3rd meeting in the OECD advisory group
for molecular screening and toxicogenomics
(Paris, Oct. 26-27)

Ecotoxicogenomics in predictive risk assessment – potential use of *in vitro* and *in vivo* transcriptomics for assessing toxicological effects of chemicals

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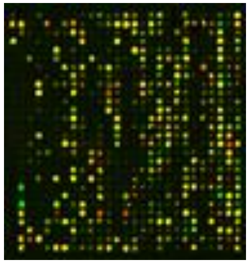
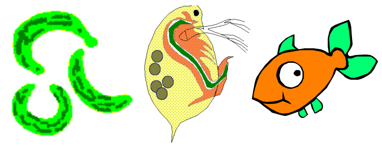
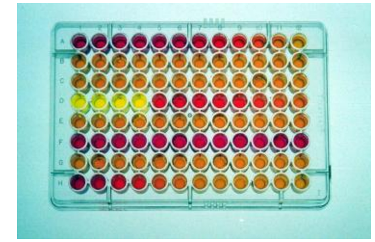
Ecotoxicology and risk assessment



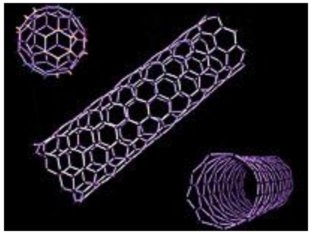




Ecotoxicological testing

Molecular toxicology

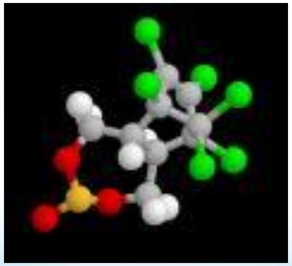
