# Non-animal (alternative) testing methods in Aquatic toxicology

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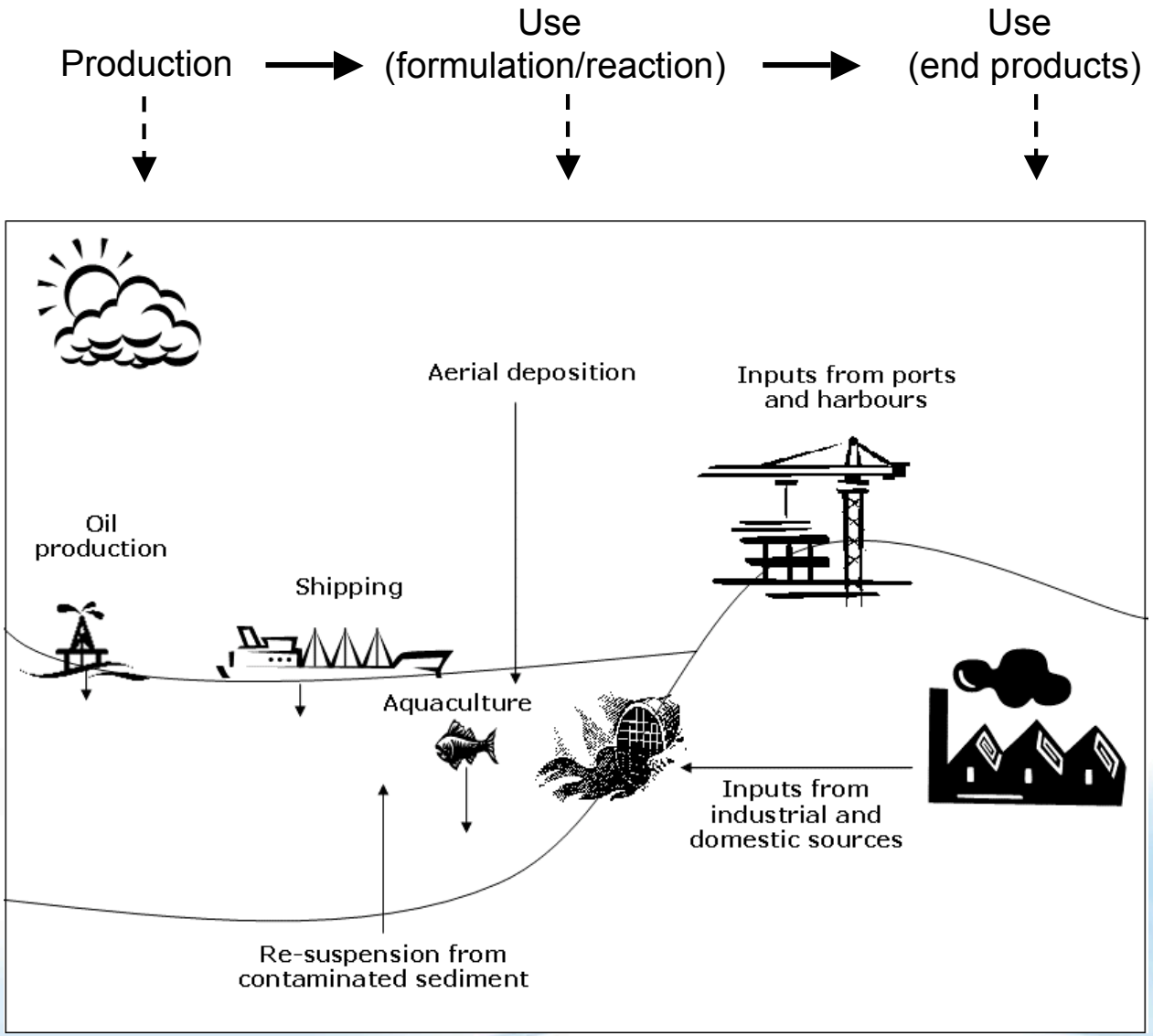
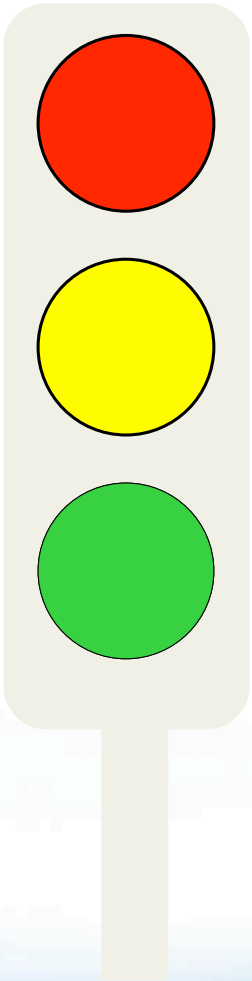
# Aquatic pollutants

Chemical
risk assessment
(single substances)

