Centrum pro výzkum toxických látek v prostředí

# BIOMARKERS AND TOXICITY MECHANISMS

# 01 - INTRODUCTION

Luděk Bláha, PřF MU, RECETOX
www.recetox.cz

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

EVROPSKÁ UNIE esf

MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY

OP Vzdělávání pro konkurenceschopnost

UNIVERSITAS MASARYKIANA BRUNENSIS

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**

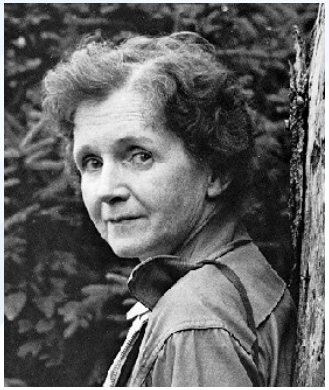
- Membrane toxicity, enzyme inhibitions, oxidative stress, genotoxicity, Nuclear Receptors (AhR, ER, AR ….) etc.
- Methods to determine toxicity mechanism

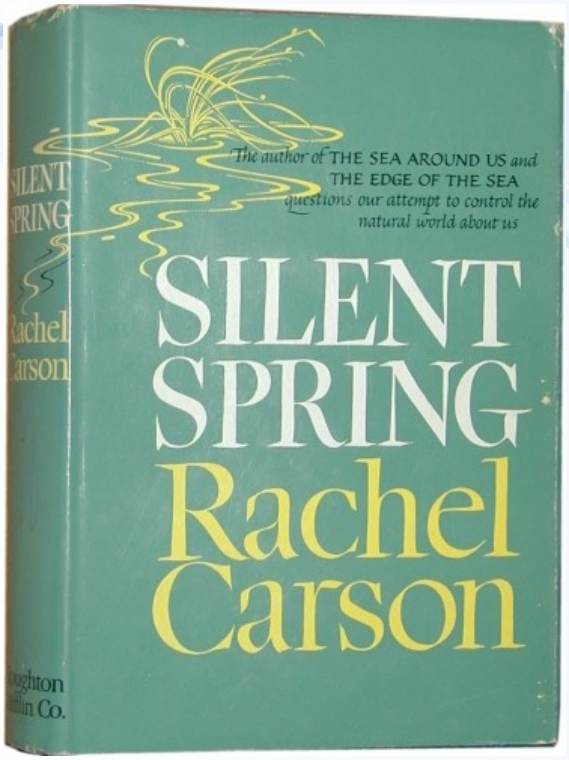
**3) Biomarkers**

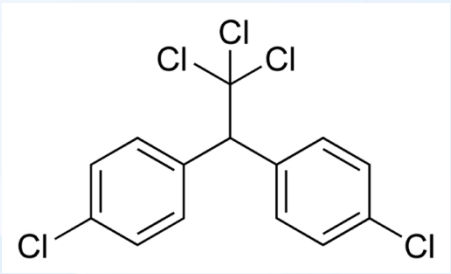
- What it is and how to find (identify) suitable biomarker(s)?
- The overview of the most important biomarker classes
- Methods of biomarker assessment

# The importance of understanding to toxicity mechanisms

# 1962







© Patuxent Wildlife Refuge, MA, USA

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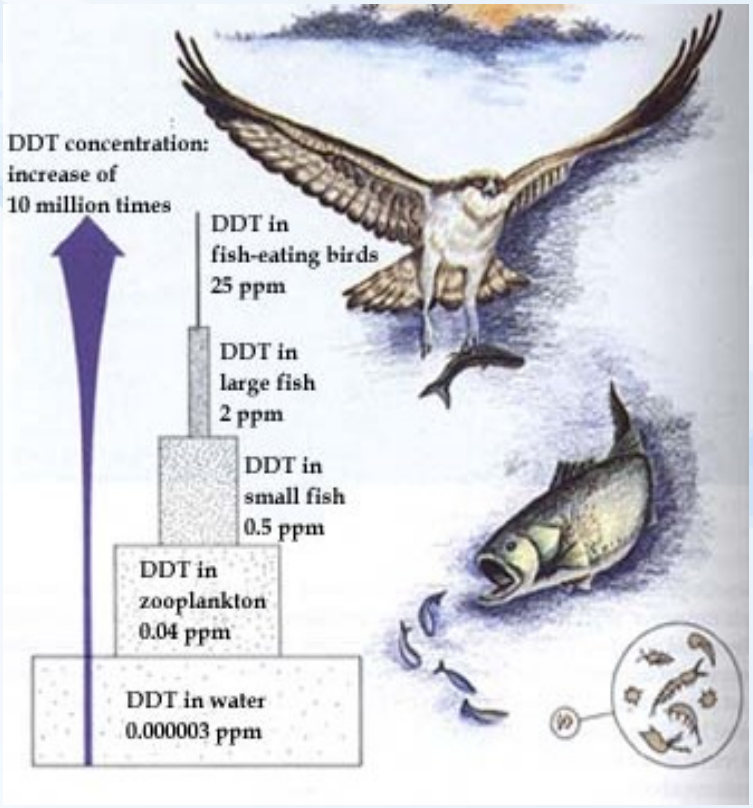
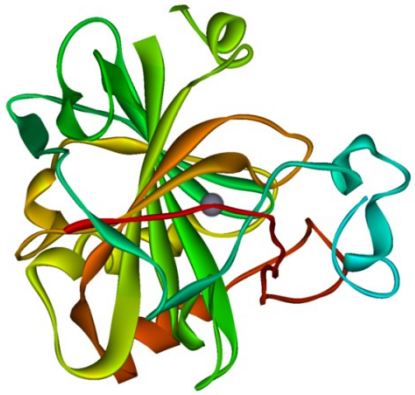
http://www2.ucsc.edu/scpbrg/

**In vivo:** shell thinning

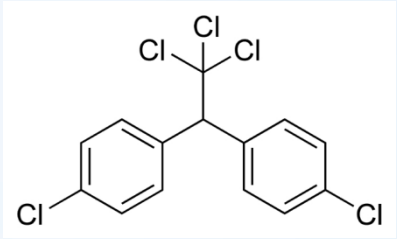
**In situ:** bioaccumulation
**-> bird population decline**



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

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

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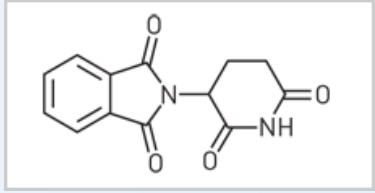
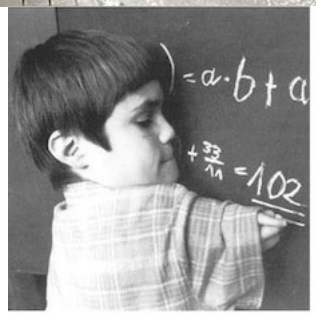
# Thalidomide



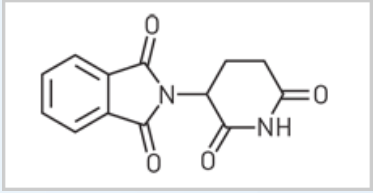

- Originally marketed in 1957 as sedative / hypnotic
  - also curing anxiety, gastritis, tension
  - against nausea and morning sickness of pregnant
    - TERATOGENICITY → Develoment of phocomelia = limb malformations (10 000 children worldwide / 40% survived)

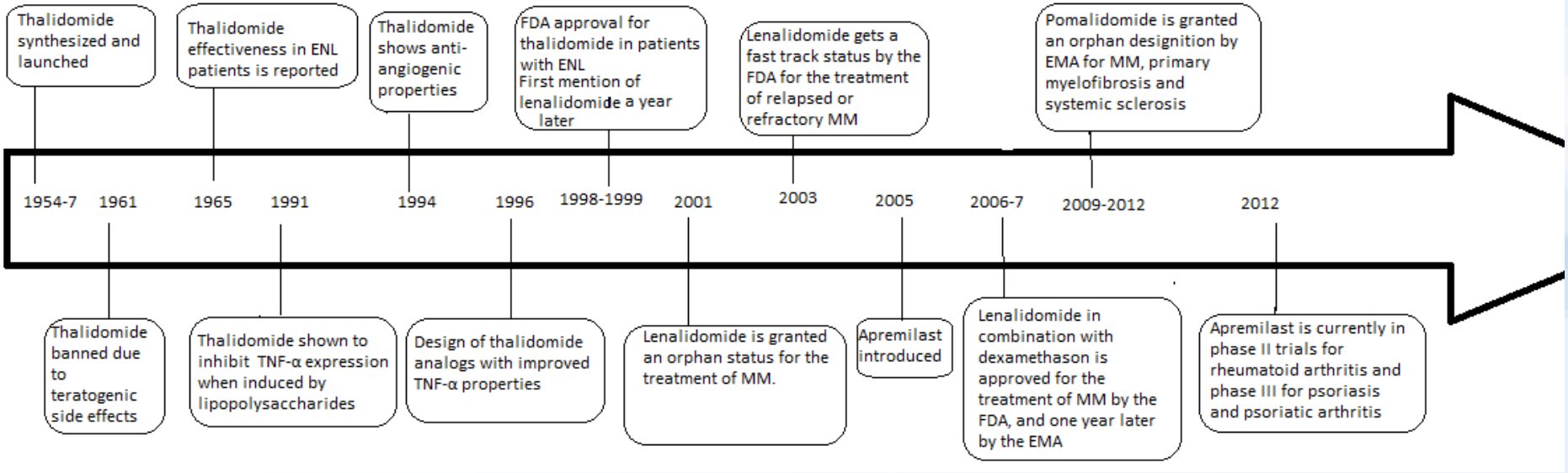


| | Teratogenic Manifestations of Thalidomide<br>Number of Days Past Last Menstruation |
|---|---|
| | 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 |
| Ear missing (anotia) | |
| Thumbs missing or deformed (aplasia) | |
| One or both arms missing (amelia) | |
| Both arms shortened (phocomelia) | |
| Hip dislocation | |
| Ears deformed | |
| Legs Missing (amelia) | |
| Both Legs shortened (phocomelia) | |
| Thumbs malformed (triphalangism) | |
| Humerus missing or deformed (ectromelia) | |
| Femur missing or deformed (ectromelia) | |
| Chart Based on Nowack[106] | |

