



Centrum pro výzkum  
toxických látek  
v prostředí

# Ekotoxikologie – závěr přednášek nové přístupy a poznatky

Luděk Bláha, PŘF MU

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



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

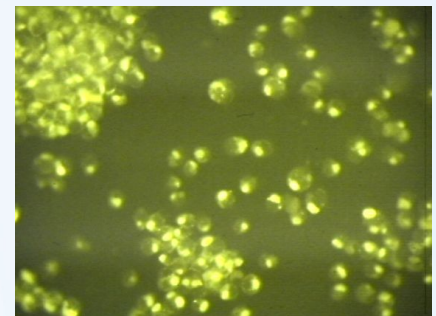
## Přehled závěrečné přednášky

- Moderní přístupy experimentální ekotoxikologie
  - In vitro modely
  - Biomarkery a „MOA“ (mode-of-action / omics) techniky
- Modely v ekotoxikologii
  - SAR a QSAR
  - AOP / PBPK / TOXCAST
- „Nové“ problémy v ekotoxikologii
  - Nanočástice
- Novinky a zajímavosti

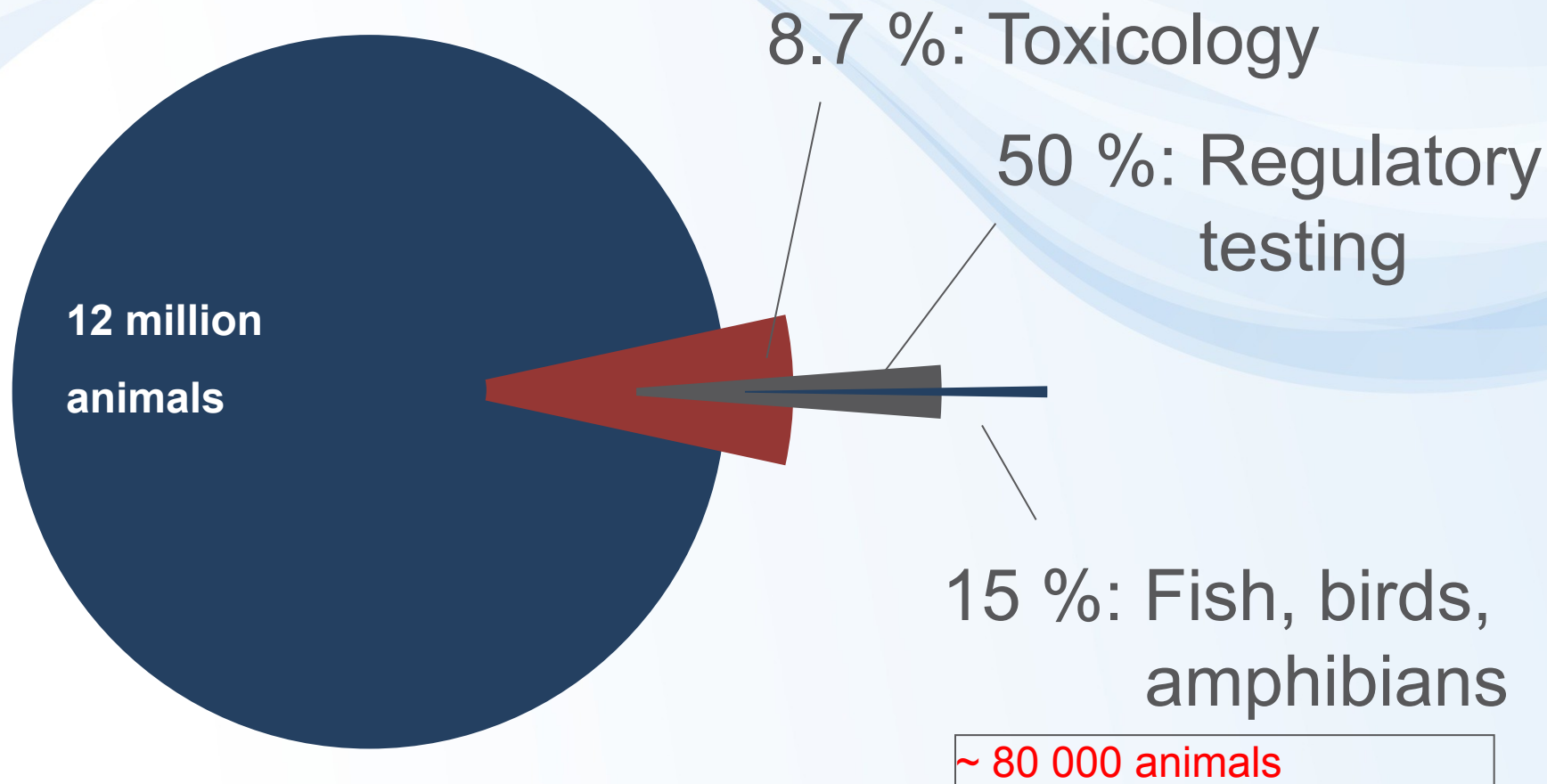


# Výzkum mechanismů toxicity in vitro modely a biotesty

- Zjišťování účinků (Biologie, Toxikologie a Ekotoxikologie) - existuje velké množství modelů
- Účinky na celých organismech
  - Standardní biotesty in vivo: legislativa
  - „Nestandardní“ biotesty in vivo: experimentální výzkumná práce
- Pochopení **a identifikace specifických mechanismů** působení
  - In vitro modely: Orgánové / Tkáňové / Buněčné
  - Výhody
    - Mechanistické porozumění
    - **Šetření experimentálních zvířat (etické principy „3R“)**
  - Nevýhody
    - „Jen“ in vitro, chybí komplex a interakce v organismu



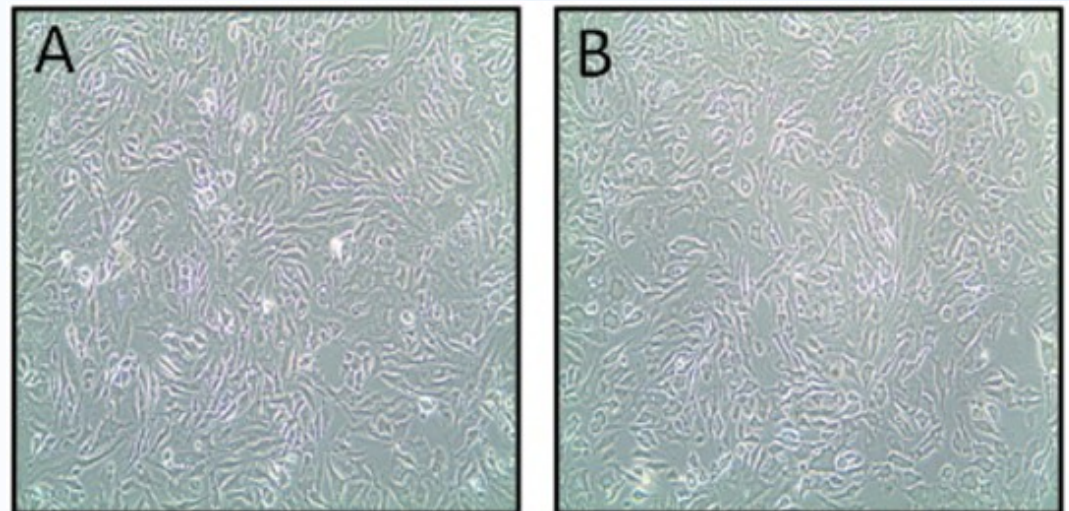
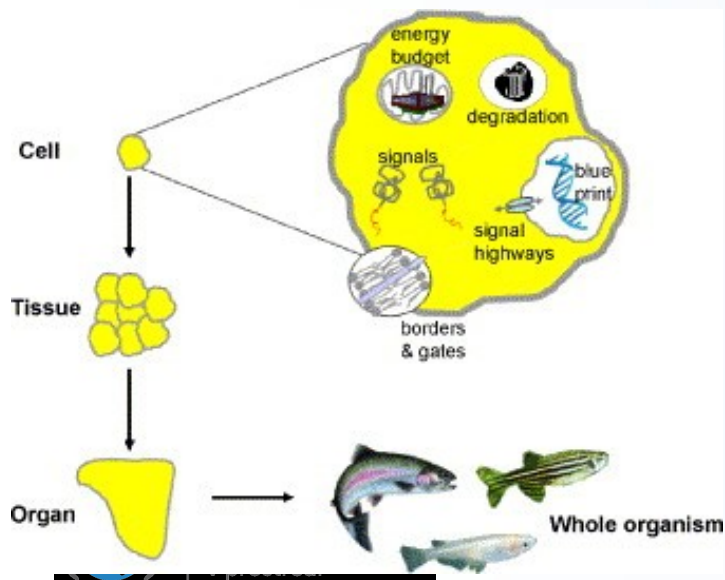
# Počty obratlovců používaných pro hodnocení chemických látek v Evropě



Commission of the European Communities, 2010

# In vitro models v ekotoxikologii 1 – rybí buňky

- Rybí buňky in vitro
  - Relativně snadná izolace buněk a udržování v kultuře (na rozdíl od savčích primárních linií se rybí buňky in vitro chovají jako immortalizované)
  - Příklady linií
    - **RTL-W1 (Rainbow Trout Liver - W1)**
    - RTgill (Rainbow Trout Gill)
  - Využití např. pro testování akutní toxicity (snaha o nahrazení testů in vivo)
    - podobná citlivost s in vivo modely → *validace / standardizace*



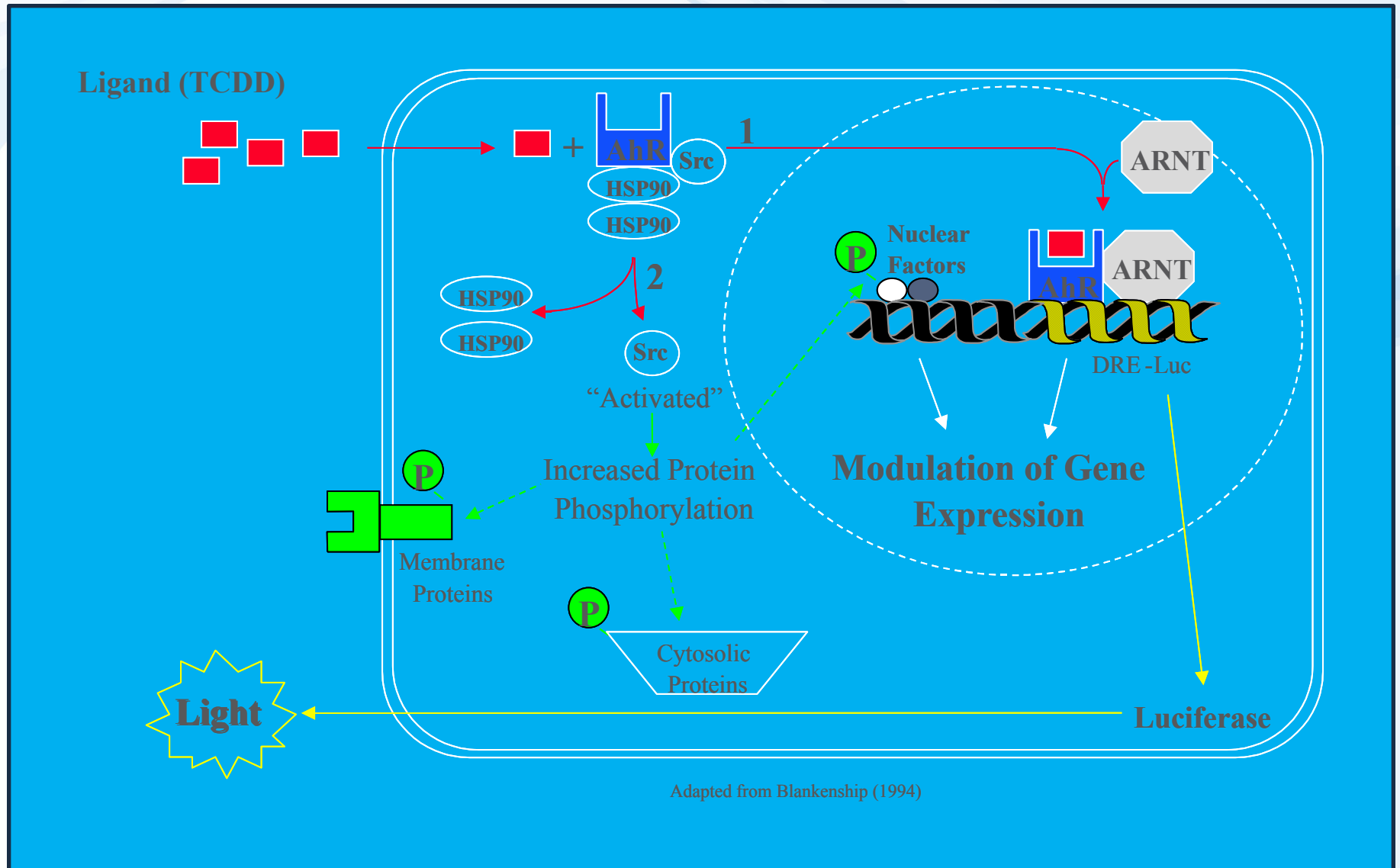
# Reporterové testy

## analýza účinků závislých na jaderných receptorech

- Specificky vytvořené buněčné linie
- Původně odvozené z lidských, potkaních, rybích či jiných tkání
- Následná úprava („GMO“)
  - stabilní transfekce specifickými geny, které se v buňkách normálně nevyskytují
  - Luciferáza (ze světlušky), Beta-galaktosidáza
  - Vložení do DNA v místech, která jsou kontrolována příslušným receptorem (AhR, ER...)
- Princip – viz obrázek
  - Měření světla z luciferázy ~ množství dioxinově aktivních látek
- Někdy označované „**CALUX**“ (**Chemical Assisted Luciferase Expression**)
  - jde o komerční název některých buněk, ale v mnoha laboratořích (včetně RECETOX) se užívají principiálně stejné „nekomerční“ buňky (např. H4IIE.luc / MVLD / MDAkb2)



