NIFES

Evaluation of toxicological effects of POPs using primary fish cell cultures

Liv Søfteland, Invar Eide and Pål A. Olsvik

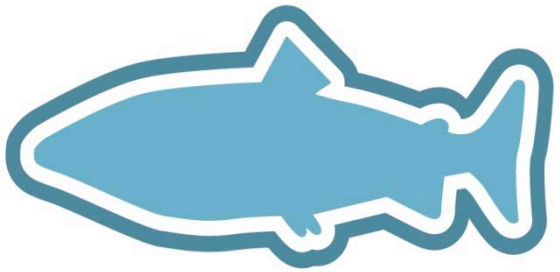
National Institute of Nutrition and Seafood Research

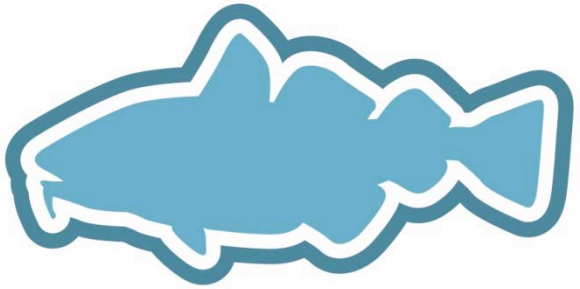
www.des.umd.edu

www.cleantechnologies.co.

Primary fish cells

- 90% of all experimental animals used in research in Norway are fish
- Important to develop *in vitro* fish bioassays
  - refine and replace existing fish experimental protocols
  - reduce the number of fish used in research
- It is important to develop species specific *in vitro* systems
  - especially important for farmed fish species with commercial interest



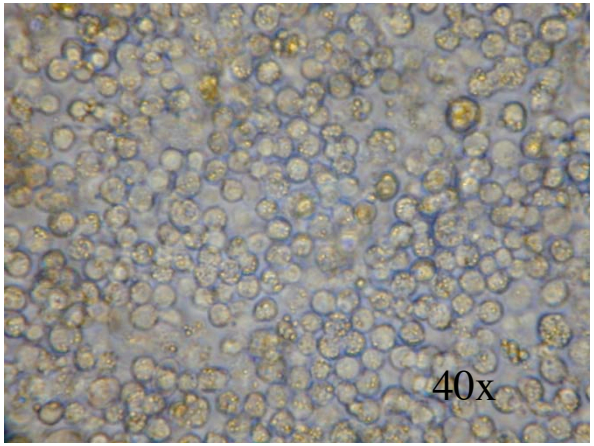


The nutritional composition of the media could have an effect on toxicological responses

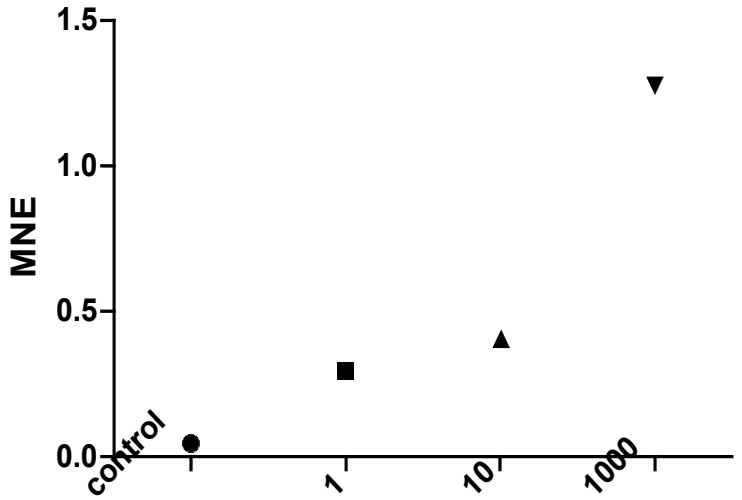
- **Supplementation of serum vs. serum-free media**
  - Serum
    - Content of nutrients, growth factors, hormones, minerals and lipids are of importance for cell maintenance
    - Concentration and action of the constituents of serum are not fully determined
    - Problem with standardization of experiments and between laboratories due to batch effects and accessibility of different serum
    - Use of high serum concentration can reduce chemical bioavailability and toxic responses. Can have an effect on downstream processing
  - Serum-free media
    - More control on proliferation and differentiation, and growth optimization for specific cell types
    - Reduced cell growth and selection of resilient cells
- **Fish cultures**:
  - FBS (fetal bovine serum) is frequently used
  - Use of fish serum might be a better alternative
  - Need for standardization of experiments and between laboratories

