



Centrum pro výzkum  
toxických látek  
v prostředí

# BIOMARKERS AND TOXICITY MECHANISMS 01 - INTRODUCTION

Luděk Bláha, PŘF MU, RECETOX  
[www.recetox.cz](http://www.recetox.cz)

Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

# Course summary

## 1) Introduction

- Intro and overview of the mechanisms beyond the toxicity  
(*with special respect to environmental contaminants*)
- Intro and concept of biomarkers

## 2) Details on selected important toxicity mechanisms

- Membrane toxicity, enzyme inhibitions, oxidative stress, genotoxicity, Nuclear Receptors (AhR, ER, AR ....) etc.
- Methods to determine toxicity mechanism

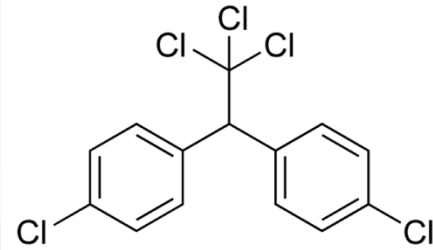
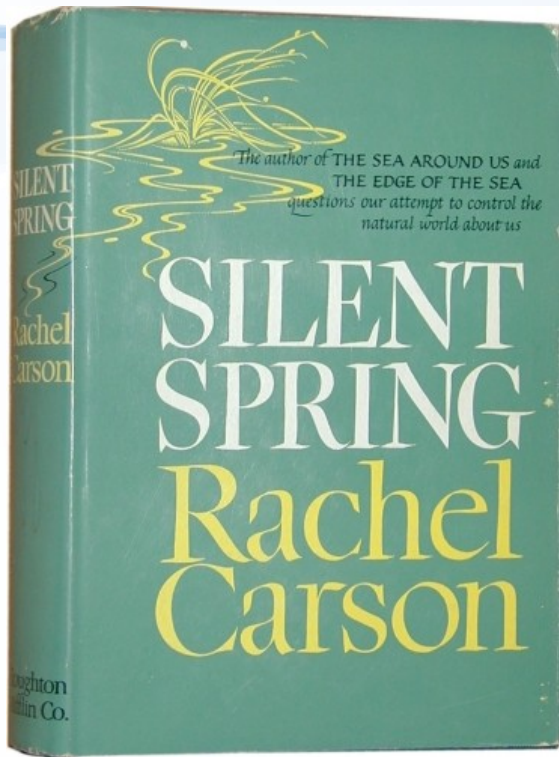
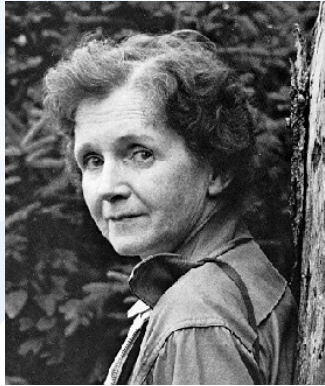
## 3) Biomarkers

- What it is and how to find (identify) suitable biomarker(s)?
- The overview of the most important biomarker classes
- Methods of biomarker assessment

# The importance of understanding toxicity mechanisms



1962



© Patuxent Wildlife Refuge, MA, USA

"DDT is good for me-e-e!"

The great expectations held for DDT have been realized. During 1946, exhaustive scientific tests have shown that, when properly used, DDT kills a host of destructive insect pests, and is a benefactor of all humanity.

Pennsalt produces DDT and its products in all standard forms and is now one of the country's largest producers of this amazing insecticide. Today, everyone can enjoy added comfort, health and safety through the insect-killing powers of Pennsalt DDT products . . . and DDT is only one of Pennsalt's many chemical products which benefit industry, farm and home.

**GOOD FOR STEERS**—Beef grows meatier nowadays . . . for it's a scientific fact that—compared to untreated cattle—beef-steers gain up to 50 pounds extra when protected from horn flies and many other pests with DDT insecticides.

**GOOD FOR THE HOME**—helps you to make healthier, more comfortable homes . . . protect your family from dangerous insect pests. Use Knox-Out DDT Powders and Sprays as directed . . . then watch the bugs "bite the dust!"

**GOOD FOR DAIRIES**—Up to 20% more milk . . . more butter . . . more cheese . . . tests prove greater milk production when dairy cows are protected from the annoyance of many insects with DDT insecticides like Knox-Out Stock and Barn Sprays.

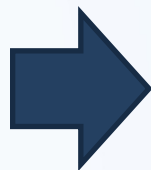
**GOOD FOR FRUITS**—Bigger apples, juicier fruits that are free from unsightly worms . . . all benefits resulting from DDT dusts and sprays.

**GOOD FOR ROW CROPS**—25 more barrels of potatoes per acre . . . actual DDT tests have shown crop increases like that! DDT dusts and sprays help truck farmers pass these gains along to you.

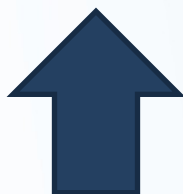
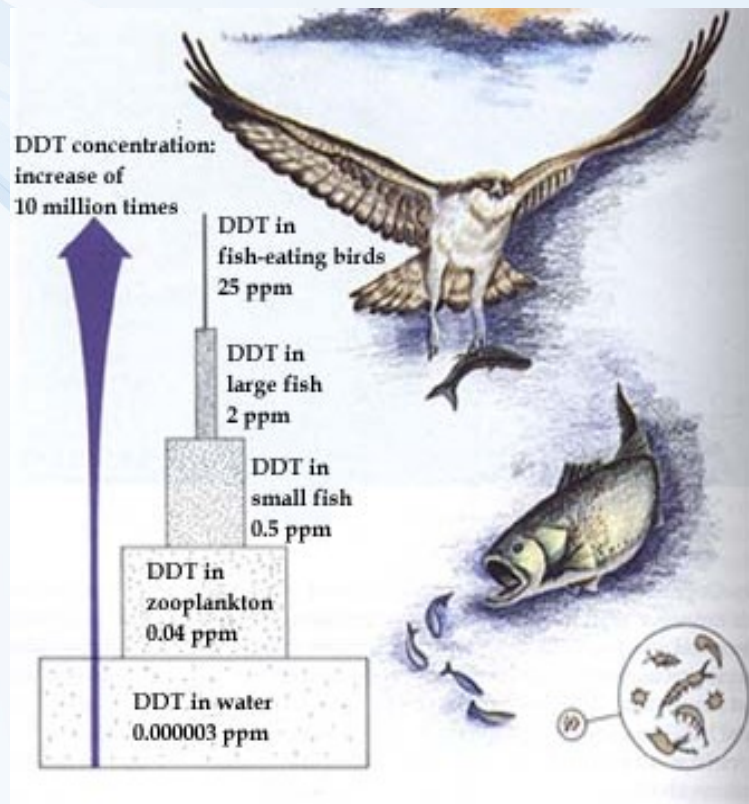
**KNOX FOR INDUSTRY**—Food processing plants, laundries, dry cleaning plants, hotels . . . dozens of industries gain effective bug control, more pleasant work conditions with Pennsalt DDT products.

**PENN SALT**  
CHEMICALS  
87 Years' Service to Industry • Farm • Home  
PENNSYLVANIA SALT MANUFACTURING COMPANY  
WIDENER BUILDING, PHILADELPHIA 7, PA.

**In vivo:** shell thinning

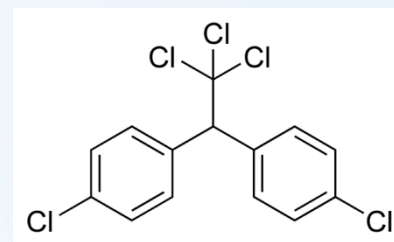


**In situ:** bioaccumulation  
-> **bird population decline**

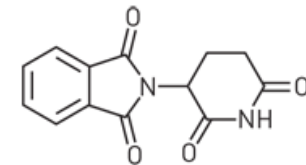


Biochemistry discovered in 1970s:  
**Bird** carbonate dehydratase

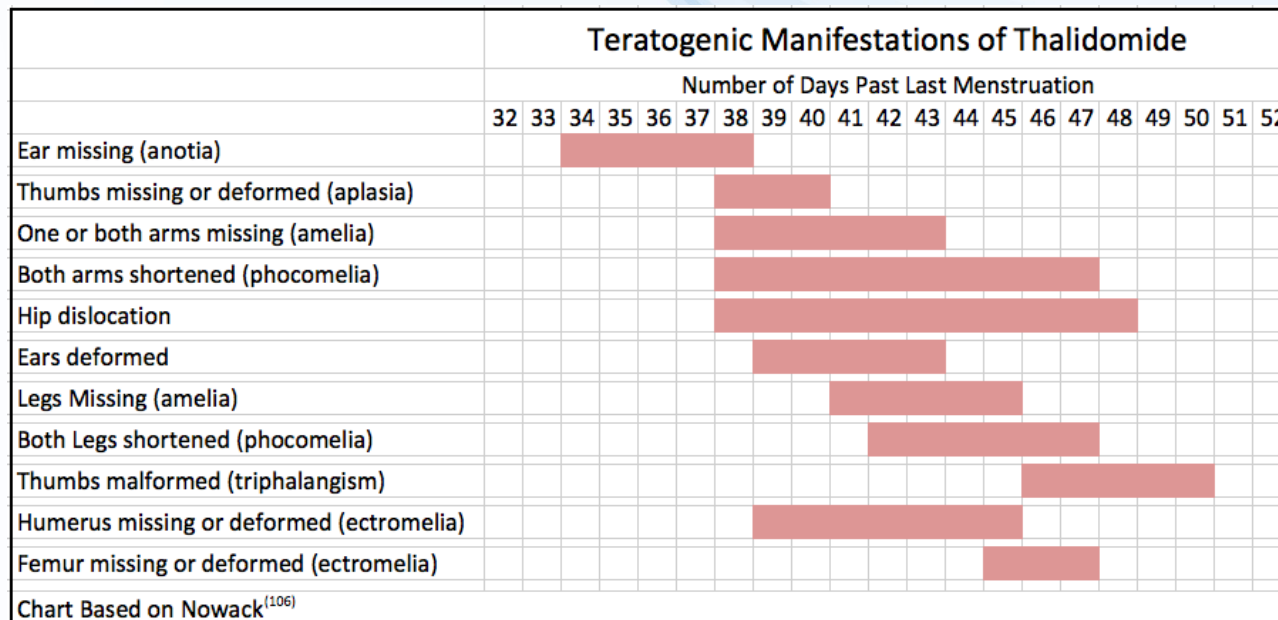
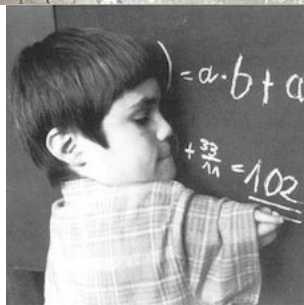
Bitman et al. *Science* 1970,  
168(3931): 594