Biomarker research



Cellular toxicology

Chemistry & Ecotoxicology

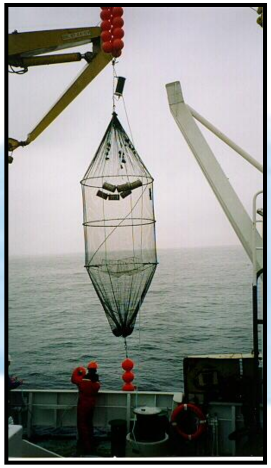
Effluent monitoring



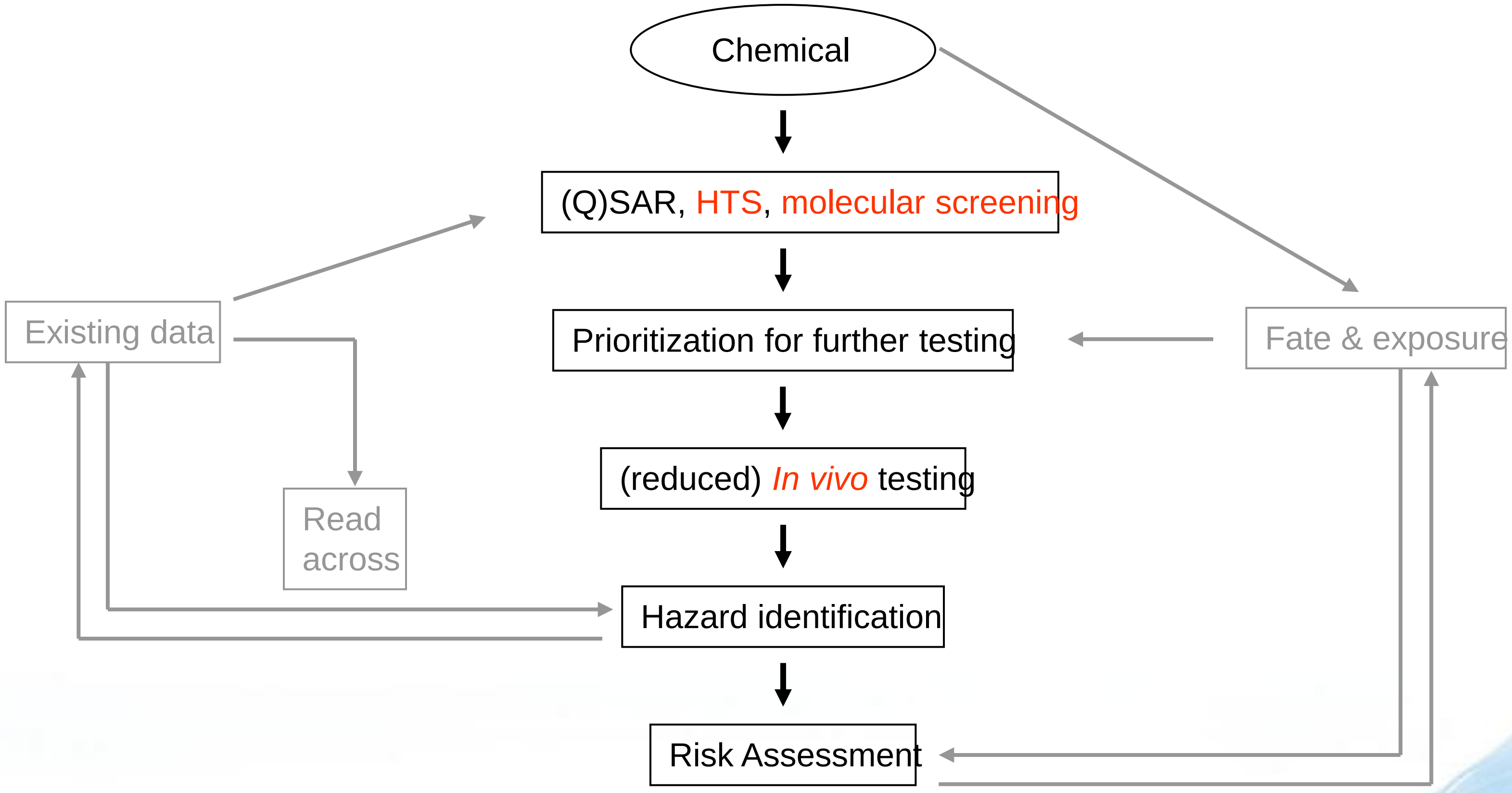
Ecotoxicological consulting

Environmental monitoring

Risk assessment

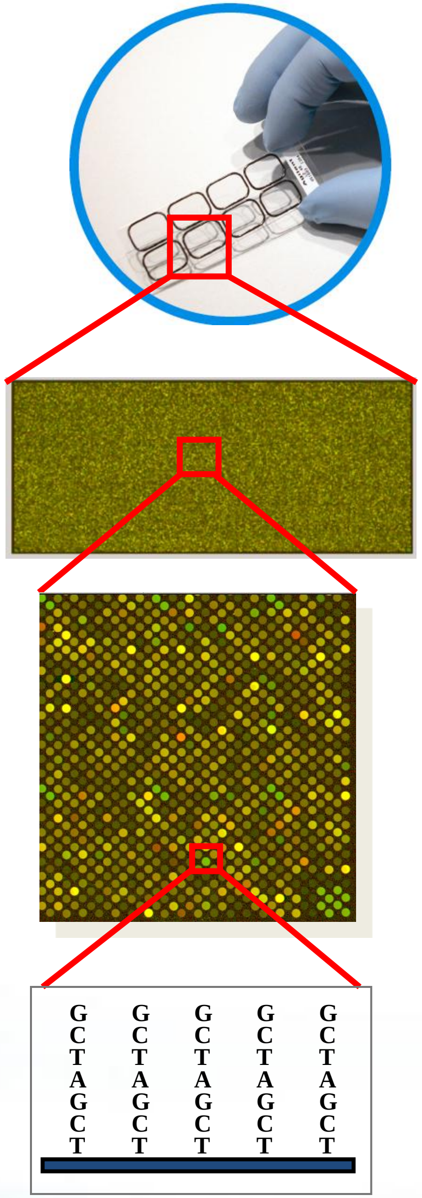


Integrated testing strategies



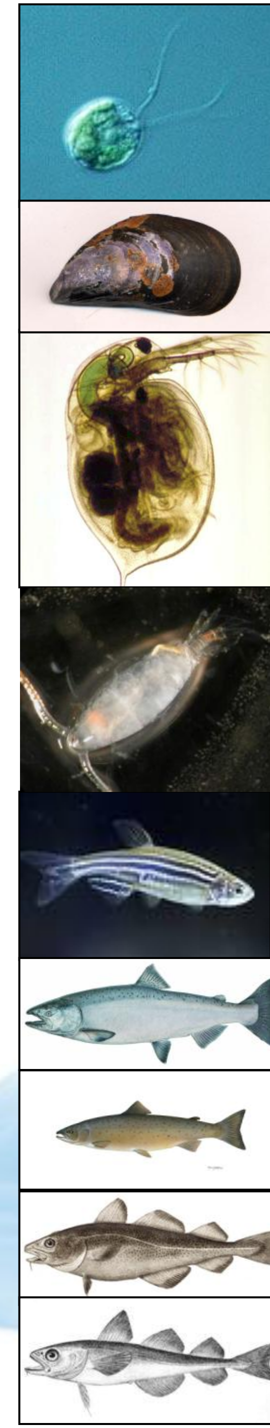
Objectives

- Develop transcriptomic tools for aquatic toxicity testing
- Apply technology to *in vitro* and *in vivo* test systems
- Screen for potential adverse effects of chemicals & mixtures
- Determine whether technology is applicable to risk assessment