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

# Thalidomide

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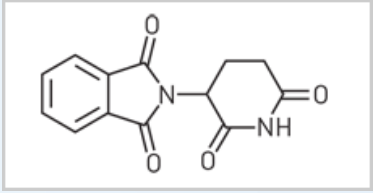
# Thalidomide

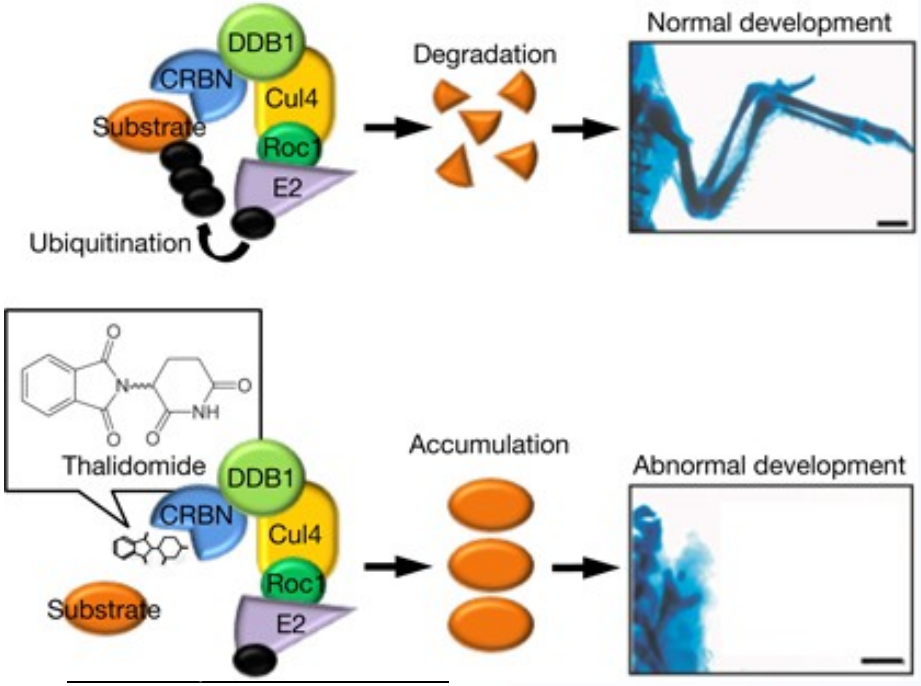
## ... mechanisms of action



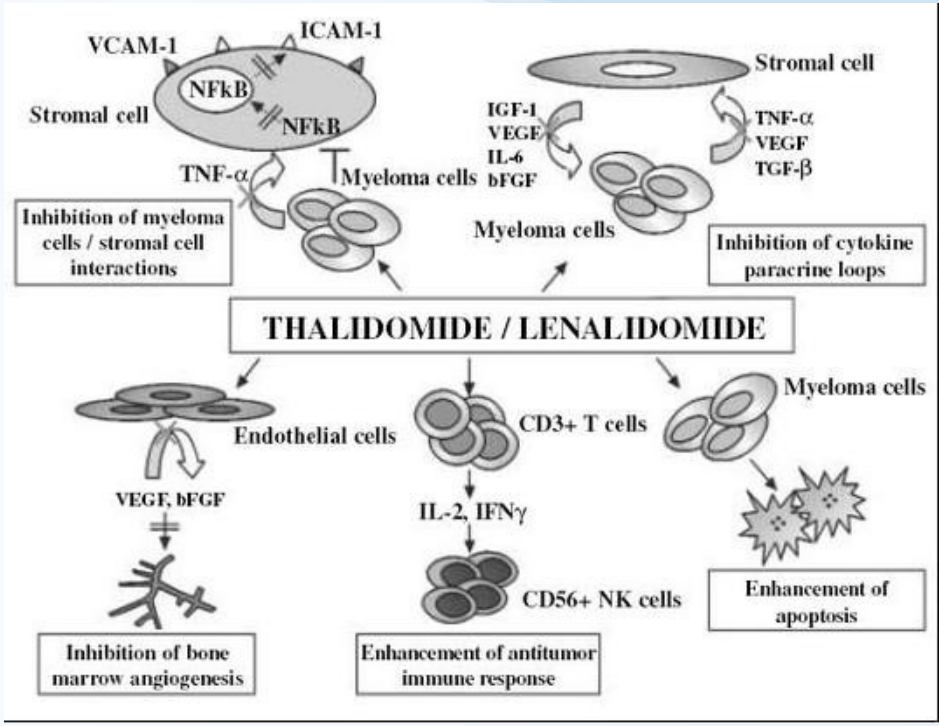

**(1) Sedative effects**

... mechanism unknown

**(2) Teratogenicity**

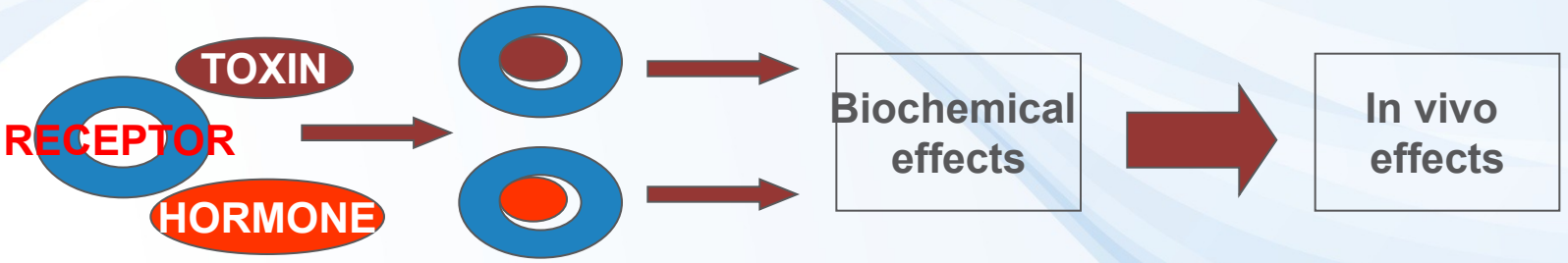



**(3) Anticancer**



# MECHANISMS of chronic toxicity

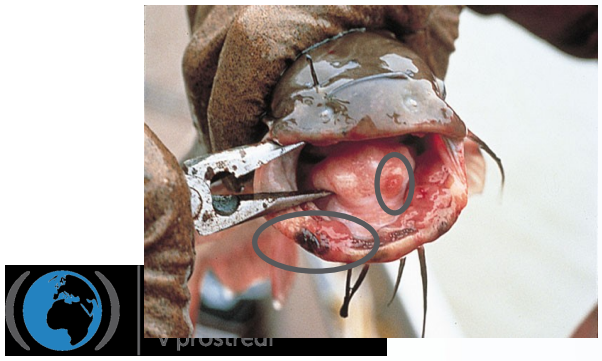
- **Various chronic effects have uniform biochemical basis**




  - 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

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# Toxicity - concept

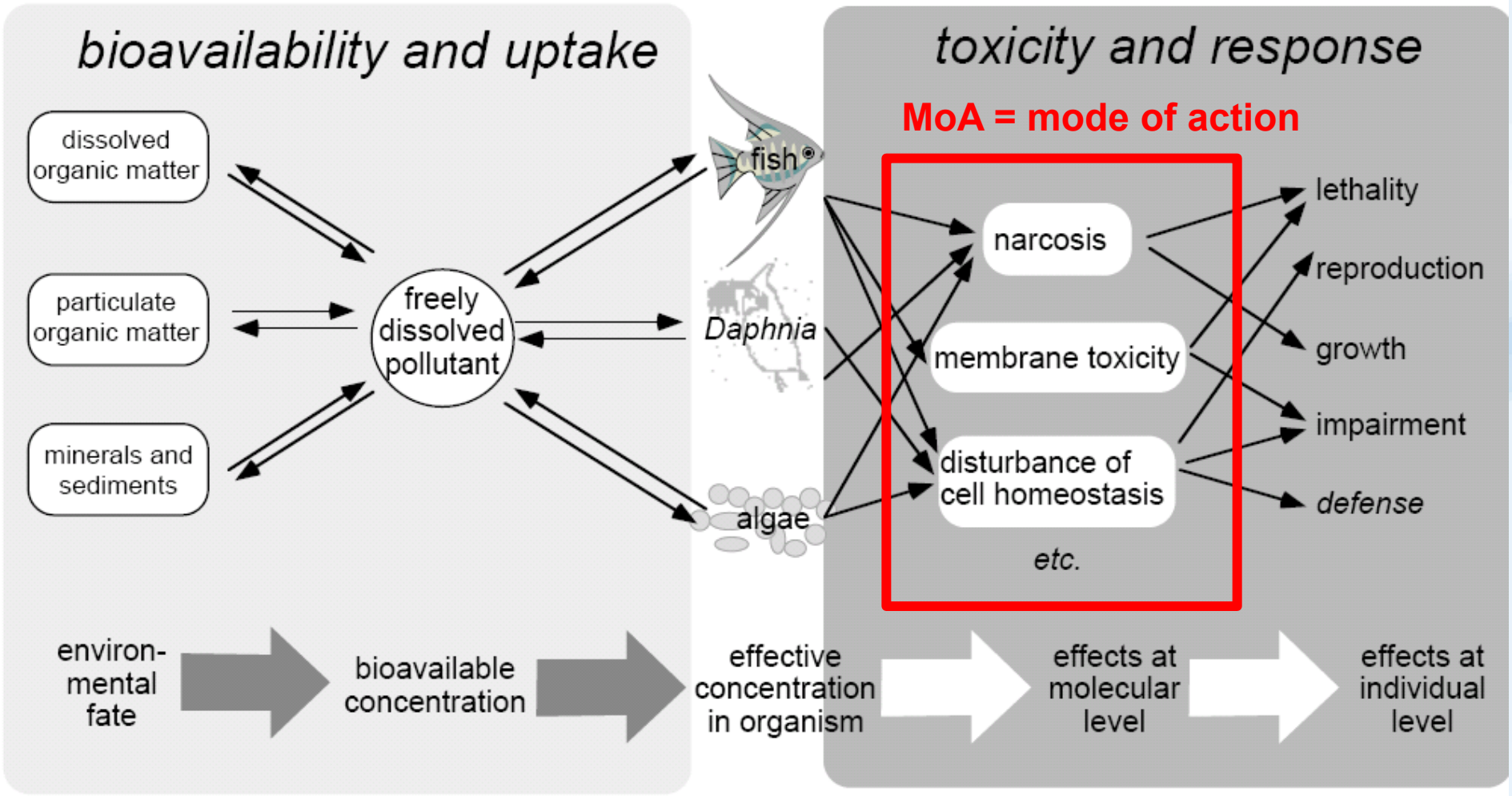


***Figure 1*** *The effective concentration of a pollutant in an organism (e.g. fish, daphnia, algae) or at the target site inside the organism is the link between the environmental fate of a pollutant and its toxic effect.*

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Escher, B. I., Behra, R., Eggen, R. I. L., Fent, K. (1997), "Molecular mechanisms in ecotoxicology: an interplay between environmental chemistry and biology", *Chimia,* **51**, 915-921.

