

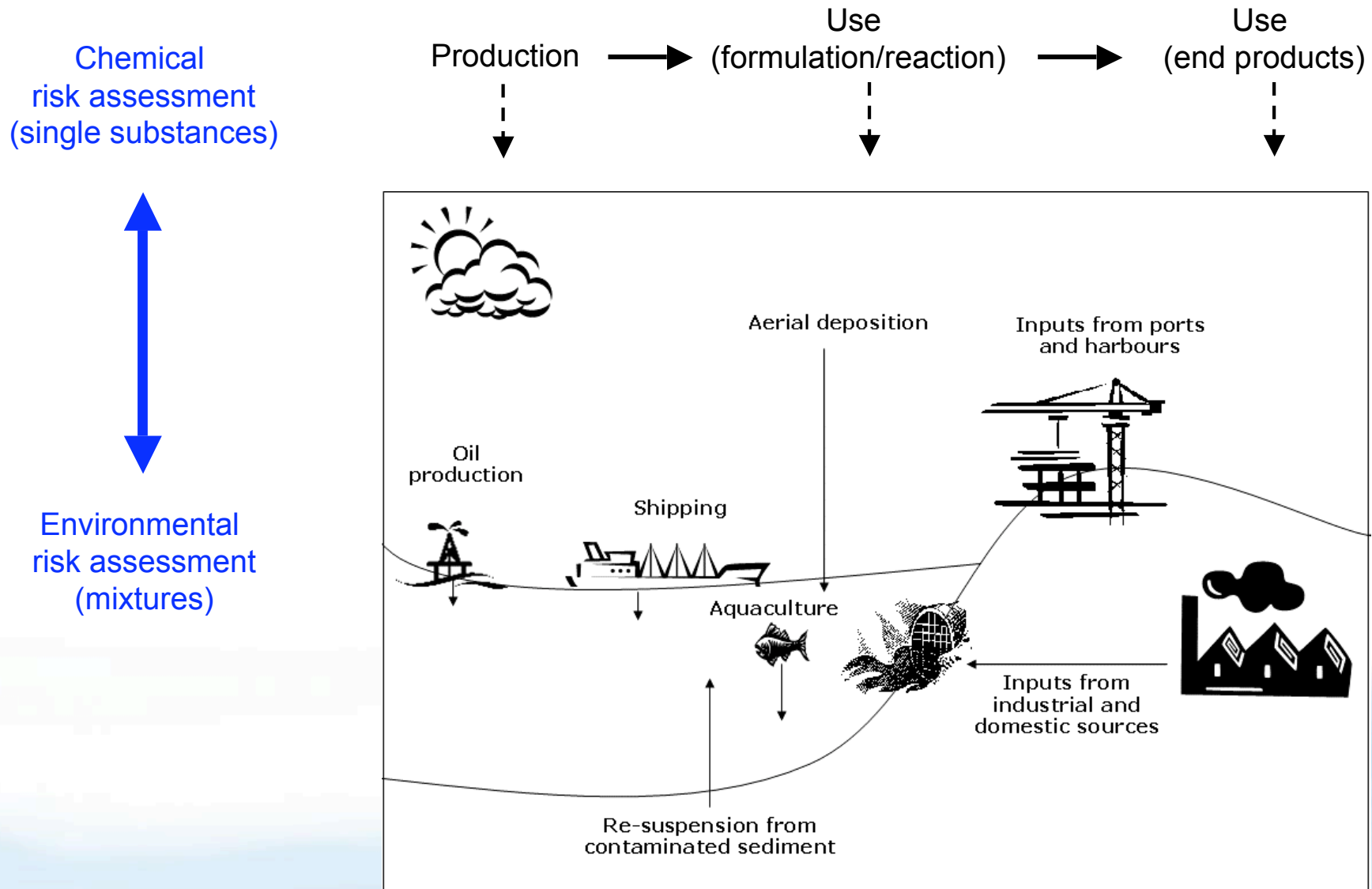
Non-animal (alternative) testing methods in Aquatic toxicology

Knut Erik Tollefsen

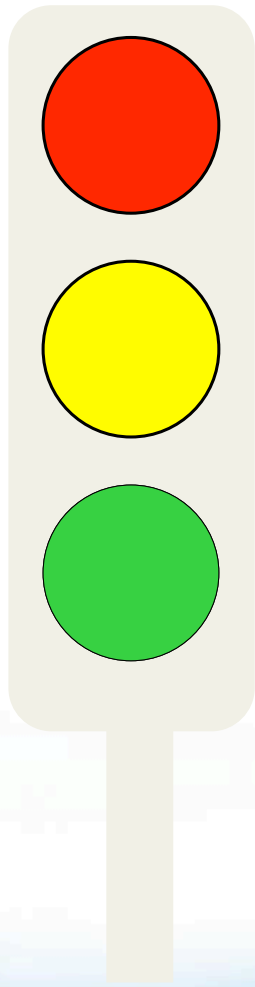
Norwegian Institute for Water Research (NO)
University of Life Sciences (NO)