# Stanovení toxicit závislých na intracelulárních receptorech

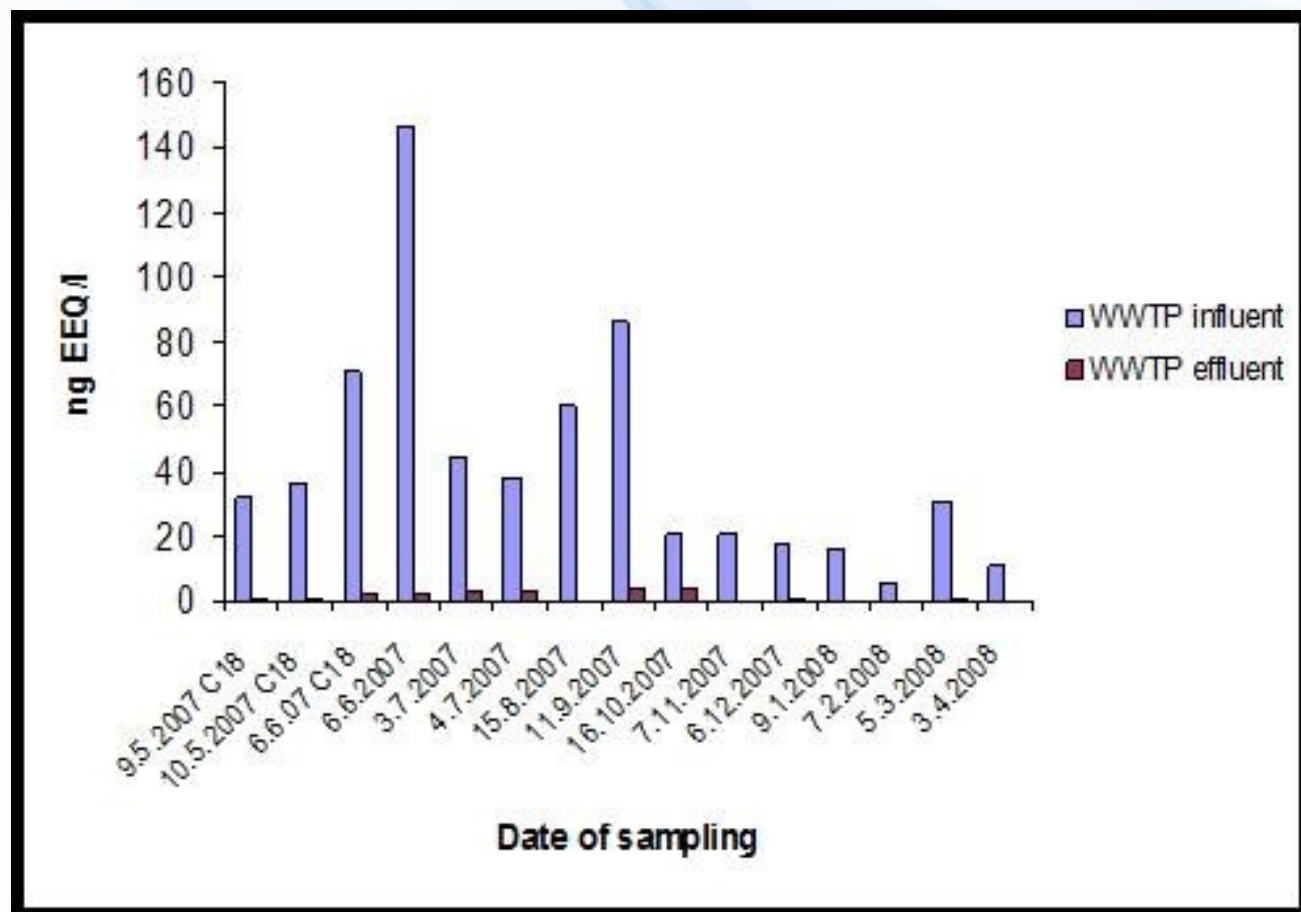


Adapted from Blankenship (1994)

# Příklad – využití reporterových testů

Hladiny estrogenních látek (stanovení pomocí MVLN testu) na  
Přítoku a Odtoku ČOV Brno-Modřice

- Velká účinnost čištění
- Výsledné koncentrace (až 5 ng/L) jsou i tak biologicky účinné !



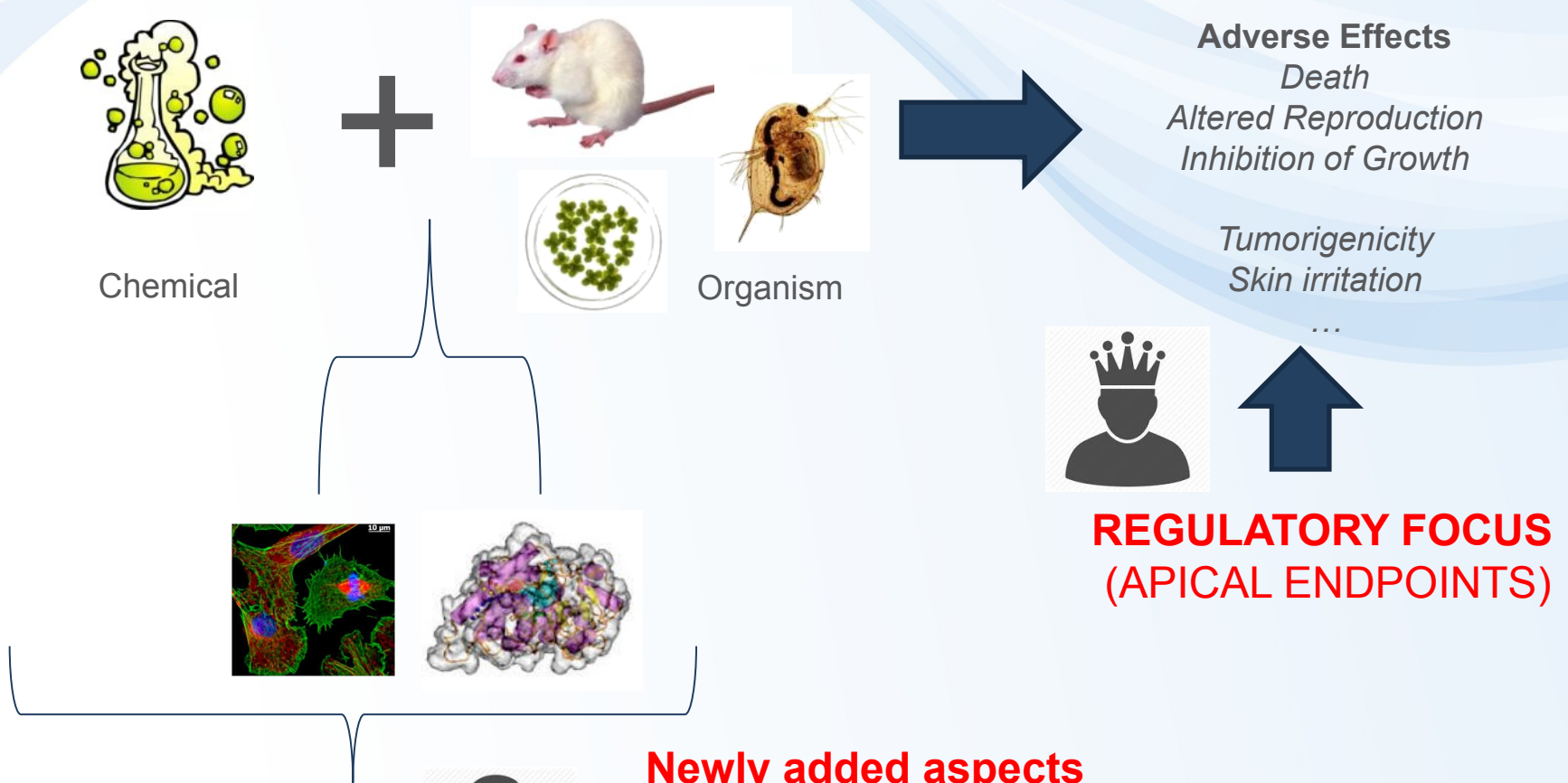


# Budoucnost ekotoxikologie: Big data a „omics“



# Hazard assessment

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

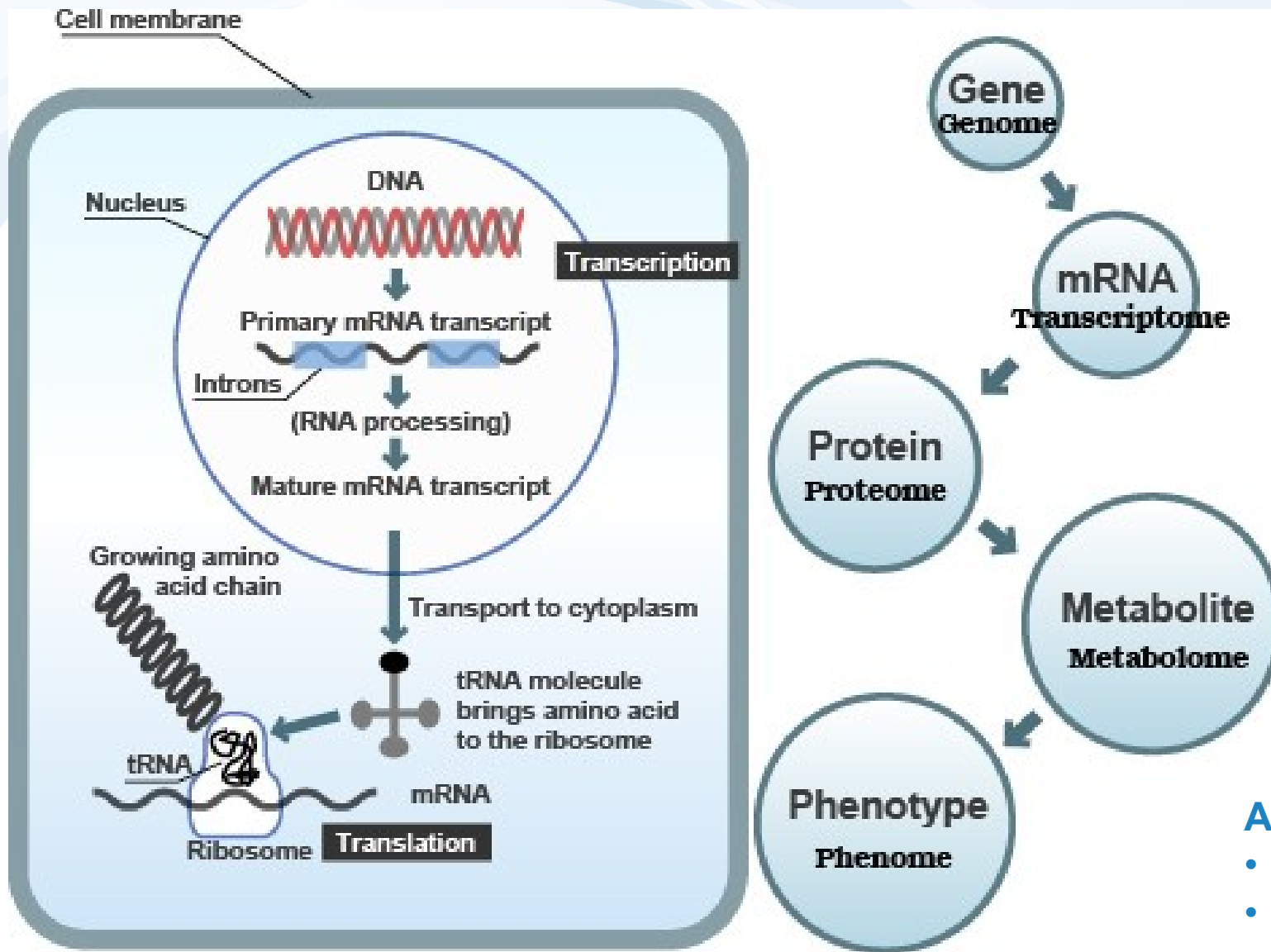


## Newly added aspects

What are the mechanisms (MoA – mode of action)?  
Can mechanisms serve for predictions?



# Extrémní rozvoj analytických technologií → „OMICS“



A další „ómy“

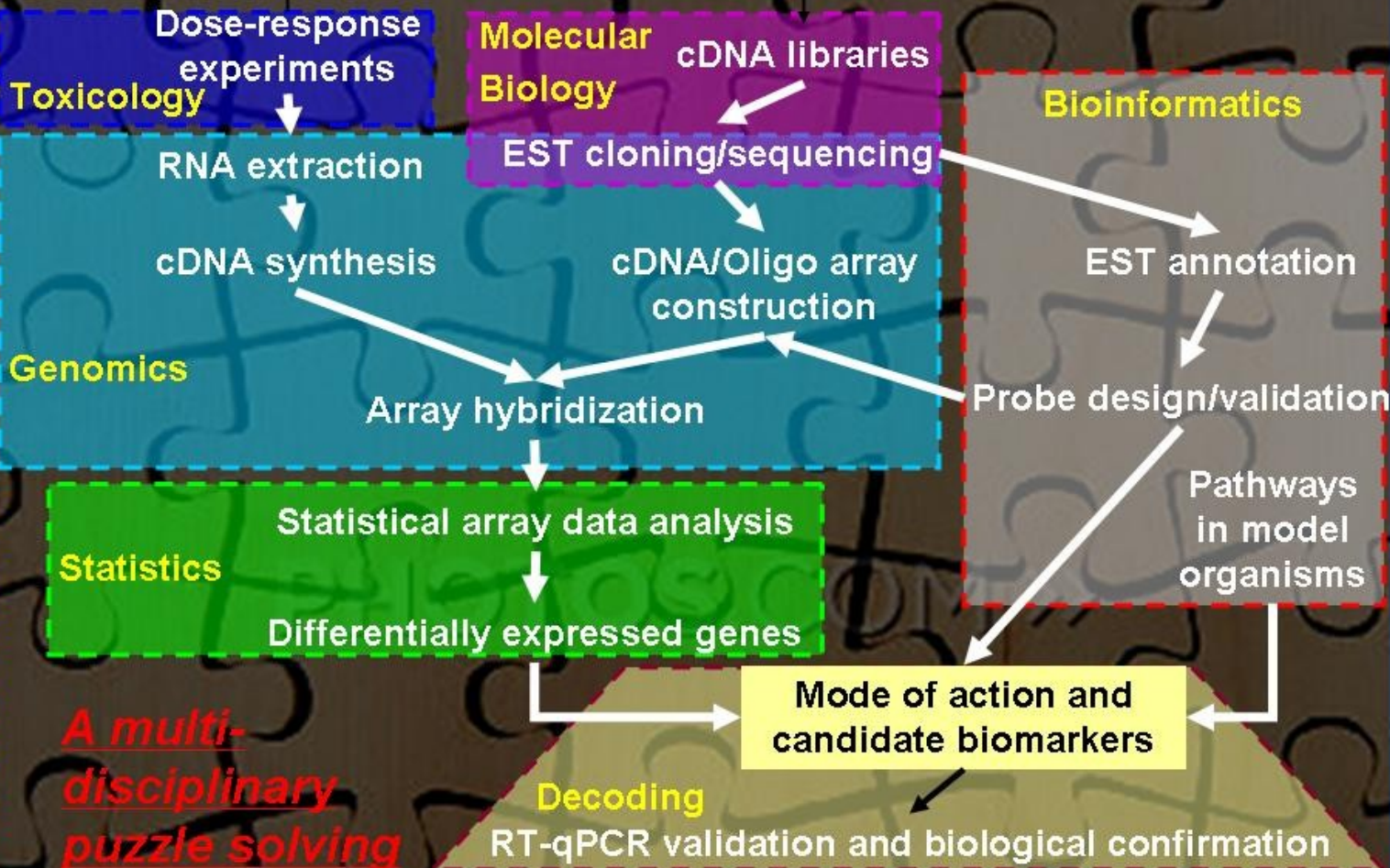
- Lipidóm
- Mikrobióm ...



