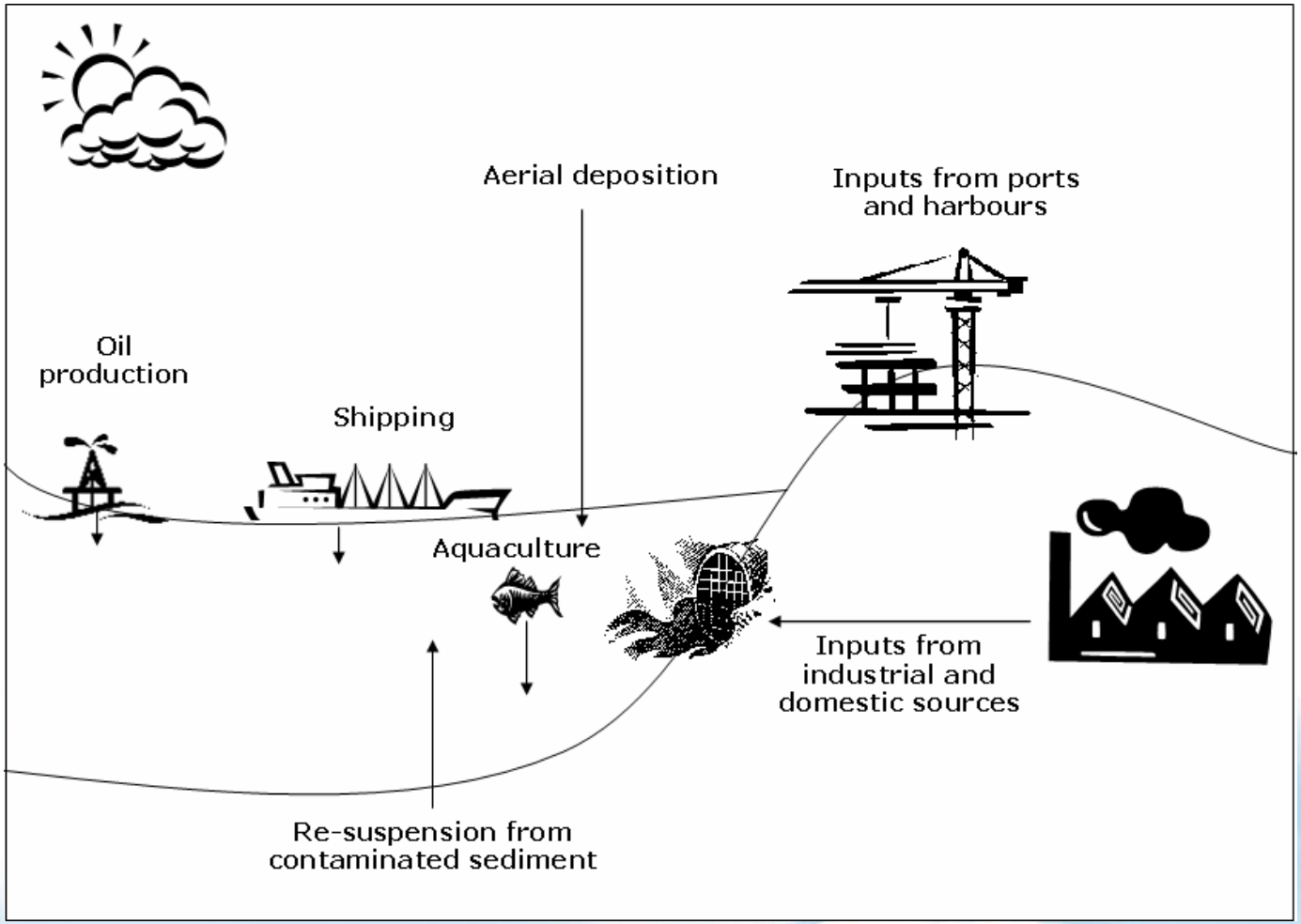
Effects of contaminants and combination effect measurements

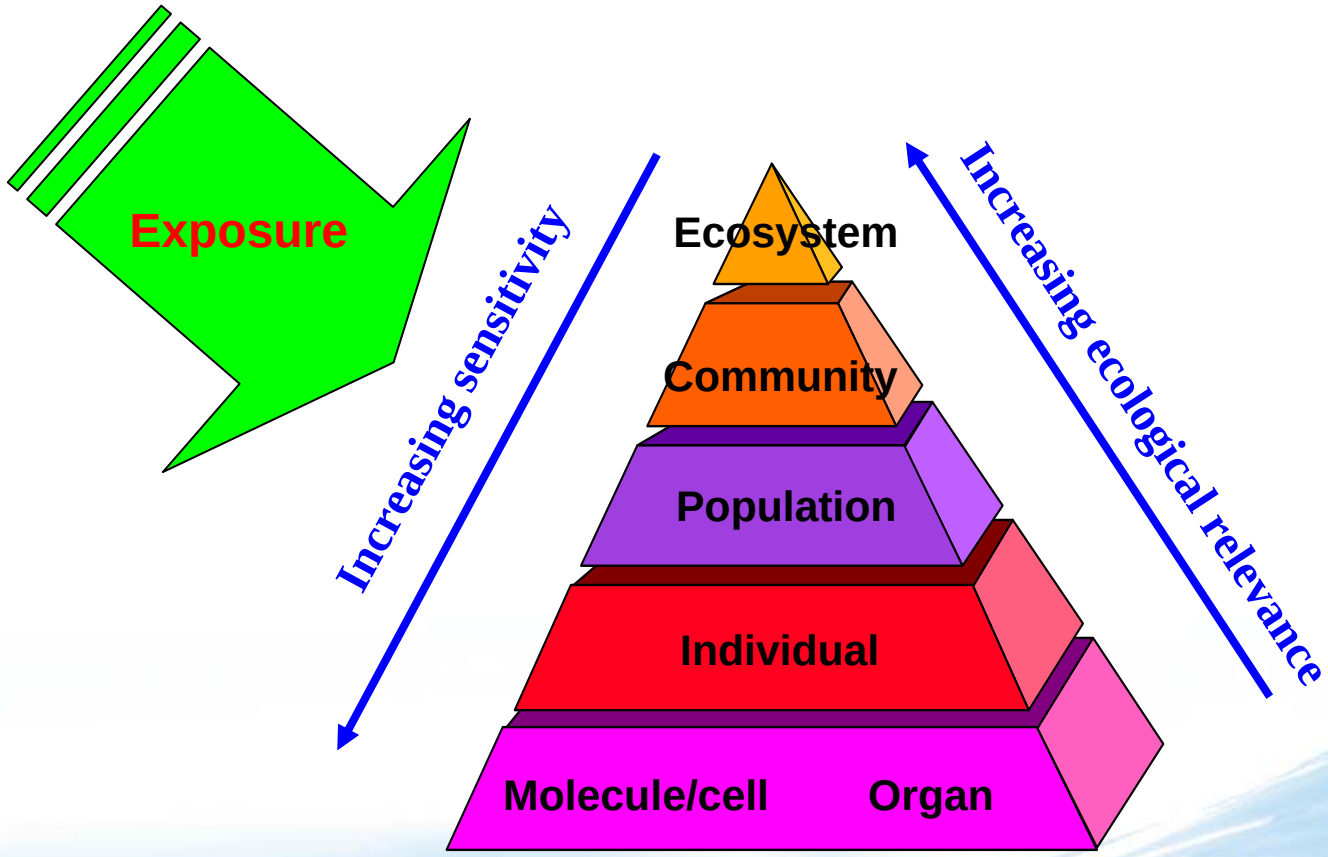
Knut Erik Tollefsen[1,2] and Kevin V. Thomas[1]
(ket@niva.no/kth@niva.no)