Primary Atlantic cod hepatocyte cultures

- Important to develop primary hepatocyte cultures since liver is the main biotransformation organ
- It has been problematic to establish cod hepatocyte cultures due to the high fat content in cod liver
- Intact cod hepatocytes have been isolated from mature individuals
  - 1,2,3,7,8'-PeCDD exposed cod cells induced CYP1A



CYP1A

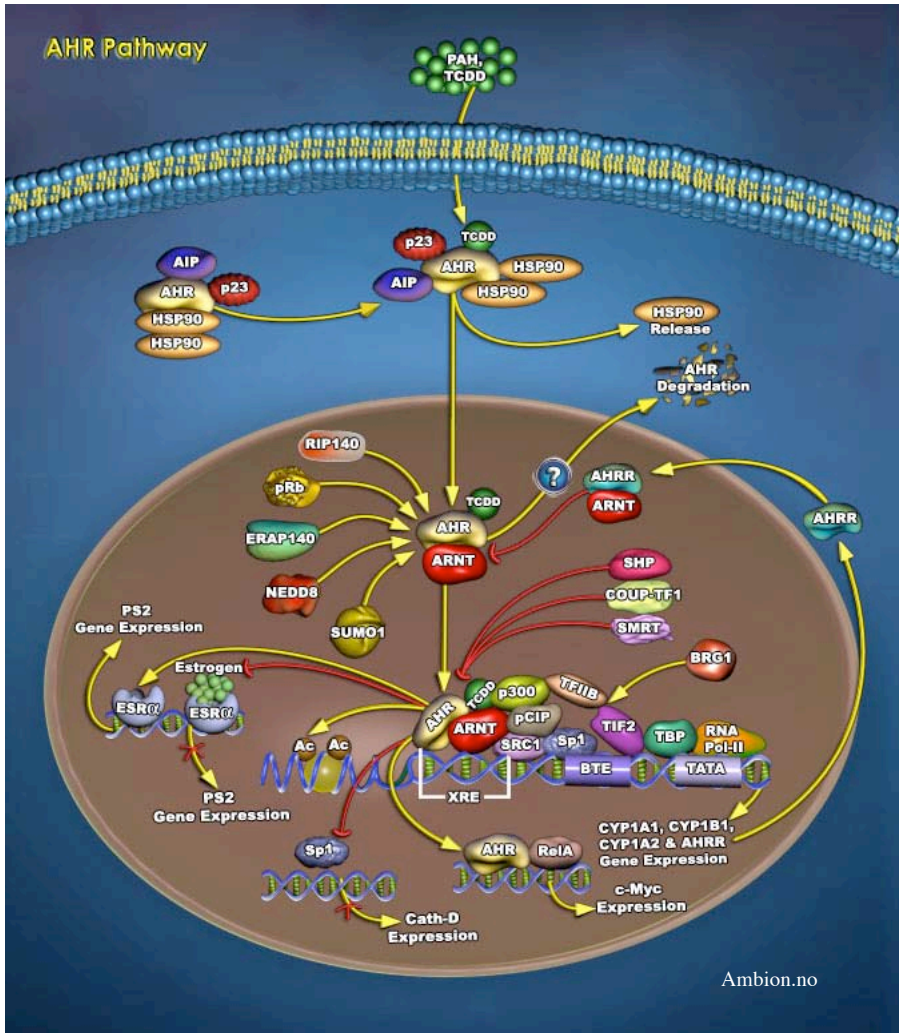


Factorial design applied for multiple-endpoint toxicity evaluation in Atlantic salmon (Salmo salar L.) hepatocytes

The organic pollutants Polychlorinated dibenzo-*p*-dioxins (PCDDs), Polychlorinaed dibenzofurans (PCDFs) and Polychlorinated bifenyles (PCBs)

- **PCDD/Fs and PCBs**
  - They are still of great concern regards to food safety
    - Relative high concentrations have been measured in farmed fish

- **Mechanism of toxicity**
  - **Dioxins and dioxin-like PCBs**
    - Toxicity are induced through the aryl hydrocarbon receptor (AhR)
    - Binding of ligand to AhR results in transcriptional up-regulation of CYP1A and the AhR gene battery (e.g. UDPGT = UGT)
  - **Non-dioxin-like PCBs**
    - Toxic responses are induced through other mechanisms