# From mechanisms (or modes of action) to biomarkers

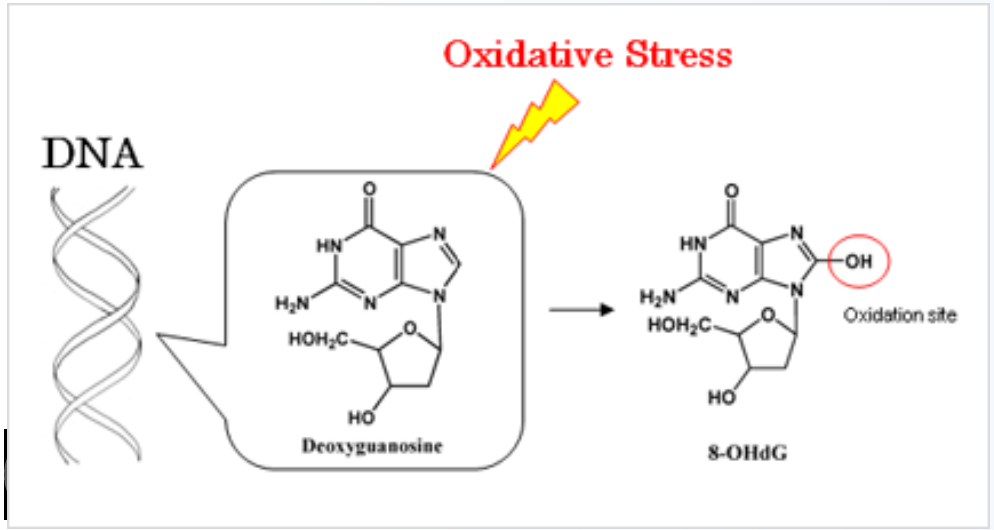
- Chemical enters organism
  + may be metabolized/detoxified, transported, released ...

→ **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

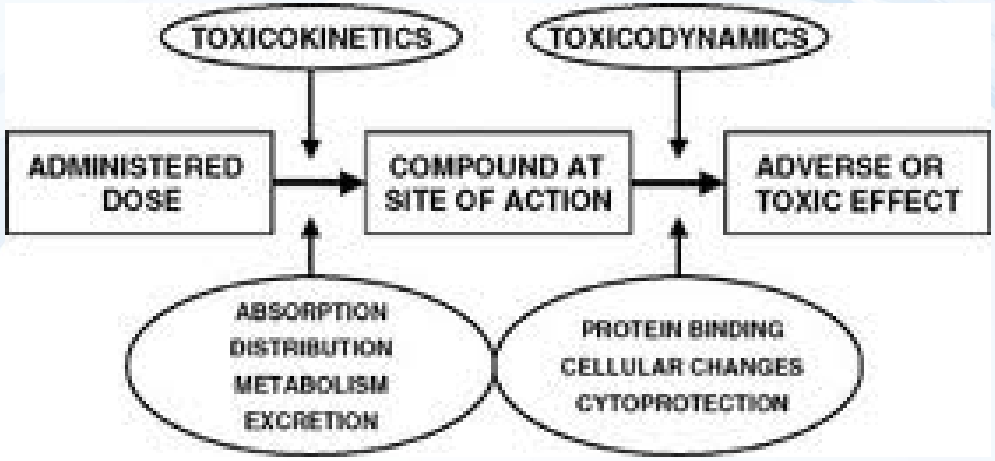
## Paracelsus (1493 - 1541)


'What is there which is not a poison?

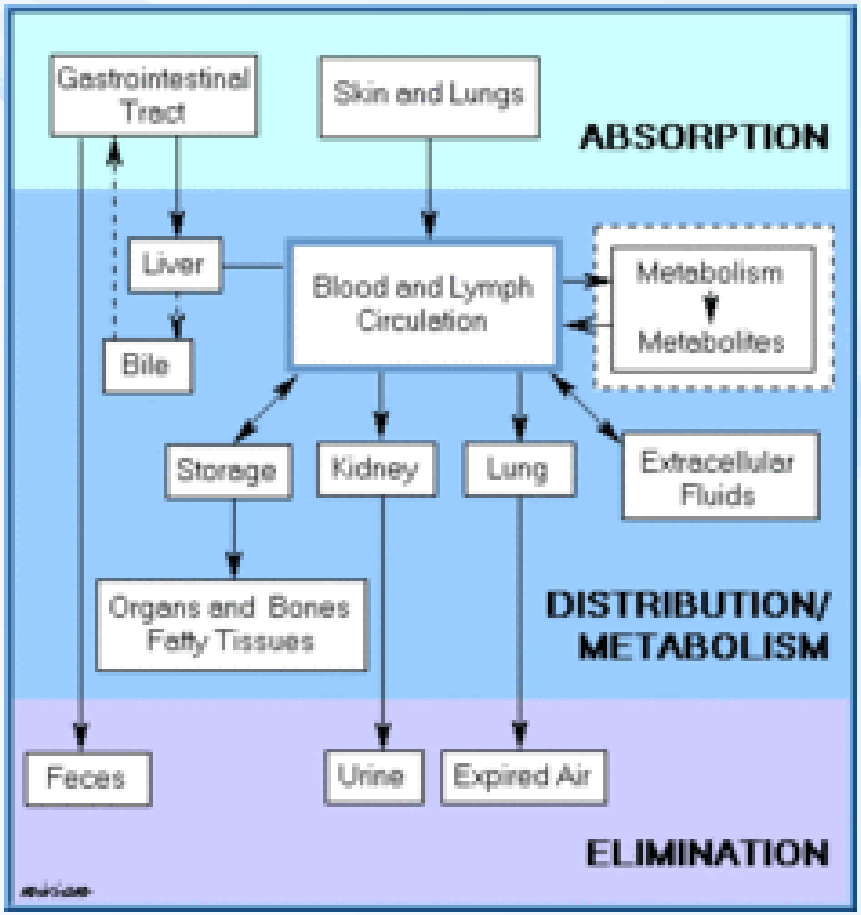
- All things are poison and nothing without poison.
- Solely **the dose determines** that a thing is not a poison.

- *Toxicology – the science of doses*

# What processes are beyond toxicokinetics?




**ADME**




***Toxicokinetics ...***

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

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# Toxicokinetics in fish

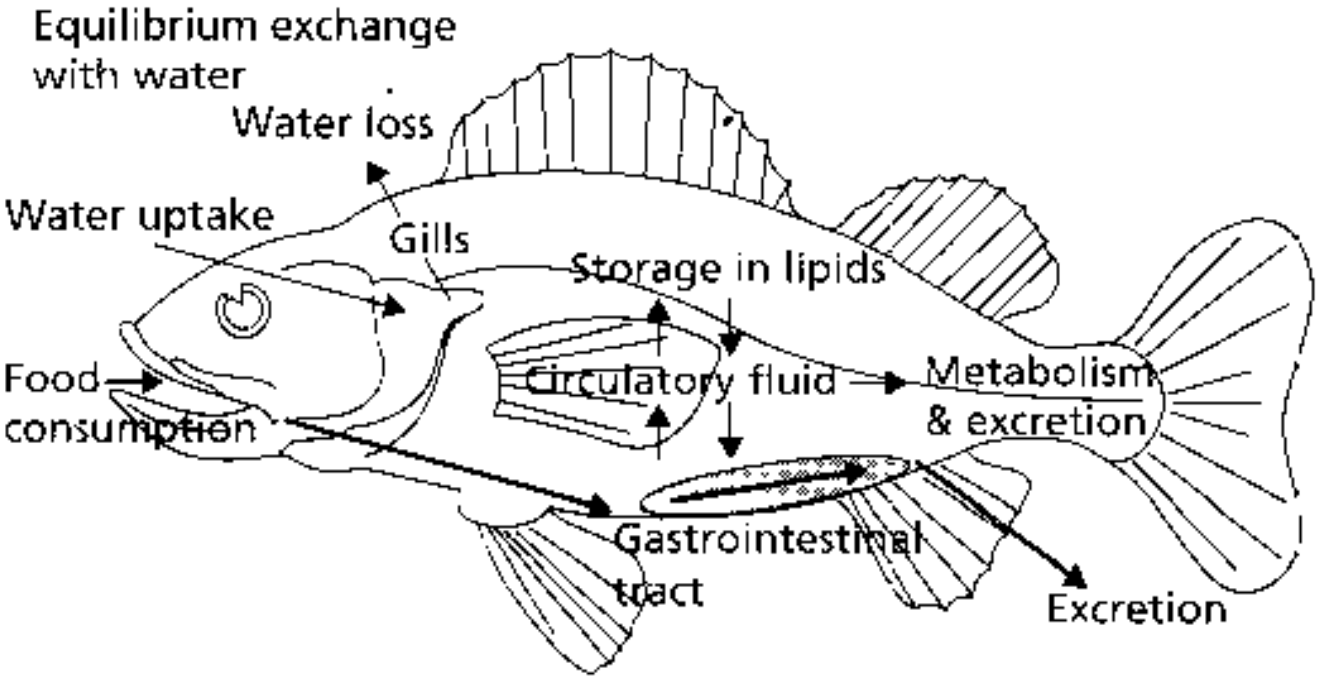
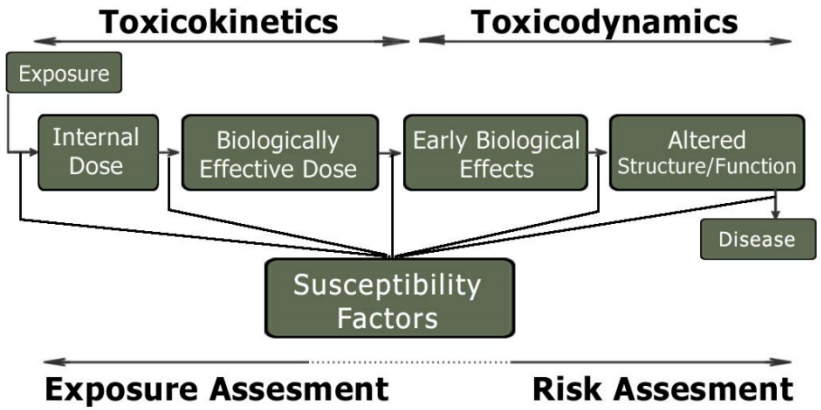



