Alternative techniques in fish toxicity testing

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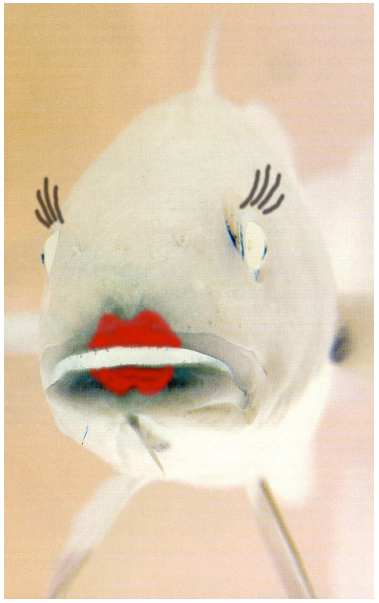
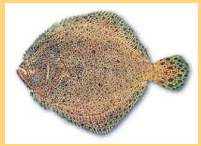


- Biomarkers
- Non-invasive methods
- In vitro testing

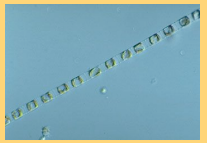
"Ecotoxicological testing of chemicals"

ACUTE TOXICITY



Turbot
Fish

Corophium v.
Sediment reworker



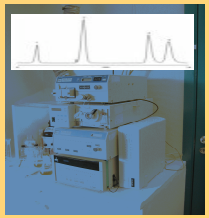
Skeletonema c.
Green algae

Acartia tonsa

BIOACCUMULATION POTENTIAL

Partition octanol-water

or

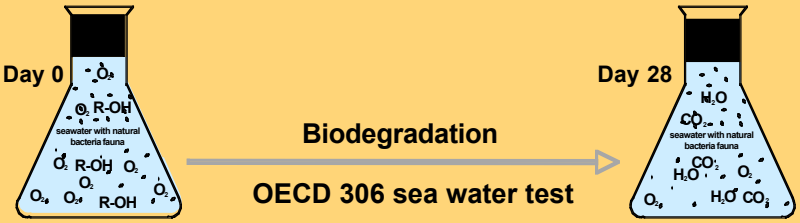


HPLC

Log Pow

$$\text{Log}\ \frac{\text{partition in octanol}}{\text{partition in water}}$$

BIODEGRADATION

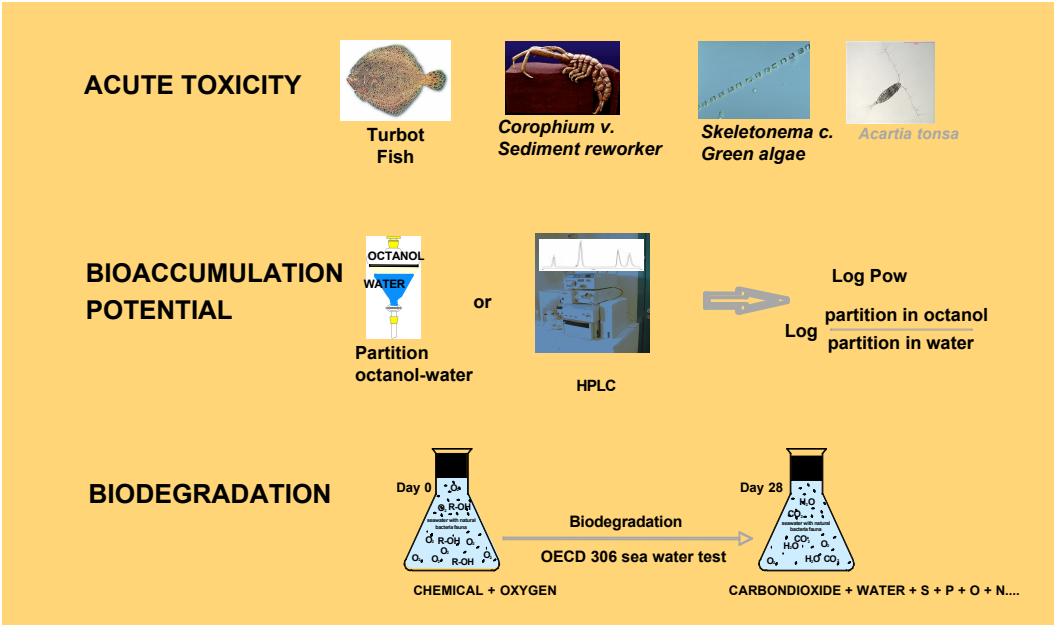


CHEMICAL + OXYGEN

CARBONDIOXIDE + WATER + S + P + O + N....