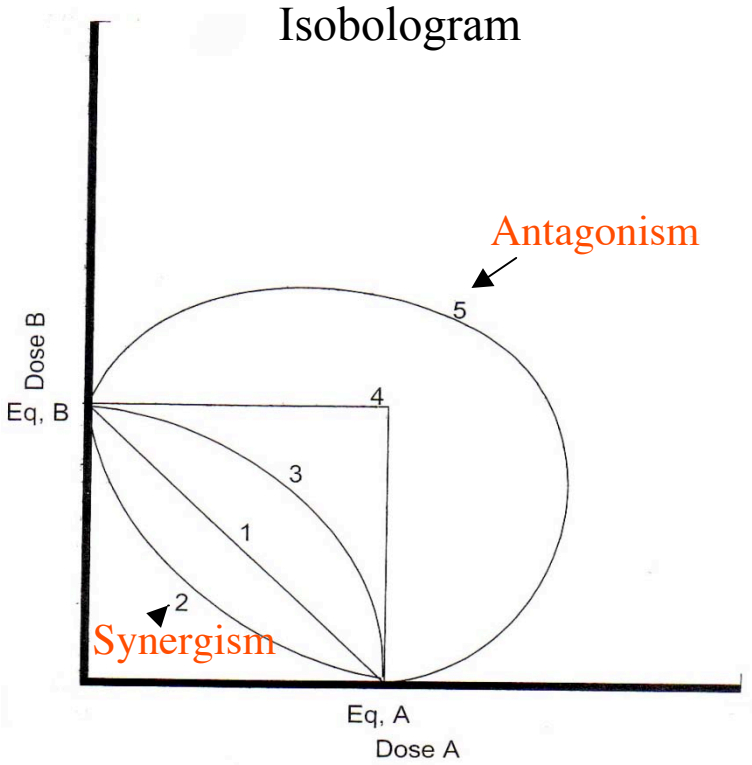


The TEQ method can underestimate toxicity

- **The toxic equivalent (TEQ) method:**
  - Interaction between chemicals in mixtures are detected by comparing the theoretical TEQ and experimental TEQ
  - Assumptions:
    - Identical dose-response curves
    - dose additivity

  Usually not achieved in complex mixtures
- Essential to develop a alternative method to evaluate occurrence of combine effects between chemicals in mixtures

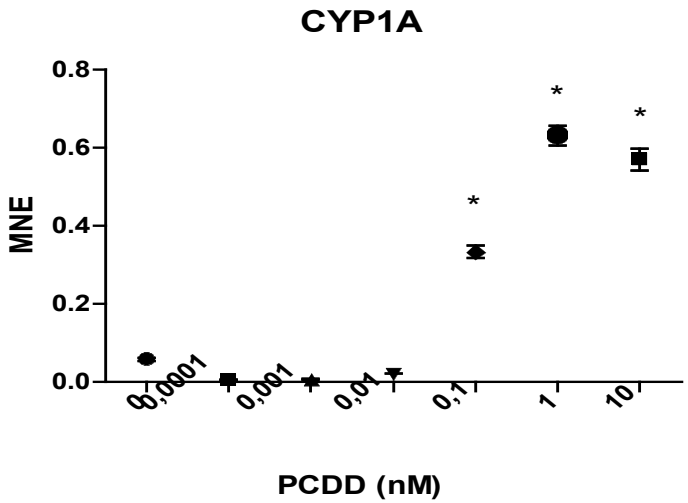
Denison et al.. 2004; Toyoshiba et al.. 2004