# Earthworm Toxicogenomics



US Army Engineer Research & Development Center



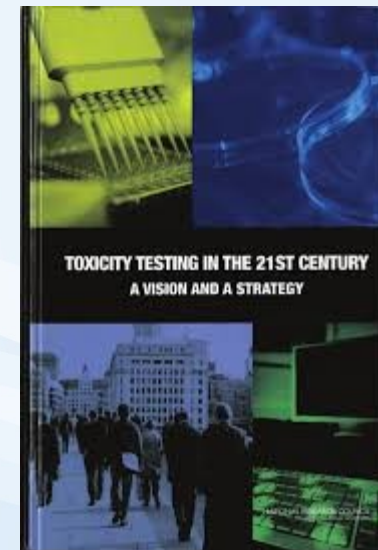
**A multi-disciplinary puzzle solving exercise**

# Sběr omics podporují strategické dokumenty & projekty

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

US National Academies of Sciences

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



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### Computational Toxicology Research

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#### Key Links

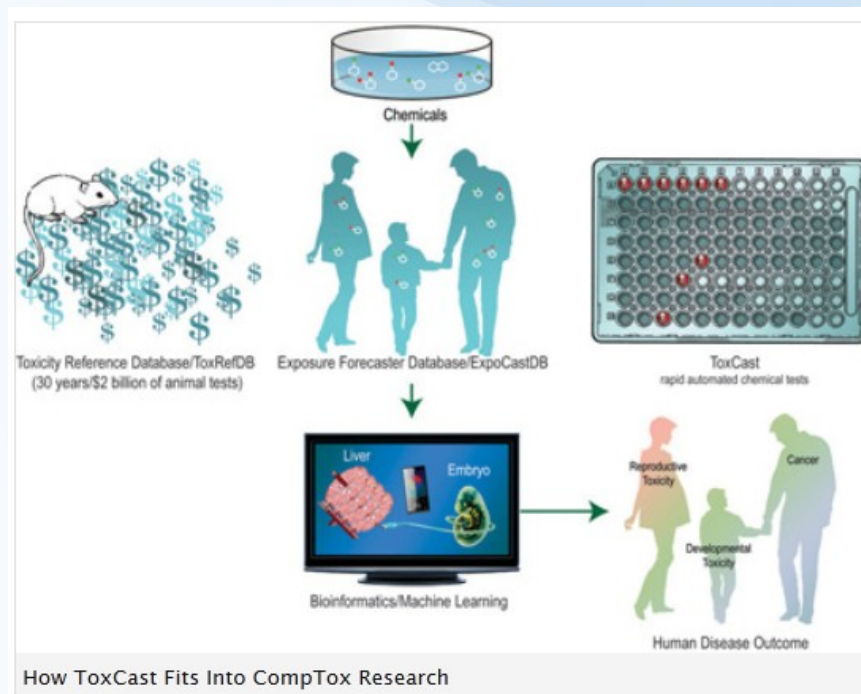
[CompTox Home](#)  
[Basic Information](#)  
[Organization](#)

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

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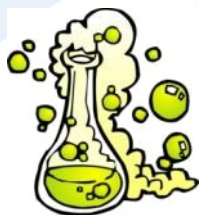
## ToxCast™

Screening Chemicals to Predict Toxicity Faster and Better



# Hazard assessment

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



Chemical

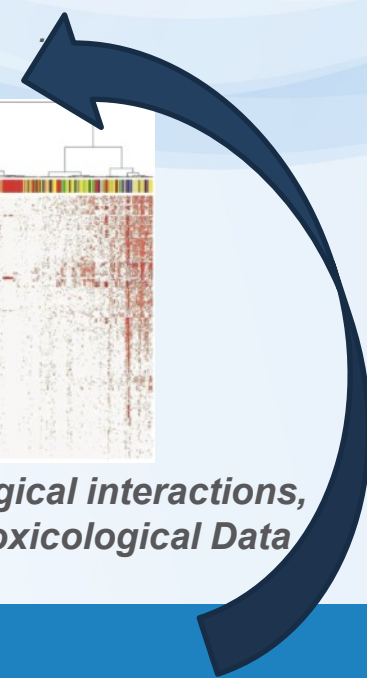


Organism



**Adverse Effects (EC50)**

- Death
- Inhibition of Growth
- Altered Reproduction
- Tumor
- Skin irritation



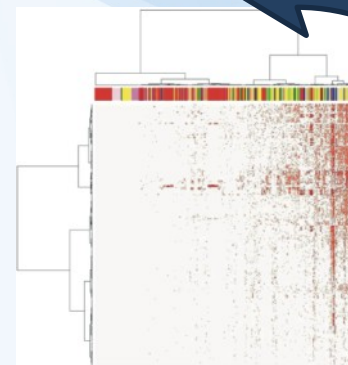
**New** – Ex vivo / in vitro / In chemico / In silico Methods



10<sup>4</sup> Chemicals



HTS



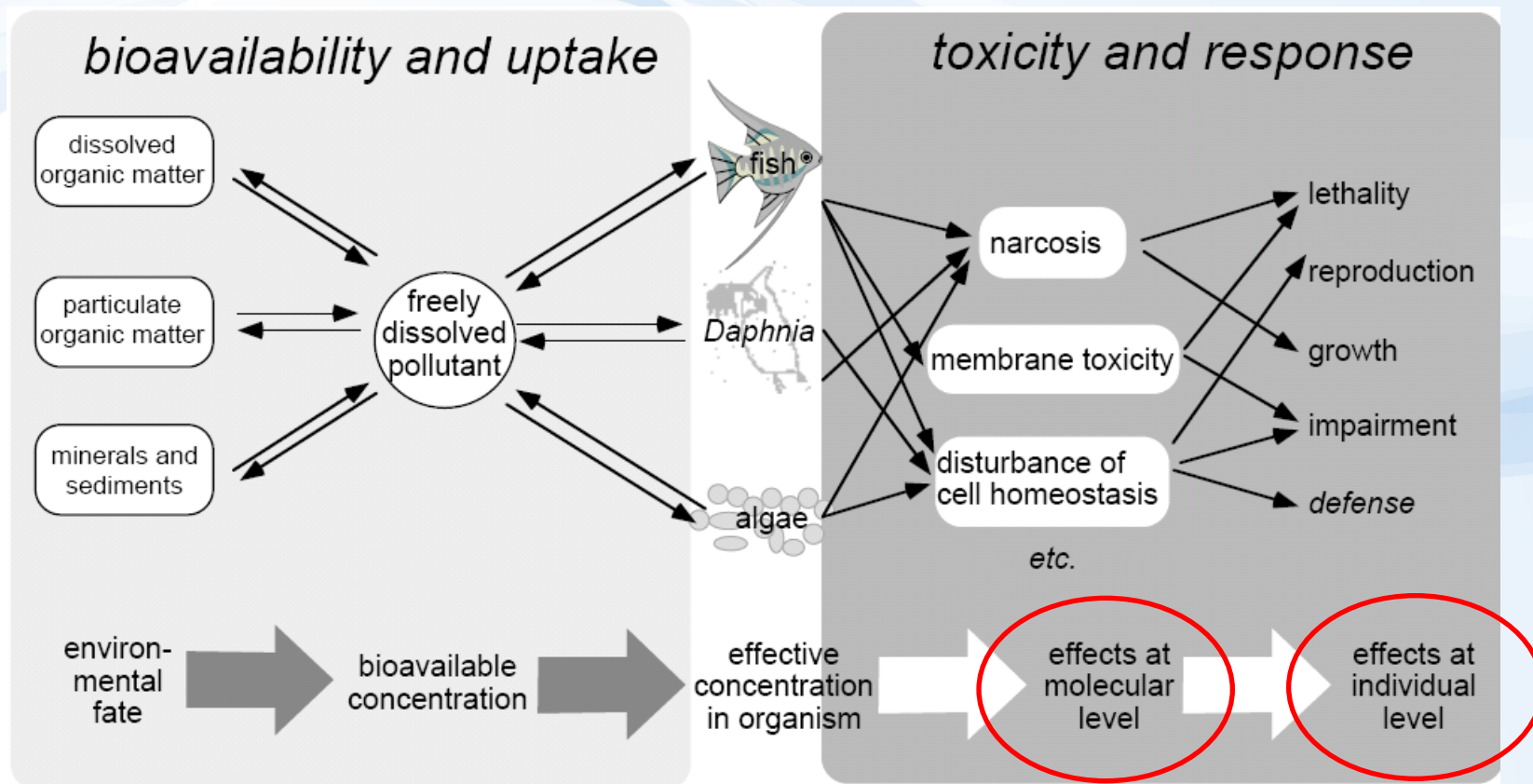
**Chemical-biological interactions,  
Mechanistic Toxicological Data**

**Key task/question:**

How to link MECHANISTIC INFORMATION with APICAL ENDPOINTS ?

# Využití mechanistických dat → AOPs

# Ecotoxicity is a consequence of sequence of events

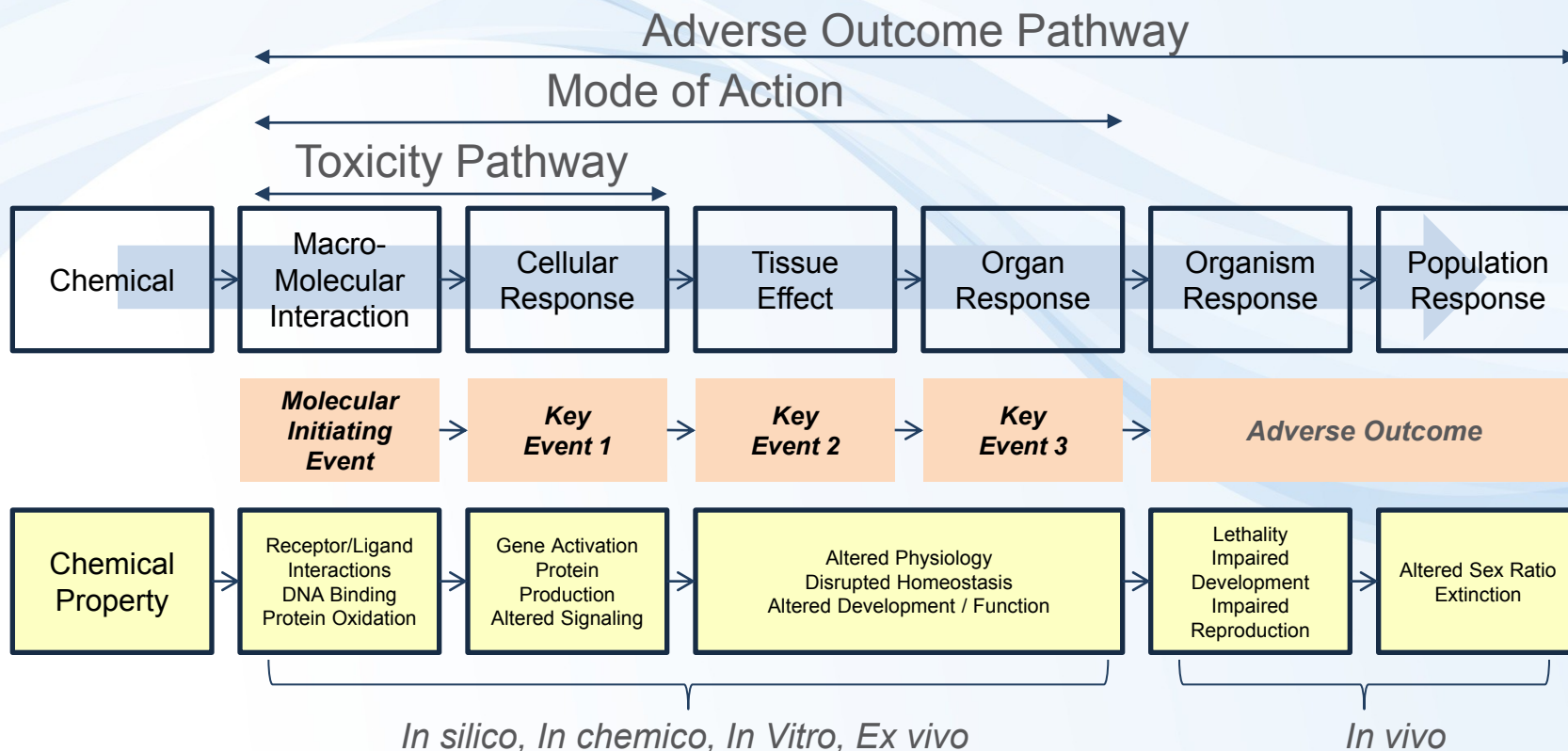


**Figure 1** The effective concentration of a pollutant in an organism (e.g. fish, daphnia, algae) or at the target site inside the organism is the link between the environmental fate of a pollutant and its toxic effect.

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.