[1]NIVA-Norwegian Institute for Water Research (NO)
[2]UMB-University of Life Sciences (NO)

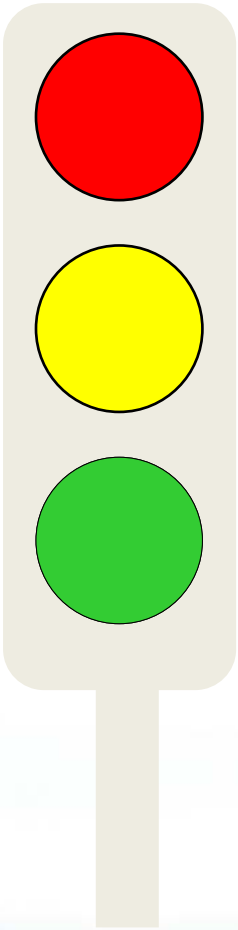
Multiple contaminants and sources



Biological effects at different organisational level



Chemical risk assessment



Toxicity (NOEC<1 mg/L) => **Hazard**

Bioaccumulation (LogK_{ow}>3)

Persistence (not readily biodeg. <30% in 28d)

Environmental risk !

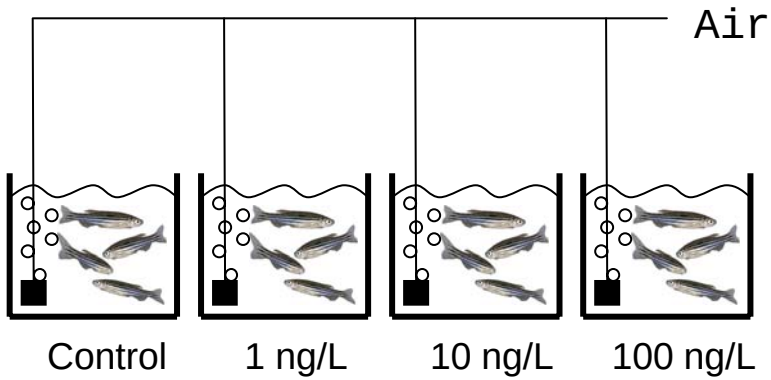
Objectives

<u>Ecotoxicological screening tests and risk assessment</u>

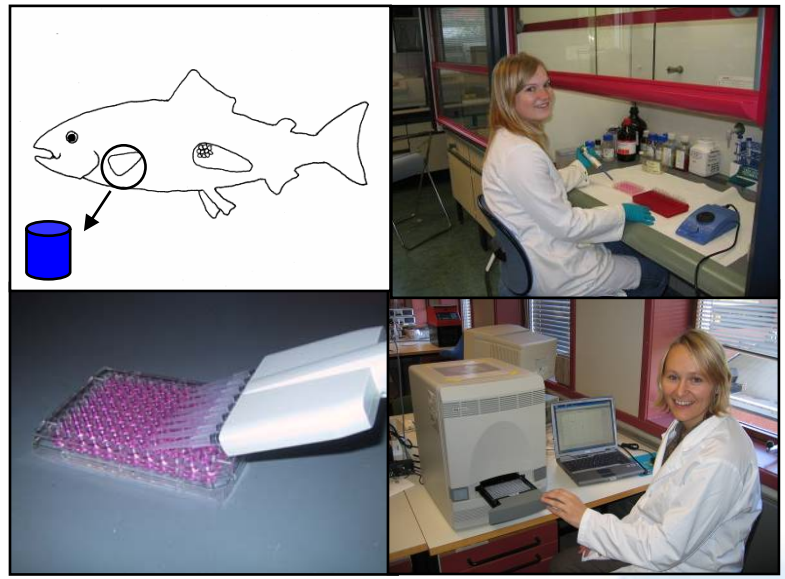
- (Priority/existing pollutants)
- Compounds of emerging concern (Ag-NPs, sucralose)
- Complex mixtures (estrogen mimics)
- Integrated testing strategies
- Alternative testing/screening methods
- Future directions

Experimental models

In vivo
(1-100 days exposure)



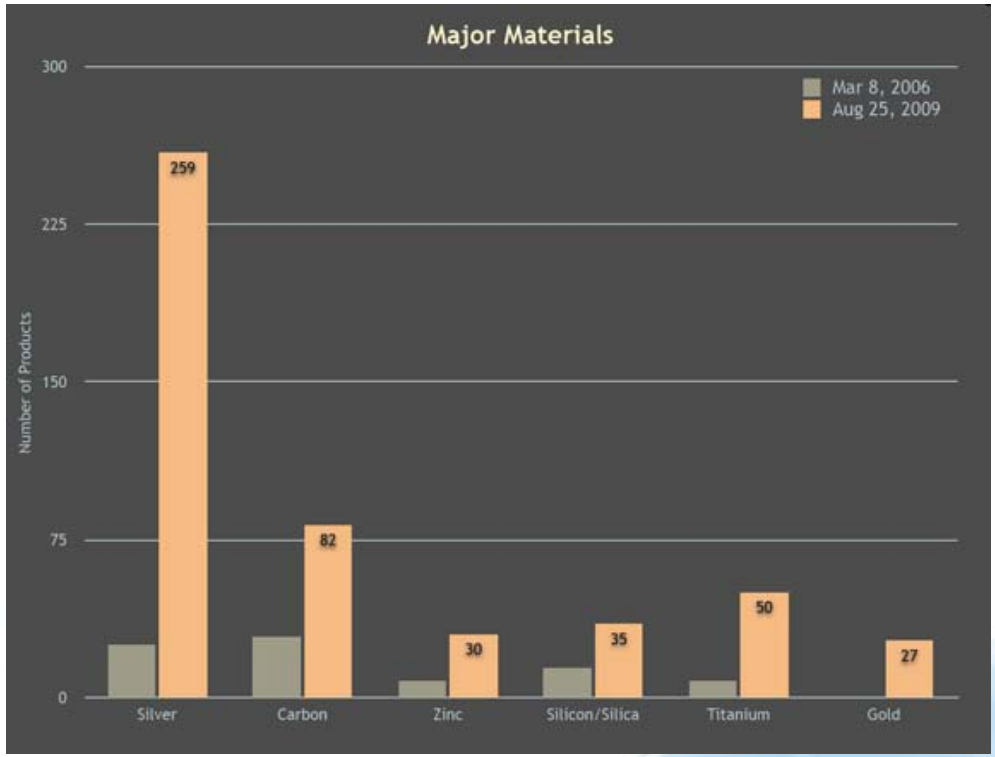
In vitro
(1-4 days exposure)



Engineered nanoparticles

Gold (Au NP)

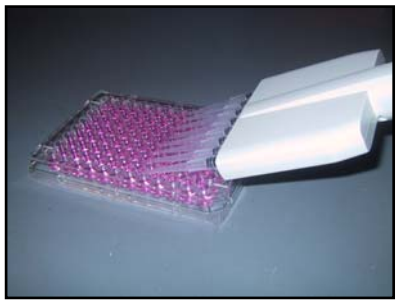
medical application
consumer products



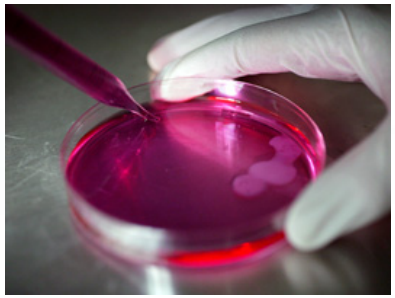
http://www.nanotechproject.org/inventories/consumer/analysis_draft/)