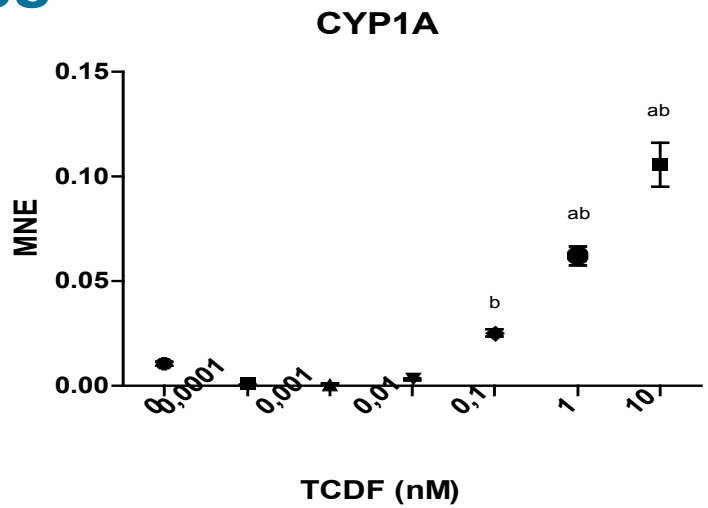


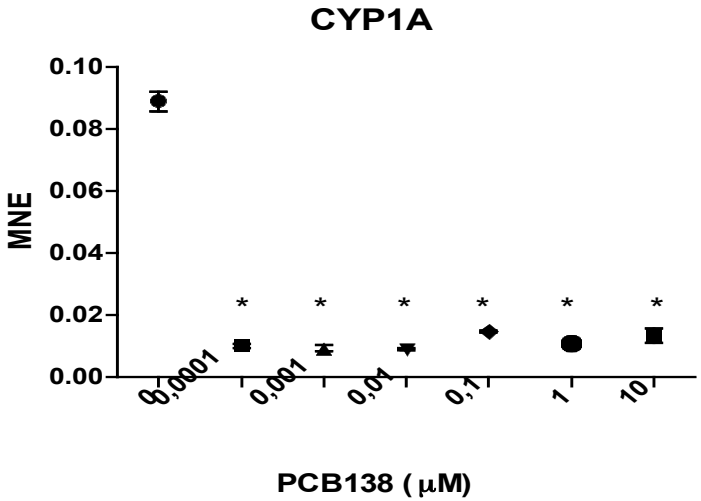
Combined effects between chemicals in mixtures (Cassee et al.,1999):

- Additivity: Dose additivity (1) and response additivity (3)
- Interaction: Synergism (2) and antagonism (5)

CYP1A dose-response curves in hepatocytes exposed to 1,2,3,7,8-PeCDD, 2,3,7,8-TCDF and PCB138







Factorial design

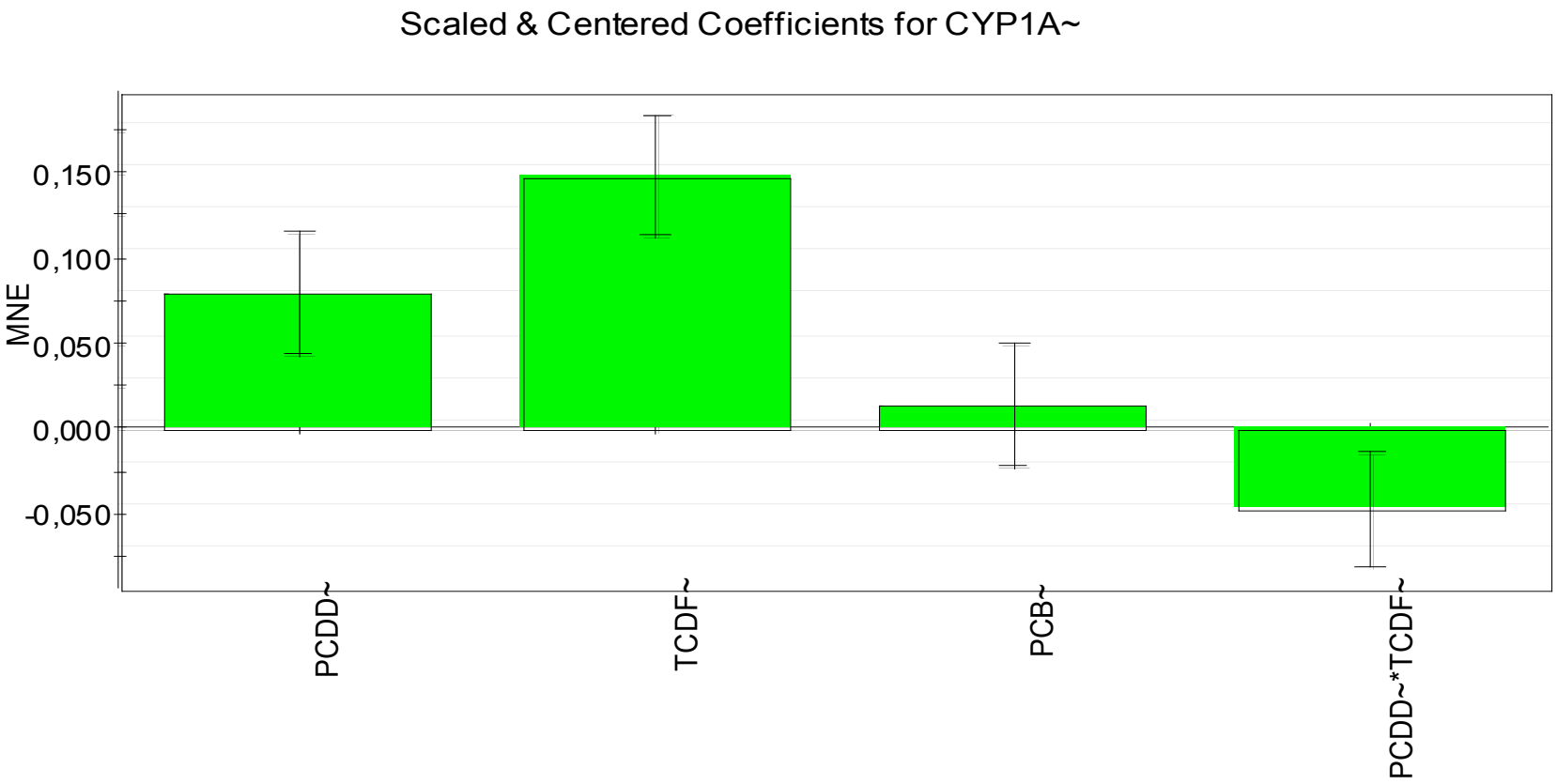
Ex.no.	PCDD (nM)	TCDF (nM)	PCB138 (µM)
1	0.03	0.1	0.05
2	0.1	0.1	0.05
3	0.03	1	0.05
4	0.1	1	0.05
5	0.03	0.1	1
6	0.1	0.1	1
7	0.03	1	1
8	0.1	1	1
9	0.065	0.55	0.525
10	0.065	0.55	0.525
11	0	0	0

