Assessment of toxic effects

Ludek Blaha et al.

Outline

- **Why** toxicity assessment
- **Who** wants the assessment
- **When**
- **Where** the assessment is needed
- **What** to assess for toxicity
- **How** to assess toxicity

- **What if not** do we need tests? alternatives

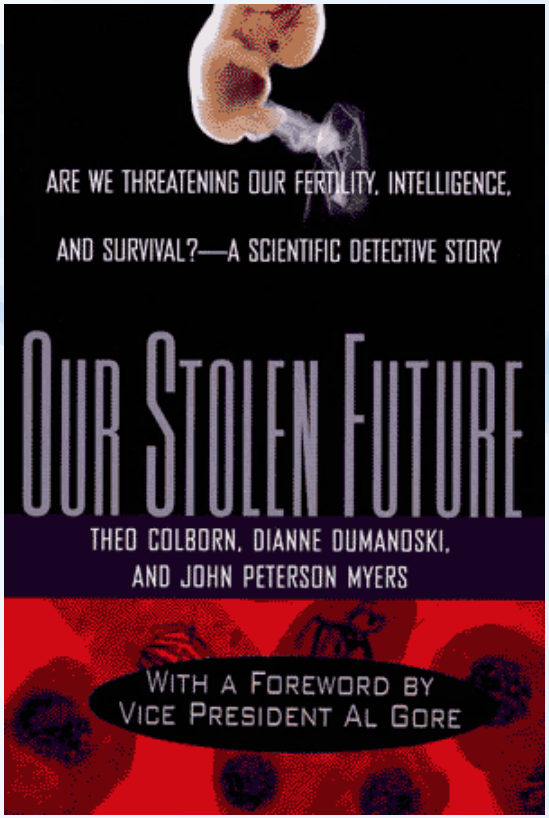
1996 - Chemicals in the environment

Do you believe that chemicals in products sold to consumers have been proven safe?

Think again

most chemicals in modern use have simply not been tested for their impacts on
human, even very basic effects.

… what about the effects in nature, then ?



How we stand 20 years later?

news@nature.com
The best in science journalism

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Published online: 21 October 2005; | doi:10.1038/news051017-16

Pollution makes for more girls

The stress of dirty air skews sex ratios in Sao Paulo.

Erika Check

Toxic fumes favour the fairer sex, a group of researchers in Brazil has found.

Babies born in highly polluted areas are more likely to be girls.

theguardian

World news

Man-made chemicals blamed as many more girls than boys are born in Arctic

- High levels can change sex of child during pregnancy
- Survey of Greenland and east Russia puts ratio at 2:1

Paul Brown in Nuuk, Greenland

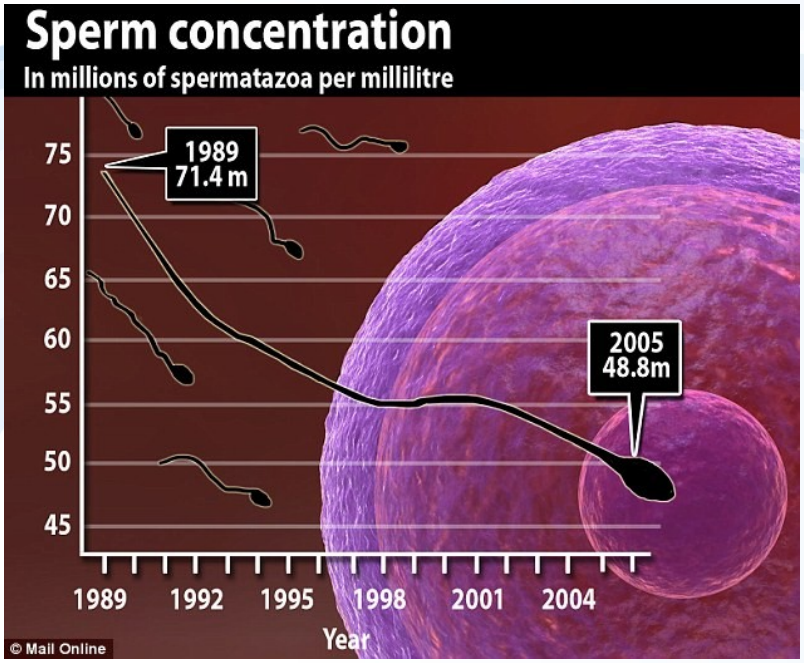
Wednesday 12 September 2007
03.00 BST

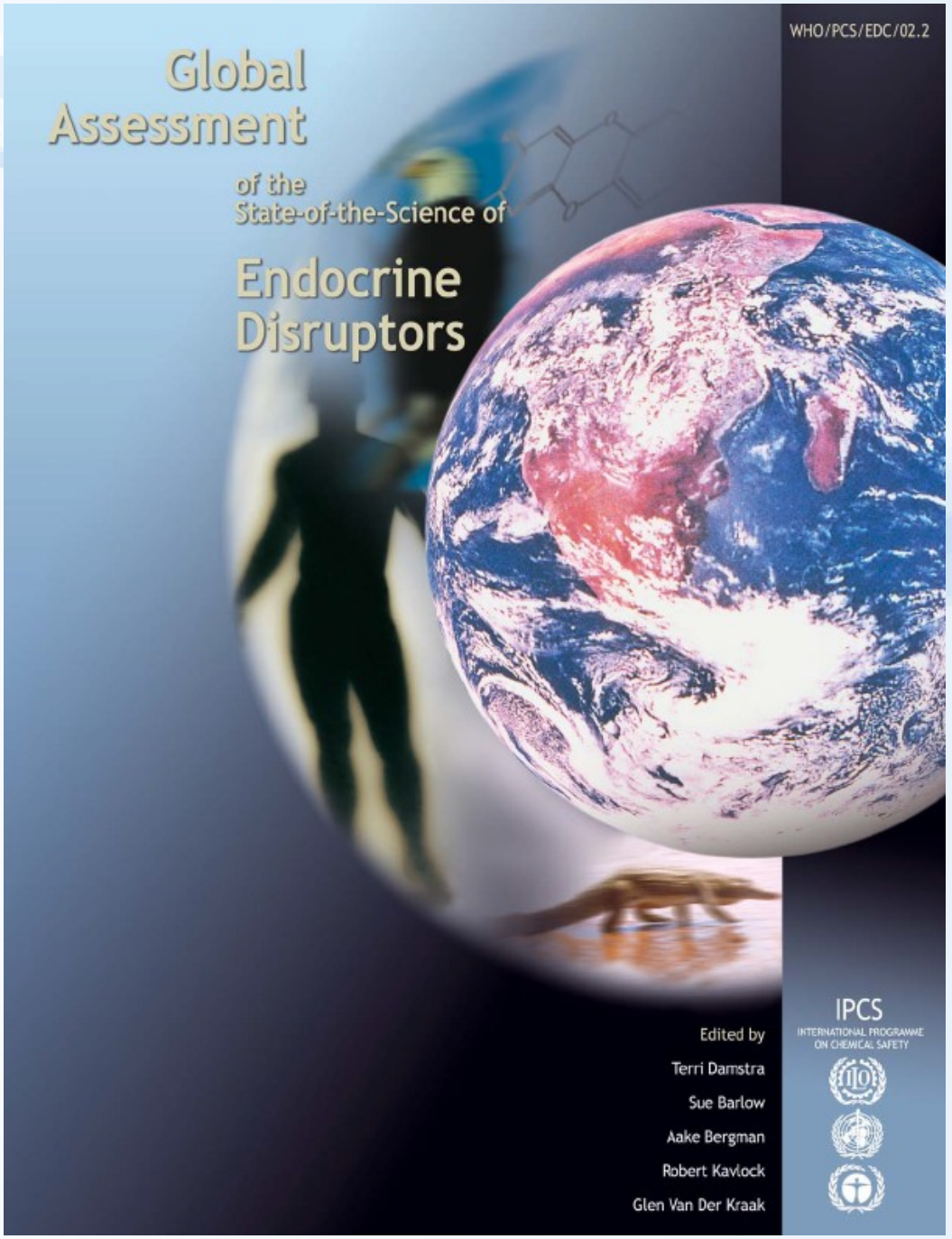
This article is 8 years old

Shares
79

Save for later

An Inuit child in a traditional parka. Photograph: Joel Sartore/Getty/National Geographic


Sperm concentration
In millions of spermatazoa per millilitre
1989
71.4 m
2005
48.8m
75
70
65
60
55
50
45
1989
1992
1995
1998
2001
2004
Year
© Mail Online


WHO/PCS/EDC/02.2
Global Assessment
of the State-of-the-Science of
Endocrine Disruptors
Edited by
Terri Damstra
Sue Barlow
Aake Bergman
Robert Kavlock
Glen Van Der Kraak
IPCS
INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

Impacts on biota → global effects

Mixing oceans

→ cooling the atmosphere

[Nature 447, p.522, May 31, 2007]

Marine life supplies up to 50% of the mechanical energy required worldwide to mix waters from the surface to deeper cool layers

[Dewar, Marine Res 64:541 (2006)]

[Katija a Dabiri, Nature 460:624 (2009)]

Who

wants the assessment ?

Who wants the toxicity assessment?

Who wants the toxicity assessment?

	Researcher	Government
Goal	To understand with joy!	To survive (law or jail? $$ or hunger?)

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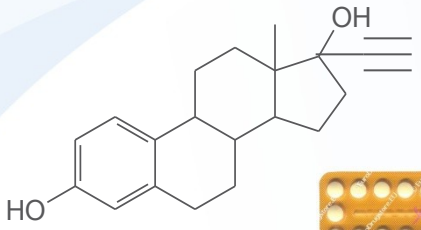
Approach	Why rules?	Strict and tough rules

Who wants the toxicity assessment?

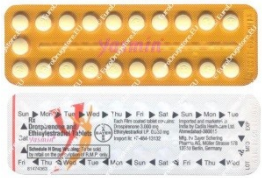
	Researcher	Government
Goal	To understand with joy!	To survive (law or jail? $$ or hunger?)
Approach	Why rules?	Strict and tough rules
Stakeholders	Any? (… other scientists?)	Many! • **Businesses** … providing jobs • **People** … wanting jobs but also health

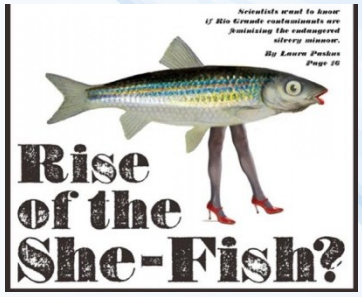
Scientific approach – EE2 (part 1/2)

1. Problem definition

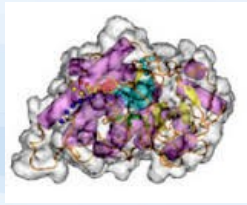


Ethinylestradiol (EE2)

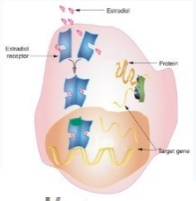


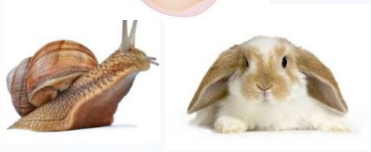


2. Hypotheses!

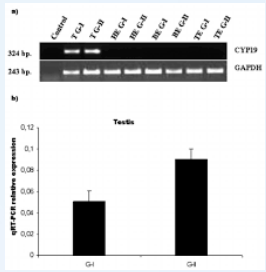


Estrogen receptor?
in fish?
snails?
rabbits?

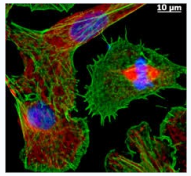




More proteins?
yolk? vitellogenin?
her2neu?
cyp19a?



More cell proliferation?
cancer? gut?
stem cell?

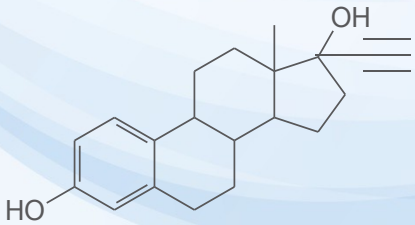


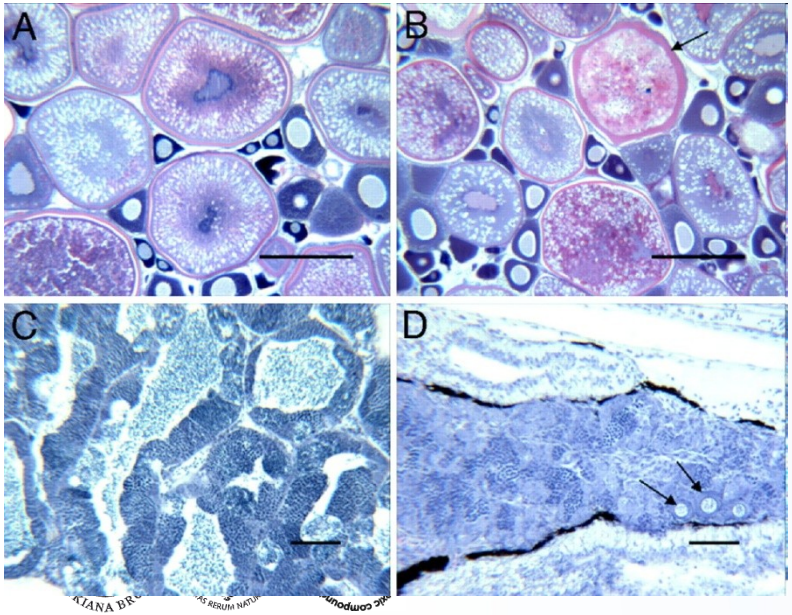
Adverse effects?
Death?
Infertility?
Hyperactivity?