In vitro experimental model

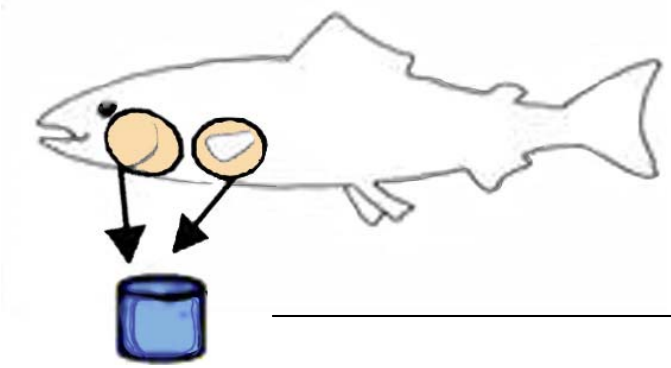
What are the possible effects of nanoparticles on fish cells used as an *in vitro* test system?



Primary hepatocytes



Primary gill epithelium cells



Liver

Gill

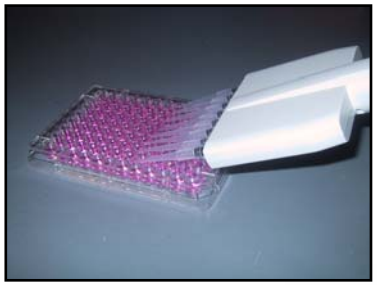
Exposure

(48h)

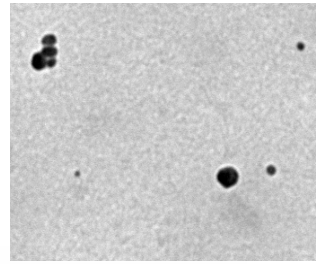
Cytotoxicity

Reactive oxygen species (ROS) production

Epithelial integrity

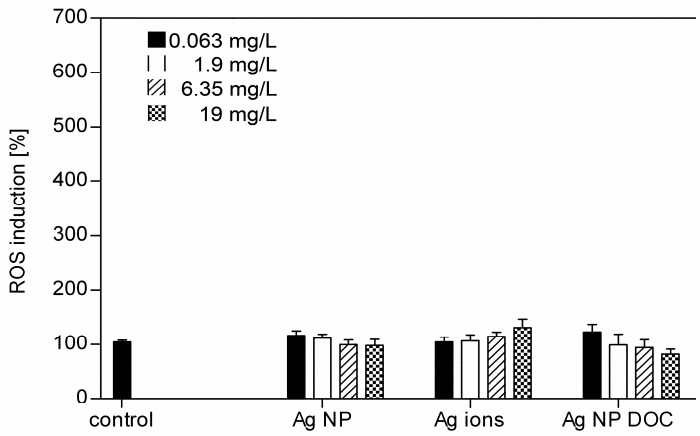


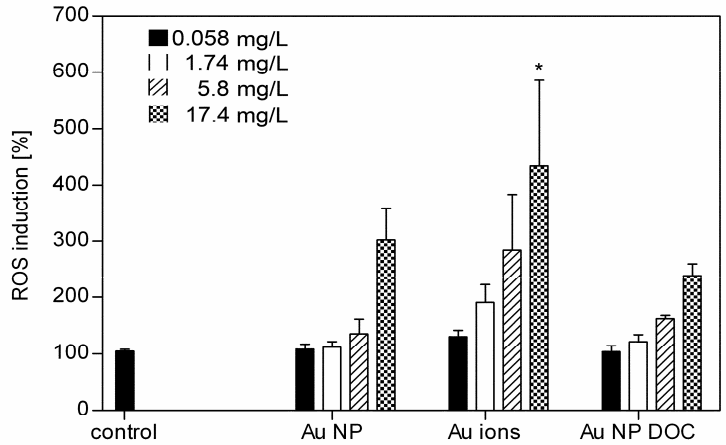
ROS & Cytotoxicity (hepatocytes)



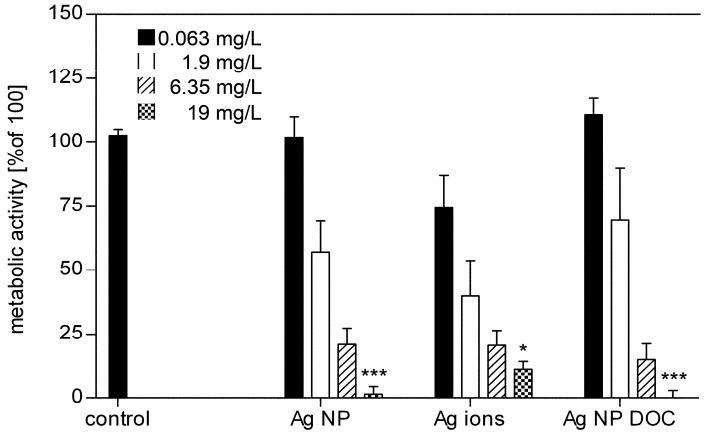
Silver NPs **Gold NPs**

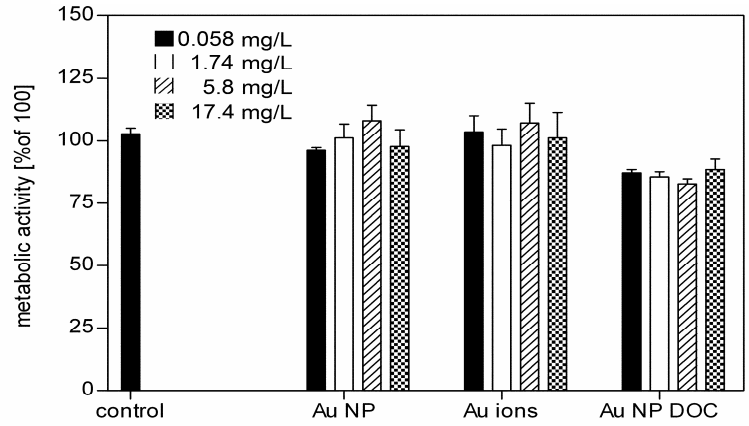
ROS





Cytotoxicity





Farkas et al. 2010. Effects of silver and gold nanoparticles on rainbow trout (Oncorhynchus mykiss) hepatocytes. Aquatic toxicol. 96: 44-52.

