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

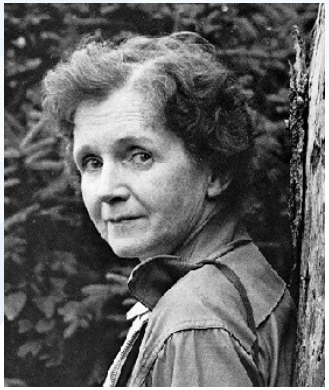
- Membrane toxicity, enzyme inhibitions, oxidative stress, genotoxicity, Nuclear Receptors (AhR, ER, AR ….) etc.
- Methods to determine toxicity mechanism

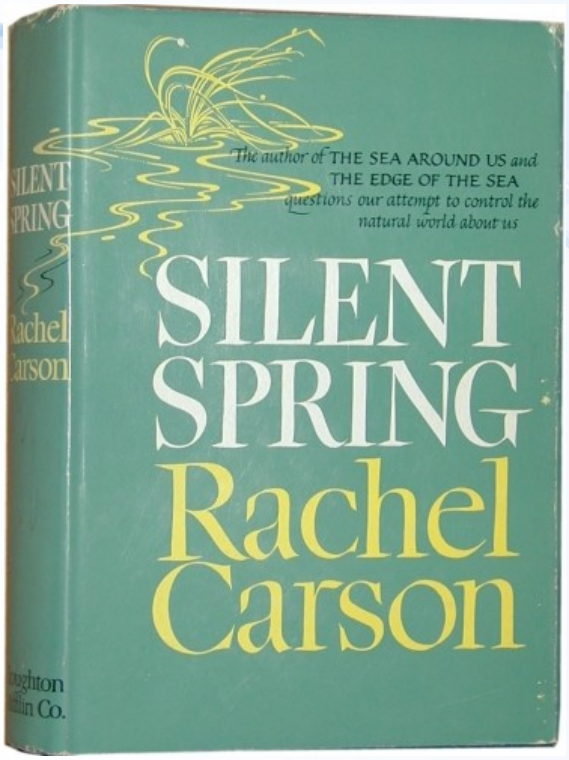
3) Biomarkers

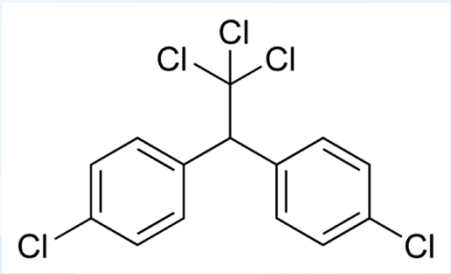
- What it is and how to find (identify) suitable biomarker(s)?
- The overview of the most important biomarker classes
- Methods of biomarker assessment

The importance of understanding to toxicity mechanisms

1962







© Patuxent Wildlife Refuge, MA, USA

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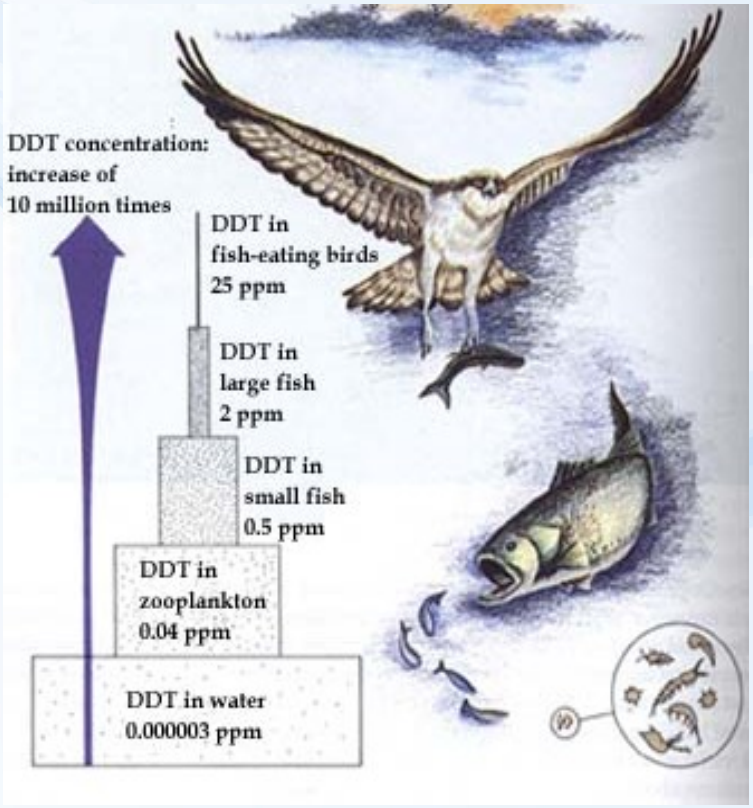
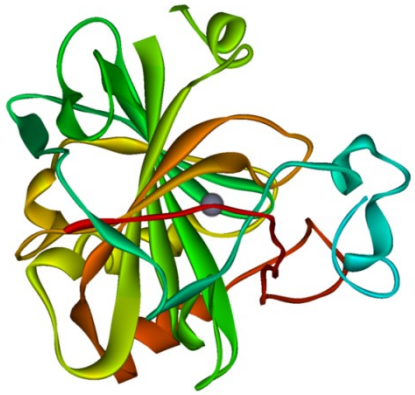
http://www2.ucsc.edu/scpbrg/

In vivo: shell thinning

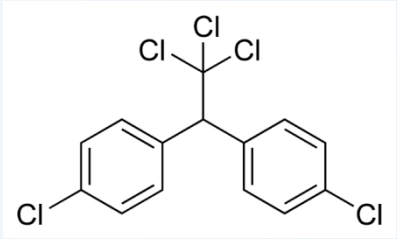
In situ: bioaccumulation
-> bird population decline





Biochemistry discovered in 1970s:
Bird carbonate dehydratase

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



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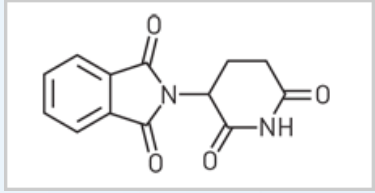
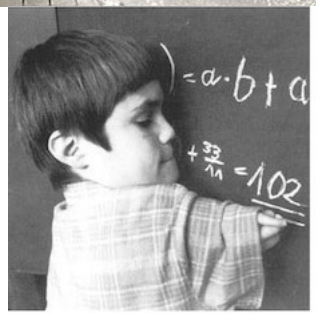
Thalidomide



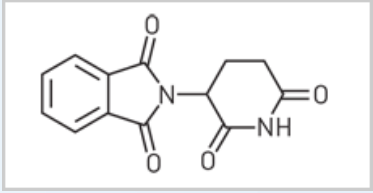
- Originally marketed in 1957 as sedative / hypnotic
  - also curing anxiety, gastritis, tension
  - against nausea and morning sickness of pregnant
    - TERATOGENICITY → Develoment of phocomelia = limb malformations (10 000 children worldwide / 40% survived)

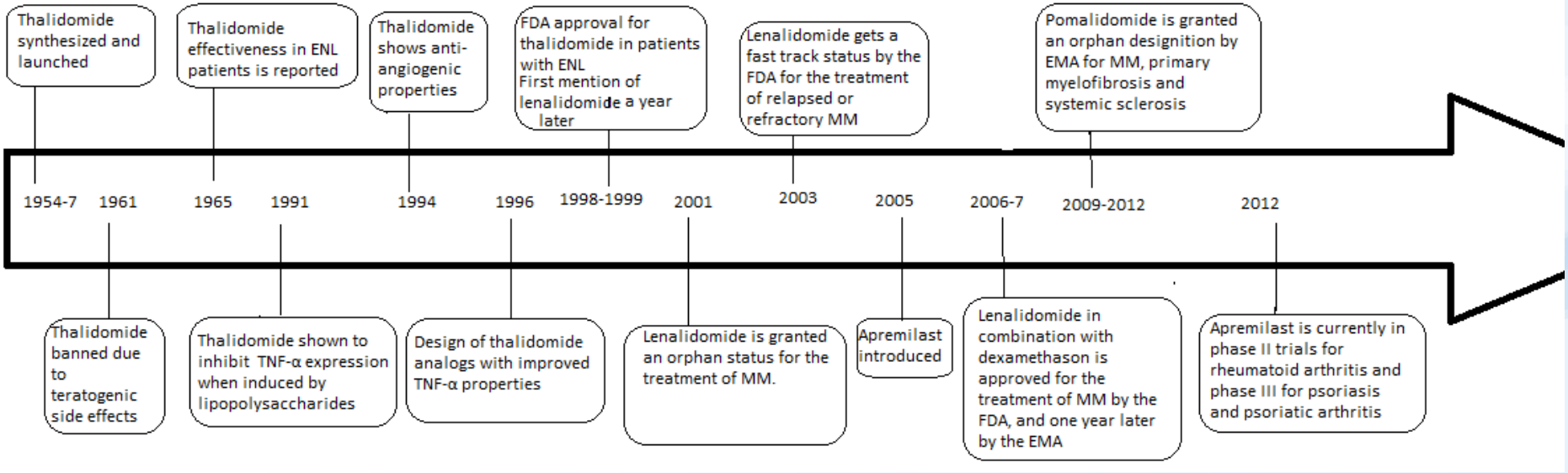


	Teratogenic Manifestations of Thalidomide Number of Days Past Last Menstruation
	32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52
Ear missing (anotia)	
Thumbs missing or deformed (aplasia)	
One or both arms missing (amelia)	
Both arms shortened (phocomelia)	
Hip dislocation	
Ears deformed	
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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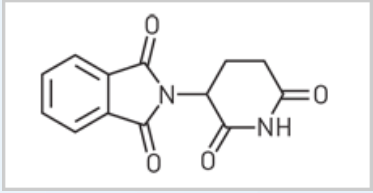
Thalidomide