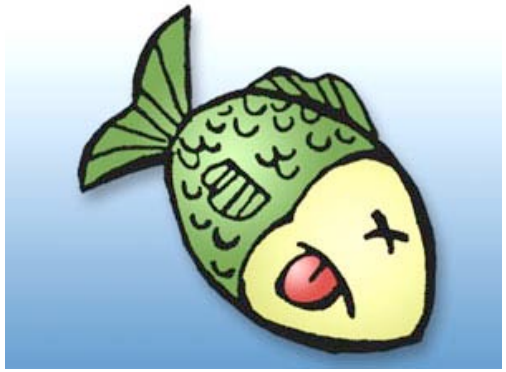
Environmental
risk assessment
(mixtures)



# Chemical risk assessment

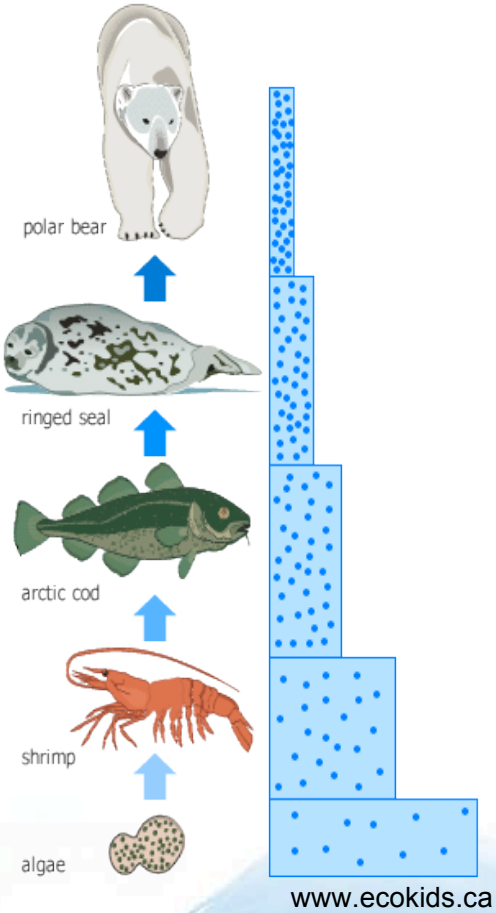


**Toxicity (Hazard)**



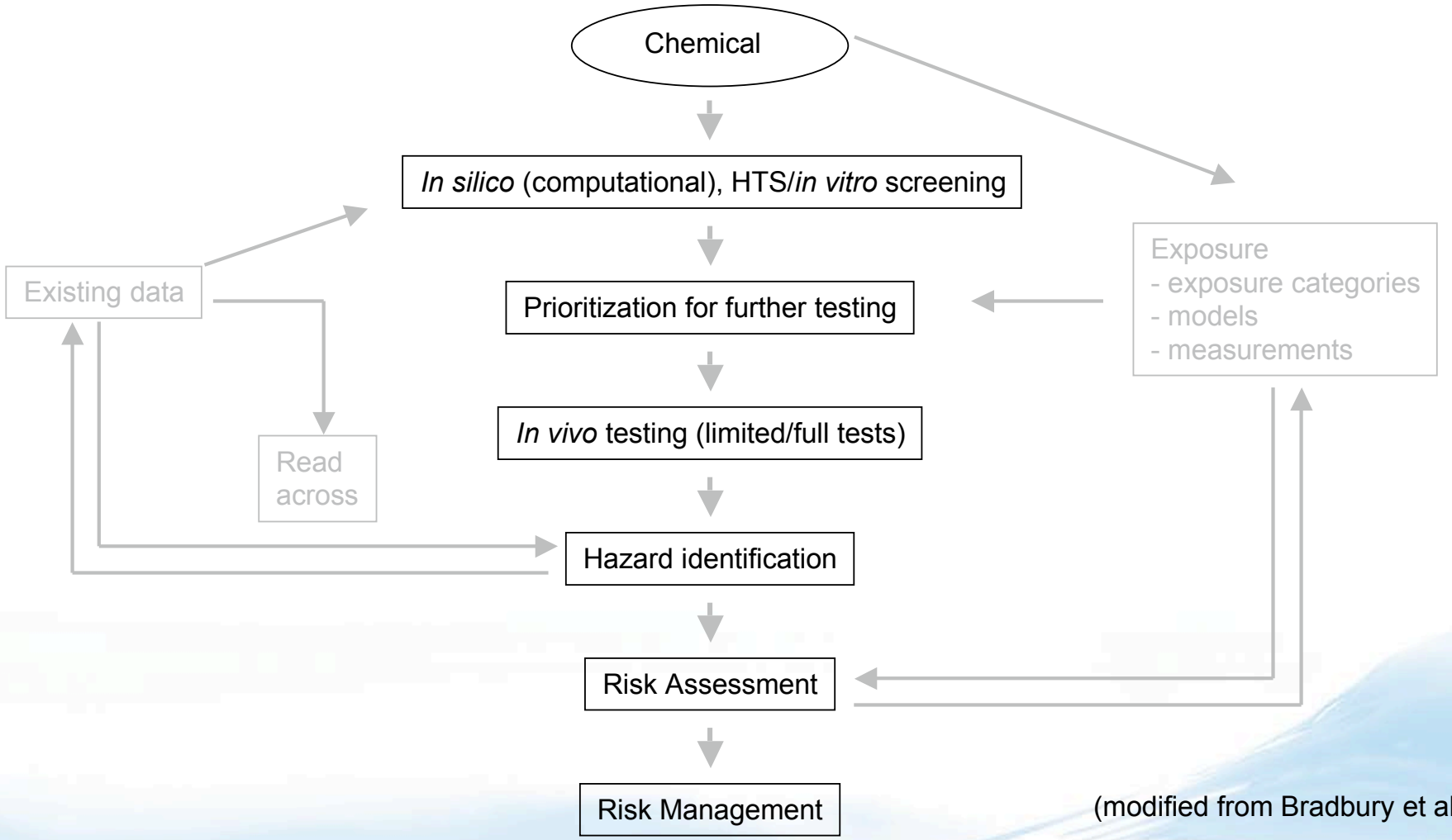
**Bioaccumulation**

**Persistence**

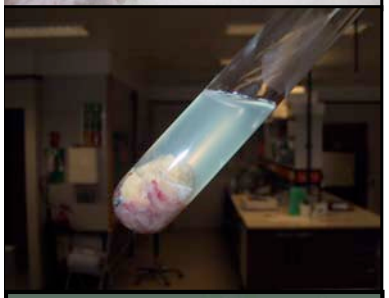