In vivo study with salmon

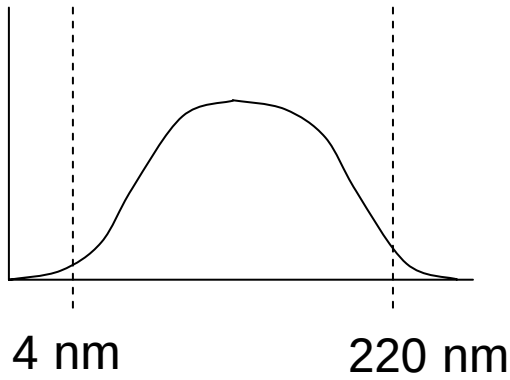
Contr 1 µg/L 20 µg/L 100 µg/L 20 µg/L 100 µg/L

Commercial NP solution | Ag^+ | HM NP

Salmon (~25g, n=15) exposed to Ag+ and AgNPs for 2x24h (48h)

NP characterisation

220 nm ultrafiltration
4 nm hollow fibre X-flow ultrafiltration
ICP-MS

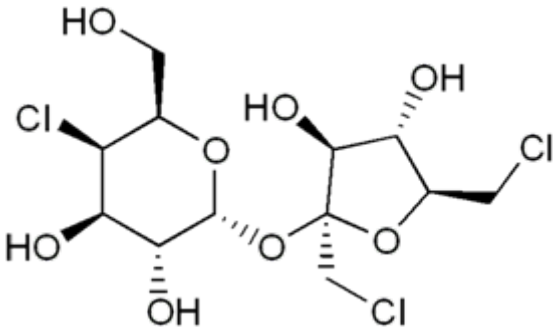


Biological endpoints

Gill accumulation
Gill histopathology
Gill genetic stressmarkers (qPCR)
Plasma ions and glucose
Mortality

Microarray

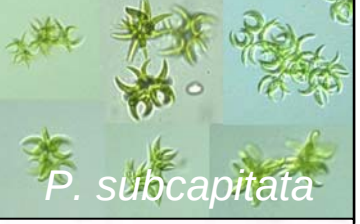
Sucralose



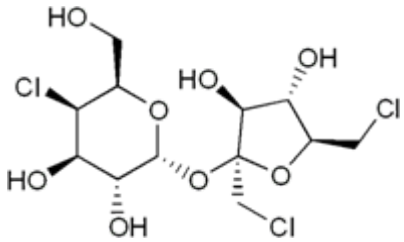
Sucralose

- 600 times sweeter than sucrose
- Chemically stable (inert)
- pH and UV stable
- High water-solubility
- Low fat-solubility
- Slow biodegradation potential in STW

	Concentrations (μgL^{-1})	References
STW effluents	*1.8-119*	*(Brorström-Lunden et al., 2008; Green et al., 2008; Loos et al., 2009; Mead et al., 2009; Scheurer et al., 2009)*
Lake/riverine water	0.004-3.6	*(Brorström-Lunden et al., 2008; Loos et al., 2009; Mead et al., 2009; Scheurer et al., 2009)*
Sea water	0.001-0.39	*(Green et al., 2008; Loos et al., 2009; Mead et al., 2009)*



Bioaccumulation tests (OECD 305)



Exposure
2 concentrations (10 and 100 mg/L)
Control

Duration
48 hours (Zebrafish 48h dep.)

Sampling
Time-specifc sampling

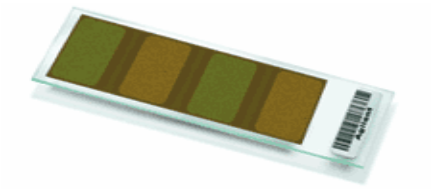
Chemical analysis
Biota and exposure media

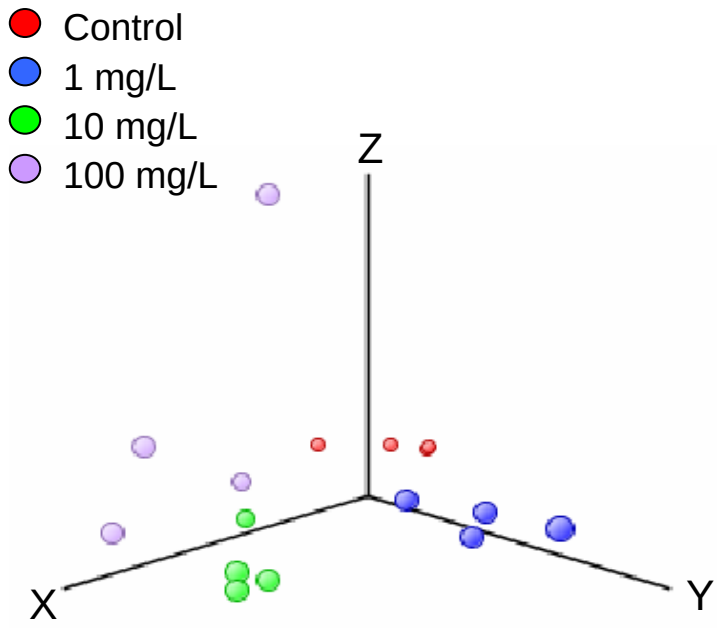
Bioconcentration factor (BCF)

Test conc (mg/L)	***P. subcapitata***	**Daphnia magna**	**Zebrafish**
10 mg/L	0.10	2.2	0.72
100 mg/L	0.15	1.6	0.47

Lillicrap, A. et al. (In press). Bioconcentration of sucralose in a multitrophic battery of aquatic organisms *Environ. Toxicol. Chem.*

Zf-liver gene expression





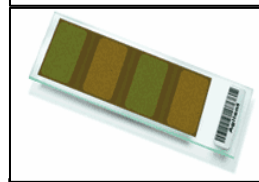
Agilent Zebrafish oligoarray

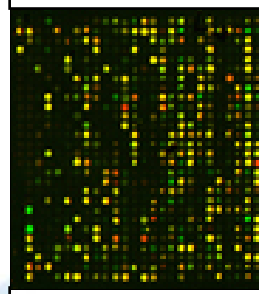
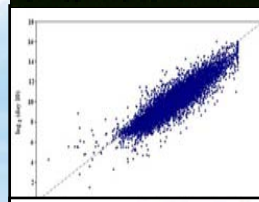
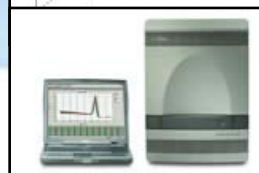
- High density oligoarray
- 22k (V1)/44k (V2) *D. rerio* probes
- Highly annotated
- Sequences (RefSeq, UniGene, TIGR, Ensemble & UCSC Zv7)

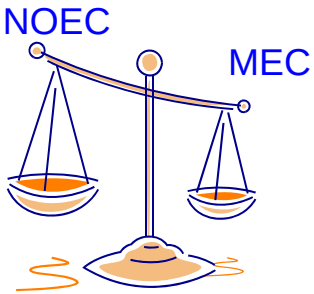
RUN and FYVE domain containing 2 , **ARNT-like 1A,** 1 unknown

basic helix-loop-helix domain containing, class B, 3 like
cryptochrome 1b, zinc finger CCCH-type containing 10, 2 unknown







Effects to be expected?

Sucralose

MEC= ~200 ng/L (effluent: 3-10 µg/L)

BCF_{fish}= <1

$NOEC_{acute}$ for fish ≥ 1800 mg/L

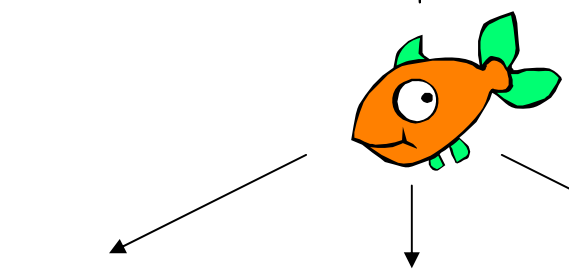
$NOEC_{gene}$: ≥100 mg/L (known toxic mechanisms)

$NOEC_{Lemma}$: ≥ 100 mg/L (growth inhibition)

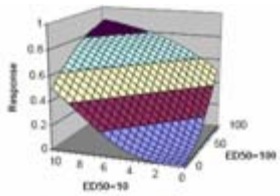
$MEC/NOEC_{gene}$: ≤ $5x10^{-5}$ (Effluent: **≤** $0.3\text{-}1x10^{-4}$)

Combined (joint) toxicity

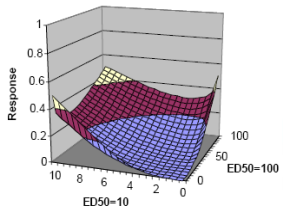
Comp A Comp B Comp C Comp D…..