Scientific approach – EE2 (part 2/2)

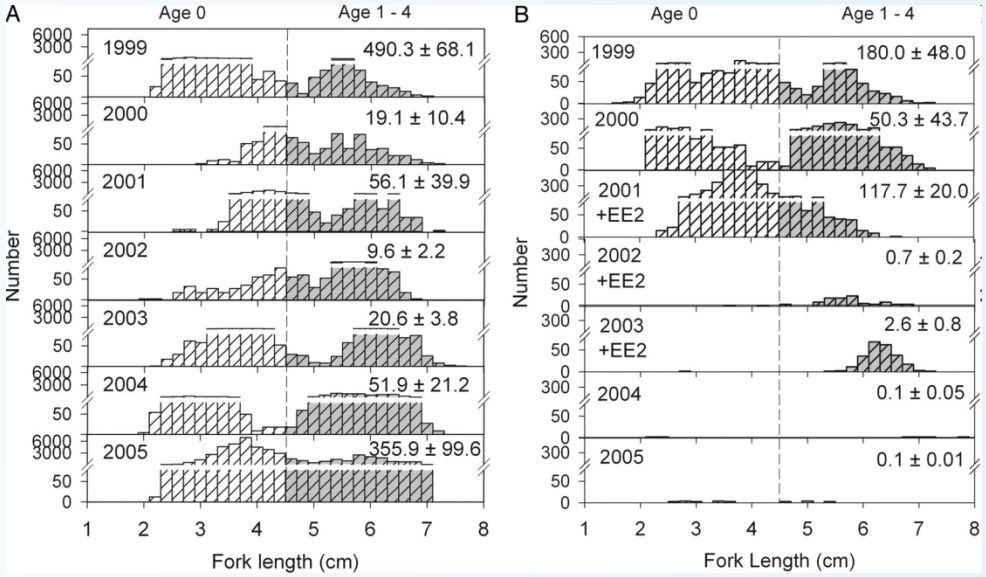
Kidd, K.A. et al. 2007. **Collapse of a fish population** following exposure to **a synthetic estrogen**. *Proceedings of the National Academy of Sciences* 104(21):8897-8901

5 ng/L (!)
7 years





Controls **+Ethinylestradiol**



Regulatory approach: risk assessment and management

Hazard

Exposure assessment
Dose (or PEC)

Effect assessment
TDI (or PNEC)

Risk characterisation

RQ = Dose / TDI
(or PEC / PNEC)

RQ < 1

RQ > 1

Risk **management**

Regulatory approach: risk assessment and management

Hazard

Exposure assessment
Dose (or PEC)

Effect assessment
TDI (or PNEC)

Risk characterisation

RQ = Dose / TDI
(or PEC / PNEC)

DW in city of Bruno …
atrazine 0.15 μg/L

RQ < 1

RQ > 1

Risk **management**

RQ = 0.15/0.1 = **1.5**

EU Directive 98/83/EC
(in addition to others)
pesticide in drinking water

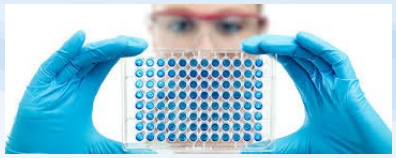
No pesticide
in DW
>0.1 μg/L

DWTP company
$$ for penalty
$$ for DWTP improvement
$$ lobbying to affect
legislation

Summary on **Who?**

- Toxicity assessment depends on goal
  - approaches, standardization, demands on quality etc
- Science vs Regulatory/business reality
  are different worlds with specific requirements

- (Eco)Toxicological research is exciting and important:
  - if relevant (and if accepted by the society / polititans) the results continuously improve quality of life



- Risk assessment concept integrates is central to decision making
  - Integrates eco/toxicity testing (= „effect“ assessment or dose-response assessment)

When

Where

the assessment of toxicity is needed ?

What

to assess for toxicity ?

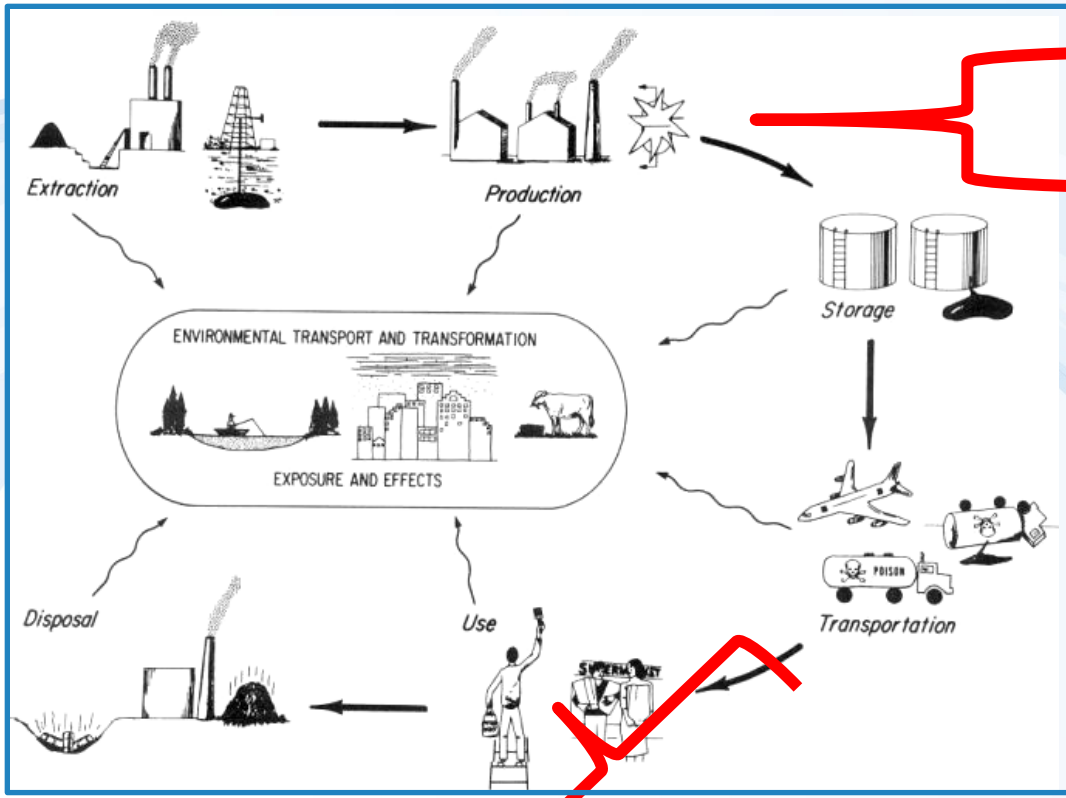
When & where the toxicity assessment is needed?

Anytime!

… depending on researcher's budget

As the law says!

… what are the law(s)? →



Chemical laws („bulk“)

- Industrial chemicals
- Cosmetics
- PPP (pesticides)
- Biocides
- Human pharmaceuticals
- Veterinary pharmaceuticals

MIXTURES!

nano

nano

nano

nano

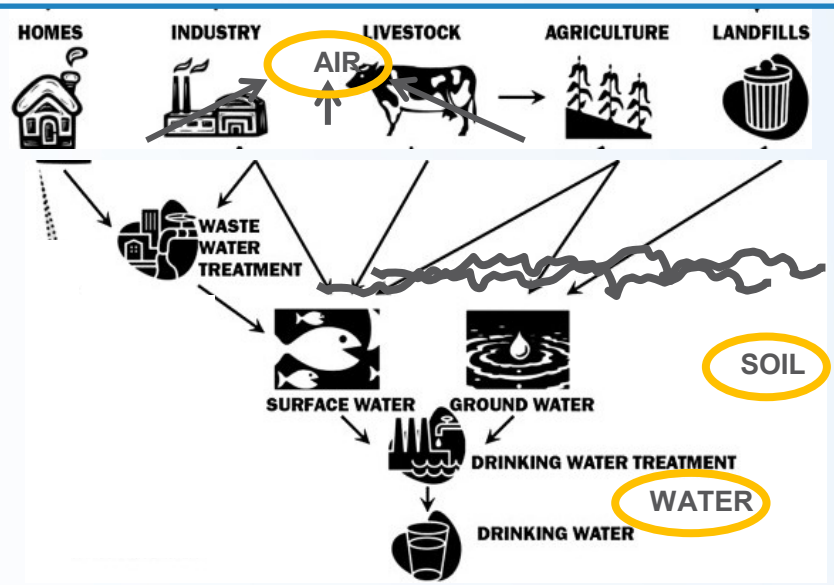
REACH (ECHA)

PPP (EFSA)

MPs (EMA)

Two approaches:

- Prospective (chemicals…)
- Retrospective (mixtures …)



WFD – surface w.
GWD – ground w.

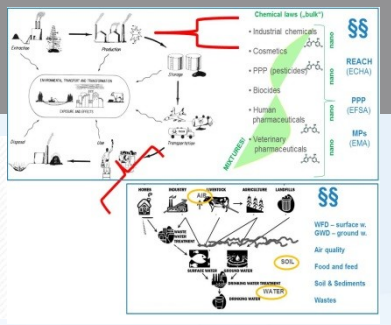
Air quality

Food and feed

Soil & Sediments

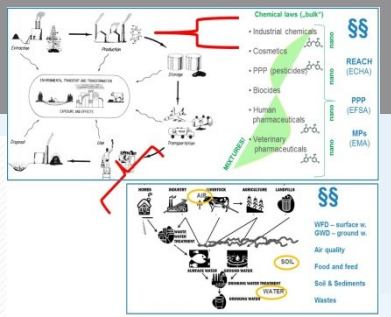
Wastes

What to assess for toxicity?



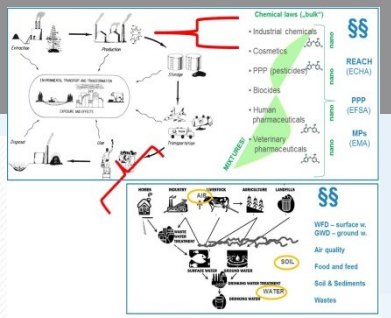
	Current research topics	As required by law
Individual chemicals		
Mixtures		
Contaminated samples		

What to assess for toxicity?



	Current research topics	As required by law
Individual chemicals	Engineered **nanomaterials**/particles **Ecological effects** (e.g. of pharmaceuticals) **Endocrine** disruption & **chronic** diseases	Industry & biocides (REACH) PPPs = pesticides Pharmaceuticals Cosmetics
Mixtures		
Contaminated samples		

What to assess for toxicity?



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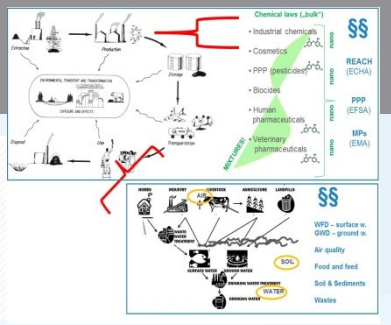
Mixtures	**Multistressors** +T°C, salinity, pathogens, irradiation, food **Exposome**	
Contaminated samples		

What to assess for toxicity?



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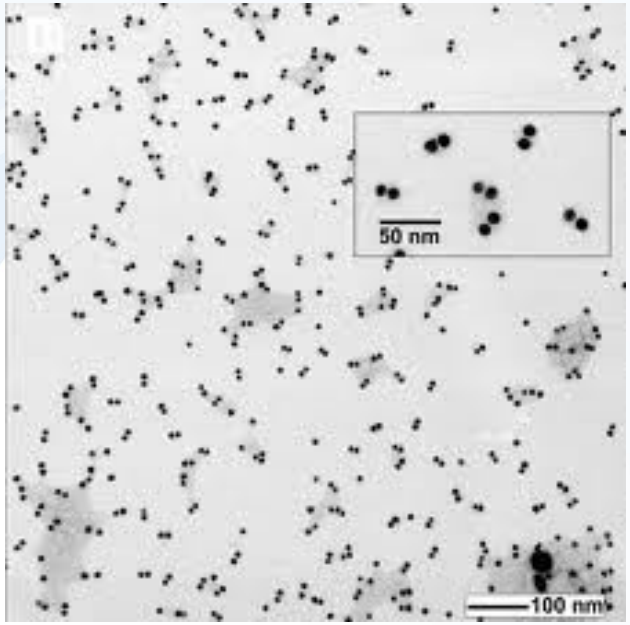
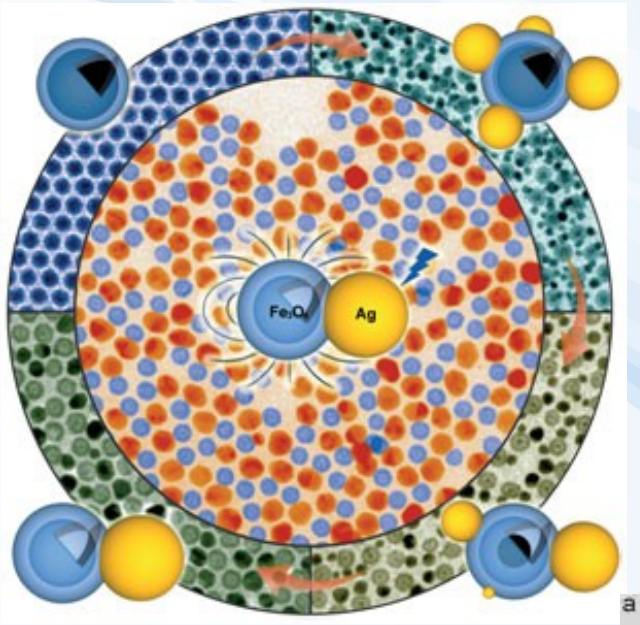
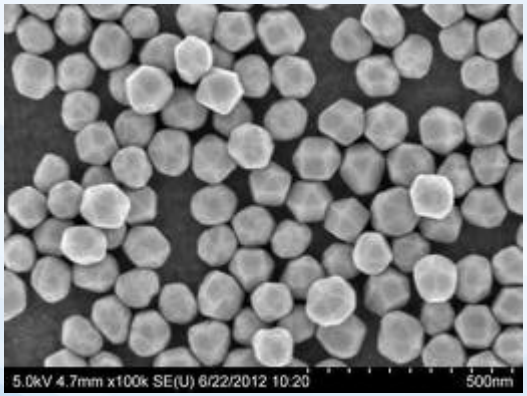
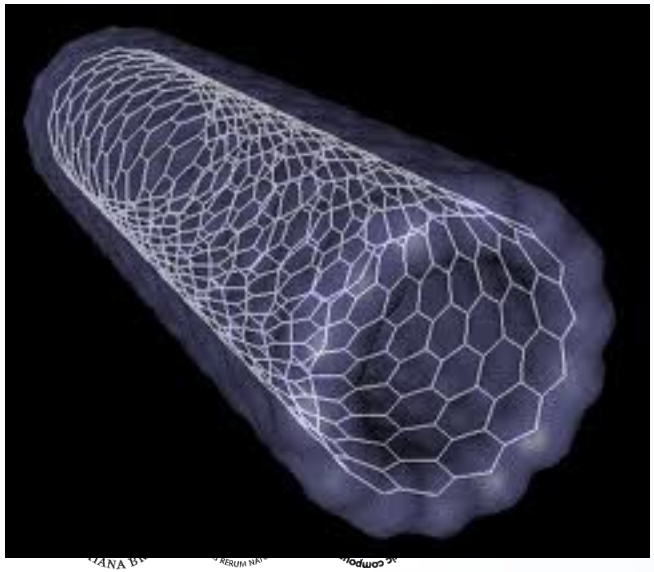
Mixtures	**Multistressors** +T°C, salinity, pathogens, irradiation, food **Exposome**	
Contaminated samples	Can analyzed chemicals explain observed effects ?	**Chemical analyses & limits** **Effect testing rare**: Remediation, dredged sediments (CZ), effluents (DE)