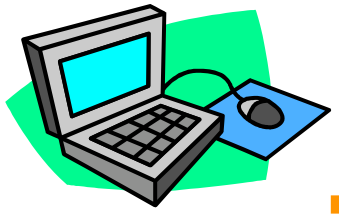
Hazard and risk modelling - DREAM

OSPAR standard test program



Risk assessment

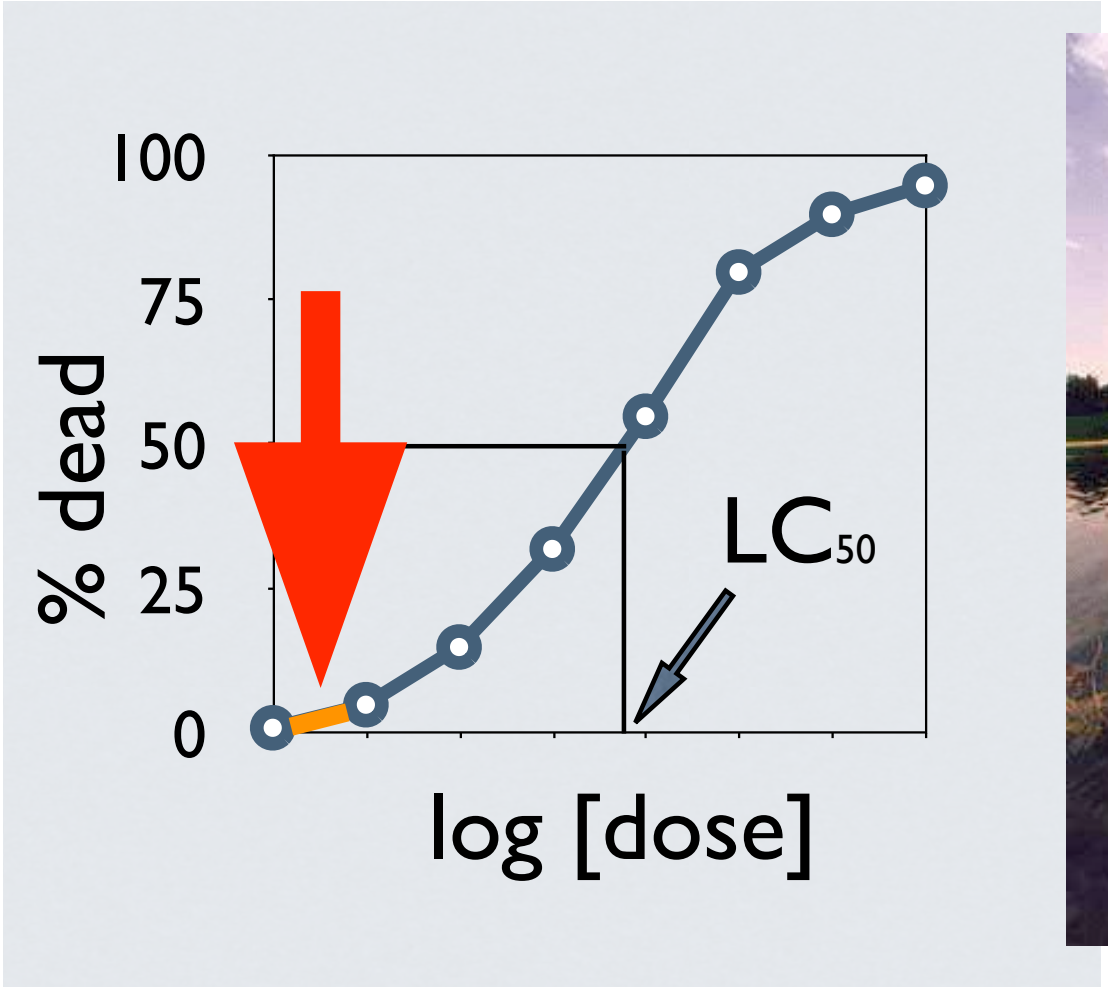
Dosage
OSPAR data
Oil/water /distribution
Long term studies
Physical data
Site information
Sea currents
Etc.



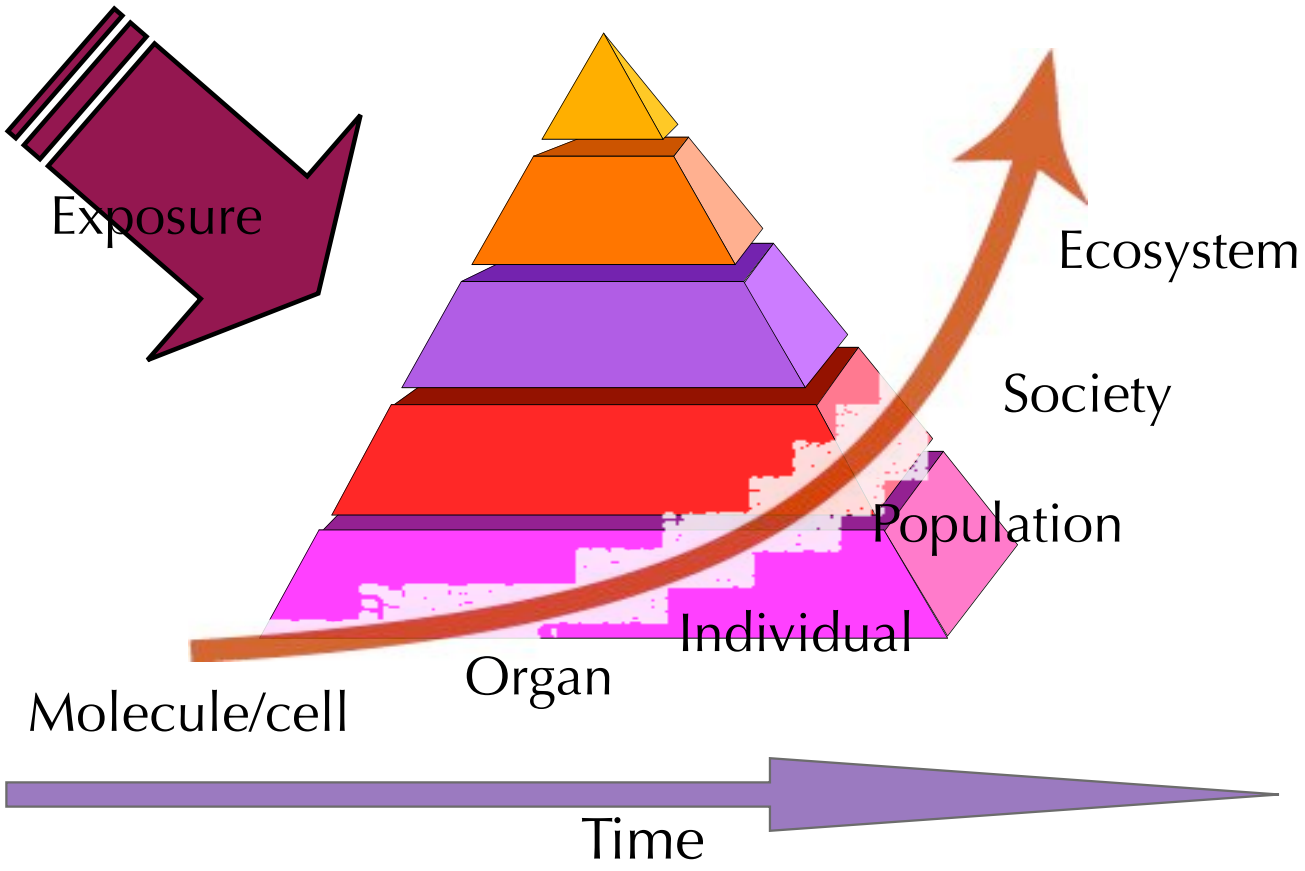
DREAM
Dose-related Risk and Effect Assessment Model

EIF (Environmental impact factor)