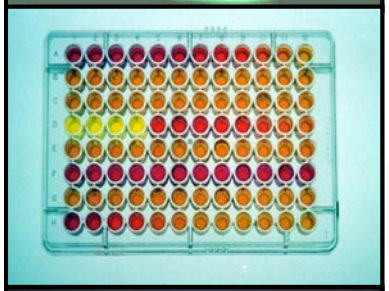
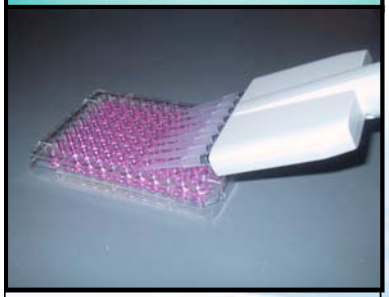

Environmental risk !

# Integrated testing strategies (ITS)



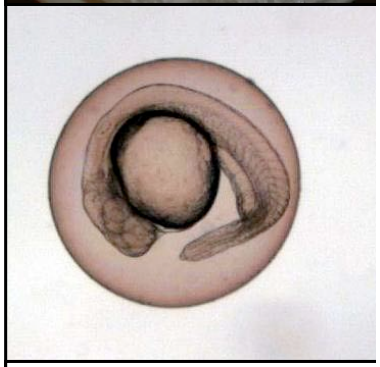
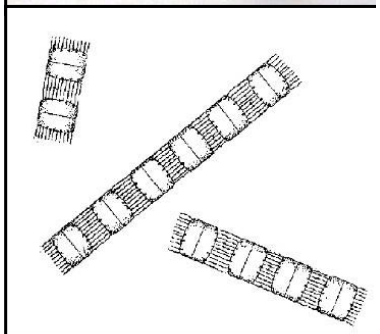
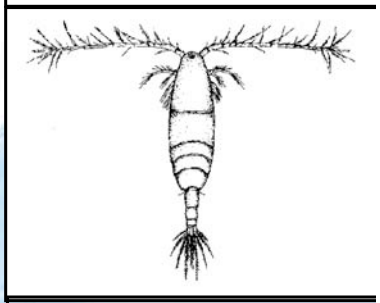

(modified from Bradbury et al. 2004)