- High
- Middle, center points
- Low
- DMSO control

qPCR:

- Phase 1 and II enzymes: CYP1A, UDPGT and GST
- Cellular stress: HSP70, GR, GPX and Mn SOD
- Apoptosis: p53
- Control genes: Beta-actin, EL and ARP

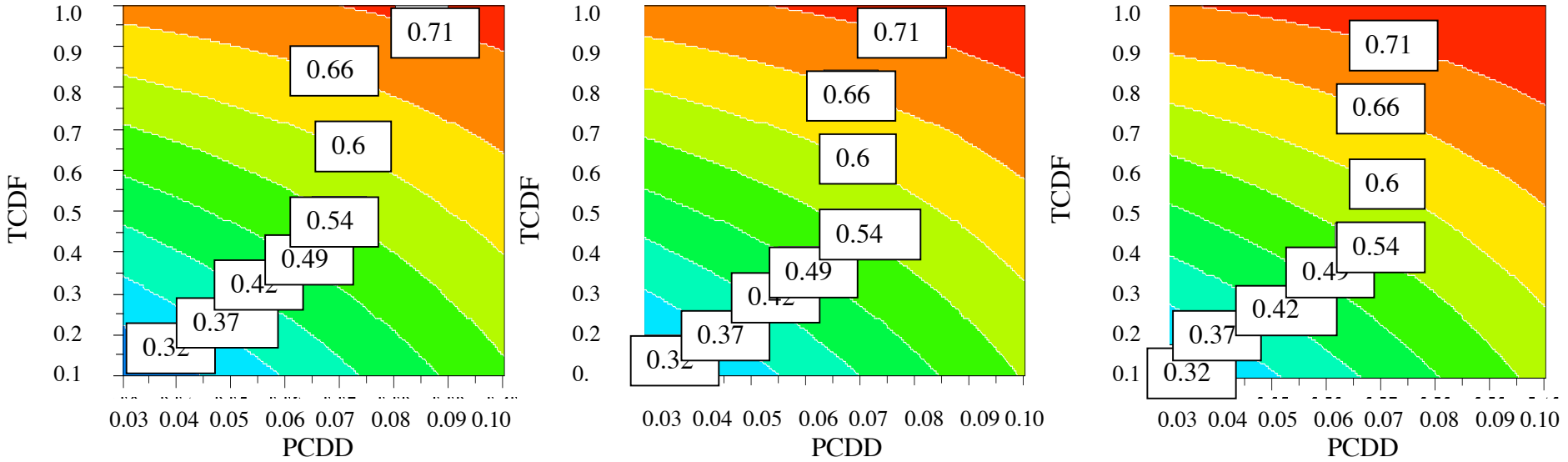
PLS analysis of CYP1A gave a good model with good prediction capability



$R^2 = 0.97$ $Q^2 = 0.768$ Conf. lev. = 0.95 1 Comp.