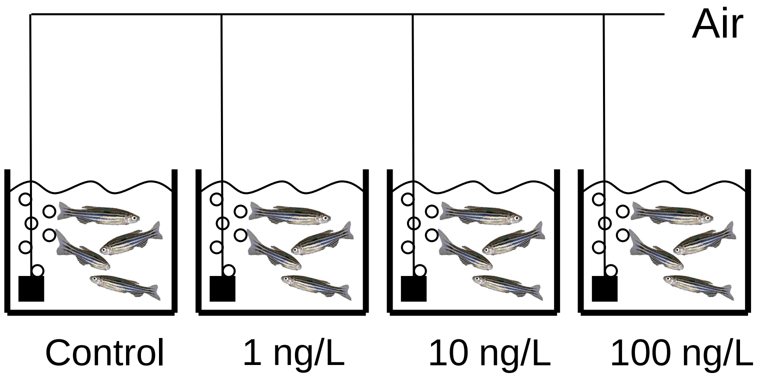
Oligo microarrays

- Flexible (custom & standard) microarrays
- 60-mer oligonucleotides printed on array (15-244k)
- Chip technology accomodates up to 1 million gene probes (features)
- Gene expression detected by laser scanner technology
- Bioinformatics translate gene expression to meaningful biological information
- Full genome sequence available for some species
- Currently applied for *in vitro* and/or *in vivo* testing (zebrafish, salmonids, cod, halibut, calanus, mussels)
- Implemented in environmental monitoring (salmonids, cod, calanus, mussels)



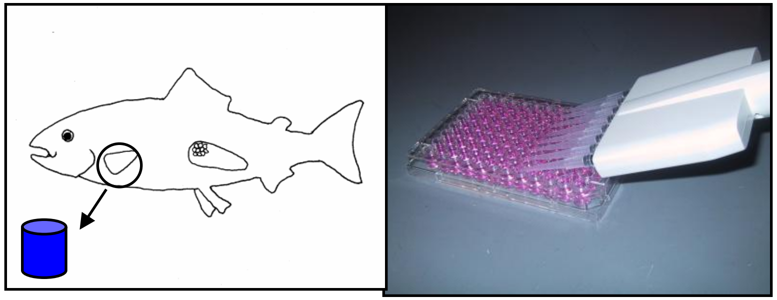
Experimental models

In vivo
(1-4 days exposure)



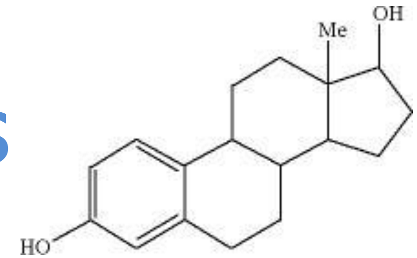
Studies
(xeno)estrogen (E2, EE2)
PAH (BaP)
Persistent compounds (HBCD)
Pharmaceuticals (Diclofenac)
Artificial sweetener (Sucralose)
Metals (Copper)
Nanoparticles (Silver NPs)
Road run-off/tunnel wash water

In vitro
(1-4 days exposure)

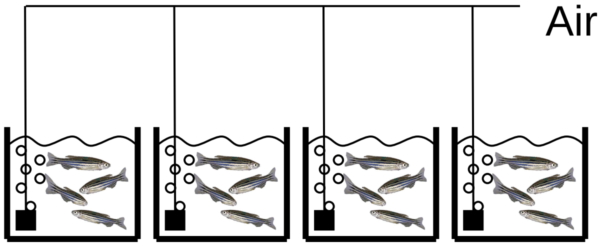


Studies
(xeno)estrogen (EE2)
Persistent compounds (TCDD)
Direct mutagens (1-NQO)
Pesticides (paraquat)
Mixtures