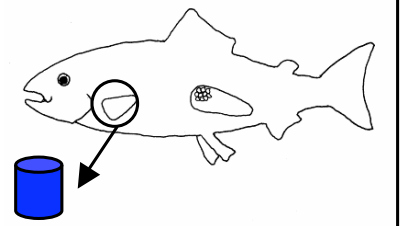



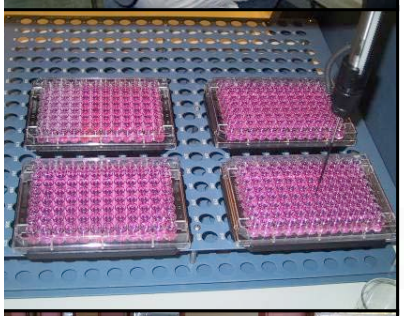



# *In vitro methods*

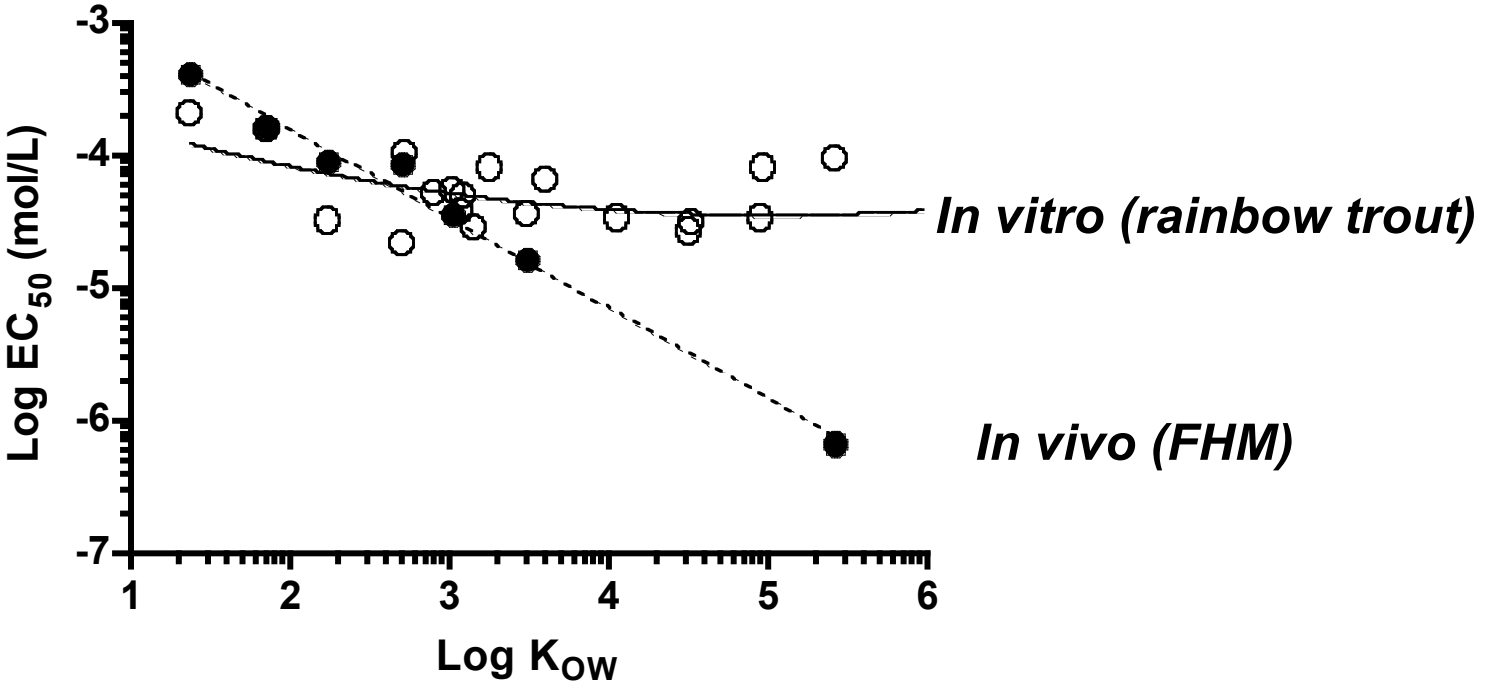
- Cell free systems (receptors)
- Tissue homogenates (enzymatic activity)
- Luminescent bacteria (microtox)
- Recombinant bacteria (mutatox/SOS test)
- Recombinant cells (reporter cell lines)
- Primary cultures of cells (hepatocytes)
- Continuous cell lines (hepatoma cells)
- Embryonic models (embryos/cell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