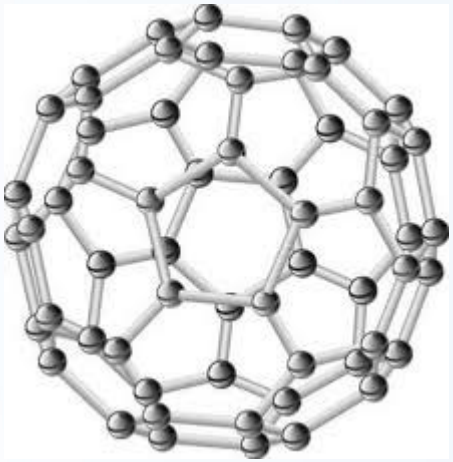
Nanoparticles - examples

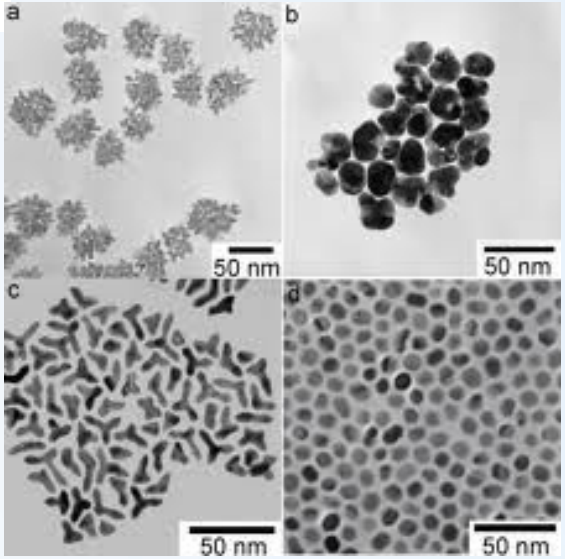




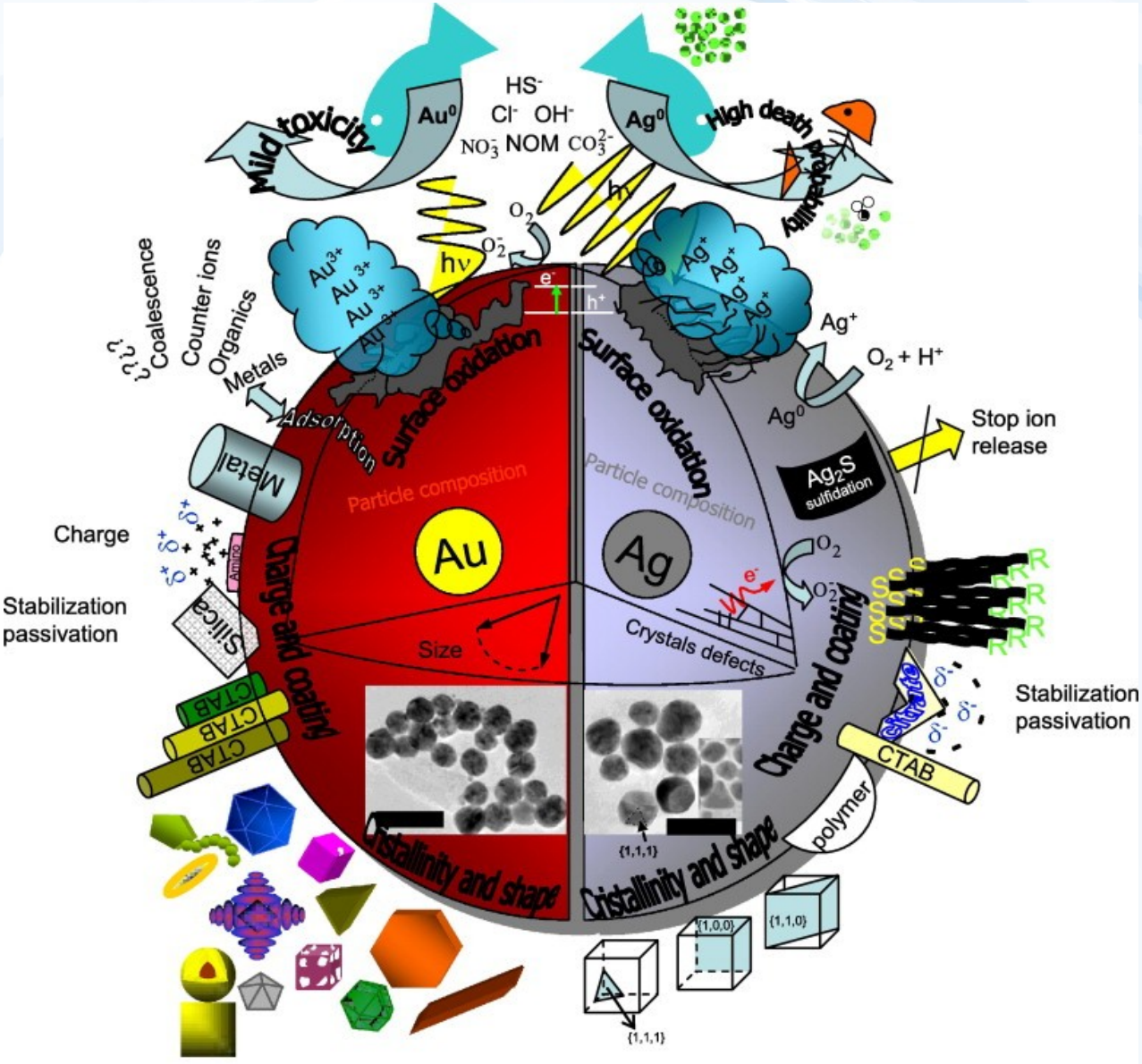








Toxicity of nanoparticles …



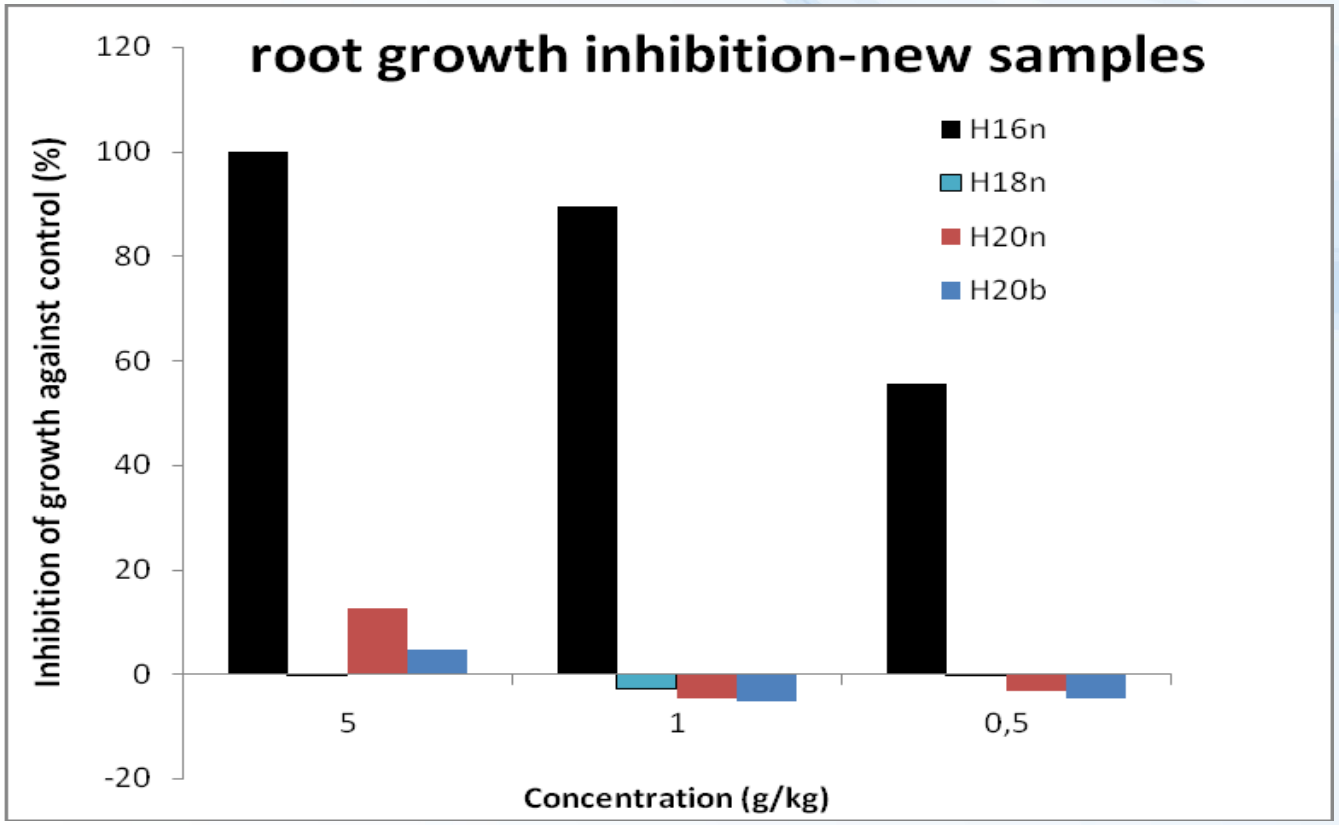
(Mostly unknown) Parameters may Affect ecotoxicity

Composition (chemical)
Surface (size, area)
Charge
Reactivity
Interactions with ions, other chemicals…

➔ Effects on environmental Fate and toxicity

Ecotoxicity of nanoparticles – RECETOX example

Comparison of toxicity - 4 „appeared to be the same“ particles
(one producer – 4 different lots)
(zerovalent iron – ZVI – Fe^0)



?? Why is H16 so toxic ??
… despite of detailed investigation never revealed

PHARMACEUTICALS

R&D and Manufacturing

Storage Transport

Distribution

Storage Transport

Consumption

Storage Transport

Waste management

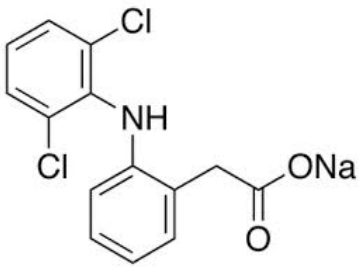
Manufacturing waste

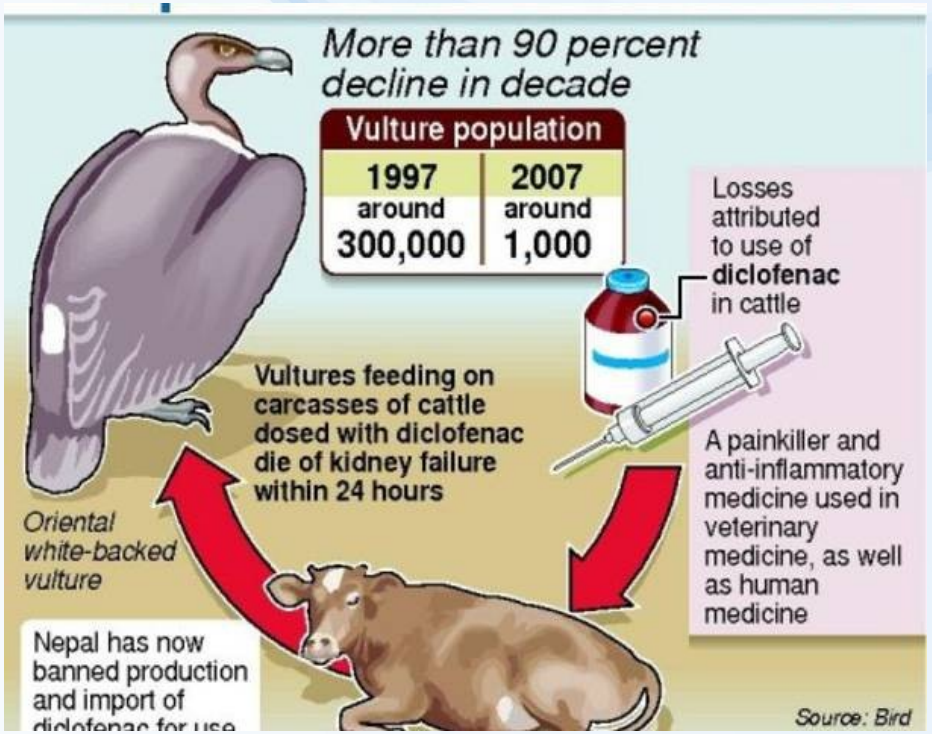
Possible releases to the environment

Example 1 - DICLOFENAC

Unexpected effects at NON-TARGET species

- **nephrotoxicity** at vultures
- Relevant also in EU (ESP, EL,CY)