Endocrine disruptors

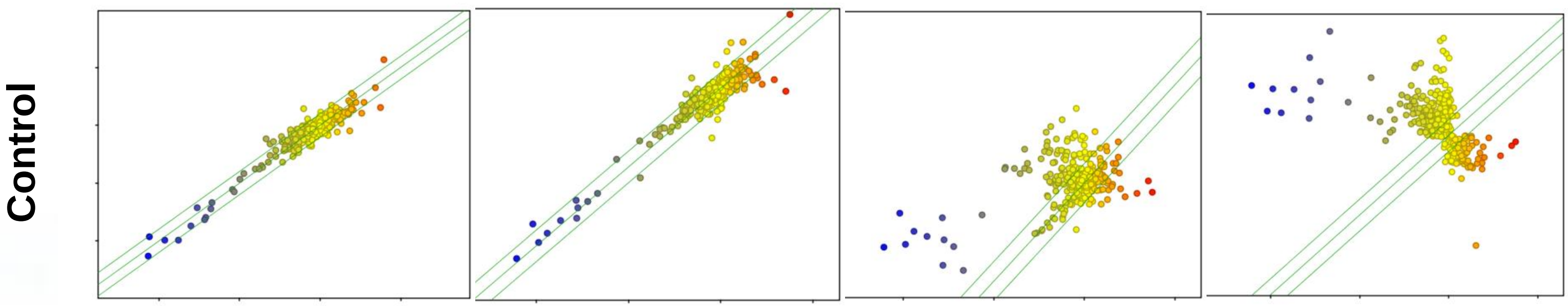


Ethynylestradiol



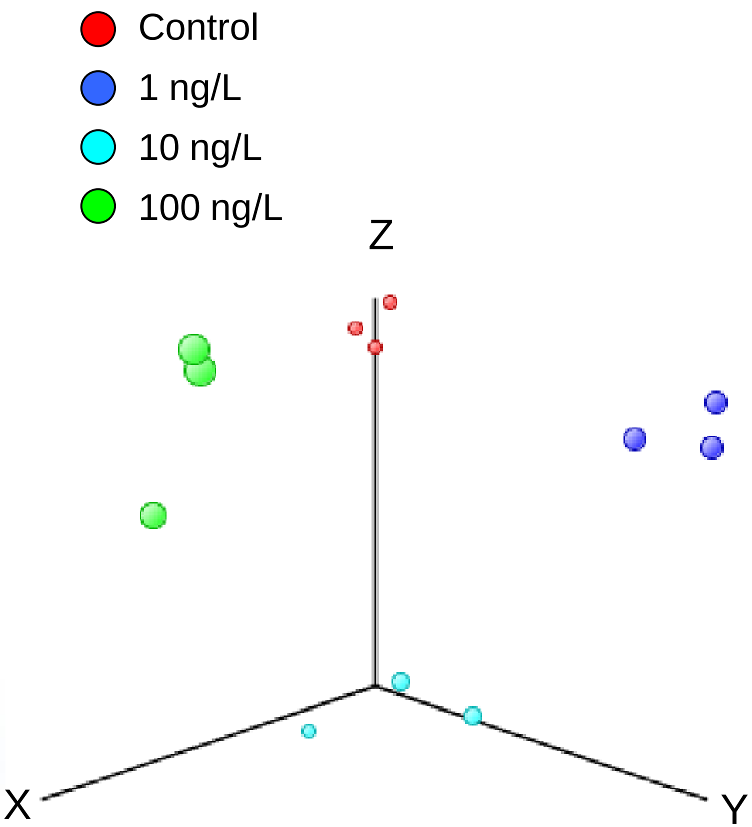
17α-ethynylestradiol (EE2)

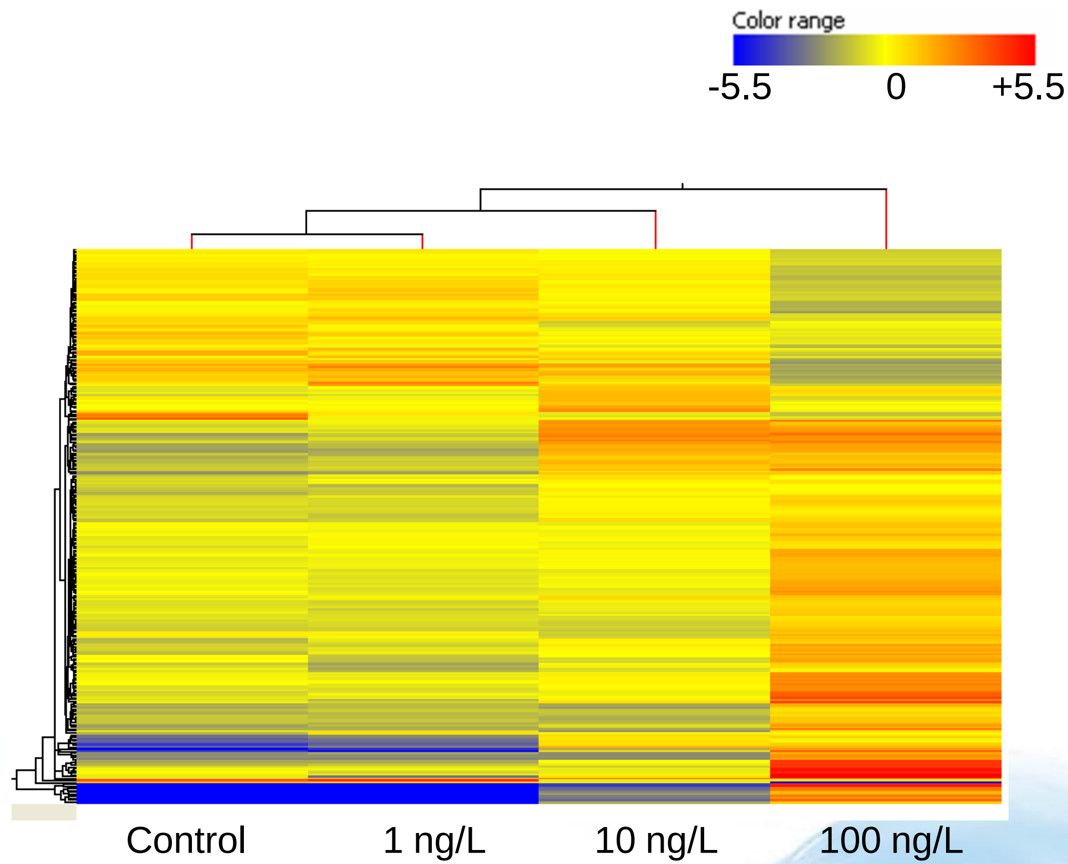
Control | **1 ng/L** | **10 ng/L** | **100 ng/L**