# Adverse Outcome Pathways – Dráhy škodlivého účinku

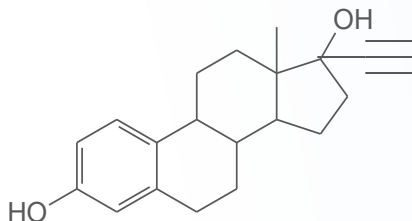


The **EXISTING KNOWLEDGE** is used **to link the** two anchor points: **Molecular Initiating Event (MIE)** and **Adverse Outcome (AO)** **via a series** of intermediate steps: **Key Events**

# AOP Example:

## Fish population decline due to estrogen receptor modulation

### Ethinylestradiol (EE2)

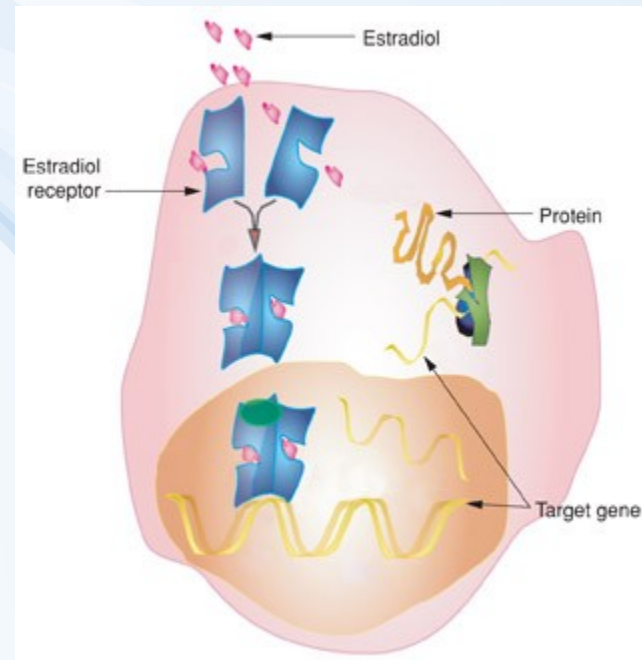


### MIE

Binding to ESTROGEN RECEPTOR

### KEs - Activation Target genes

- Proliferation/Apoptosis (sexual organs)
- Synthesis of egg yolk (fish, amphibia)



### KEs - Effects

- Females: reproduction regulation
- Males: feminization  
(+ e.g. cancer promotion, development, immunomodulation)

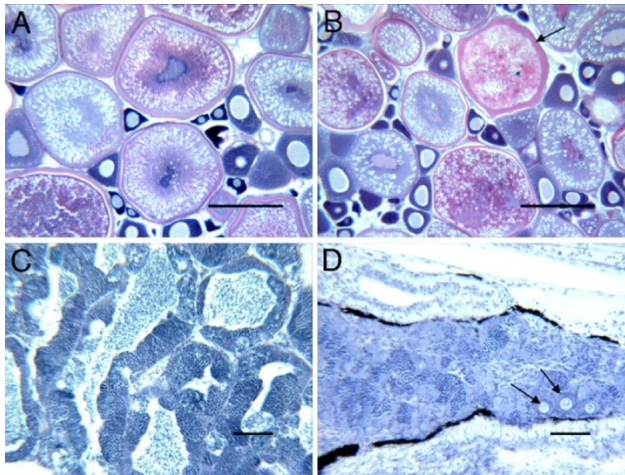
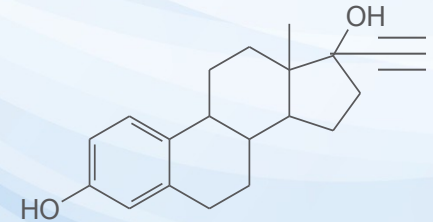


# Adverse outcome – fish population decline

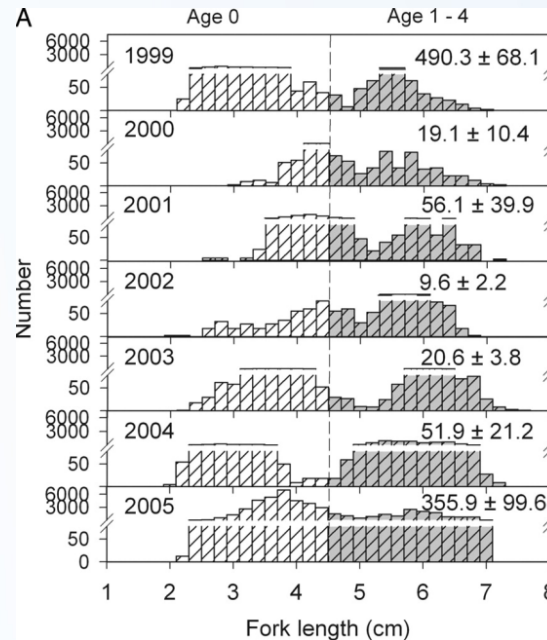
Kidd, K.A. et al. 2007. **Collapse of a fish population following exposure to a synthetic estrogen.** PNAS 104(21):8897-8901



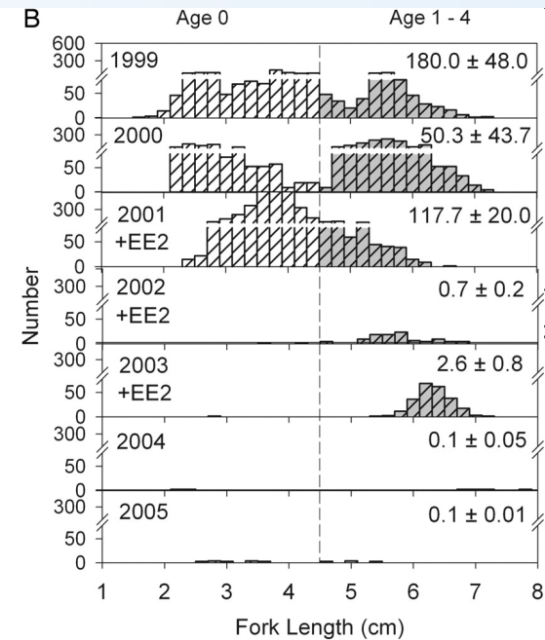
**EE2 - 5 ng/L (!)  
7 years**



## Control lake



## lake with EE2




# AOP a regulatorní prediktivní toxikologie

## OECD AOP Knowledge Base



# Strategické směřování – podpora OECD



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- > 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.

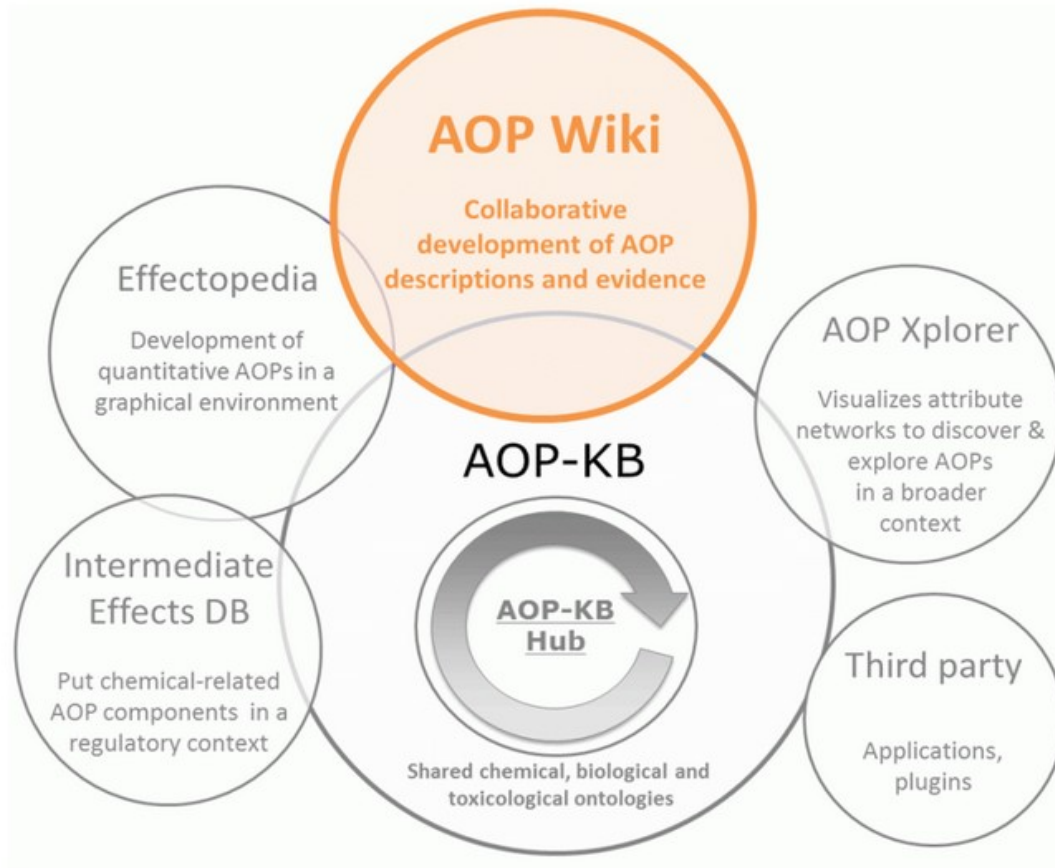
The survey is mainly for chemical safety regulators who are experiencing a transition in their work towards an increased use of 'alternative' methods and AOPs. However, stakeholders that come from the regulated community and environmental NGOs are also welcome to participate.

> **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**

# Listing AOPs

Name	MIE	AO
EGFR Activation Leading to Mucus Hypersecretion	EGFR	
5-hydroxytryptamine transporter (5-HTT; SERT) inhibition leading to increased predation	5-HTT	increased predation
Acetylcholinesterase inhibition leading to acute mortality	AChE	acute mortality
Glucocorticoid Receptor (GR) Mediated Adult Leydig Cell Dysfunction Leading to Decreased Male Fertility	GR	decreased male fertility
AhR activation leading to embryo toxicity in fish	AhR	embryo toxicity
Protein Alkylation leading to Liver Fibrosis	Protein alkylation	liver fibrosis
NR1I2 (Pregnane X Receptor, PXR) activation leading to hepatic steatosis	PXR	hepatic steatosis

# AOP Example: MIE aromatase inhibition

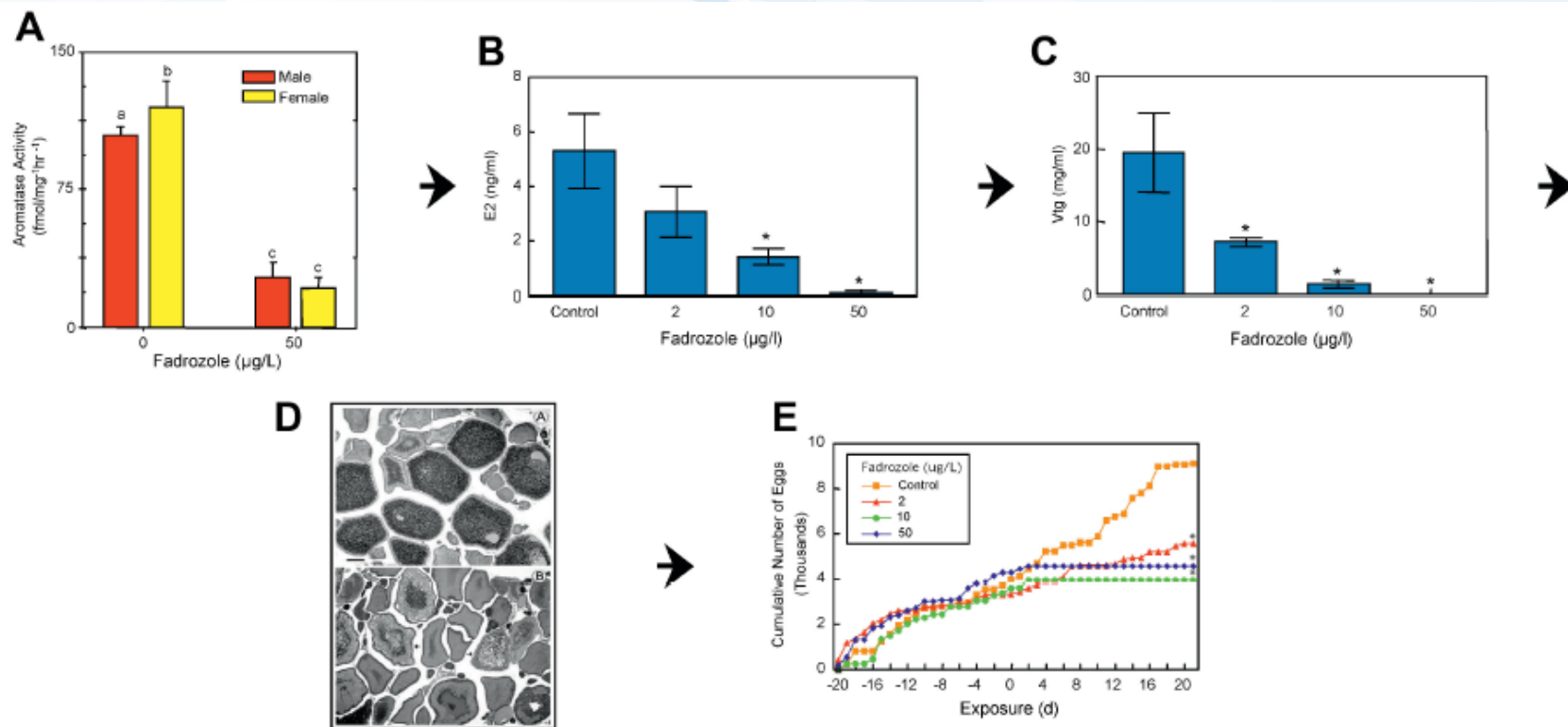


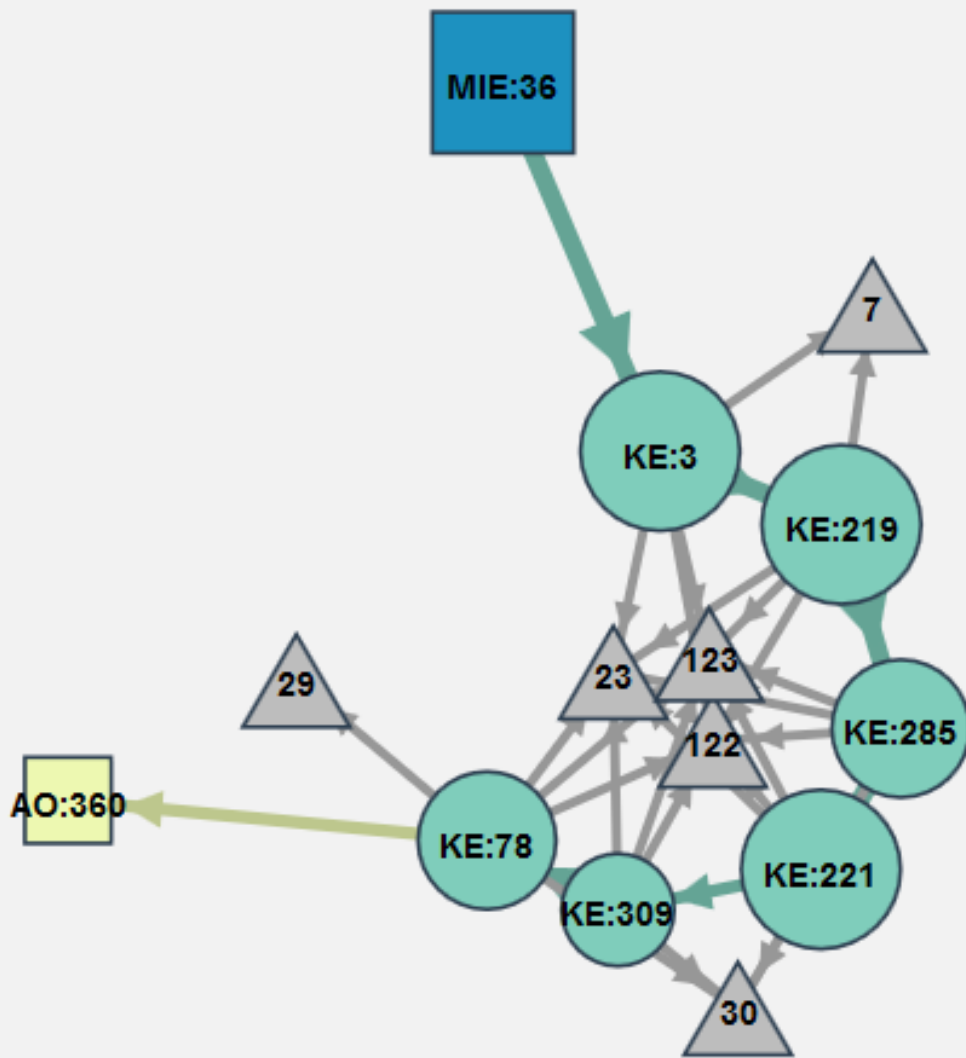
Fig. 3. An adverse outcome pathway in fish [2,50]. Aromatase inhibitor example. (A) Aromatase inhibition by fadrozole; (B) Reduction in circulating estradiol; (C) Reduction in circulating vitellogenin (Vtg); (D) Histopathology of ovarian tissue, top panel normal ovary, bottom panel fadrozole treated;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