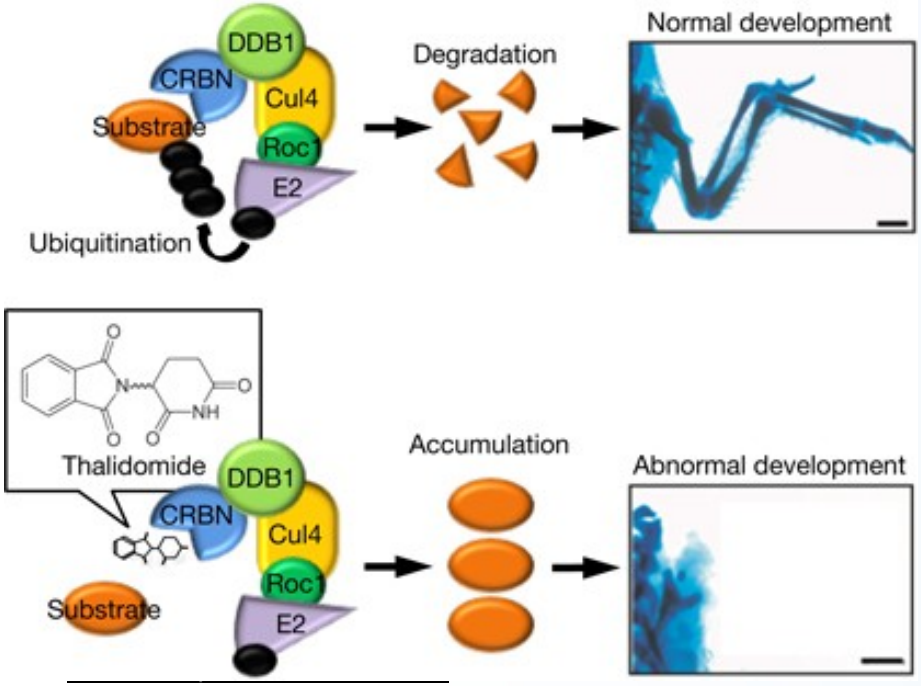
... mechanisms of action



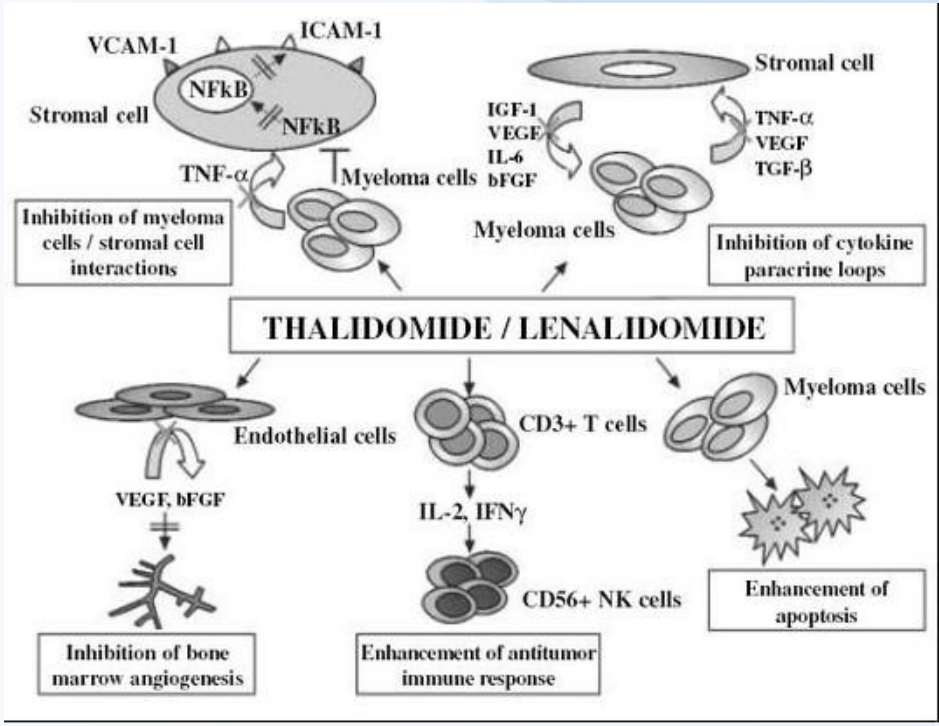
(1) Sedative effects

... mechanism unknown

(2) Teratogenicity

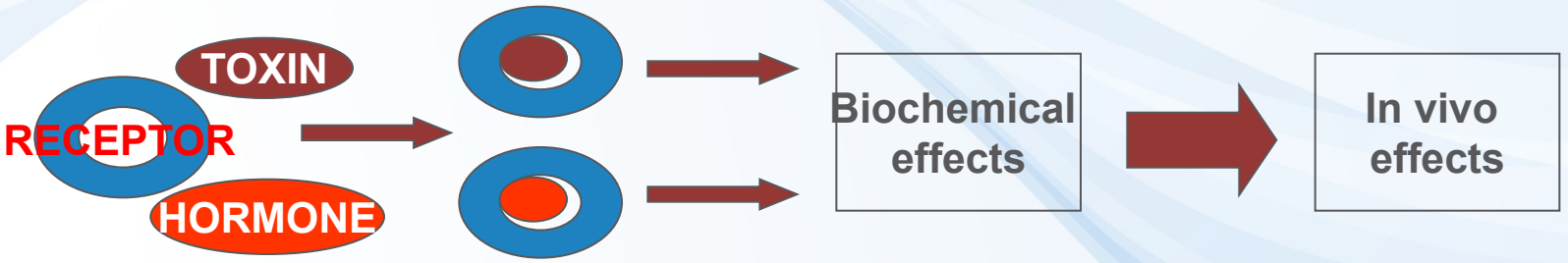


(3) Anticancer



MECHANISMS of chronic toxicity

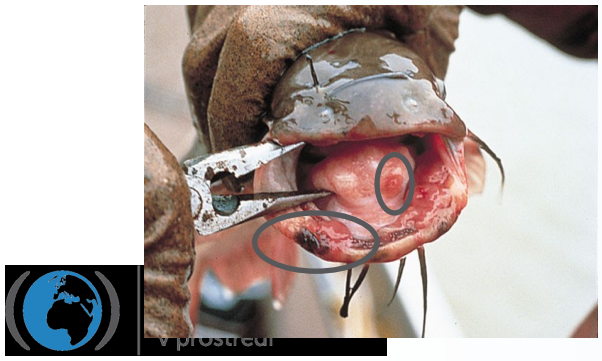
- **Various chronic effects have uniform biochemical basis**



  - 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

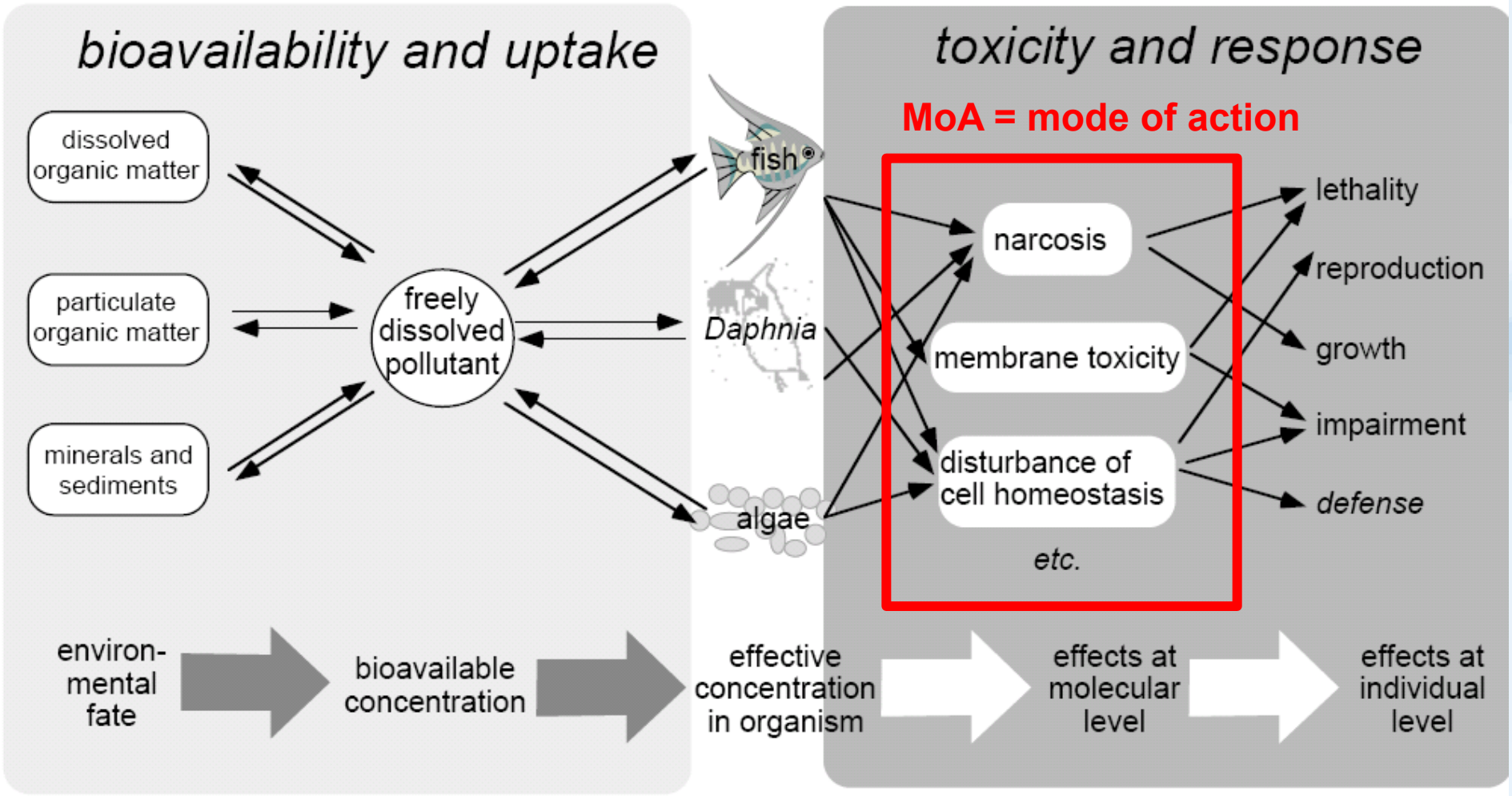

Figure 1 *The effective concentration of a pollutant in an organism (e.g. fish, daphnia, algae) or at the target site inside the organism is the link between the environmental fate of a pollutant and its toxic effect.*