**Fig. 3.5** Uptake, accumulation and loss processes for a toxicant in the ambient water with fish.

# ToxicoDYNAMICS




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

**MoA**

... and measurable **EFFECTS**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# 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, nefrotoxicity ...)
  - Developmental toxicity
  - Reproduction toxicity

# Principles of toxicity testing

1) Define and know **biological target** (molecule, cell, organism, population) and its properties

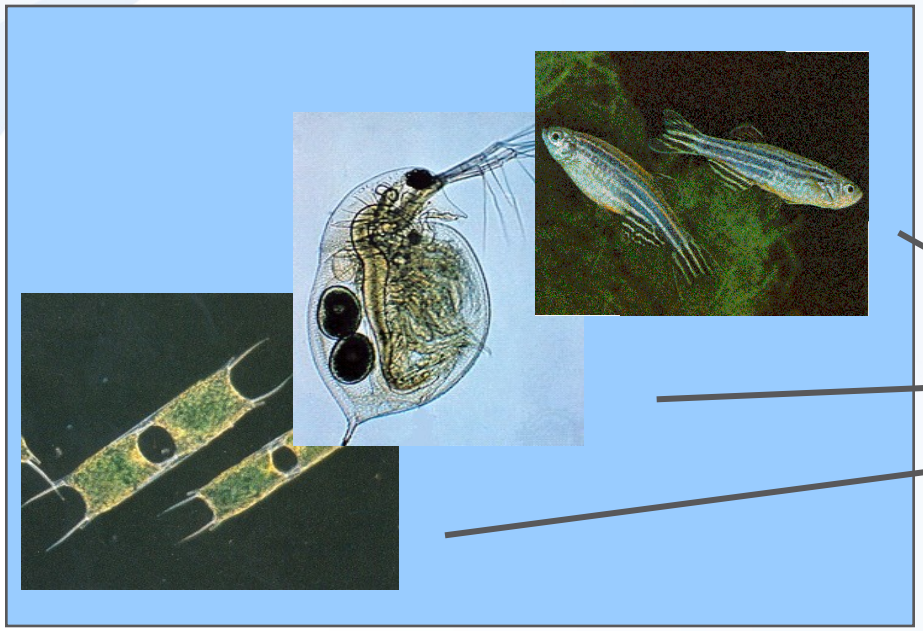
2) Define and know **chemical** and its properties

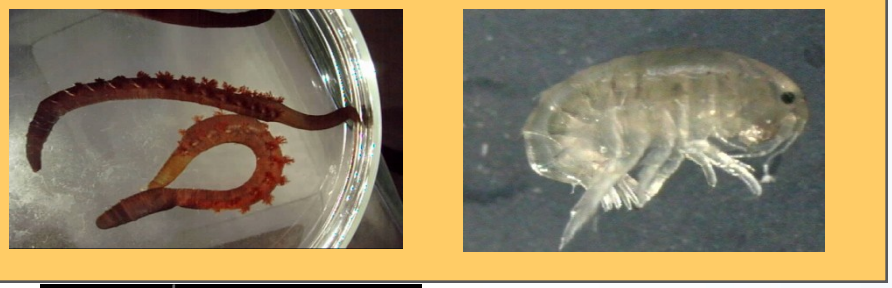
3) **Define exposure** of biological system to a chemical
   - variable concentrations
   - defined or variable duration (time)
   - conditions (T, pH, life stage ....)

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):
   LDx, ICx, ECx, LOEC/LOEL, MIC ...

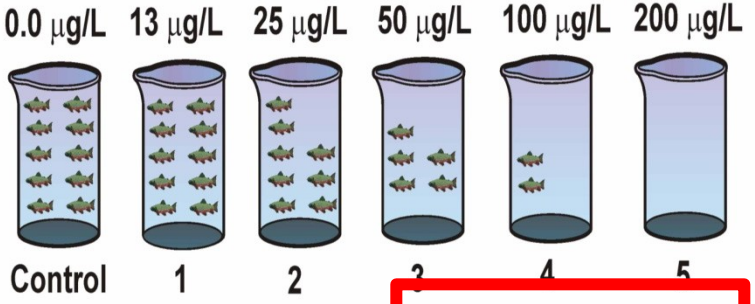
# Effect assessment - procedure





Cu addition

**Concentration:**




96-hour LC50 = 50 µg/L

Effect concentrations expressed in total/dissolved Cu

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# 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)

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# Hazard assessment

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

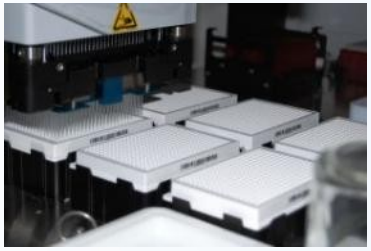
Chemical

Organism

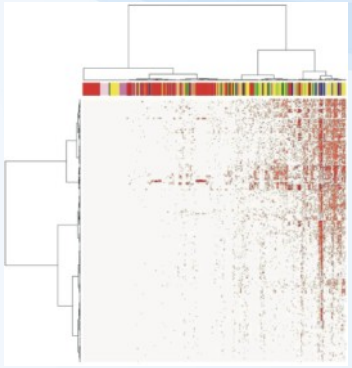
**Adverse Effects**
*Death*
*Inhibition of Growth*
*Altered Reproduction*
*Tumor*
*Skin irritation*
*…*