Contact: ket@niva.no

Aquatic pollutants



Chemical risk assessment



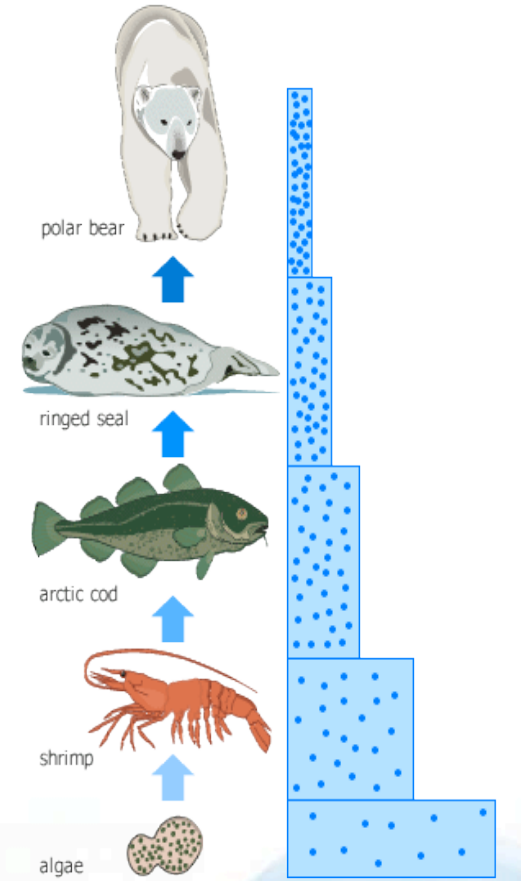
Toxicity (Hazard)