# Thalidomide

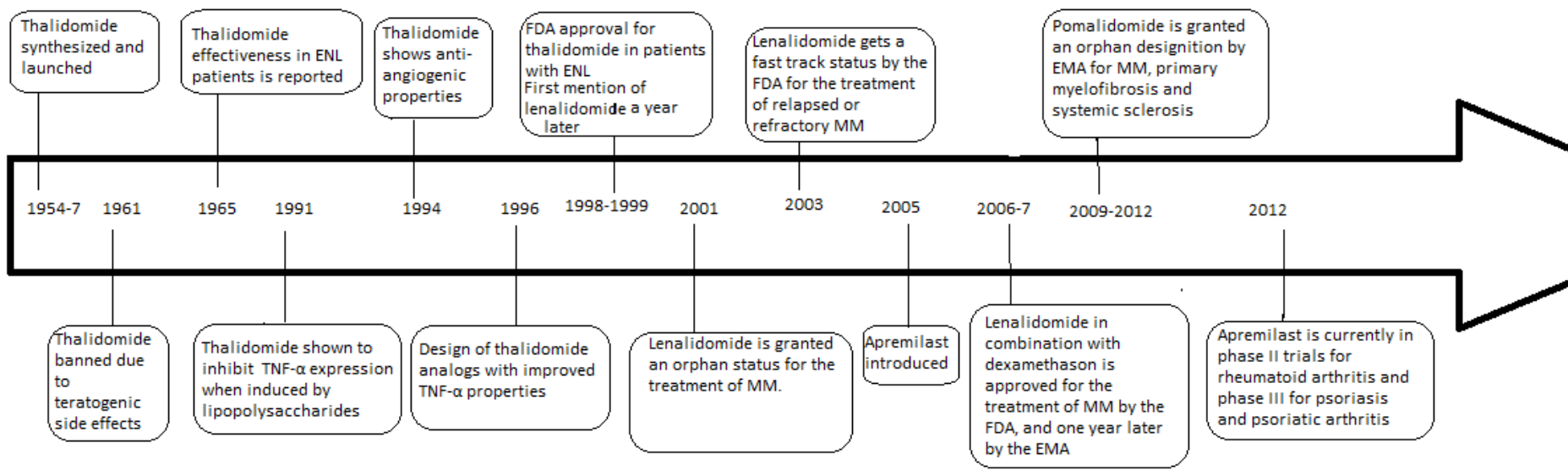
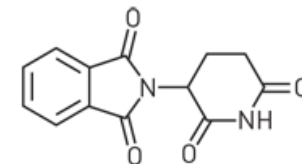


- Originally marketed in 1957 as sedative / hypnotic
  - also curing anxiety, gastritis, tension
  - against nausea and morning sickness of pregnant
    - TERATOGENICITY → Development of phocomelia = limb malformations (10 000 children worldwide / 40% survived)



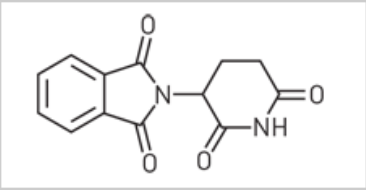
- Currently still in use - completely different targets
  - : anticancer (multiple myeloma), antileprosis, immunosuppression

# Thalidomide



# Thalidomide

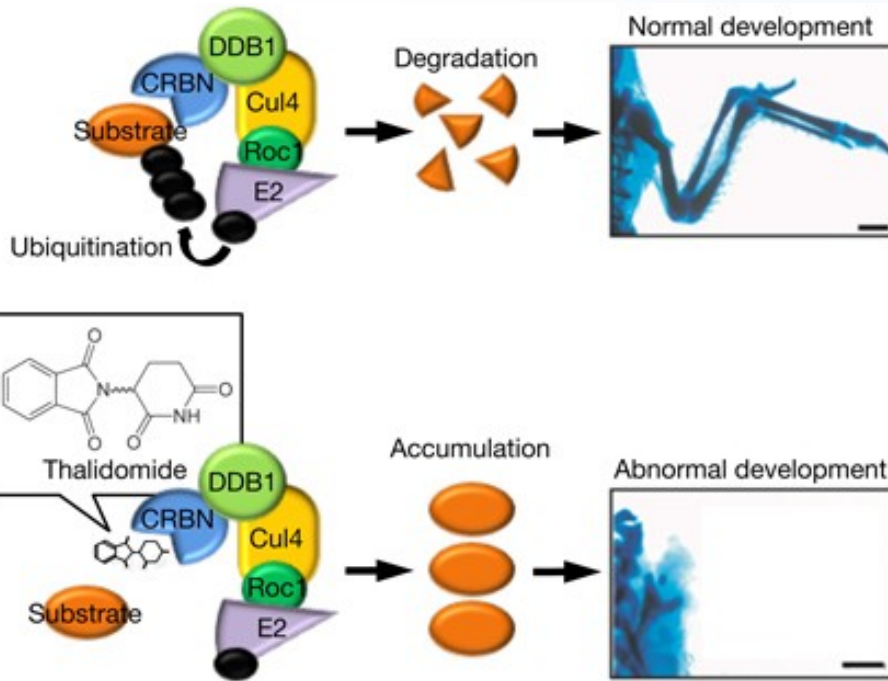
## ... mechanisms of action



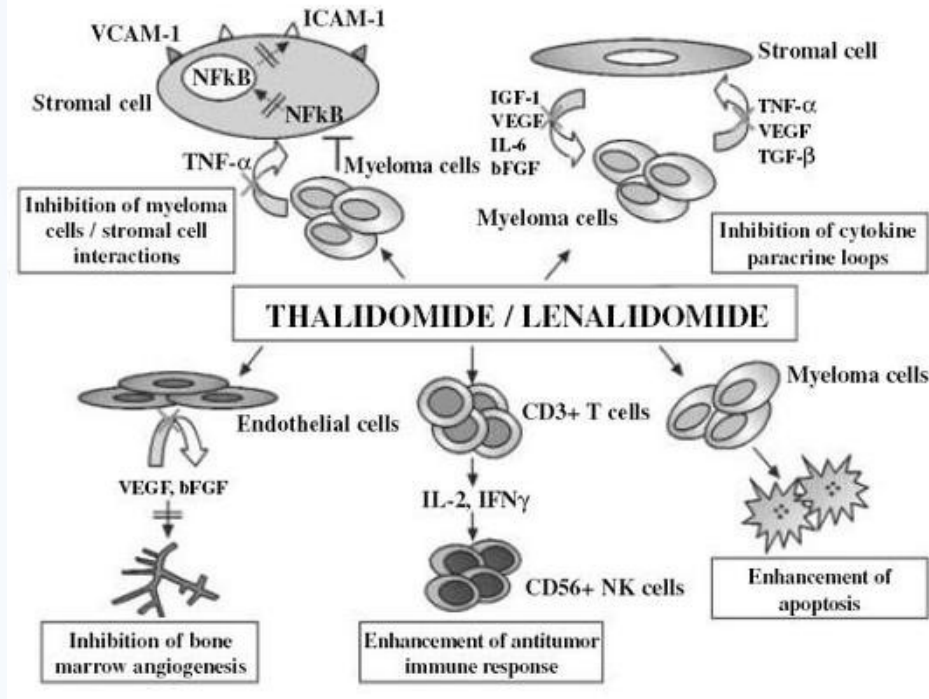
### (1) Sedative effects

... mechanism unknown

### (2) Teratogenicity



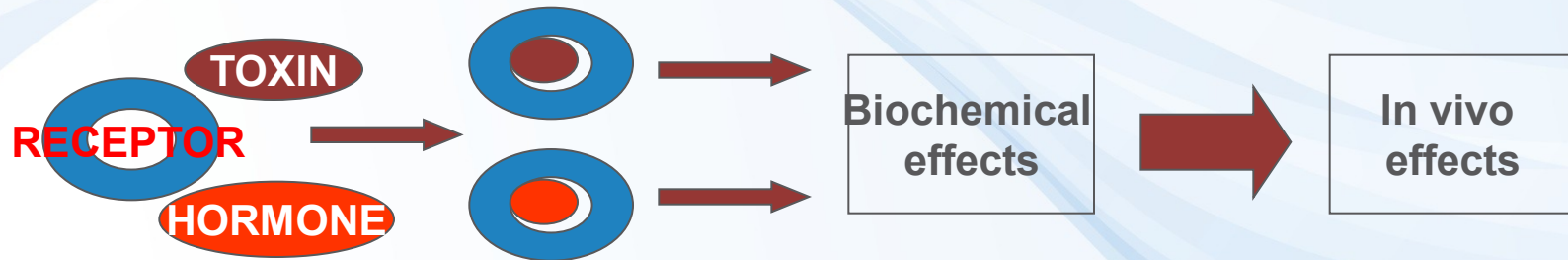
### (3) Anticancer





# MECHANISMS of chronic toxicity

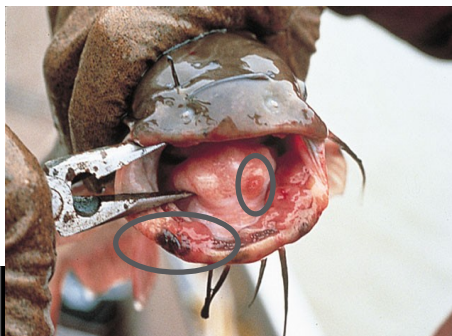
- Various chronic effects have uniform biochemical basis



- principle studies with mechanistically based *in vitro* techniques
- estimation of *in vitro* effects of individual compounds