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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

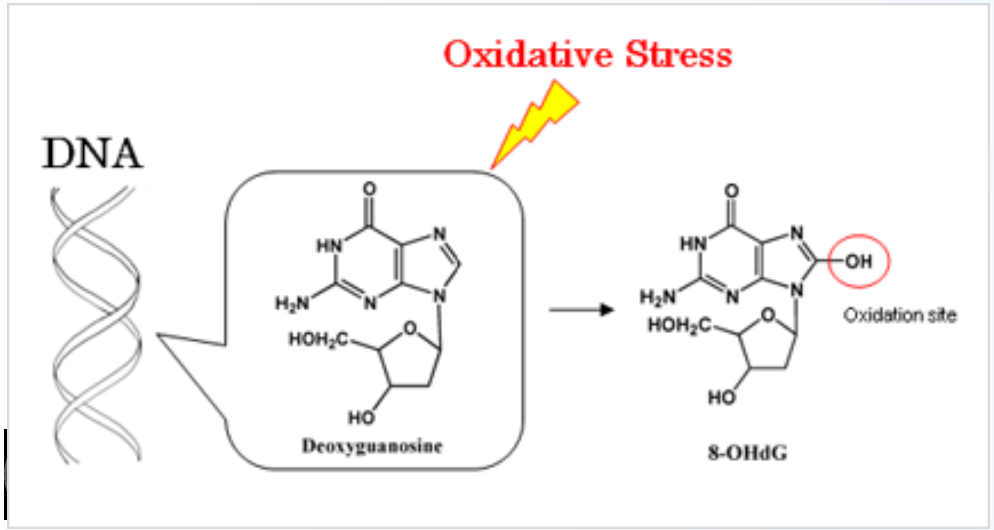
- Chemical enters organism
  + may be metabolized/detoxified, transported, released ...

→ **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

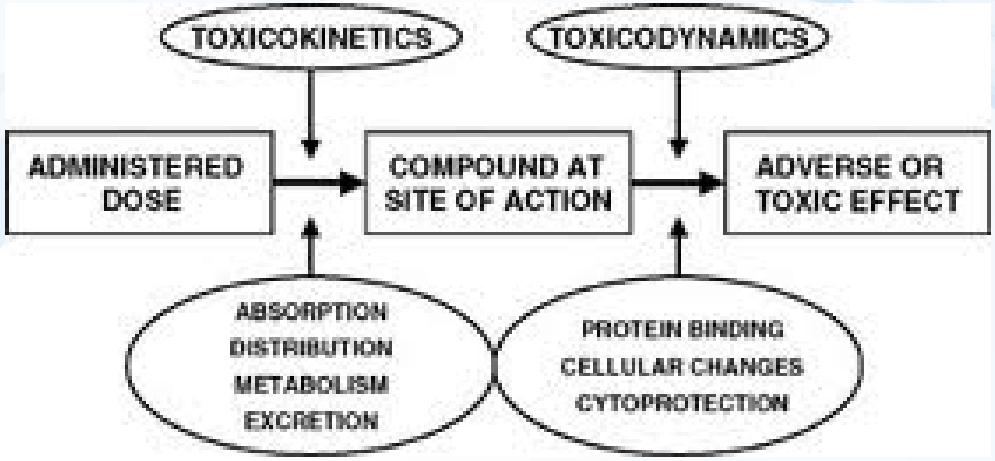
Paracelsus (1493 - 1541)

'What is there which is not a poison?

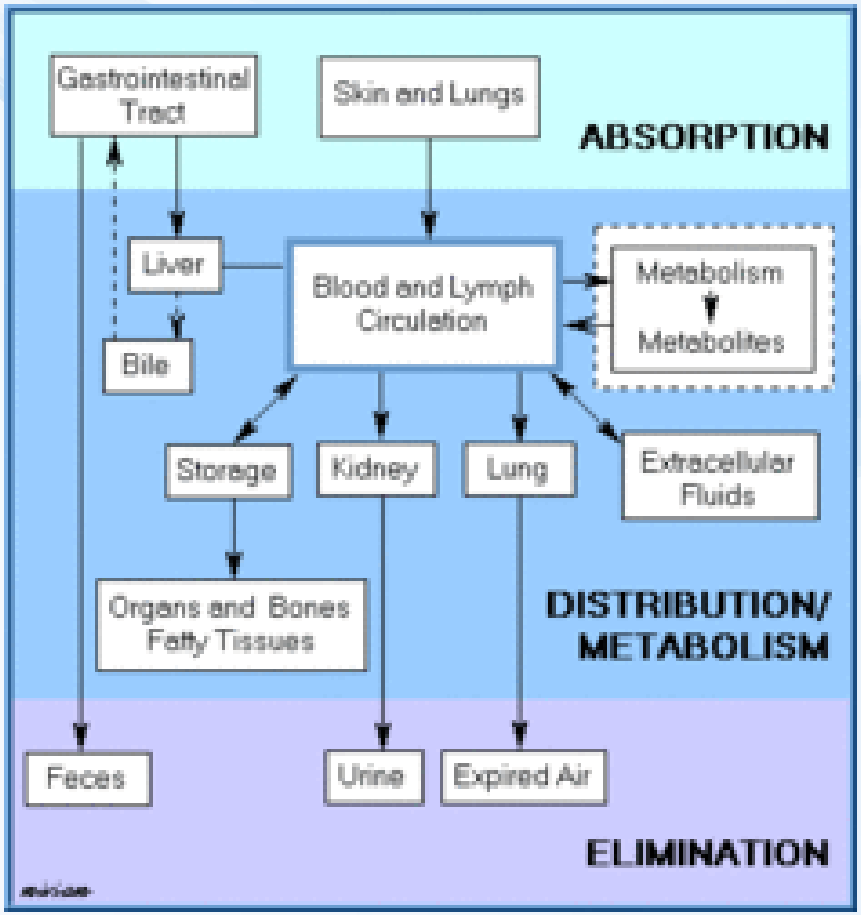
- All things are poison and nothing without poison.
- Solely **the dose determines** that a thing is not a poison.

- *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

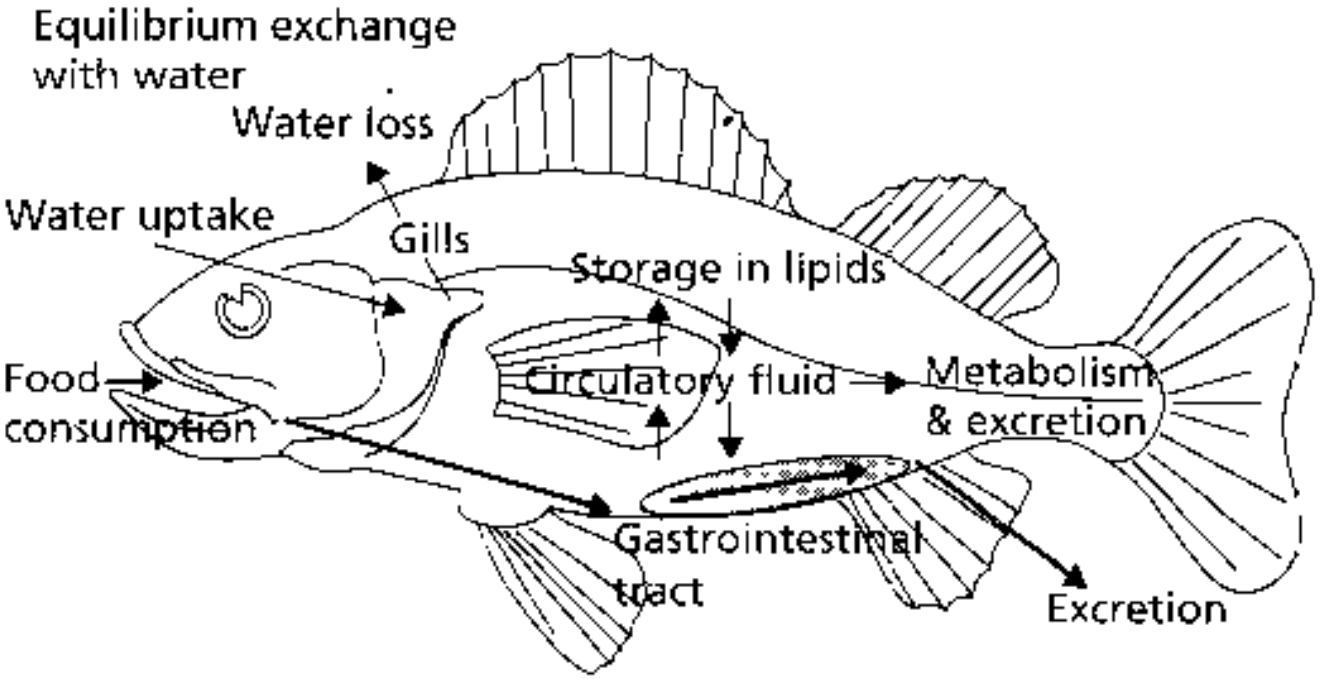
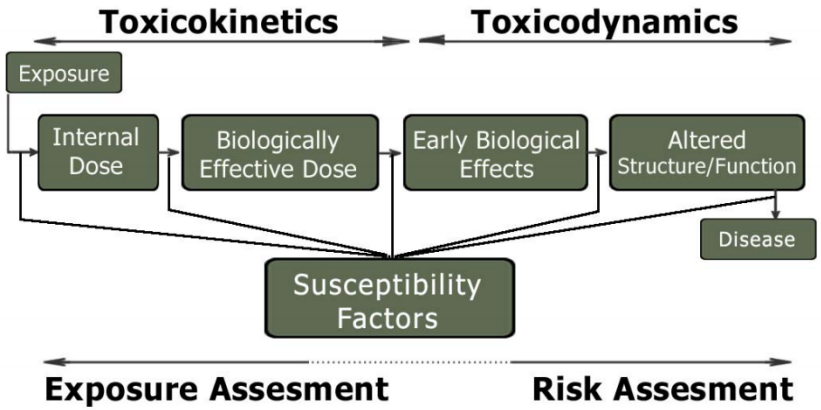