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

10^4 Chemicals



HTS
High-Throughput-Screening



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

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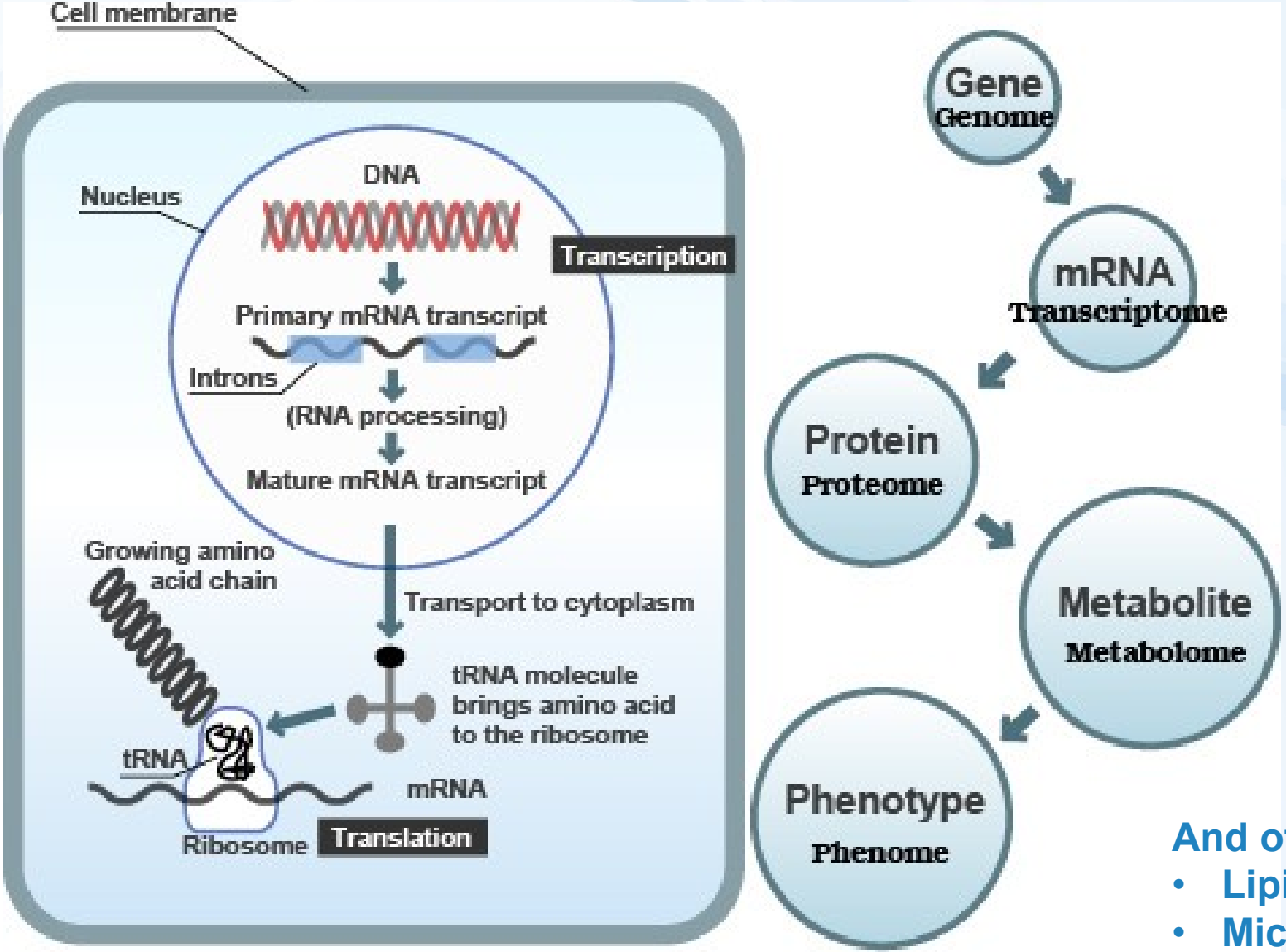
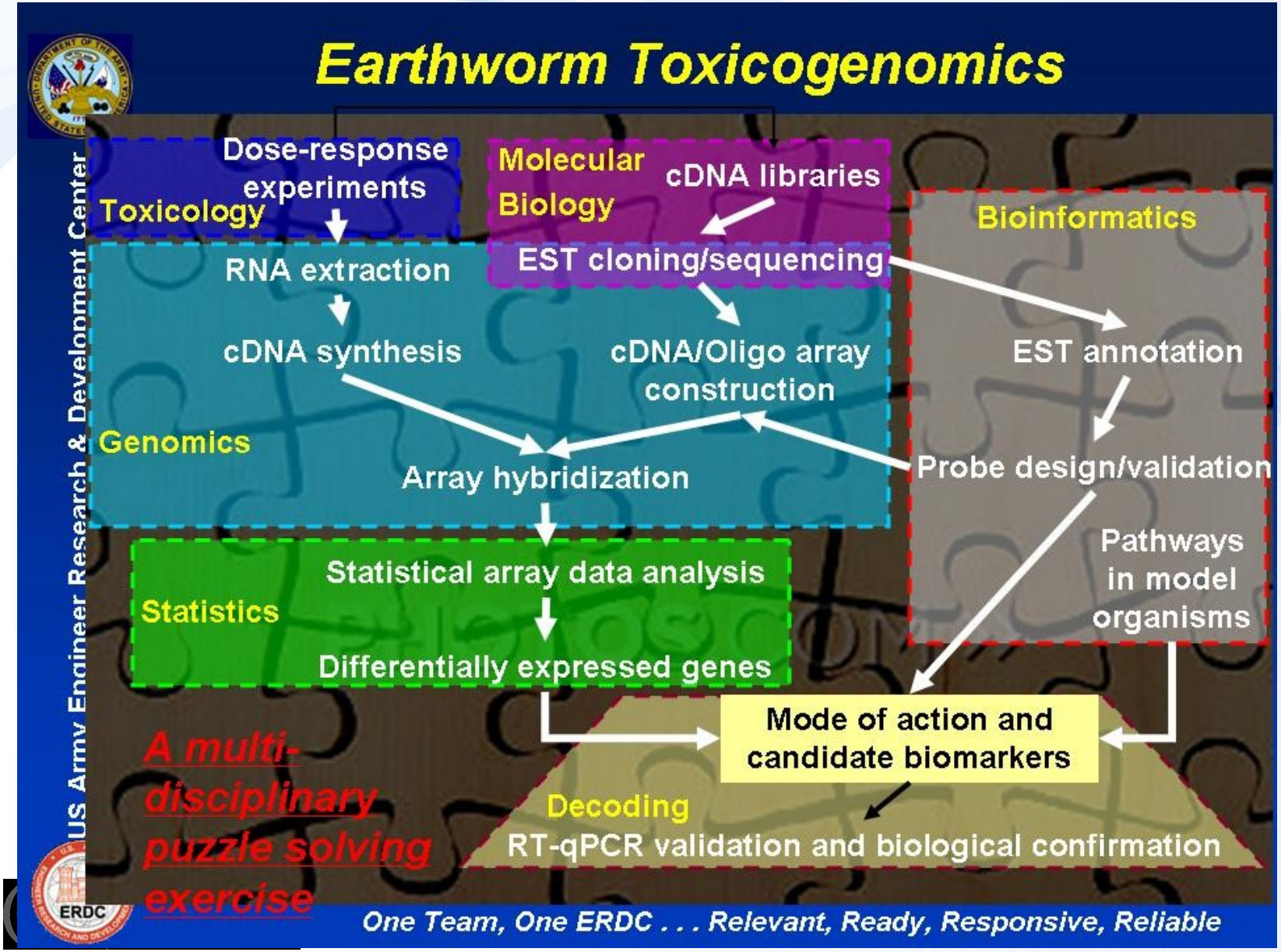
# Mode of Action (omics) toxicity testing




**And other „omes“**

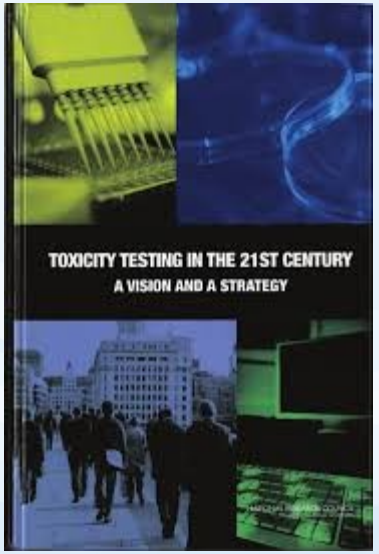
- **Lipidome**
- **Microbiome …**

# Omes is not only for humans …

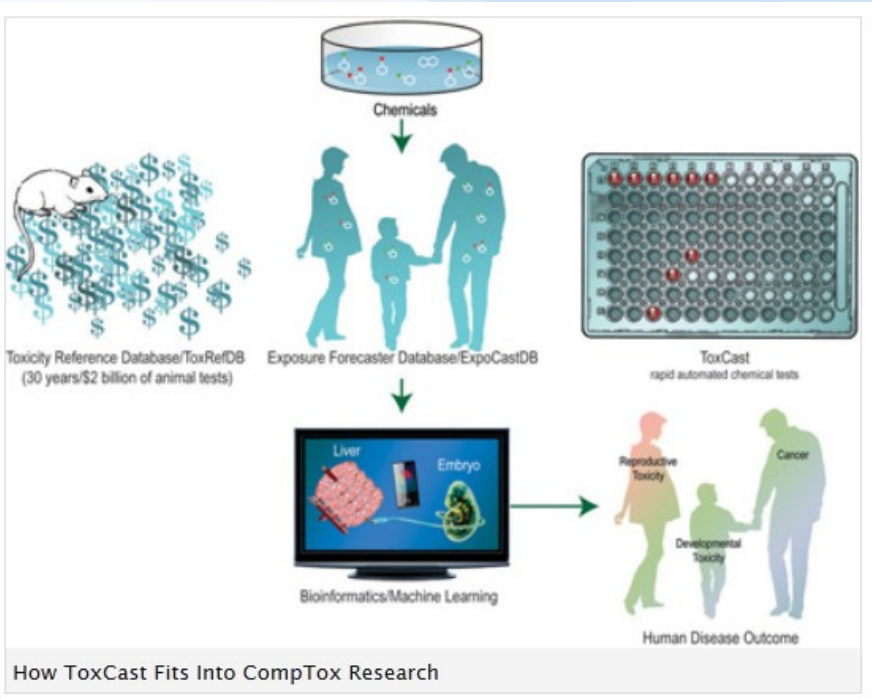


# MoA and omics are supported by strategic documents & organizations

**Toxicity Testing in the 21st Century: A Vision and a Strategy**
US National Academies of Sciences
http://www.nap.edu/catalog/11970.html