Differential gene expression in male zebrafish livers after 48h exposure (323 features)

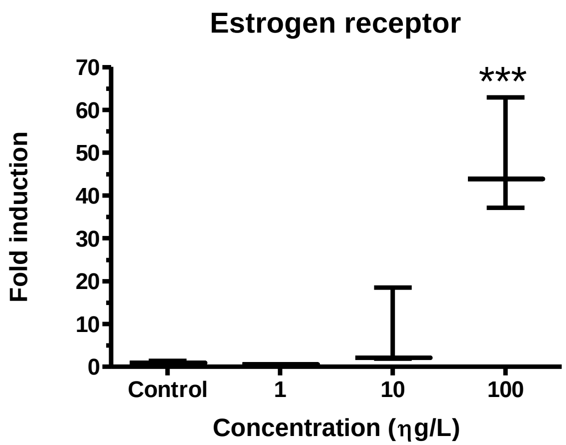
Global clustering (EE2)

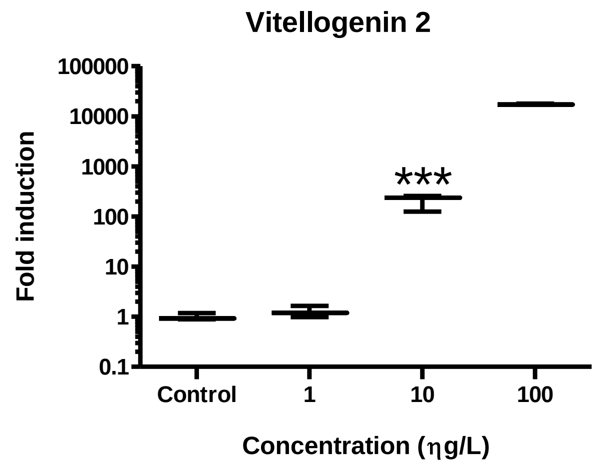




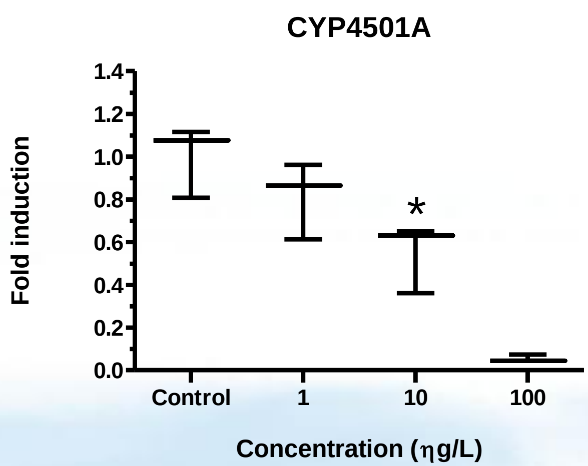
Single gene expression (EE2)