# Multiple challenges !



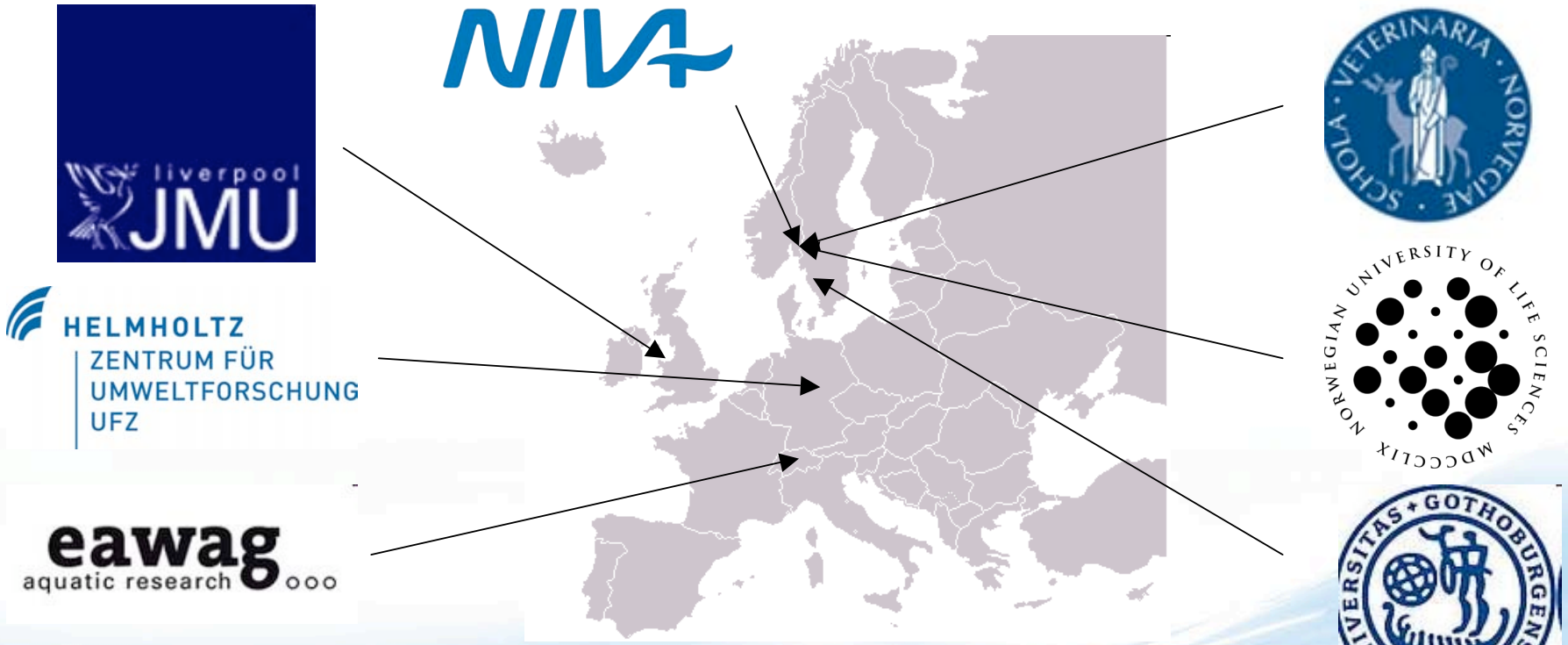

***In vitro* bioassays may not be predictive of *in vivo* toxicity due to different toxic targets and dissimilar behavior in the tests systems!**

**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.

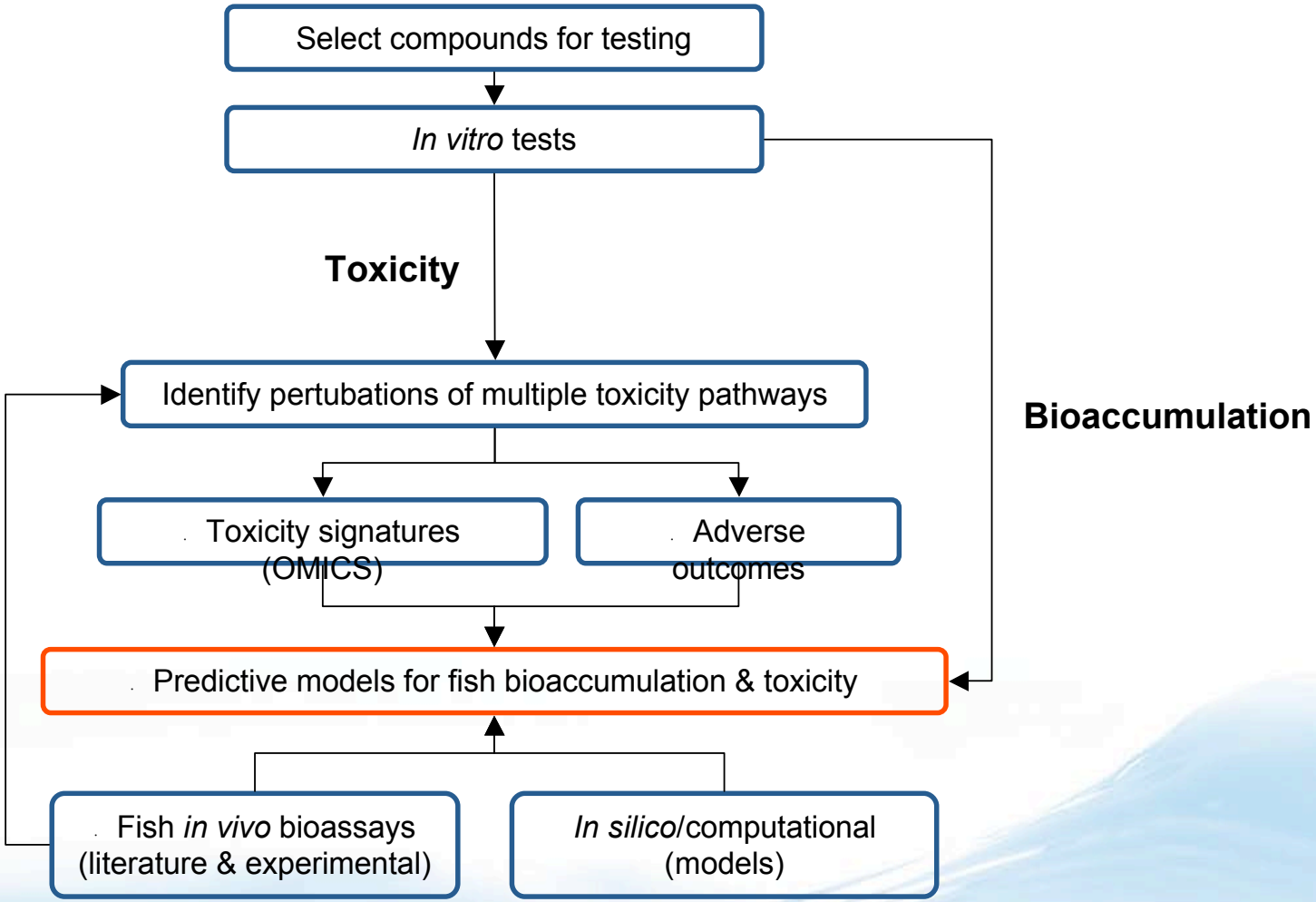
# Non-animal (alternative) testing methods for REACH (alterREACH)

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**)