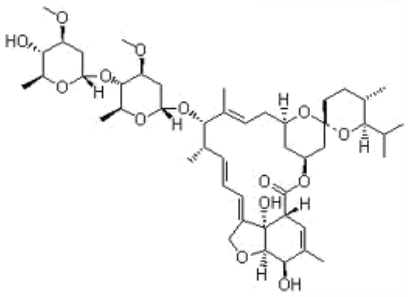


Example 2 – AVERMEKTIN-like antiparasitics

Moxidectin – used e.g. in home „spot on" products

Ivermectin – antiparasitics in large herds

- Used **2-times per season** per sheep/cow
- **Kills 100% parasites** in sheep
- Released in dung - **kills 80-90% larvae of dung flies**
- High concentrations in dung (released 2 days post application)
- **Persistent in the soil** (half-life 30 days)
- Can be washed into adjacent streams (highly toxic to water insects)



POLICYFORUM

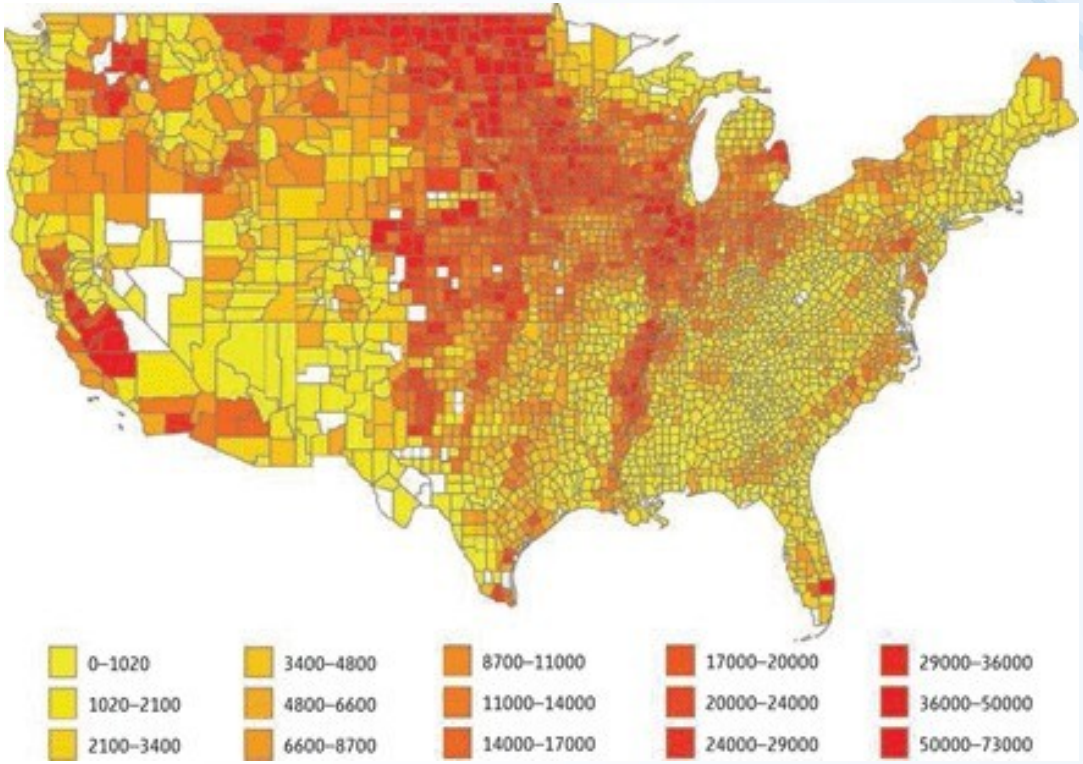
Science
AAAS

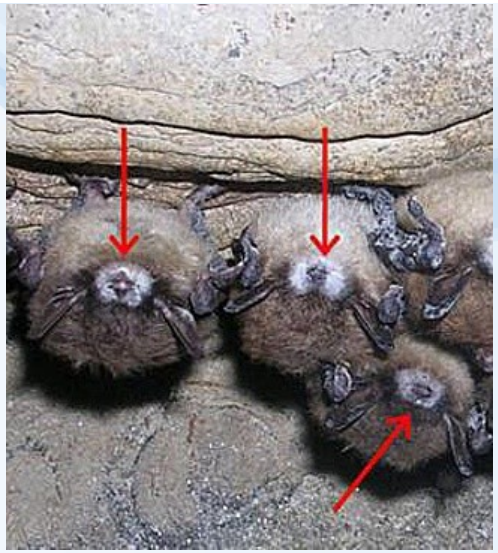
CONSERVATION

Economic Importance of Bats in Agriculture

Justin G. Boyles,[1]* Paul M. Cryan,[2] Gary F. McCracken,[3] Thomas H. Kunz[4]

Insectivorous bat populations, adversely impacted by white-nose syndrome and wind turbines, may be worth billions of dollars to North American agriculture.





Boyles et al. (2011) Science 332 (60251) 41-42

biology letters
Animal behaviour

Biol. Lett.
doi:10.1098/rsbl.2012.0685
Published online

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

Daniel P. Roche, Katie E. McGhee* and Alison M. Bell

School of Integrative Biology, University of Illinois, Urbana, IL 61801, USA
**Author for correspondence (kemcghee@illinois.edu).*

Stress → multigeneration effects

Epigenetics → DNA methylations

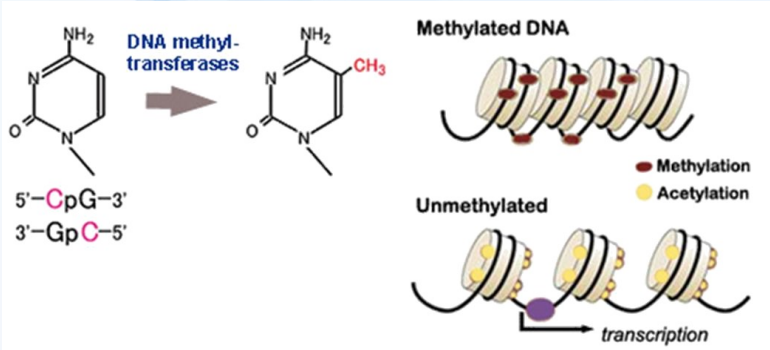


Table 1. Behaviours (mean ± s.e.) of the offspring from the maternal treatments.

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

2x difference

Neuroscience and Biobehavioral Reviews 32 (2008) 1073–1086

Contents lists available at ScienceDirect

Neuroscience and Biobehavioral Reviews

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

Review

The long-term behavioural consequences of prenatal stress

Marta Weinstock*

Department of Pharmacology, Hebrew University, Medical Centre, Ein Kerem, Jerusalem 91120, Israel

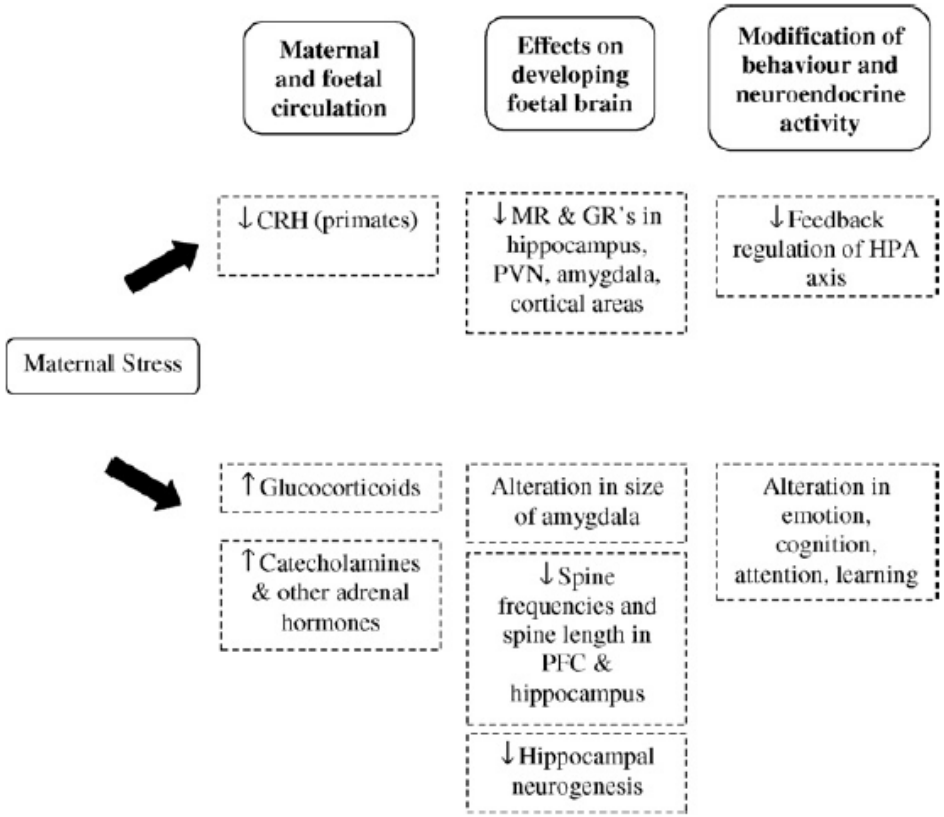


Fig. 2. Routes by which maternal stress hormones may induce changes in the foetal brain in the programming of offspring behaviour. The developing foetal brain is sensitive to the actions of excess amounts of glucocorticoids and other hormones. These may alter the structure and function of the limbic system and HPA axis resulting permanent changes in behaviour and neuroendocrine regulation in the offspring. ↑ = increase; ↓ = decrease.

International ring test (2012-13)

Testing comparability of existing and innovative bioassays for water quality assessment

Main questions:

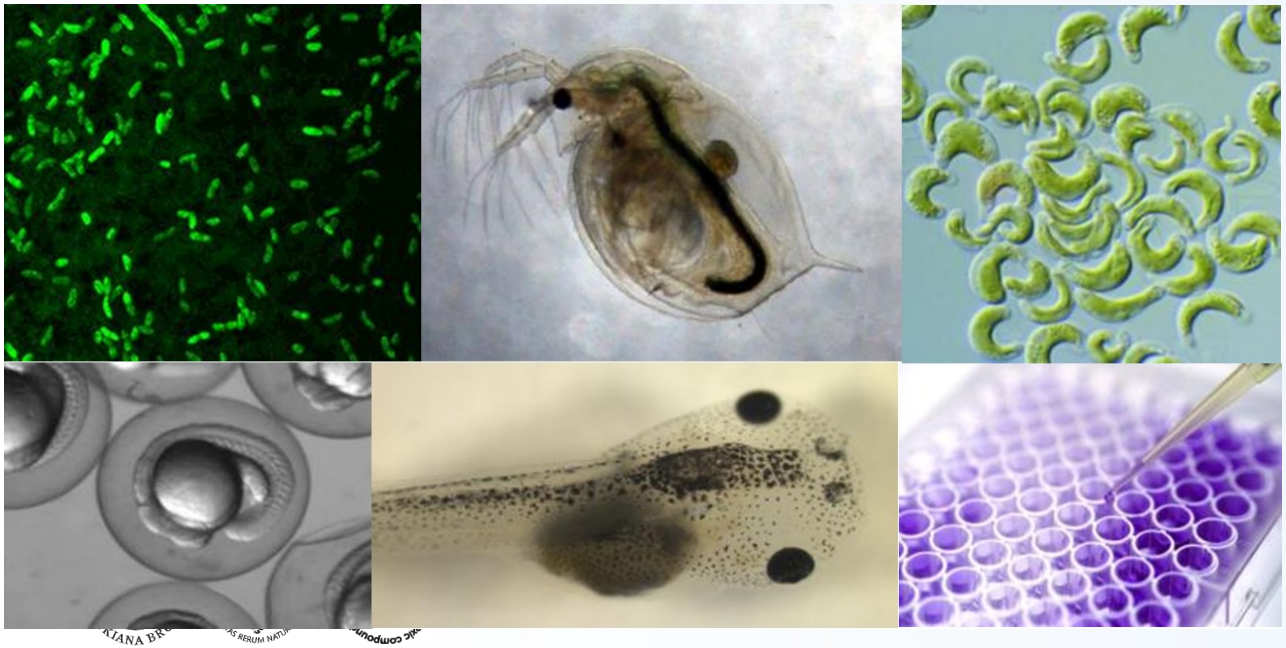
Are current limits (for individual compounds) safe?

Relevance of **"Something from Nothing"** phenomenon ?