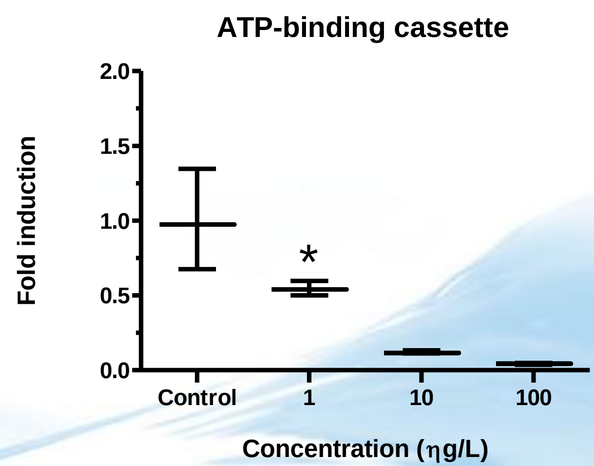
Up-regulation



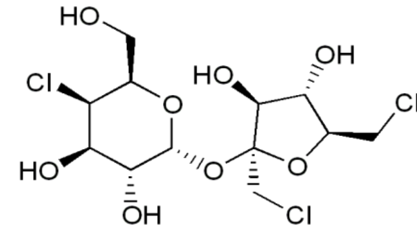


Down-regulation

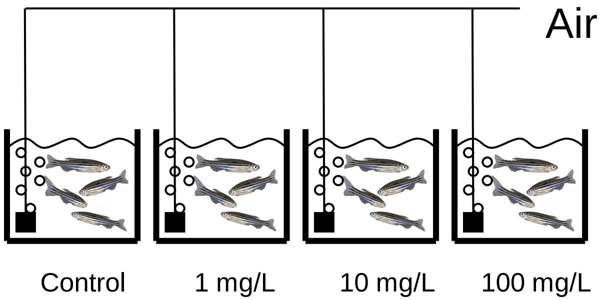


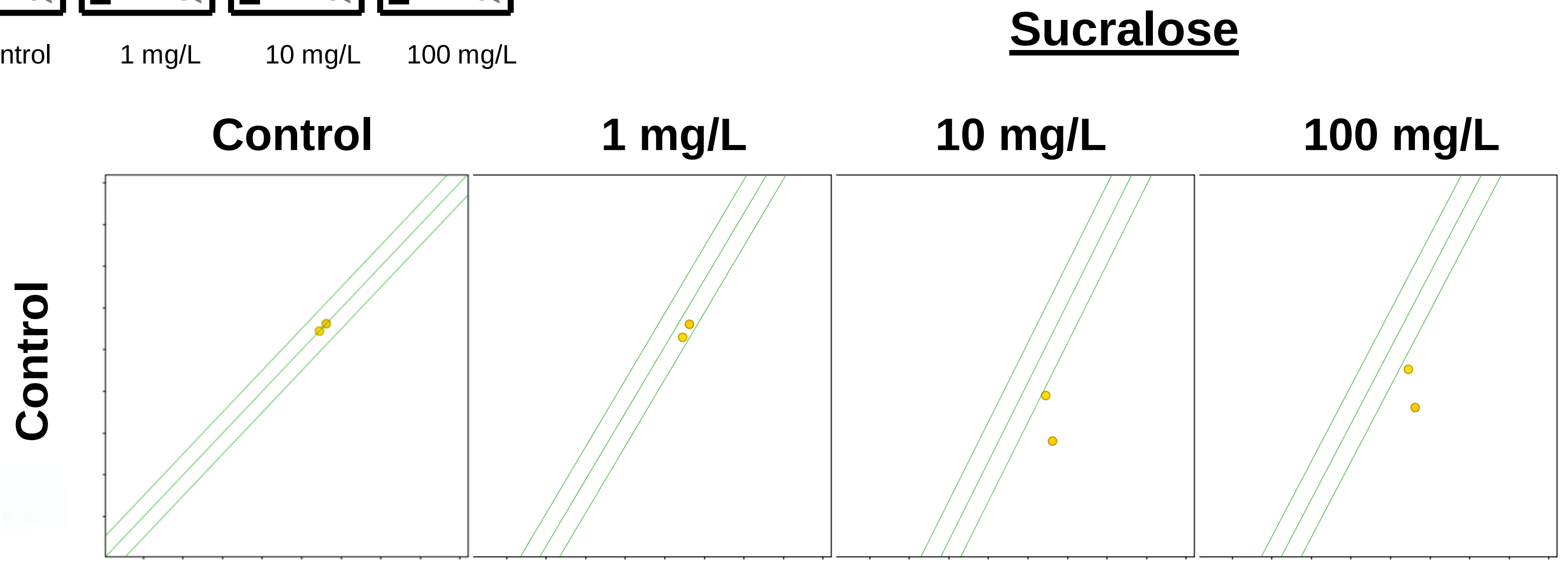


Intense sweetener

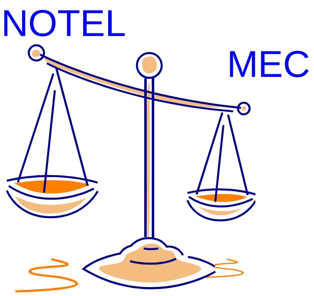


Sucralose





Differential gene expression in female zebrafish livers after 48h exposure (2 features)



Effects to be expected?