Fig. 3.5 Uptake, accumulation and loss processes for a toxicant in the ambient water with fish.

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

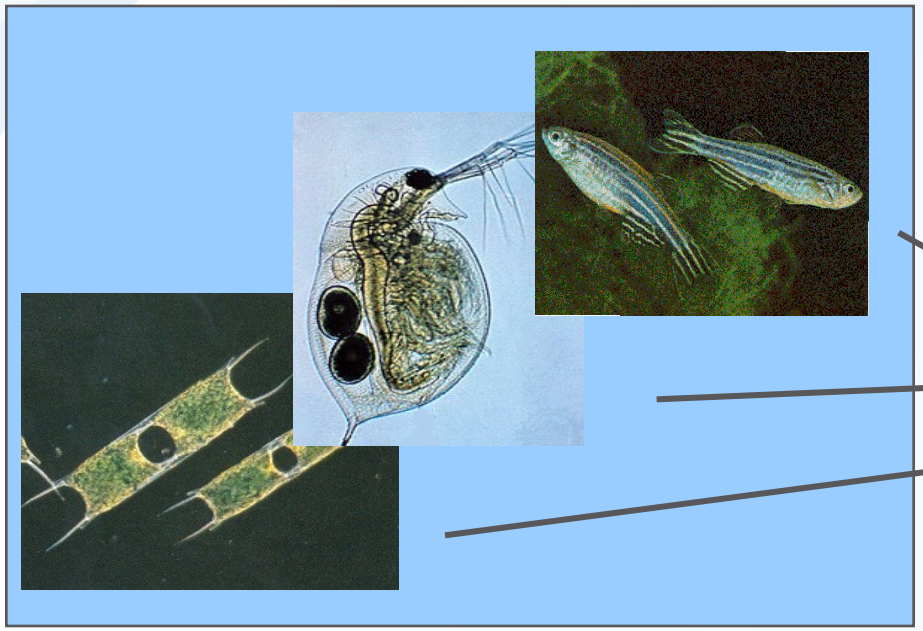
2) Define and know **chemical** and its properties

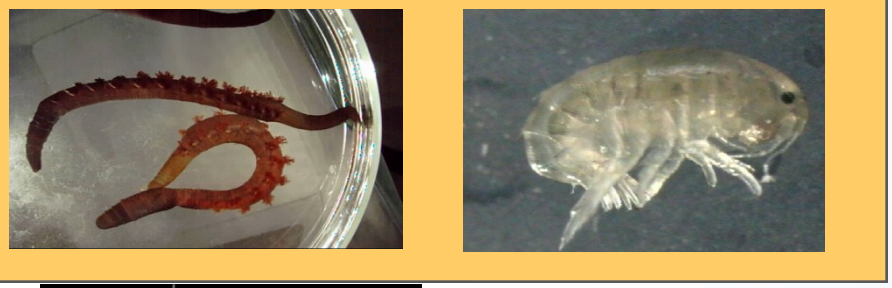
3) **Define exposure** of biological system to a chemical
   - variable concentrations
   - defined or variable duration (time)
   - conditions (T, pH, life stage ....)

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 ...

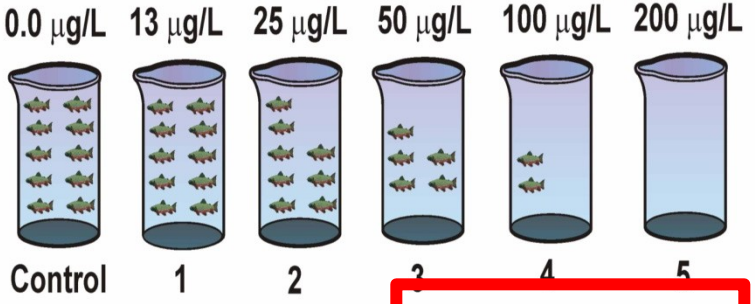
Effect assessment - procedure





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

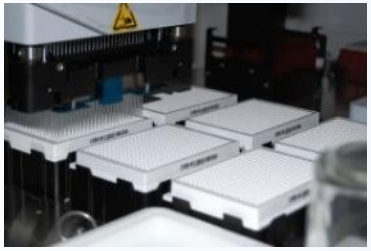
Chemical

Organism

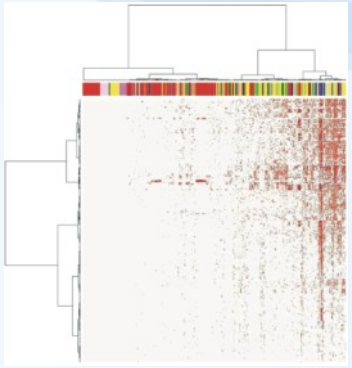
Adverse Effects
Death
Inhibition of Growth
Altered Reproduction
Tumor
Skin irritation
…

