



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.



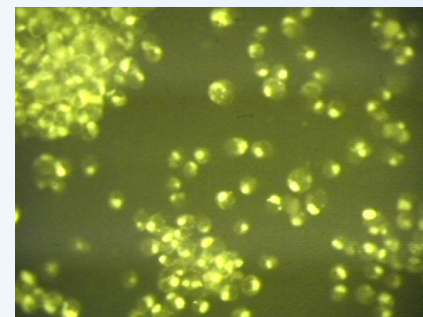
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Přehled závěrečné přednášky

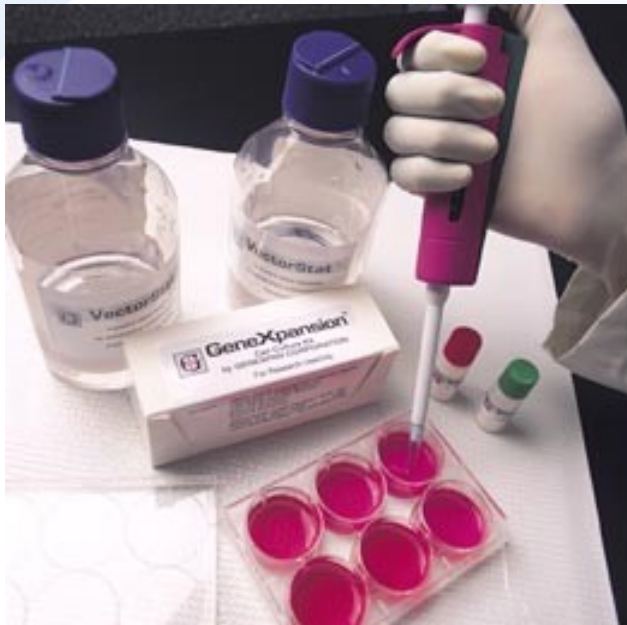
- Moderní přístupy experimentální ekotoxikologie
 - In vitro modely
 - Biomarkery a „MOA“ (mode-of-action / omics) techniky
- Modely v ekotoxikologii
 - SAR a QSAR
 - AOP / PBPK / TOXCAST
- „Nové“ problémy v ekotoxikologii
 - Nanočástice
- Novinky a zajímavosti

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



Výzkumy mechanismů toxicity in vitro modely a biotesty



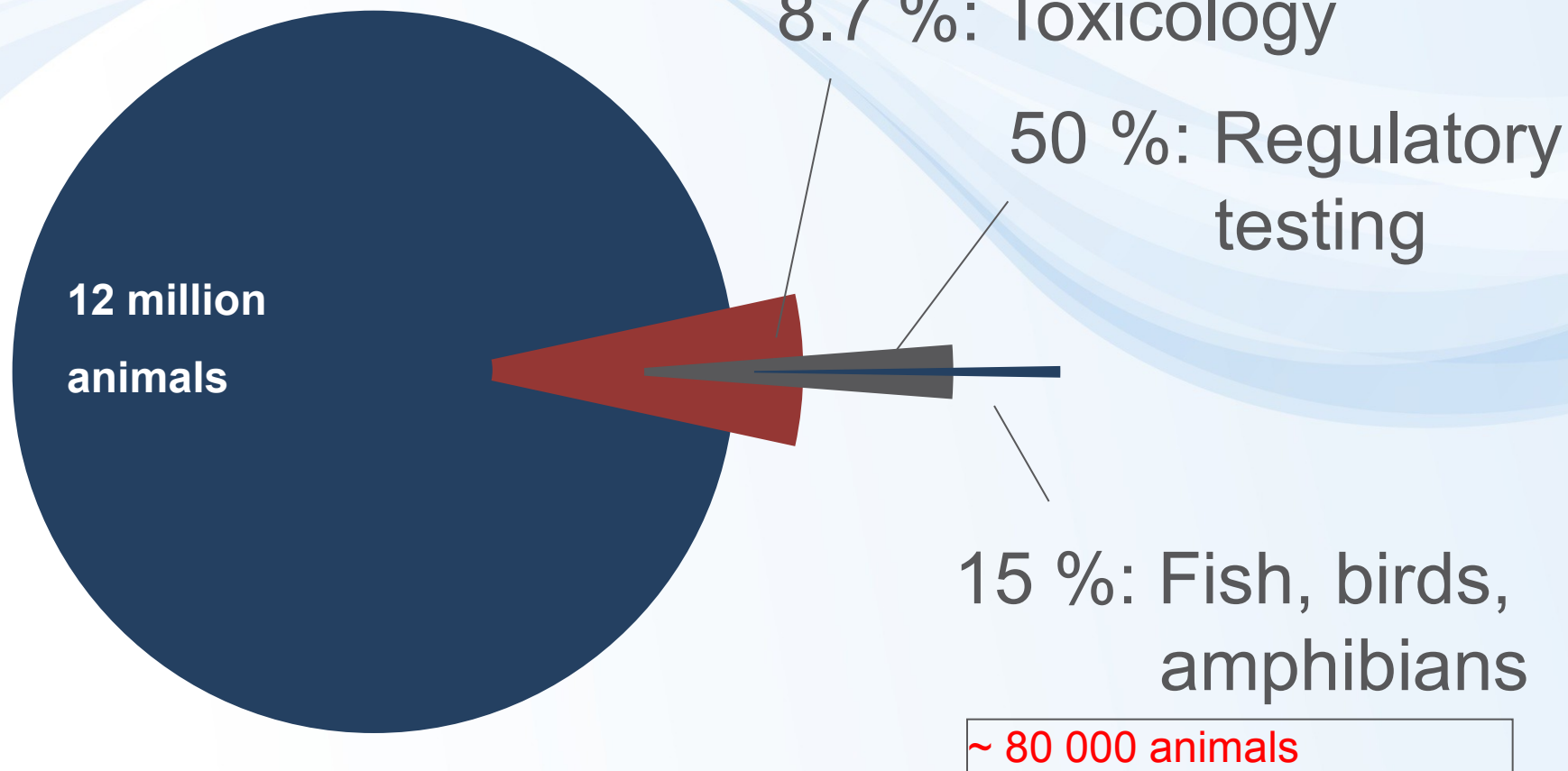
Práce s in vitro kulturami
(kultivační / expoziční
nádoby a média)



Sterilní práce s modely in
vitro



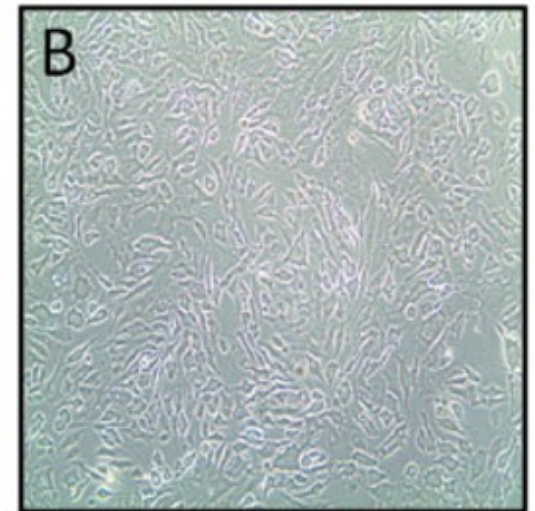
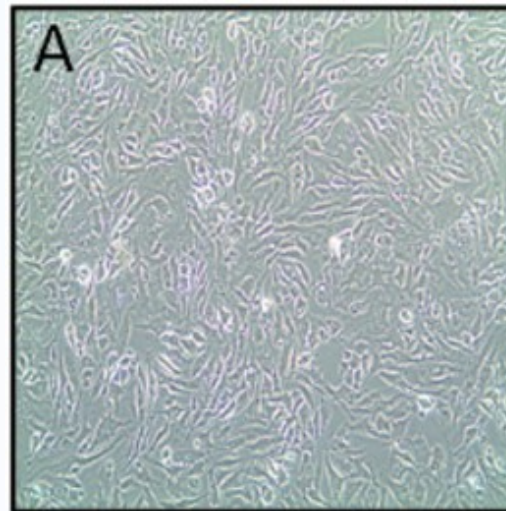
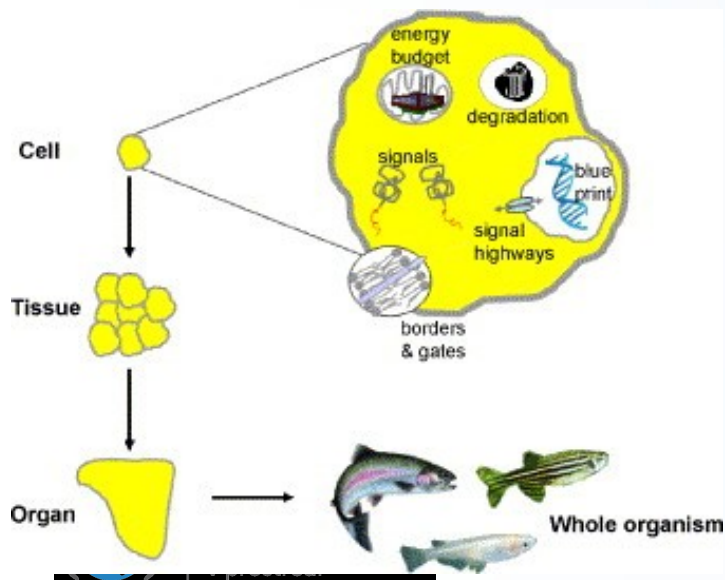
Počty obratlovců používaných pro hodnocení chemických látek v Evropě



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*



In vitro modely – stanovení specifických účinků

Jaderné receptory – ER, AhR

- Toxicita závislá na jaderných receptorech → velký význam v ekotoxicitě (viz jinde v přednáškách)
- Jaderné receptory = transkripční faktory
 - Aktivace receptoru → produkce nových proteinů (zvýšené hladiny = marker expozice)
 - Možnosti měření
 - Měření aktivity (např. oxidace – CYP450 – viz dále)
 - Měření celých proteinů (např. WesternBlotting nebo ELISA)
 - Měření na úrovni mRNA – genová exprese - PCR nebo kvantitativní PCR
 - Sleování „reporterových genů“ (viz dále)
- **Měření aktivity AhR → indukce detoxikačních enzymů – CYPs**
 - **Nejčastěji - měření oxidázové aktivity CYP (metoda EROD)**
 - (využívají se i další analýzy – Wblotting, mRNA ...)
- Měření aktivity ER → indukce ER-závislých proteinů
 - **Vitellogenin (prekurzor žloutkového proteinu u vejcorodých – ryby, ptáci ..):** měření WBlottin, ELISA nebo exprese mRNA



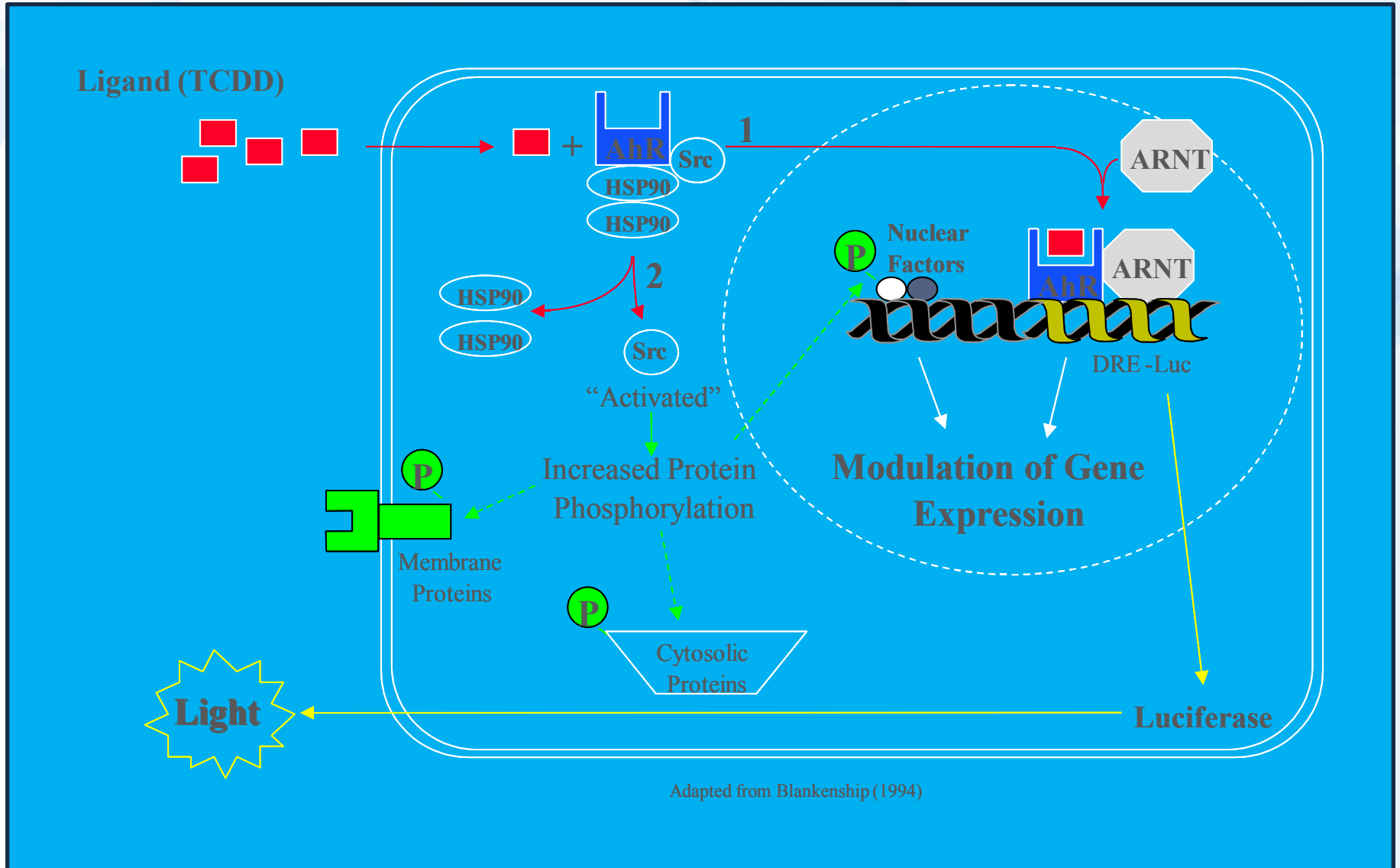
Reporterové testy

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)