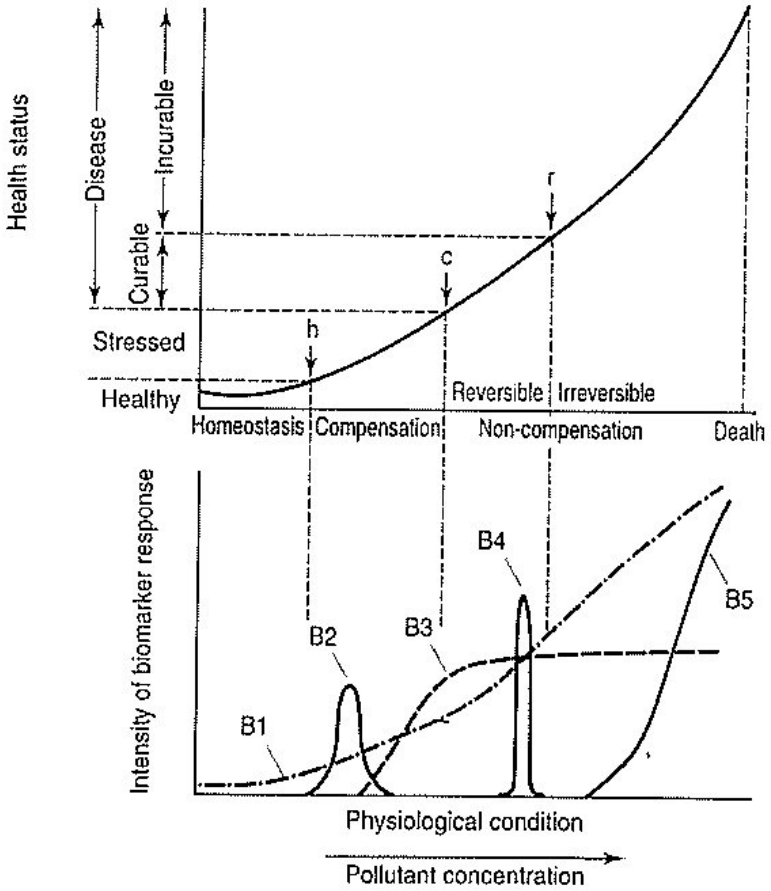
The relevance of LC50



Effects on a biological system



Exposure, health status and biomarker responses



Biomarkers

- various definitions have been described
- e.g. “any biological response (...) at the individual level or below demonstrating a departure from the normal status”*
- used in ecotoxicology, human toxicology and human medicine

* Walker et al. 2001: Principles of Ecotoxicology

Biomarkers

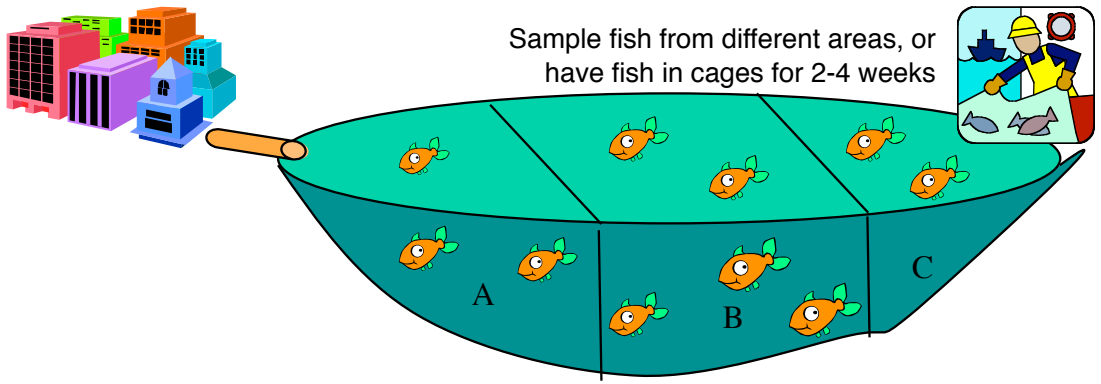
- Biomarkers of exposure
  - levels of compound/metabolites in the organism
  - e.g. PAH: bile-fluorescence
- Biomarkers of effect/response
  - mRNA/enzyme/protein levels in target organs or body fluids
  - e.g. PCB/dioxin/PAH: CYP1A; Cd, Zn, Hg, Cu: MT; DNA-damage
- Biomarkers for susceptibility
  - genetic polymorphisms, physiological condition
  - e.g. CYP2D6 in drug metabolism in humans

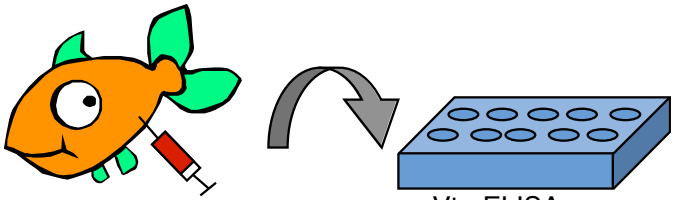
Specificity of biomarkers

Biomarker	Pollutant
Inhibition of ALAD	Pb
Induction of MT	Cd, Hg, Cu, Zn
Inhibition of AChE	OPs, carbamates
Induction of CYP1A	Dioxins, PCBs, PAHs
Porphyrin profiles	Several OCs
Retinol profiles	OCs
DNA and hemoglobin adducts	Largely PAHs
Induction of Vtg	Estrogenic chemicals
Other serum enzymes	Metals, OCs, PAHs
Stress proteins	Metals, OCs
Immune responses	Metals, OCs, PAHs

Biomarkers in environmental monitoring:

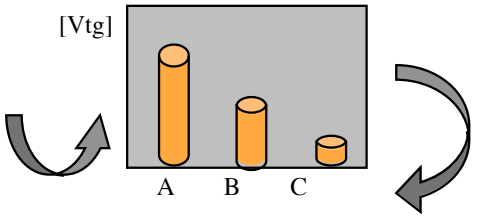
Is area A influenced by effluents with endocrine disrupting effects?





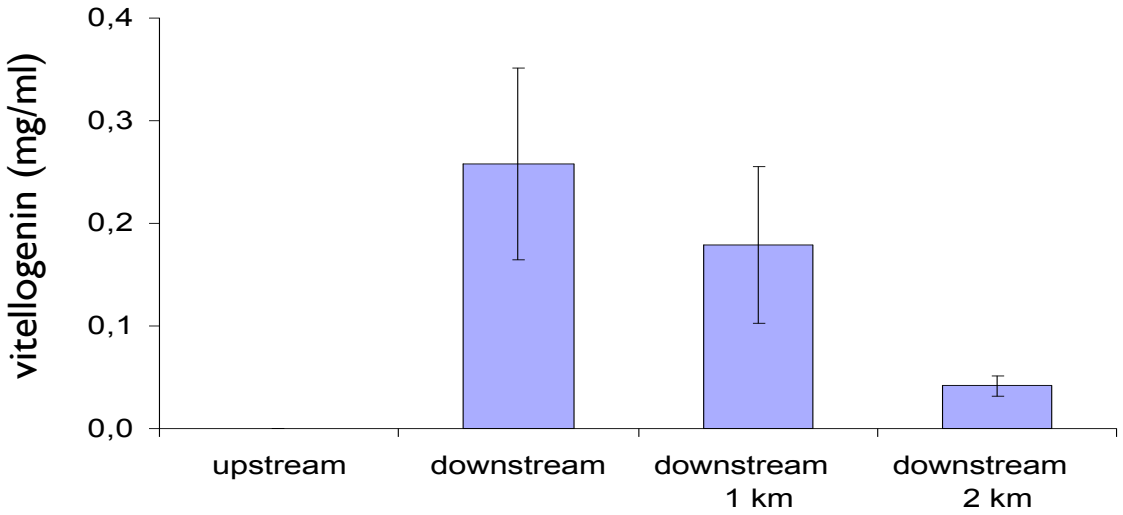
Take blood samples and
analyze in Vtg-ELISA