Understanding MoA ... may predict higher-level effects

Organism

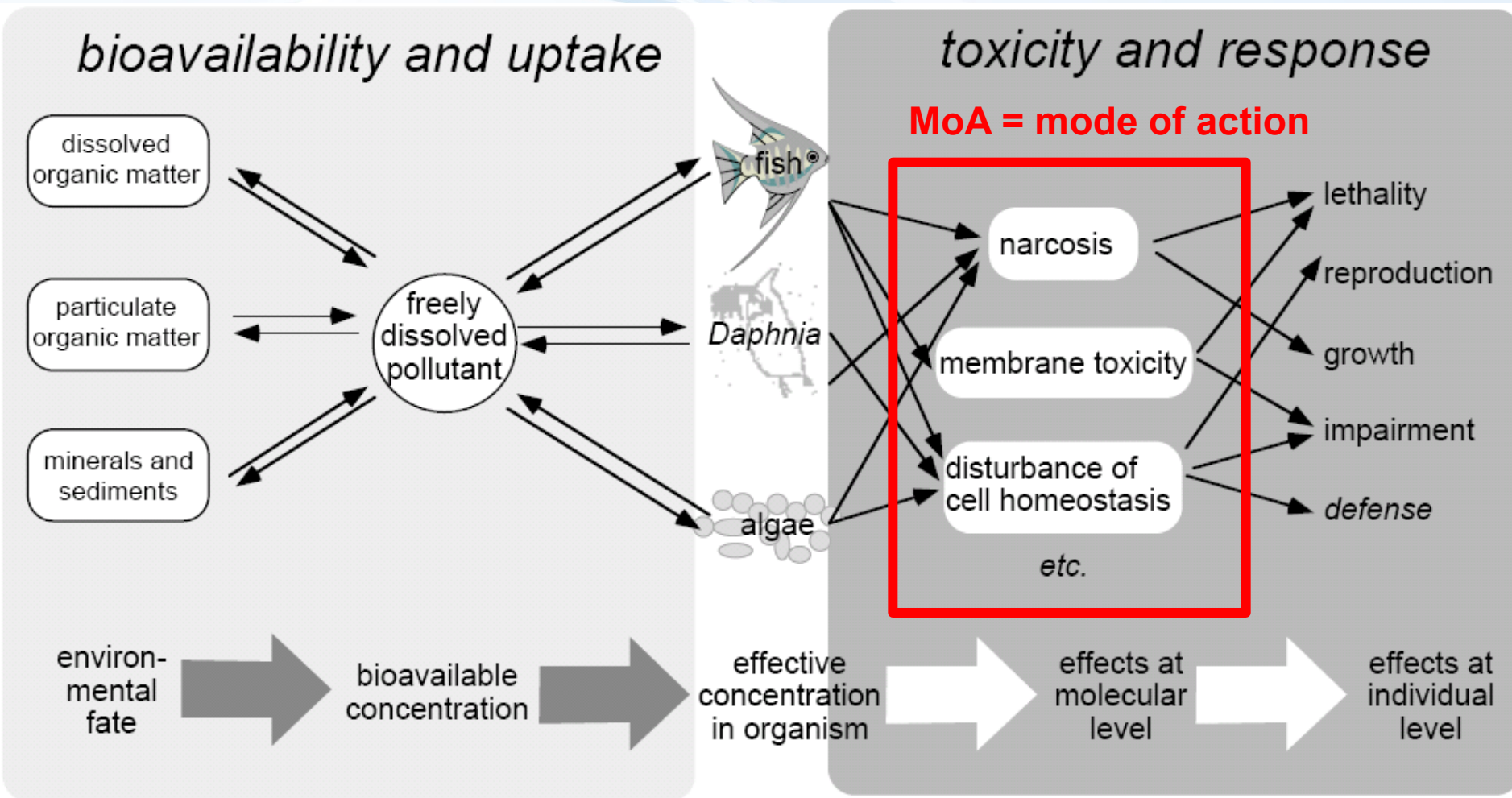


Population & beyond



# Basics and keywords from toxicology

# Toxicity - concept



**Figure 1** The effective concentration of a pollutant in an organism (e.g. fish, daphnia, algae) or at the target site inside the organism is the link between the environmental fate of a pollutant and its toxic effect.

# From mechanisms (or modes of action) to biomarkers

- Chemical enters organism  
+ may be metabolized/detoxified,  
transported, released ...

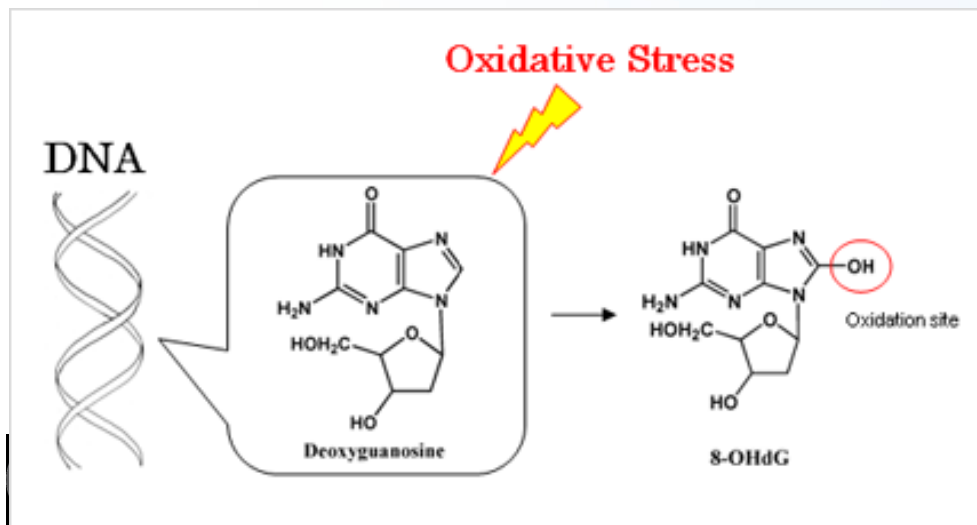
→ **Toxicokinetics**

- Chemical reacts with target (e.g.  
DNA) and changes a specific  
nucleotide (e.g. G → de-oxo-G)

→ **Toxicodynamics**

= **toxicity mechanisms (MoA)** and following **toxic effects** (e.g. mutation, cancer ...)

- Elevated **de-oxo-G** in blood



→ **(Selective) biochemical marker (biomarker)**  
= information about exposure and/or effect

# Toxicity – the cause-effect paradigm

## Paracelsus (1493 - 1541)

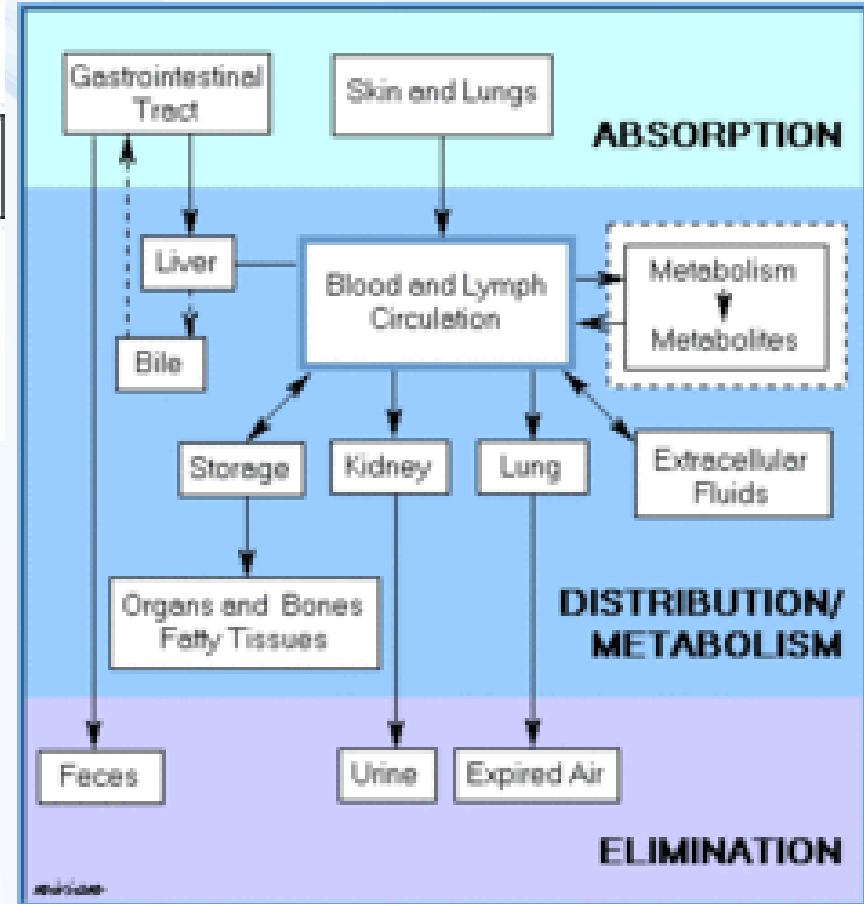
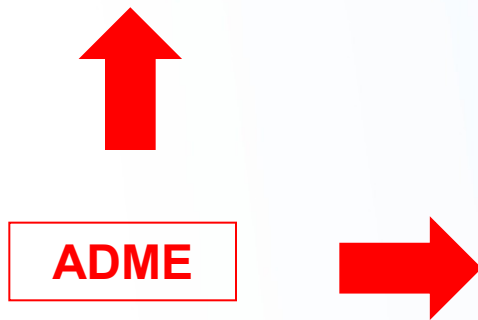
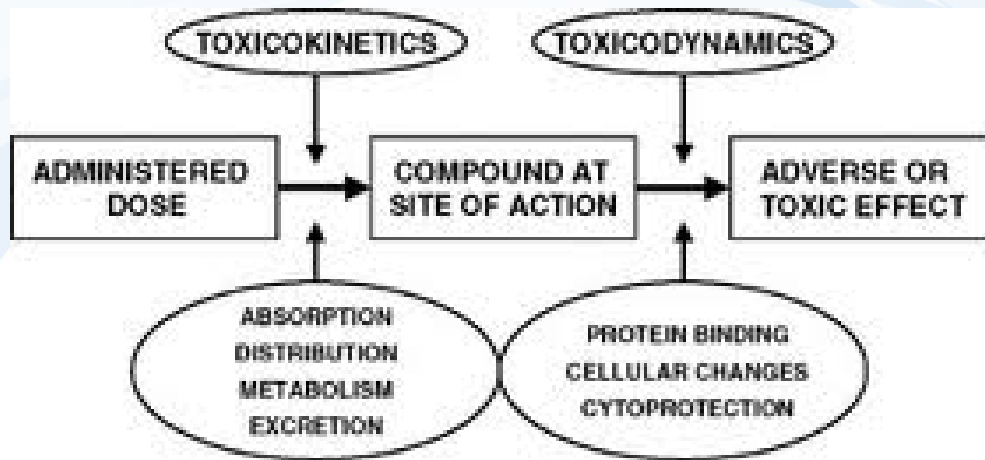


‘What is there which is not a poison?’

- All things are poison and nothing without poison.
- Solely the dose determines that a thing is not a poison.
- *Toxicology – the science of doses*



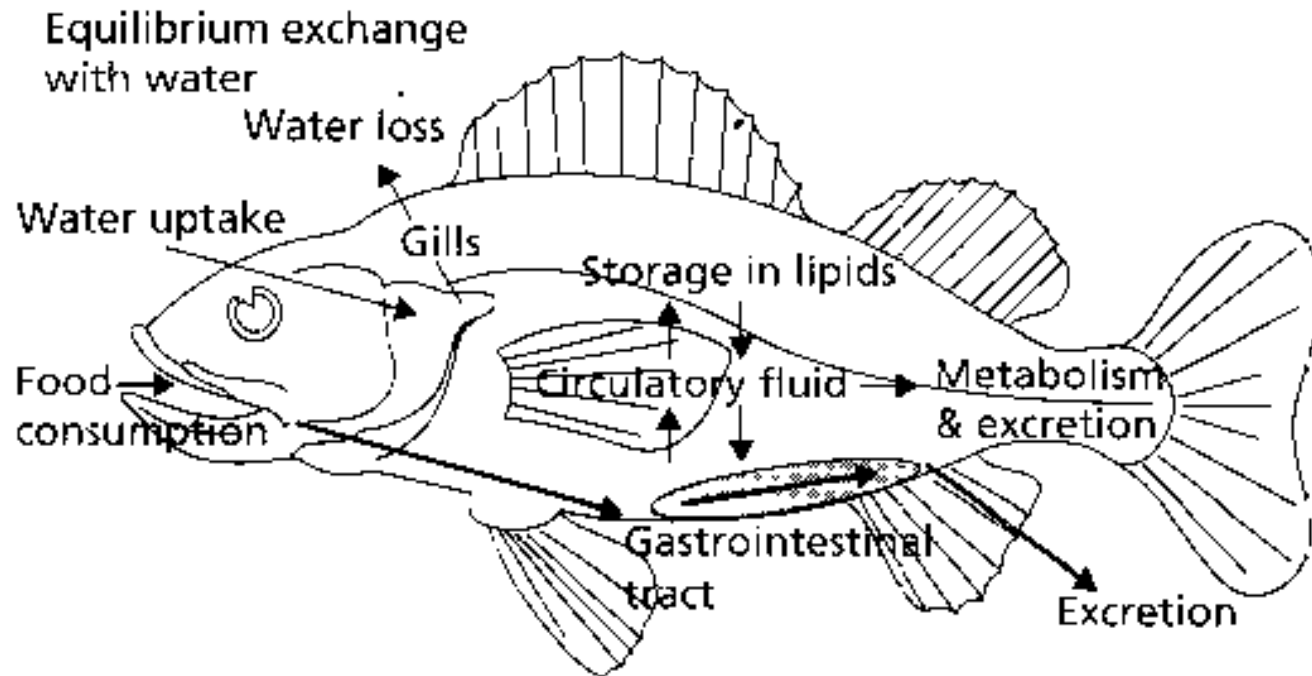
# What processes are beyond toxicokinetics?