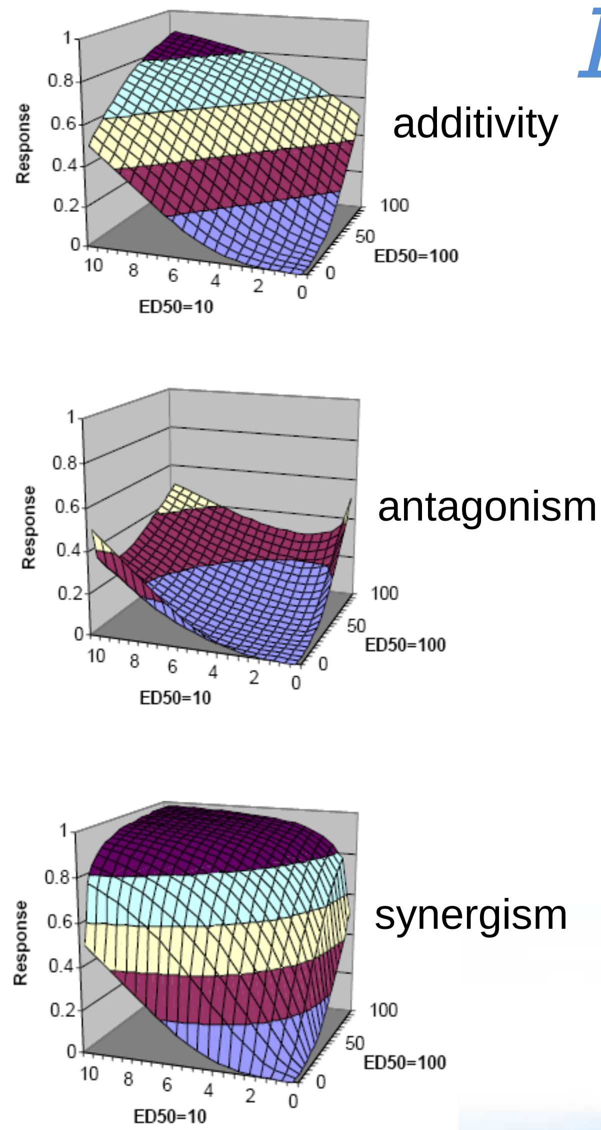
17α-ethynylestradiol (EE2)	Sucralose
Environment (MEC)= 1-15 ng/L	**Environment (MEC)= ~**50 ng/L (effluent: 3 ug/L)
$NOEC_{acute}$ for fish ≥ 1 μg/L	**$NOEC_{acute}$** for fish ≥ 1800 mg/L
NOTEL: 1-10 ng/L	**NOTEL:** 10-100 mg/L
MEC/NOTEL ratio: 1-15	**MEC/NOTEL ratio:** $0.5\text{-}5 \times 10^{-7}$ (Effluent: $0.3\text{-}3 \times 10^{-4}$)

MEC: measured environmental concentration, NOTEL: No Observed Transcriptional Effect Level

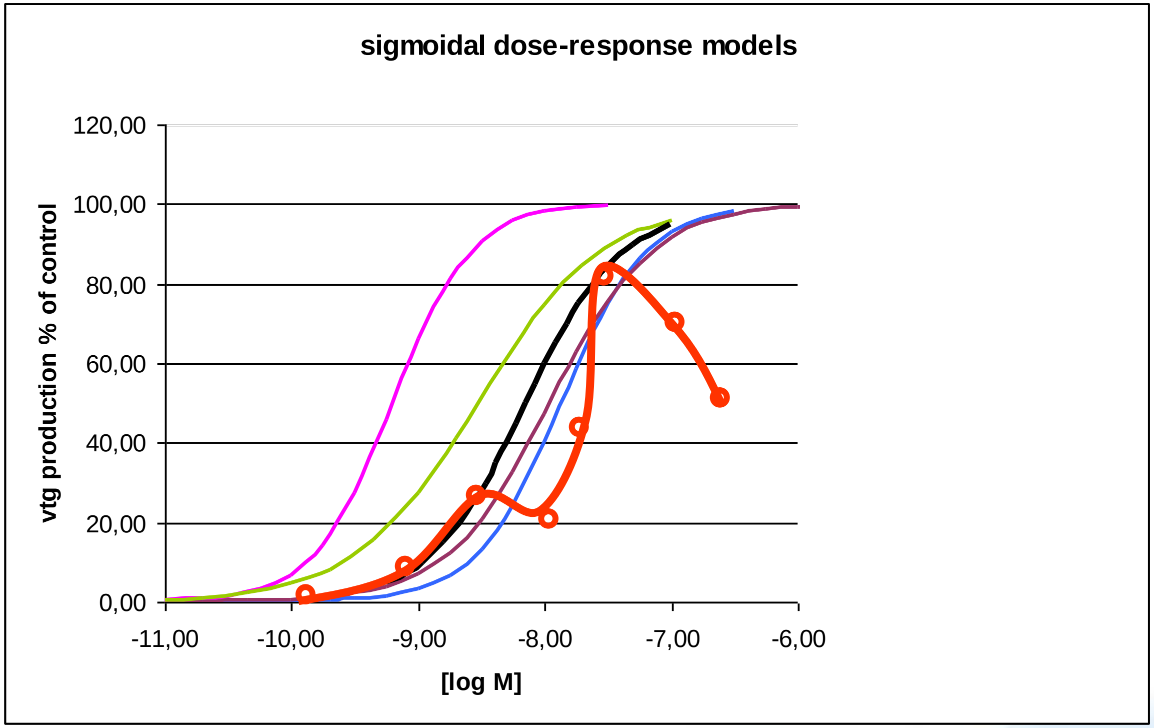
Conclusion

- MEC/NOTEL ratio for EE2 indicating transcriptional responses possible
- MEC/NOTEL ratio for Sucralose indicating large margin of safety