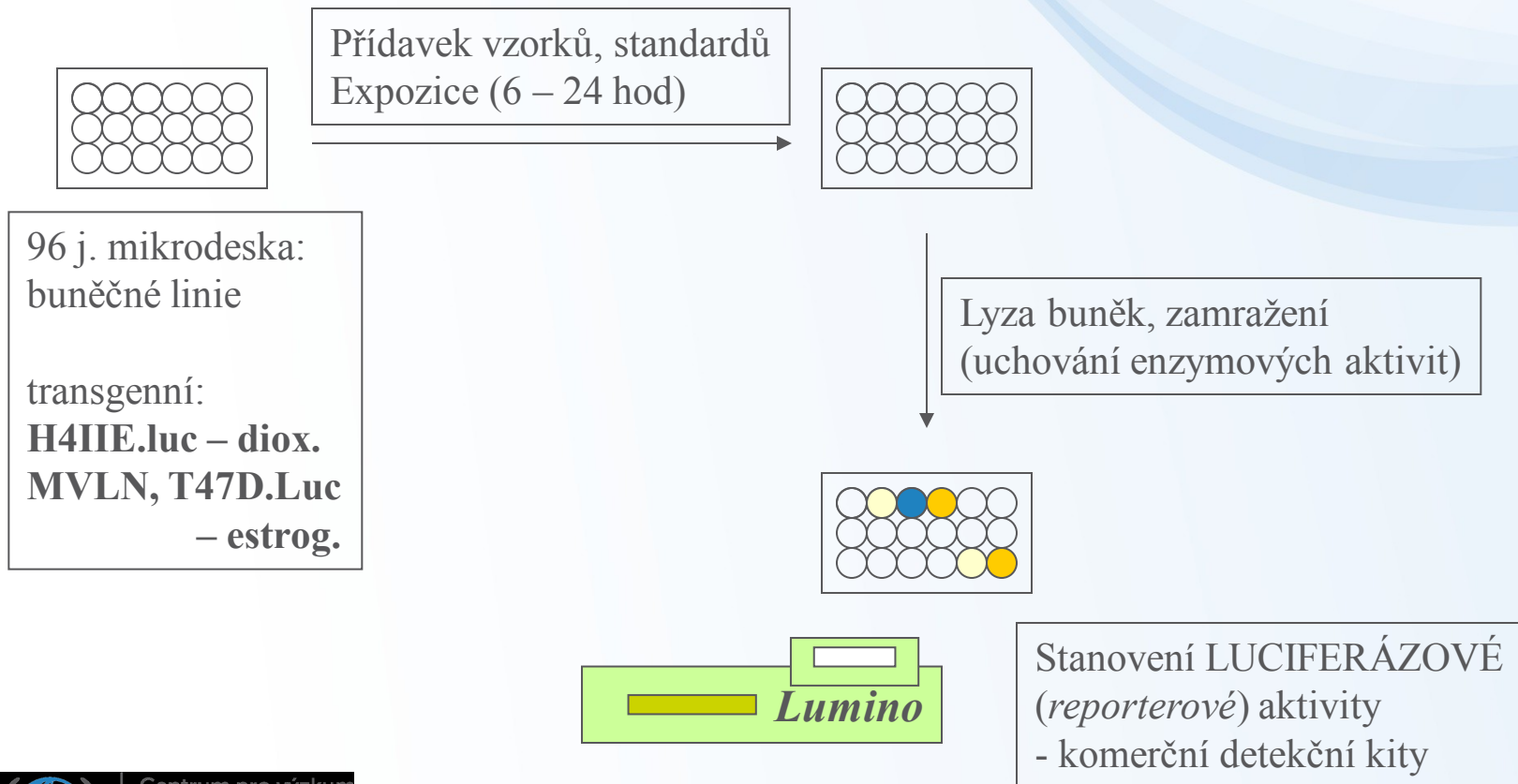


Stanovení toxicit závislých na intracelulárních receptorech



Stanovení aktivit/toxicit závislých na intracelulárních receptorech

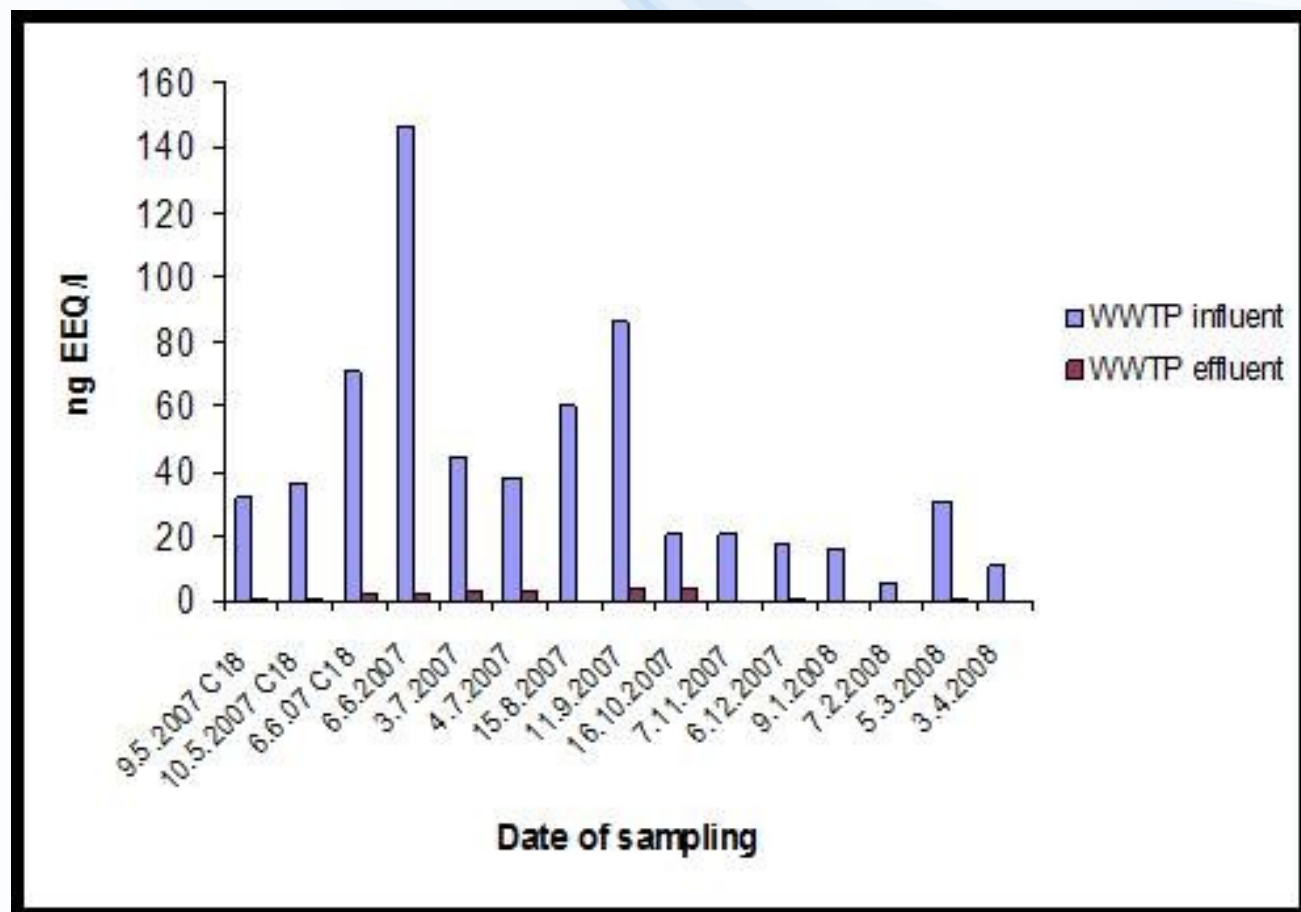
AhR, ER – experimentální design



Příklad – využití reporterových testů

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

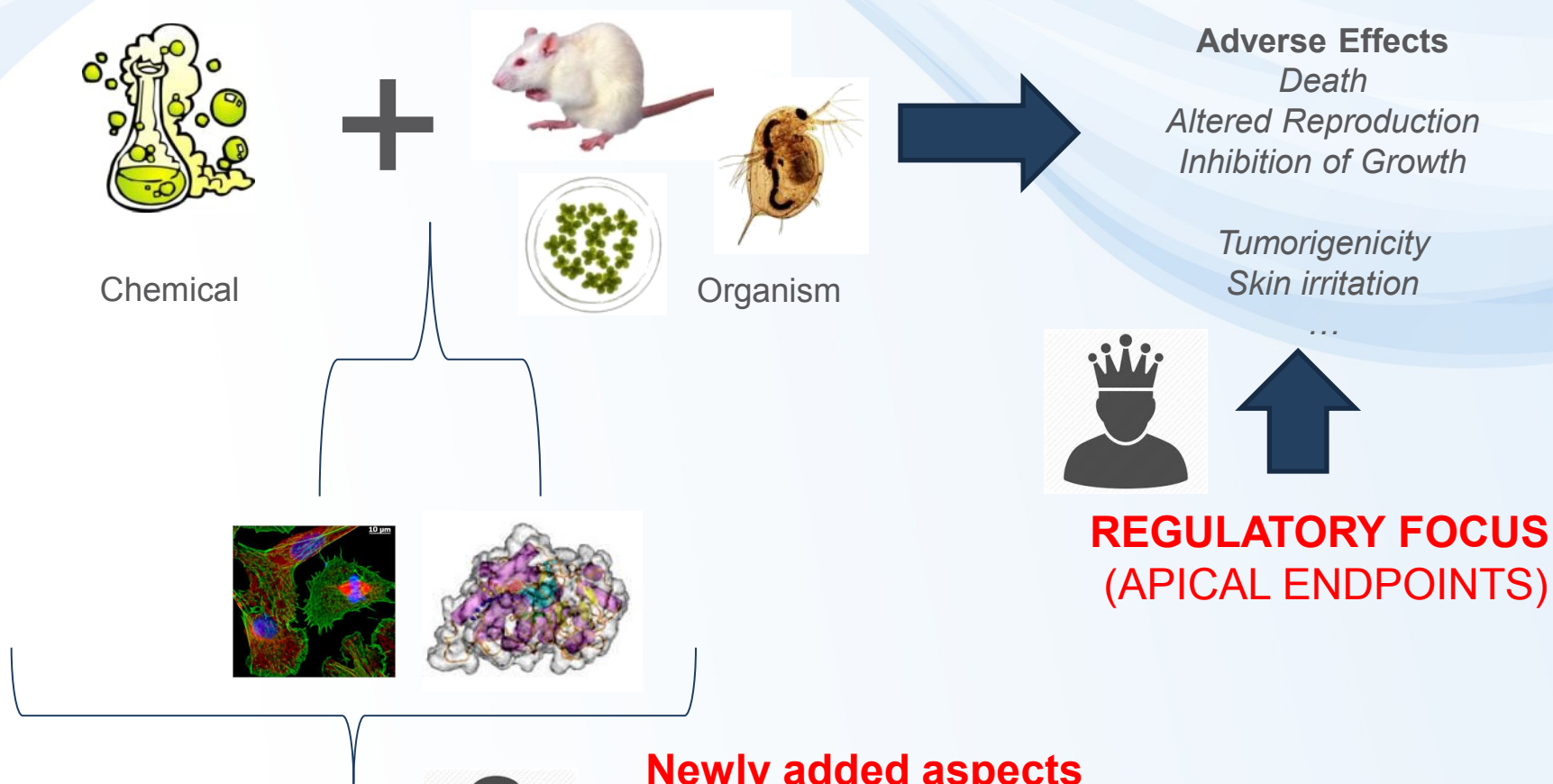
- Velká účinnost čištění
- Výsledné koncentrace (až 5 ng/L) jsou i tak biologicky účinné !



Budoucnost ekotoxikologie: Big data a „omics“

Hazard assessment

Traditionally – Evaluation of adverse effects using the whole organism models

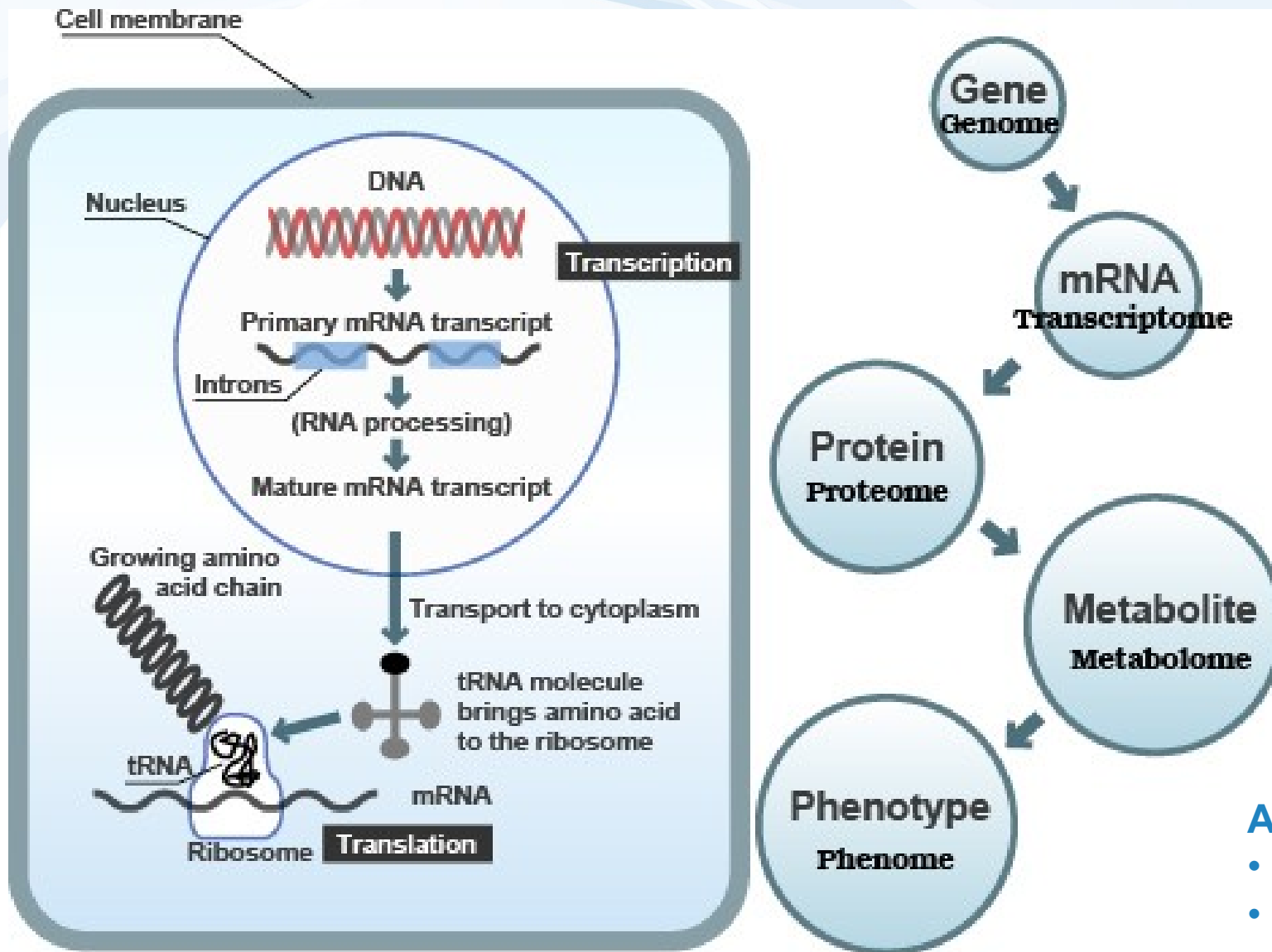


Newly added aspects

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



Extrémní rozvoj analytických technologií → „OMICS“



A další „ómy“

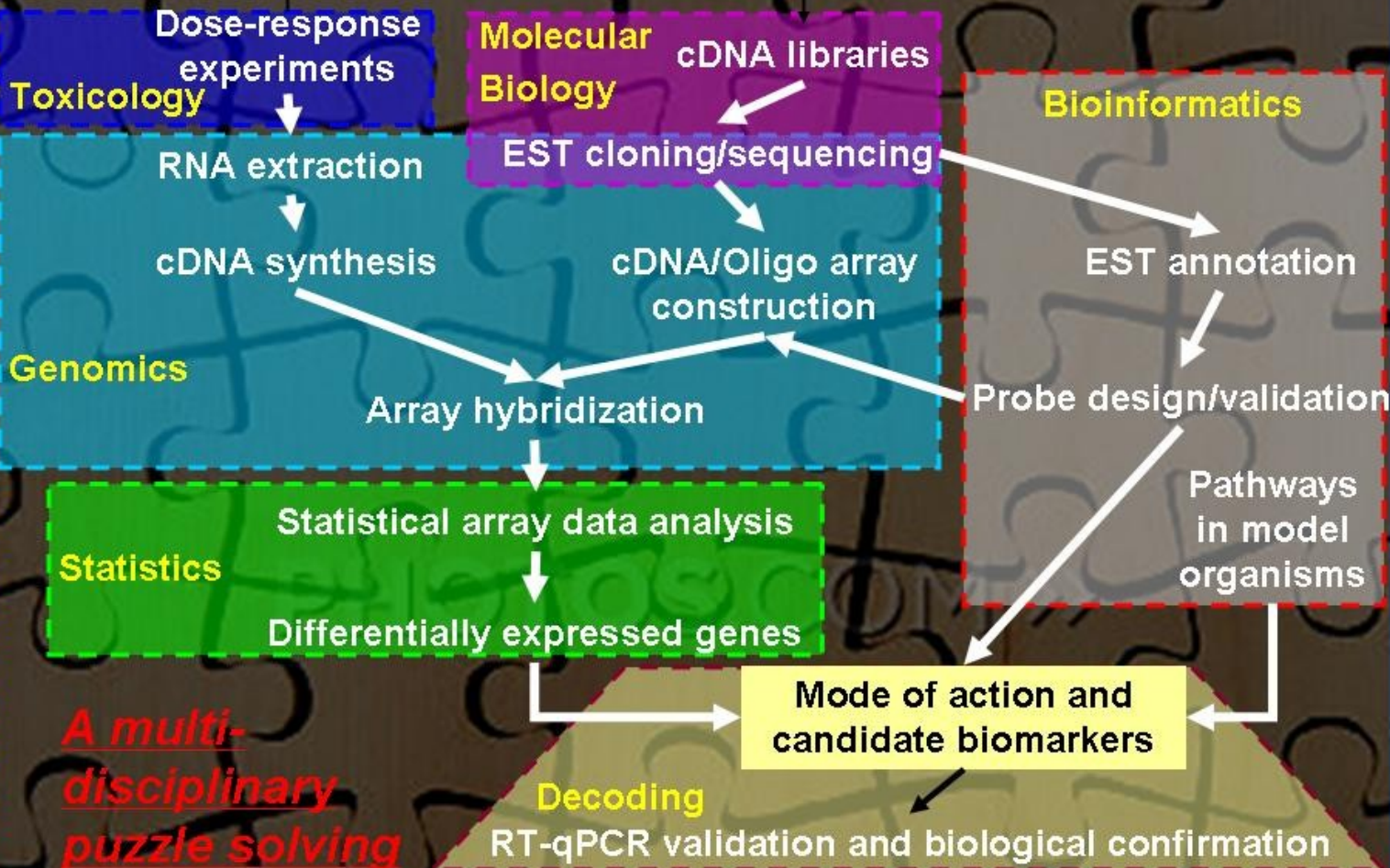
- Lipidóm
- Mikrobiom ...