*Toxicokinetics ...*

*... EXPOSURE phase → Determines the final dose*

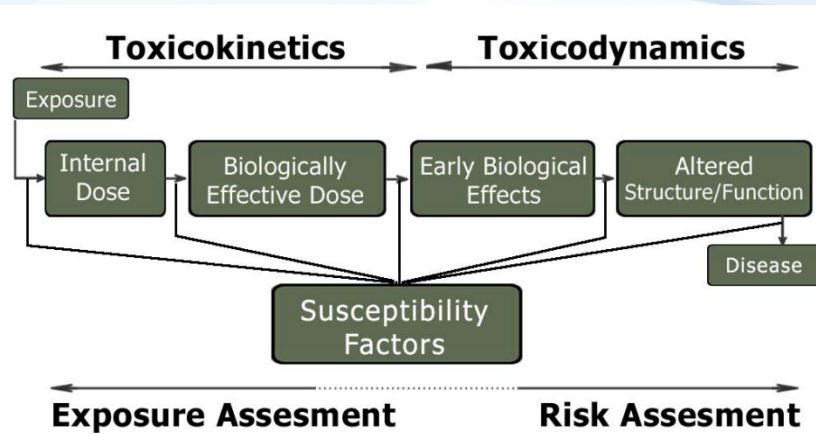
# Toxicokinetics in fish



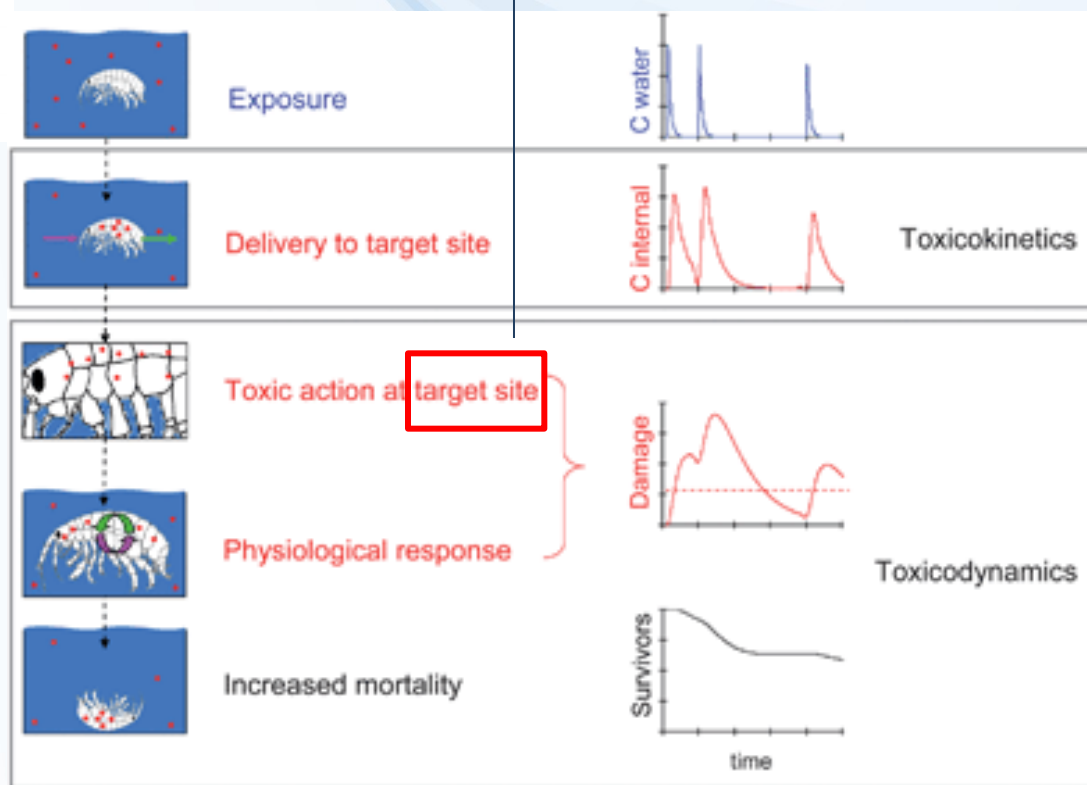
**Fig. 3.5** Uptake, accumulation and loss processes for a toxicant in the ambient water with fish.



# ToxicoDYNAMICS



**TARGETS = macromolecules**  
(DNA/RNA, proteins, membrane lipids)



MoA →



... and measurable EFFECTS →



# What is toxicity? What are the types of effects?

- **Toxicity**
  - degree to which a substance (at certain dose) can damage an organism
- **Exposure & toxicity**
  - **acute** (immediate, high doses, days)
  - **chronic** (sublethal / low doses, long-term)
- **Effect & toxicity**
  - **lethal (acute)**
    - mortality – definitive endpoint / high doses
    - easy to determine (single endpoint – death)
  - **nonlethal, sublethal (chronic)**
    - endocrine disruption, reproduction toxicity, immunotoxicity, tumor induction etc.
    - difficult to determine (multiple endpoints)
    - more specific – low concentrations / longer exposures
    - **often reflected by specific biochemical changes (biomarkers)**
- **Systems and organ & toxicity**
  - Systemic lethal toxicity
  - Organ-specific toxicity (neurotoxicity, hepatotoxicity, nephrotoxicity ...)
  - Developmental toxicity
  - Reproduction toxicity

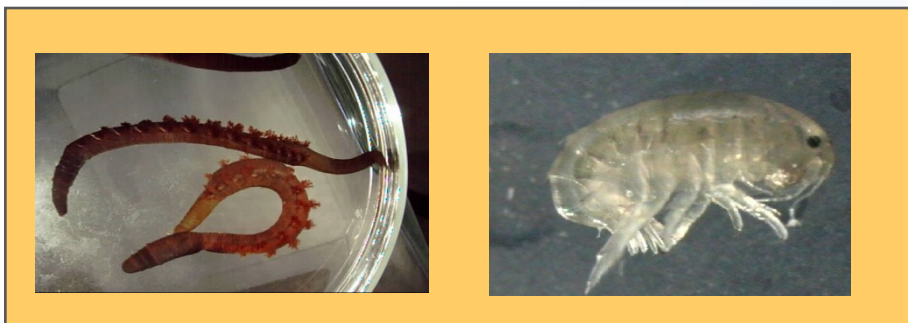
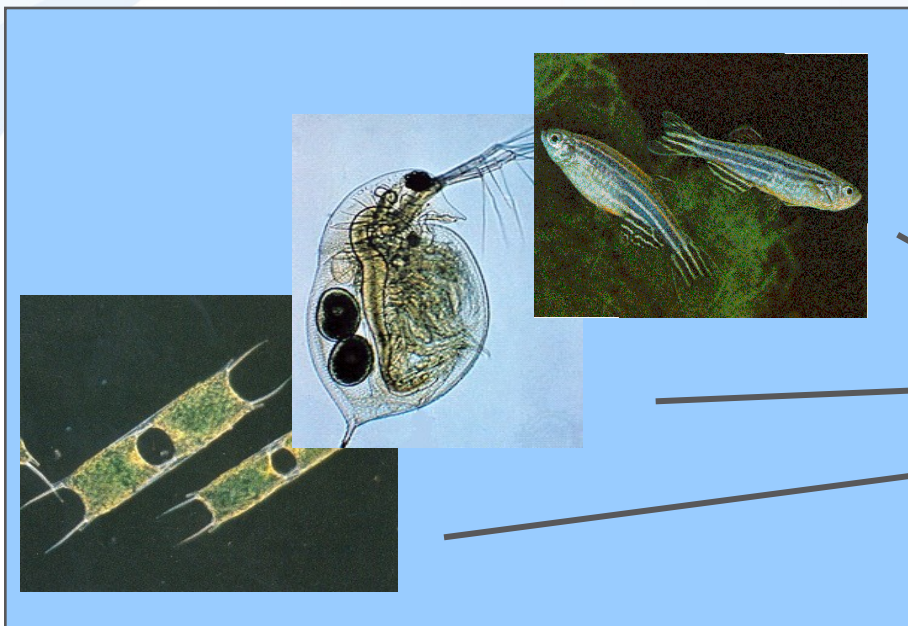


# Principles of toxicity testing

- 1) Define and know **biological target** (molecule, cell, organism, population) and its properties
- 2) Define and know **chemical** and its properties
- 3) **Define exposure** of biological system to a chemical
  - variable concentrations
  - defined or variable duration (time)
  - conditions (T, pH, life stage ....)
- 4) **Assess effects**, i.e. Changes in measurable parameter in relationship to variable doses
- 5) **Dose-response evaluation** & estimation of the toxicity value (i.e. concentration or dose):  
LD<sub>x</sub>, IC<sub>x</sub>, EC<sub>x</sub>, LOEC/LOEL, MIC ...



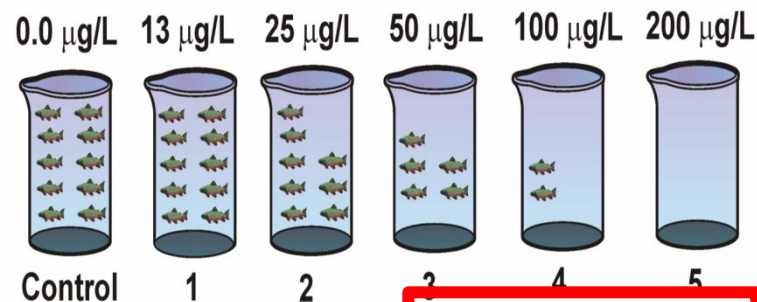
# Effect assessment - procedure



Cu addition



Concentration:



96-hour LC50 = 50  $\mu\text{g/L}$

Effect concentrations expressed in total/dissolved Cu



# How to study (chronic) toxicity ?

- **In vitro studies (biochemical mechanisms)**

- + easy to perform, short-term
- + highly controlled conditions
- + lower amounts of chemicals needed  
(new cmpnds screening)

- ecotoxicological relevancy
- mostly with vertebrate cells

- **In vivo biotest testing**

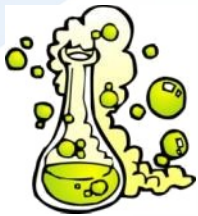
- + unique whole organisms
- + controlled conditions
- + better ecological interpretation

- only few (ecologically nonrelevant) organisms used
- mostly ACUTE assays
- chronic: long exposures

- **Field and *in situ* observations, epidemiological studies**

# Hazard assessment

**Traditionally** – Evaluation of adverse effects using the whole organism models



Chemical



Organism



## Adverse Effects

*Death*  
*Altered Reproduction*  
*Inhibition of Growth*

*Tumorigenicity*  
*Skin irritation*

...