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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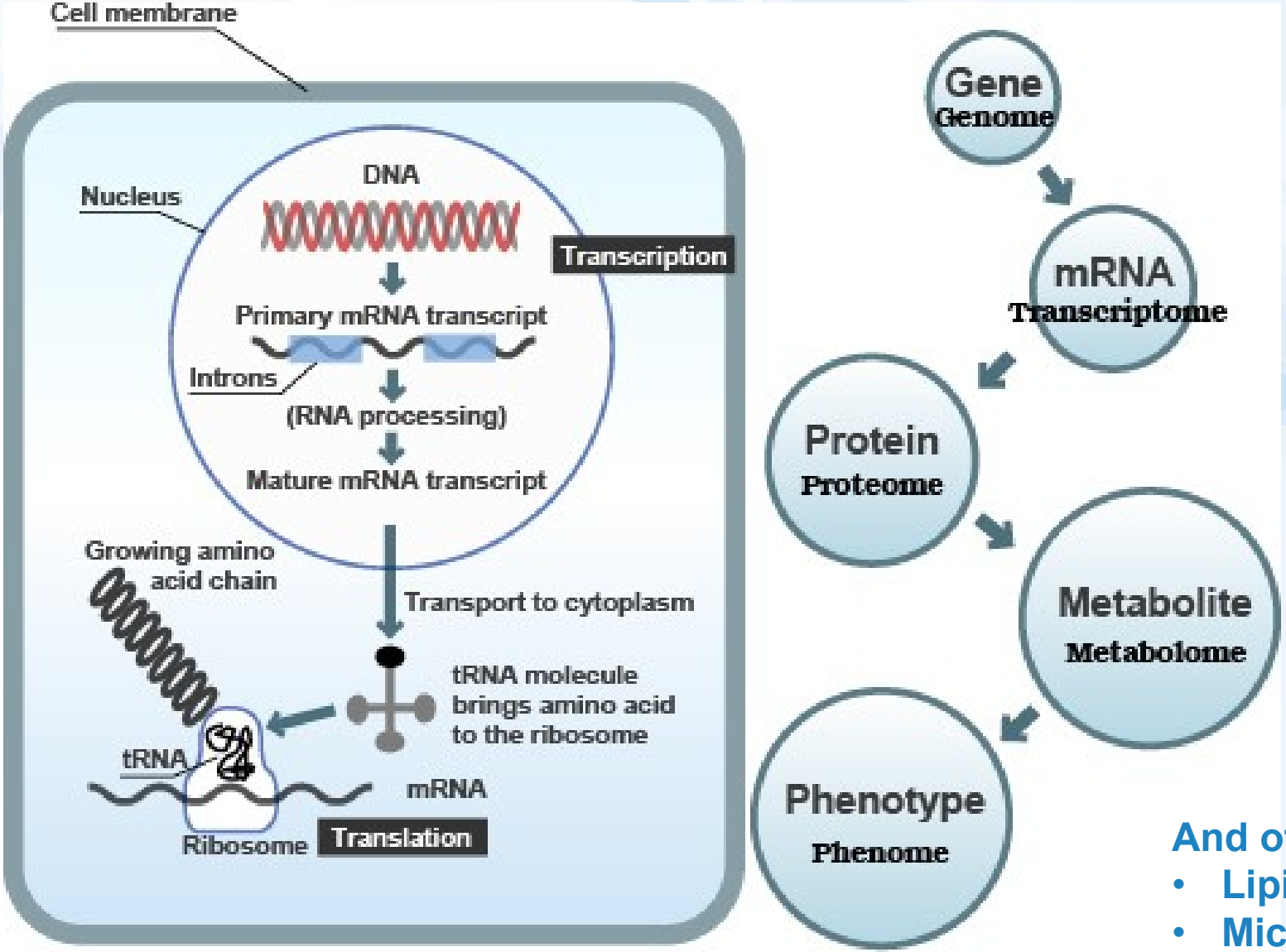
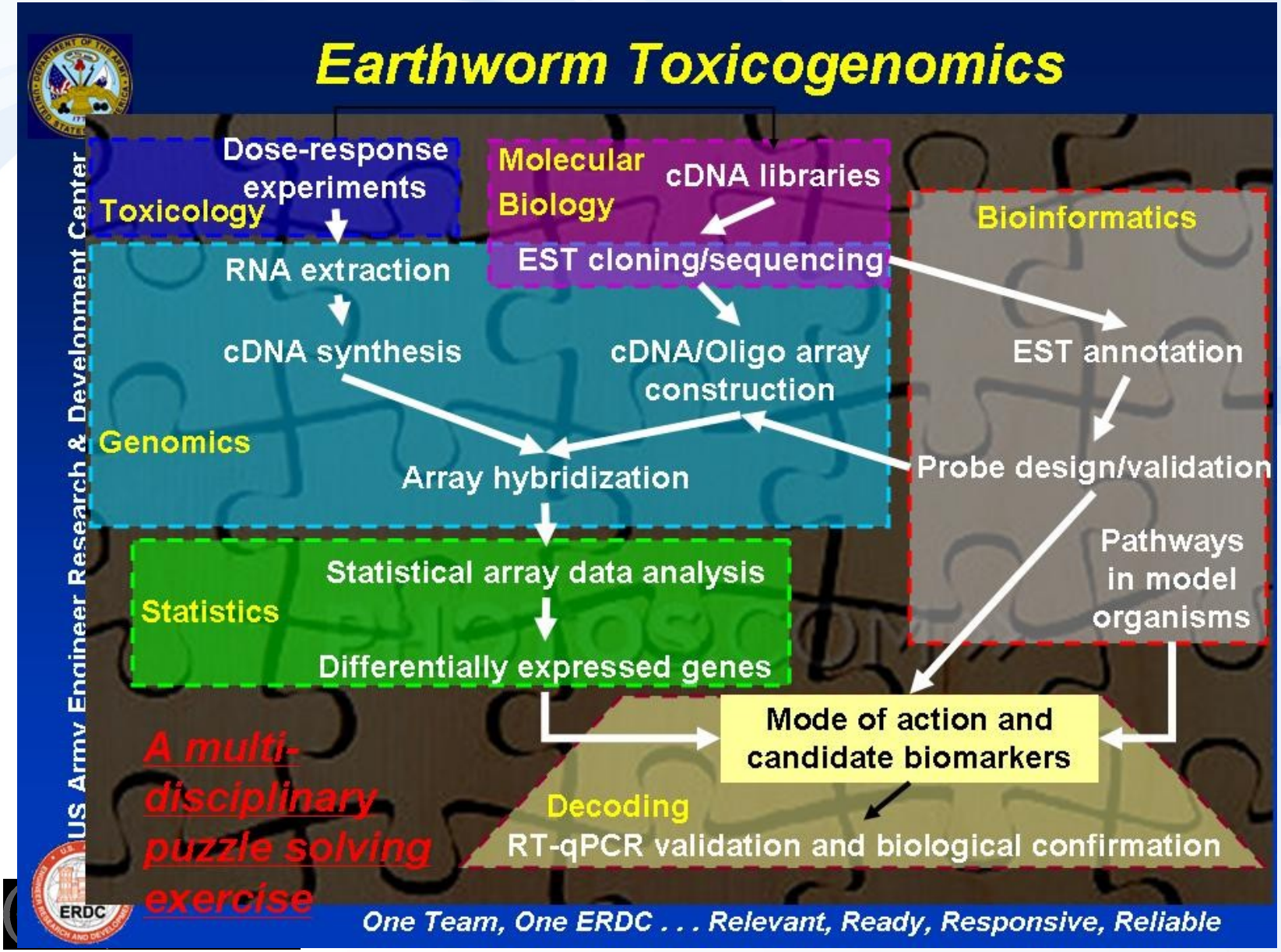
Mode of Action (omics) toxicity testing