Bioaccumulation

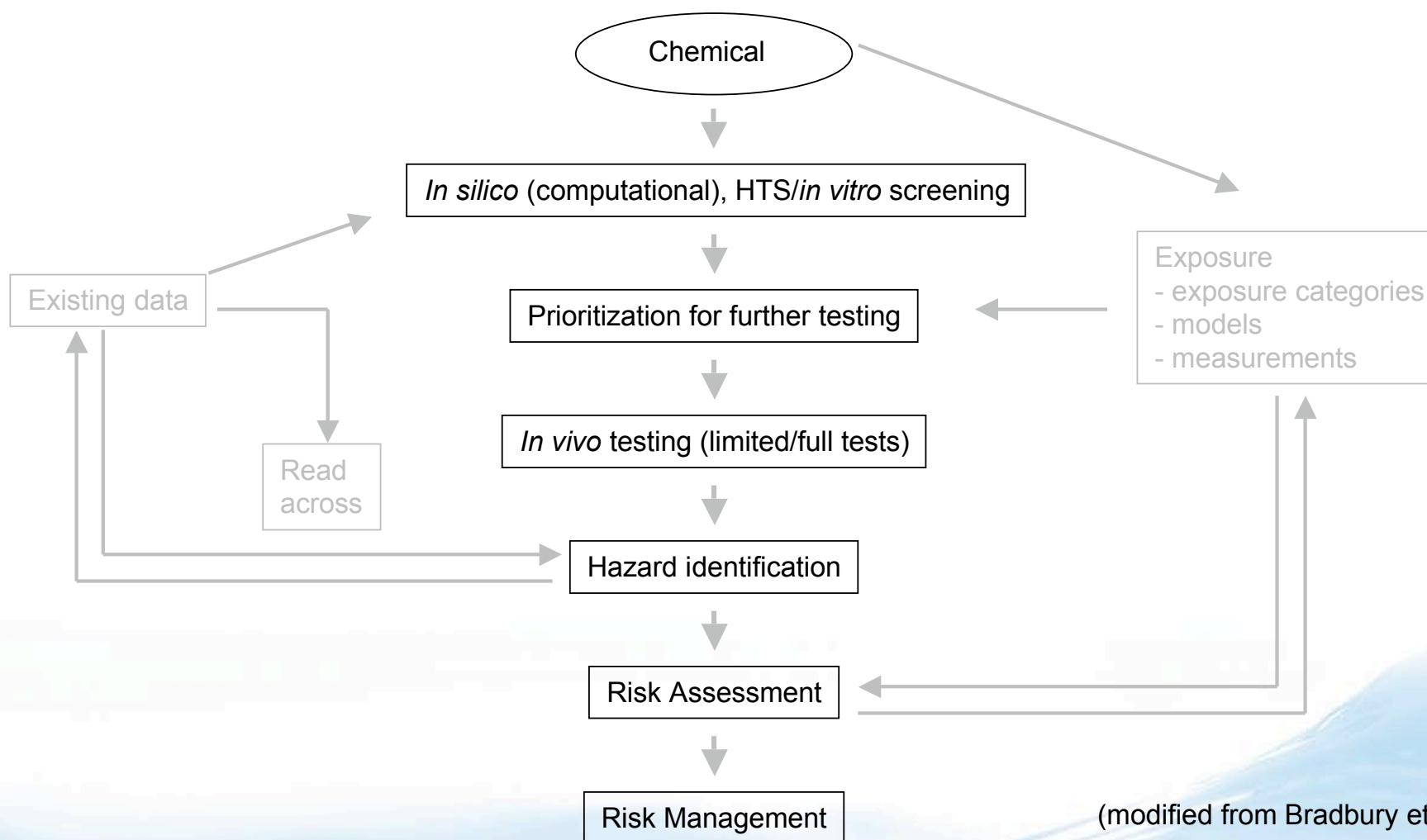


Persistence



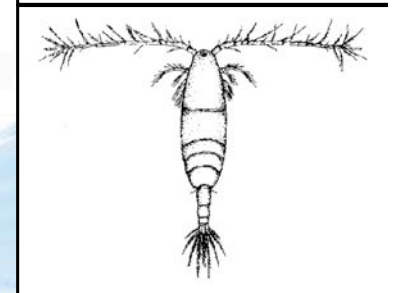
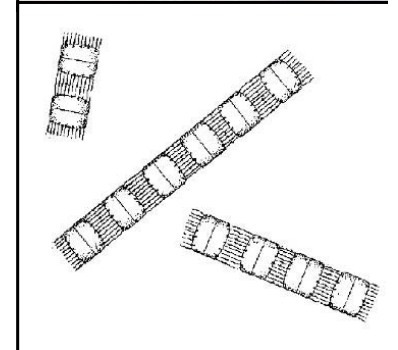
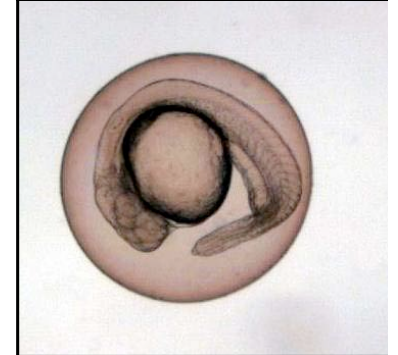
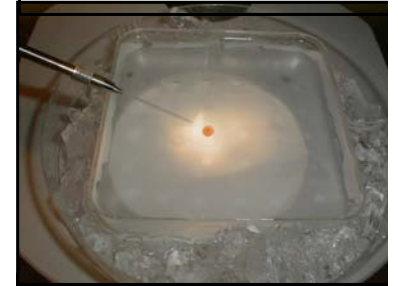
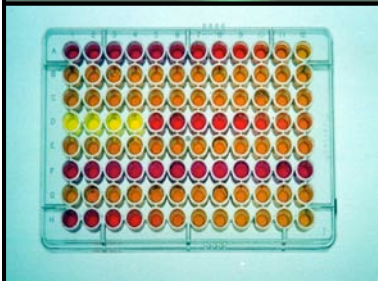
Environmental risk !

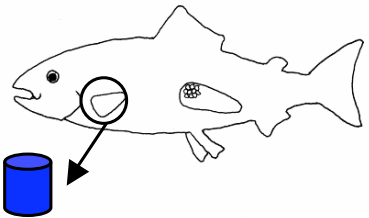
Integrated testing strategies (ITS)



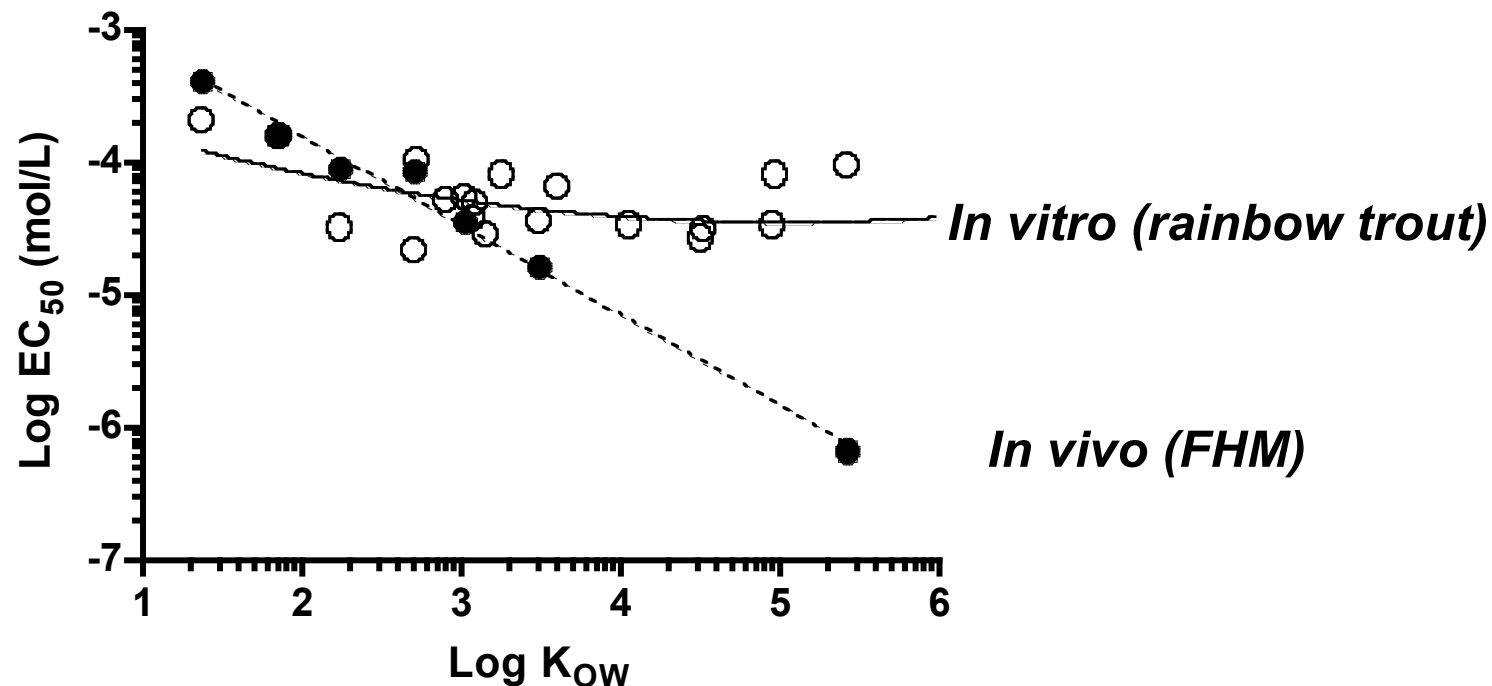
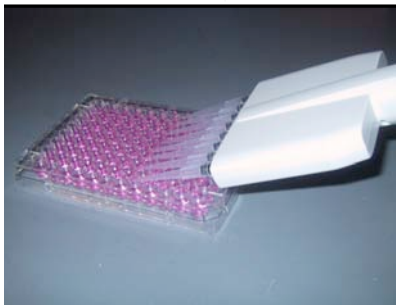
In vitro methods