Earthworm Toxicogenomics



US Army Engineer Research & Development Center



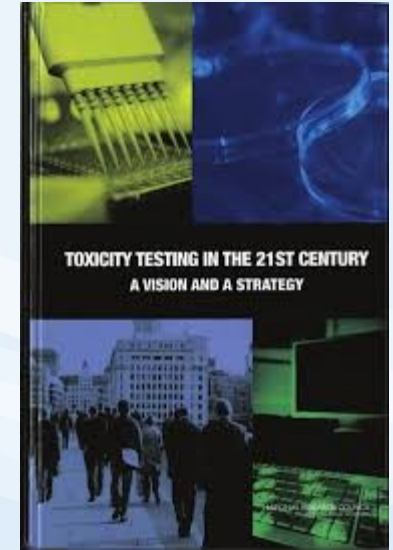
A multi-disciplinary puzzle solving exercise

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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Key Links

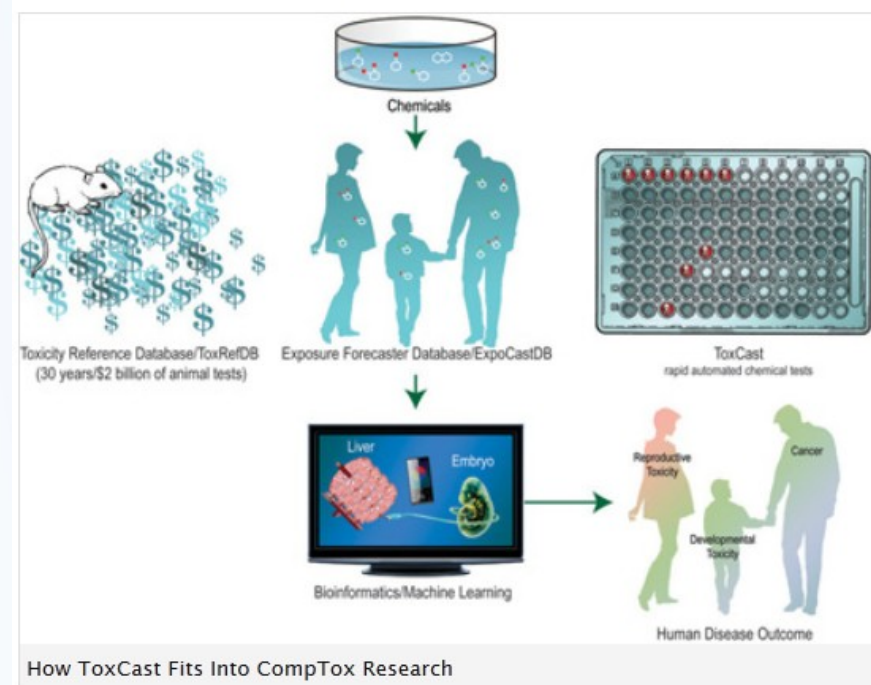
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[Organization](#)

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

R
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C

ToxCast™

Screening Chemicals to Predict Toxicity Faster and Better



Hazard assessment

Traditionally – Evaluation of adverse effects using the whole organism models



Chemical

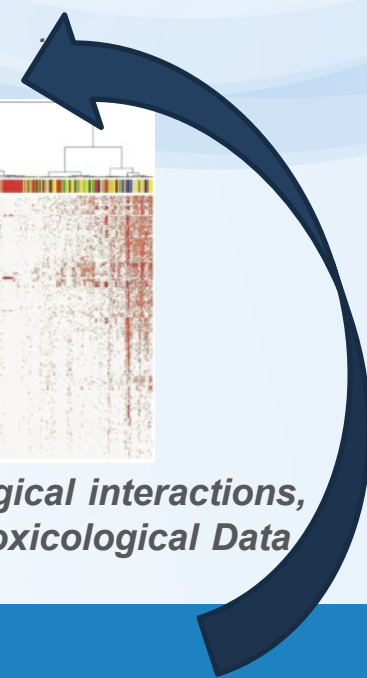


Organism



Adverse Effects (EC50)

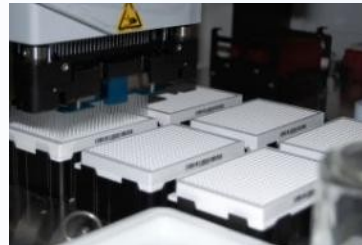
Death
Inhibition of Growth
Altered Reproduction
Tumor
Skin irritation



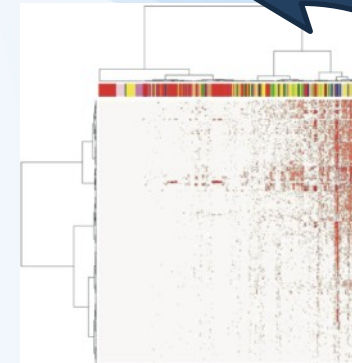
New – Ex vivo / in vitro / In chemico / In silico Methods



10⁴ 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

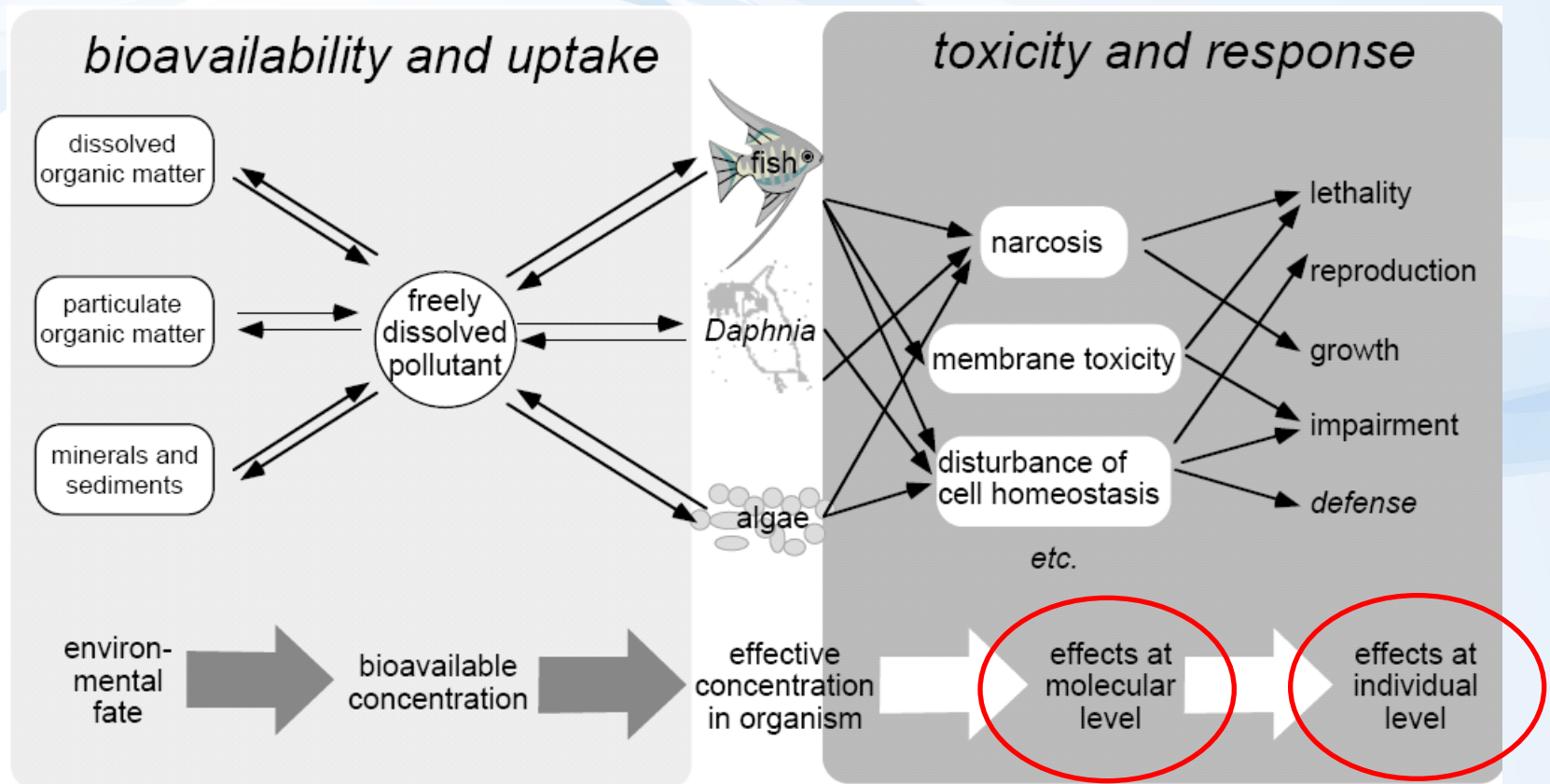
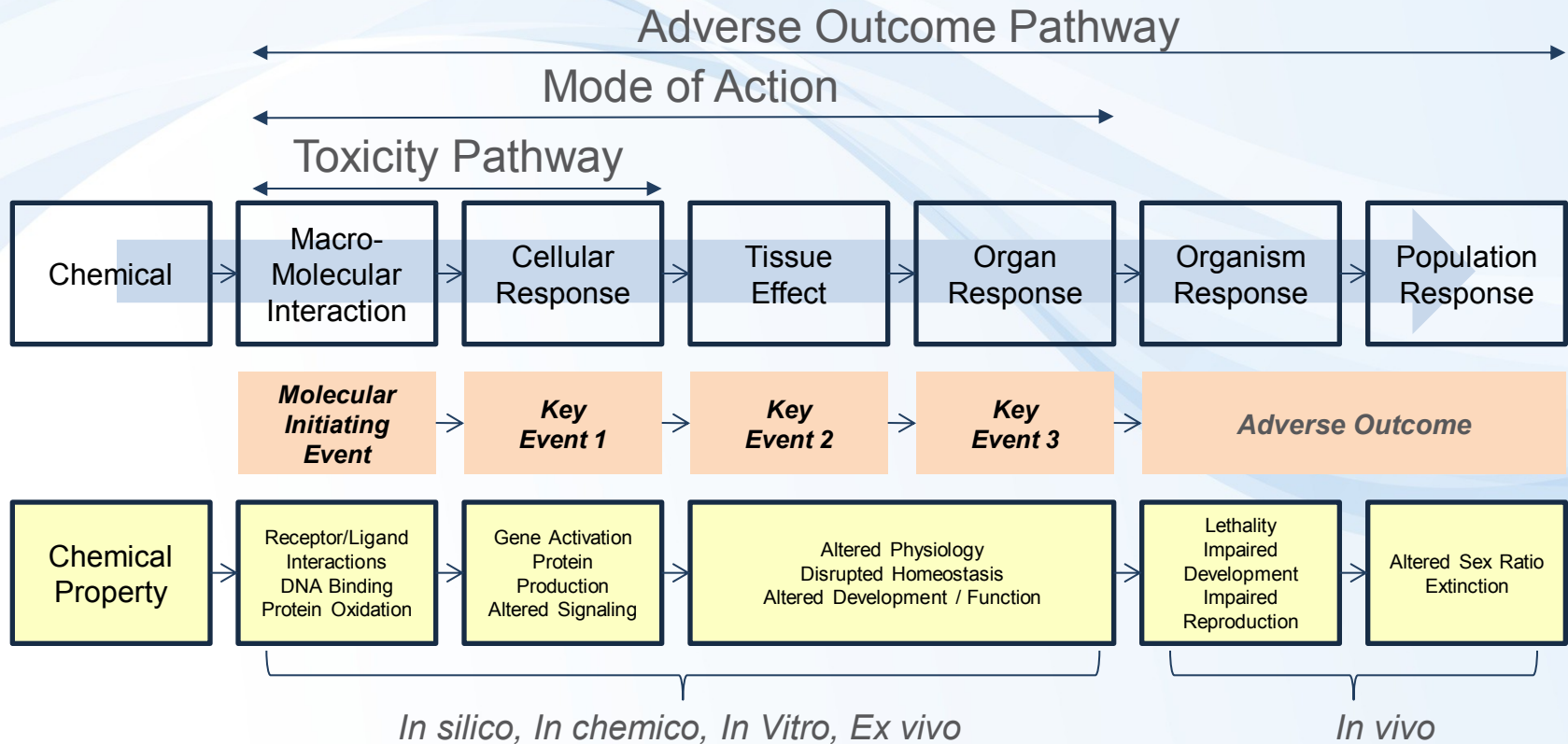


Figure 1 The effective concentration of a pollutant in an organism (e.g. fish, daphnia, algae) or at the target site inside the organism is the link between the environmental fate of a pollutant and its toxic effect.

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

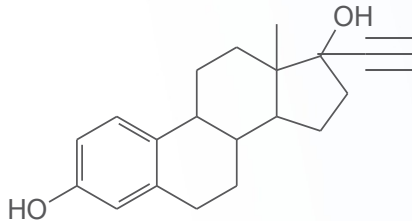


The **EXISTING KNOWLEDGE** is used **to link the** two anchor points: **Molecular Initiating Event (MIE)** and **Adverse Outcome (AO)** **via a series** of intermediate steps: **Key Events**

AOP Example:

Fish population decline due to estrogen receptor modulation

Ethinylestradiol (EE2)

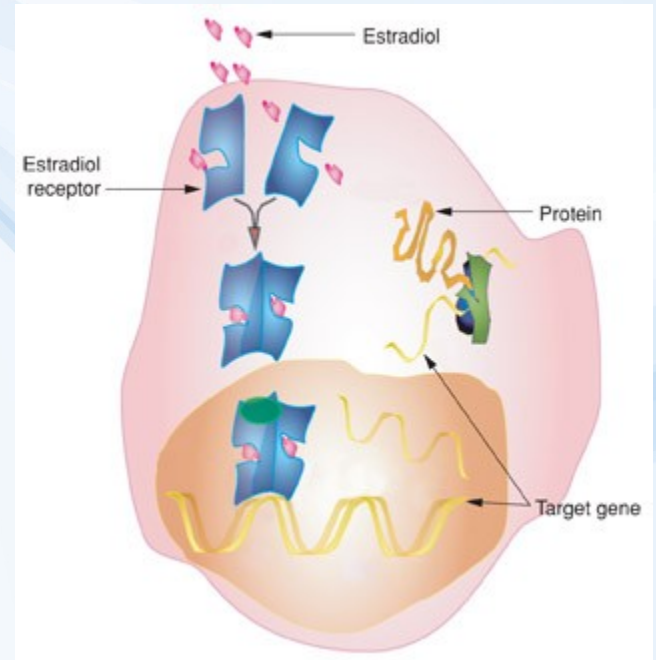


MIE

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)

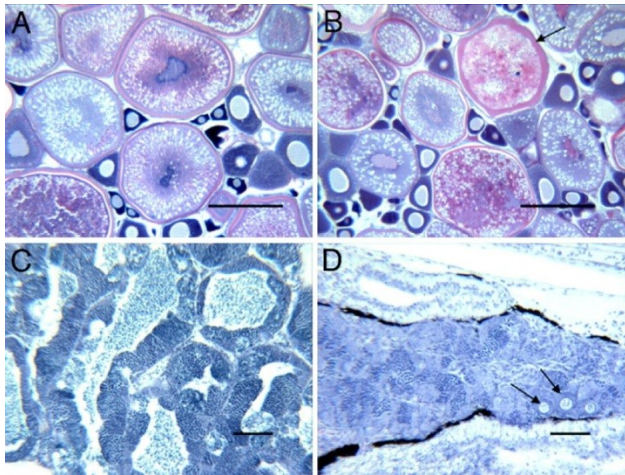
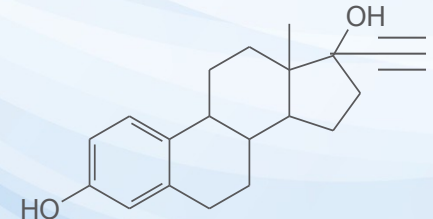


Adverse outcome – fish population decline

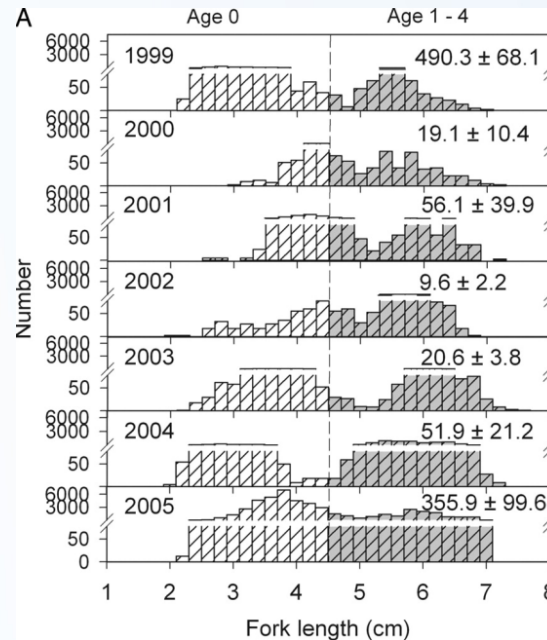
Kidd, K.A. et al. 2007. **Collapse of a fish population following exposure to a synthetic estrogen.** PNAS 104(21):8897-8901



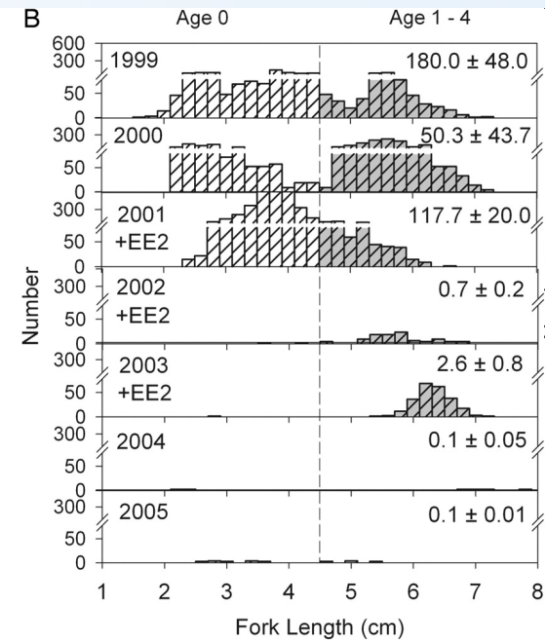
**EE2 - 5 ng/L (!)
7 years**



Control lake




lake with EE2



AOP a regulatorní prediktivní toxikologie


OECD AOP Knowledge Base

Strategické směřování – podpora OECD



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- > Assessment of chemicals
- > Risk management of chemicals
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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.