And other „omes“

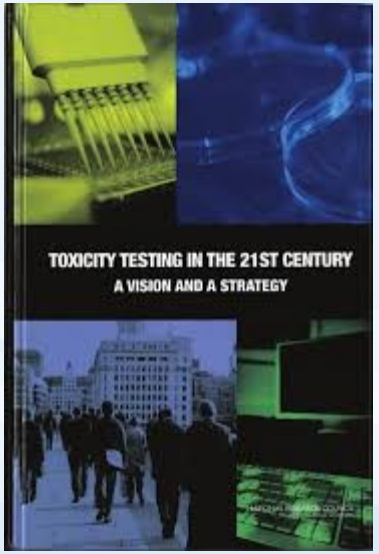
- **Lipidome**
- **Microbiome …**

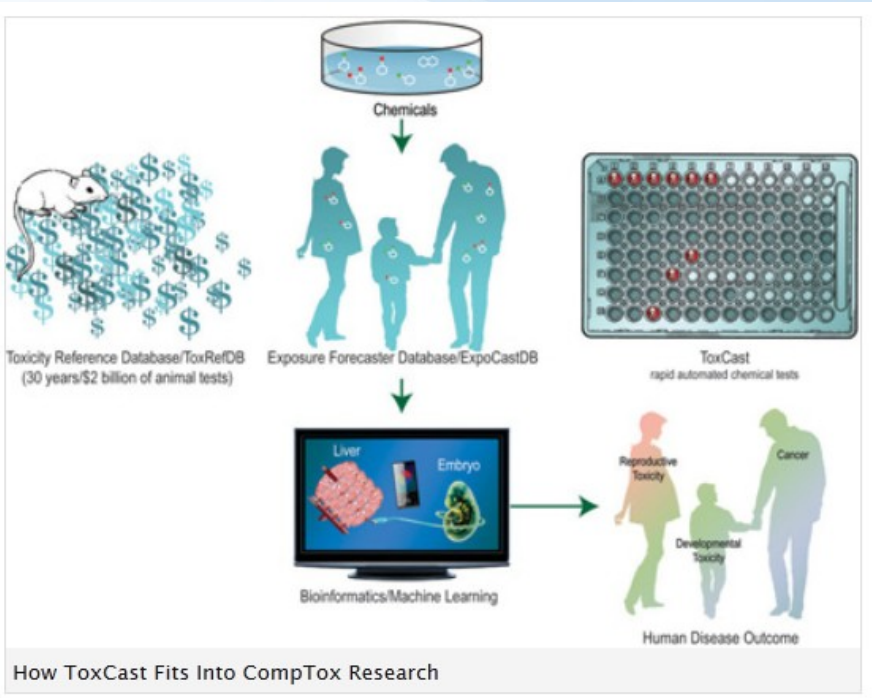
Omes is not only for humans …



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

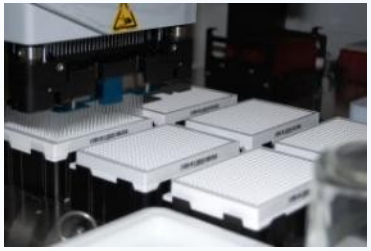
Chemical

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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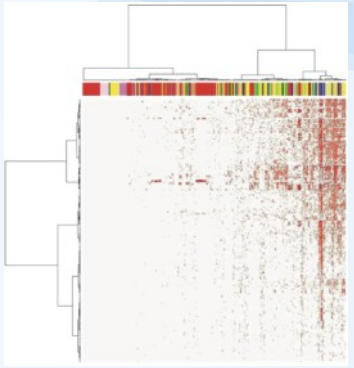
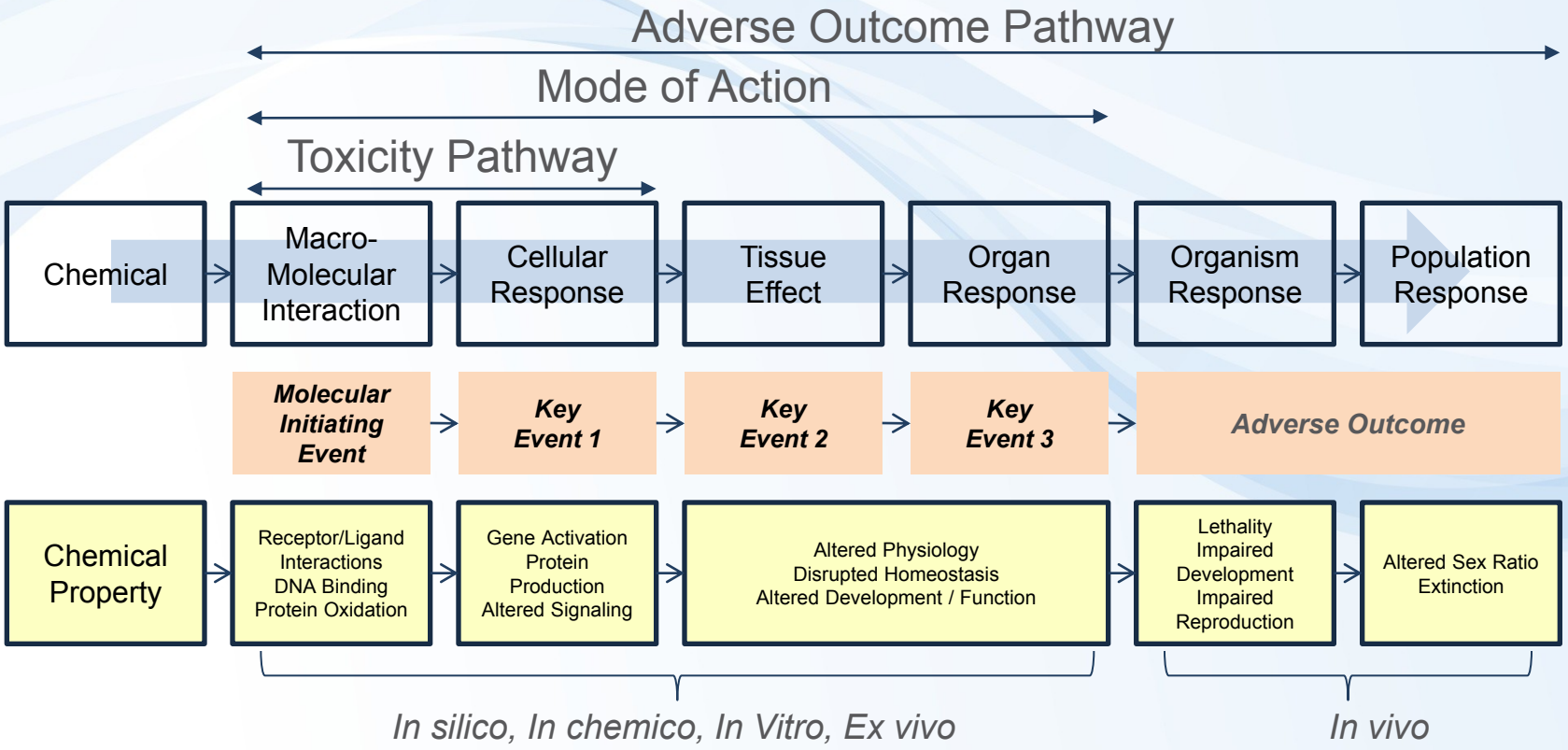


Chemical-biological interactions, Mechanistic Toxicological Data

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

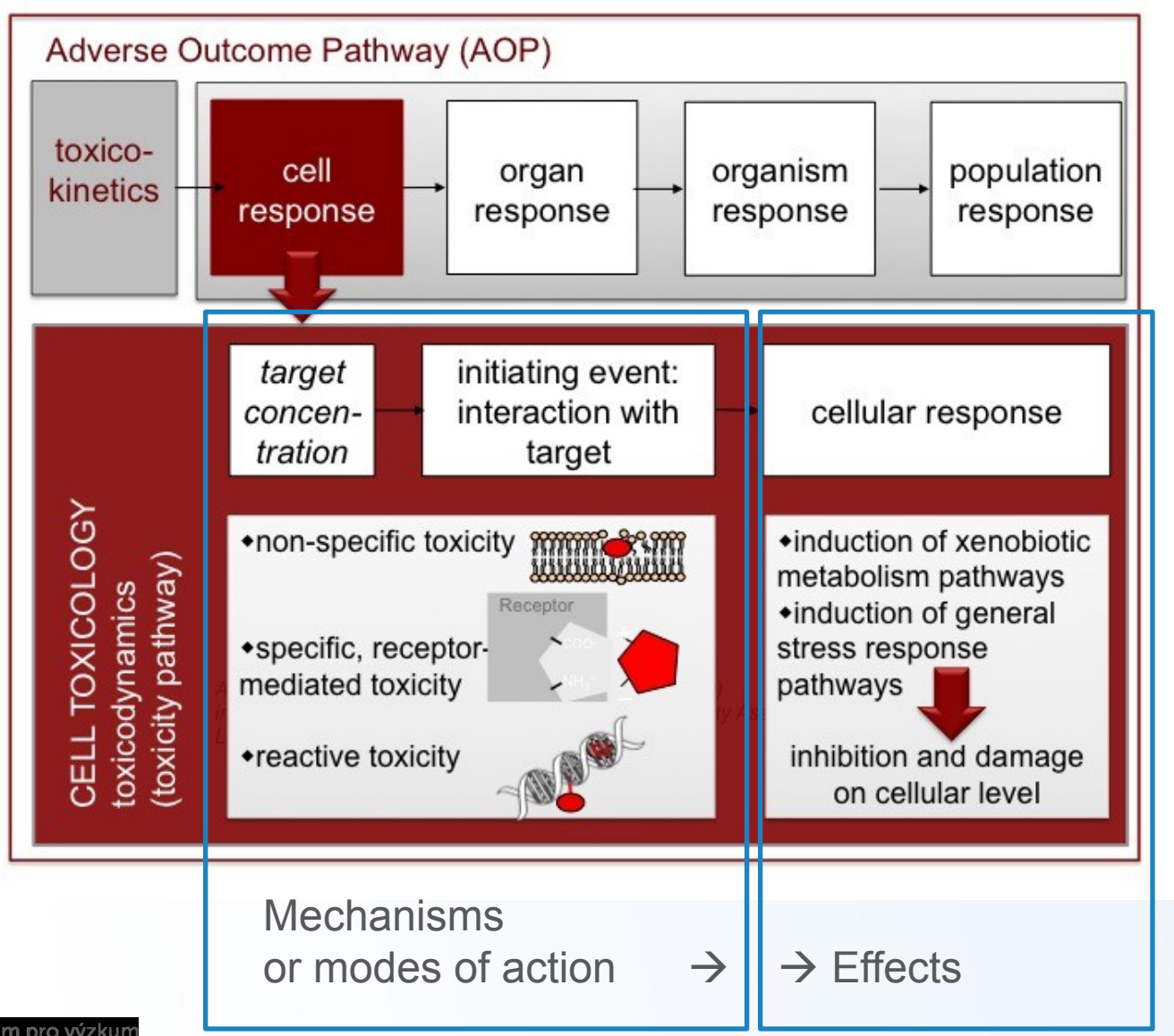
Adverse Outcome Pathways



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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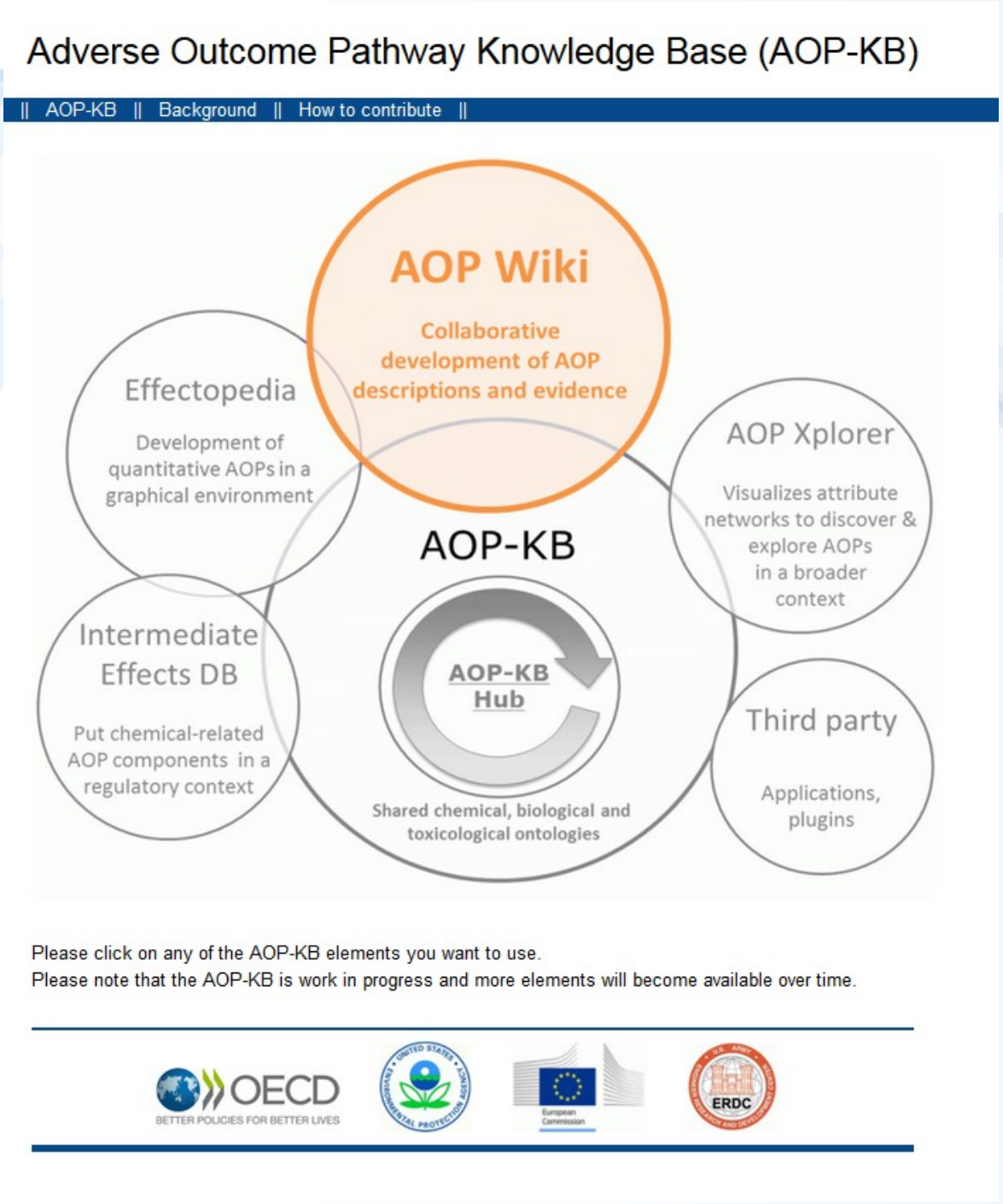

http://aopkb.org/

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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v prostředí