Vtg-ELISA



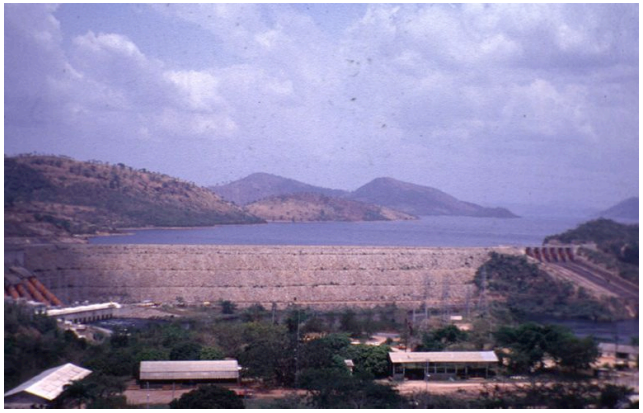
Concl.: Yes, area A is contaminated with
environmental estrogens

Vitellogenin induction downstream sewage treatment works

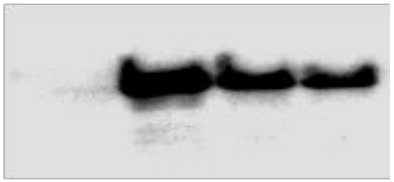


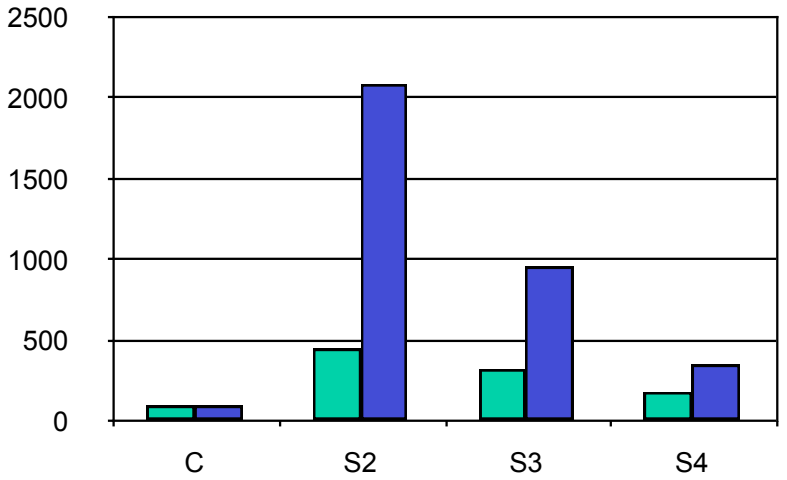
Vitellogenin (mean ± SEM; mg/ml) in plasma from rainbow trout caged immediately upstream or downstream and one and two km downstream from a sewage treatment works.
From Parkkonen et al., 2000.

CYP1A responses in liver of caged tilapia, Volta River, Ghana



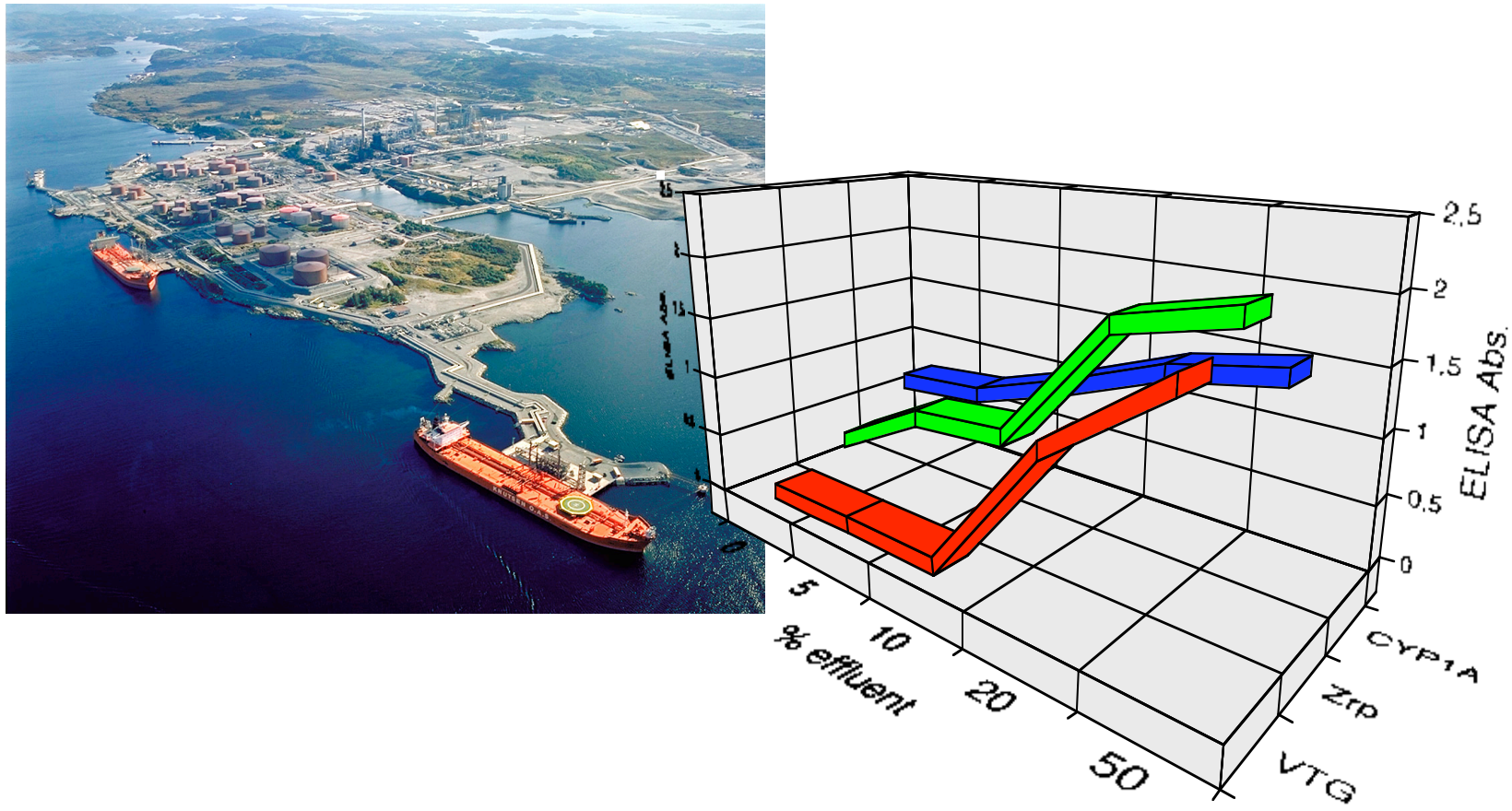
CYP1A protein





Gadagbui & Goksøyr (1996)