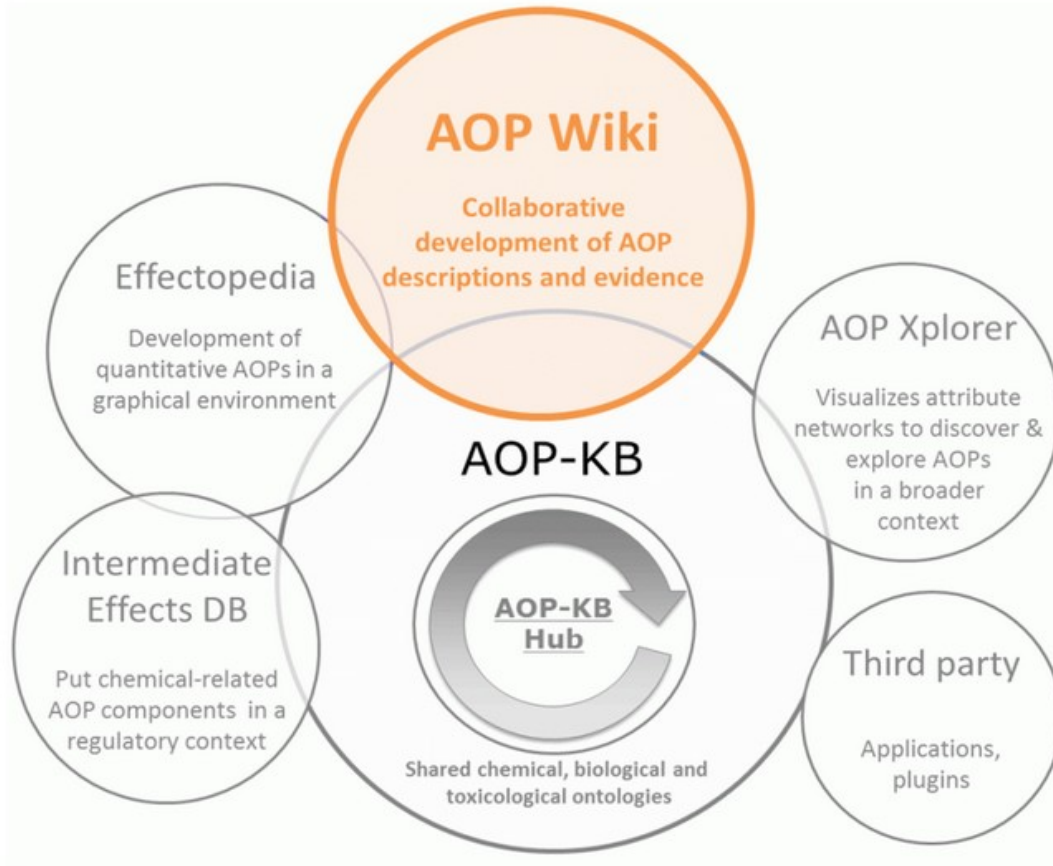
The survey is mainly for chemical safety regulators who are experiencing a transition in their work towards an increased use of 'alternative' methods and AOPs. However, stakeholders that come from the regulated community and environmental NGOs are also welcome to participate.

> **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

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



AOP Development

- Identification of the chemical-biological interaction - **Anchor 1**
- Understanding of the **apical outcome** elicited by the MIE - **Anchor 2**
- Identification of **intermediate key events** depends on the level of knowledge about this outcome



E.g. semi-automated literature searches, semantic & network methods
http://www.oecd.org/env/ehs/testing/AOP%20process_10%20June%202013.pdf

Key Events

- Are seminal **intermediate events** that are toxicologically relevant to the apical outcome.
- The basis for hypothesis development and testing => must be **experimentally quantifiable**
- Are **often assessed by rapid screening methods**

AOP Assessment

- Completeness (reliability & robustness)
- Qualitative Understanding
- **Weight of Evidence** supporting AOP (Bradford-Hill criteria)
- Quantitative Understanding



TABLE 1. Primary Components of an Adverse Outcome Pathway (AOP)

Key event (KE)	<ul style="list-style-type: none">• A measurable change in biological state that is essential, but not necessarily sufficient for the progression from a defined biological perturbation toward a specific AO.• Represented as nodes in an AOP diagram or AOP network.• Provide verifiability to an AOP description.
Key event relationship (KER)	<ul style="list-style-type: none">• Define a directed relationship between a pair of KEs, identifying one as upstream and the other as downstream.• Supported by biological plausibility and empirical evidence.• Represented as a directed edge (i.e., an arrow) in an AOP diagram or AOP network.• Unit of inference or extrapolation within an AOP.
Molecular initiating event (MIE)	<ul style="list-style-type: none">• A specialized type of KE.• Defined as the point where a chemical directly interacts with a biomolecule to create a perturbation—as such, by definition occurs at the molecular level.• Anchors the “upstream” end of an AOP.
Adverse outcome (AO)	<ul style="list-style-type: none">• A specialized type of KE.• Measured at a level of organization that corresponds with an established protection goal and/or is functionally equivalent to an apical endpoint measured as part of an accepted guideline test.• Generally at the organ level or higher.• Anchors the “downstream” end of an AOP.

AOPs and their links ...

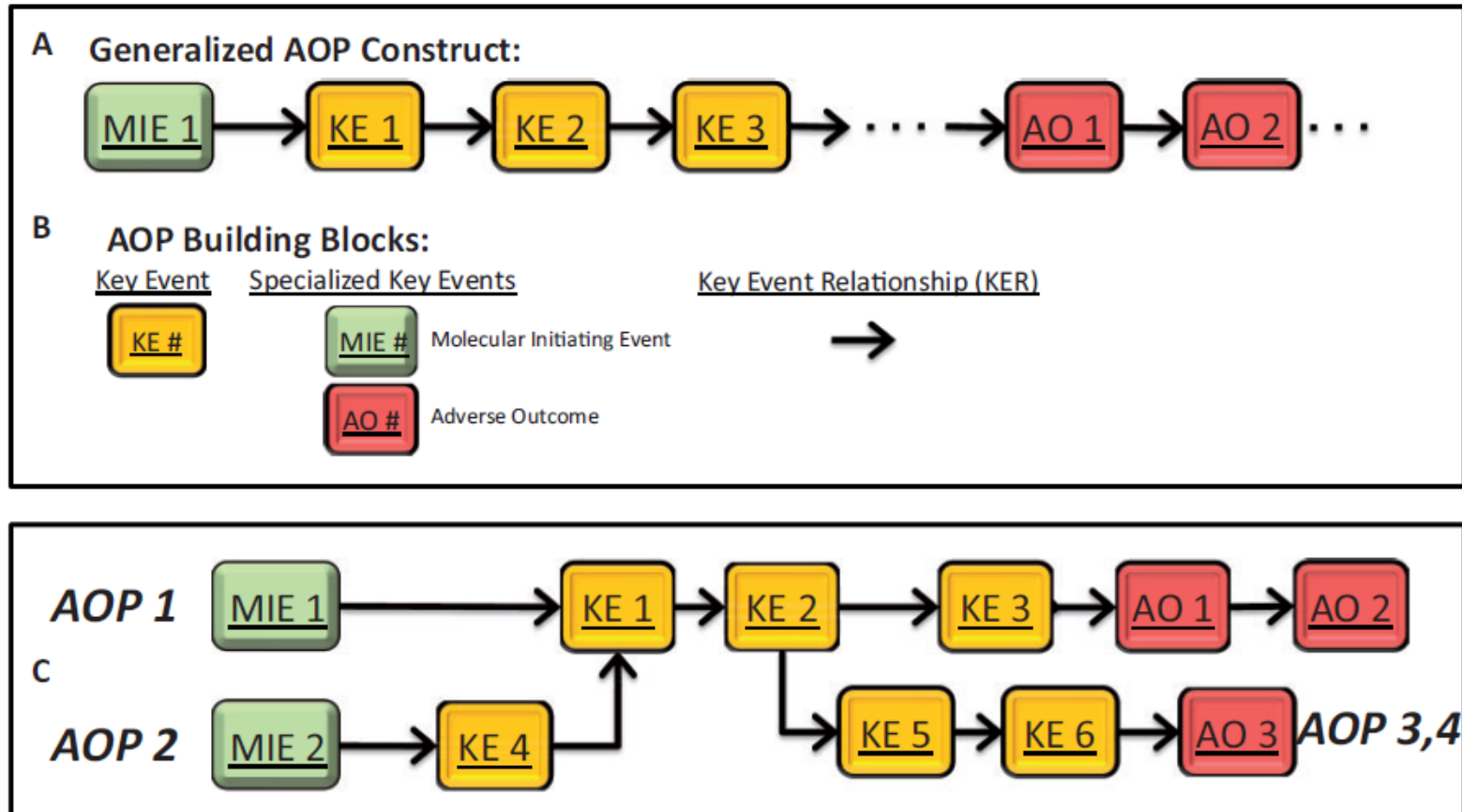
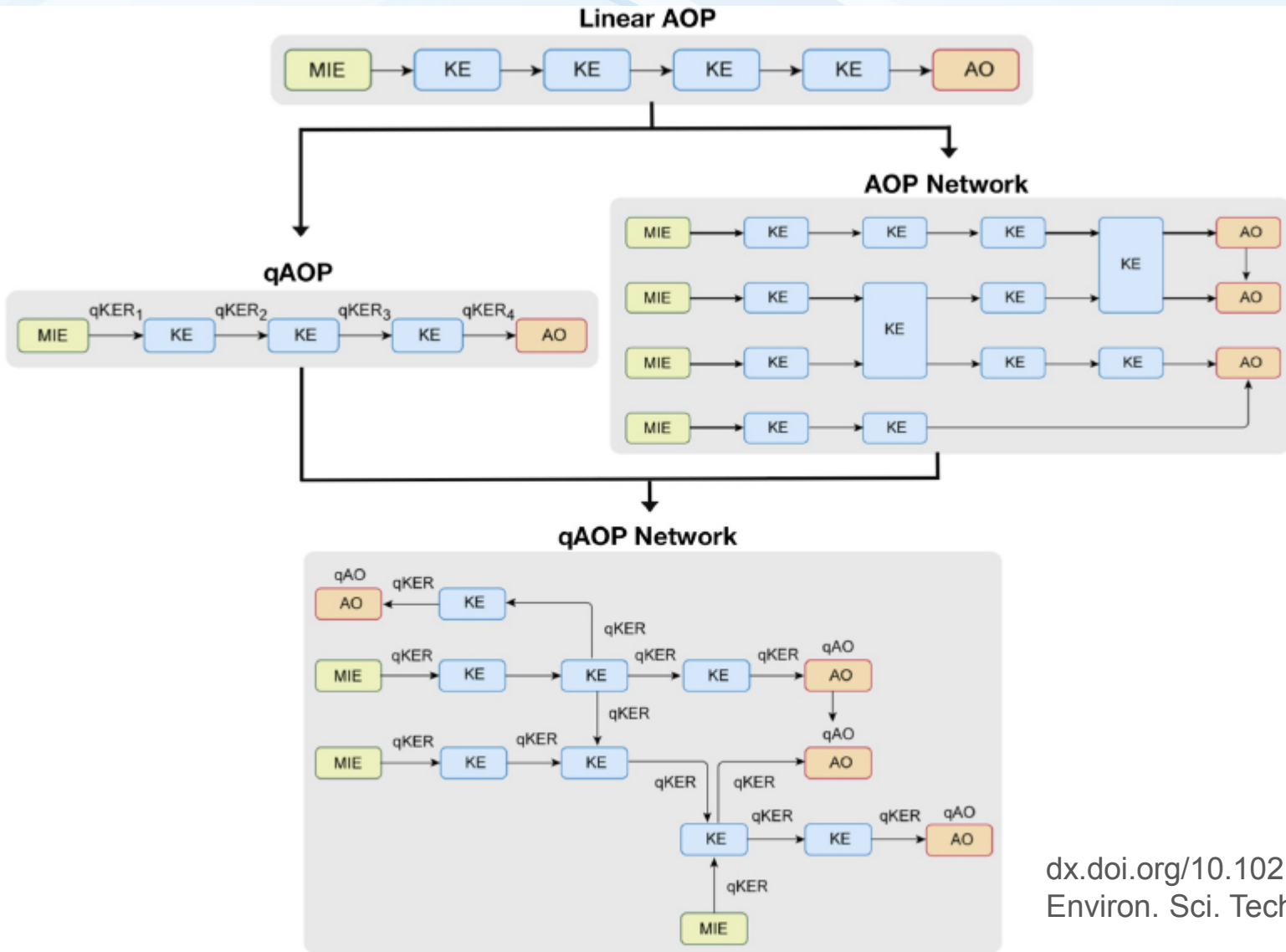


FIG. 1. Graphical representation of a generalized adverse outcome pathway (AOP; A). Each AOP is composed of two key components (B), key events (KEs) and key event relationships (KERs). Additionally, there are two specialized KEs, molecular initiating events (MIEs) and adverse outcomes (AOs) that anchor an AOP description. Individual AOPs sharing KEs or KERs can be represented as an AOP network (C). The AOP network depicted is composed of four individual AOPs, each representing a unique sequence of KEs linking an MIE to AO: AOP 1 [MIE1, KE1, KE2, KE3, AO1, AO2]; AOP 2 [MIE2, KE4, KE1, KE2, KE3, AO1, AO2]; AOP 3 [MIE1, KE1, KE2, KE5, KE6, AO3]; AOP 4 [MIE2, KE4, KE1, KE2, KE5, KE6, AO3]. Color image is available in the online version of the article.

... further linking of AOPs



dx.doi.org/10.1021/es504976d
Environ. Sci. Technol. 2015, 49, 3–9

Figure 1. AOPs as pragmatic units and linear structures are rapidly evolving toward AOP networks and quantitative AOPs (qAOPs). In qAOPs, the linkages between two KEs, that is, KER, will be given a weight/value, forming the quantitative KER (qKER).²⁵ The merging of qAOPs and AOP networks will eventually lead to qAOP networks.

Jaké AOPs jsou v AOP Wiki (data z května 2016)



OECD Endorsed (WNT and TFHA)	1	Covalent Protein binding leading to Skin Sensitisation
EAGMST Approved	6	1x ecotoxicology: Aromatase inhibition leading to reproductive dysfunction (in fish)
EAGMST Under Review	12	
EAGMST Under Development	84	
SAAOP AOP Under Development	15	

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

AOPs Ready for Commenting

OECD Endorsed (WNT and TFHA)

Click [here](#) for links to the official OECD versions

Title

[Covalent Protein binding leading to Skin Sensitisation](#)

EAGMST Approved

Click [here](#) for links to the EAGMST approved versions

Title

[Alkylation of DNA in male pre-meiotic germ cells leading to heritable mutations](#)



[Androgen receptor agonism leading to reproductive dysfunction](#)

[Aromatase inhibition leading to reproductive dysfunction \(in fish\)](#)



[Binding of agonists to ionotropic glutamate receptors in adult brain causes excitotoxicity that mediates neuronal cell death, contributing to learning and memory impairment.](#)



[Chronic binding of antagonist to N-methyl-D-aspartate receptors \(NMDARs\) during brain development induces impairment of learning and memory abilities](#)



[Protein Alkylation leading to Liver Fibrosis](#)



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

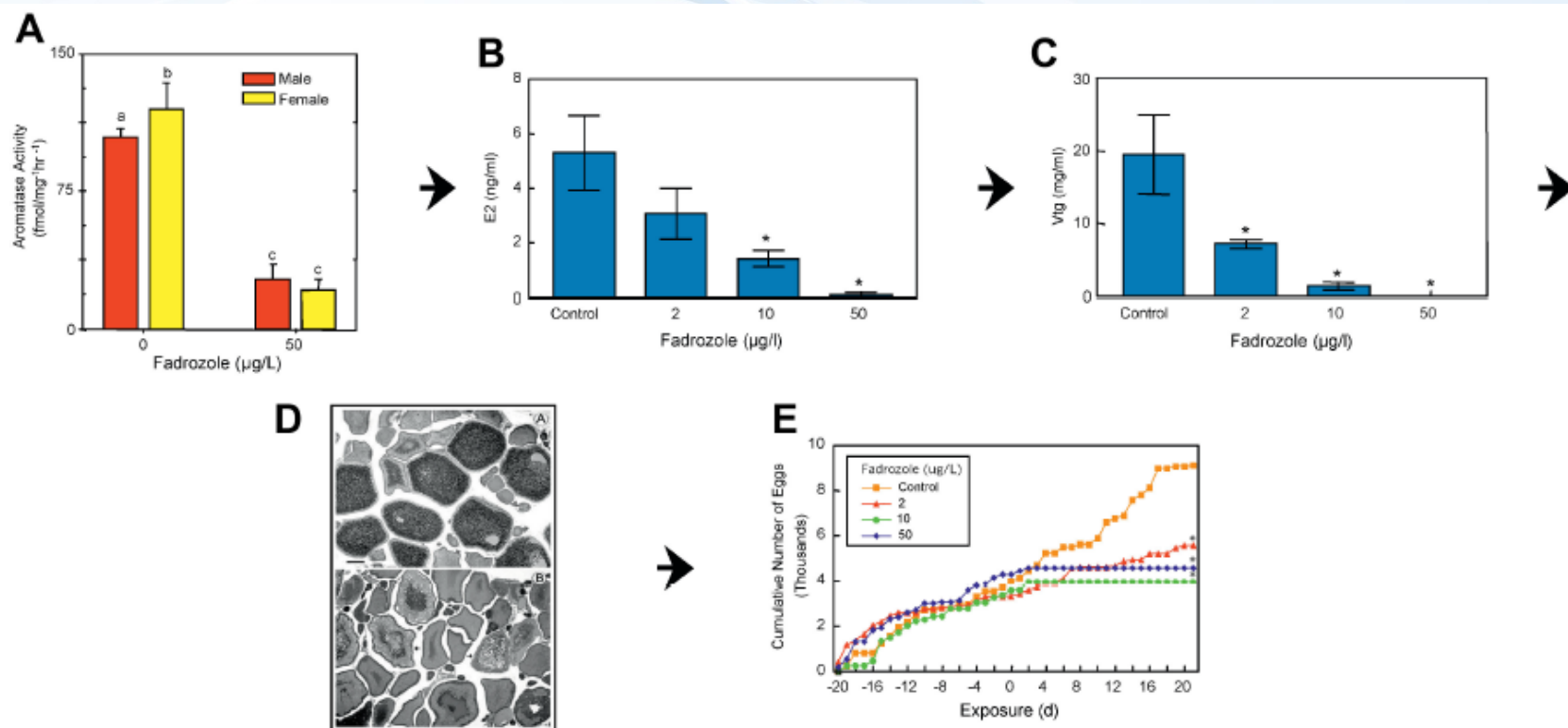
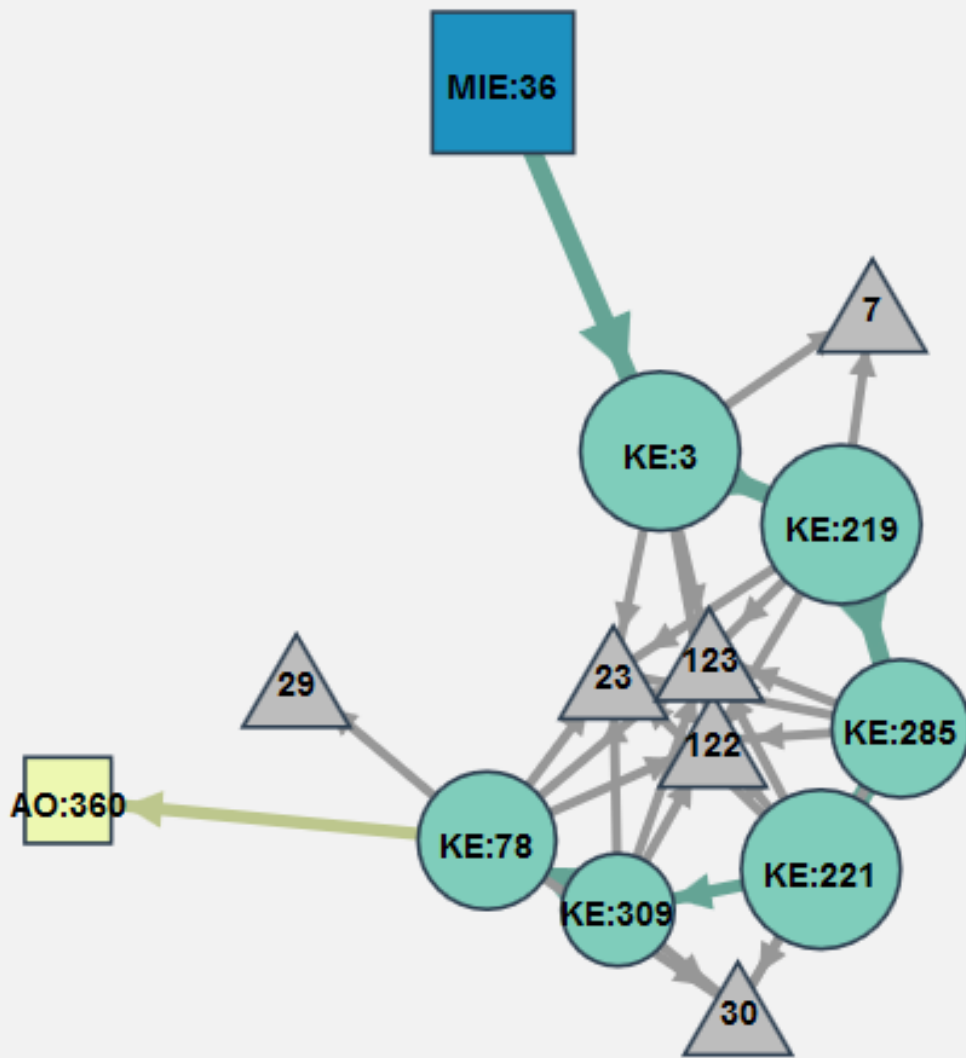