**REGULATORY FOCUS  
(APICAL ENDPOINTS)**

# Hazard assessment

**Traditionally** – Evaluation of adverse effects using the whole organism models



Chemical



Organism

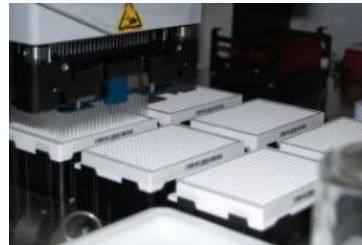


**Adverse Effects**  
Death  
Inhibition of Growth  
Altered Reproduction  
Tumor  
Skin irritation  
...

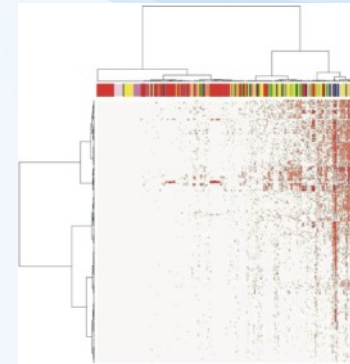
**New** – Ex vivo / in vitro / In chemico / In silico Methods



$10^4$  Chemicals

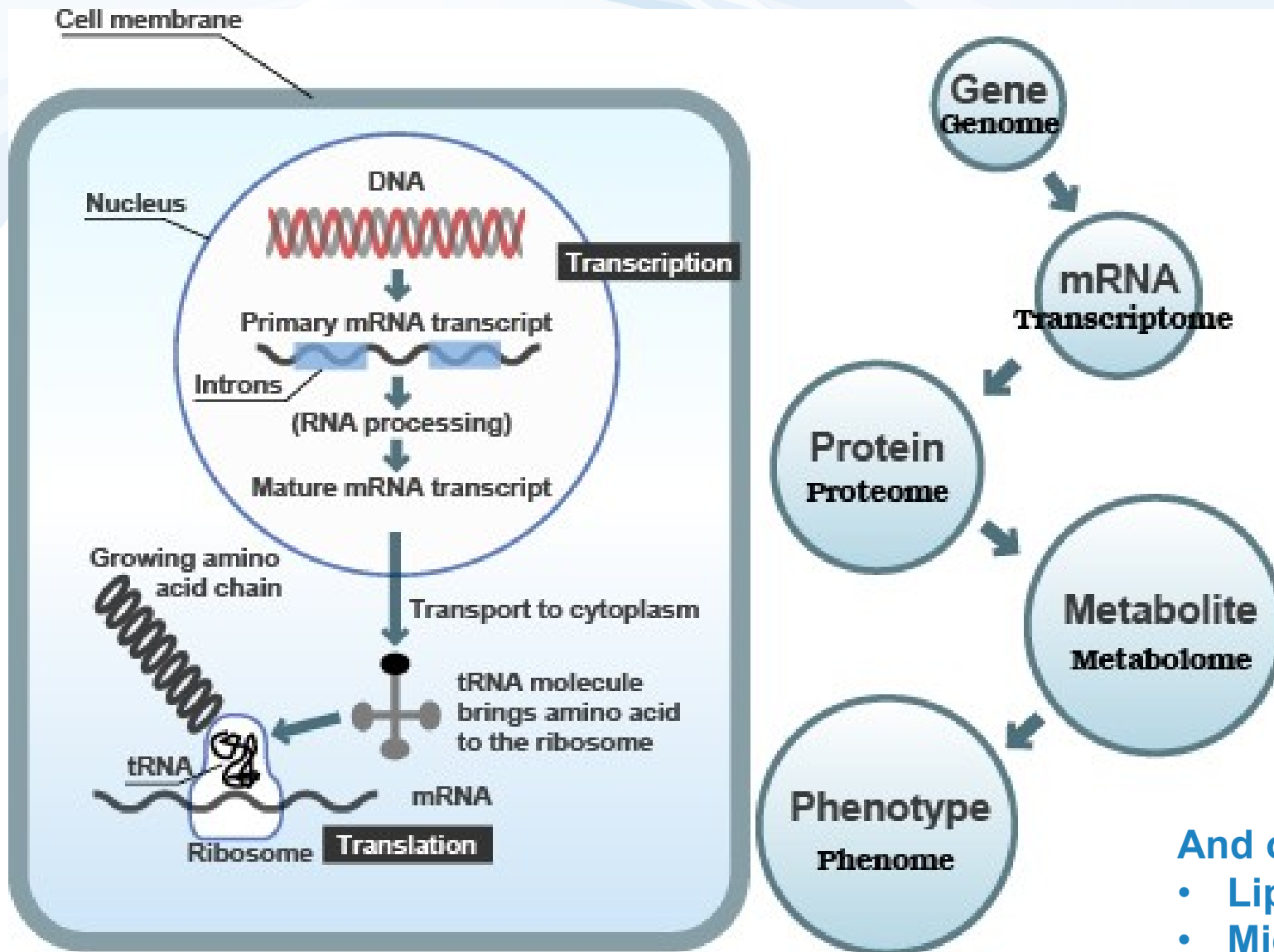


HTS  
High-Throughput-Screening



**Chemical-biological interactions,  
Mechanistic Toxicological Data**

# Mode of Action (omics) toxicity testing



And other „omes“

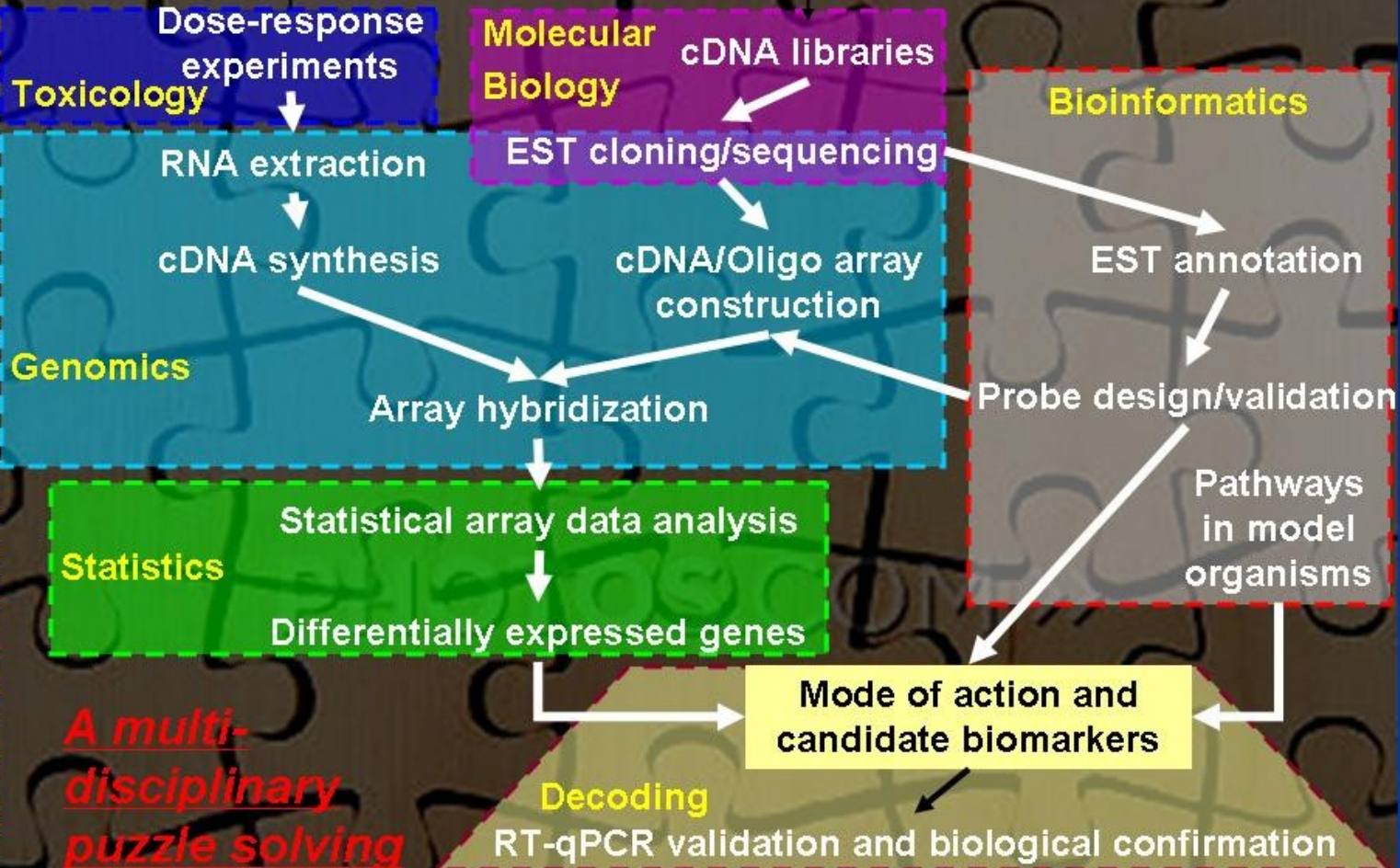
- Lipidome
- Microbiome ...

Omes is not only for humans ...

# Earthworm Toxicogenomics



US Army Engineer Research & Development Center



**A multi-disciplinary puzzle solving exercise**

One Team, One ERDC . . . Relevant, Ready, Responsive, Reliable

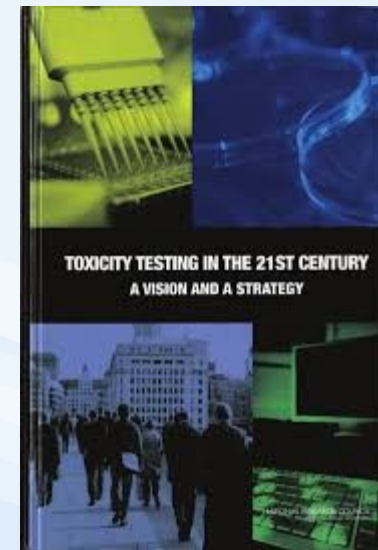


# MoA and omics are supported by strategic documents & organizations

## Toxicity Testing in the 21st Century: A Vision and a Strategy

US National Academies of Sciences

<http://www.nap.edu/catalog/11970.html>



United States Environmental Protection Agency

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### Computational Toxicology Research