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 • 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

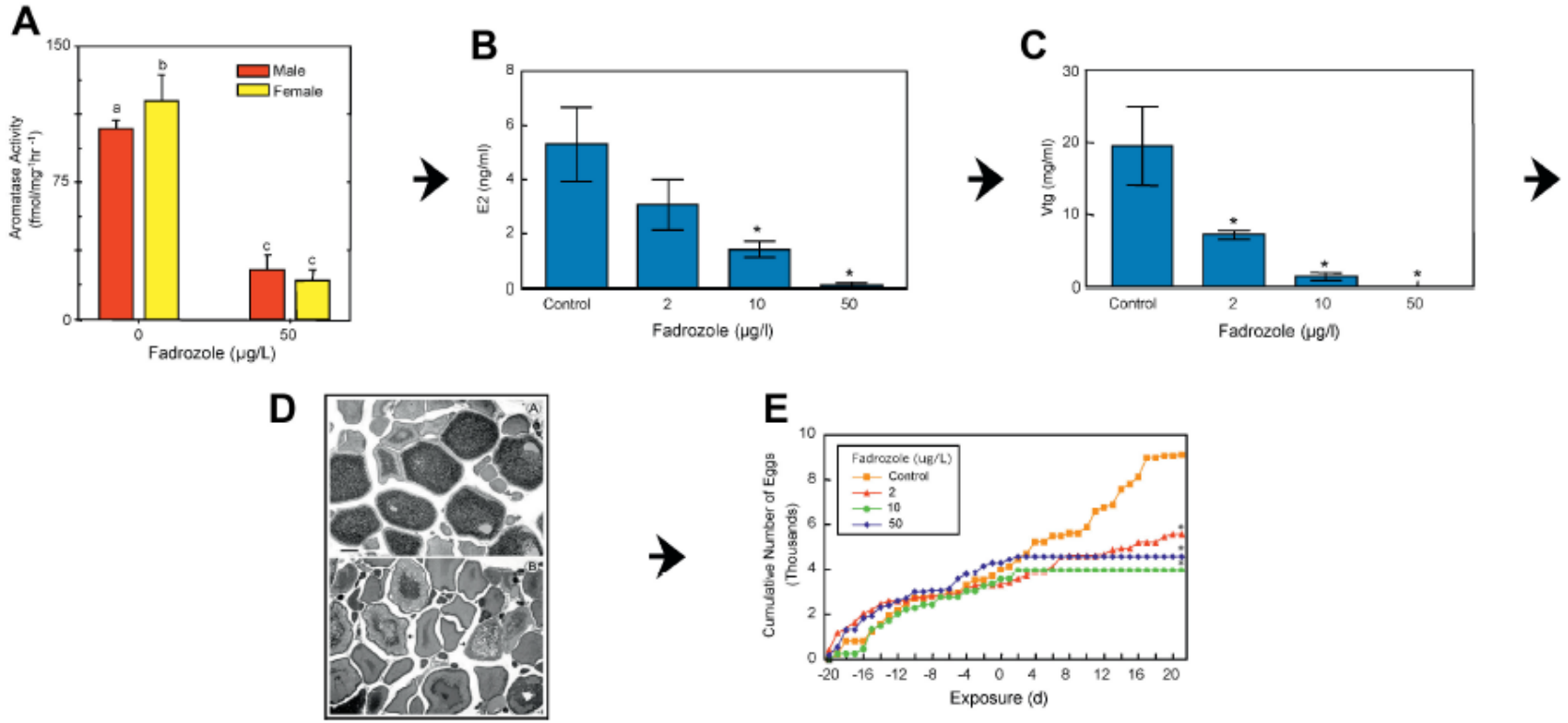
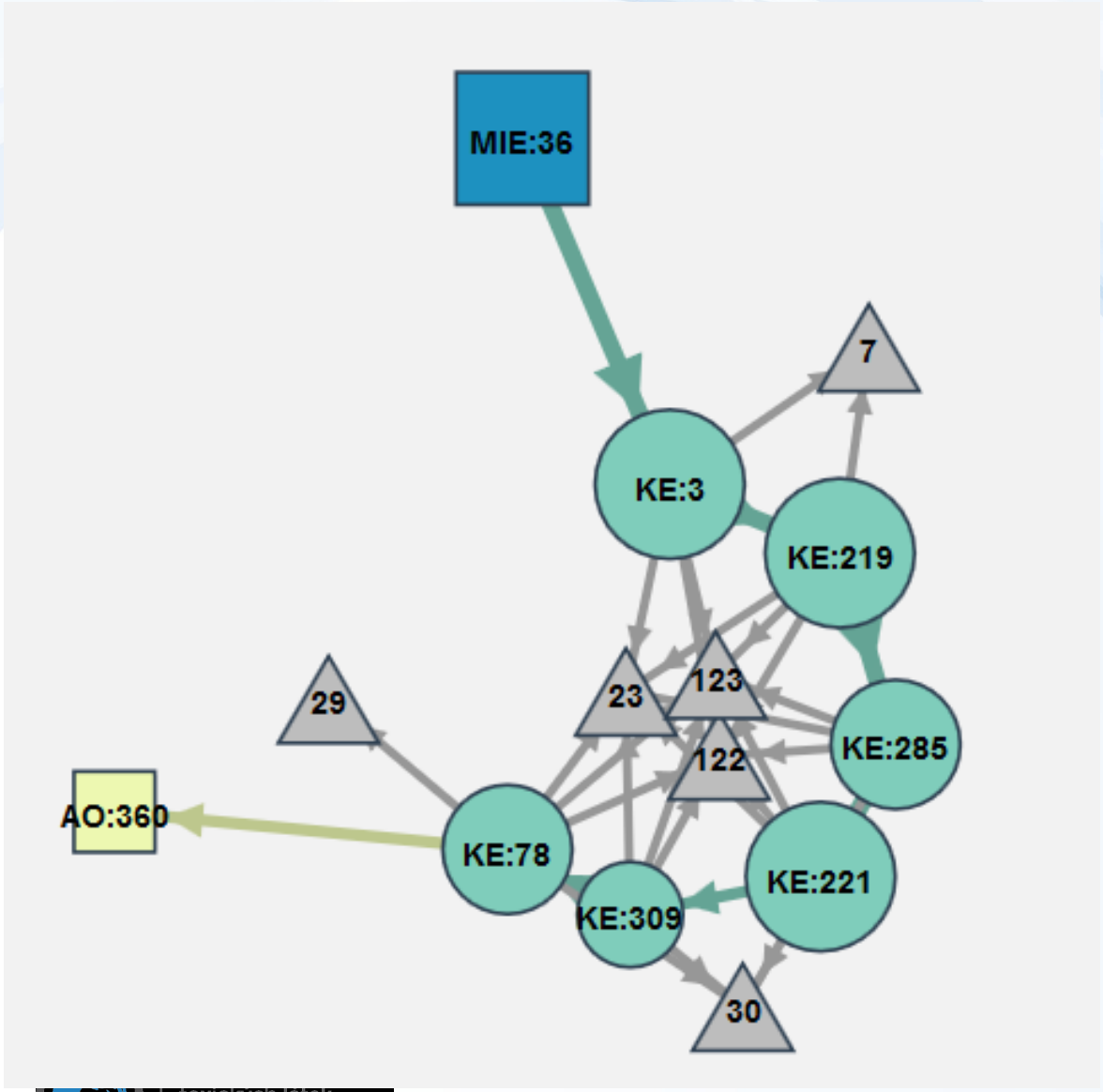


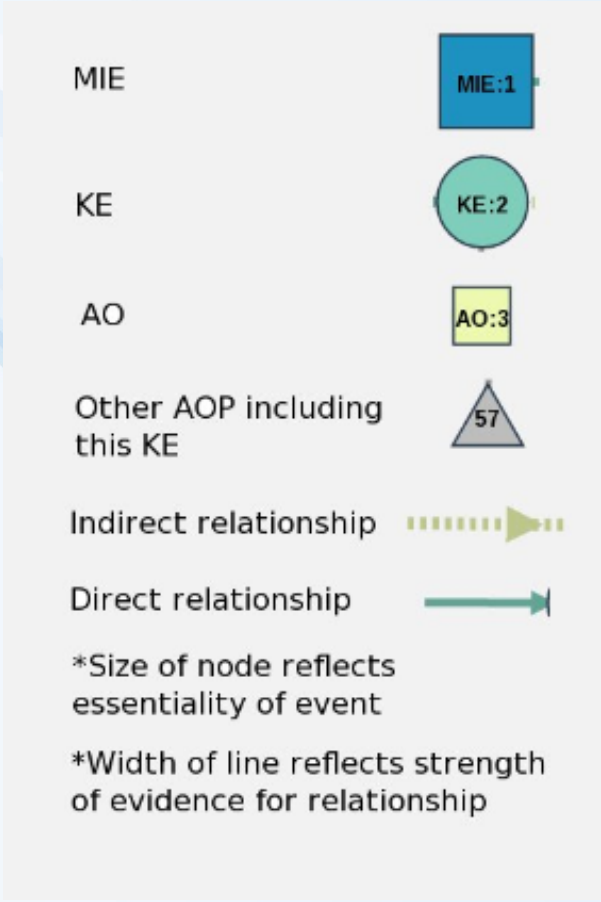
Fig. 3. An adverse outcome pathway in fish [2,50]. Aromatase inhibitor example. (**A**) Aromatase inhibition by fadrozole; (**B**) Reduction in circulating estradiol; (**C**) Reduction in circulating vitellogenin (Vtg); (**D**) Histopathology of ovarian tissue, top panel normal ovary, bottom panel fadrozole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