Fig. 3. An adverse outcome pathway in fish [2,50]. Aromatase inhibitor example. (A) Aromatase inhibition by fadrozole; (B) Reduction in circulating estradiol; (C) Reduction in circulating vitellogenin (Vtg); (D) Histopathology of ovarian tissue, top panel normal ovary, bottom panel fadrozole treated;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

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

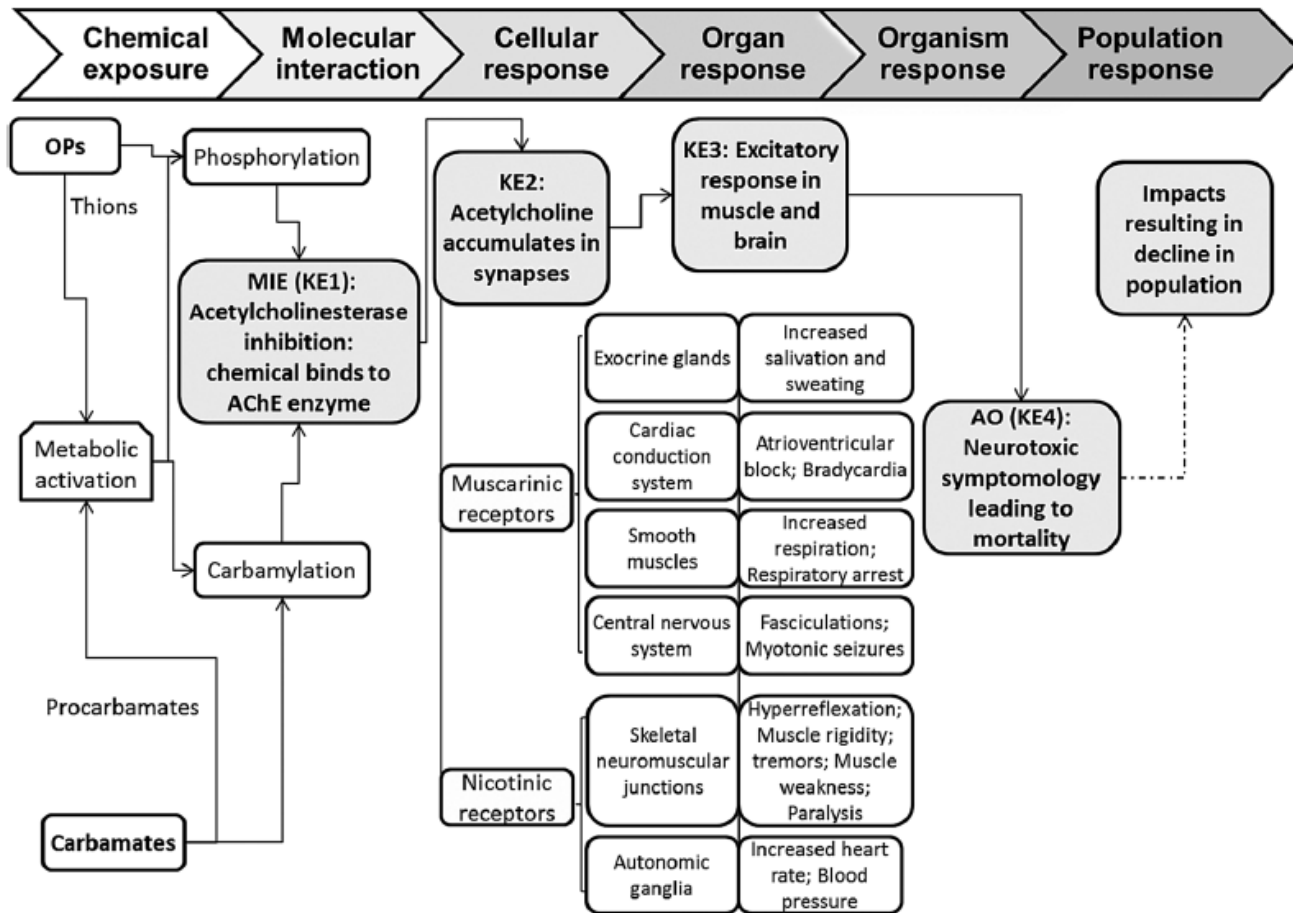


*Size of node reflects essentiality of event

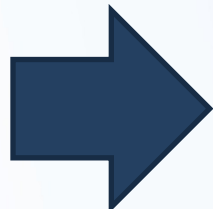
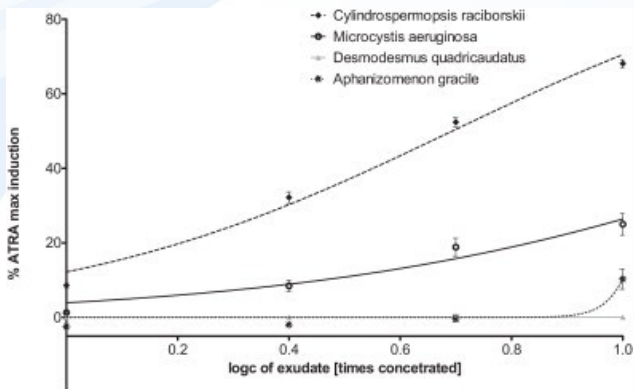
*Width of line reflects strength of evidence for relationship



AOP Example: AcChE inhibition → lethality

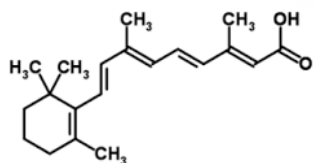


AOP Example from RECETOX: Modulation of RAR/RXR → developmental toxicity in fish

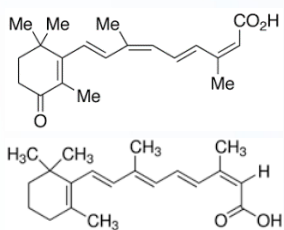


Activation of RAR/RXR

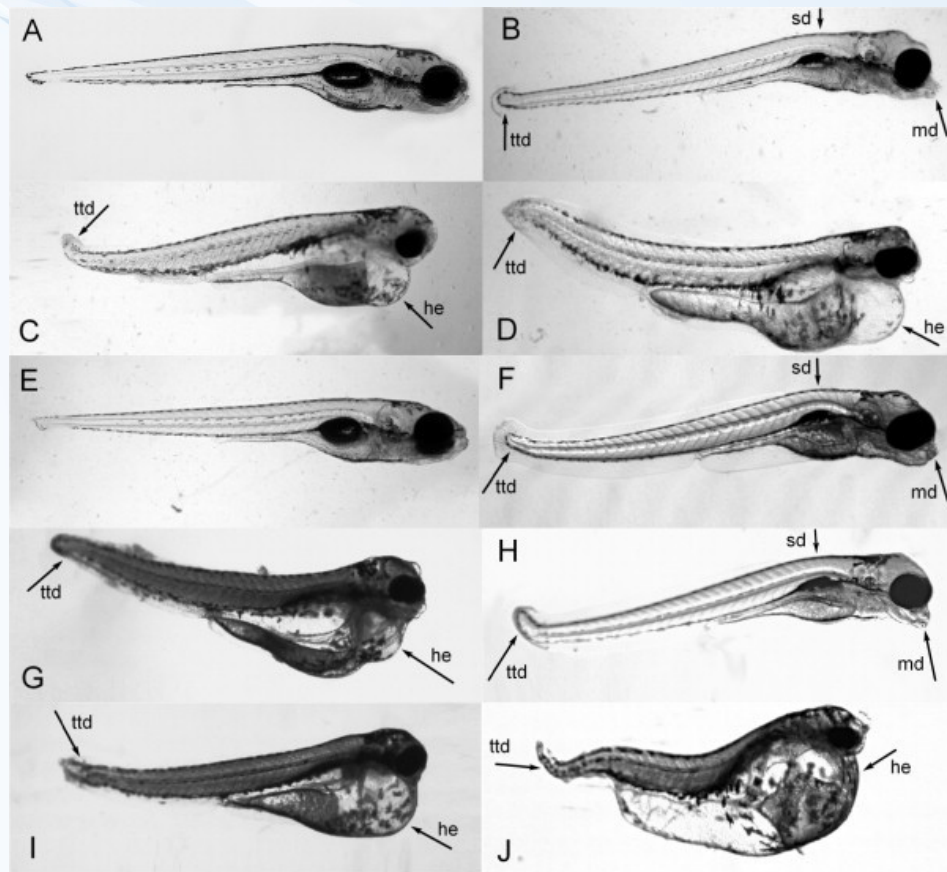
in P19/A15 cells by atRA and cyanobacterial metabolites



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).

AOPs – další poznámky

Regulatorní akceptace ... pozitivní

Regulatory Toxicology and Pharmacology 70 (2014) 629–640



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Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph



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



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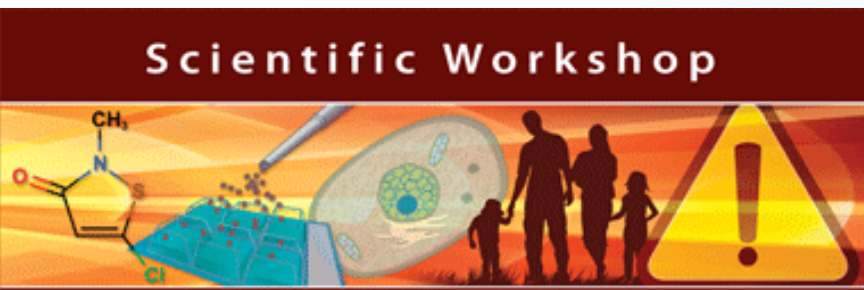
ⁱ DuPont Haskell Global Centers for Health and Environmental Sciences, Stine-Haskell 320/212, 1090 Elkton Road, Newark, DE 19711, USA



Centrum pro výzkum
toxických látek
v prostředí

Workshop – AOPs: From Research to Regulation

- **September 3-5, 2014** (NIH Campus, Bethesda, Maryland, USA)
- **150 participants** (90% USA, also Canada, UK, Germany, Italy, Belgium, Denmark...)
- **OECD, NIH (NIEHS, NICEATM, NLM, NCATS), US EPA, US FDA, EU-JRC, John Hopkins CAAT, US Army ...**
- Dow Chemical, Bayer, Syngenta, American Chemistry Council, Lorillard Tobacco, British American Tobacco, General Electric Co., ExxonMobil, Agilent, ...
- Academia – universities, research institutes



Adverse Outcome Pathways: From Research to Regulation

Regulatory Toxicology and Pharmacology

Volume 76, April 2016, Pages 39–50



Workshop report

Adverse outcome pathways: From research to regulation scientific workshop report

Nicole C. Kleinstreuer^a,  , Kristie Sullivan^b, David Allen^c, Stephen Edwards^d, Donna L. Mendrick^e, Michelle Embry^f, Joanna Matheson^g, J. Craig Rowlands^h, Sharon Munnⁱ, Elizabeth Maul^a, Warren Casey^a



International QSAR Foundation

Effectopedia

The Online Encyclopedia of Adverse Effect Pathways

- <http://www.effectopedia.org/> -> link to program download
- Visually Expresses AOPs in their **biological context**:
 - Life-stage, Taxonomy, Gender, Time-to-effect..
- **Quantitative Relationships**
- **ADME** (Absorption, Distribution, Metabolism, Excretion)
- Open-knowledge, crowd-sourcing
- Formal approval not required to enter / modify
- Credit to authors / reviewers
- Even fragments of information are welcome (any contribution)
- Export<->Import from/to AOP Wiki & others

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

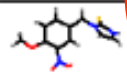
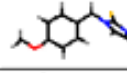
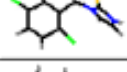
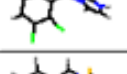
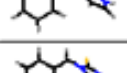
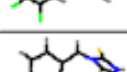

- Techniky QSAR původně vyvinuty pro design farmak
- **Aplikace SAR, QSAR v ekotoxikologii**
 - **předpovědi environmentálně významných parametrů látek**
 - *logK_{ow}*
 - *biokoncentrace, bioakumulace*
 - *předpovědi biodegradability a metabolismu*
 - *odhady rychlosti degradace t_{1/2}, vznikající 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)
 - KOWWIN (esc.syrres.com)
(www.epa.gov/oppt/exposure/docs/episuitedi.htm)
 - SLIPPER (www.ibmh.msk.su/qsar/molpro)
 - KlogP (www.multicase.com)
 - ABSOLV (www.sirius-analytical.com)
 - ProLogP (www.compudrug.com)
 - SPARC (ibmlc2.chem.uga.edu/sparc)
 - IA (www.interactiveanalysis.com)
 - ACD (www.acdlabs.com)
 - QikProp (www.schrodinger.com)
 - AP-Algorithms (www.ap-algorithms.com)
 - ProPred (www.capec.kt.dtu.dk)
 - Cerius² (www.accelrys.com)
 - QMPRPlus (www.simulations-plus.com)



SAR, QSAR - příklady

– Předpověď' biokoncentrace

Modely doporučené TGD (*technical guidelines*) při registraci nových chemických látek v EU:

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

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

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

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

– 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 ₂) or CH ₂ (CH ₂) _{n-1} 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

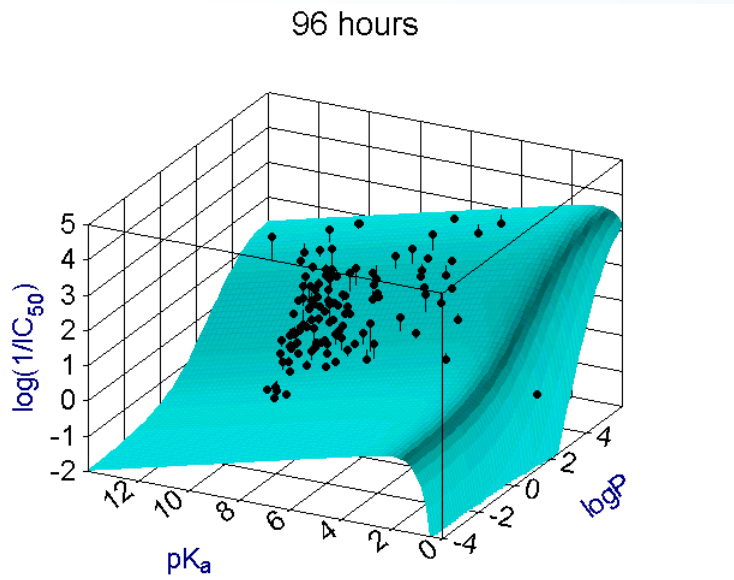
SAR, QSAR - příklady

- Předpovědi toxicity (také označováno – QSTR – Quantitative STRUCTURE-TOXICITY relationships)