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#### Key Links

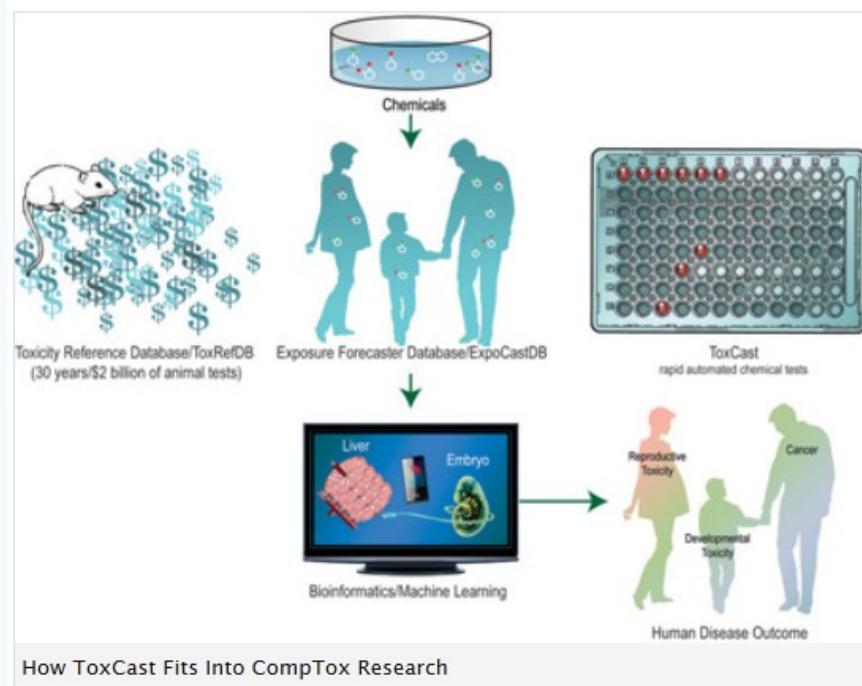
[CompTox Home](#)  
[Basic Information](#)  
[Organization](#)

[Research Projects](#)  
[Chemical Databases](#)  
[CompTox Events](#)

R  
S  
C

## ToxCast™

Screening Chemicals to Predict Toxicity Faster and Better



# Hazard assessment

**Traditionally** – Evaluation of adverse effects using the whole organism models



Chemical



Organism



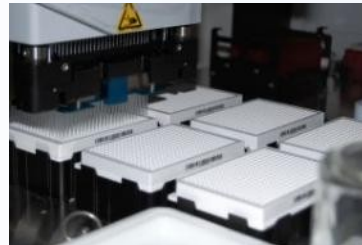
**Adverse Effects**  
Death  
Inhibition of Growth  
Altered Reproduction  
Tumor  
Skin irritation  
...



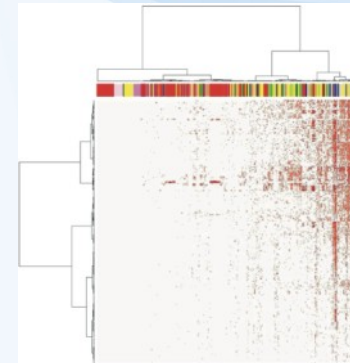
**New** – Ex vivo / in vitro / In chemico / In silico Methods



$10^4$  Chemicals



HTS  
High-Throughput-Screening

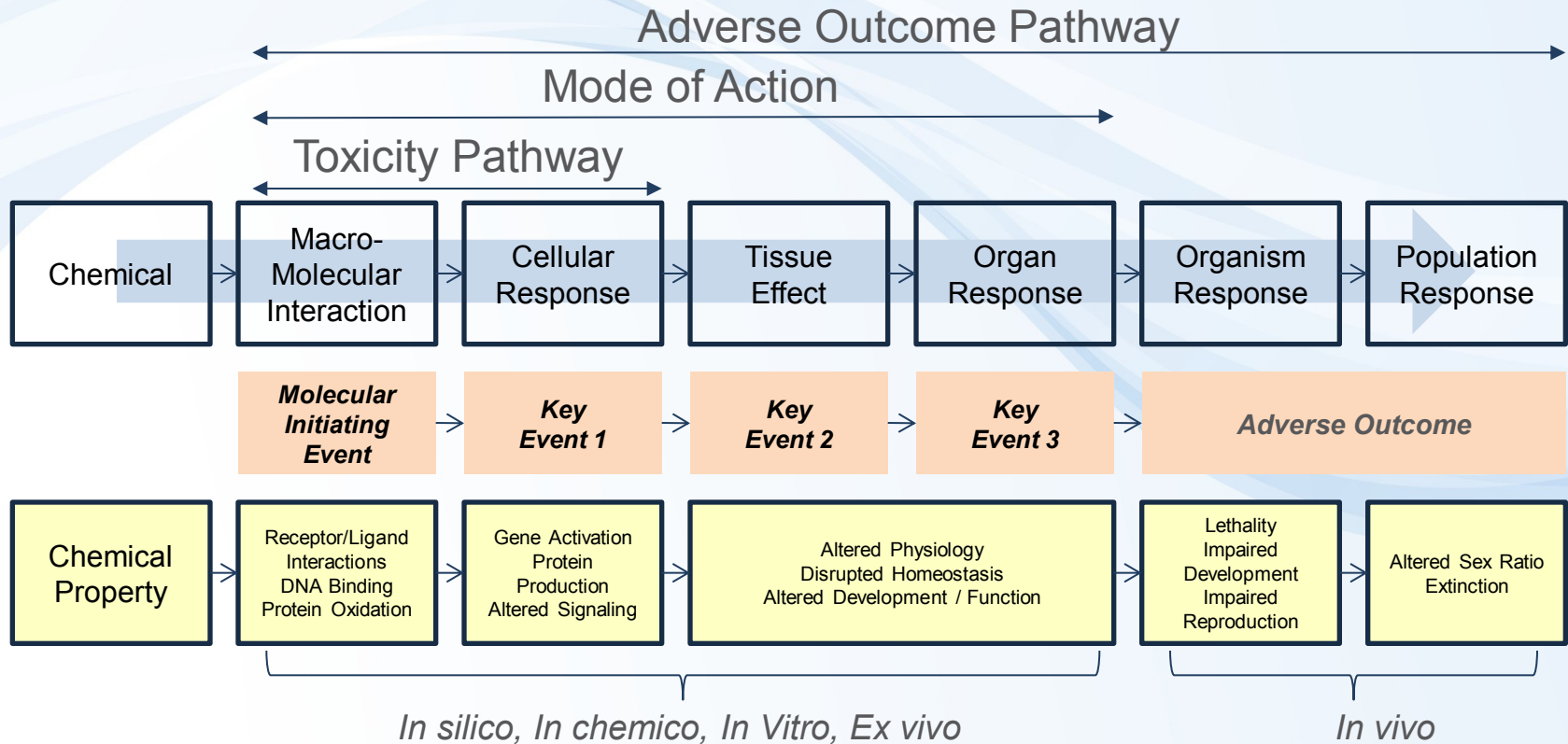


**Chemical-biological interactions,  
Mechanistic Toxicological Data**

**Key task/question:**

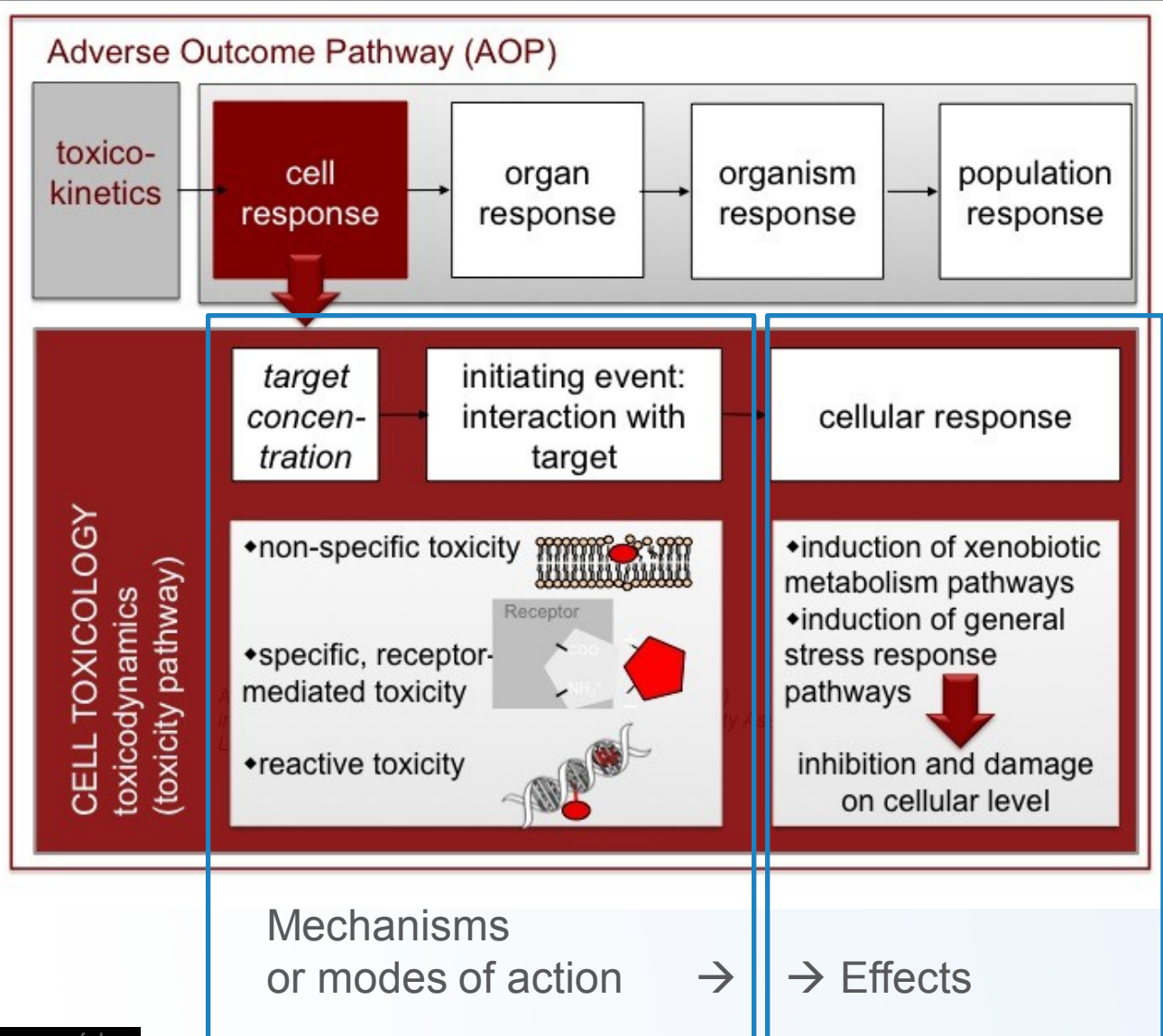
How to link MECHANISTIC INFORMATION with APICAL ENDPOINTS ?

# Adverse Outcome Pathways



The **EXISTING KNOWLEDGE** is used **to link the** two anchor points: **Molecular Initiating Event (MIE)** and **Adverse Outcome (AO)** **via a series** of intermediate steps: **Key Events**

# Concept of “Adverse Outcome Pathway” (AOP)



# AOP = Global strategy with support from OECD, EU, USA



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> Testing of chemicals

> Assessment of chemicals

> Risk management of chemicals

> Chemical accident prevention, preparedness and response

> Pollutant release and transfer register

> Safety of manufactured nanomaterials

> Agricultural pesticides and biocides

> Biosafety - BioTrack

## Adverse Outcome Pathways, Molecular Screening and Toxicogenomics

### WHAT'S NEW

#### SURVEY ON ADVERSE OUTCOME PATHWAYS (AOPS) TO IDENTIFY DEVELOPMENT PRIORITIES

The OECD has launched a survey to explore the utility of AOPs for regulatory assessment of chemicals and to identify development priorities. The objective is to collect feedback on how the AOP concept and/or existing AOPs are already being used for regulatory purposes, to understand where they fall short regarding their utility, and to identify what directions and priorities future AOP development work should embrace to increase their impact on regulatory toxicology and chemical risk assessment.