3 samples

→ 12 European laboratories – different bioassays

→ ČR – RECETOX: 11 bioassays



Carvalho, R. et al. (2014) Mixtures of chemical pollutants at European legislation safety concentrations: how safe are they? *Toxicol Sci* **141(1): 218-233**

International ring test (2012-13)

Testing comparability of existing and innovative bioassays for water quality assessment

EU WFD priority substances

Different concentrations

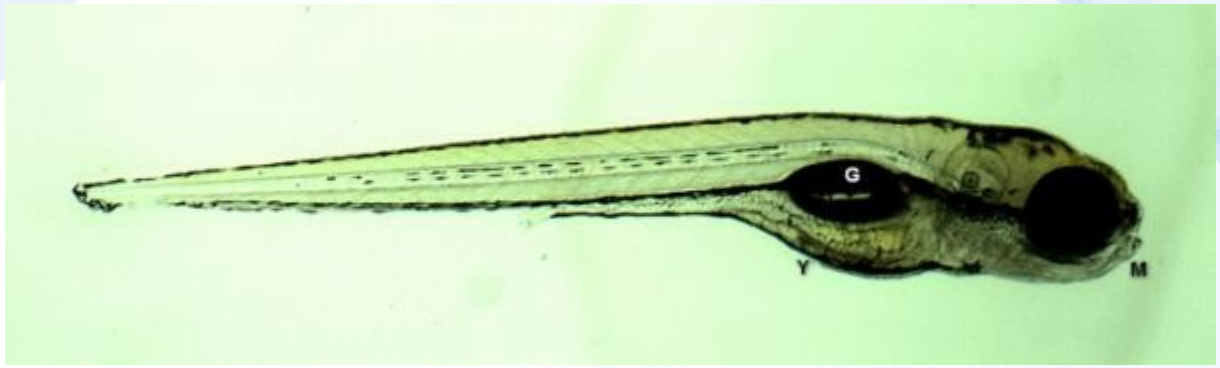
EQS = limit *(Environmental Quality Standard)*

	RM 1[a]	RM 2[a]	RM 3[a]
Priority substances mg/L	around or >EQS	< EQS	< EQS
Atrazine	6	0.6	0.6
BaP	0.0017	0.00017	0.00017
Cadmium[b]	0.8	0.08	0.08
Chlorfenvinphos	1	0.1	0.1
Chlorpyrifos	0.3	0.03	0.03
DEHP (Bis(2-ethylhexyl) phthalate)	13	1.3	1.3
Diclofenac	1	0.1	0.1
diuron	2	0.2	0.2
17beta-estradiol	0.004	0.0004	0.0004
fluoranthene	0.063	0.0063	0.0063
Isoproturon	3	0.3	0.3
Ni[b]	40	4	4
4-Nonylphenol	3	0.3	0.3
Simazine	10	1	1
Carbamazepine	-	-	0.5
Sulfamethoxazole	-	-	0.6
Triclosan (Irgasan)	-	-	0.02
DEET	-	-	41
Bisphenol A	-	-	1.5

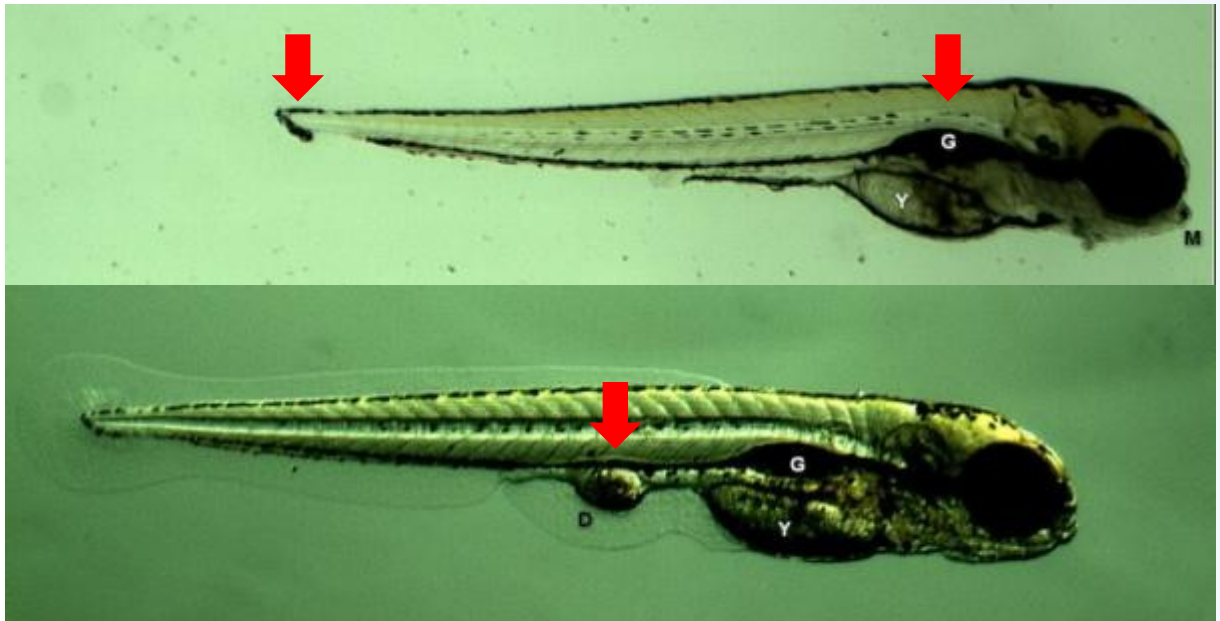
International ring test (2012-13)

Testing comparability of existing and innovative bioassays for water quality assessment

Example: Effects of mixtures on *D. rerio* fish embryos



Control



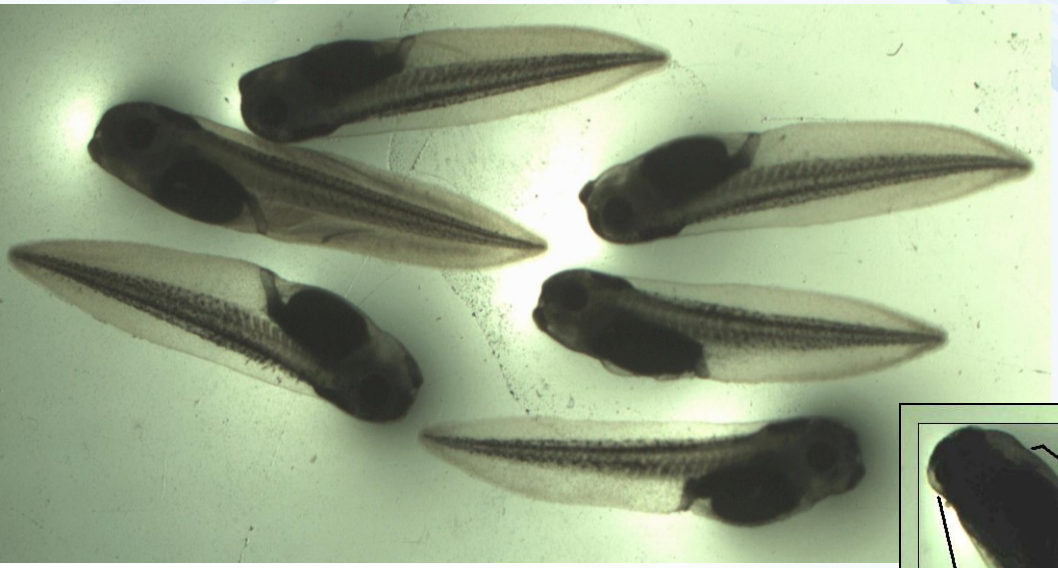
Effects of RM 3 (i.e. safe) mixtures

Carvalho, R. et al. (2014) Mixtures of chemical pollutants at European legislation safety concentrations: how safe are they? *Toxicol Sci* **141(1): 218-233**

International ring test (2012-13)

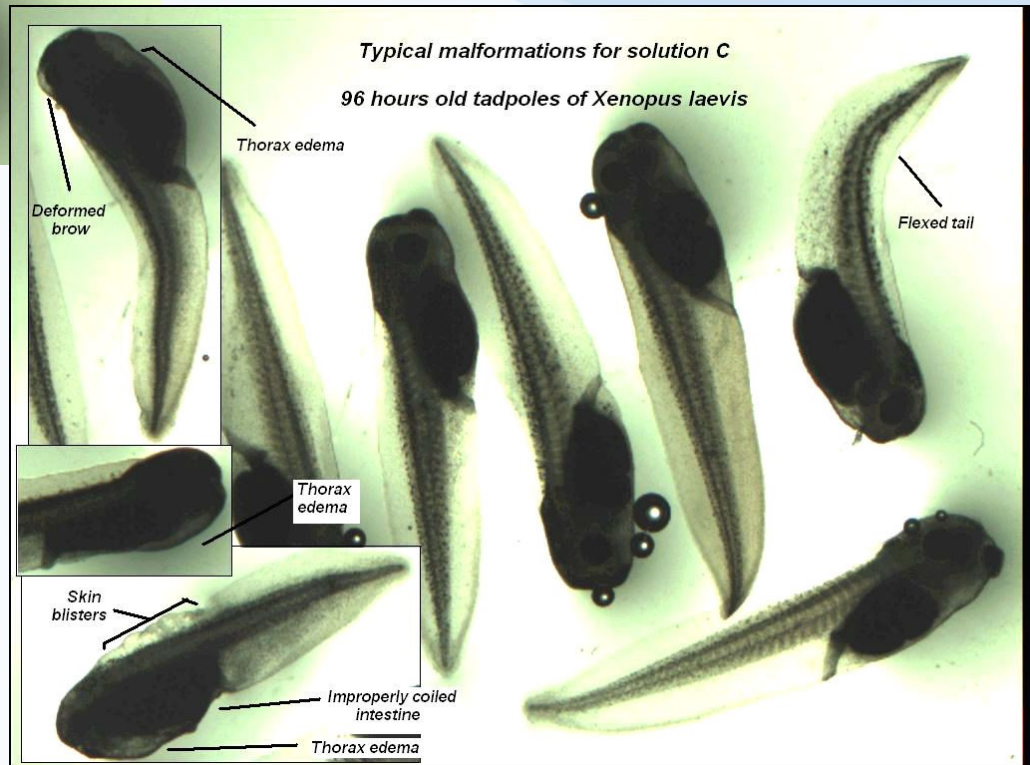
Testing comparability of existing and innovative bioassays for water quality assessment

Example: Effects of mixtures on *X. laevis* frog embryos



Controls

Effects of RM 3 (i.e. safe) mixtures



Carvalho, R. et al. (2014) Mixtures of chemical pollutants at European legislation safety concentrations: how safe are they? *Toxicol Sci* **141(1): 218-233**

Biotest	A	B	C
Microtox	26 and 36% stimulation of luminescence in 15 and 30 mins of exposure, respectively	18 and 35% stimulation of luminescence in 15 and 30 mins of exposure, respectively	22 and 39% stimulation of luminescence in 15 and 30 mins of exposure, respectively
Algae growth inhibition test 96-h exposure	31% inhibition of growth compared to solvent control	20% inhibition of growth compared to solvent control	16% inhibition of growth compared to solvent control
Acute immobilization test with *D. magna*	90% immobilization after 48 hours of exposure; 25% immobilization occurred in 50% concentration - not statistically significant	no effect observed	no effect observed
Reproduction test with *D. magna* (21-d exposure)	100% mortality after 3 days of the test, no reproduction could be evaluated	31 +/- 37 % inhibition of reproduction, not statistically significant	23 +/- 24 % inhibition of reproduction, not statistically significant
FETAX (96-h exposure)	62 +/- 10 % of malformed embryos; no effect on embryo length observed	43 +/- 12 % of malformed embryos; no effect on embryo length observed	34 +/- 14 % of malformed embryos; no effect on embryo length observed
FET (120-h exposure)	effects observed in number of defected embryos - absence of gas bladder, (head) deformities and underdeveloped embryos were observed the most often.	no significant effects observed	effects observed in number of defected embryos, number of underdeveloped embryos and length
In vitro - cytotoxicity	no effect observed compared to solvent control	no effect observed compared to solvent control	no effect observed compared to solvent control
In vitro - estrogenicity	effect under LOQ	effect under LOQ	effect under LOQ
In vitro - dioxin-like toxicity	effect under LOQ	effect under LOQ	effect under LOQ
In vitro - androgenicity	effect under LOQ	effect under LOQ	effect under LOQ
In vitro - antiandrogenicity	effect under LOQ	effect under LOQ	effect under LOQ

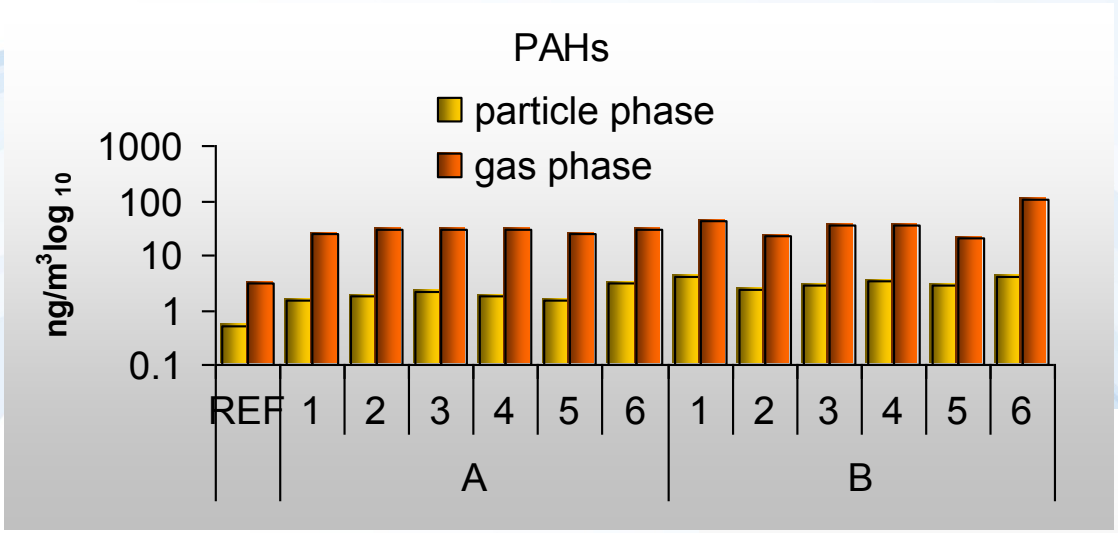
Contaminated samples? Case study **“air“**