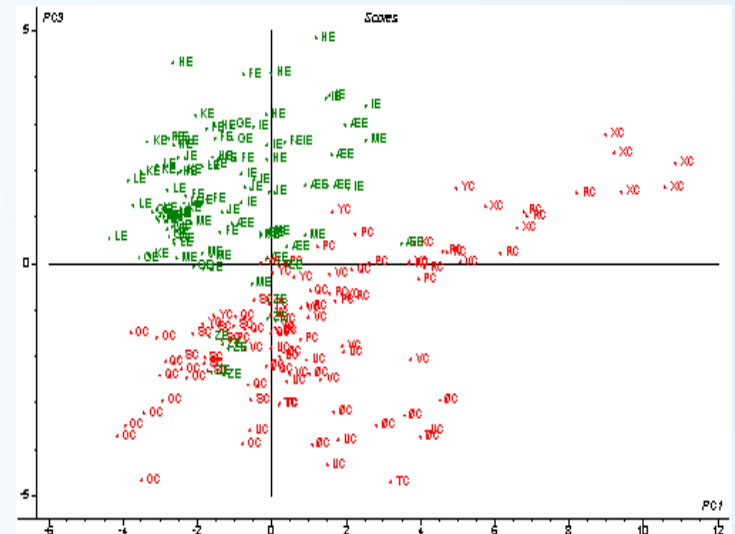
Př 1 – toxicita narkotických látek pro ryby (využívána např. i pro účely REACH)

$$\log(1/LC50) = 0.907 \cdot \log Kow - 4.94$$

Př 2 – toxicita fenolů pro koryše – vícenásobná regrese
(nepolární narkoza – $\log P$, polární toxicita – pK_a)



Př 3 – vícerozměrné modelování:
výsledek analýzy hlavních komponent (PCA)
- výstup : odlišení toxických a netoxických látek



Moderní výpočetní toxikologie



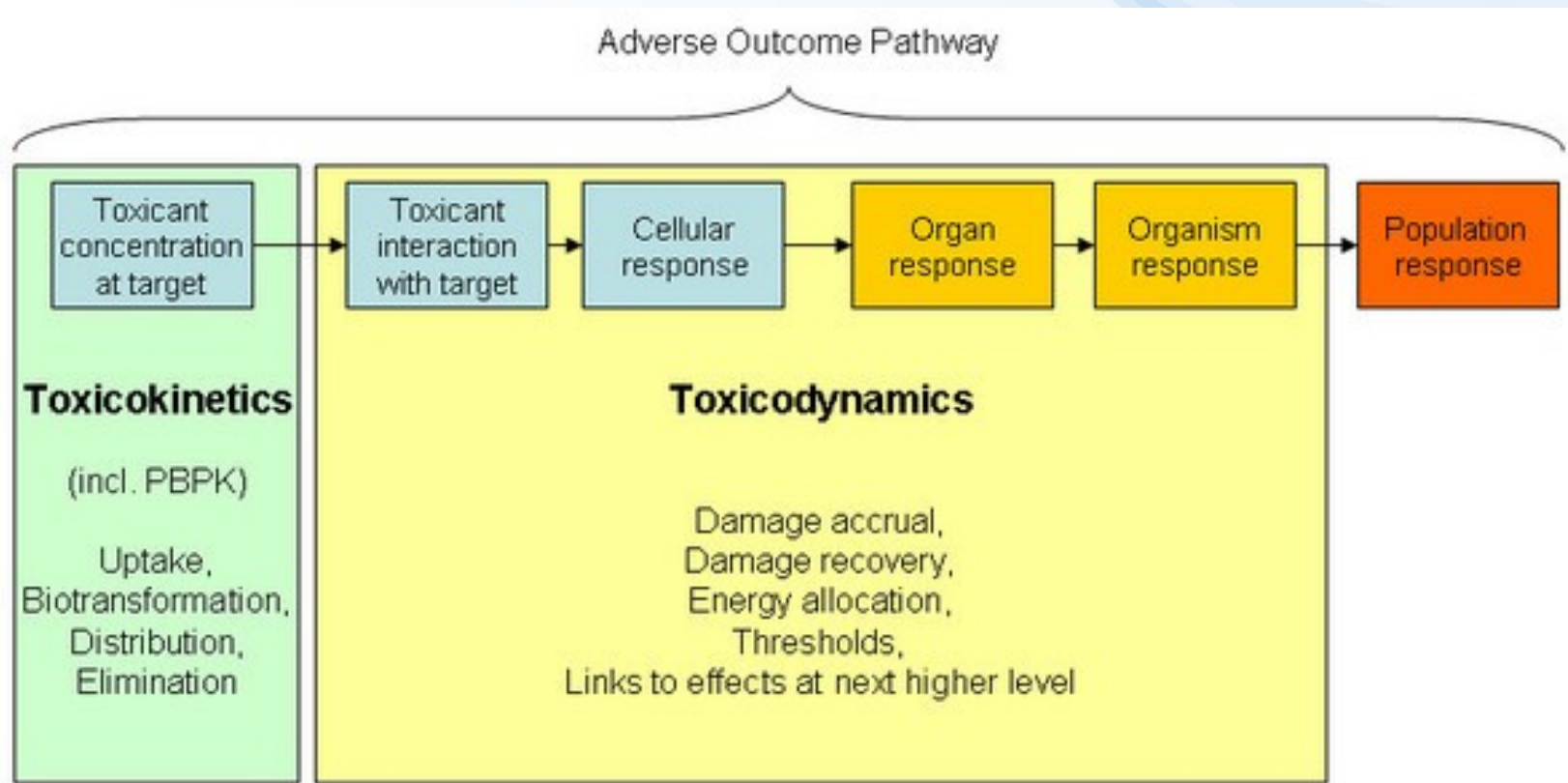
Adverse outcome pathways

Viz také dříve v přednáškách:

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

Základem je dokonalé porozumění

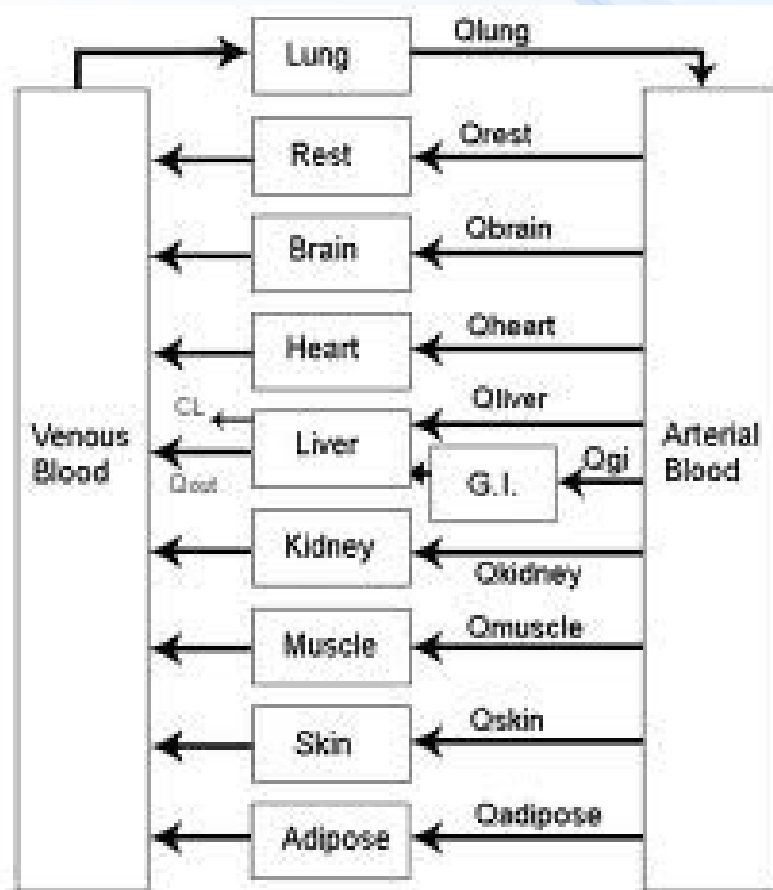
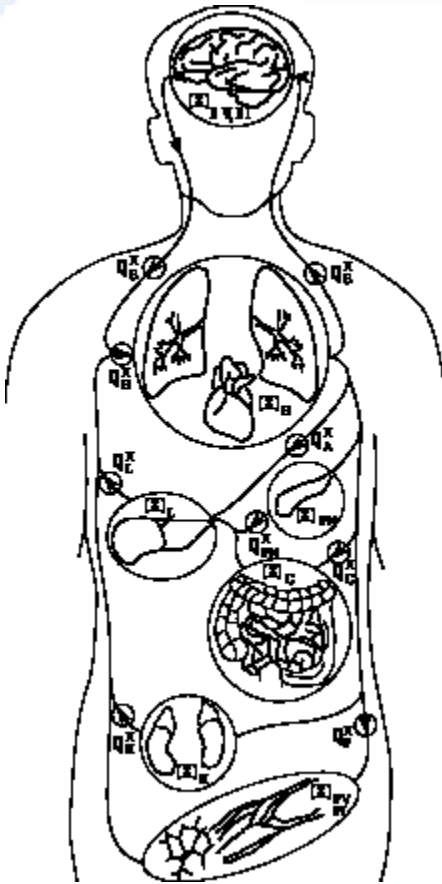
1) toxikokinetice (modely PBPK – viz dále) a 2) následně mechanismům (dynamika)



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

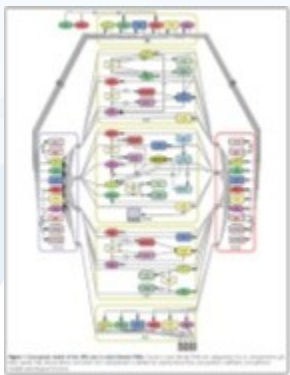
Open Access

A computational model of the hypothalamic - pituitary - gonadal axis in female fathead minnows (*Pimephales promelas*) exposed to 17α -ethynylestradiol and 17β -trenbolone

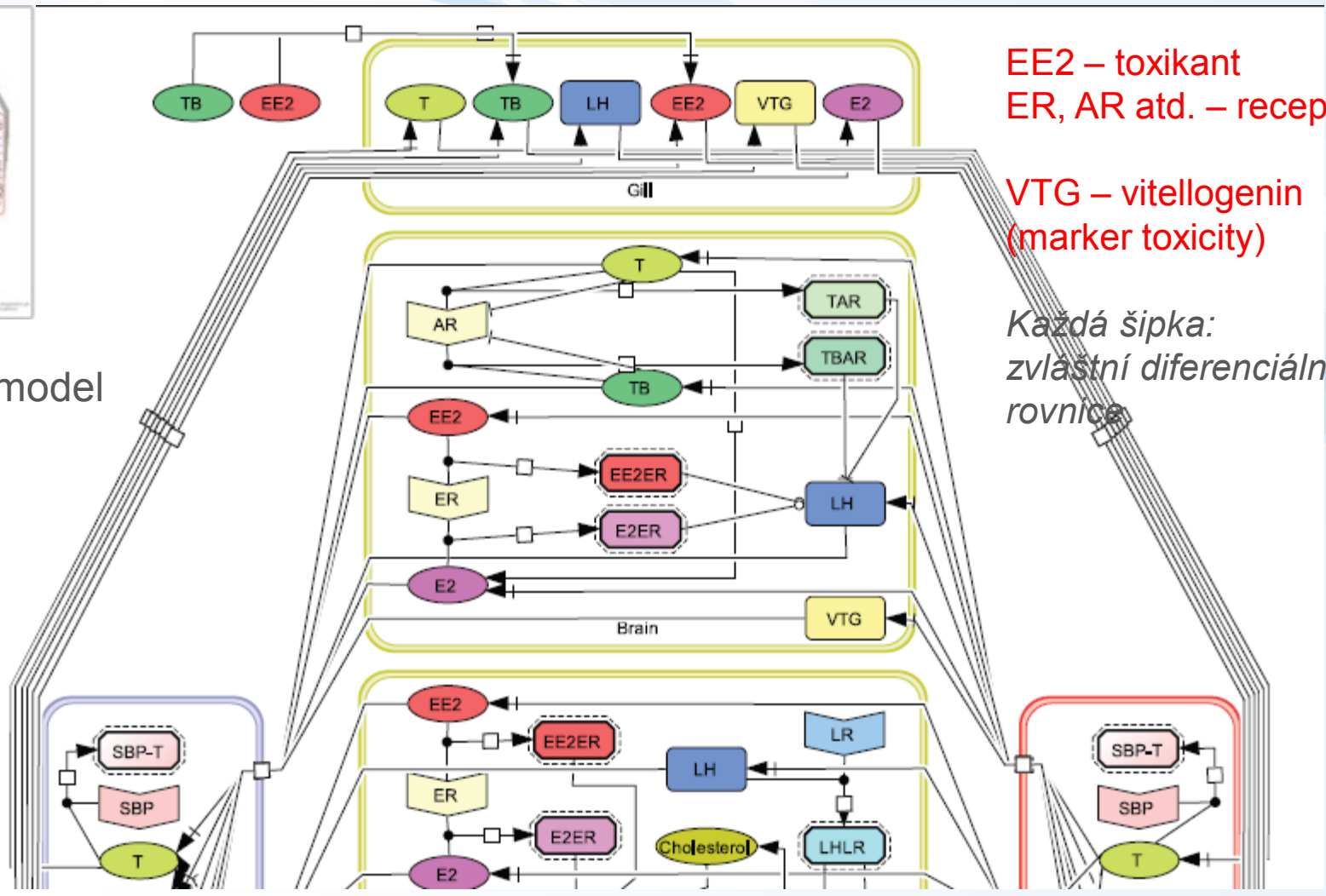
Zhenhong Li¹, Kevin J Kroll², Kathleen M Jensen³, Daniel L Villeneuve³, Gerald T Ankley³, Jayne V Brian⁴, María S Sepúlveda⁵, Edward F Orlando⁶, James M Lazorchak⁷, Mitchell Kostich⁷, Brandon Armstrong⁸, Nancy D Denslow² and Karen H Watanabe^{1*}



Li (2011) BMC Systems Biology



Konceptní model
→ ZOOM



EE2 – toxikant
ER, AR atd. – receptory

VTG – vitellogenin
(marker toxicity)

*Každá šipka:
zvláštní diferenciální
rovnice*

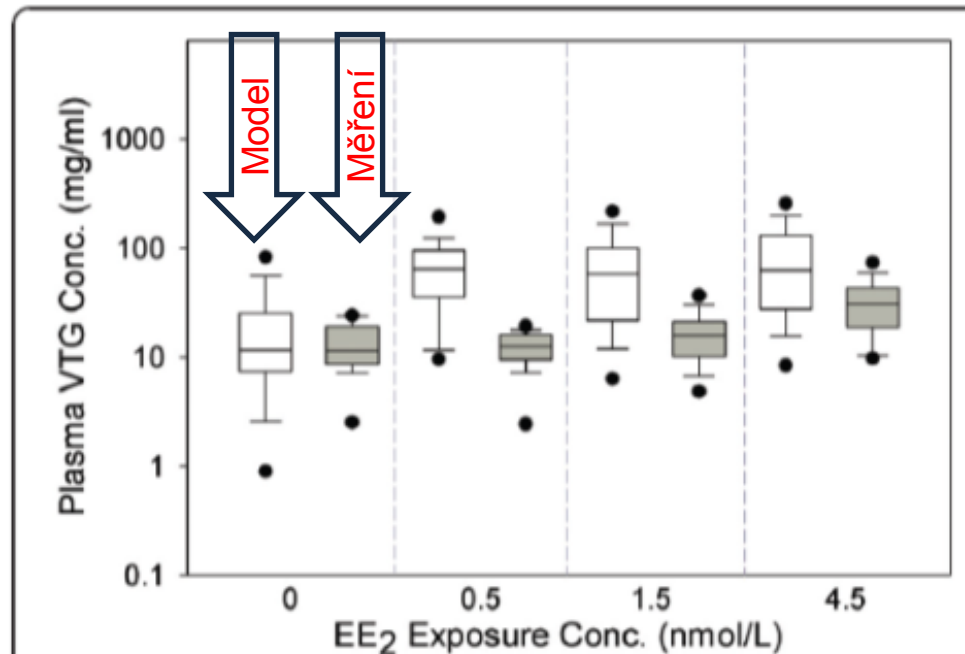


Figure 6 Comparison of model predictions with measured data in female FHMs exposed to EE₂. $n = 28$ at each sampling time. White boxes represent model predictions, and grey boxes represent measured data [42]. The x-axis represents EE₂ concentrations in ng/L. The solid line within the box marks the median; the boundary of the box farthest from zero indicates the 75th percentile; the boundary of the box closest to zero indicates the 25th percentile; the whisker (error bar) farthest from zero marks the 90th percentile; whisker (error bar) closest to zero marks the 10th percentile; the circle farthest from zero marks the 95th percentile; and the circle closest to zero marks the 5th percentile.