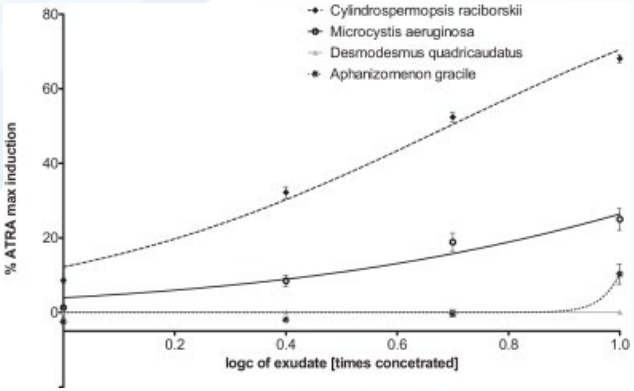
https://aopwiki.org/wiki/index.php/Aop:25





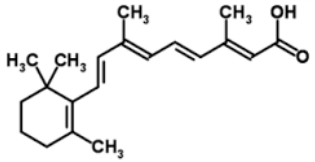
toxických látek v prostředí

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

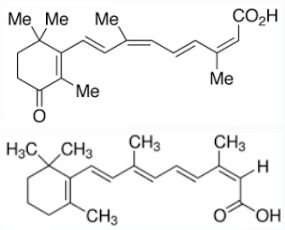


Activation of RAR/RXR

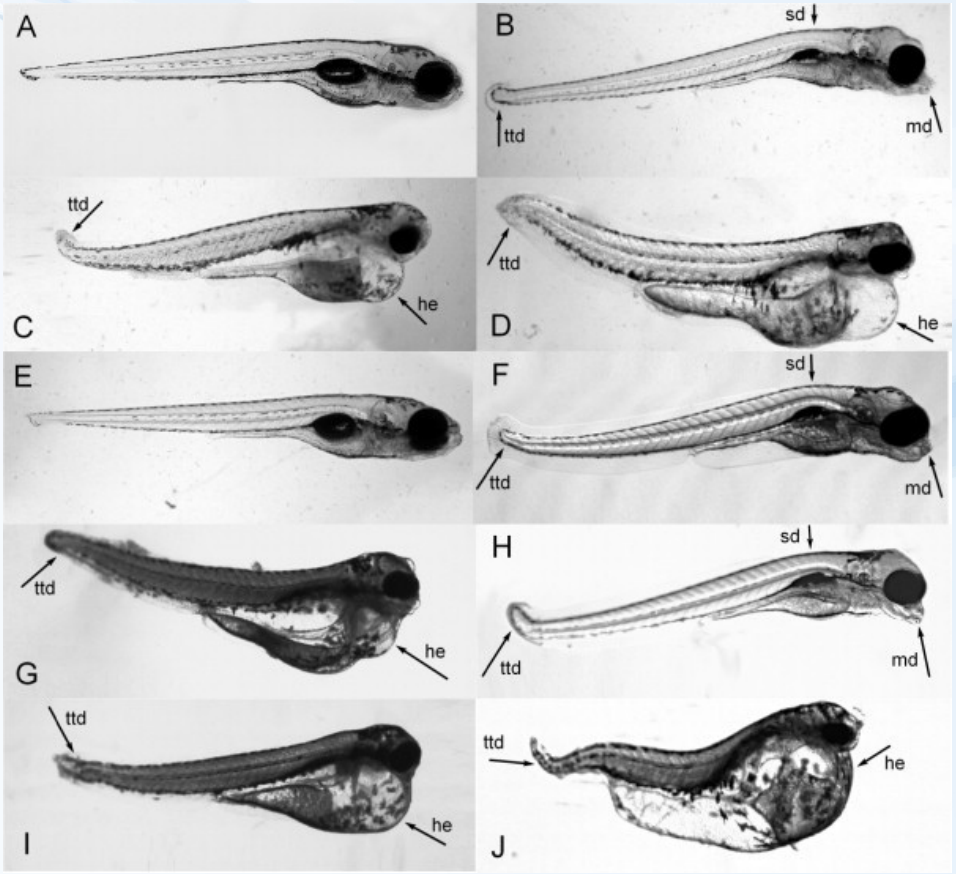
in P19/A15 cells by atRA and cyanobacterial metabolites



atRA



other RAs in cyanos



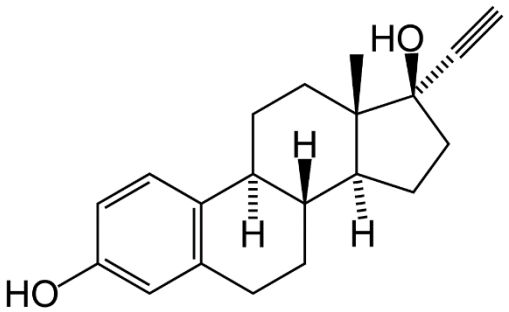
ZF exposed to ATRA and cyanobacterial (120 hpf) - Control (A), exudates of *C. raciborskii* 3.3 (B) and 10 (C), *M. aeruginosa* 10 (D) and *D. quadricaudatus* 17 (E). ATRA 4 µg/L (13.3 nM) (F), 12 µg/L (40 nM) ((G) and (H)), 36 µg/L (I) and 108 µg/L (J).

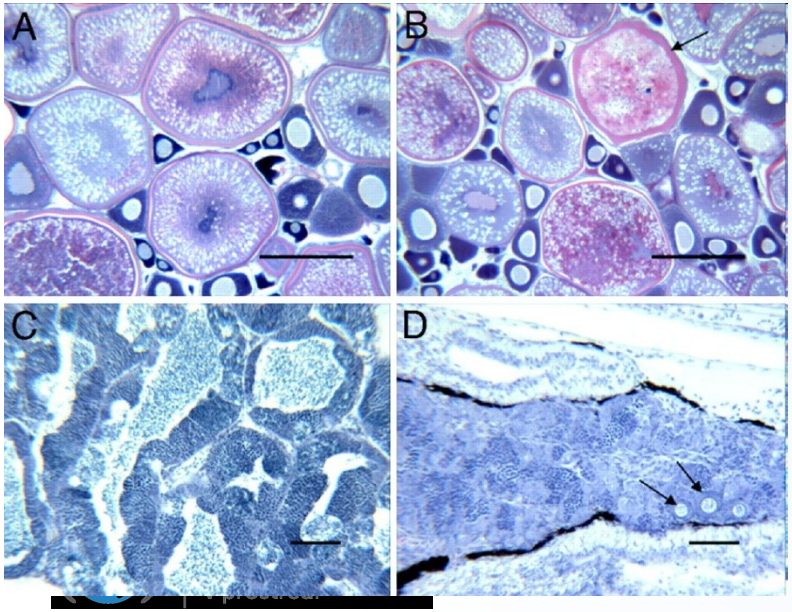
Jonáš et al. 2014 Aquatic Toxicology
http://dx.doi.org/10.1016/j.aquatox.2014.06.022

Centrum pro výzkum toxických látek v prostředí

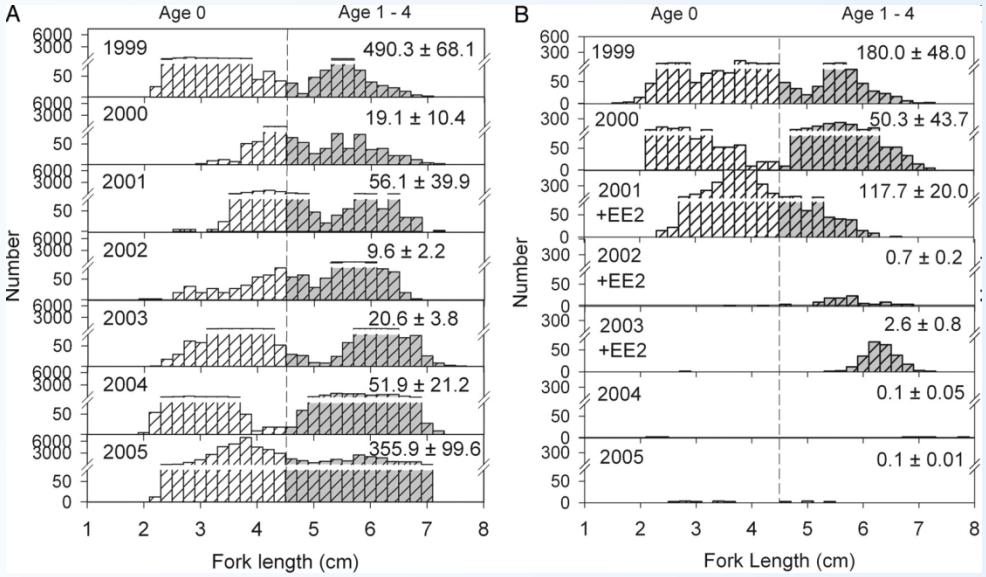
Kidd, K.A. et al. 2007. **Collapse of a fish population** following exposure to **a synthetic estrogen**. *Proceedings of the National Academy of Sciences* 104(21):8897-8901

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?