- Cell free systems (receptors)
- Tissue homogenates (enzymatic activity)
- Luminescent bacteria (microtox)
- Recombinant bacteria (mutatox/SOS test)
- Recombinant cells (reporter cell lines)
- Primary cultures of cells (hepatocytes)
- Continuous cell lines (hepatoma cells)
- Embryonic models (embryos/cell lines)





Multiple challenges !



In vitro bioassays may not be predictive of in vivo toxicity due to different toxic targets and dissimilar behavior in the tests systems!

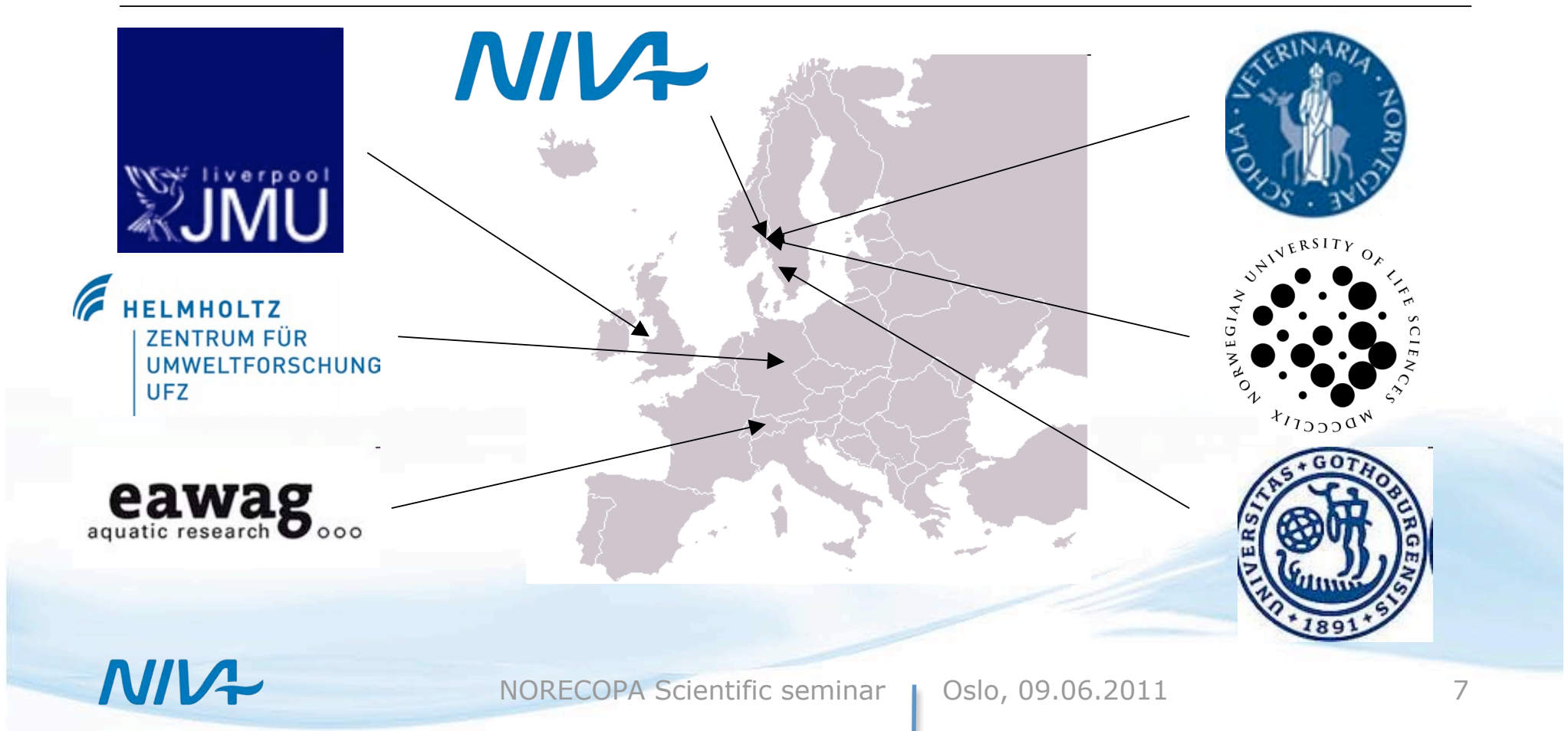
Tollefsen, K. E., Blikstad, C., Eikvar, S., Finne, E.F., Gregersen, I.K (2008). Cytotoxicity of alkylphenols and alkylated non-phenolics in a primary culture of rainbow trout (*Onchorhynchus mykiss*) hepatocytes. *Ecotoxicol. Environ. Saf.* **69**: 64-73.