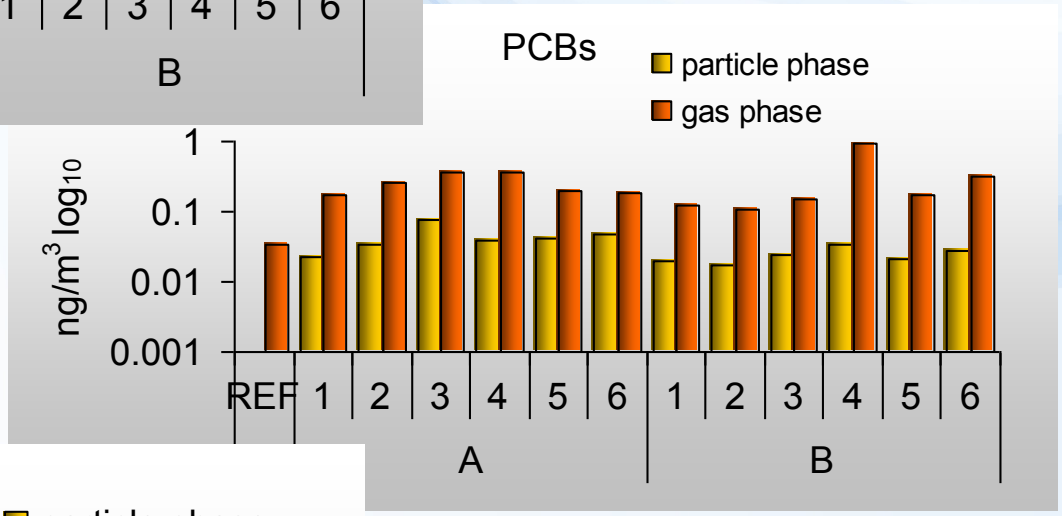
Active sampling
particles *vs* gaseous phase

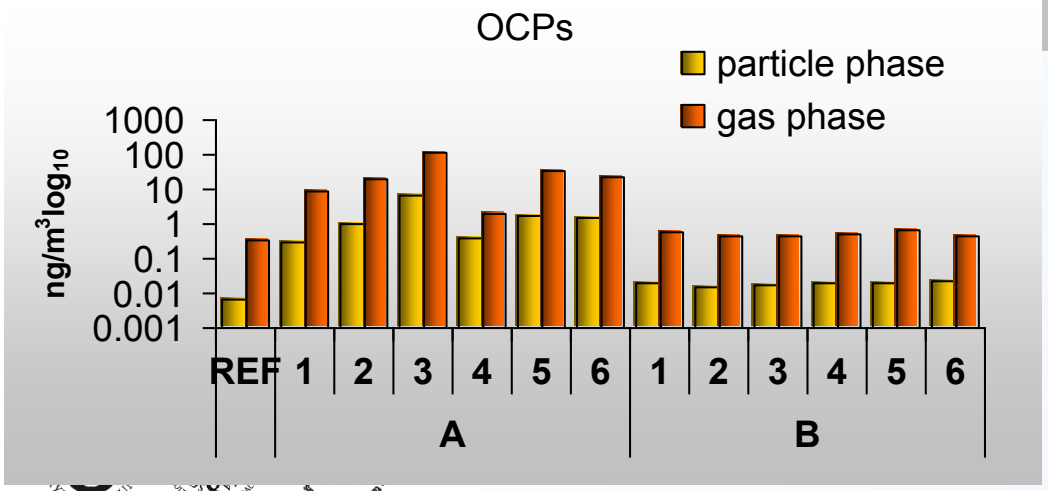
- **Reference locality –** agriculture (Košetice observatory)
- **Region A** – industrial (historically OCPs production)
- **Region B** – combined: industry, agriculture, traffic

Novák et al. (2009) Environment International

Chemical analyses

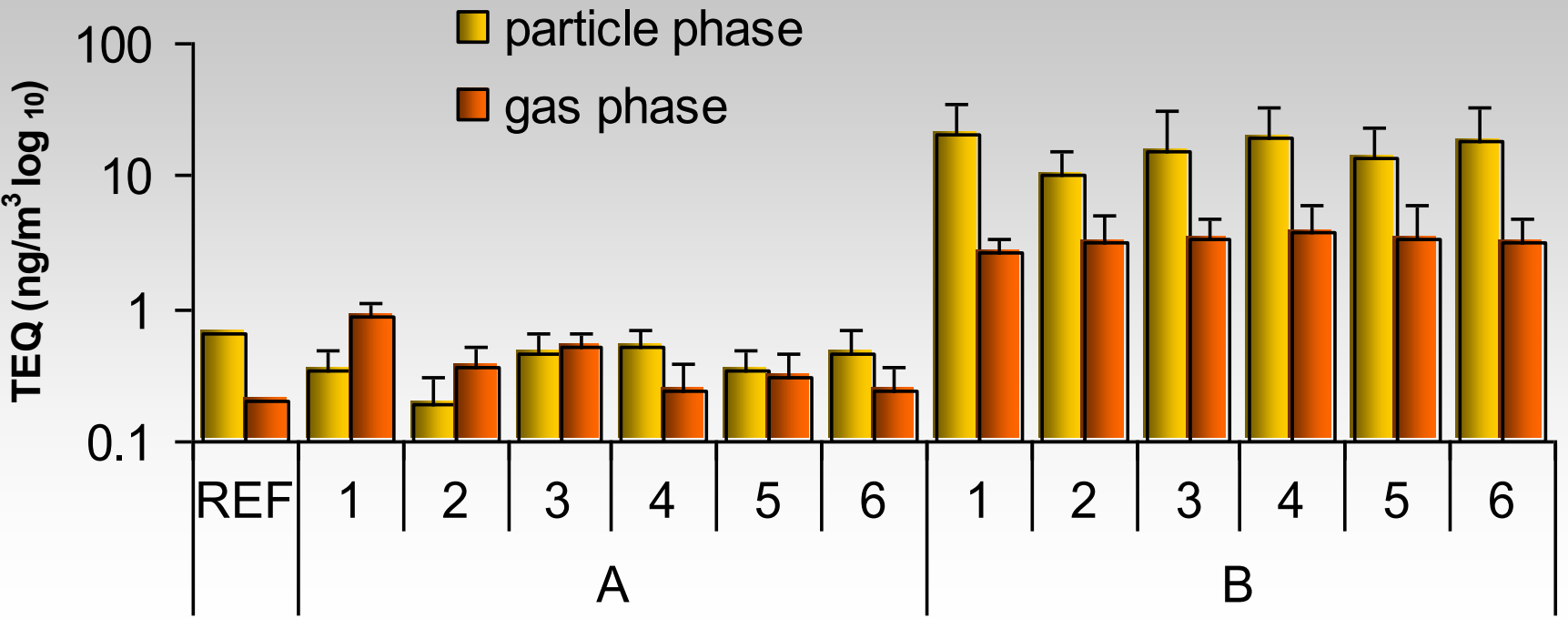






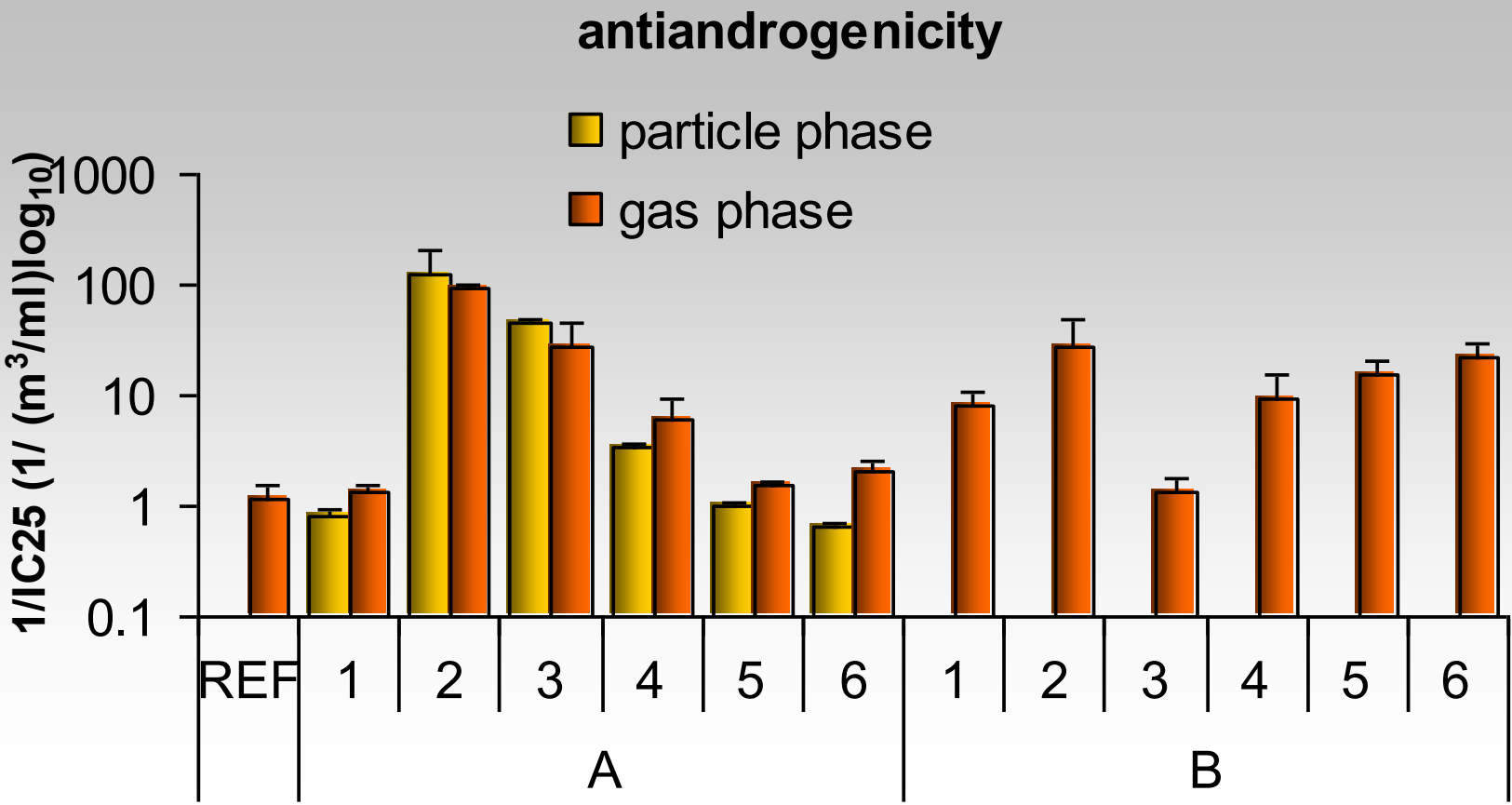
Dioxin-like effects

Labs
on Wed + Thu



- Difference B>A
- Difference B vs A – particles vs gas

Antiandrogenic effects



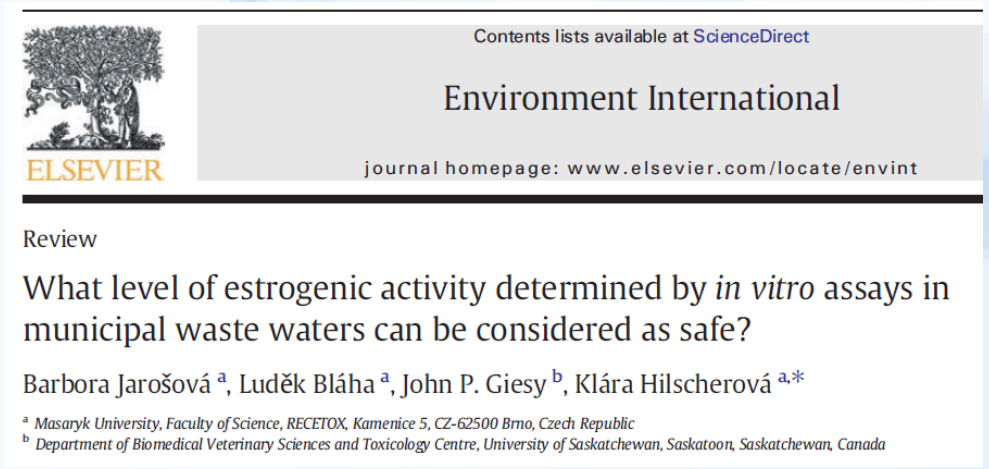
- Quantitative – comparable
- Clear differences in patterns … no effects on particles in „B“ (?)

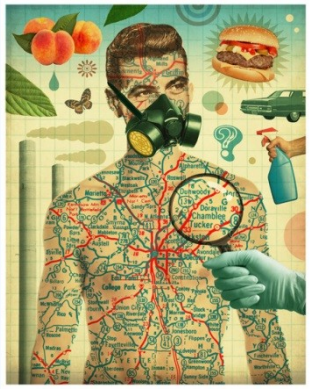
Summary on When, Where, What

- **Regulatory world**
  - Assessment of „chemicals“!