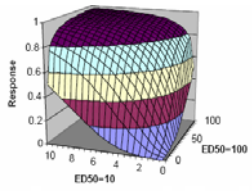
Additivity
(1+1=2)



Antagonism
(1+1<2)



Synergy
(1+1>>2)



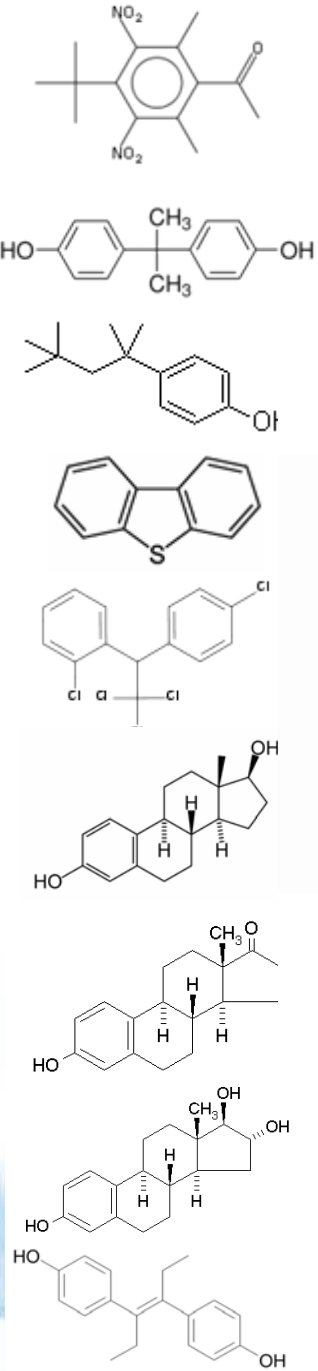
Concentration addition
(similar MoA)

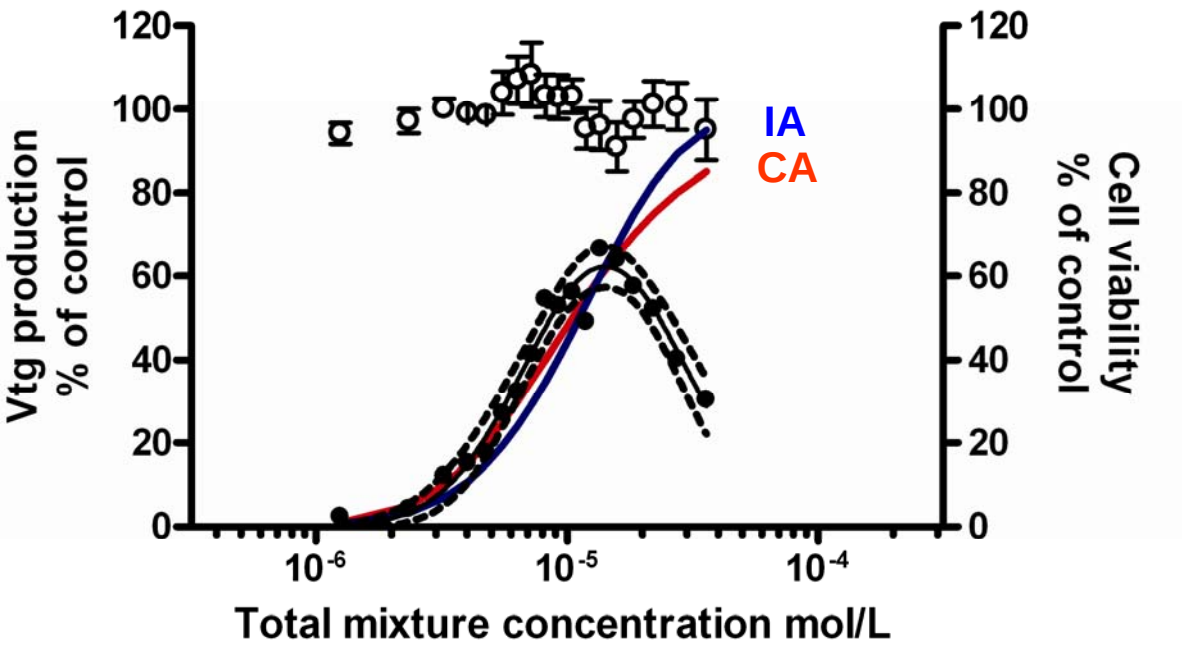
$$ECx_{(Mix)} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1}$$

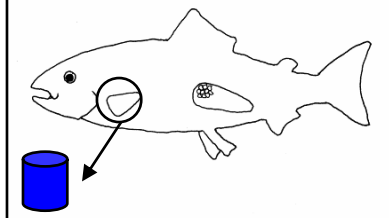
Independent action
(dissimilar MoA)

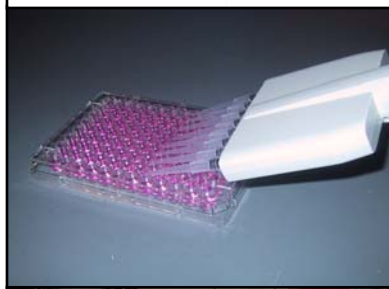
$$E_{Mix} = 1 - \prod_{i=1}^{n} (1 - E_i)$$