Výsledek:

Srovnání
MODEL vs. MĚŘENÍ






 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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[Methods](#)

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

 Check for updates

Subject Areas



Kvantitativní mechanistické modelování

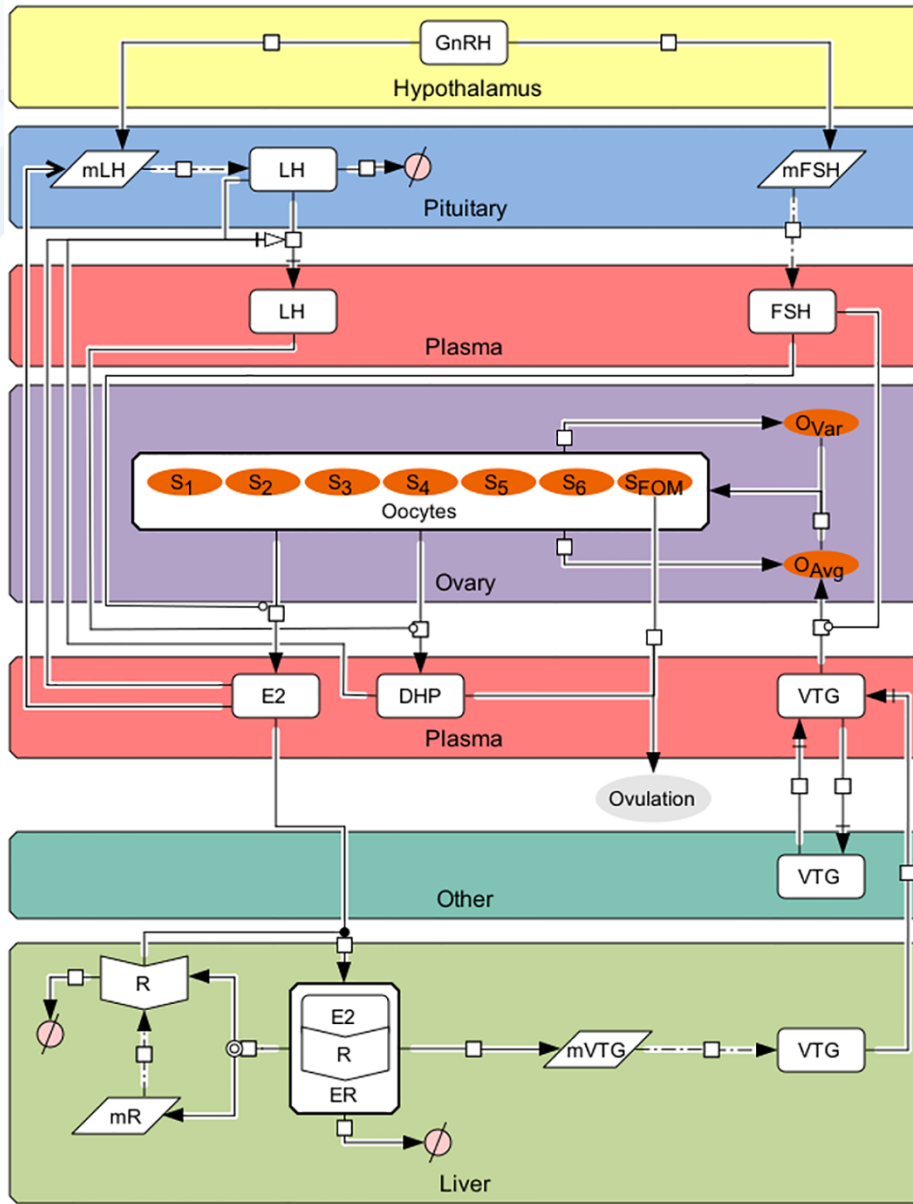
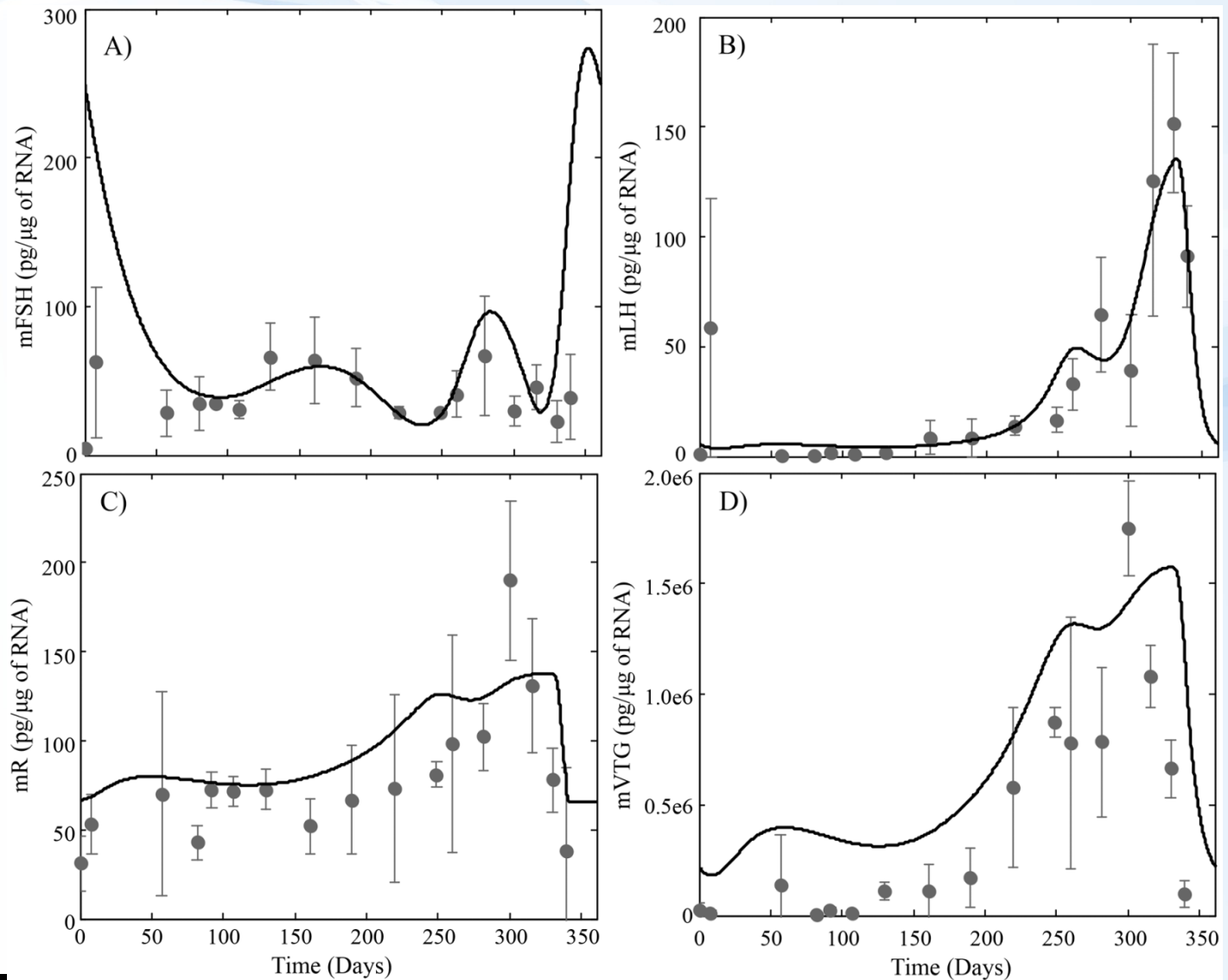


Fig 1. The HPOL signaling network in rainbow trout as formulated in our model.

Arrows and symbols on graph follow CellDesigner vs. 4.4 notation (www.celldesigner.org). 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_L. Secreted VTG then travels from the liver to the ovaries via the plasma (VTG_P) 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_P, 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_P 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

- tvar a struktura částic
 - Kulovité nebo nepravidelné částice, trubičky, vlákna, destičky
 - Homogenní částice (chemická individua)
 - Kompozitní nanomateriály (jádro a obal)
 - Nanočástice 3. a 4. generace (budoucnost: různé komponenty se specializovanými funkcemi („nanodevices“))



Základní charakteristiky vyráběných NM

Kovy

- stříbro
- zlato
- železo
- *další*

Oxidy kovů

- TiO_2
- Al_2O_3
- SiO_2
- ZnO
- ZrO_2
- *další*

Uhlíkové NM

- nanotrubičky
- fullereny
- saze
- nanodiamanty

Další anorganické NM

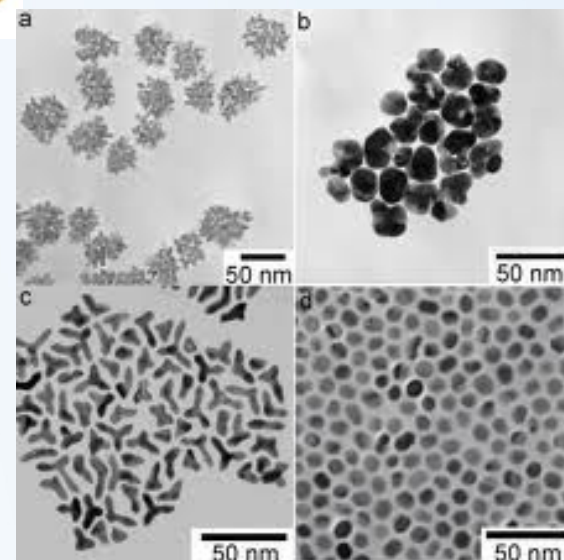
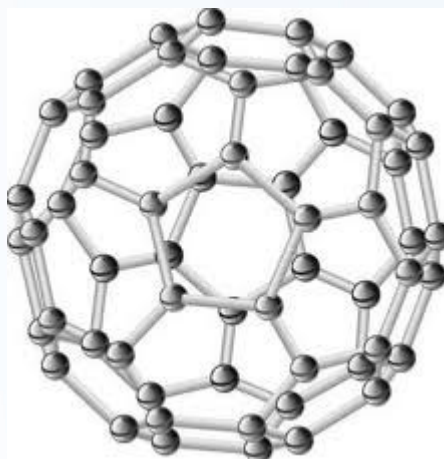
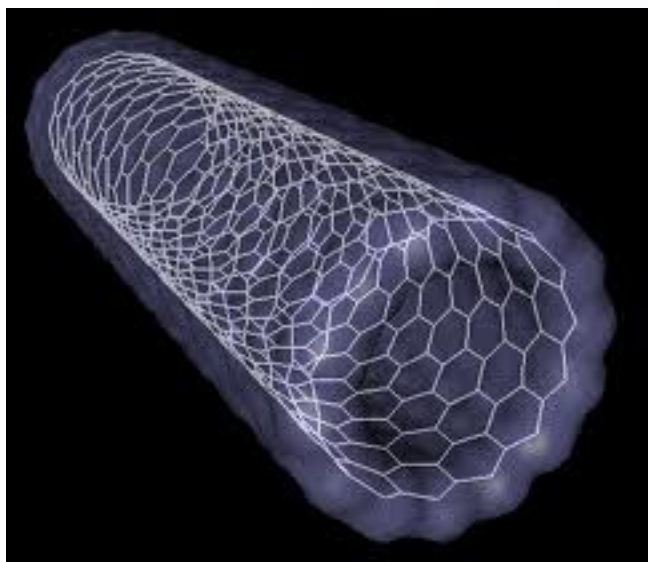
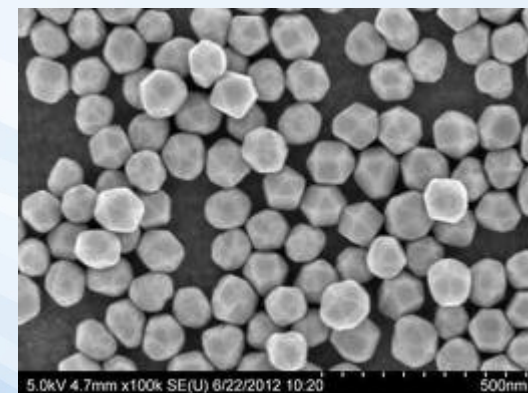
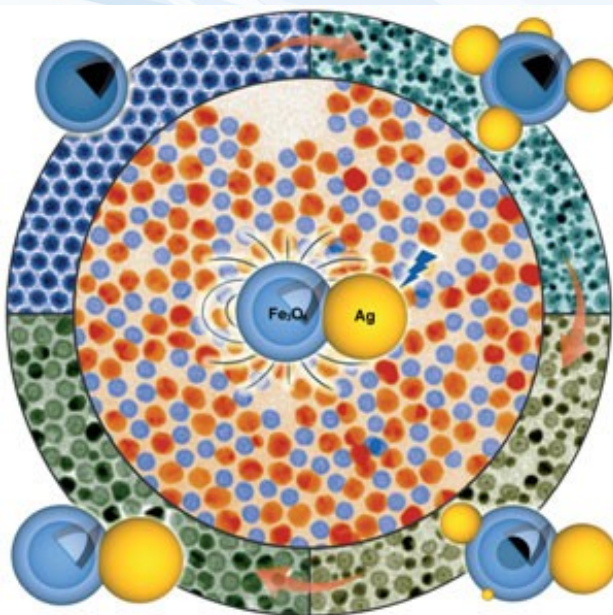
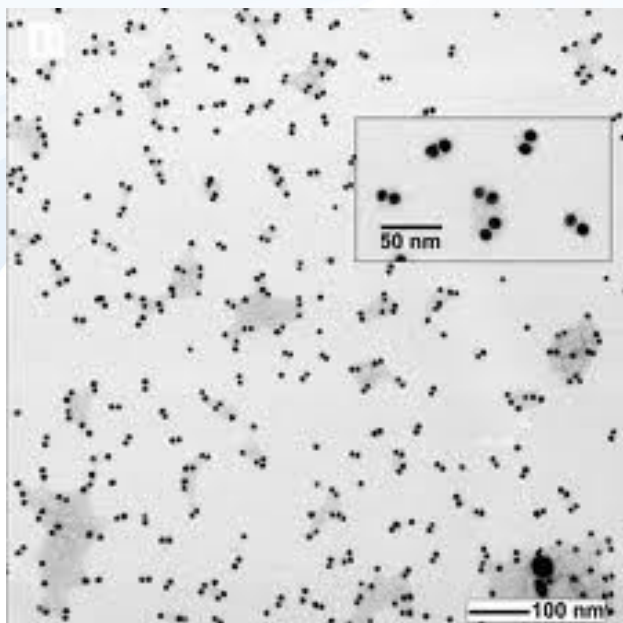
- magnetické materiály
- kompozitní nanomateriály
- kvantové tečky
- silikáty, zeolity, jíly
- anorganická nanovlákná

Organické NM

- nanovlákná polymerů
- dendrimery
- polystyren



Příklady - nanočástice



Nanoparticle movement through the environment

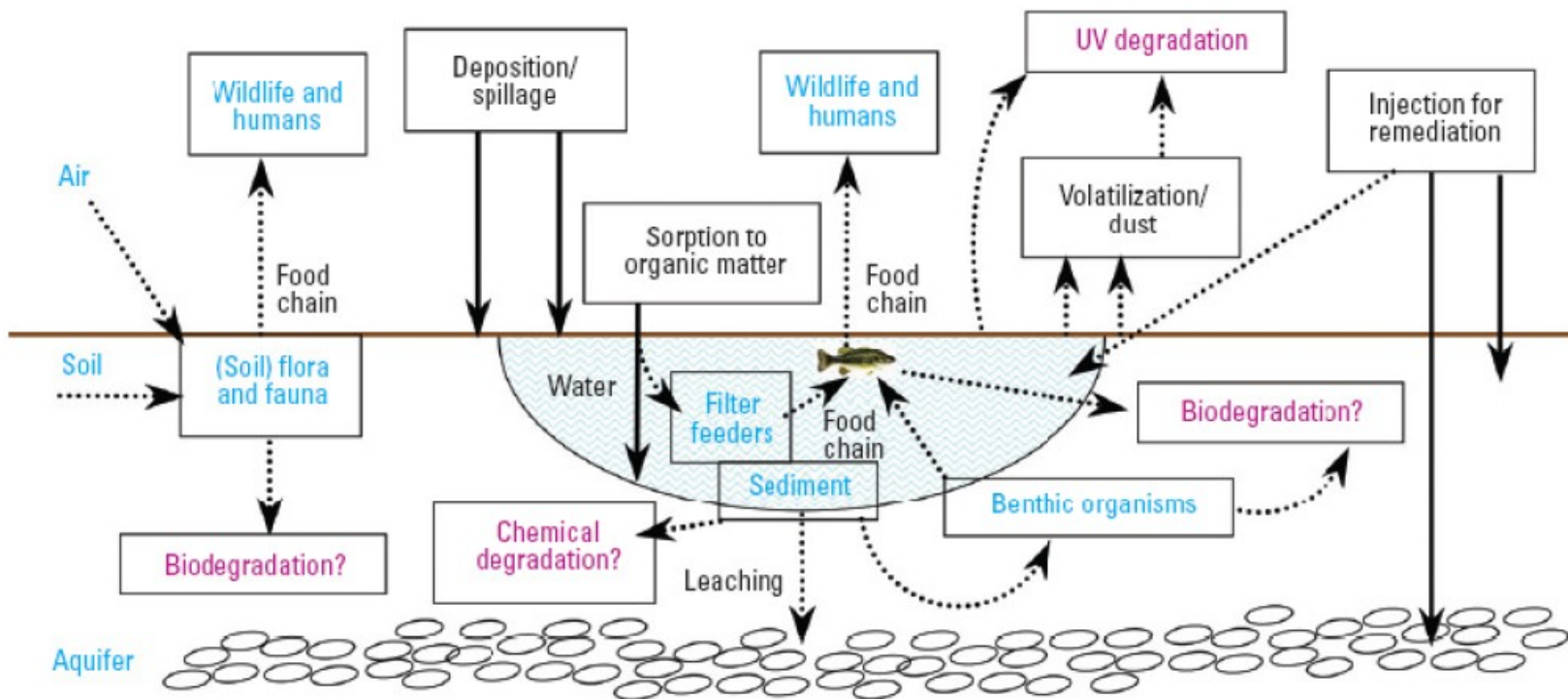
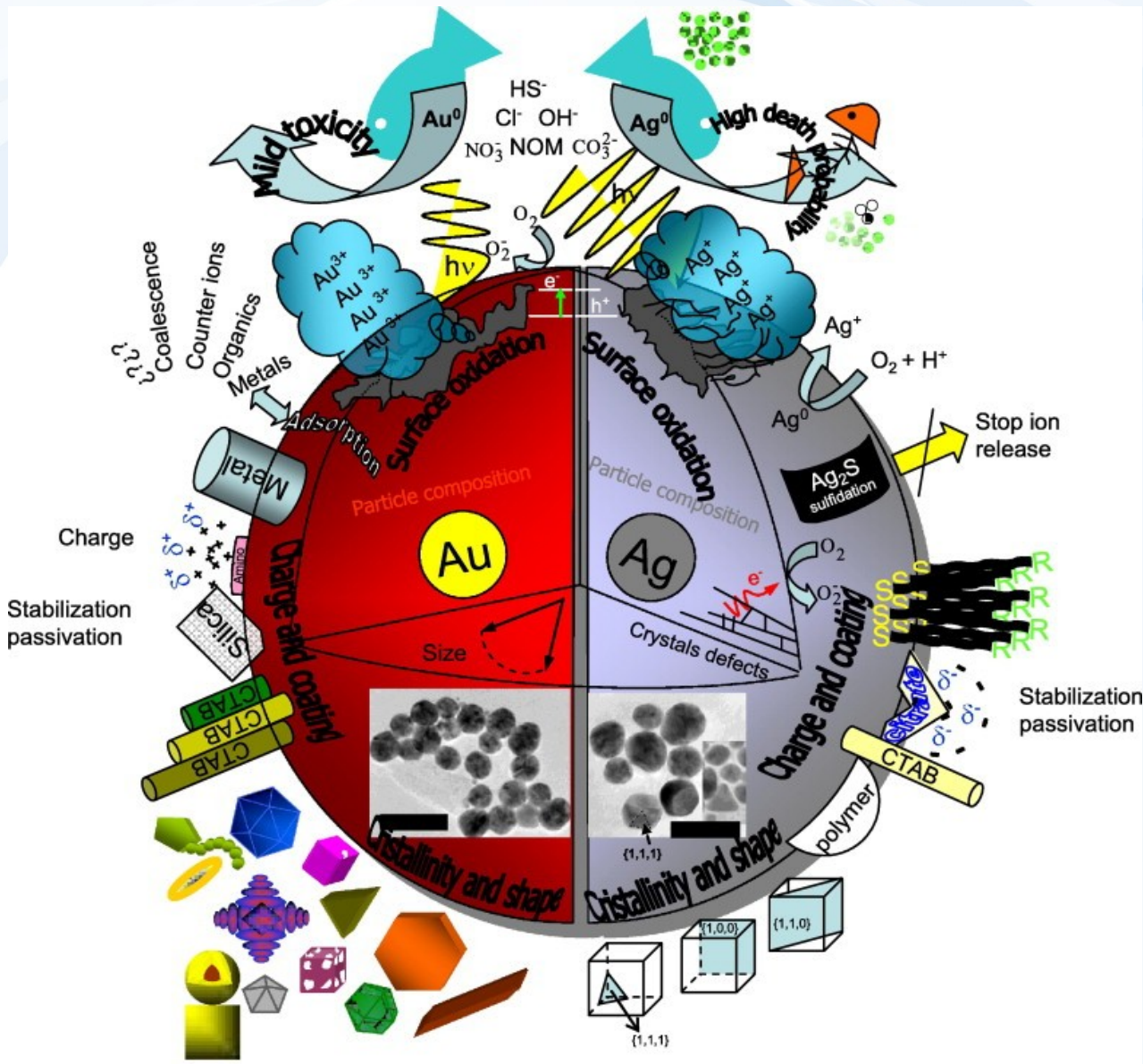