Effluent testing with salmon - water treatment plant at oil terminal



Vtg ELISAs for OECD fish species

Zebrafish
(*Danio rerio*)

Biosense develops quantitative Vtg-analyses based on robust, sensitive, and specific immunoassays

Medaka
(*Oryzias latipes*)

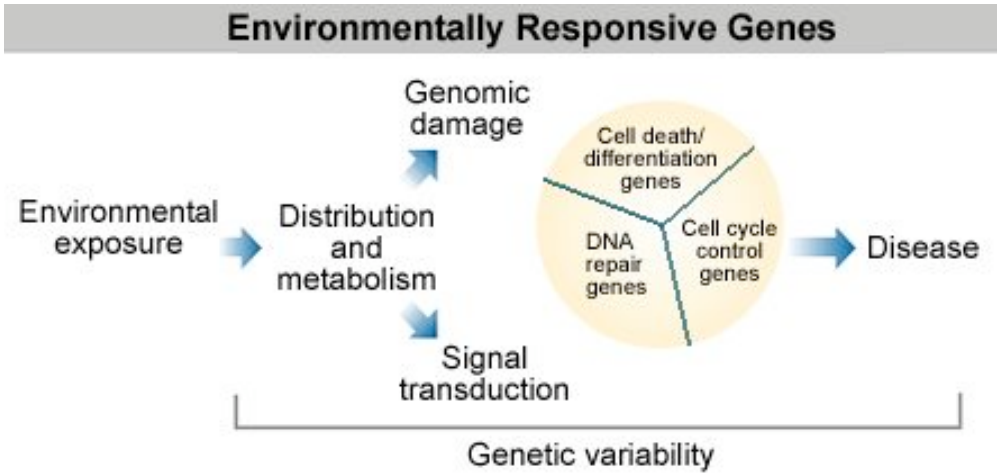
Fathead minnow
(*Pimephales promelas*)

Rainbow trout
(*Oncorhynchus mykiss*)

Carp
(*Cyprinus carpio*)

bi sense

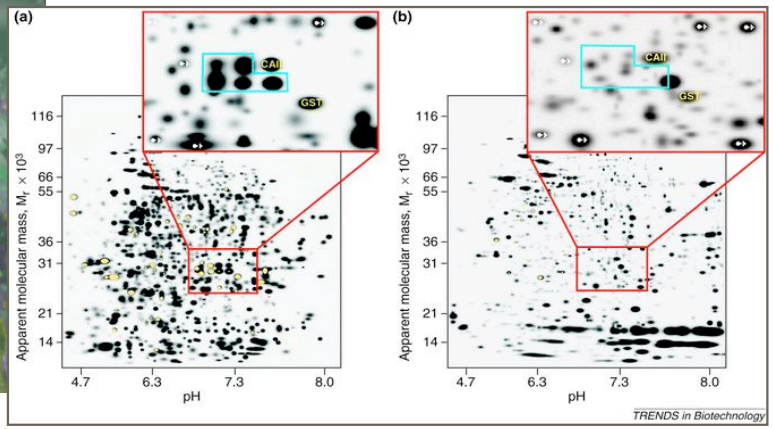
Environmental toxicogenomics and toxicoproteomics



Environmental toxicogenomics is a new approach to environmental toxicology. Environmental toxicogenomics allows us to identify and characterize genomic signatures of environmental toxicants as gene and protein expression profiles. A major application of gene expression profiling is to understand (human) genetic variability and susceptibility to disease.

Concept Statement, National Centre for Toxicogenomics, NIEHS (USA)

The proteome - the functional genome



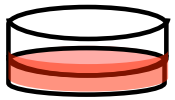
- The proteome is the full picture of proteins expressed by the genome of a cell under given conditions
- Can give us new knowledge about molecular interactions, metabolic processes, and novel biomarkers
- Full exploitation must be based on genome information, but also on basal biological knowledge (molecular, physiological, ecological)

EASYRING = Environmental Agent Susceptibility Assessment Utilising Existing and Novel Biomarkers As Rapid Non-Invasive Testing Methods.

Strategy:

Xenopus laevis

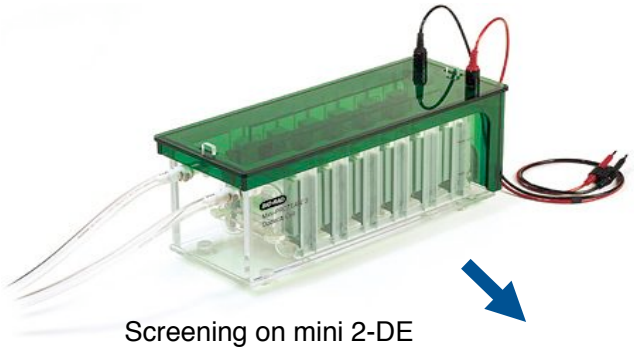
Cyprinus carpio



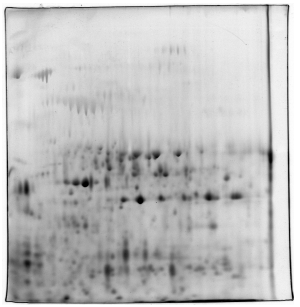
MCF-7/MVLN cells

In vitro and in vivo exposures

Individual samples

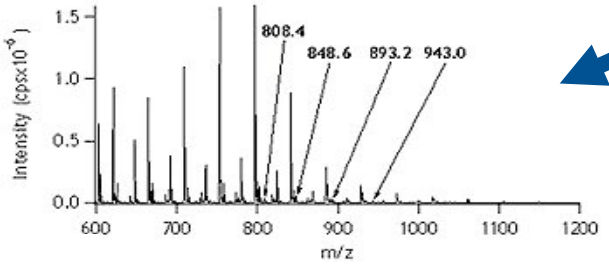


Screening on mini 2-DE



Preparative 2-DE on large gels

ELISA assay and kit development

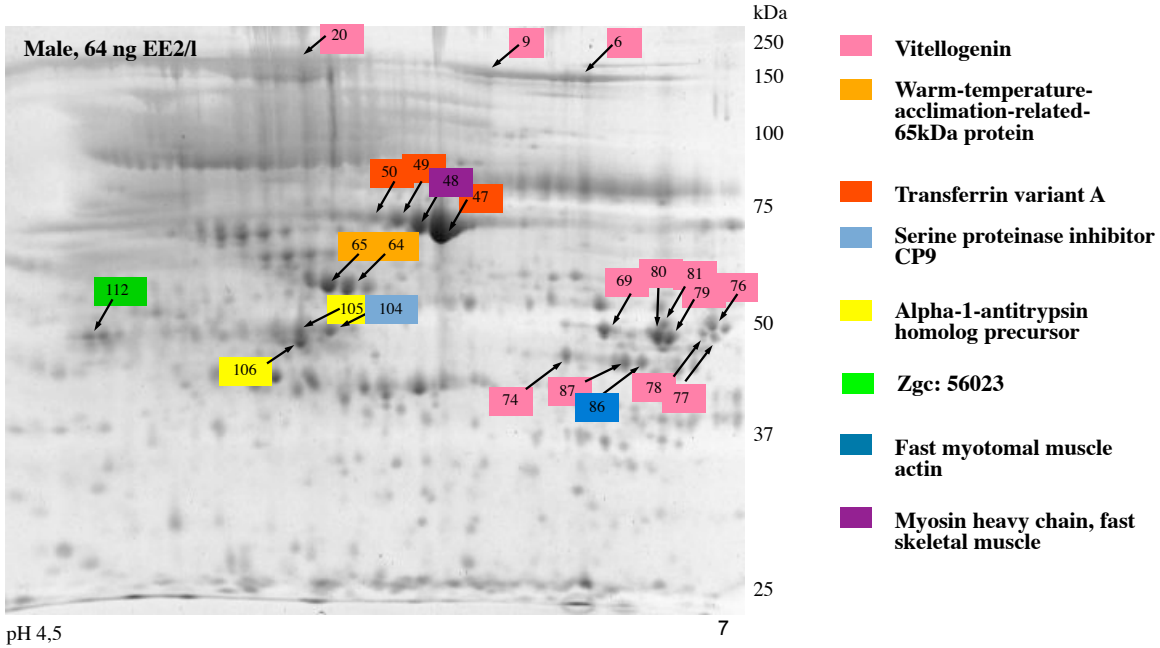


Sequencing using ESI and MALDI

Identification by database searches

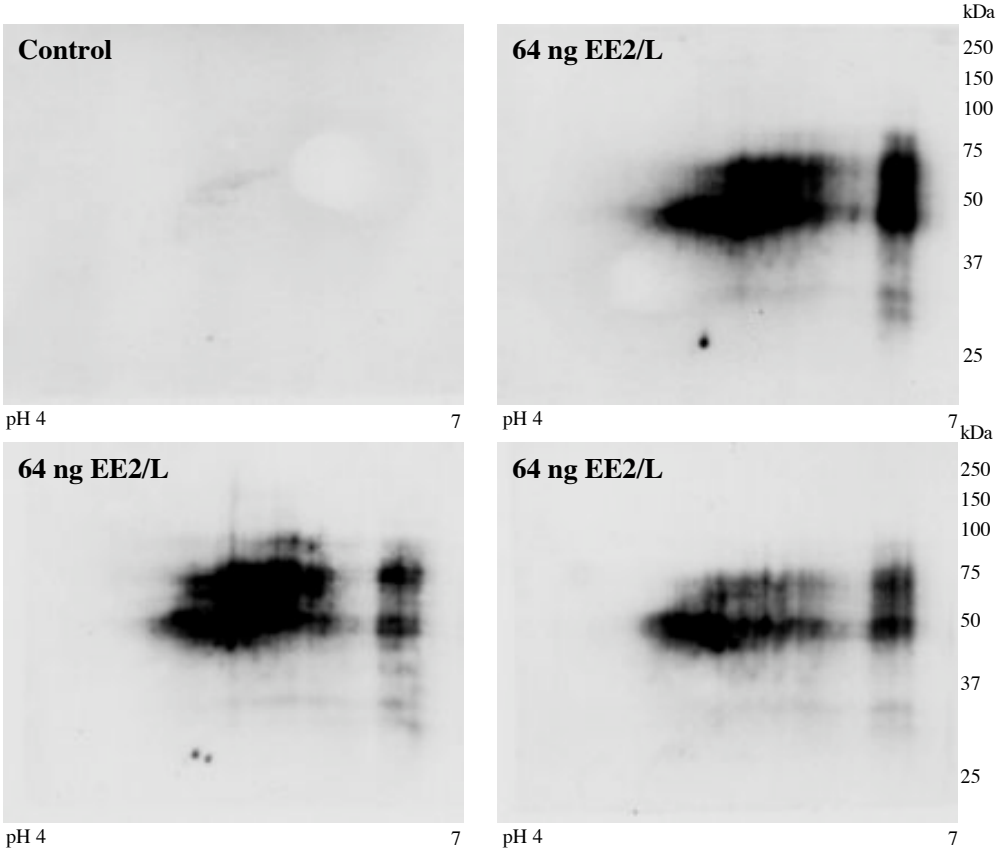
Prepare synthetic peptide antibodies

Preparative 2-DE and MALDI-TOF MS - carp plasma



Tolfsen, Grøsvik et al., in prep.

2-DE western of Vtg in mucus from carp



Vtg is present in mucus of EE2-exposed carp

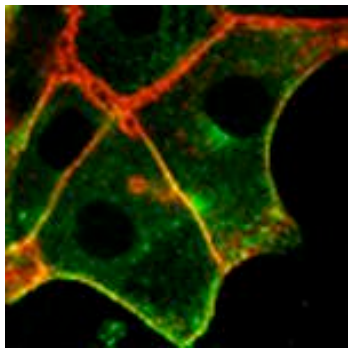
Eidem et al.

Development of dipstick for endocrine disruption monitoring - non-disruptive sampling of fish mucus

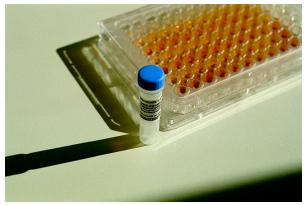
In vitro alternatives

- Many aspects of toxicity can be studied in *in vitro* systems
- Primary culture vs cell lines vs reporter cells (e.g. CALUX)
- Screening, mechanistic studies
- QSAR - in silico
- Important to know the limitations of the systems!