Joint toxicity - estrogens



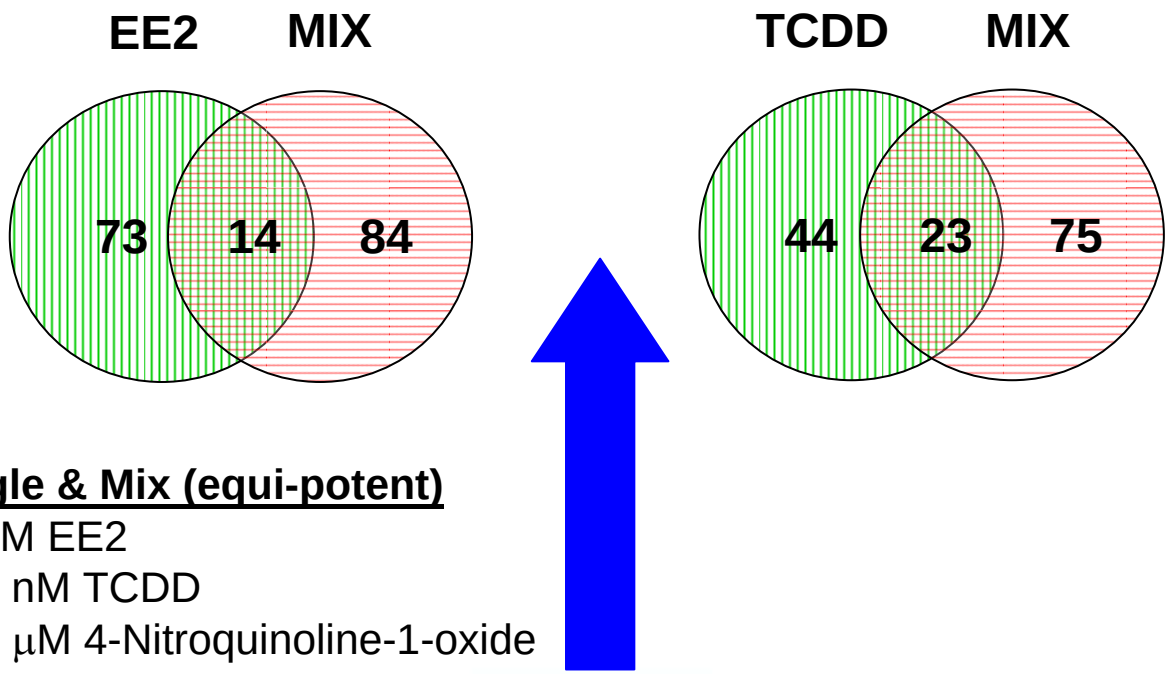




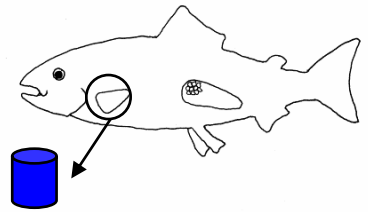


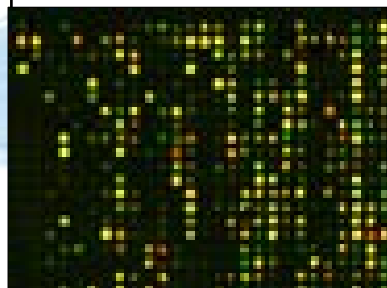
Petersen, K. & Tollefsen, K.E. (In press). Assessing combined toxicity of estrogen receptor agonists in a primary culture of rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Aquatic. Toxicol.*

Mixtures – gene expression



Single & Mix (equi-potent)
10 nM EE2
0.75 nM TCDD
0.75 μM 4-Nitroquinoline-1-oxide
100 μM Paraquat





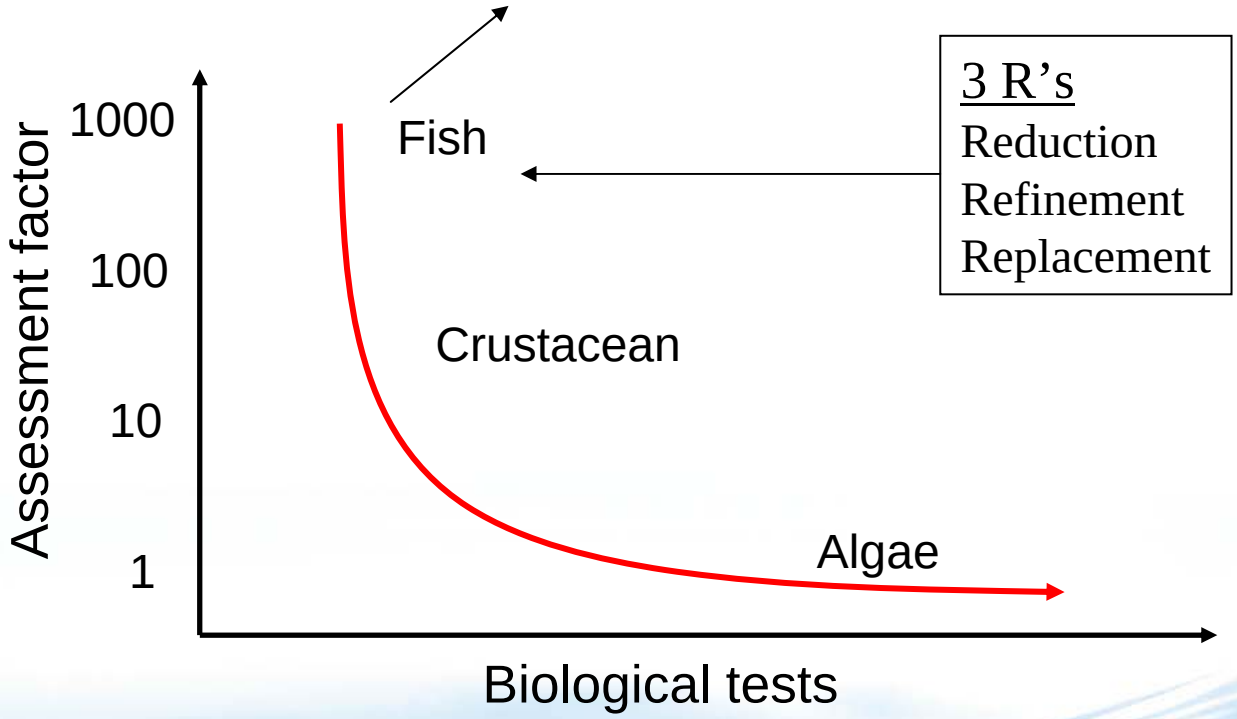
Finne, E. F., et al. (2007). Toxicogenomic responses in rainbow trout (*Oncorhynchus mykiss*) hepatocytes exposed to model chemicals and a synthetic mixture. *Aquat. Toxicol* .**81**: 293-303.