MODDE 7 - 09.09.2008 15:46:30

CYP1A contour plot: Antagonism at high concentrations

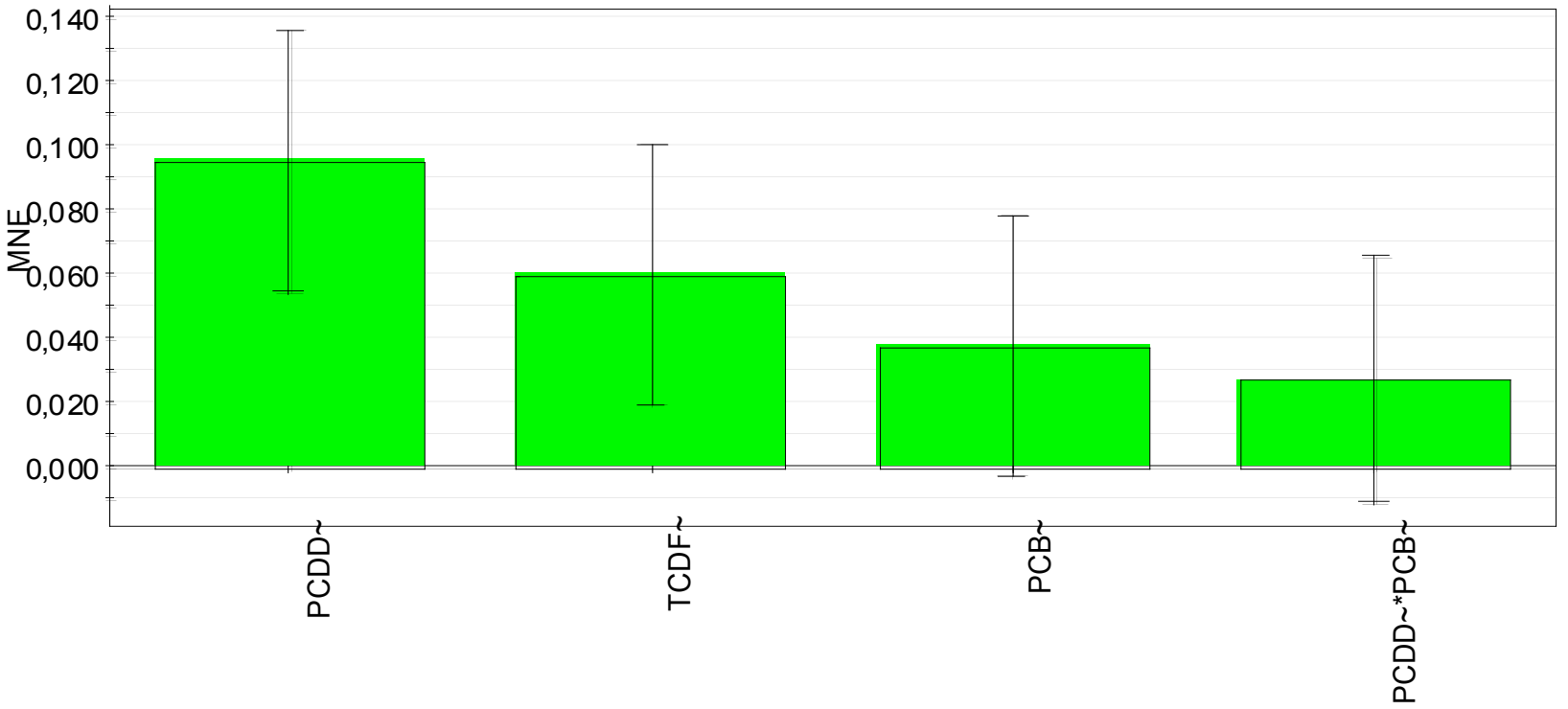


PCB 138~ 0.05

PCB 138~ 0.525

PCB 138~ 1

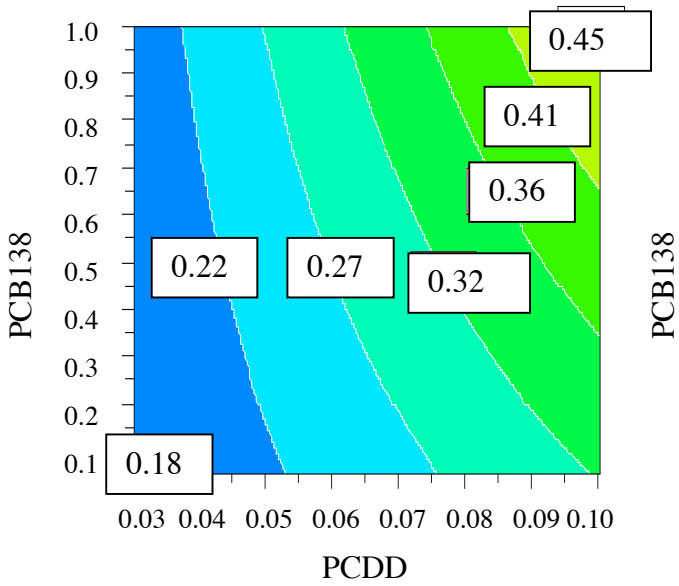
PLS analysis of UDPGT gave a good model with good prediction capability