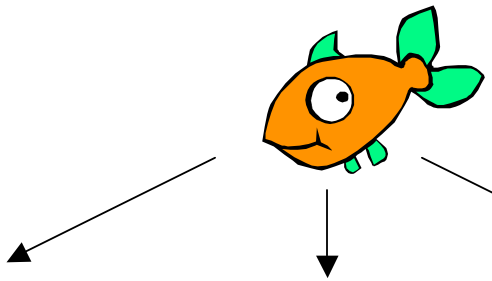


# Multi-diciplinary approach

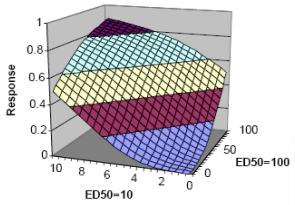


# Predicting mixture toxicity

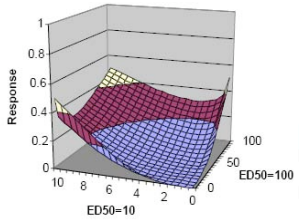
Comp A  Comp B  Comp C  Comp D…..



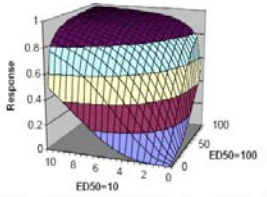
Additivity
(1+1=2)




Antagonism
(1+1<2)




Synergy
(1+1>>2)



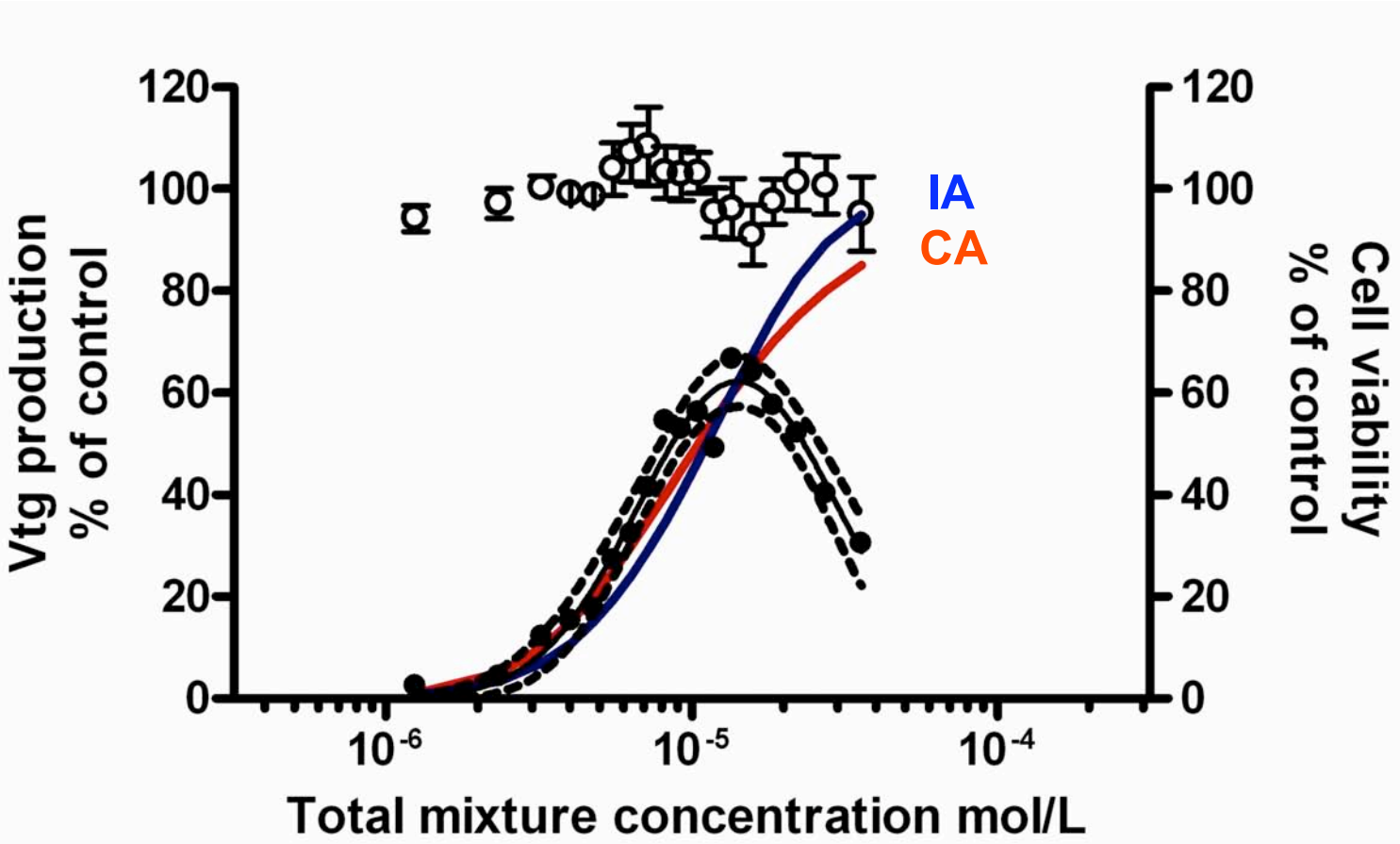

**Concentration addition**
(similar MoA)

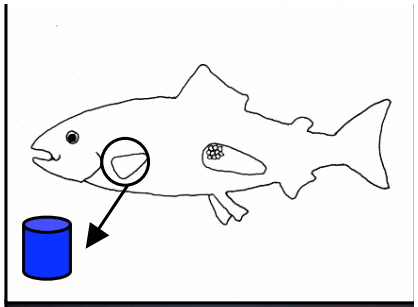
$$ECx_{(Mix)} = \left( \sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1}$$