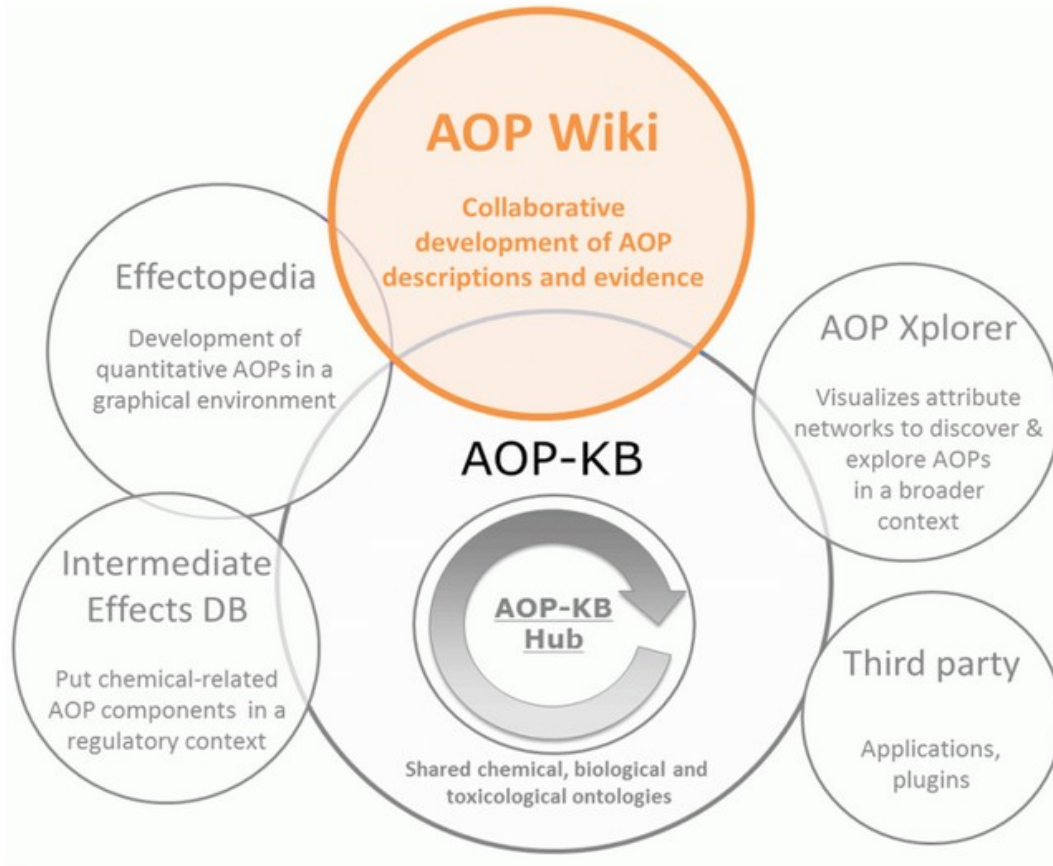
The survey is mainly for chemical safety regulators who are experiencing a transition in their work towards an increased use of 'alternative' methods and AOPs. However, stakeholders that come from the regulated community and environmental NGOs are also welcome to participate.

> **The survey is now closed. Thank you for your submissions.**

<http://www.oecd.org/chemicalsafety/testing/projects-adverse-outcome-pathways.htm>



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Please click on any of the AOP-KB elements you want to use.

Please note that the AOP-KB is work in progress and more elements will become available over time.

<http://aopkb.org/>

## Key documents

**OECD Guidance document and a template for developing and assessing adverse outcome pathways (Series No. 184, Series on Testing and Assessment)**

**Handbook for AOP developers**

# AOP Wiki

- [https://aopkb.org/aopwiki/index.php/Main\\_Page](https://aopkb.org/aopwiki/index.php/Main_Page)
- Wiki-based platform for development of AOPs
- Only members of an OECD AOP development project can create / edit AOPs



# What AOPs are now in AOP Wiki (July 2017) ?



OECD Endorsed (WNT and TFHA)	6	<ul style="list-style-type: none"> <li>Alkylation of DNA in male pre-meiotic germ cells leading to heritable mutations</li> <li>Aromatase inhibition leading to reproductive dysfunction</li> </ul>
EAGMST Approved	1	<ul style="list-style-type: none"> <li>Androgen receptor agonism leading to reproductive dysfunction</li> </ul>
EAGMST Under Review & for comments	18	
EAGMST Under Development	84	
SAAOP AOP Under Development	130+	

- OECD Extended Advisory Group on Molecular Screening and Toxicogenomics (EAG MST)
- The Working Group of the National Coordinators of the Test Guidelines Programme (WNT)



# AOP Example: MIE aromatase inhibition

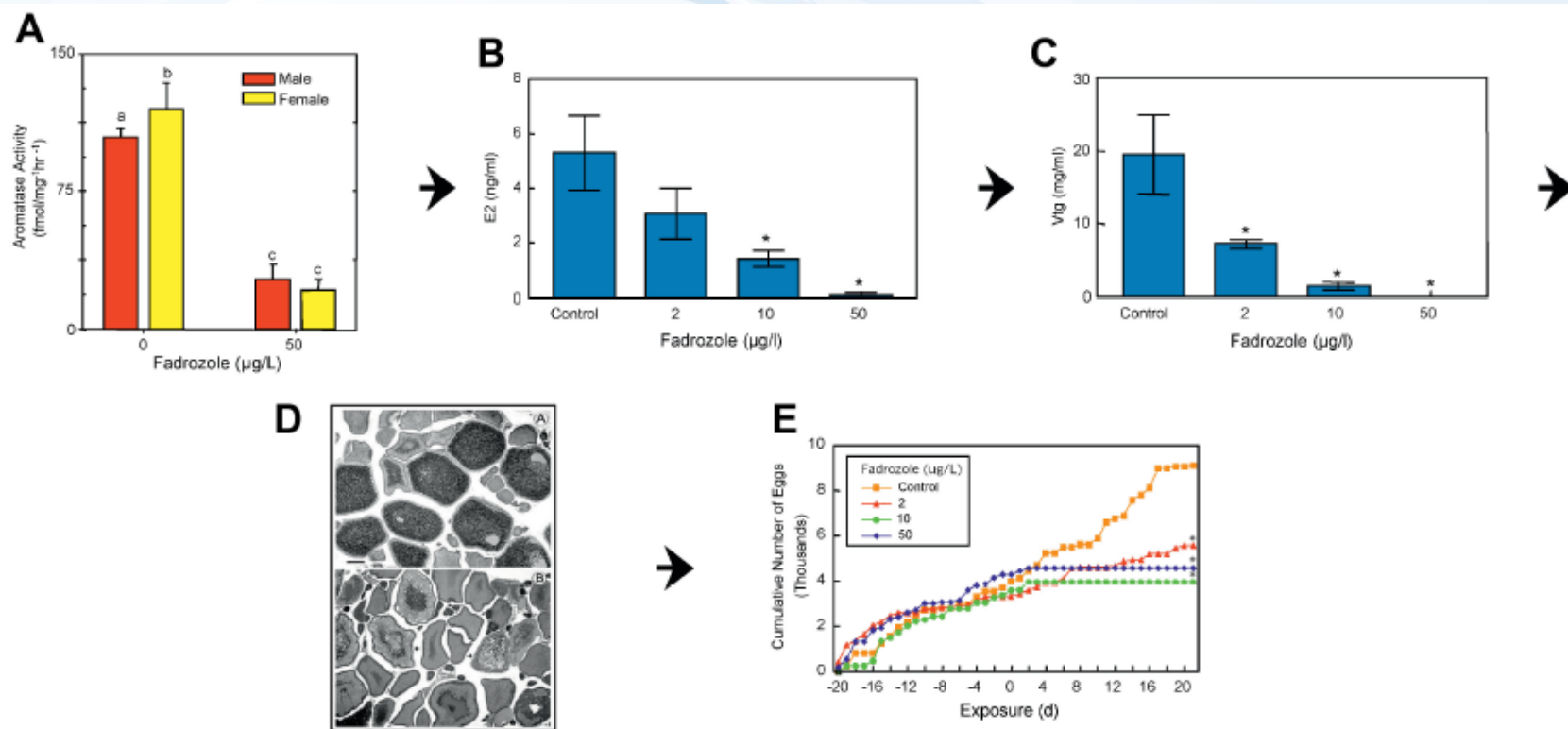
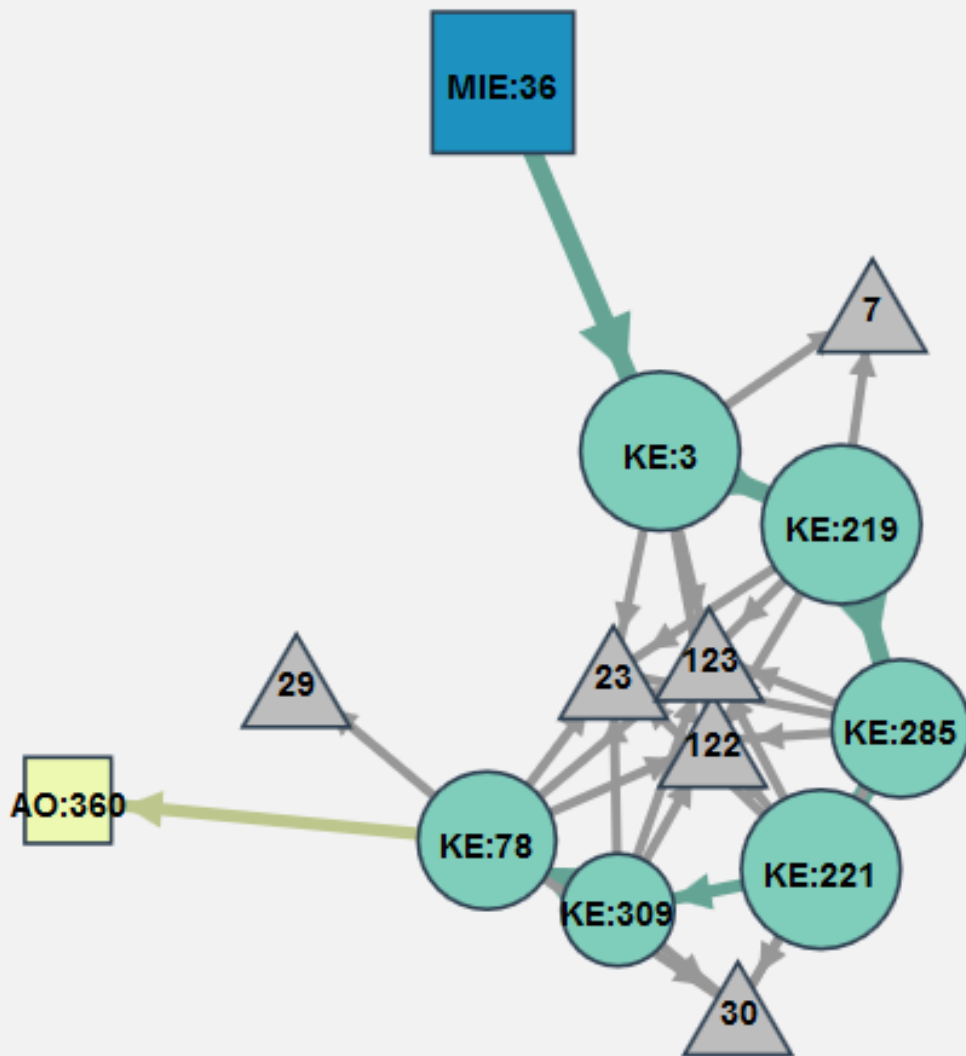