Figure 5. Routes of exposure, uptake, distribution, and degradation of NSPs in the environment. Solid lines indicate routes that have been demonstrated in the laboratory or field or that are currently in use (remediation). Magenta lettering indicates possible degradation routes, and blue lettering indicates possible sinks and sources of NSPs.

(Eko)toxicita nanočástic – specifické vlastnosti



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

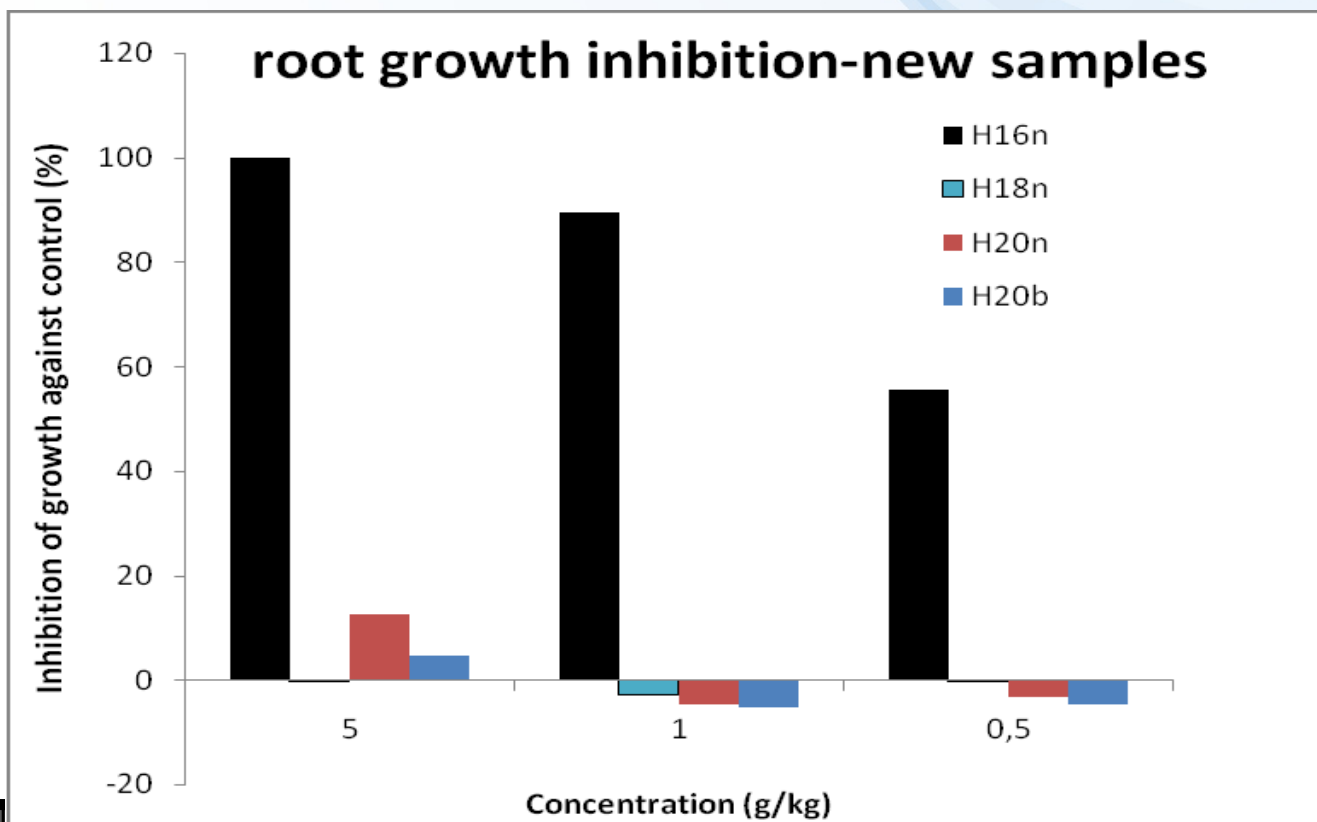
Složení (chemické)
Povrch (velikost, tvar)
Náboj
Stabilita
Agregace částic
Interakce s chemikáliemi
Interakce s ionty

Vliv na osud látek
Přímá toxicita

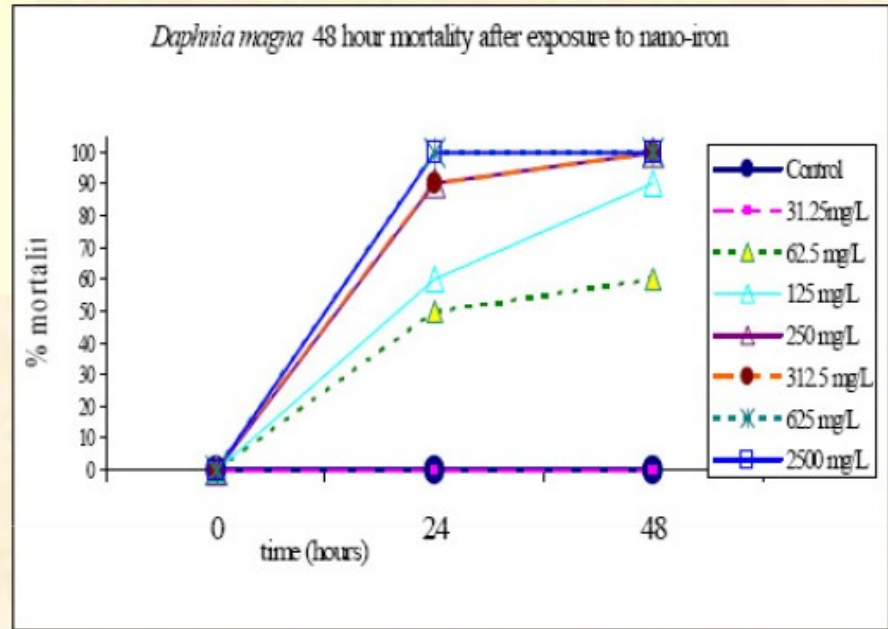
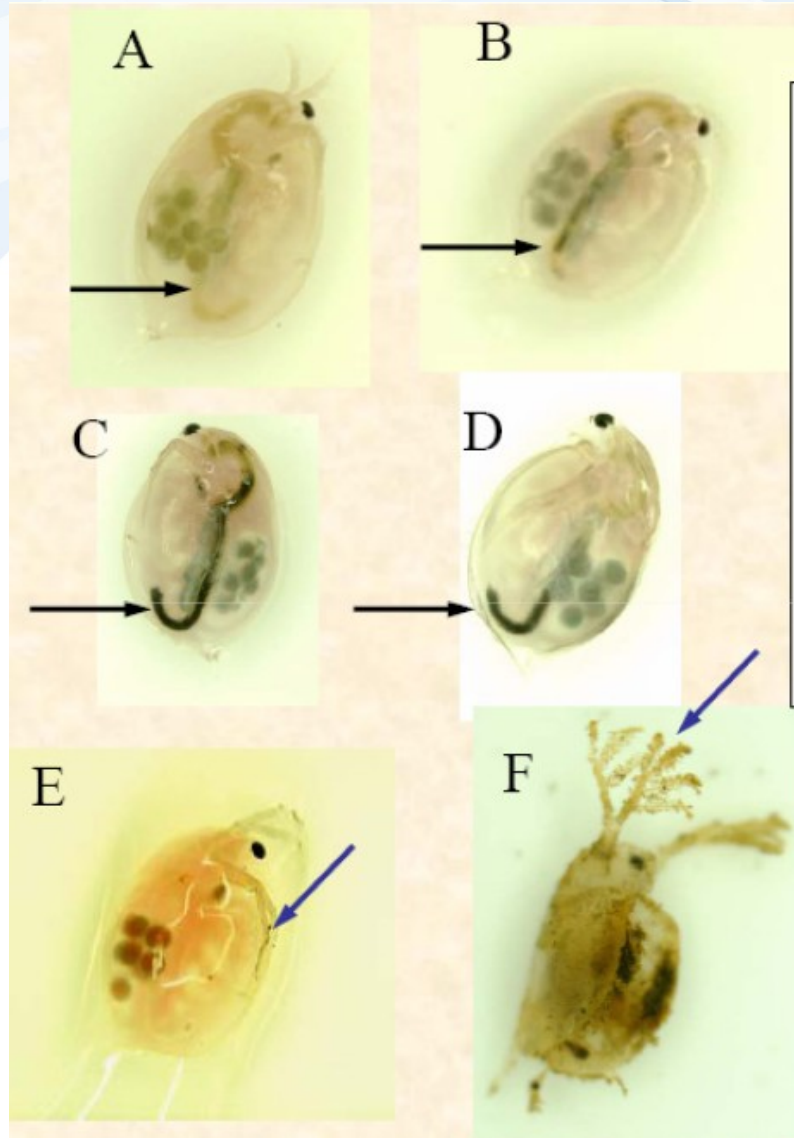
(Eko)toxická nanočástic – příklad RECETOX

Toxicita – srovnání - 4 „stejně“ částice (jeden výrobce – 4 různé šarže)
(zerovalent iron – ZVI – Fe⁰)

Opakovaně pozorována toxicita u částic H16 – příčina neznámá (žádné změny pH, rozpouštění železa či dalších příměsí ...)



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



Maternal predator-exposure has lifelong consequences for offspring learning in threespined sticklebacks



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Koljušky (ryby), které byly v době kladení vajíček ve stresu (predátor)

→ Snížená schopnost učení u potomků

! Transgenerační přenos

Table 1. Behaviours (mean \pm s.e.) of the offspring from the maternal treatments.

	offspring of predator-exposed mothers (s)	offspring of unexposed mothers (s)
initial exploratory behaviour (day 1: 09.00):		
latency to first begin moving	49 \pm 30	56 \pm 20
latency to enter either chamber for the first time	330 \pm 70	326 \pm 78
learning the colour association:		
day 1 (09.00): latency to find food reward	426 \pm 65	427 \pm 61
day 3 (09.00): latency to find food reward	533 \pm 48	304 \pm 74
day 5 (09.00): latency to find food reward	337 \pm 61	158 \pm 68



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.

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

Ekotoxicita pesticidů – nové poznatky

- Pesticidy – registrace před použitím
- Povinné testy účinků na včely
 - Odvození bezpečného dávkování pro použití
- Nově zjištěné problémy
 - Jak se projeví „bezpečné koncentrace“ více pesticidů, pokud budou působit současně ?
 - Jak se projeví u jiných druhů opylovačů než u včel ?

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

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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.*¹⁰ 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

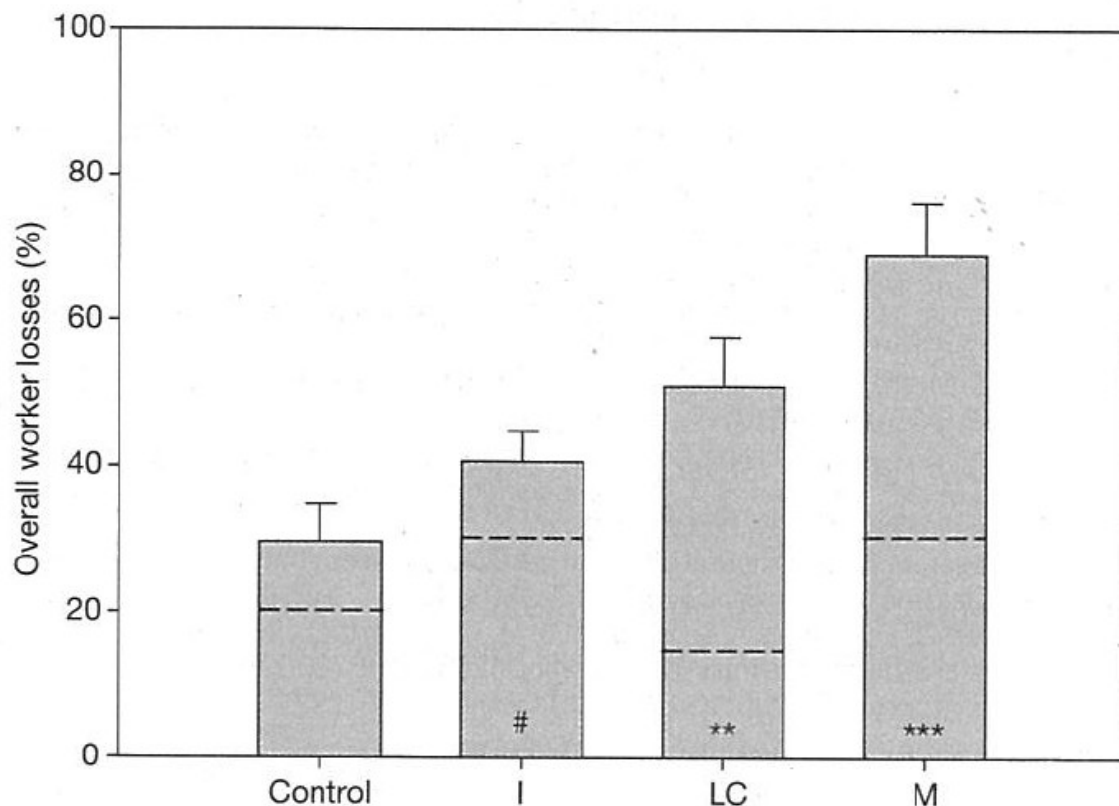


Figure 3 | Overall worker losses. Mean (\pm s.e.m.) overall percentage of workers lost per colony, including workers lost outside (below the dashed line) and worker mortality (dead workers found in nest box; above the dashed line), during the 4-week experiment. $n = 40$ colonies. # $P \leq 0.1$, ** $P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

Vliv pesticidů na čmeláky – polní studie: aplikovány povolené dávky

- 2 individuální látky „I“ a „LC“
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Table 1 | Summary of observed pesticide effects for each treatment group (I, LC or M) in comparison to the control group

Effect level	Effect type	I	LC	M
Effects on individual behaviour	Number of foragers	+	ND	+
	Foraging bout frequency	ND	ND	–
	Amount of pollen collected	–	ND	–
	Duration of pollen foraging bouts	+	ND	+
Effects at colony level	Worker production	–	ND	–
	Brood number	–	ND	–
	Nest structure mass	ND	ND	ND
	Worker mortality	ND	+	+
	Worker loss	+	–	+
	Worker mortality & loss	ND	+	+
	Colony failure (<i>n</i> failed/ <i>n</i> survived)	0/10	0/10	2/8

Significant decrease (–), significant increase (+) and no detected effect (ND) at the 5% significance level.