# Regulatorní akceptace ... pozitivní

Regulatory Toxicology and Pharmacology 70 (2014) 629–640



ELSEVIER

Contents lists available at [ScienceDirect](#)

## Regulatory Toxicology and Pharmacology

journal homepage: [www.elsevier.com/locate/yrtph](http://www.elsevier.com/locate/yrtph)



### Applying Adverse Outcome Pathways (AOPs) to support Integrated Approaches to Testing and Assessment (IATA)



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## Related Projects & Studies & Databases

- **TOXNET** - <http://toxnet.nlm.nih.gov/>
  - searching databases on toxicology, hazardous chemicals, environmental health, and toxic releases
- **Tox21** - <http://www.epa.gov/ncct/Tox21/>
  - 10,000 chemicals
  - 14 concentrations, 4 logs, 3 replicates
  - 1536 well plates, 2-8 uL volumes
  - 50+ assays
- **ToxCast** - <http://www.epa.gov/ncct/toxcast/>
  - App. 2000 chemicals
  - 700+ assay, 300 signaling pathways
  - DATA AVAILABLE iCSS Dashboard
    - <http://actor.epa.gov/dashboard>
    - <http://ww.epa.gov/ncct/toxcast/data.html>



## Related Projects & Studies & Databases

- **ToxRefDB (Toxicity Reference Database)**
  - *in vivo* toxicological data
  - <http://actor.epa.gov/toxrefdb/faces/Home.jsp>
- **ExpoCast**
  - information on human exposures
  - <http://www.epa.gov/ncct/expocast/>
- **Human Toxome Project**
  - information on human exposures
  - <http://www.ewg.org/sites/humantoxome/>
- **Agriculture Health Study**
  - Occupational Exposure to Pesticides – a cohort study
  - <http://aghealth.nih.gov/>

# Modely SAR a QSAR



# SAR, QSAR

- **SAR = Structure Activity Relationships**
  - hledání vztahů mezi STRUKTUROU a AKTIVITOU látek (*struktura -> eko-toxicita*)
  - předpovědi efektů bez nutnosti experimentálních testování
- **Řada přístupů**
  - kvalitativní
    - přítomnost určité charakteristiky implikuje aktivitu (*vlastnost*)
      - *dlouhý alifatický řetězec -> afinita k membránám*
  - kvantitativní (=QSAR – Quantitative SAR)
    - matematický popis vztahů
      - jednorozměrné vztahy – korelace, regresní vztahy ...
      - vícerozměrné modelování (*PCA, PLS*), neuronové sítě ...



# SAR, QSAR v ekotoxikologii

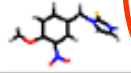

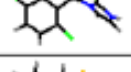

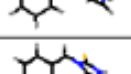
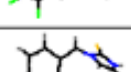

- Techniky QSAR původně vyvinuty pro design farmak
- **Aplikace SAR, QSAR v ekotoxikologii**
  - **předpovědi environmentálně významných parametrů látek**
    - *logK<sub>ow</sub>*
    - *biokoncentrace, bioakumulace*
    - *předpovědi biodegradability a metabolismu*
      - *odhady rychlosti degradace t<sub>1/2</sub>, vznikající metabolity*
  - **předpovědi toxicity**



# Princip vývoje modelu QSAR

- **1) Vstupní data – ZNÁMÉ údaje**
  - Skupina podobných látek
  - Známa (změřená) vlastnost – např. aktivita / toxicita
  - Známa fyz-chem data (stovky různých parametrů)
- **2) Hledání modelu ve známých datech**
  - Např.  $\text{Aktivita} = a * \text{parametr1} + b * \text{parametr2} + c$
  - (viz příklady dále)
- **3) Využití modelu pro předpověď „Aktivity“ neznámé látky**
  - Fyz-chem parametry  $\rightarrow$  dosazení do modelu  $\rightarrow$  výpočet „toxicity“

*Příklad – vstupní data pro QSAR*

	Structure	Activity [Y1]	Apol [X1]	Area [X2]	Dipole [X3]	Energy [X4]	Fl
1.		3.150	1.06E+04	270.566	7.139	133.002	
2.		3.450	9.55E+03	242.417	2.056	100.681	
3.		4.130	1.17E+04	252.990	1.037	103.760	
4.		3.450	1.17E+04	257.214	2.313	109.687	
5.		3.690	8.65E+03	215.372	1.028	90.970	
6.		4.010	1.17E+04	242.563	2.286	93.813	
7.		4.280	1.17E+04	251.587	1.558	100.894	





# SAR, QSAR - příklady

- Předpovědi environmentálně významných parametrů chemických látek (*viz také úvod přednášek*)
  - Fyzikálně chemické parametry
- **Log P (log Kow)**  Příklady software, různé modely a principy výpočtu
  - ClogP ([www.biobyte.com](http://www.biobyte.com))
  - KOWWIN ([esc.syrres.com](http://esc.syrres.com))  
([www.epa.gov/oppt/exposure/docs/episuitedi.htm](http://www.epa.gov/oppt/exposure/docs/episuitedi.htm))
  - SLIPPER ([www.ibmh.msk.su/qsar/molpro](http://www.ibmh.msk.su/qsar/molpro))
  - KlogP ([www.multicase.com](http://www.multicase.com))
  - ABSOLV ([www.sirius-analytical.com](http://www.sirius-analytical.com))
  - ProLogP ([www.compudrug.com](http://www.compudrug.com))
  - SPARC ([ibmlc2.chem.uga.edu/sparc](http://ibmlc2.chem.uga.edu/sparc))
  - IA ([www.interactiveanalysis.com](http://www.interactiveanalysis.com))
  - ACD ([www.acdlabs.com](http://www.acdlabs.com))
  - QikProp ([www.schrodinger.com](http://www.schrodinger.com))
  - AP-Algorithms ([www.ap-algorithms.com](http://www.ap-algorithms.com))
  - ProPred ([www.capec.kt.dtu.dk](http://www.capec.kt.dtu.dk))
  - Cerius<sup>2</sup> ([www.accelrys.com](http://www.accelrys.com))
  - QMPRPlus ([www.simulations-plus.com](http://www.simulations-plus.com))



# SAR, QSAR - příklady

## – Předpověď' biokoncentrace

Modely doporučované TGD (*technical guidelines*) při registraci nových chemických látek v EU (REACH)

*(není třeba testovat biokoncentraci – stačí model – šetření zvířat)*

$$\log K_{ow} < 6$$

$$\log BCF = 0.85 \log K_{ow} - 0.7$$

$$\log K_{ow} \text{ values } 6 - 10$$

$$\log BCF = -0.2 \log K_{ow}^2 + 2.74 \log K_{ow} - 4.72$$

## – Předpověď' biodegradability *také někdy: QSBR*

- jednoduché korelace degradability-chemický parametr
- sčítání vlivu charakteristických subdomén na degradabilitu ("+" degradace, "-" stabilita)  
-> *suma pro celou molekulu = degradability score*

- vícerozměrné modelování

Table 11. Molecular structures and groups used by Geating (1981) to evaluate biodegradability

Description	Coefficient"
Single occurrence of sulfur in a ring	-13.9
More than two carbocyclic rings	-10.5
Alkyl chain (CH <sub>2</sub> ) <sub>n</sub> or CH <sub>3</sub> (CH <sub>2</sub> ) <sub>n-1</sub> where n = 10 or more (chain fragment)	5.03
One benzene ring	3.94
More than one —N= or HN= group (substituent fragment)	-12.1
One —C=O group (sub. fragment)	4.71
Atoms other than C, H, O, N, S, or halogen	5.01
One —OH group (sub. fragment)	3.03
Substituent hydroxylamine	-16.4
Single occurrence of carbonyl in a ring	6.16
Substituent primary amide	-11.0
Presence of suffix	-4.80
More than one —O— group (chain fragment)	-5.44

# Moderní výpočetní toxikologie



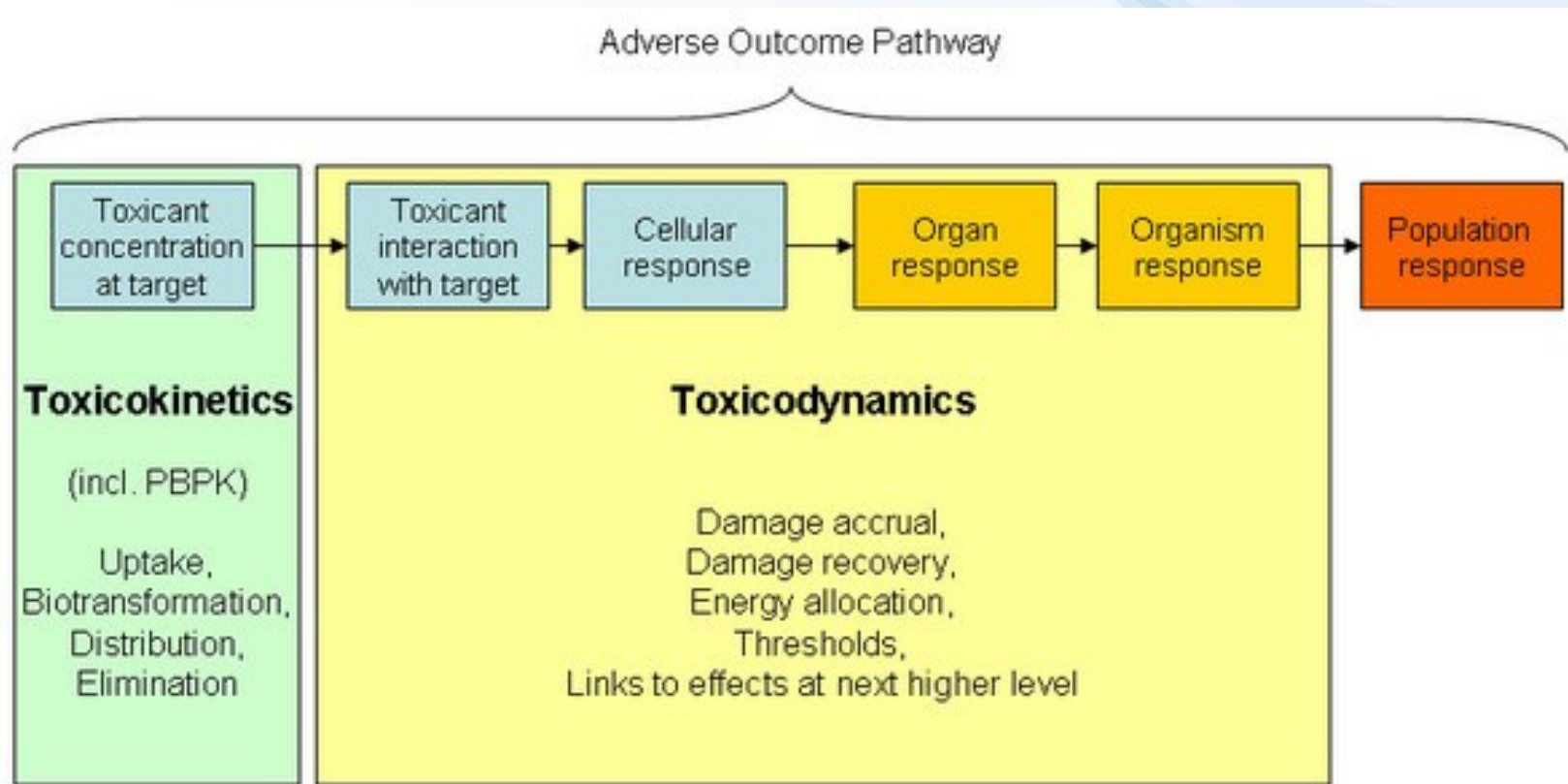
# Adverse outcome pathways

Viz také dříve v přednáškách:

**Dokážeme z koncentrace látky v prostředí předpovědět (matematicky) účinky ?**

Základem je dokonalé porozumění

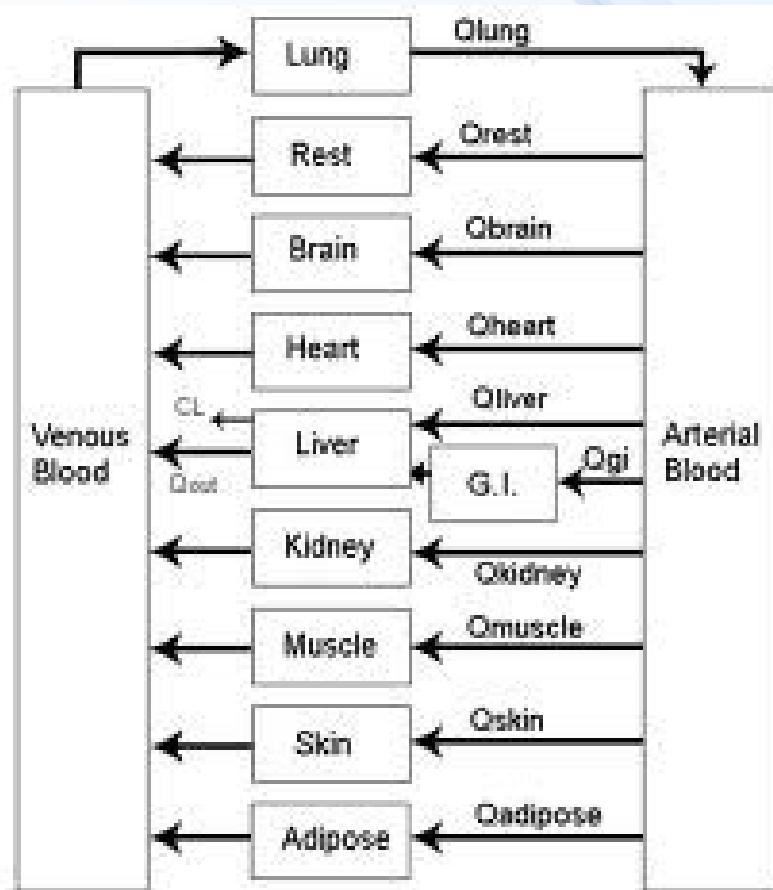
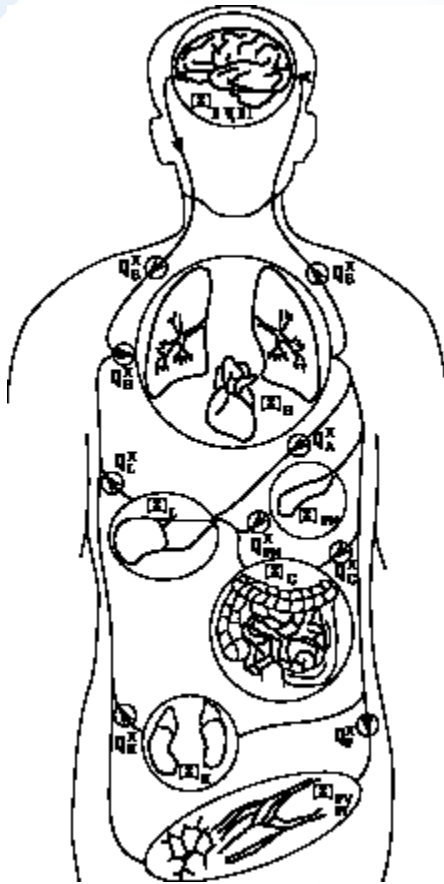
1) toxikokinetice (modely PBPK – viz dále) a 2) následně mechanismům (dynamika)



# PBPK modely

PBPK (PBTK)

Physiologically based pharmacokinetic (toxicokinetic) models



Vnitřní „rozdělení“ organismu a parametrizace běžících procesů

→ Složitý model : Predikce koncentrací v jednotlivých tkáních



Li et al. *BMC Systems Biology* 2011, 5:63  
<http://www.biomedcentral.com/1752-0509/5/63>



RESEARCH ARTICLE

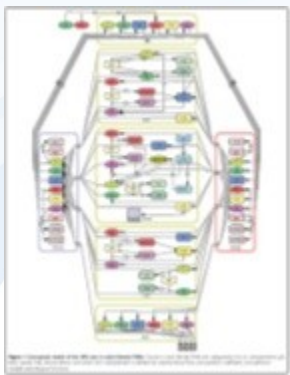
Open Access

## A computational model of the hypothalamic - pituitary - gonadal axis in female fathead minnows (*Pimephales promelas*) exposed to $17\alpha$ -ethynylestradiol and $17\beta$ -trenbolone

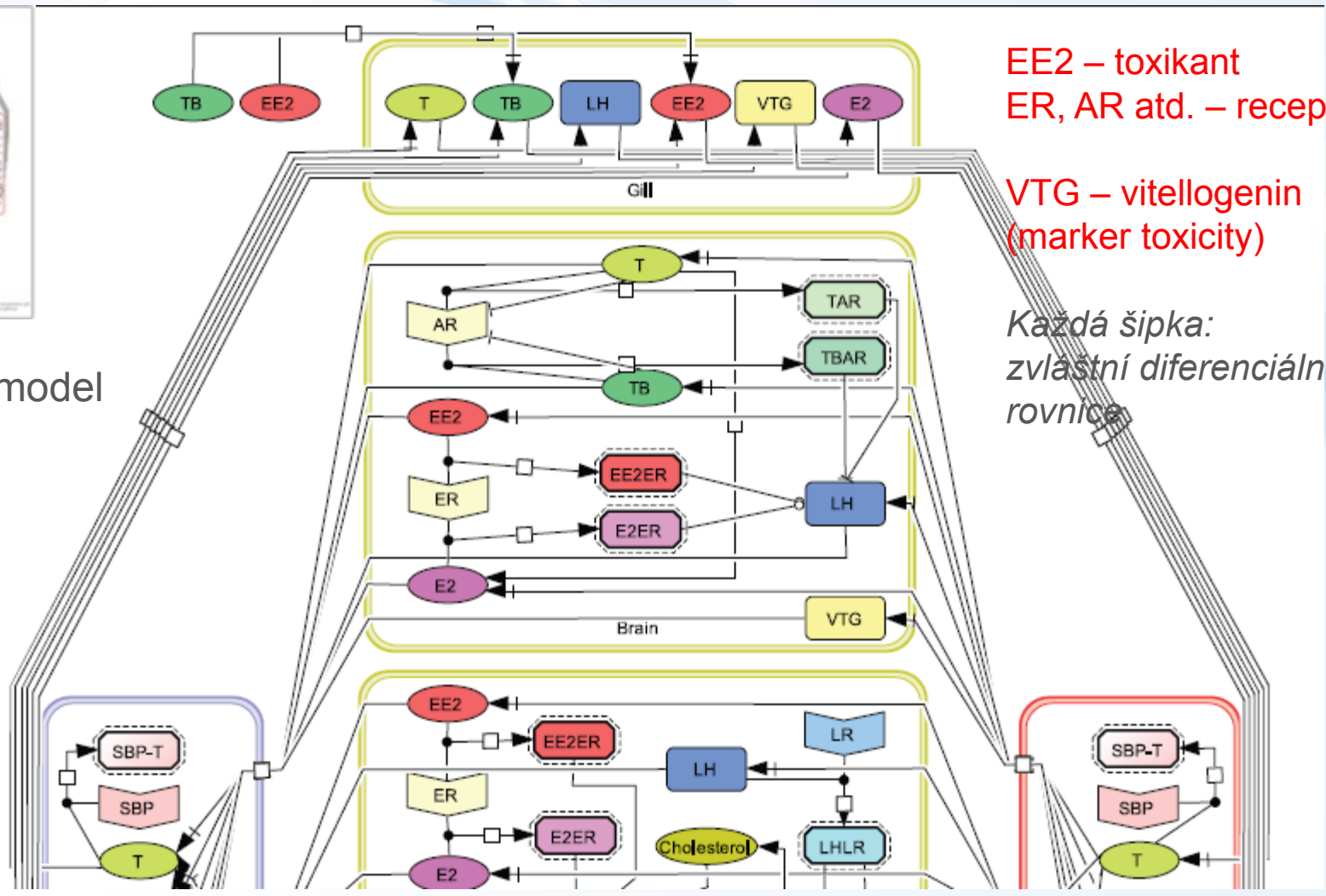
Zhenhong Li<sup>1</sup>, Kevin J Kroll<sup>2</sup>, Kathleen M Jensen<sup>3</sup>, Daniel L Villeneuve<sup>3</sup>, Gerald T Ankley<sup>3</sup>, Jayne V Brian<sup>4</sup>, María S Sepúlveda<sup>5</sup>, Edward F Orlando<sup>6</sup>, James M Lazorchak<sup>7</sup>, Mitchell Kostich<sup>7</sup>, Brandon Armstrong<sup>8</sup>, Nancy D Denslow<sup>2</sup> and Karen H Watanabe<sup>1\*</sup>



# Li (2011) BMC Systems Biology



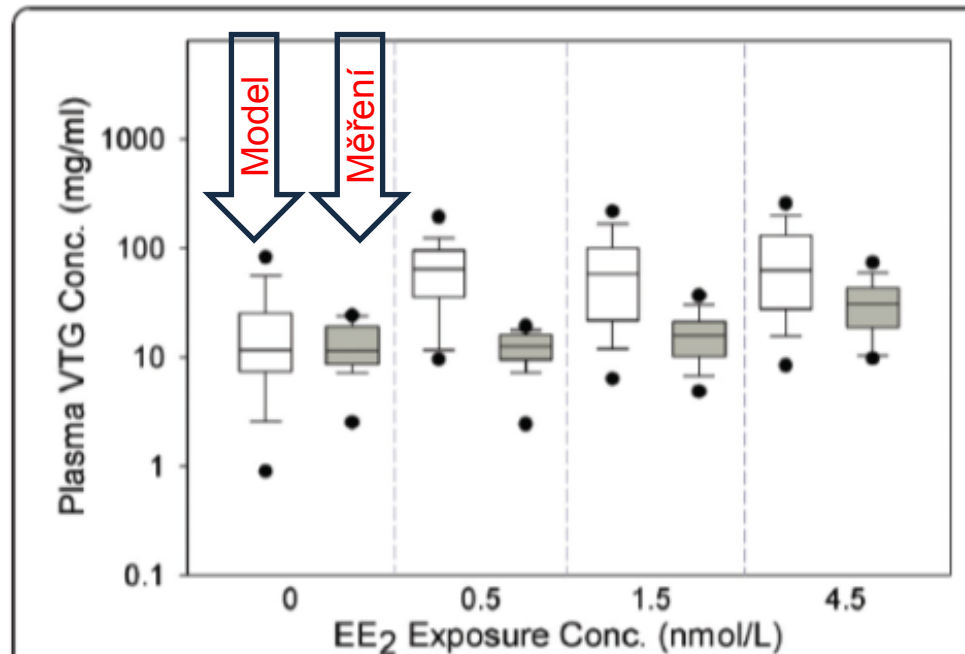
Konceptní model  
→ ZOOM



EE2 – toxikant  
ER, AR atd. – receptory

VTG – vitellogenin  
(marker toxicity)

*Každá šipka:  
zvláštní diferenciální  
rovnice*



**Figure 6** Comparison of model predictions with measured data in female FHMs exposed to EE<sub>2</sub>.  $n = 28$  at each sampling time. White boxes represent model predictions, and grey boxes represent measured data [42]. The x-axis represents EE<sub>2</sub> concentrations in ng/L. The solid line within the box marks the median; the boundary of the box farthest from zero indicates the 75<sup>th</sup> percentile; the boundary of the box closest to zero indicates the 25<sup>th</sup> percentile; the whisker (error bar) farthest from zero marks the 90<sup>th</sup> percentile; whisker (error bar) closest to zero marks the 10<sup>th</sup> percentile; the circle farthest from zero marks the 95<sup>th</sup> percentile; and the circle closest to zero marks the 5<sup>th</sup> percentile.

Výsledek:

Srovnání  
MODEL vs. MĚŘENÍ






# Kvantitativní mechanistické modelování



 OPEN ACCESS  PEER-REVIEWED

RESEARCH ARTICLE

## A Computational Model of the Rainbow Trout Hypothalamus-Pituitary-Ovary-Liver Axis

Kendall Gillies, Stephen M. Krone, James J. Nagler, Irvin R. Schultz 

Published: April 20, 2016 • <https://doi.org/10.1371/journal.pcbi.1004874>

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### Abstract

Reproduction in fishes and other vertebrates represents the timely coordination of many endocrine factors that culminate in the production of mature, viable gametes. In recent years