How ToxCast Fits Into CompTox Research

# Hazard assessment

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

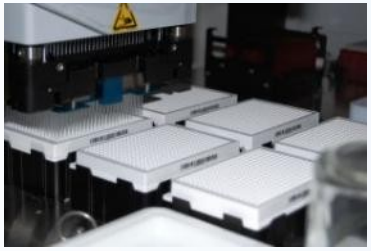
Chemical

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



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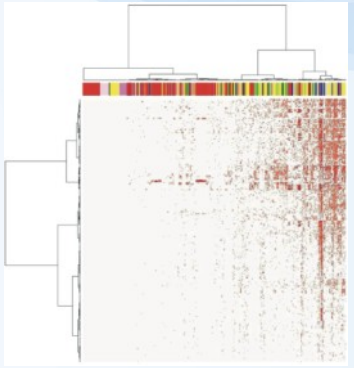
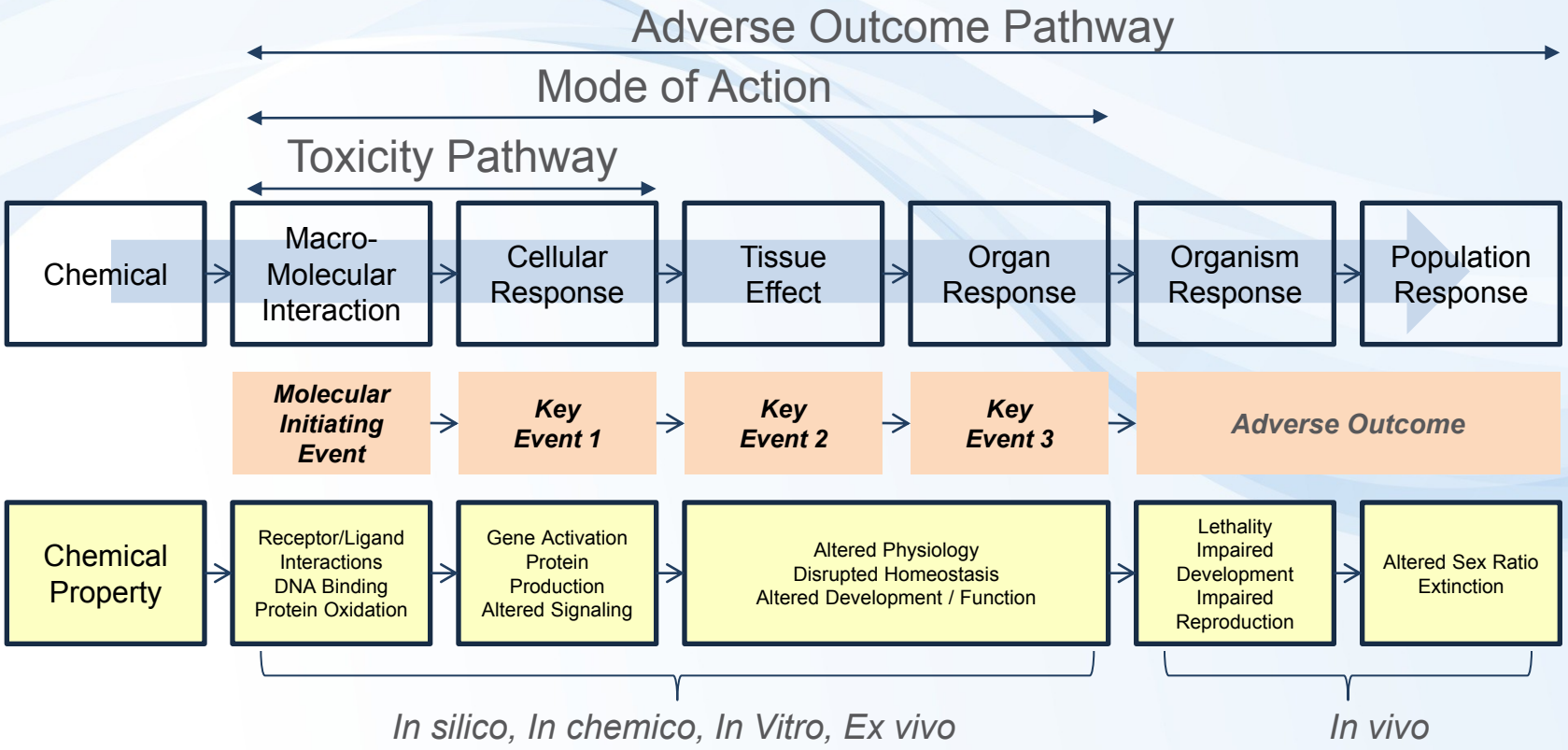


***Chemical-biological interactions, Mechanistic Toxicological Data***

**Key task/question:**
How to link MECHANISTIC INFORMATION with APICAL ENDPOINTS ?

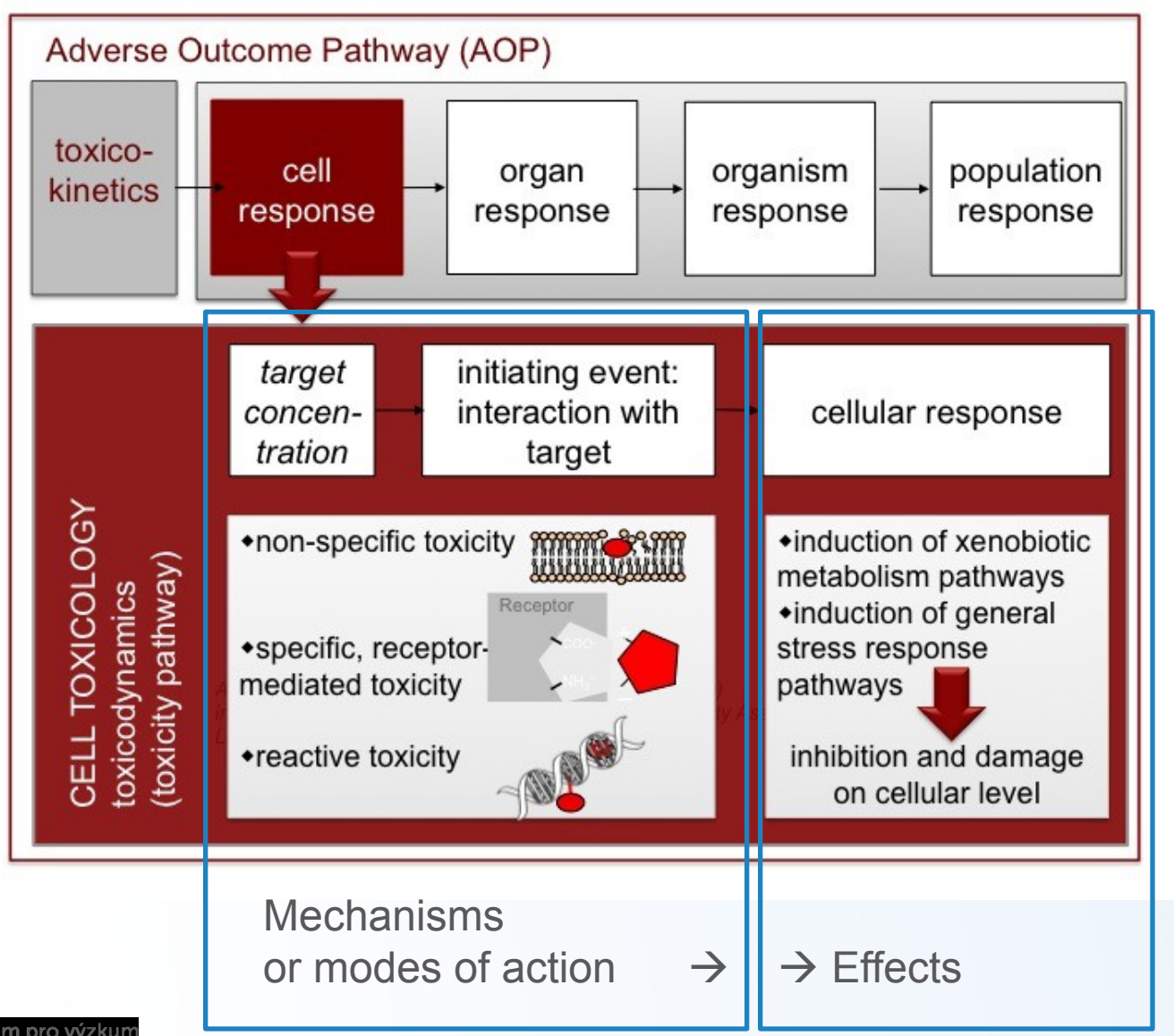
# Adverse Outcome Pathways




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**

Ankley, G. T., R. S. Bennett, et al. (2010). "Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment." Environmental Toxicology and Chemistry **29**(3): 730-741.

# Concept of “Adverse Outcome Pathway” (AOP)

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


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

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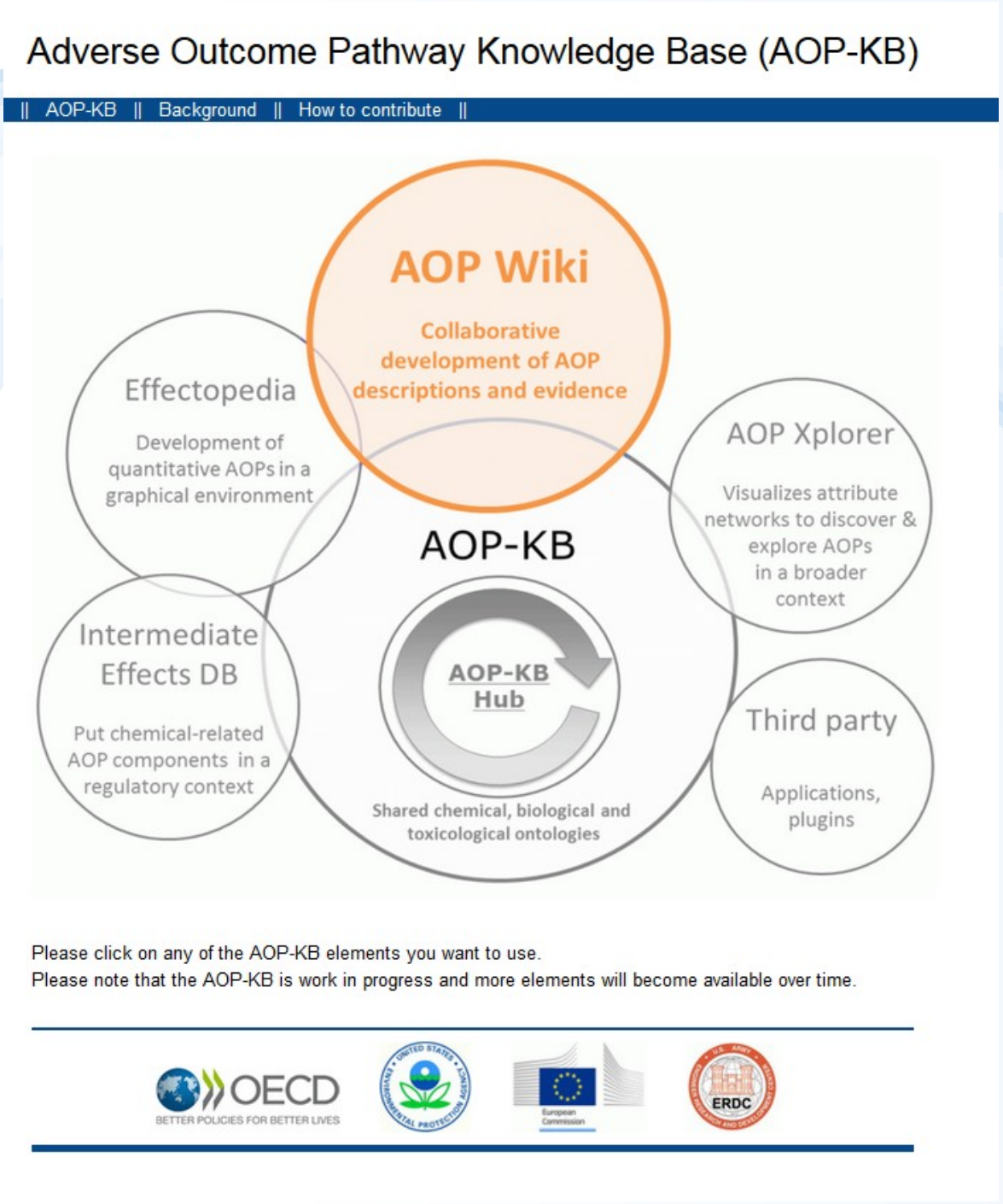


http://aopkb.org/

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
- Wiki-based platform for development of AOPs
- Only members of an OECD AOP development project can create / edit AOPs


