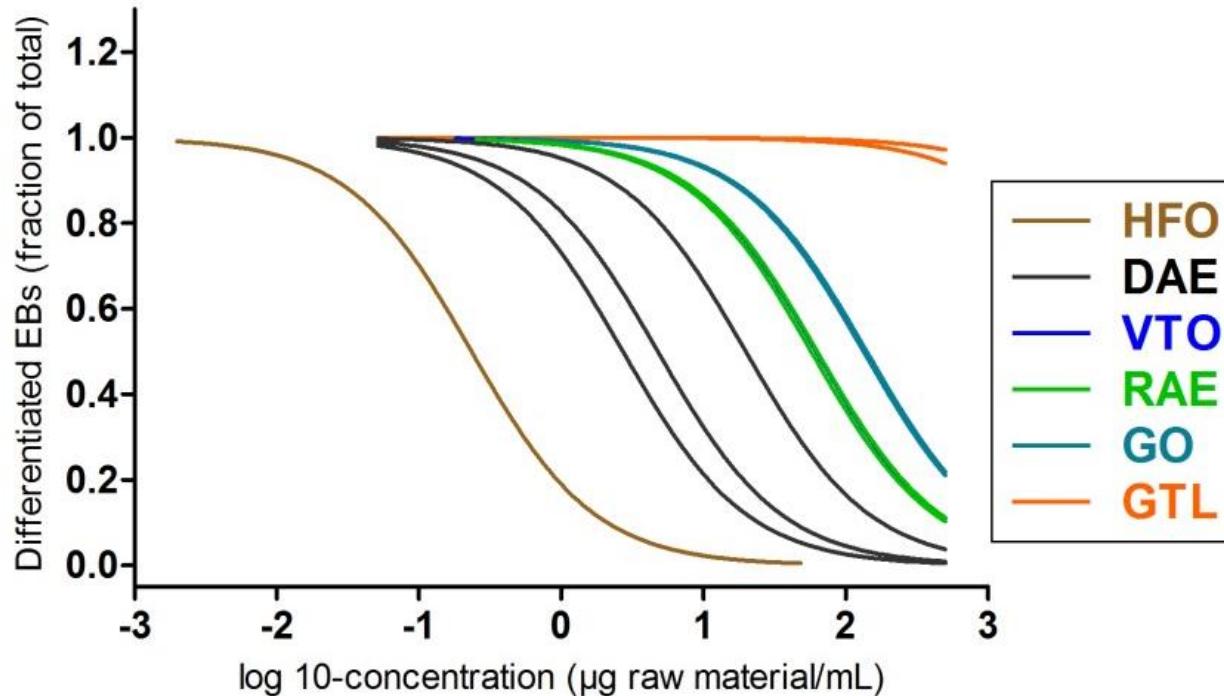


#34 - Heavy fuel oil (HFO)



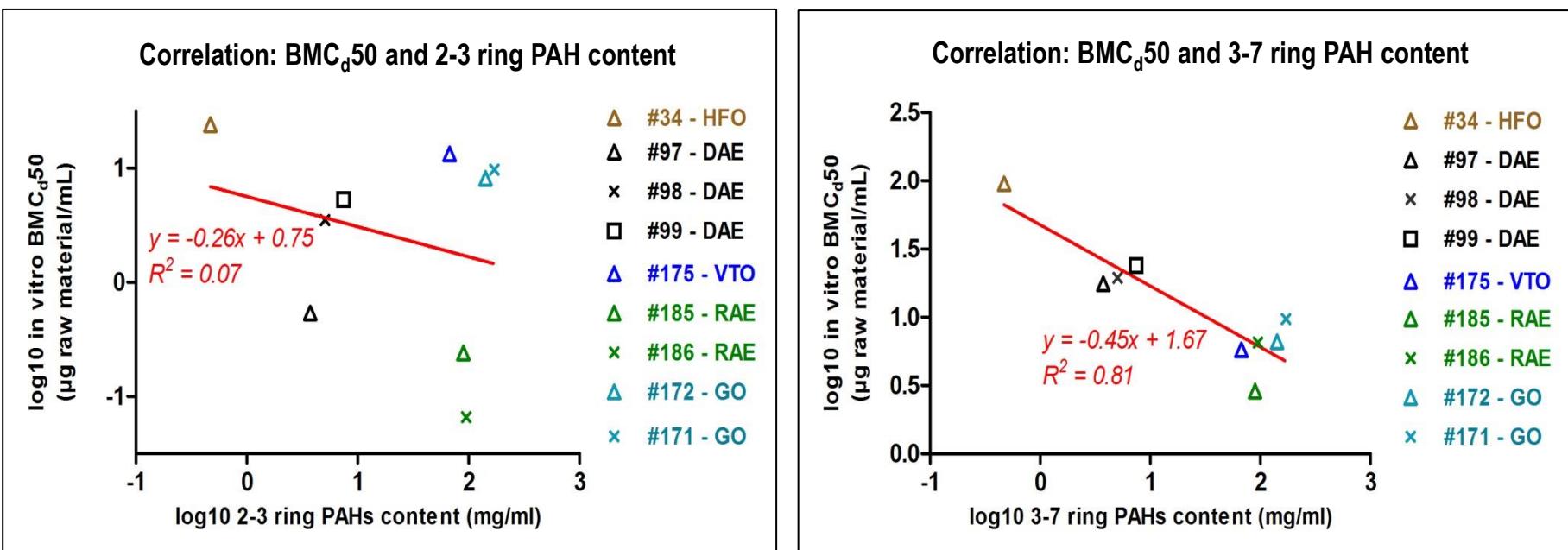
→ Petroleum substances:  
concentration-dependent  
inhibition of ES-D3 cell  
differentiation.