Non-animal (alternative) testing methods for REACH (alterREACH)

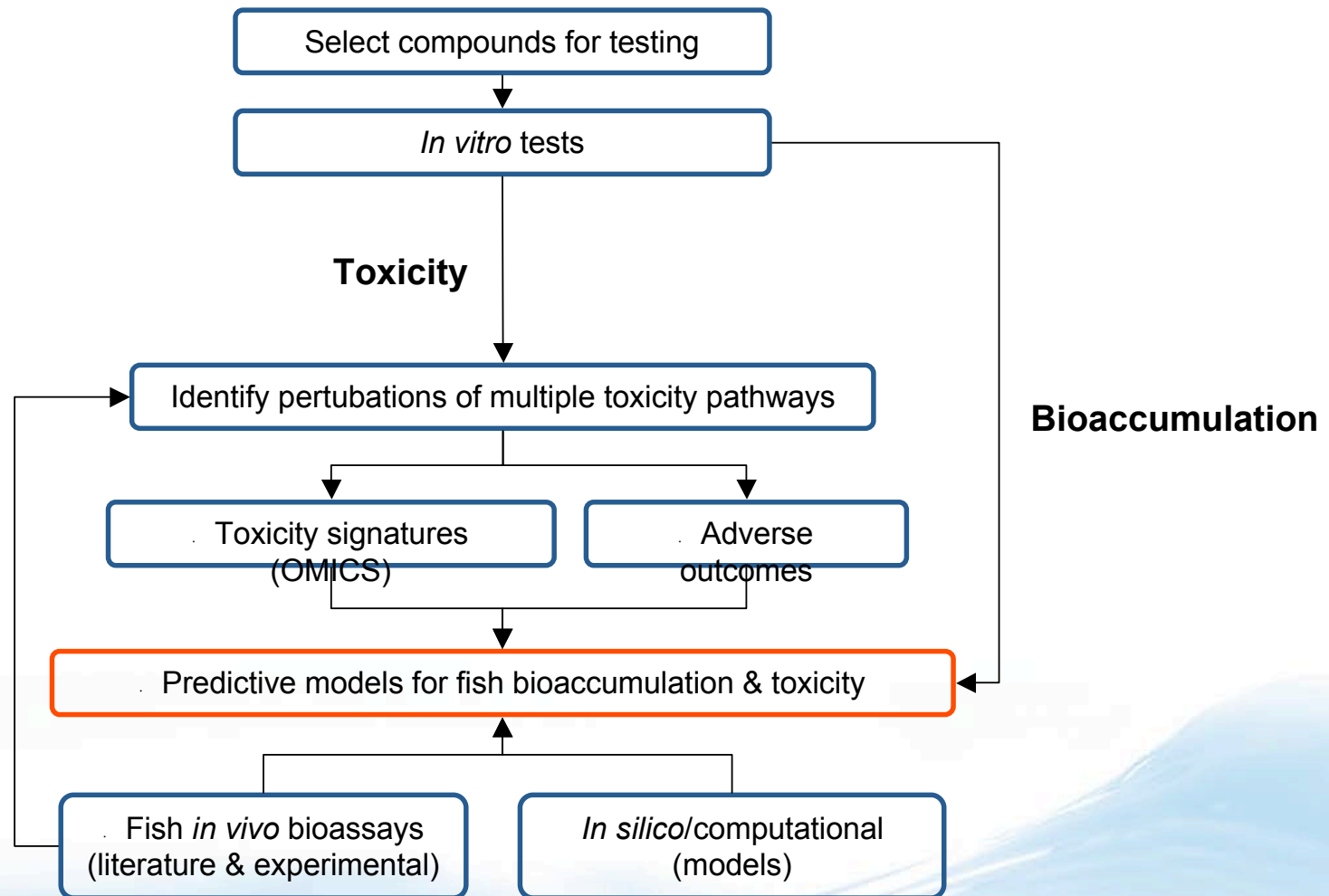
K.E. Tollefsen, P. Aleström, M. Cronin, Ø. Evensen, M. Hultman, Langford, A. Lillicrap,
B. O. Rosseland, K. Schirmer, S. Scholz, J. Sturve, K. Thomas.