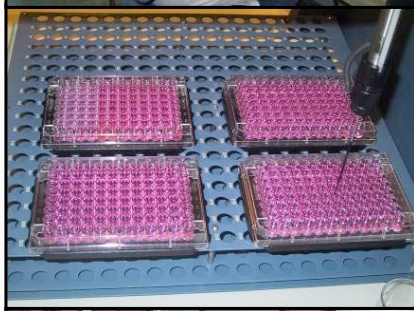
**Independent action**
(dissimilar MoA)

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

# Combined effects 9 estrogens

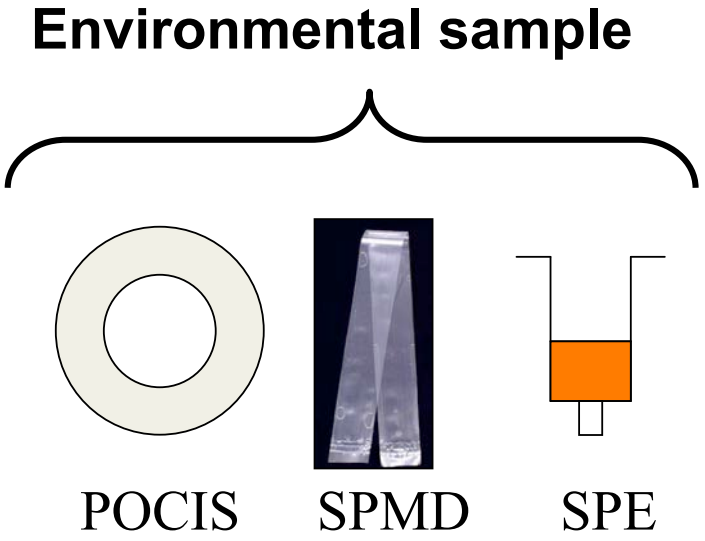


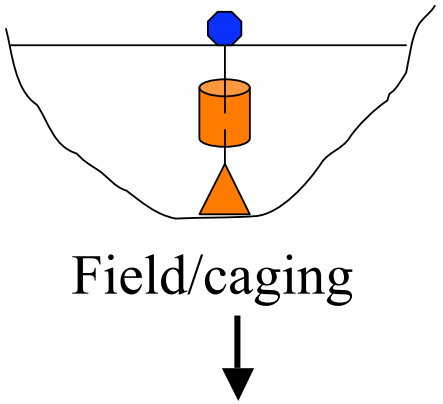



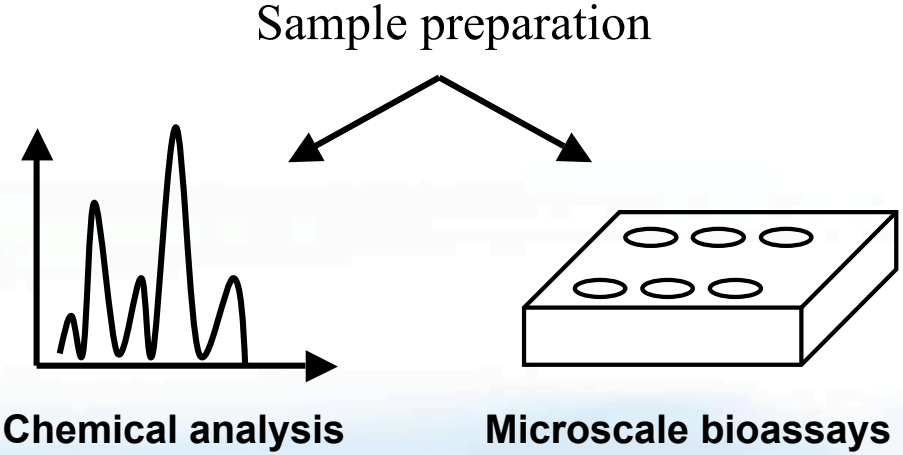


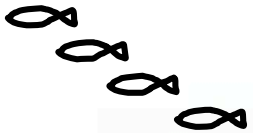
Petersen and Tollefsen (2011). Assessing combined toxicity of estrogen receptor agonists in a primary culture of rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Aquatic Toxicology 101, 186–195