- Contaminated samples
  - effects rarely tested
  - **Great value of bioassays** in assessment of contaminated samples
    - Effects observed (!)
    - **How to set the „limits“?**

- **Research issues and questions**
  - Nanomaterials, Pharmaceuticals, EDCs
  - Mixtures!
  - Exposome





How

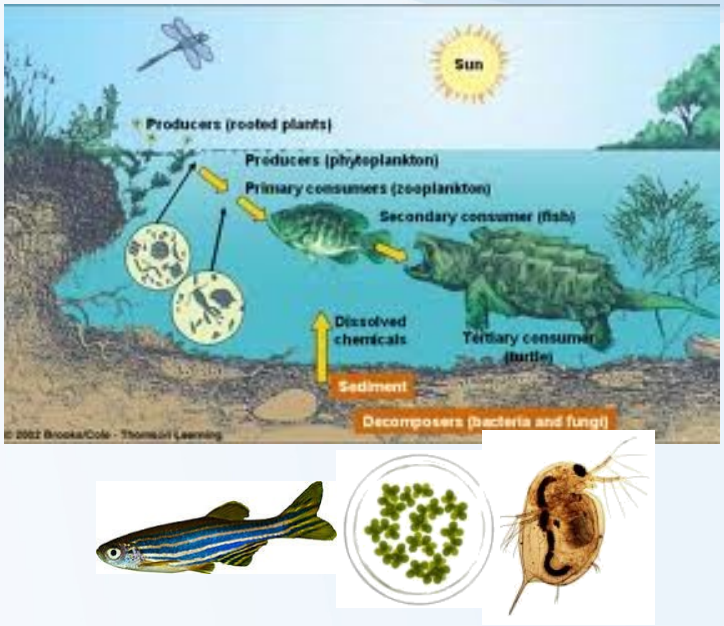
to assess the toxicity ?

Assessment of chemical hazards

…to…

Humans (**TOXICOLOGY**)

Other organisms (**ECO**toxicology)



(Eco)Toxicology – science of „doses“

Paracelsus (1493 - 1541)

‘What is there which is not a poison?

„Cause-effect paradigm“

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

Toxicology – ultimate goal ?

To identify (or predict)
safe *vs* hazardous levels

Hazard assessment

Traditionally – Evaluation of adverse effects using the whole organism models

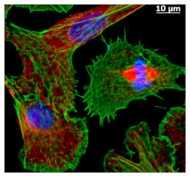
Chemical

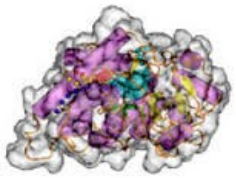
Organism

Adverse Effects

Death
Altered Reproduction
Inhibition of Growth

Tumorigenicity
Skin irritation
…





REGULATORY FOCUS
(APICAL ENDPOINTS)

What assays and how exactly?

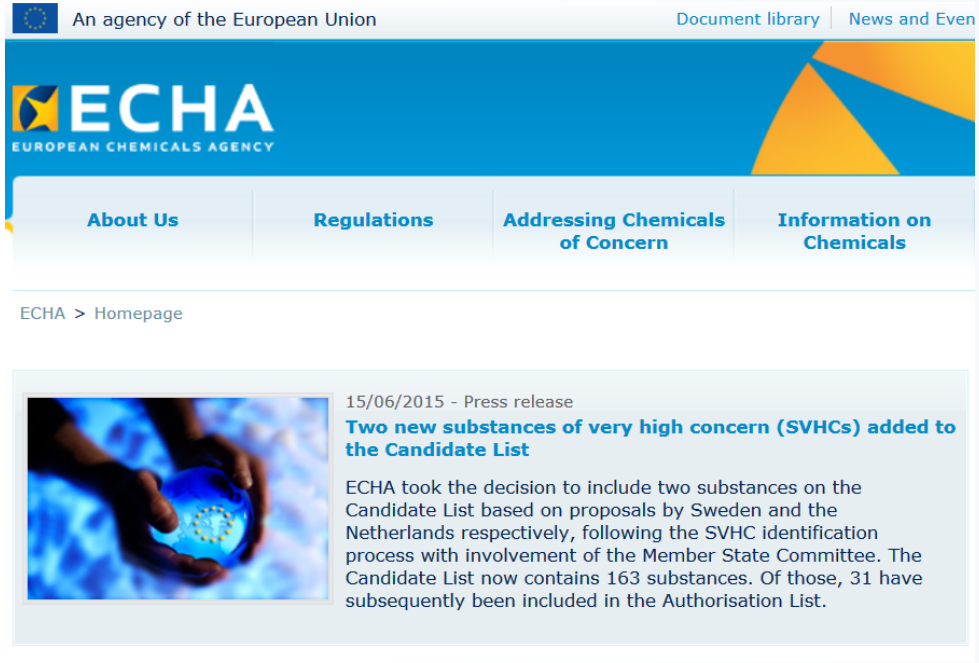
Depends on legislation (… of course !)

… but current EU legislations tend to be harmonized (use similar approaches)

→ **example of REACH**

REACH
Registration, Evaluation and Authorisation of Chemicals

- 27-2-2001: White Paper on the Strategy for Future Chemicals Policy
- 23-10-2003: Commission's proposal REACH
- December 2008: Pre-registration mandatory (all chemicals in EU must be registered at ECHA

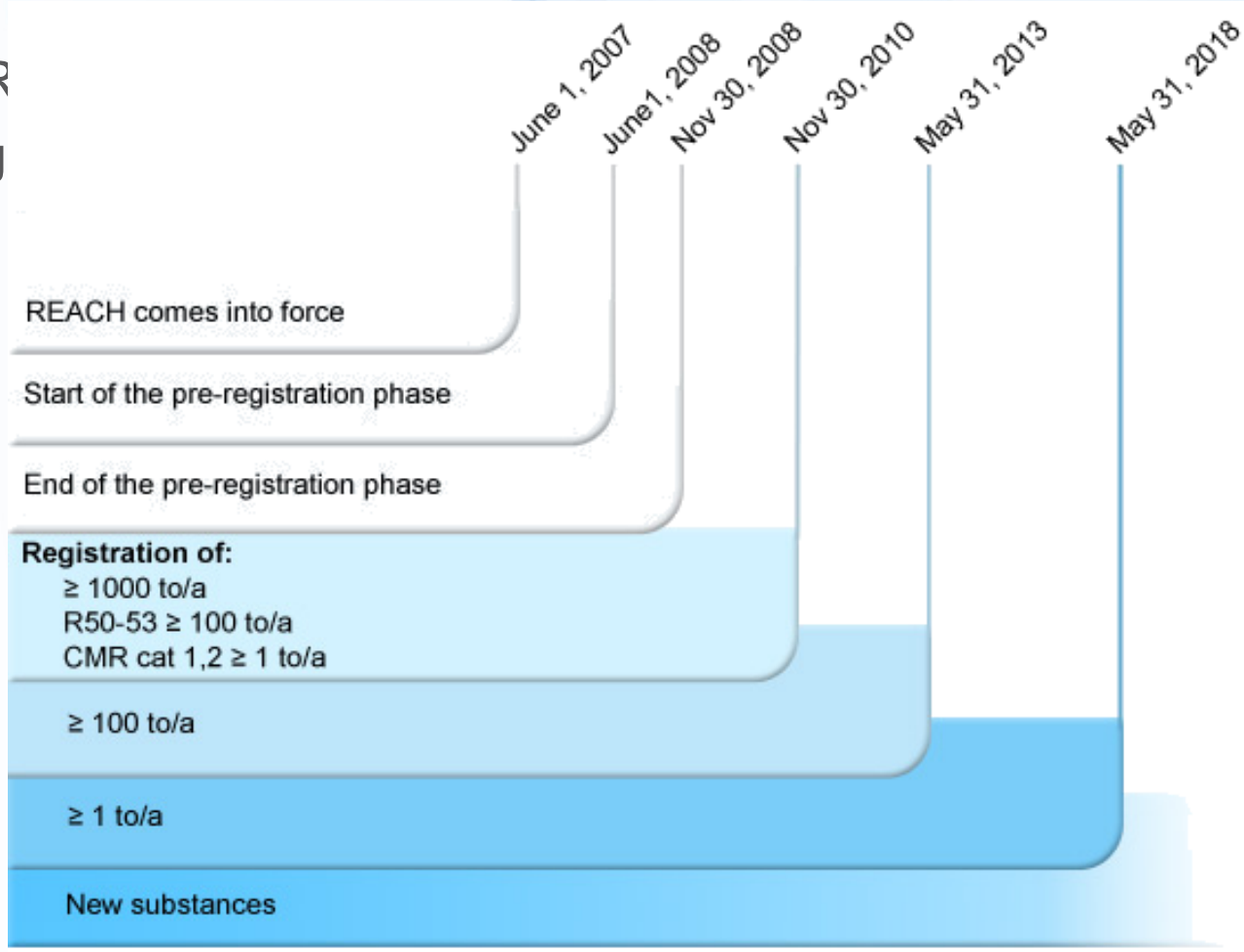


European Chemicals Agency (http://echa.europa.eu)

Existing substances and REACH

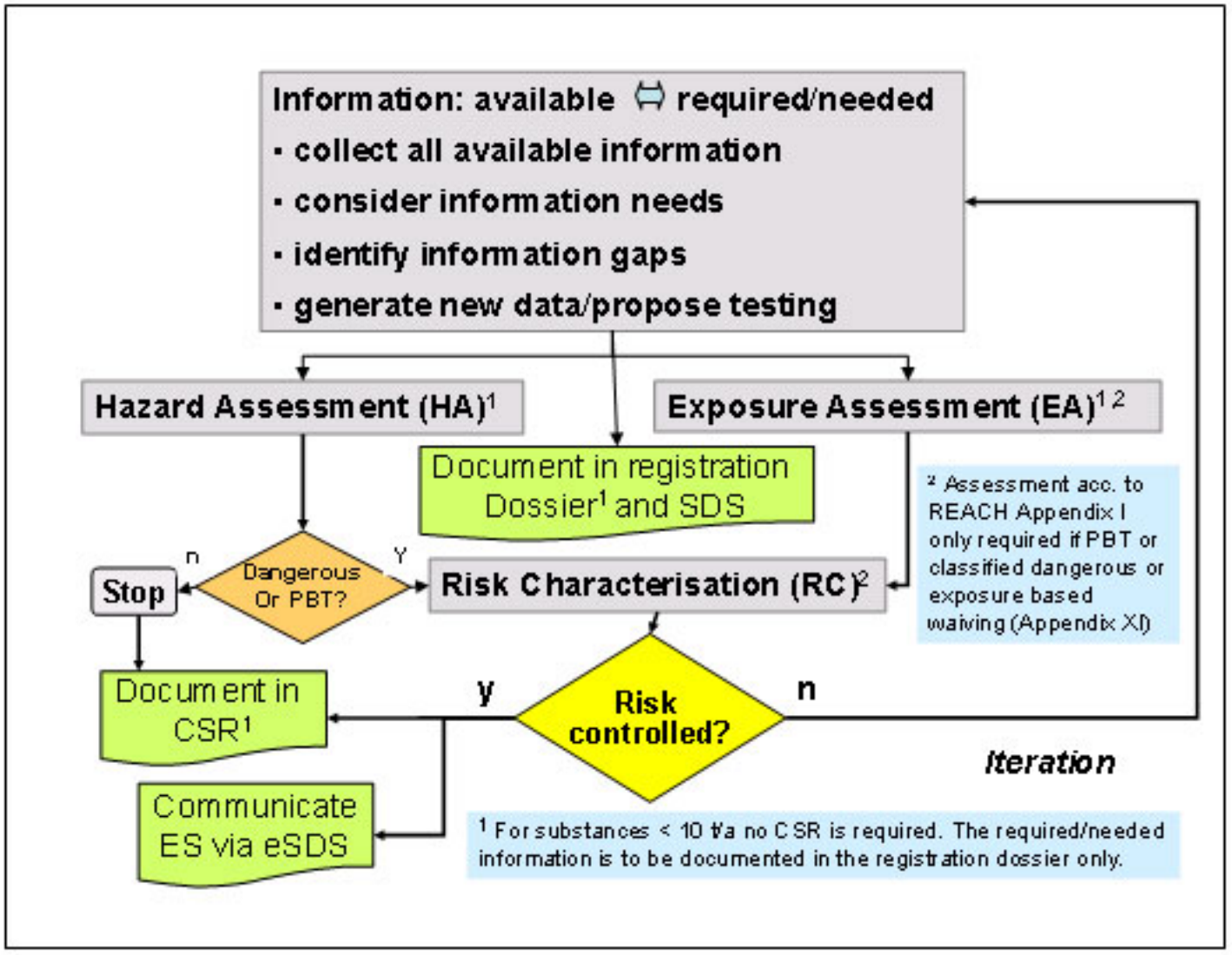
> 95,000,000 known chemicals
(…and counting http://www.cas.org/)

– 100,000 substances in EINECS (i.e. commercial use)
– 30,000 relevant for R
– cc 3000 HPVCs (Hig Volume Chemicals)



REACH legislation in EU

Registration, Evaluation and Authorisation and Restriction of Chemicals



REACH: what data type must be registered?

- **Physico-chemical properties, e.g.:**
  - Vapour pressure, boiling point, Kow,…
- **Human toxicology, e.g.:**
  - Acute and chronic toxicity, skin irritation, carcinogenity,…
- **Environment/ Ecotoxicological information, e.g.:**
  - Acute and/or chronic toxicity for aquatic organisms, biodegradation, …

REACH: testing

Classification categories	Test requirements in REACH			
	>1t		>10t	>100t
	New or prioritised substance			
Reproductive toxicity (a generation test)	no	no	no	no
Chronic toxicity and cancer	no	no	no	(yes)
90-day study	no	no	no	(yes)
28-day study	no	no	(yes)	yes
Acute toxicity (a second route of exposure)	no	no	yes	yes
Acute toxicity	no	yes	yes	yes
Skin allergy	no	yes	yes	yes
Skin and eye irritation	no	yes	yes	yes
Mutageneicity (in vitro)	no	yes	yes	yes
Further ecotoxicity studies (ind long term tests)	no	no	no	yes
Acute toxicity: fish	no	no	yes	yes
Acute toxicity: algae	no	yes	yes	yes
Acute toxicity: Daphnia	no	yes	yes	yes
Biotic degradation	no	yes	yes	yes

- Total costs: 2,8 to 5,6 billion € (industry pays)
- Testing costs (50-60% of total): 86% for Human, 14% Ecotox

What assays and how exactly?