Project web page: www.niva.no/alterREACH

Contact: Knut Erik Tollefsen (ket@niva.no)

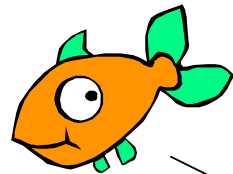


Multi-disciplinary approach

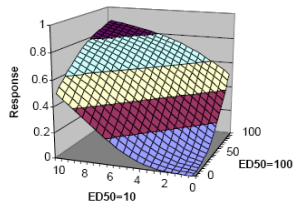


Predicting mixture toxicity

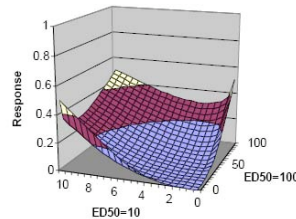
Comp A Comp B Comp C Comp D.....



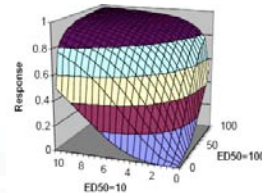
Additivity
(1+1=2)



Antagonism
(1+1<2)



Synergy
(1+1>>2)



Concentration addition

(similar MoA)

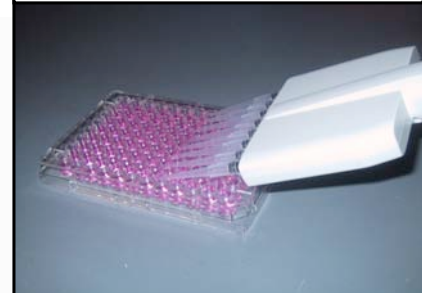
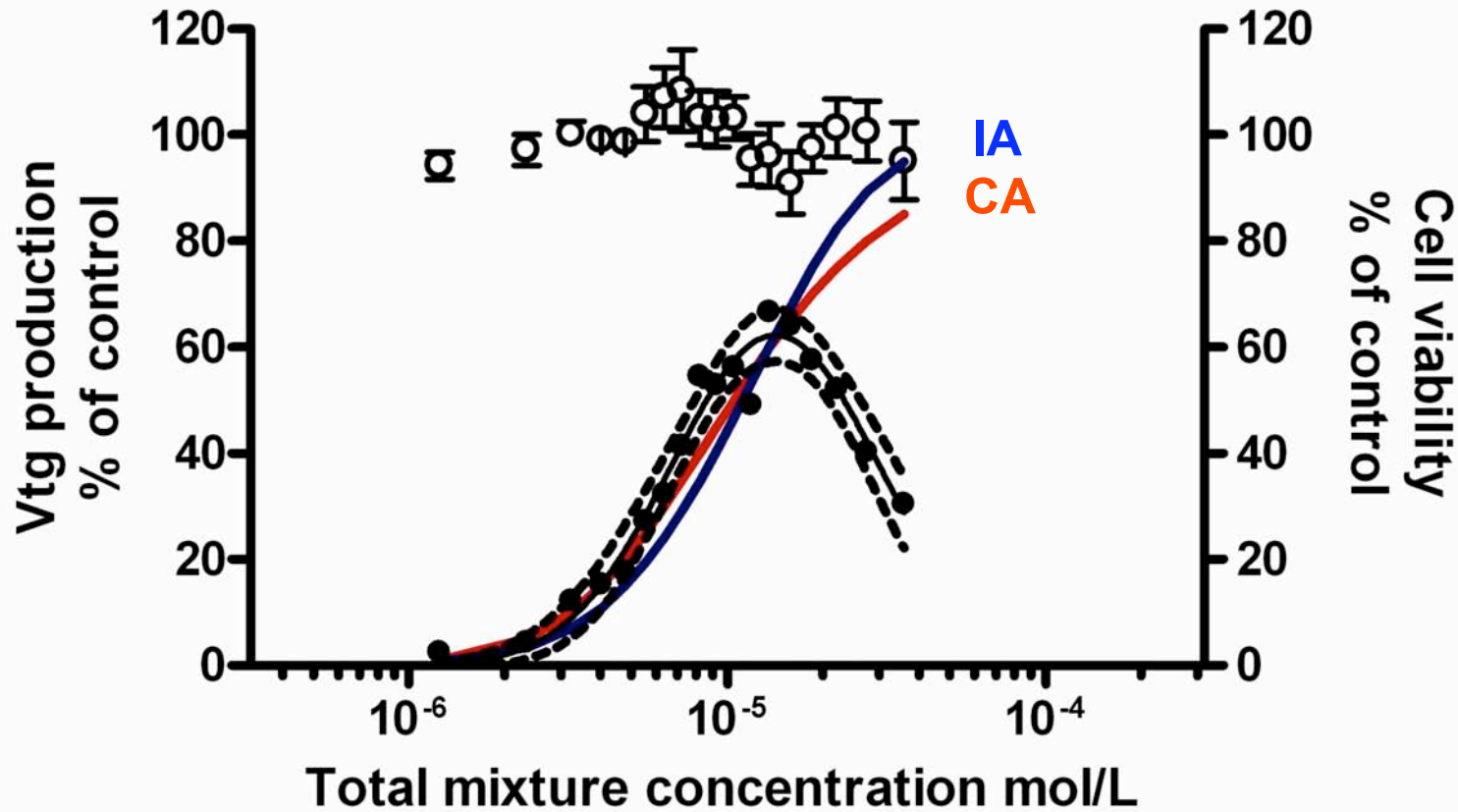
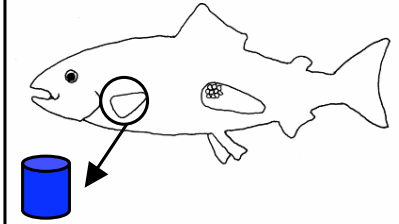
$$ECx_{(Mix)} = \left(\sum_{i=1}^n \frac{P_i}{ECx_i} \right)^{-1}$$