Risk assessment

Estimated animal usage

Base set (1-100 t/y): >1 million fish

Level 1 production (100-1000 t/y): >2 million fish



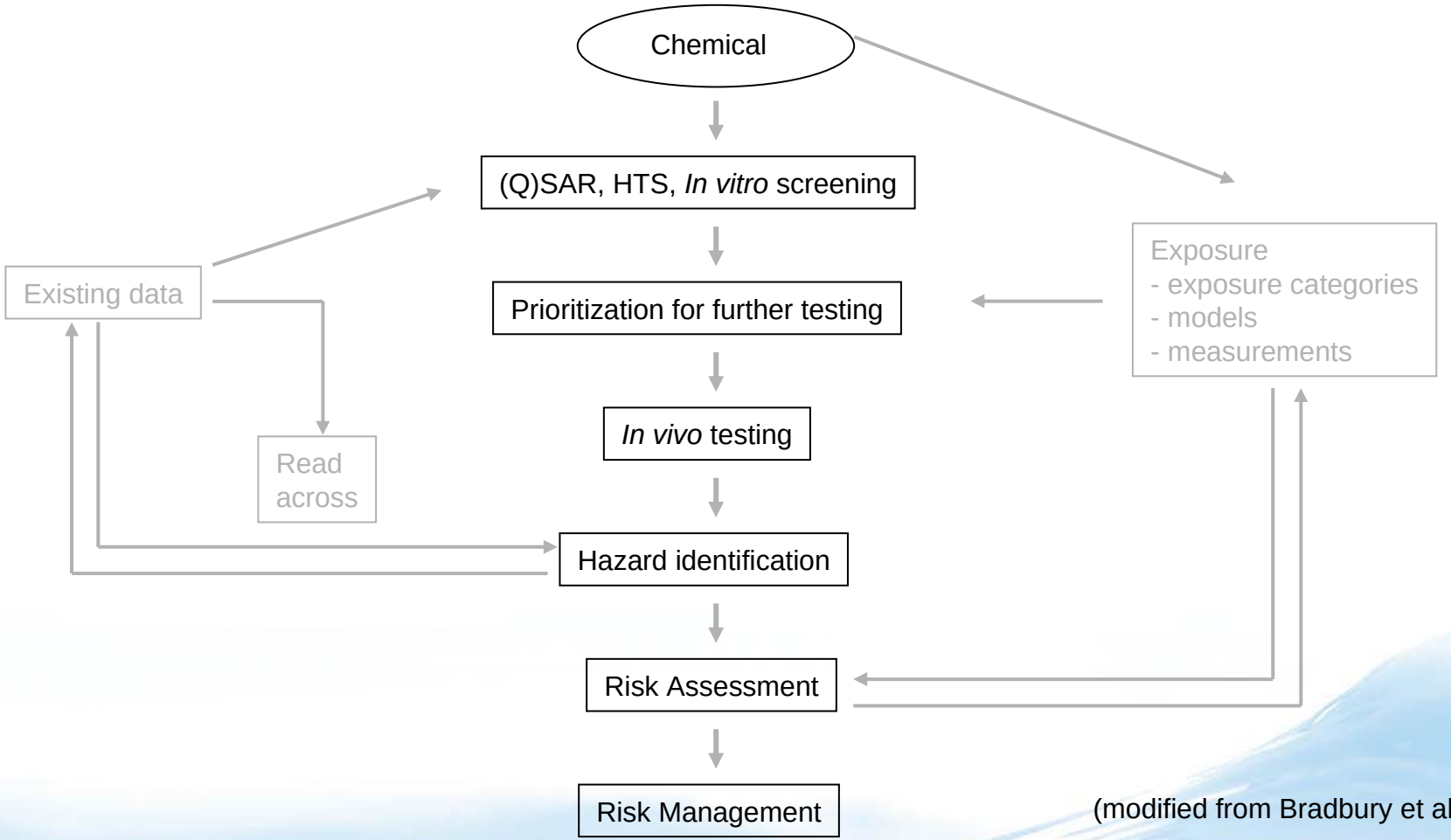
3 R's

Reduction

Refinement

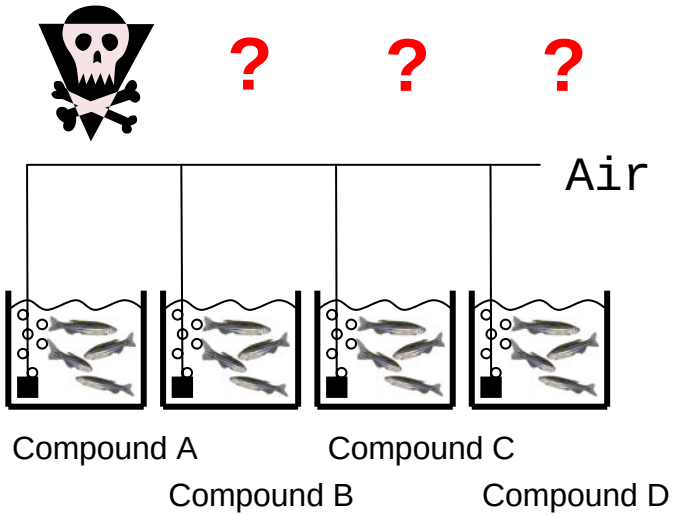
Replacement

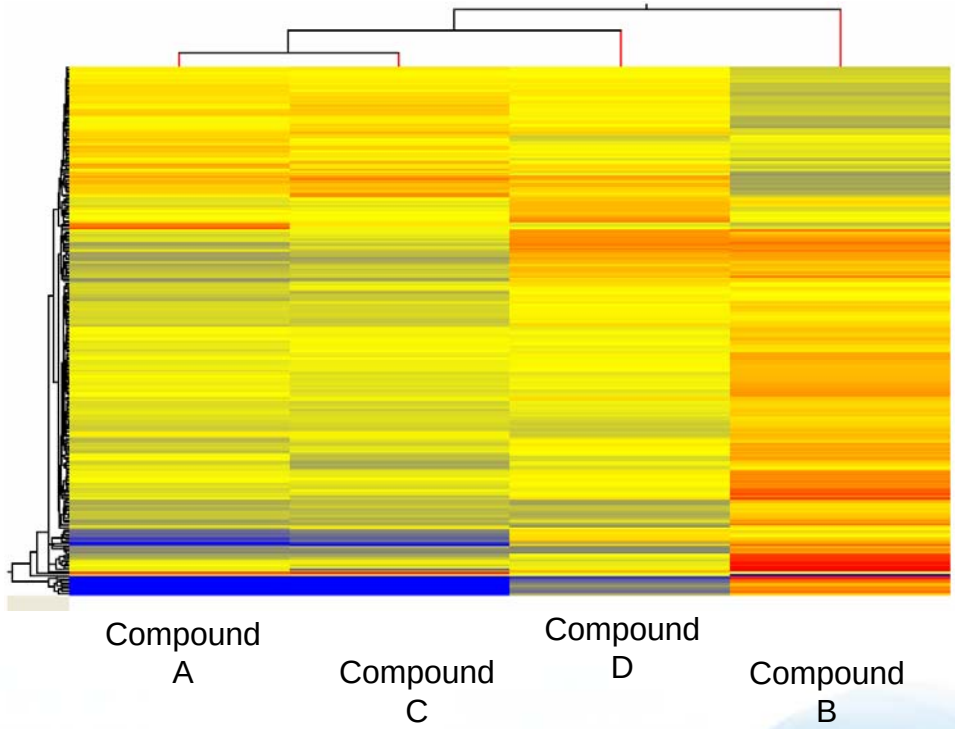
Integrated testing strategies



(modified from Bradbury et al. 2004)