Centrum pro výzkum  
toxických látek  
v prostředí

# Kvantitativní mechanistické modelování

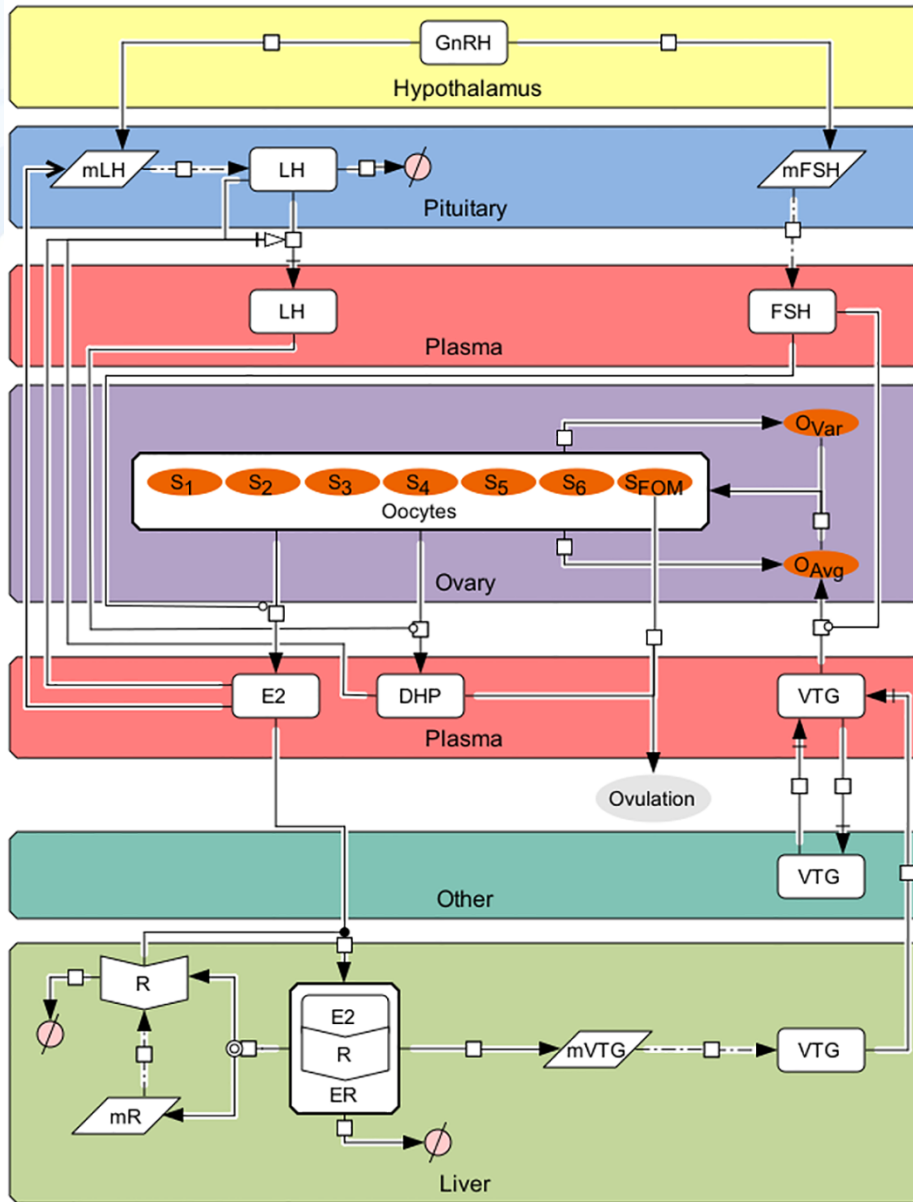
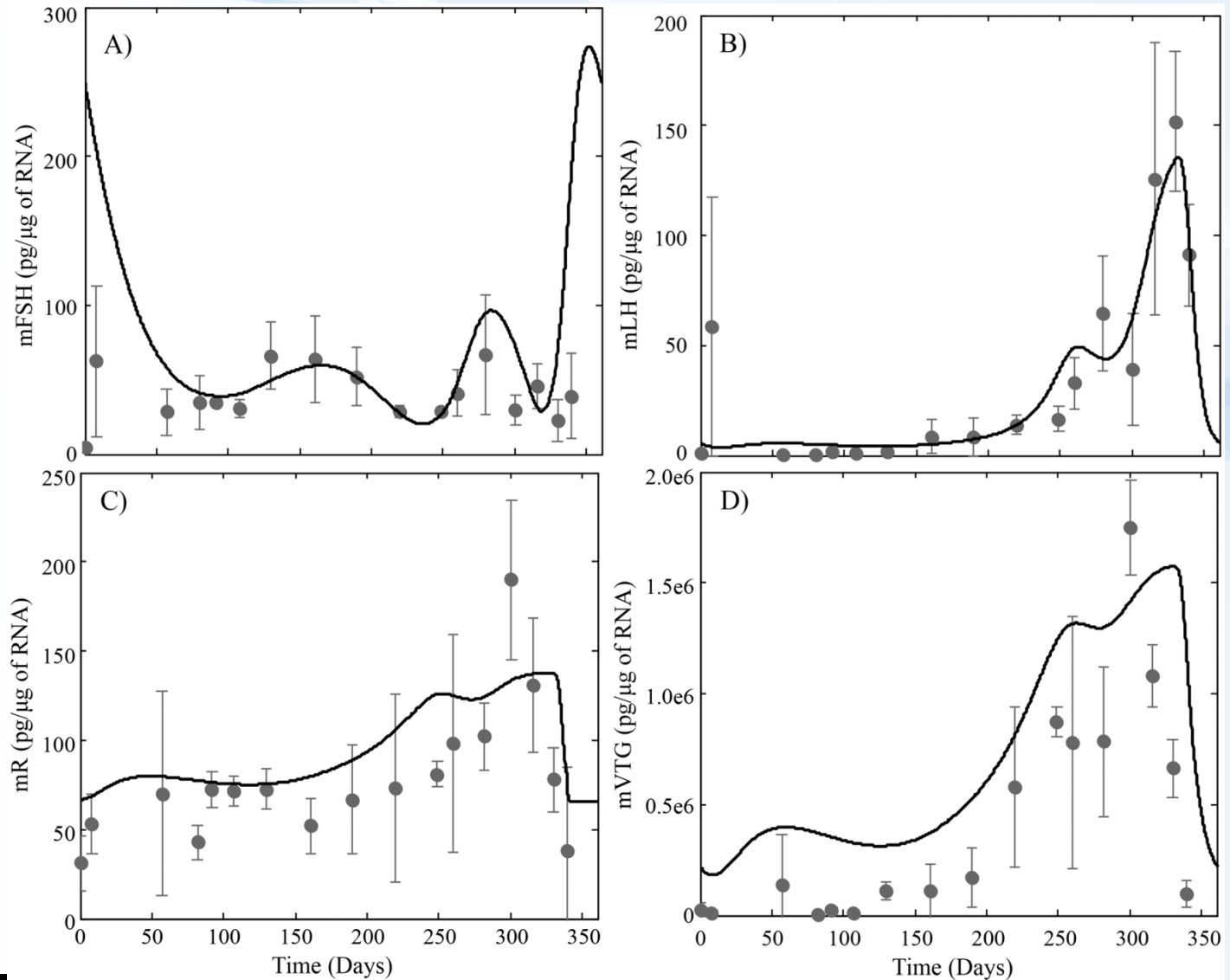


Fig 1. The HPOL signaling network in rainbow trout as formulated in our model.

Arrows and symbols on graph follow CellDesigner vs. 4.4 notation ([www.celldesigner.org](http://www.celldesigner.org)). GnRH is secreted from the hypothalamus into the pituitary stimulating the production of mFSH and mLH, which then leads to formation of FSH and LH, respectively. FSH, which is being continuously secreted from the pituitary, travels to the ovaries to stimulate production of E2. E2 then travels to the liver to bind with E2 receptors (R; translated from mR) to form ER. ER then stimulates the production of mVTG, which produces VTG<sub>L</sub>. Secreted VTG then travels from the liver to the ovaries via the plasma (VTG<sub>P</sub>) where it is absorbed by follicles in stages 3 through 6 (the proportion of follicles in these stages are denoted by  $S_j$ ,  $j = 3, 4, 5$ , and 6) during vitellogenesis, the rate of which is affected by FSH<sub>P</sub>, to promote oocyte growth ( $O_{Avg}$ ). Oocyte growth then progresses the oocytes through the stages using a Weibull distribution created from  $O_{Avg}$  together with  $O_{Var}$ . In the later stages LH<sub>P</sub> stimulates the oocytes to produce DHP. Finally, oocytes undergo final maturation ( $S_{FOM}$ ) and combined with DHP, determine when the fish ovulates

# Kvantitativní mechanistické modelování

Fig 3. HPOL **model predictions** for (A) pituitary levels of FSH $_{\beta}$  subunit mRNA, (B) pituitary levels of LH $_{\beta}$  subunit mRNA, (C) Hepatic levels of E2 receptor mRNA and (D) Hepatic levels of VTG mRNA  
**Observed data** (dark grey circles; mean TG mRNA = 3)



# Nano-eko-toxikologie



# NANOČÁSTICE

- „NANO“ – relativně nová oblast, řada praktických využití
- **ALE: unikátní vlastnosti**
  - Vlastnosti nanočástic (včetně toxicity) nelze odvodit z vlastností částic z téhož materiálu o větších rozměrech a ani z vlastností chemikálie, ze které je materiál tvořen
- Definice
  - **Nanočástice** (nanoparticles): alespoň jeden rozměr <100 nm
  - **Nanočástice přírodního původu** - „ultrafine particles“ přítomné v přírodních aerosolech nebo jako vedlejší produkt lidské činnosti (prach, dým, kouř apod.)
  - **Vyráběné nanomateriály** (manufactured, engineered NM)
  - **Nanoaerosoly**: aerosoly jednotlivých volných nanočástic nebo nanostrukturních částic (= aglomerátů nanočástic nebo nanovláken) – přírodního původu nebo vyráběných

# Základní charakteristiky vyráběných NM

## Kovy

- stříbro
- zlato
- železo
- *další*

## Oxidy kovů

- $\text{TiO}_2$
- $\text{Al}_2\text{O}_3$
- $\text{SiO}_2$
- $\text{ZnO}$
- $\text{ZrO}_2$
- *další*

## Uhlíkové NM

- nanotrubičky
- fullereny
- saze
- nanodiamanty

## Další anorganické NM

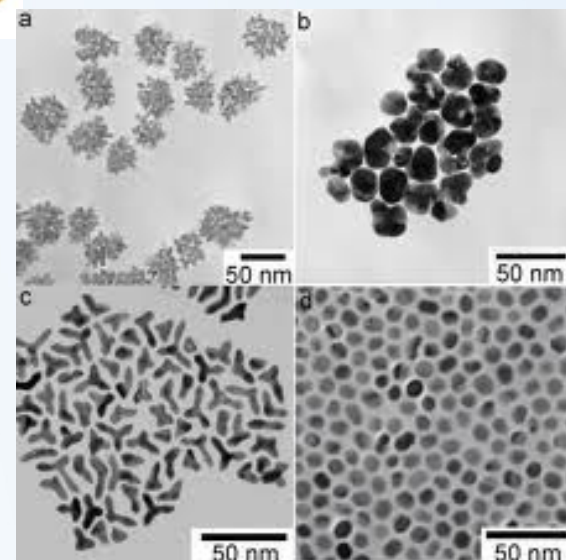
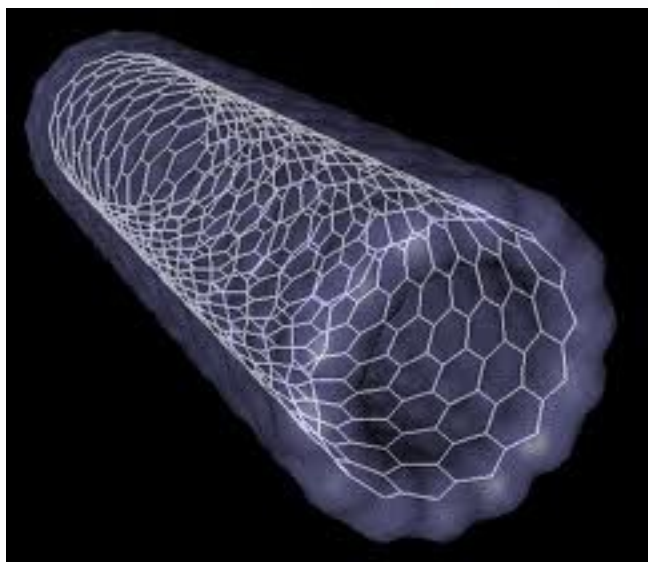
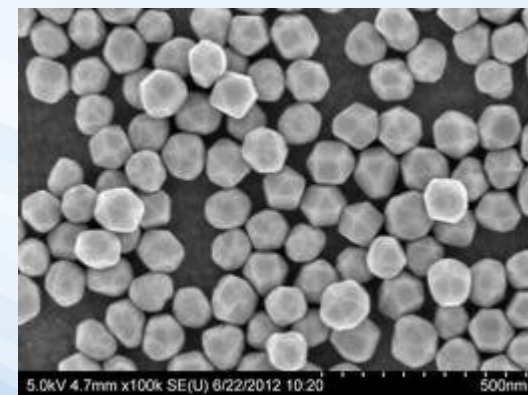
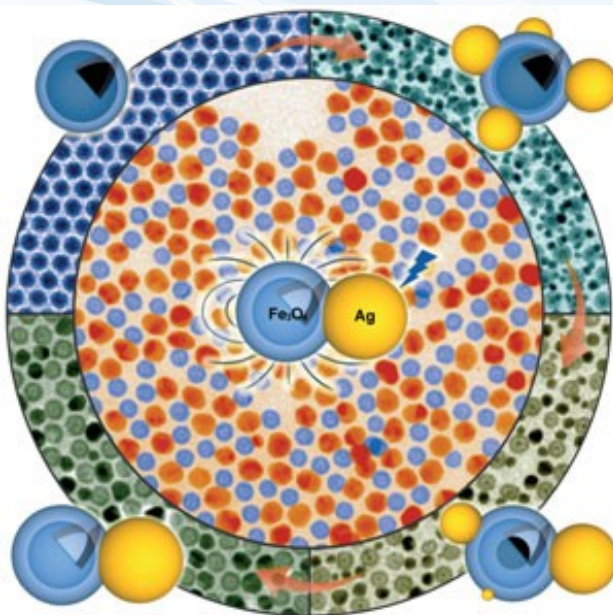
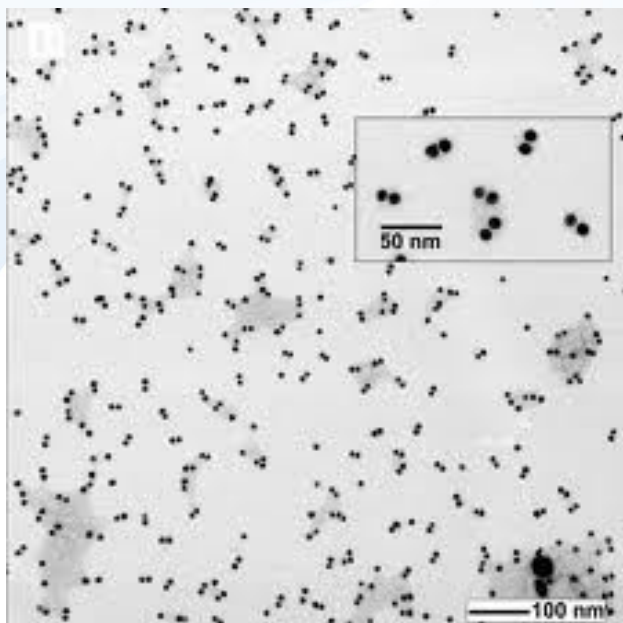
- magnetické materiály
- kompozitní nanomateriály
- kvantové tečky
- silikáty, zeolity, jíly
- anorganická nanovlákná