In vitro - mixture toxicity

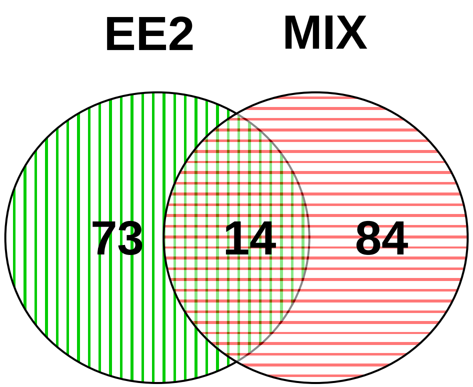


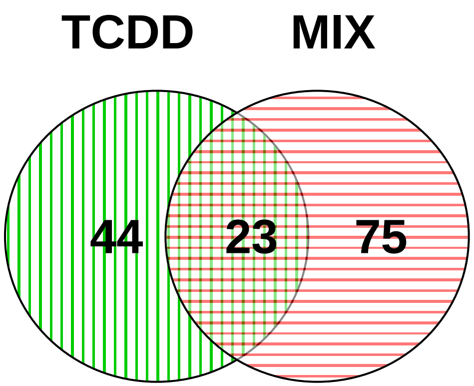
(Eide, 2004)



Multi-compound concentration-addition prediction model applied to experimental data from rainbow trout hepatocyte

In vitro gene expression

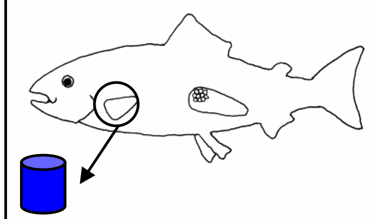


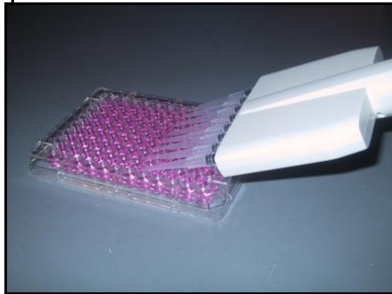


Single & Mix (equi-potent)
10 nM EE2
0.75 nM TCDD
0.75 µM 4-Nitroquinoline-1-oxide
100 µM Paraquat

Single chemical & combined toxicity assessment may require genome-wide response assessment

Finne, E.F.et al. (2007). *Aquat. Toxicol.* 81: 293-303





Regulatory positions (NO)

The Norwegian State Pollution Authority (SFT) support the EU (REACH) position on alternative testing methods:

The Commission, Member States, industry and other stakeholders should continue to contribute to the promotion of alternative test methods on an international and national level including computer supported methodologies, in vitro methodologies, as appropriate, those based on toxicogenomics, and other relevant methodologies.

(Source: REACH 40 introductory speech [fortale] for IR & CSA)

How useful are transcriptomics?

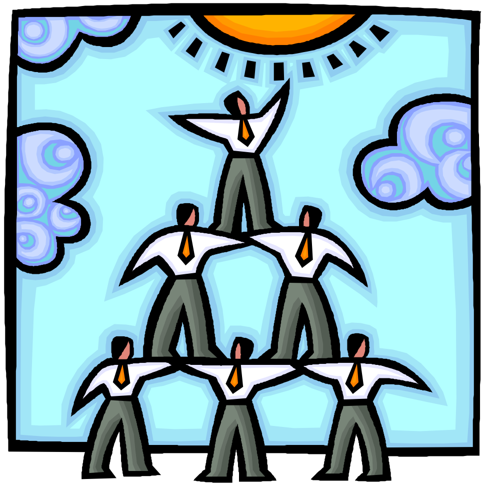
Advantages:

- High-throughput screening
- Hypothesis-generating
- Provide MoA based knowledge
- *in vivo* & *in vitro* applications
- Single chemical toxicity
- Mixture toxicity assessment
- Diagnostic/predictive tools
- Direct in-depth studies?

Disadvantages:

- Few tools for non-traditional species
- Few standardized guidelines & protocols
- Biostatistics/bioinformatics challenging
- Temporal variation in transcriptome
- Inter-assay reproducibility
- Whole organism responses
- Not regulatory validated nor accepted

Are we able to link transcriptome (biomarker) effects to adverse effects in RA?



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