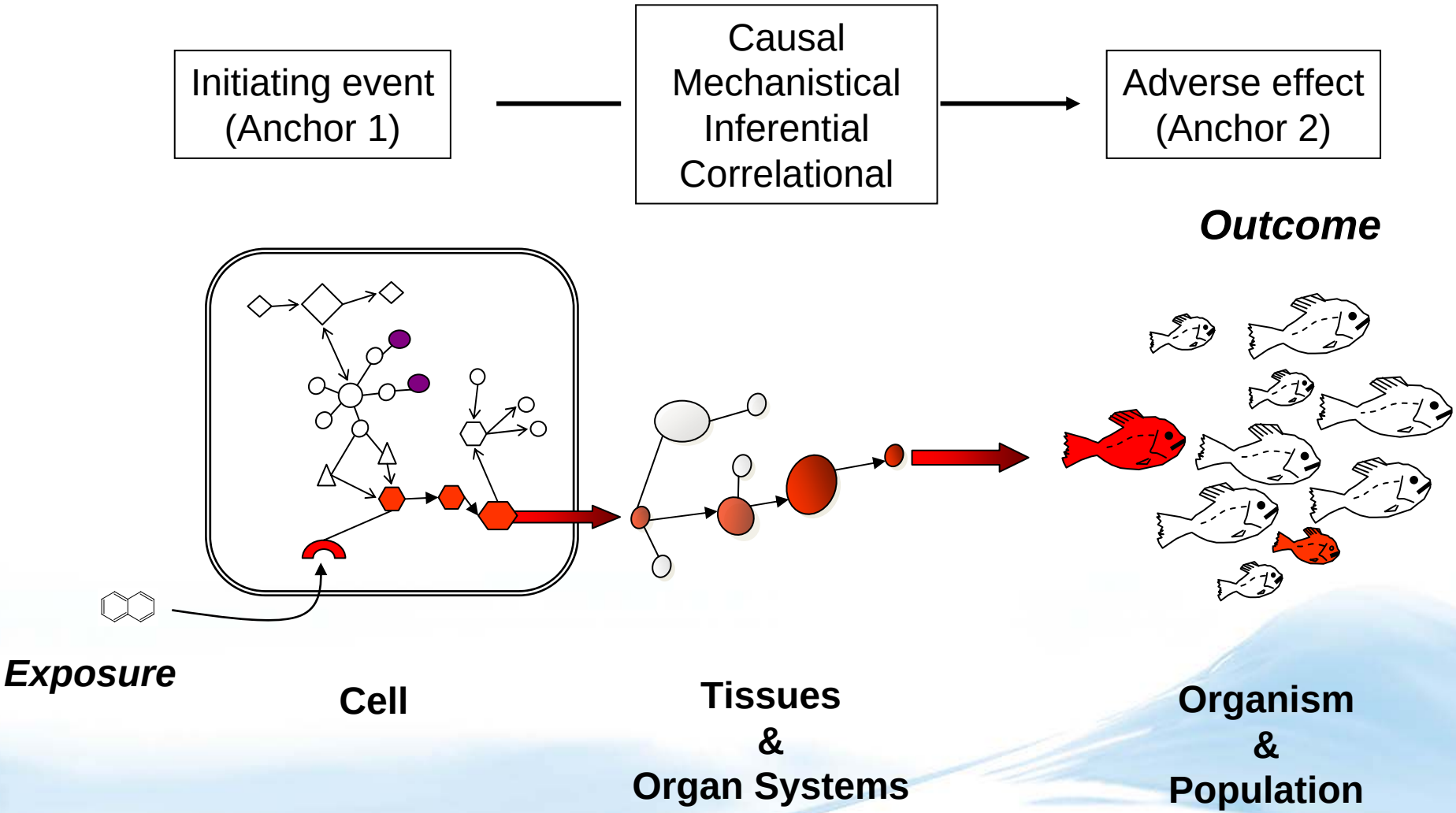
”OMICS” in toxicity screening



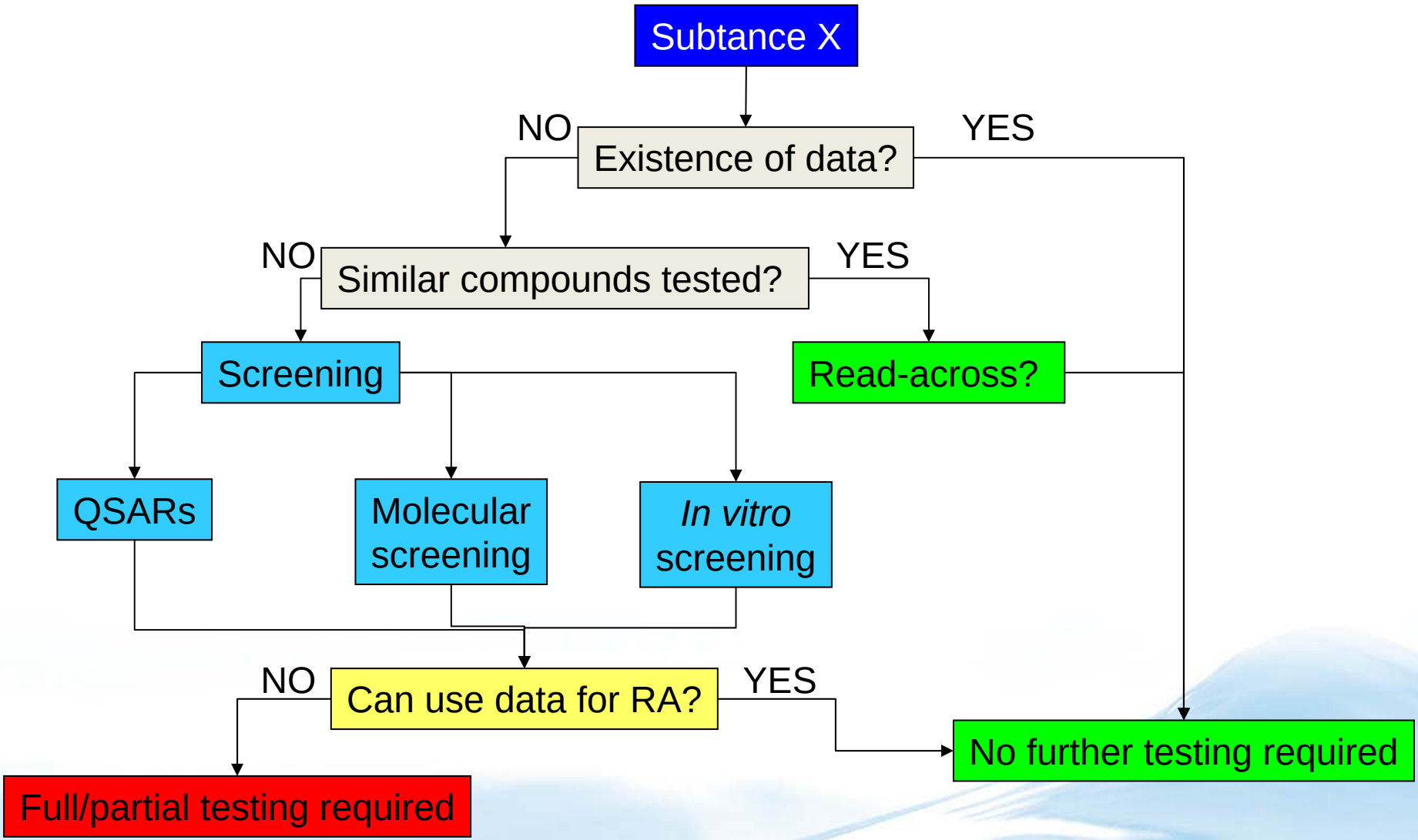


Use OMICS for preliminary screening, prioritization and read-across?

Adverse outcome pathways (AOP)

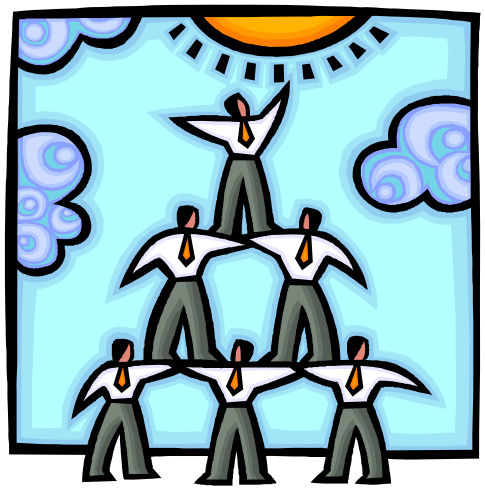


Strategy predicting PBT properties



Future directions

- Determine fate & effects of nanoparticles under realistic exposure scenarios (hard vs soft water)
- Assess toxicity of sucralose to mysid shrimp
- Determine combined (joint) toxicity to fish and crustaceans *in vivo*
- Develop/evaluate non-animal alternatives to ecotoxicological testing
  - *In silico* models, *in vitro* screening, ZF embryotest, ”Limits” test
  - AOP and OMICS approaches
- Support international initiatives to promote use of ITS approaches



Acknowledgements

NIVA
Adam Lillicrap
Eivind Farmen Finne
Julia Farkas
Karina Petersen
Kenneth McRae
Kathy Langford
Martine Musse
Steven Brooks

Norwegian Vet. College
Peter Alestrom
Jan R. Torp
Øistein Evensen

University of Oslo
Tor Fredrik Holth
Silje Kile

University of Life Sciences (Ås)
Deborah Oughton
Henrik Mikkelsen

Financial contribution
NFR-SIP 160118 NEWPOLL
NFR-project 178621 MixTox
NFR-project 182069 Fate & ecotoxicity of NPs
NFR-project NanoTrace
NFR-196318 alterREACH
Minstry of Environment (Institutional funding)
Tate & Lyle (Decatur, IL, USA)

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

The Research Council of Norway