Independent action

(dissimilar MoA)

$$E_{Mix} = 1 - \prod_{i=1}^n (1 - E_i)$$

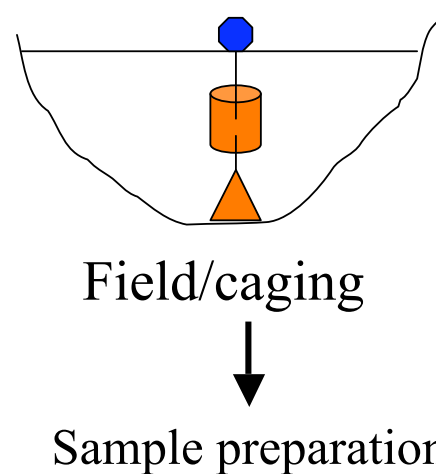
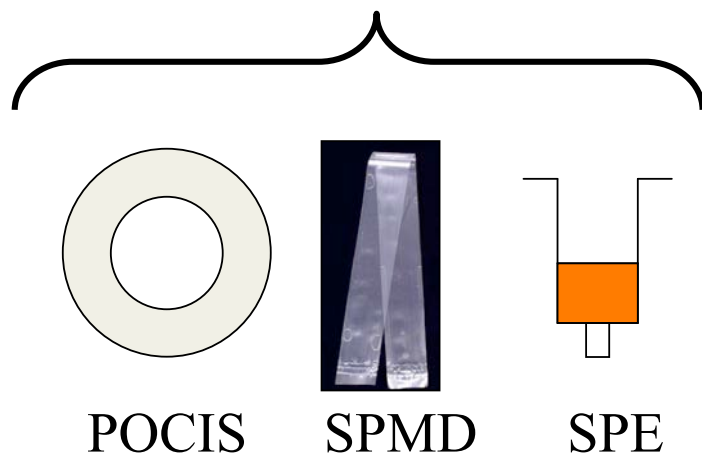
Combined effects 9 estrogens



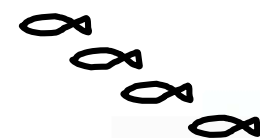
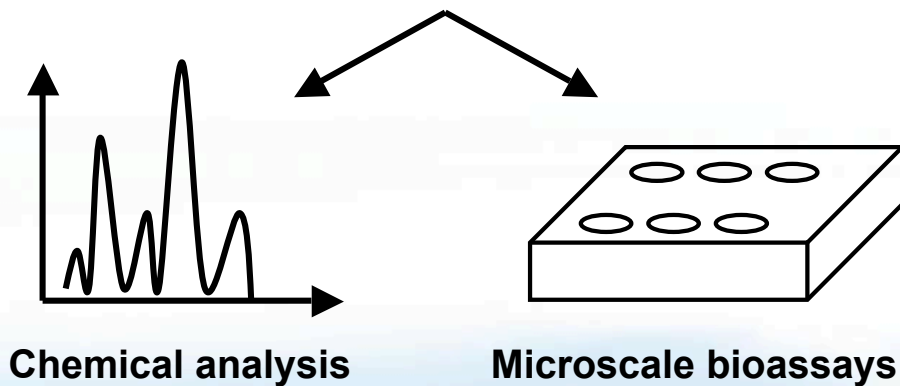
Petersen and Tollefsen (2011). Assessing combined toxicity of estrogen receptor agonists in a primary culture of rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Aquatic Toxicology* 101, 186-195