Fig. 3. An adverse outcome pathway in fish [2,50]. Aromatase inhibitor example. (A) Aromatase inhibition by fadrozole; (B) Reduction in circulating estradiol; (C) Reduction in circulating vitellogenin (Vtg); (D) Histopathology of ovarian tissue, top panel normal ovary, bottom panel fadrozole treated; note oocyte atresia; (E) Adverse outcome on egg production–fecundity (© Elsevier, Used with permission,)

Environmental Toxicology and Chemistry, Vol. 30, No. 1, pp. 64–76, 2011

# Aromatase inhibition leading to reproductive dysfunction (in fish)

<https://aopwiki.org/wiki/index.php/Aop:25>



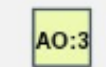
MIE



KE



AO



Other AOP including this KE



Indirect relationship



Direct relationship

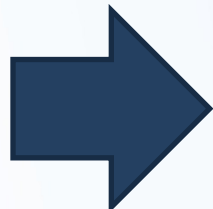
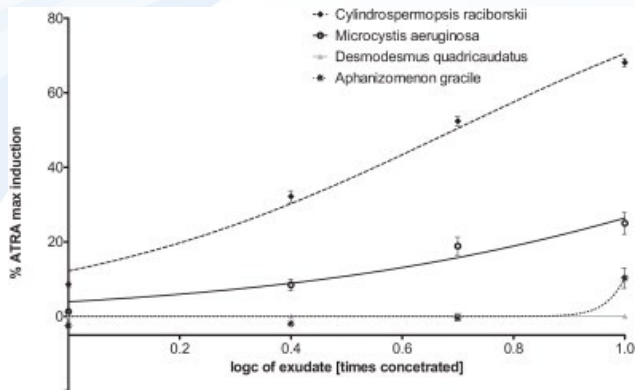


\*Size of node reflects essentiality of event

\*Width of line reflects strength of evidence for relationship

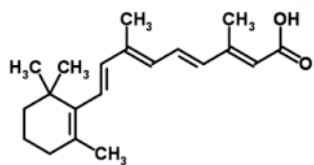


# AOP Example from RECETOX: Modulation of RAR/RXR → developmental toxicity in fish

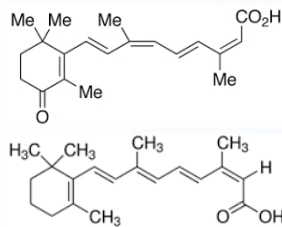


Activation of RAR/RXR

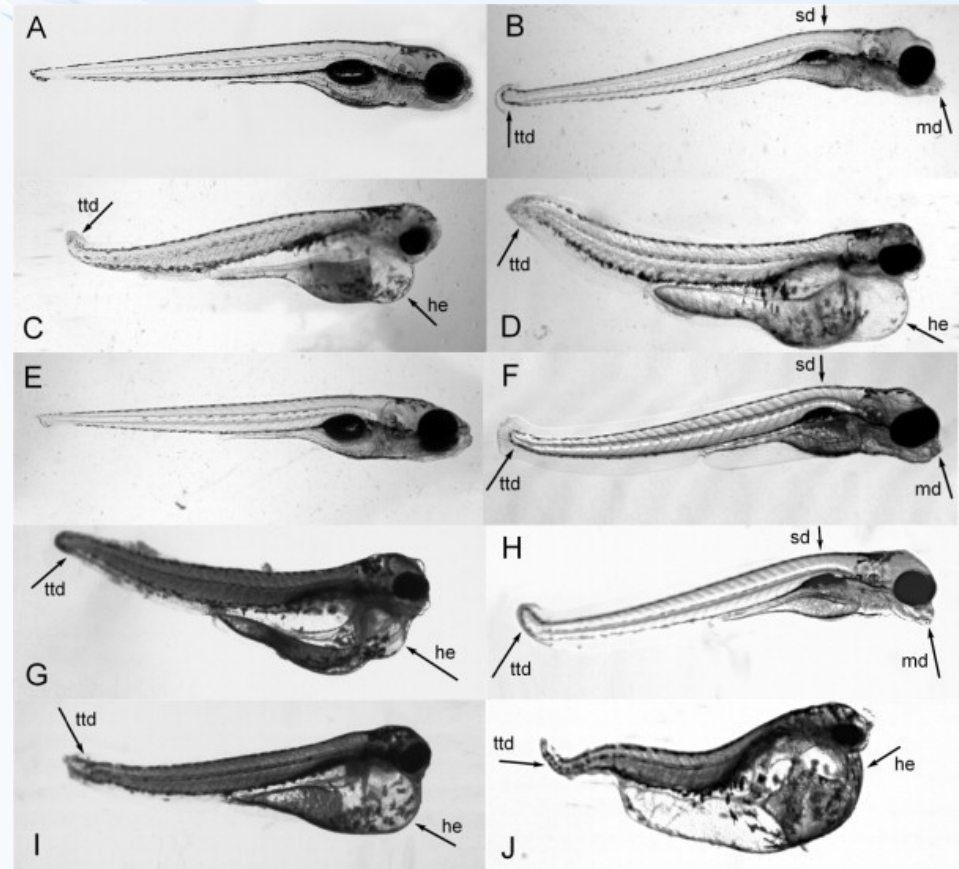
in P19/A15 cells by atRA and cyanobacterial metabolites



atRA



other RAs in cyanos

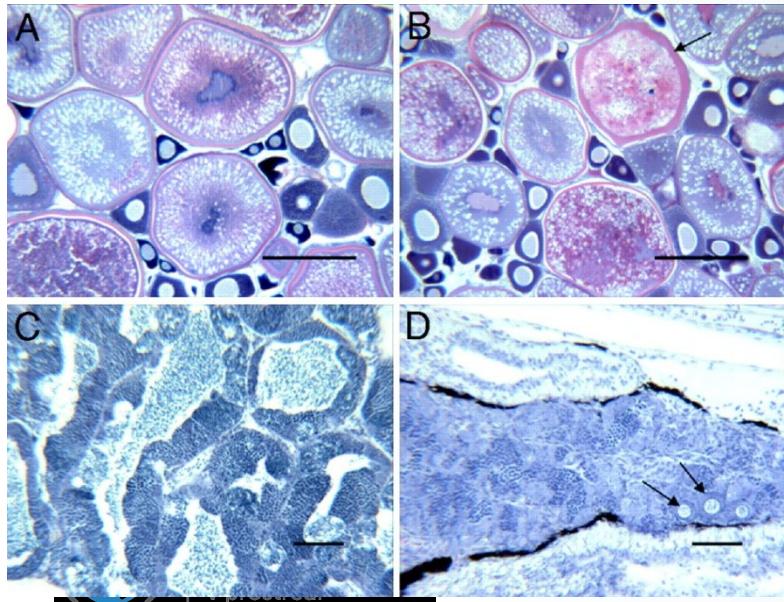
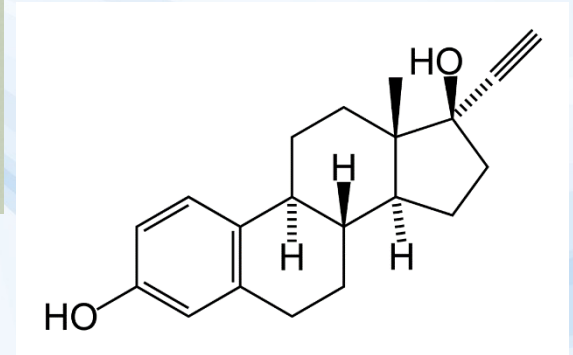


ZF exposed to ATRA and cyanobacterial (120 hpf) - Control (A), exudates of *C. raciborskii* 3.3 (B) and 10 (C), *M. aeruginosa* 10 (D) and *D. quadricaudatus* 17 (E). ATRA 4 µg/L (13.3 nM) (F), 12 µg/L (40 nM) ((G) and (H)), 36 µg/L (I) and 108 µg/L (J).

Kidd, K.A. et al. 2007. **Collapse of a fish population** following exposure to **a synthetic estrogen**. *Proceedings of the National Academy of Sciences* 104(21):8897-8901

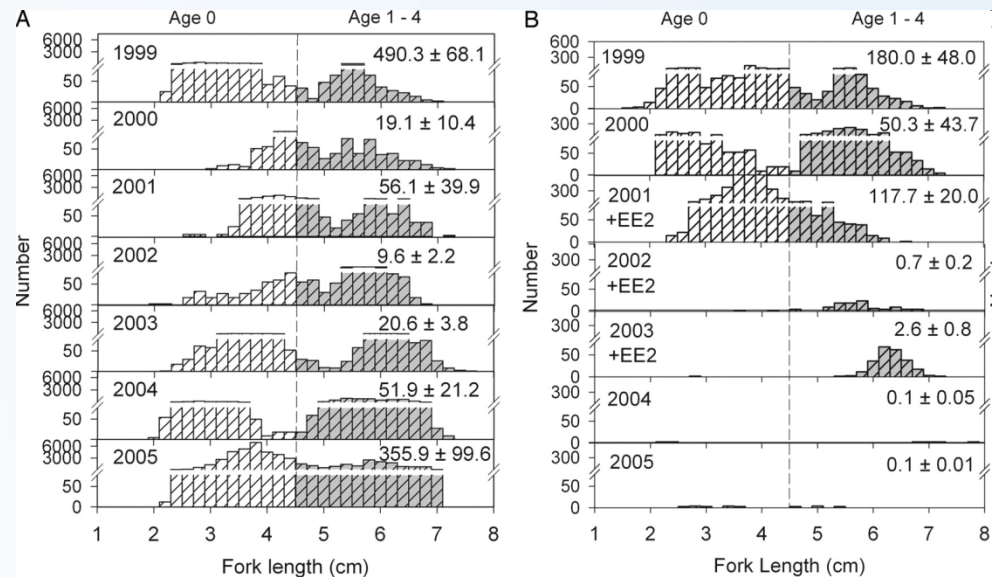


**5 ng/L (!)**  
**7 years**



**Controls**

**+ Ethinylestradiol**



# Keywords to remember and understand

- What is meant by the “mechanism of action” (or “mode of action”) in toxicology?
- Why is it necessary to understand MoAs? What is the AOP concept?
- What is toxicokinetics? What is ADME?
- What is toxicodynamics?
- What is the relationship between the exposure and the effect?
- What are the different types of toxicity?
- How can the (toxic) effect be measured / assessed?
- What types of “bioassays” are available to study toxicity and/or MoA?
- How is the result (i.e. „toxicity“) described in numbers?