Toxicity identification and evaluation (TIE) of effluents for EDC effects



Expose fish hepatocytes (in vitro) to water extracts

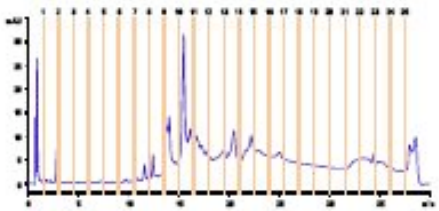


Analyze Vtg levels

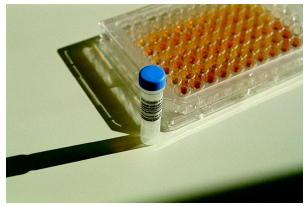
Yes/No

OK

HPLC fractionation



Analyze fractions of water extracts for Vtg effects (in vitro)/ levels of suspected compunds

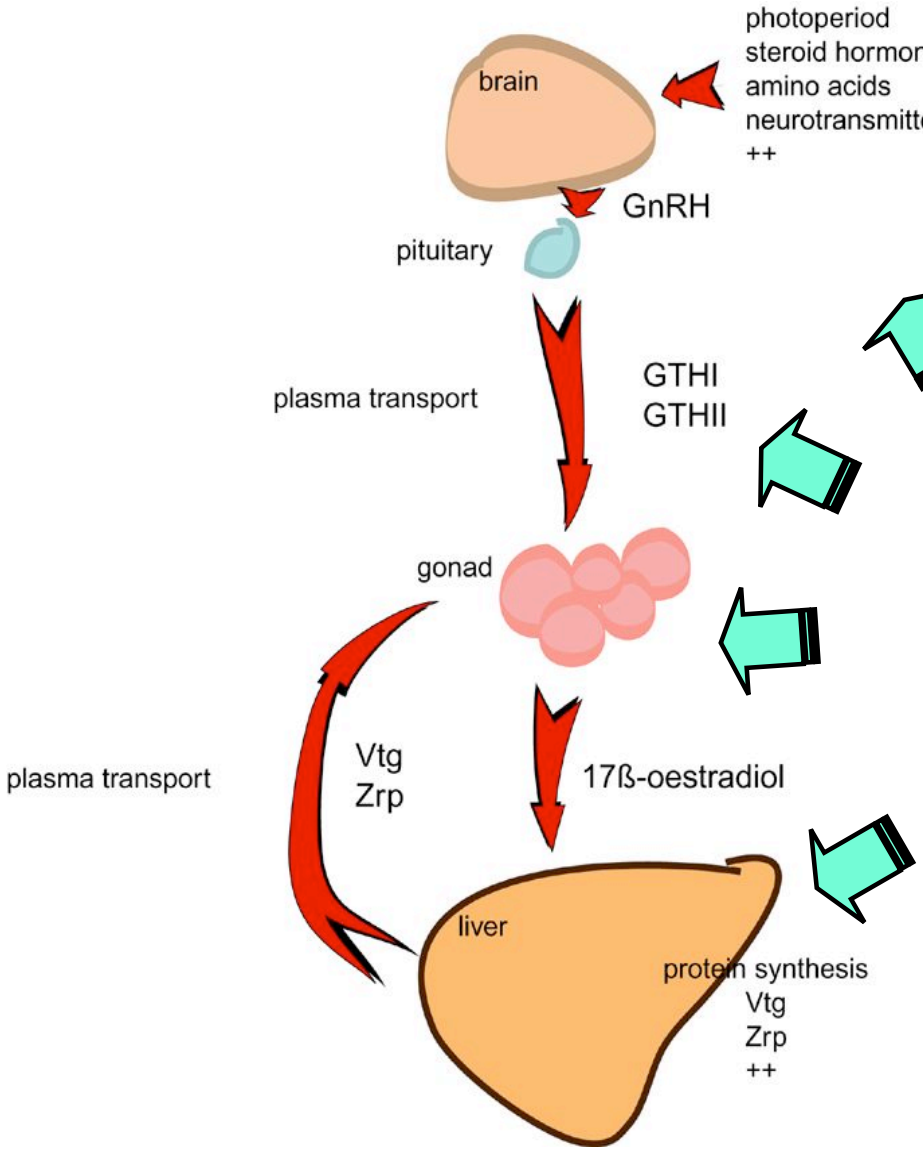


Identify sources - adjust production processes & effluent treatment processes

In vitro limitations

Folmar et al. (2002) A comparison of the estrogenic potencies of estradiol, ethynylestradiol, diethylstilbestrol, nonylphenol and methoxychlor in vivo and in vitro. Aquat. Toxicol. 60:101-110:

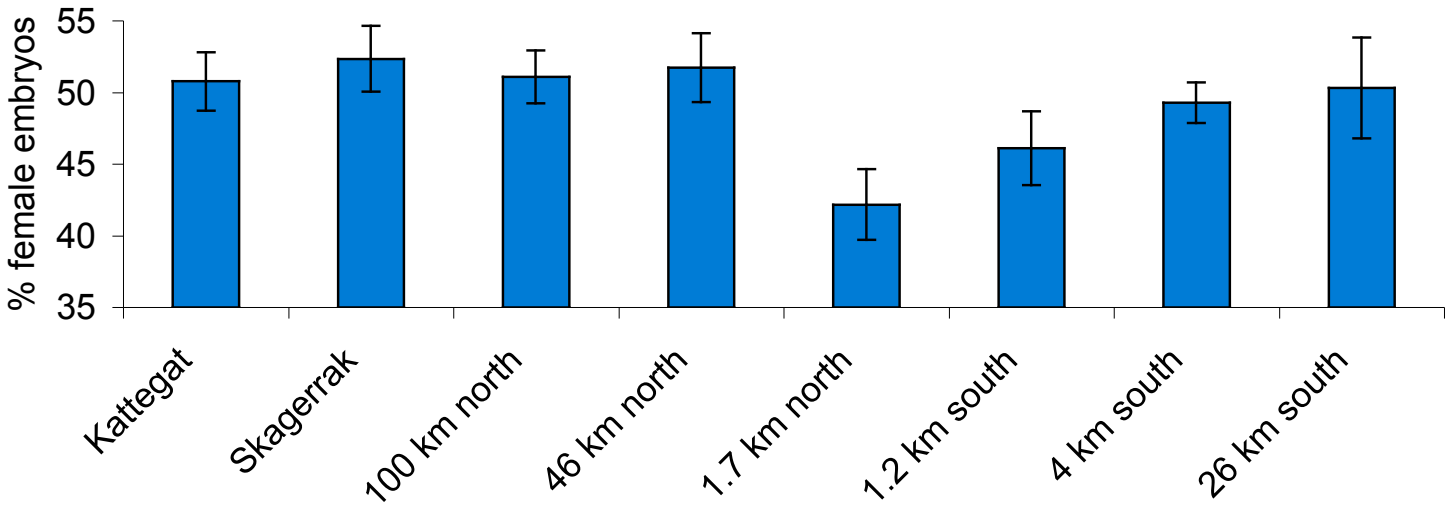
- *In vitro* assays can rank chemicals within a given test, but they cannot be extrapolated or predict whether the test chemicals will maintain the same order of potency in a live animal bioassay
- *In vitro* assessments for estrogenicity, particularly for chemicals which require metabolic activation or are capable of substantial bioaccumulation, underestimate the responses observed with *in vivo* testing
- The *in vitro* assays do not identify proestrogens, are insensitive to antiestrogens and, although they provide information on binding affinity to the ER, they do not provide any information regarding potential physiological alterations
- *In vitro* assays working at the biochemical and cellular level do not fully incorporate the signal amplification process observed in the exposure of whole organisms.