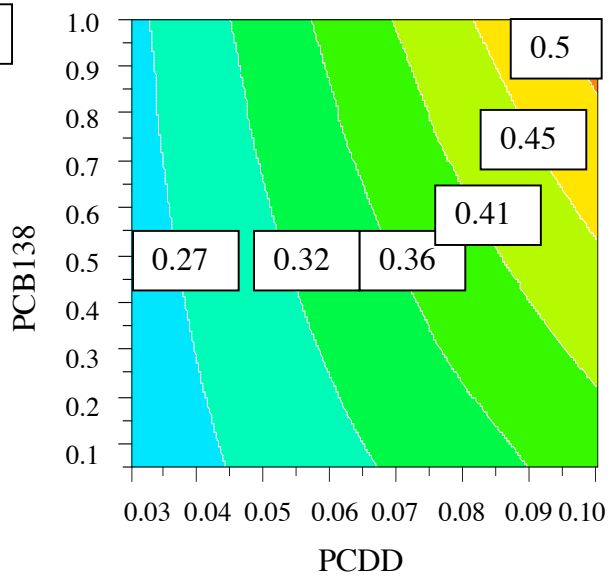
R2 = 0.922 Q2 = 0.700 Conf. lev. = 0.95 1 Comp.

MODDE 7 - 09.09.2008 16:05:30

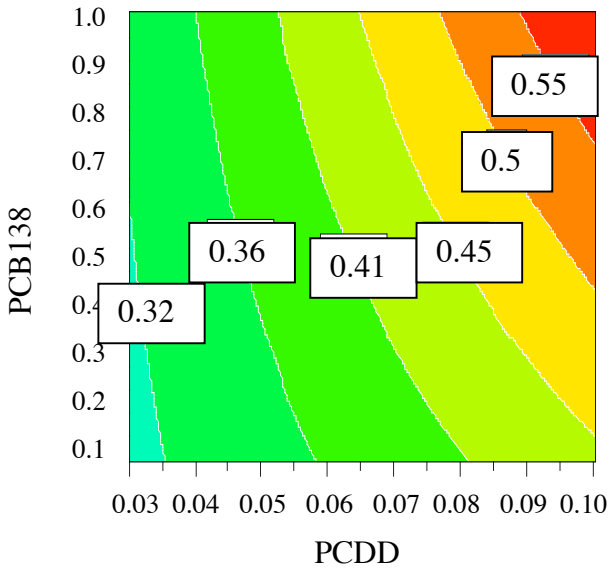
UDPGT contour plot: Synergism at low concentrations