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# What AOPs are now in AOP Wiki – Feb 2020 ?

| | | |
|---|---|---|
| OECD Endorsed (WNT and TFHA) | 14 | • Alkylation of DNA in male pre-meiotic germ cells leading to heritable mutations<br>• Aromatase inhibition leading to reproductive dysfunction |
| EAGMST Under Review & for comments | 20 | |
| EAGMST Under Development | 34 | |
| SAAOP AOP Under Development | 130+ | |

- **Total 269 AOPs**
- OECD Extended Advisory Group on Molecular Screening and Toxicogenomics (EAG MST)
- The Working Group of the National Coordinators of the Test Guidelines Programme (WNT)

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https://aopwiki.org/aops

# AOP Example: MIE aromatase inhibition

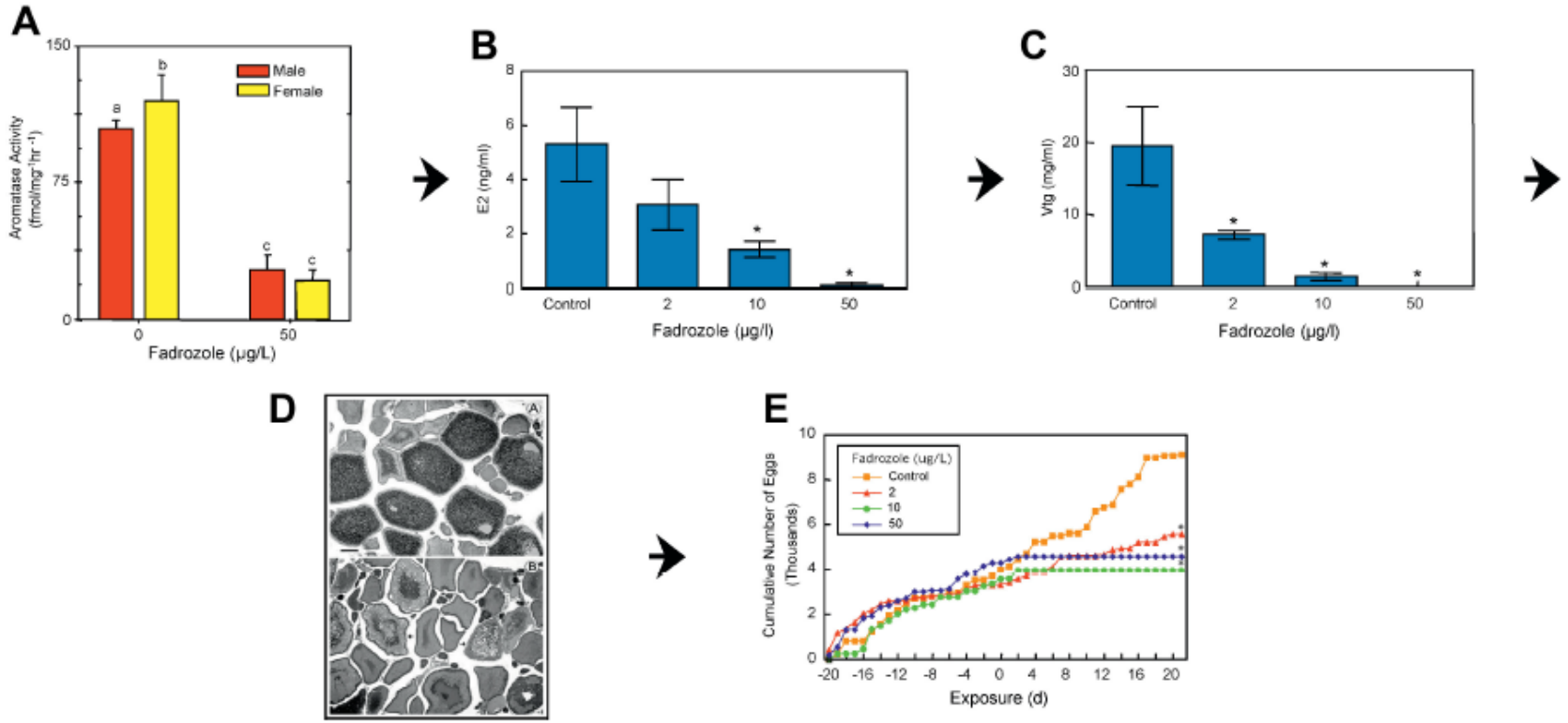
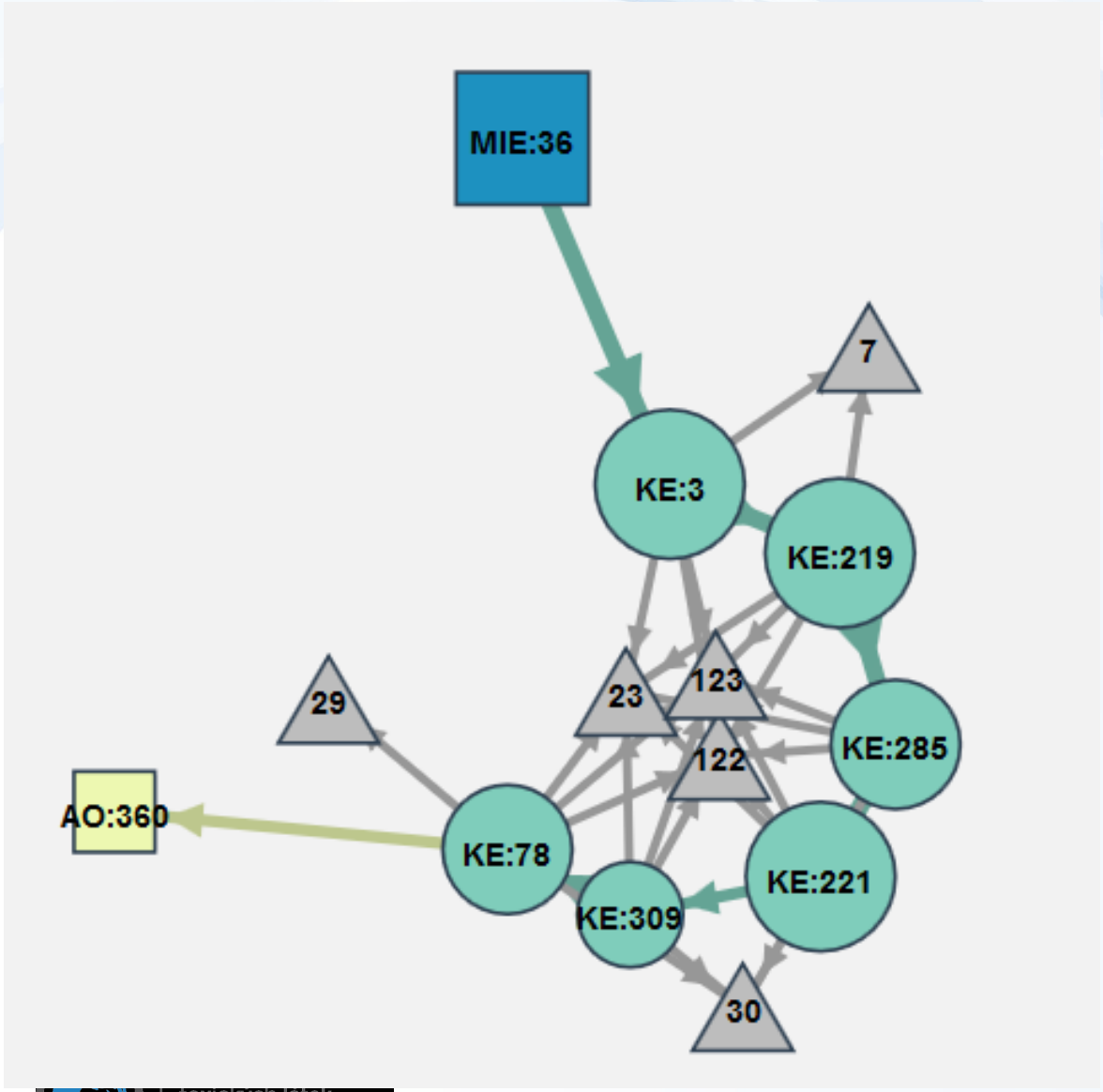


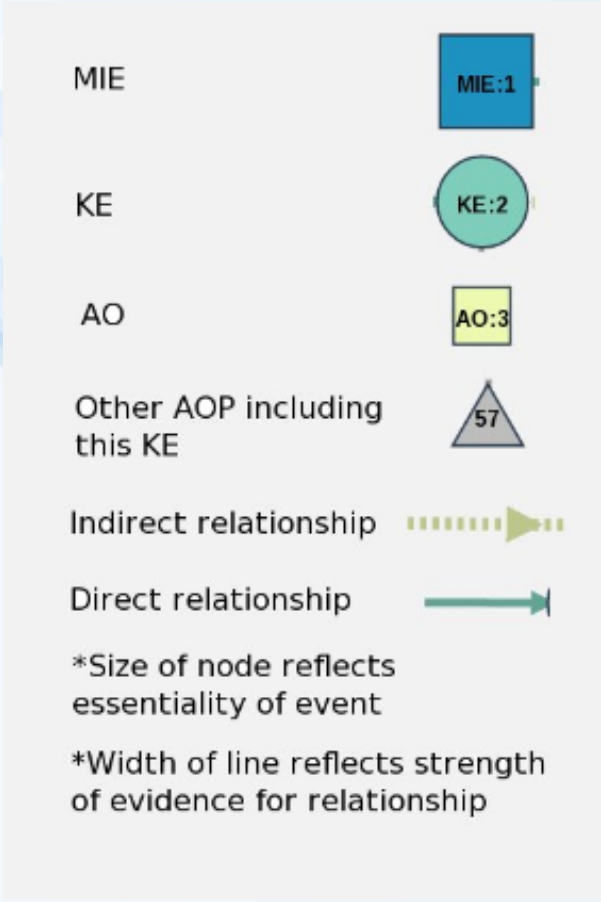

Fig. 3. An adverse outcome pathway in fish [2,50]. Aromatase inhibitor example. (**A**) Aromatase inhibition by fadrozole; (**B**) Reduction in circulating estradiol; (**C**) Reduction in circulating vitellogenin (Vtg); (**D**) Histopathology of ovarian tissue, top panel normal ovary, bottom panel fadrozole 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)