**Comparison:** concentration-dependent inhibition of ES-D3 cell differentiation upon exposure to DMSO-extracts of petroleum substances and GTL products.



\* $BMC_d50$ : the benchmark concentration for 50% inhibition of cell differentiation, which was calculated using the EPA-BMD software.

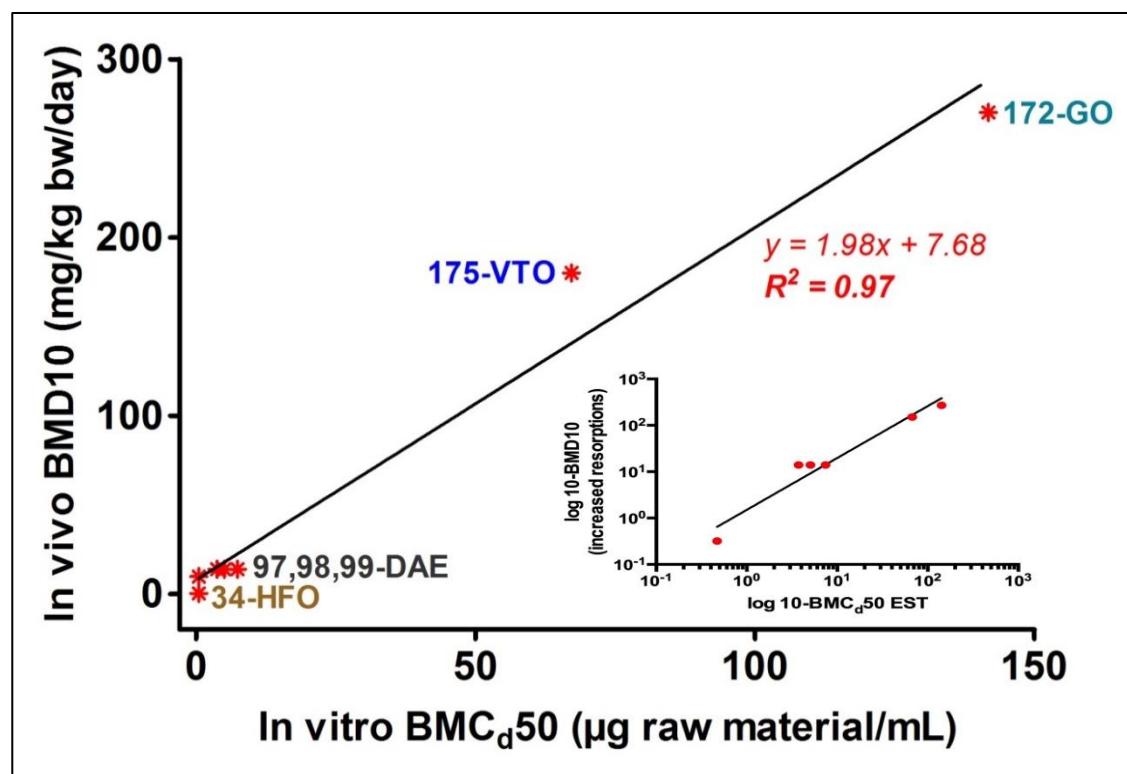
## Correlation: in vitro BMC<sub>d</sub>50 values (ES-D3 cell diff. assay) and specific PAH content



- No correlation to total 2-3 ring PAH content.
- A better correlation was obtained when BMC<sub>d</sub>50 values were plotted against the total 3-7 ring PAH content.