The brain-pituitary-gonad-liver axis is a target for endocrine disruptors

Arukwe & Goksøyr (2003) Comp Hepatol

Changes in embryonic sex ratios in eelpout caught near a pulp mill



Embryonic sex ratios in viviparous eelpout from four reference sites and four sites near a pulp mill outfall. The study comprises 3,423 embryos from 99 females.
From Larsson et al. (2000), Environ. Toxicol. Chem.

Effects of EE2 on germ cells and gonad differentiation in vasa-GFP transgenic medaka

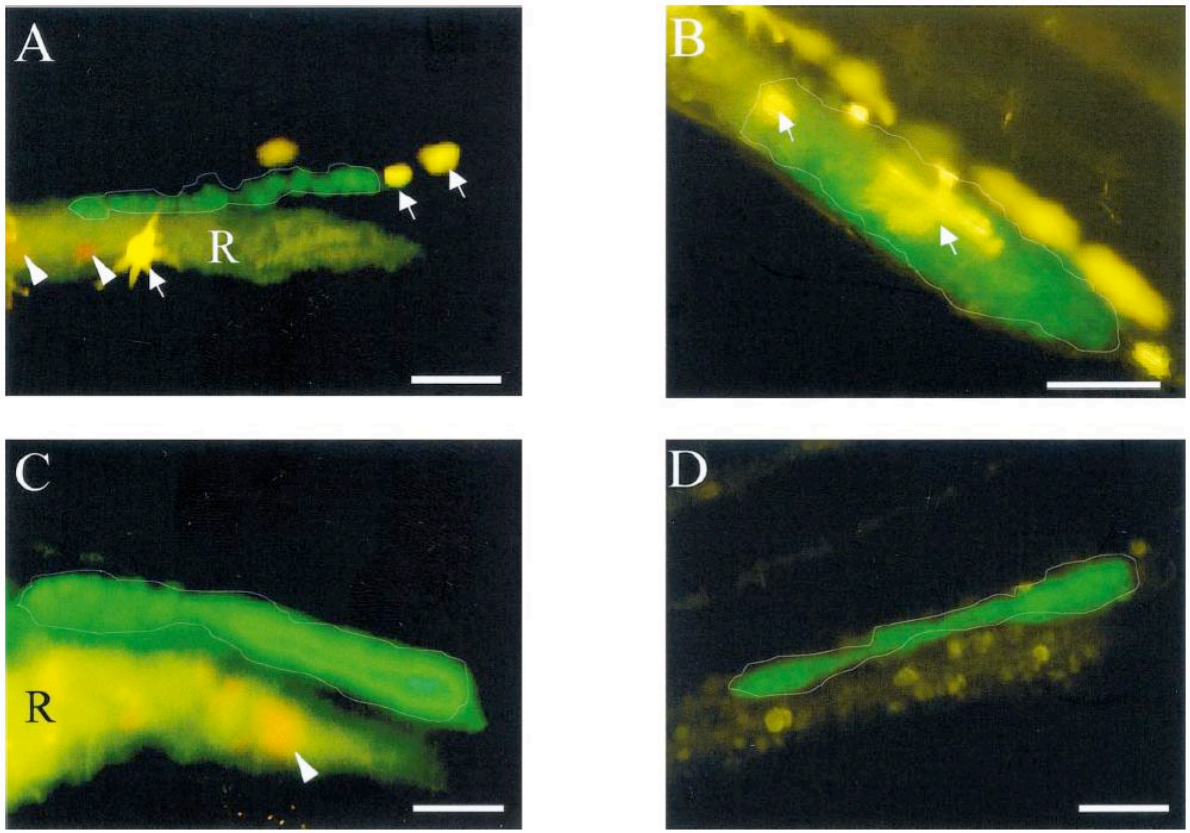
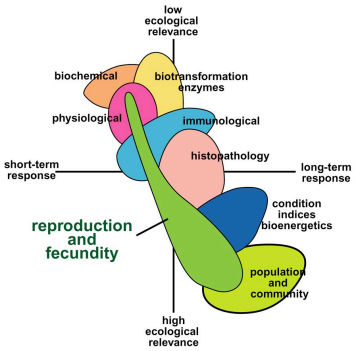


Fig. 2. Green fluorescent protein (GFP) fluorescence images captured from the lateral aspect at juveniles at 10 d posthatch. **A.** Uninjected XY males. **B.** XY males that developed from embryos injected with 0.5 ng of 17α-ethinylestradiol (EE_2). **C.** Uninjected XX females. **D.** XX females from embryos injected with 5.0 ng of EE_2. Outlined area in each photo corresponds to the undifferentiated gonad. Arrows indicate a male-specific pigment (leucophore); arrowheads indicate autologous red fluorescence of brine shrimp. R = residual food and/or discharges in the gut. Bar = 200 μm.

- EE2 injection into embryos caused abnormal gonadal development in both sexes.
- Complete sex reversal in some XY males after 0.5-, 2.5-, and 5.0-ng treatments.
- No changes in XX females after any treatment.

Hano et al., ETC 24:70-77 (2005)



Long-term effects

- Critical windows
- Accumulated effects
- Effects on fecundity and recruitment
- Transfer to and effects in offspring
- Population effects

The emergence of systems biology

The emerging field of systems biology attempts to harness the power of mathematics, engineering, and computer science to analyze and integrate data from all the “omics” and ultimately create working models of entire biological systems.

Spivey A.(2004) Systems biology: the big picture. Environ Health Perspect.

The three R's

- *Replacement* - to replace live animal studies with alternative methods, when possible
- *Reduction* - to reduce the number of animals used in the study
- *Refinement* - develop methods to ensure better animal welfare, reduced stress and improved quality of data obtained

Exit LC50 in fish toxicity testing?

- In vitro testing, when possible- replacement
- Non-destructive biomarkers - reduction
- Sublethal toxicity tests with biomarker endpoints - refinement

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