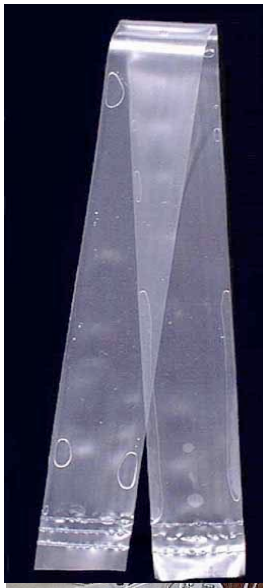
# Environmental risk assessment



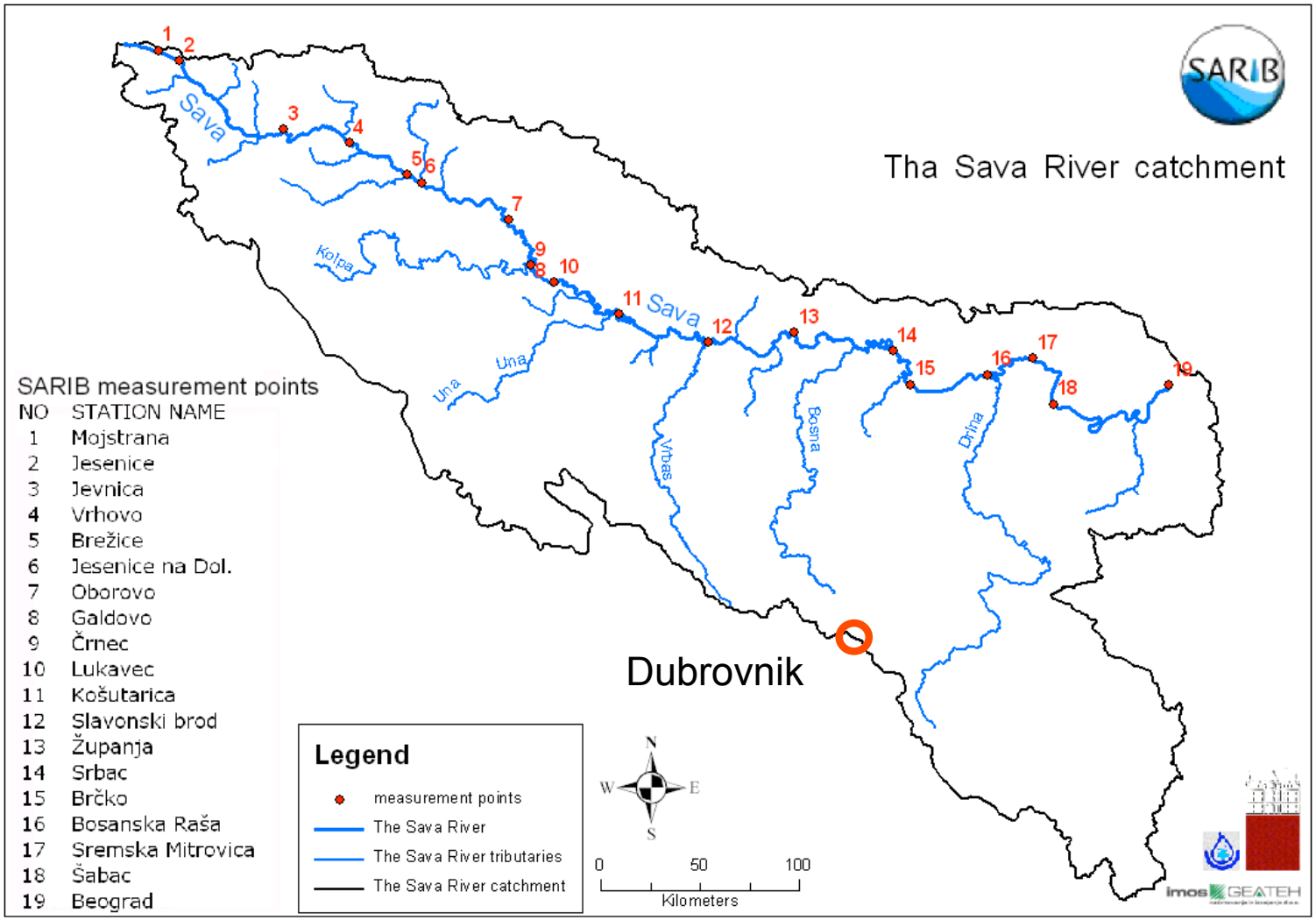



# Estrogens - Sava River basin

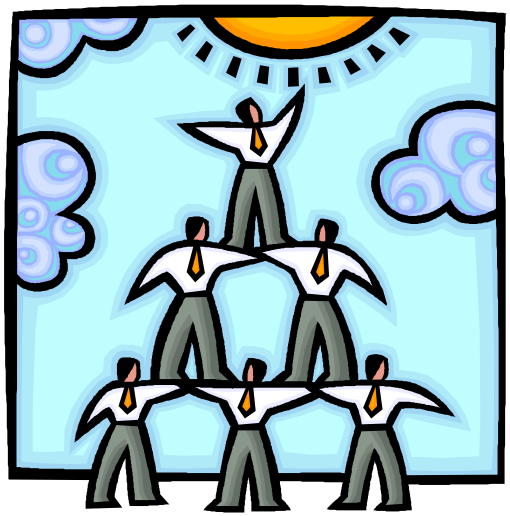



**Sampling site**

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



# Acknowledgements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Camilla Blikstad
Torsten Källqvist
Kevin V. Thomas

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