TCDD~ 0.1



TCDD~ 0.55



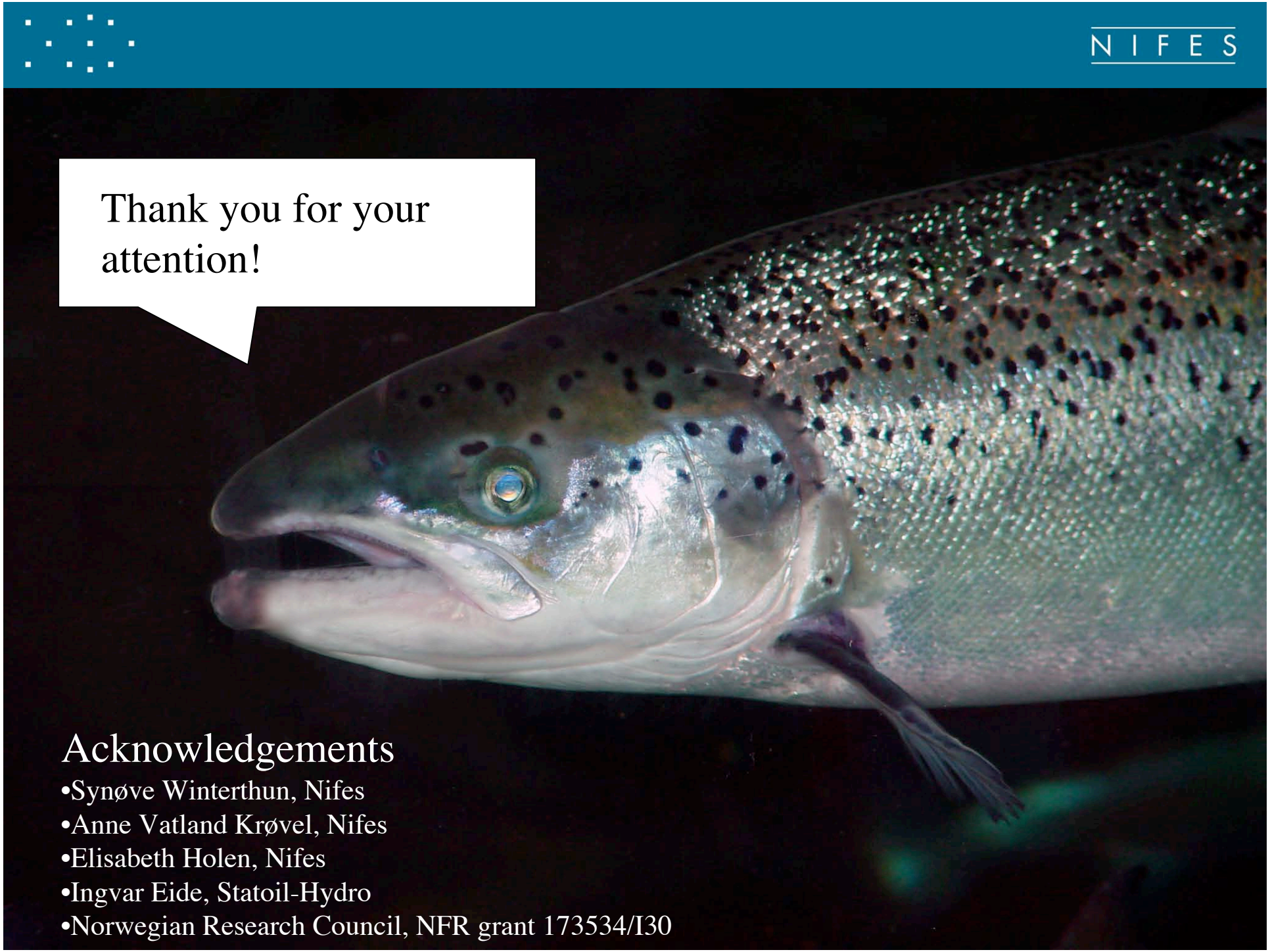
TCDD ~1

Synergism and antagonism

- PCB 138 can contribute to the measured CYP1A and UDPGT responses
- The synergistic interaction between PCB 138 and PCDD on UDPGT might counteract the CYP1A antagonistic effect of TCDF and PCDD
  - Possible mechanisms for the synergism:
    - Hepatic retention of potent CYP1A inducers (de Jongh et al., 1993)
    - Higher levels of AhR in the cells (de Jongh et al., 1993; Cherng et al., 2001)
    - And/or higher activation of AhR to a DNA binding form (Cherng et al., 2001)
- Previous studies have detected antagonistic interaction between chemicals on CYP1A at high concentration (Vamvakas et al., 1996; Østby & Krøkje, 2002)
  - Possible mechanisms antagonism:
    - Competitive inhibition (Gooch et al., 1989; Fent & Bätscher, 2000)

Conclusions

- Important to develop *in vitro* fish bioassays
- Intact Atlantic cod hepatocytes have been isolated and PCDD exposed cells induce CYP1A
- Only CYP1A and UDPGT were proved to be good biomarkers for PCDD, TCDF and PCB138
- Using primary fish cell cultures, multivariate data analysis of qPCR data are shown to be a useful tool in toxicological studies
- A multi-endpoint strategy can enhance the quality of risk evaluation of chemical compounds
- But: TEQ is a useful concept in risk assessment of mixtures with dioxin and dioxin-like compounds, until a better evaluation strategy is developed



Acknowledgements

- Synøve Winterthun, Nifes
- Anne Vatland Krøvel, Nifes
- Elisabeth Holen, Nifes
- Ingvar Eide, Statoil-Hydro
- Norwegian Research Council, NFR grant 173534/I30