Environmental risk assessment

Environmental sample



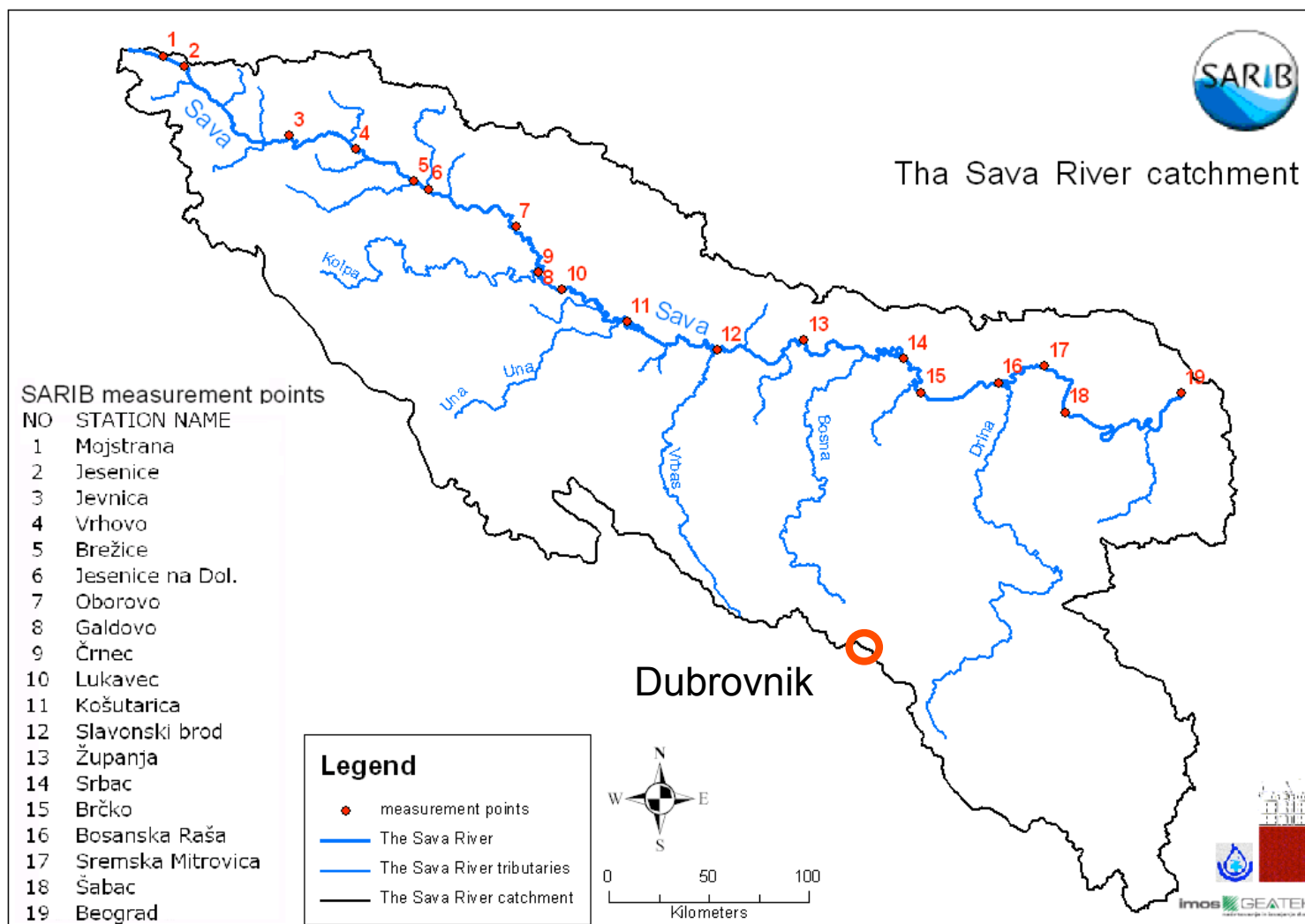
Sample preparation



In vivo bioassay



Estrogens - Sava River basin



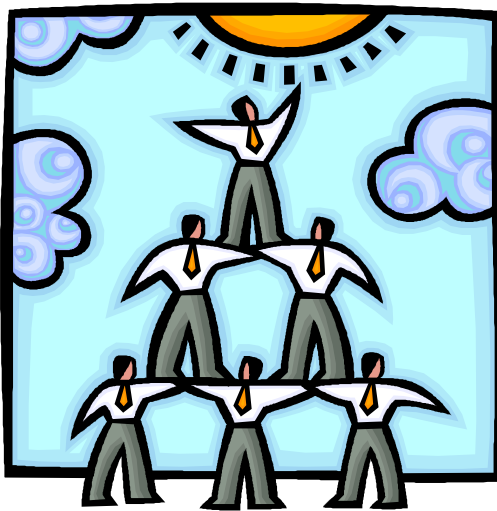
International commitments



- **OECD-workgroup on non-animal tests (OECD-NA)**
- **OECD-advisory group for molecular screening and toxicogenomics (Comparative Toxicology group)**
- **OECD *ad hoc* expert working group for development of Fish Embryo Toxicity (FET) test**



- **ILSI-HESI subcommittee on emergence of animal alternative needs in ERA**
- **ILSI-HESI subcommittee on alternatives to in vivo tests for EDCs**



Acknowledgements

UiO

Ketil Hylland
Kathrin Ellesat

UMB

Bjørn O. Rosseland
You Song

NIVA

Maria Hultman
Karina Petersen
Adam Lillicrap
Eivind Farnen Finne
Katherine Langford
Inger K. Gregersen
Cecilie Sandberg
Camilla Blikstad
Torsten Källqvist
Kevin V. Thomas

NVH

Jan R. Torp
Peter Alestrom
Øystein Evensen

Others

Kristin Schimer (EAWAG, Switzerland)
Stefan Scholz (UFZ, Germany)
Rolf Altenburger (UFZ, Germany)
Kevin Chipman (U. of Birmingham)
Mark Cronin (LJMU, UK)

Financial contribution

Norwegian Research Council
(NFR-project no. 159113, 160118, 178621, 196318)
MD – Institution funding (Basis bevilgning)

Infrastructure funding

Norwegian Research Council (NFR-AViT 183929)
FUGE – Zebrafish platform (Alestrom lab)