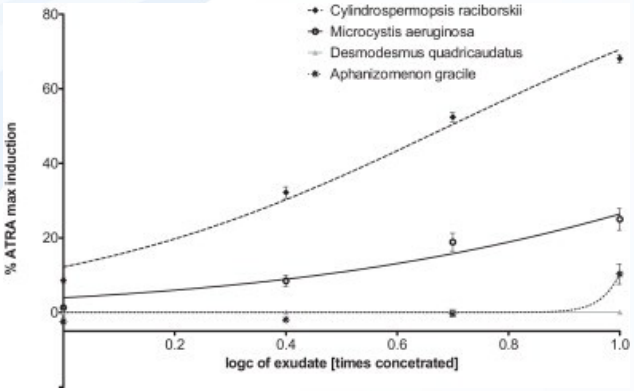
https://aopwiki.org/wiki/index.php/Aop:25



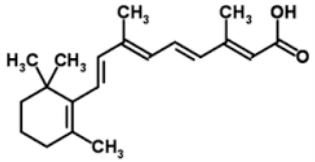

toxických látek v prostředí

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

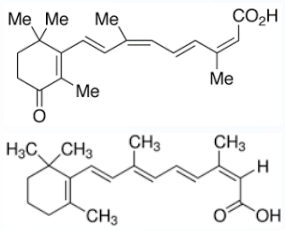



Activation of RAR/RXR

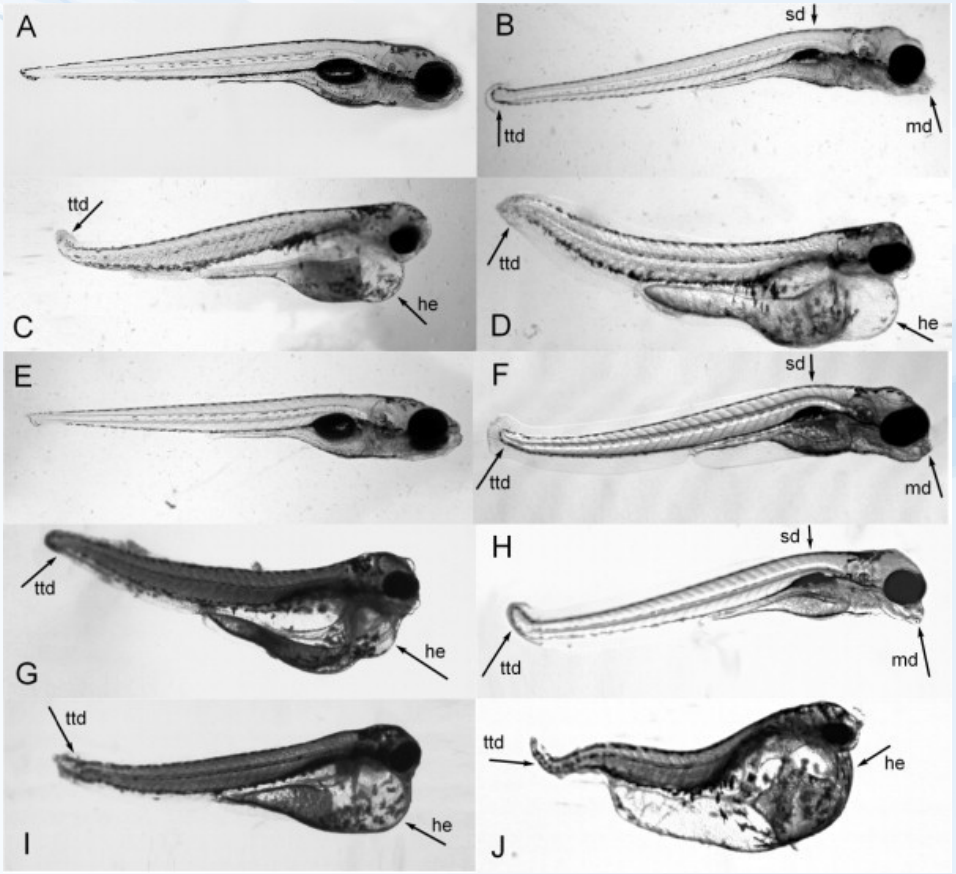
in P19/A15 cells by atRA and cyanobacterial metabolites



atRA



other RAs in cyanos



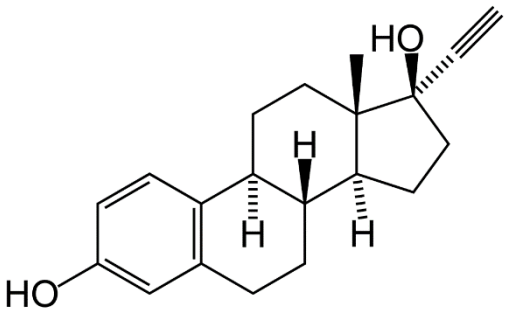

ZF exposed to ATRA and cyanobacterial (120 hpf) - Control (A), exudates of *C. raciborskii* 3.3 (B) and 10 (C), *M. aeruginosa* 10 (D) and *D. quadricaudatus* 17 (E). ATRA 4 µg/L (13.3 nM) (F), 12 µg/L (40 nM) ((G) and (H)), 36 µg/L (I) and 108 µg/L (J).

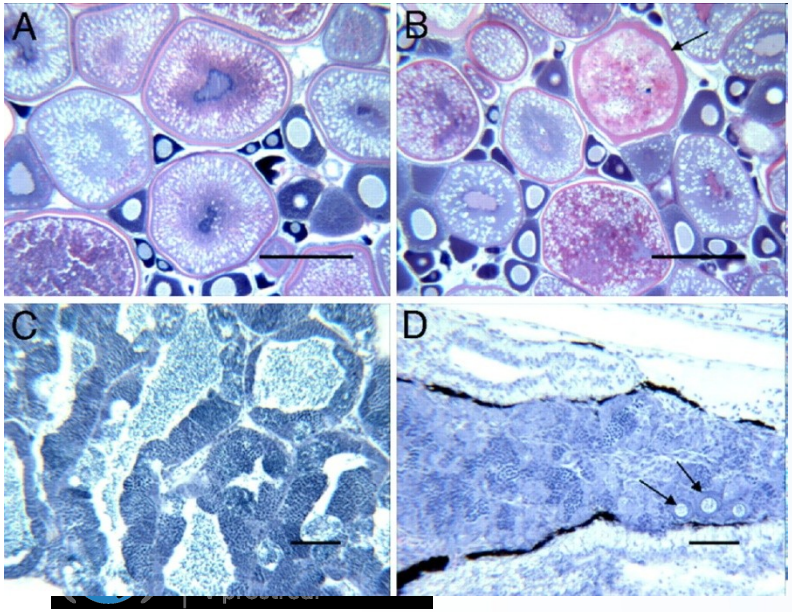
Jonáš et al. 2014 Aquatic Toxicology
http://dx.doi.org/10.1016/j.aquatox.2014.06.022

Centrum pro výzkum toxických látek v prostředí

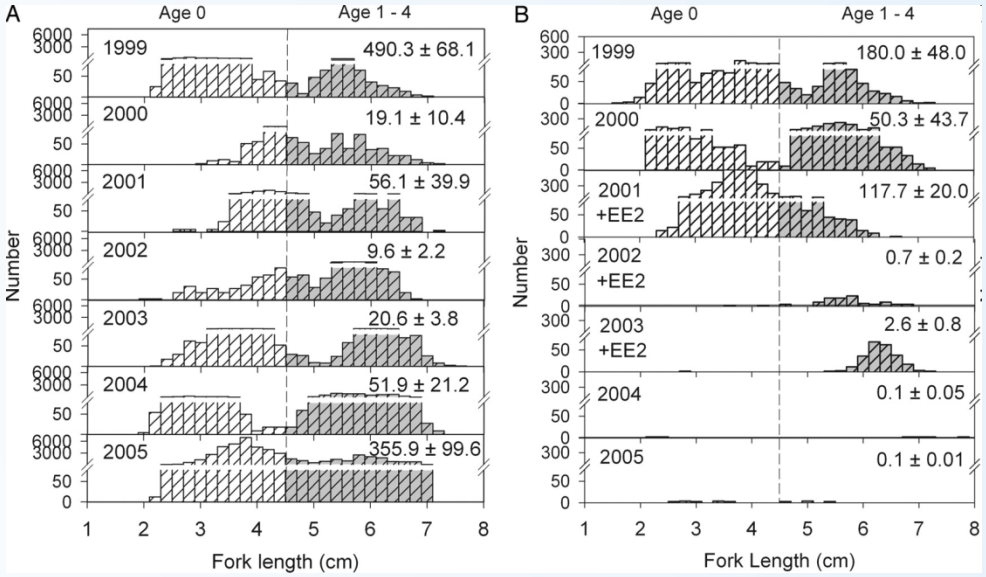
Kidd, K.A. et al. 2007. **Collapse of a fish population** following exposure to **a synthetic estrogen**. *Proceedings of the National Academy of Sciences* 104(21):8897-8901

**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?