## Organické NM

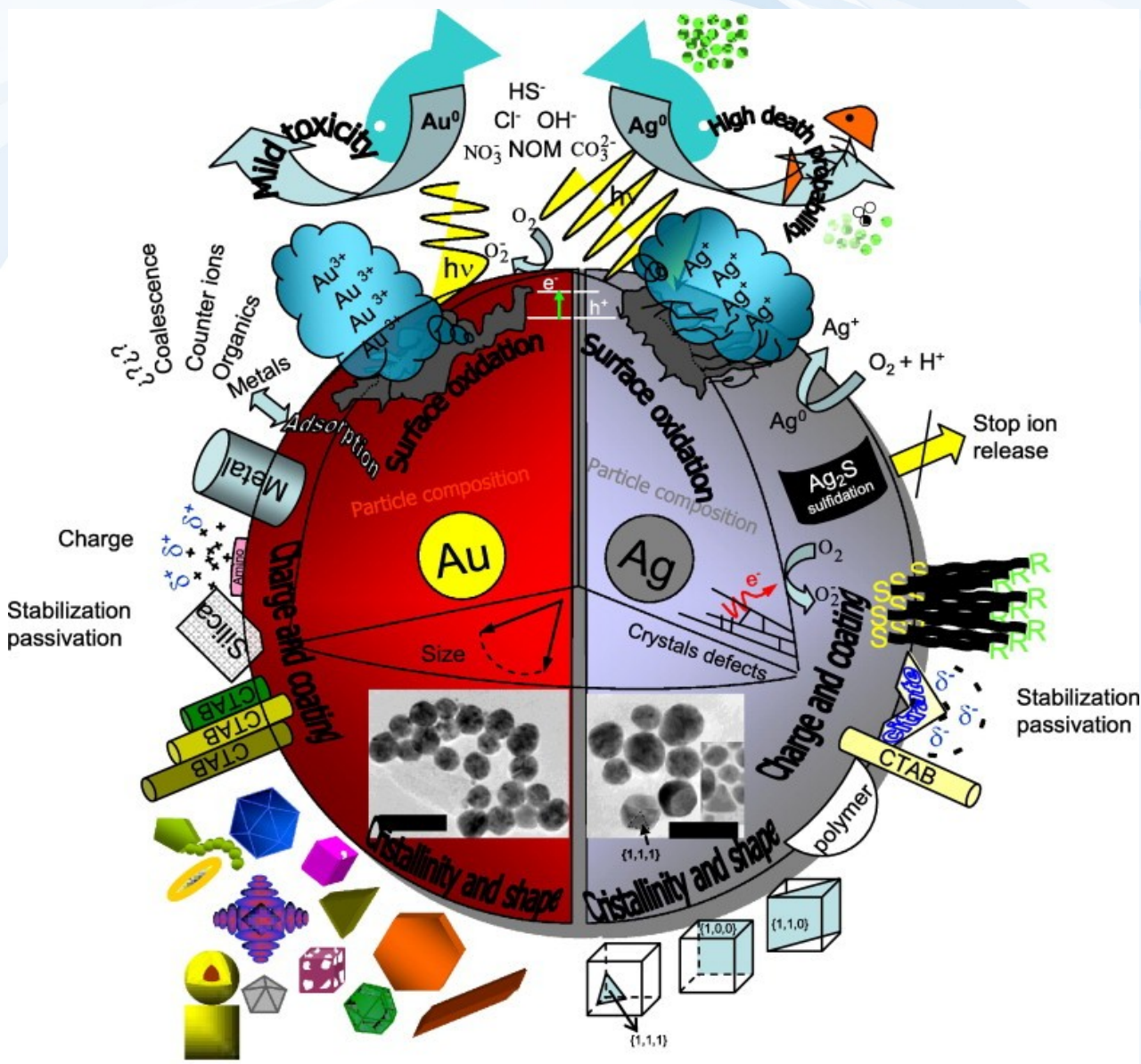
- nanovlákná polymerů
- dendrimery
- polystyren



# Příklady - nanočástice



# (Eko)toxicita nanočástic – specifické vlastnosti



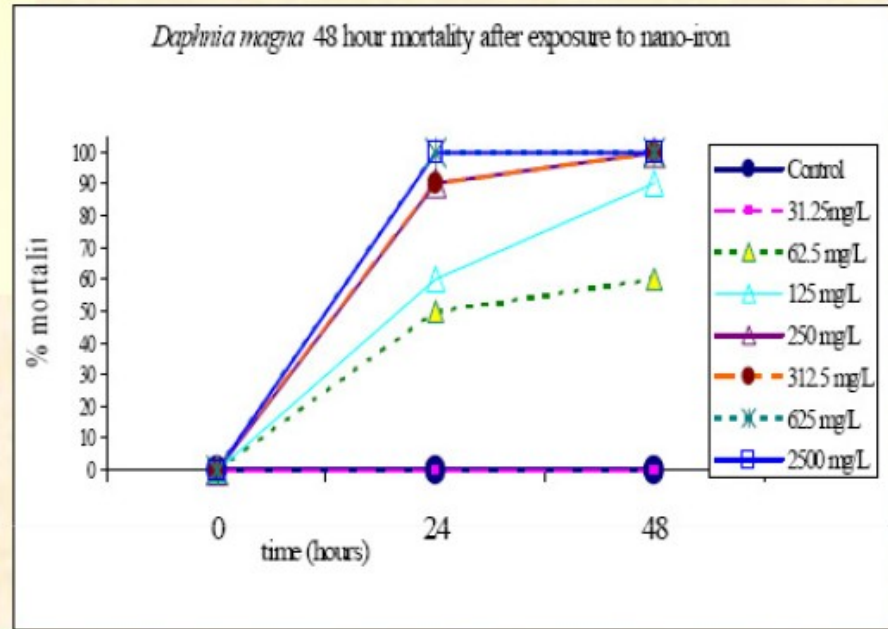
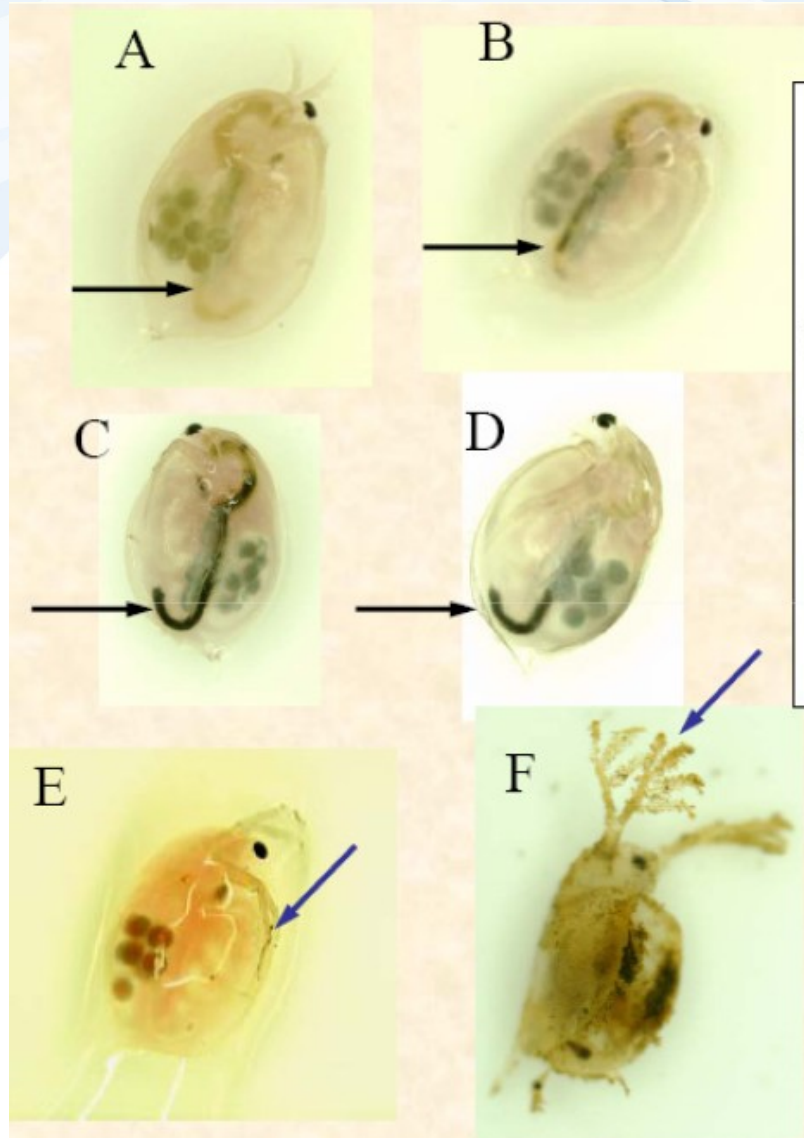
(Neznámé) parametry částic, které mohou mít vliv na toxicitu

Složení (chemické)  
Povrch (velikost, tvar)  
Náboj  
Stabilita  
Agregace částic  
Interakce s chemikáliemi  
Interakce s ionty

Vliv na osud látek  
Přímá toxicita



# Nanočástice → mechanické vlivy = toxicita



Daphnia exposed to various concentrations of nano-iron used in remediation. A = control; B = 3 mg/L; C = 7.5 mg/L; D = 15 mg/L; E = 30 mg/L; F = 125 mg/L (dead daphnid). All daphnids shown are 21 days old and eggs are visible in their brood pouches (green circles). Note the darkening of the digestive tract from A (normal greenish color) to D with increased ingestion of nano-iron particles (black arrows). Antennae become clogged with nano-iron in E and F (blue arrows). The 24 and 48 hour mortality curve is shown on the right.

# Novinky ... stresová biologie



## LETTER

### Sperm of colourful males are better protected against oxidative stress

#### Abstract

Sperm cells are highly vulnerable to free radicals, and sperm quality and male fertility are critically affected by oxidative stress. Recently, sexual ornaments, particularly carotenoid-based colourful traits, have been proposed to depend on a male's capacity to resist oxidative stress, and thus to signal sperm quality. We conducted an experimental test of this hypothesis on great tits *Parus major*, in which adults are sexually dichromatic in carotenoid-based breast plumage. We report the first evidence that ornaments and sperm quality may be linked through oxidative stress. When experimentally subjected to oxidative stress resulting from increased workload, less colourful males suffered a greater reduction in sperm motility and swimming ability, and increased levels of sperm lipid peroxidation compared to more colourful males. Moreover, the level of sperm lipid peroxidation was negatively correlated with sperm quality. Finally, carotenoid supplementation increased sperm quality of less colourful males, suggesting that pale males are deficient in carotenoid antioxidants.

Fabrice Helfenstein,<sup>1\*</sup> Sylvain Losdat,<sup>1</sup> Anders Pape Møller,<sup>2,3</sup> Jonathan D. Blount<sup>4</sup> and Heinz Richner<sup>1</sup>

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<sup>3</sup>Center for Advanced Study, Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA



**Barevnější samci sýkor**  
 → Atraktivnější pro samice ...  
 → Lepší kvalita spermatu  
 (karotenoidy brání proti oxidativnímu stresu)

# Combined pesticide exposure severely affects individual- and colony-level traits in bees

Richard J. Gill, Oscar Ramos-Rodriguez & Nigel E. Raine

[Affiliations](#) | [Contributions](#) | [Corresponding authors](#)

*Nature* 000 (2012) | doi:10.1038/nature11585

Received 19 April 2012 | Accepted 13 September 2012 | Published online 21 October 2012

- **Čmeláci a pesticidy**
  - Velice významní opylovači
  - Specifická biologie oproti včelám
    - kolonie s velmi malým počtem jedinců
  - Současné aplikace různých pesticidů na sousedních polích
    - V praxi není koordinace mezi farmáři: koexpozice



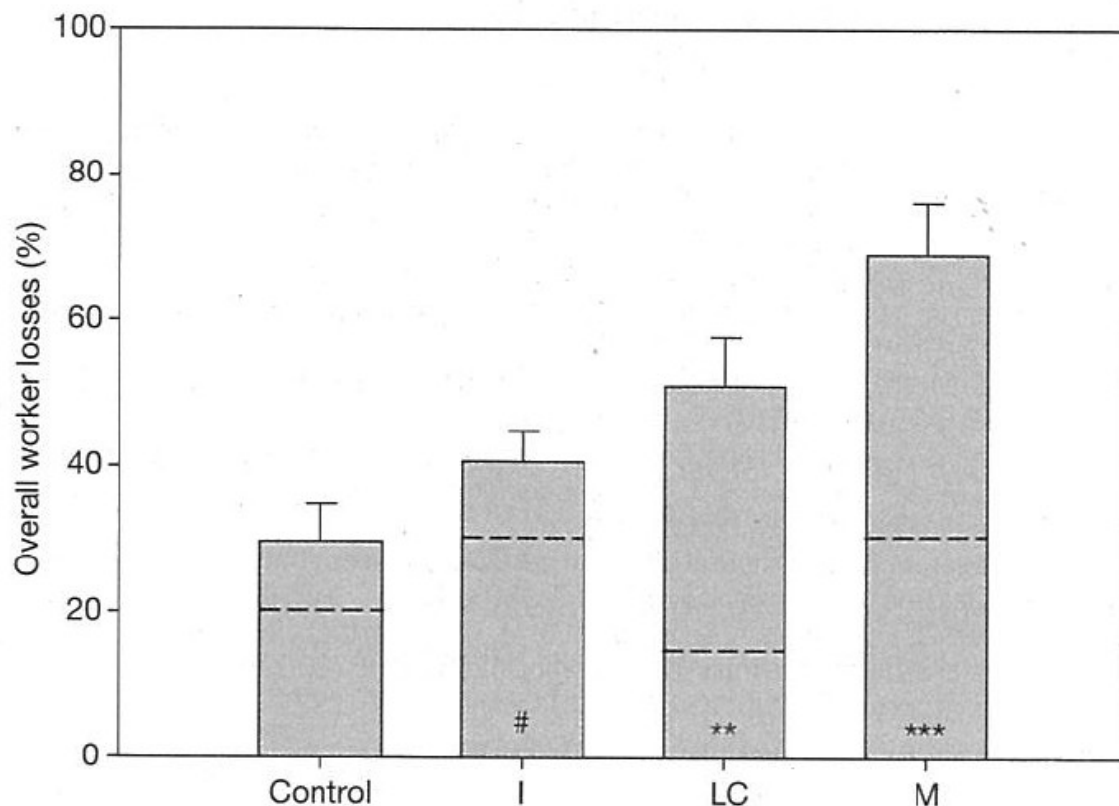


**Figure 1 | A complex exposure landscape.** In a typical agricultural setting, different crops may be sprayed with different pesticides at different times and doses. Bees will obtain food both from these crops and from wild plants, which makes it difficult to estimate their overall exposure to chemicals. Furthermore, bees returning to the colony after foraging may pass on the pesticides as they feed larvae. In an attempt to partially mimic this exposure complexity, Gill *et al.*<sup>10</sup> placed pesticide-laden feeders and filter paper (not shown) at the entrance to boxed colonies of bumblebees, which could also access flowers on crops and wild plants in the wider landscape. The researchers measured the effect of these added pesticides at both the individual-bee and colony level.

## Vliv pesticidů na čmeláky – polní studie: aplikovány povolené dávky

- 2 individuální látky „I“ a „LC“
- současná expozice „M“ (mixed)

Celkové ztráty  
dělnic v  
průběhu experimentu



**Figure 3 | Overall worker losses.** Mean ( $\pm$  s.e.m.) overall percentage of workers lost per colony, including workers lost outside (below the dashed line) and worker mortality (dead workers found in nest box; above the dashed line), during the 4-week experiment.  $n = 40$  colonies. # $P \leq 0.1$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$  (comparison with control).