**Correlation:** in vitro  $\text{BMC}_{d50}$  values (ES-D3 cell differentiation assay-EST), and in vivo  $\text{BMD}10^*$  values (based on the increased incidence in resorptions)

\* $\text{BMD}10$ : the benchmark dose for a 10% additional incidence of resorptions, which was determined using the EPA-BMD software.

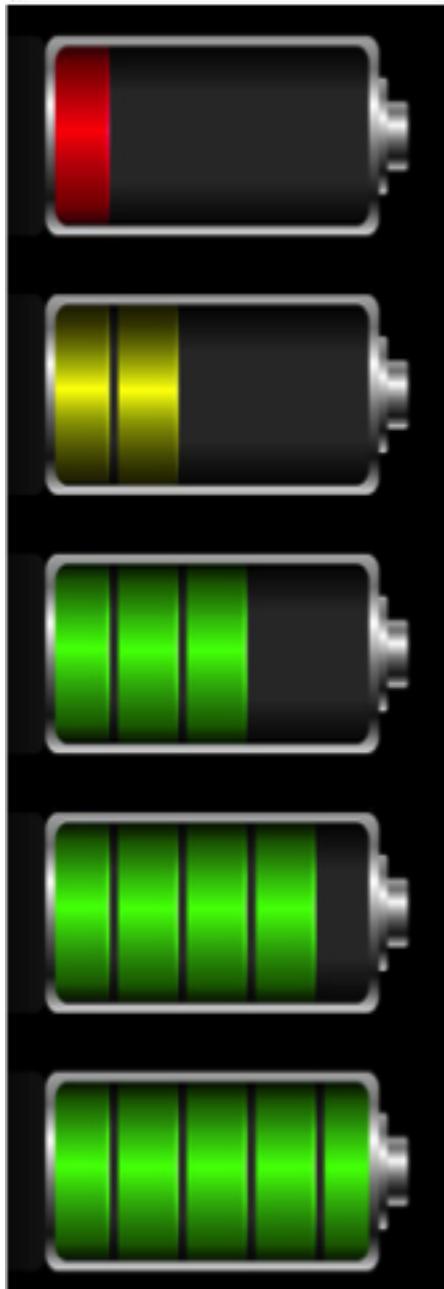


→ Good correlation: in vitro potencies to in vivo PNNT potencies.

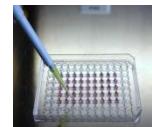
- GTL samples did not decrease viability and also did not inhibit differentiation of the ES-D3 cells, whereas petroleum substances samples did decrease the differentiation at non-cytotoxic concentrations.  
**In vitro ranking: HFO > DAE > VTO > RAE > GO**
- A good correlation was found between in vitro and in vivo PNNT potencies of petroleum substances.
- The ES-D3 cell differentiation assay of the EST was able to assess the in vitro embryotoxicity of petroleum substances (UVCBs), within and among classes, suggesting the applicability of this assay for grouping similar substances.
- **Our findings in the EST support the hypothesis that 3-7 ring PAHs are the sole inducers of PDT induced by petroleum substances.**

# Dissemination of results

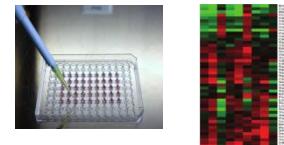
- ETS 2016 Conference, Dublin (by Lenny Kamelia)
- International Conference on Toxicity Testing Alternatives & Translational Toxicology 2016, Hangzhou (by Peter J. Boogaard)
- **Publication:** Prenatal developmental toxicity testing of petroleum substances: application of the mouse embryonic stem cell test (EST) to compare in vitro potencies with potencies observed in vivo (*submitted*).



### Embryonic stem cell test (EST)



### (+) EST + Metabolism/gene expression studies



### (+) Zebrafish embryo test (ZET)



### (+) A series of relevant cell line

EST + Metabolism/gene exp. studies  
Zebrafish embryo test (ZET)  
A series of relevant cell line  
(+) *C. elegans* with knock-in P450 genes

For a better understanding,  
&  
to unravel the association:  
**PAHs ~ PNNT**





Timothy Roy – Port Royal Research Lab

Hans Ketelslegers - Concawe

Laura de Haan,

Jochem Louisse,

Ivonne. M.C.M. Rietjens,

Peter J. Boogaard.

# Thankyou