Depends on legislation (… of course !)

… but current EU legislations tend to be harmonized (use similar approaches)

→ **example of REACH**

Assays must be STANDARDIZED for REACH should follow **OECD Guidelines**

Other standardization agencies (also include toxicity tests) e.g. ISO, ASTM

OECD guidelines for testing of chemicals

- 5 main sections
  - Section 1: Physical Chemical Properties
  - Section 2: **Effects on Biotic Systems** (i.e. Ecotoxicity)
  - Section 3: Degradation and Accumulation
  - Section 4: **Health Effects** (i.e. Toxicity)
  - Section 5: Other Test Guidelines

OECD guidelines (examples – selection)

SECTION 2 - Aquatic organisms

Test No. 201: Alga, Growth Inhibition Test	11 July 2006
Test No. 221: Lemna sp. Growth Inhabition Test	11 July 2006
Test No. 202: Daphnia sp. Acute Immobilisation Test	23 Nov 2004
Test No. 211: Daphnia magna Reproduction Test	16 Oct 2008
Test No. 203: Fish, Acute Toxicity Test	17 July 1992
Test No. 204: Fish, Prolonged Toxicity Test: 14-Day Study	04 Apr 1984
Test No. 210: Fish, Early-Life Stage Toxicity Test	17 July 1992
Test No. 212: Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages	21 Sep 1998
Test No. 215: Fish, Juvenile Growth Test	21 Jan 2000
Test No. 229: Fish Short Term Reproduction Assay	08 Sep 2009
Test No. 230: 21-day Fish Assay	08 Sep 2009
Test No. 231: Amphibian Metamorphosis Assay	08 Sep 2009

OECD guidelines (examples – selection)

SECTION 4 – Human health effects

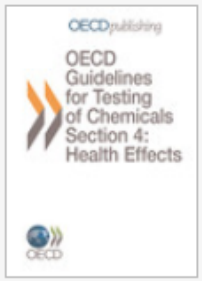
Test No. 401: Acute Oral Toxicity
Test No. 402: Acute Dermal Toxicity
Test No. 403: Acute Inhalation Toxicity
Test No. 404: Acute Dermal Irritation/Corrosion
Test No. 405: Acute Eye Irritation/Corrosion
Test No. 406: Skin Sensitisation
Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents
Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents
Test No. 409: Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents
Test No. 410: Repeated Dose Dermal Toxicity: 21/28-day Study
Test No. 411: Subchronic Dermal Toxicity: 90-day Study
Test No. 412: Subacute Inhalation Toxicity: 28-Day Study

OECD Guidelines for the Testing of Chemicals, Section 4

Health Effects

OECD Guidelines for the Testing of Chemicals

Subscribe to the feed



Hide / Show Abstract

The OECD *Guidelines for the Testing of Chemicals* is a collection of about 150 of the most relevant internationally agreed testing methods used by government, industry and independent laboratories to identify and characterise potential hazards of chemicals. They are a set of tools for professionals, used primarily in regulatory safety testing and subsequent chemical and chemical product notification, chemical registration and in chemical evaluation. They can also be used for the selection and ranking of candidate chemicals during the development of new chemicals and products and in toxicology research. This group of tests covers health effects.

Also available in French

English
ISSN: 2074-5788 (online)
DOI: 10.1787/20745788

Hide / Show all Abstracts

Mark	Date	Title	Click to Access
☐	11 Sep 2006	Summary of Considerations in the Report from the OECD Expert Groups on Short Term and Long Term Toxicology OECD	PDF READ
☐	24 Feb 1987	Test No. 401: Acute Oral Toxicity OECD	PDF READ
☐	24 Feb 1987	Test No. 402: Acute Dermal Toxicity OECD	PDF READ
☐	08 Sep 2009	Test No. 403: Acute Inhalation Toxicity OECD	PDF READ

Try it! … download and study your guideline for free!

Hazard assessment

Traditionally – Evaluation of adverse effects using the whole organism models

Chemical

Organism

Adverse Effects

Death
Altered Reproduction
Inhibition of Growth

Tumorigenicity
Skin irritation
…

REGULATORY FOCUS
(APICAL ENDPOINTS)

Hazard assessment

Traditionally – Evaluation of adverse effects using the whole organism models

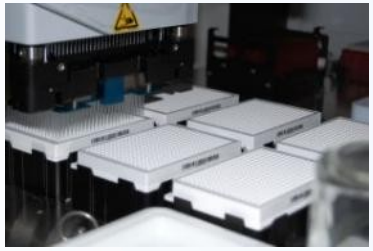
Chemical

Organism

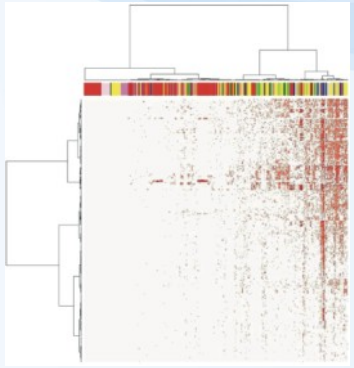
Adverse Effects
Death
Inhibition of Growth
Altered Reproduction
Tumor
Skin irritation
…

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

10^4 Chemicals



HTS
High-Throughput-Screening

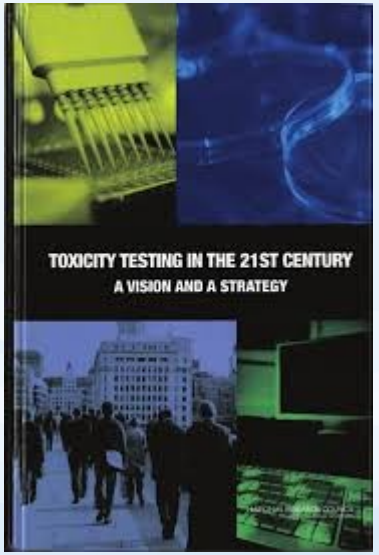


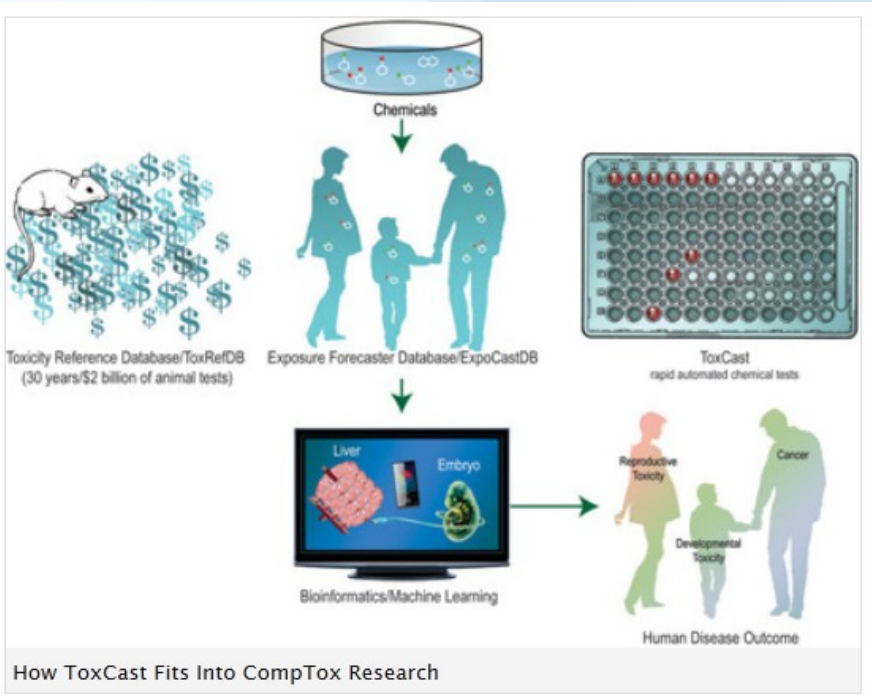
Chemical-biological interactions, Mechanistic Toxicological Data

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

MoA and omics are supported by strategic documents

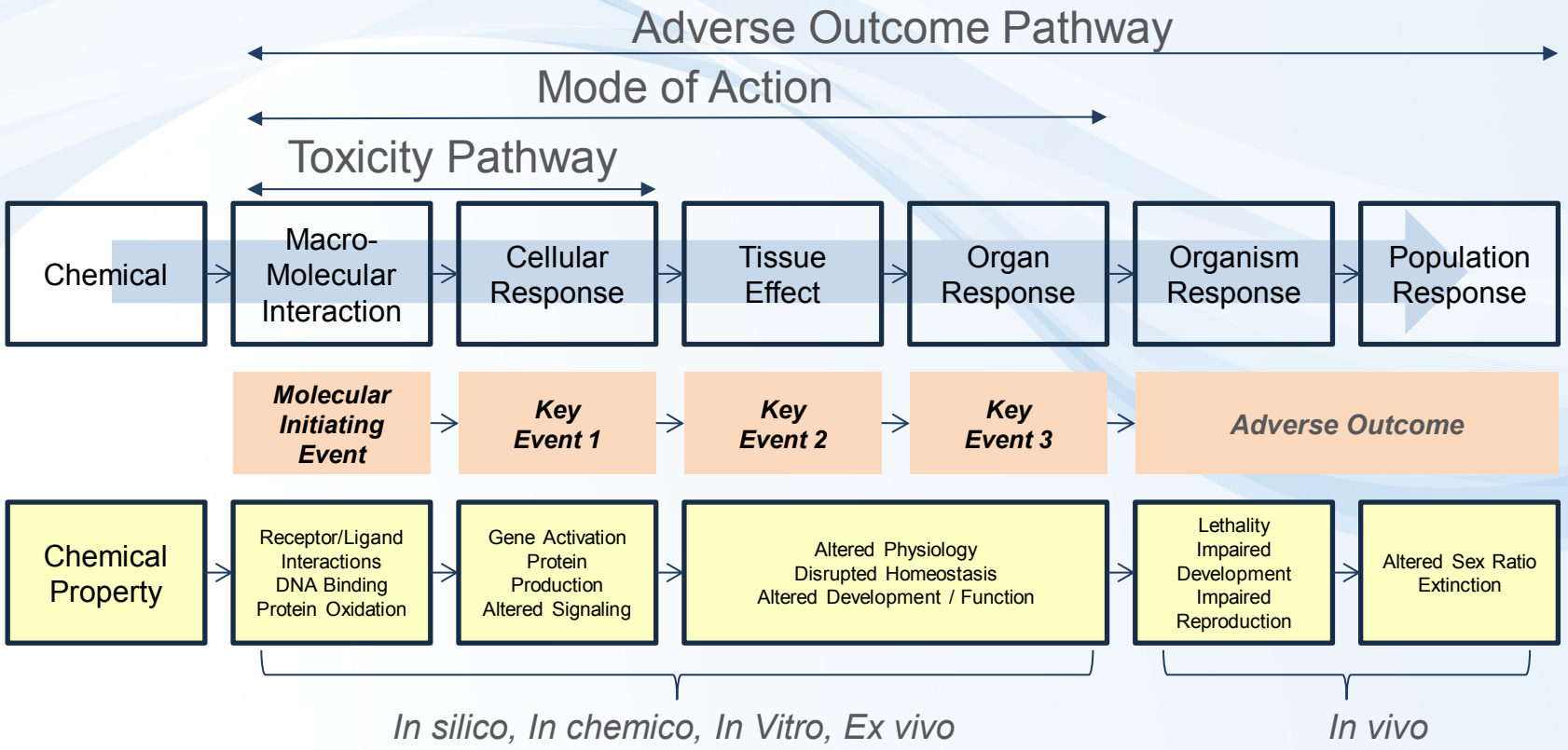
Toxicity Testing in the 21st Century: A Vision and a Strategy
US National Academies of Sciences
http://www.nap.edu/catalog/11970.html





How ToxCast Fits Into CompTox Research

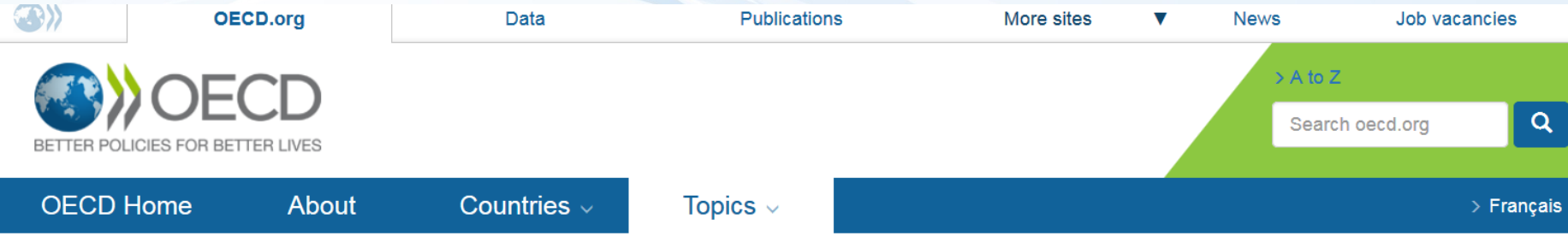
Adverse Outcome Pathways

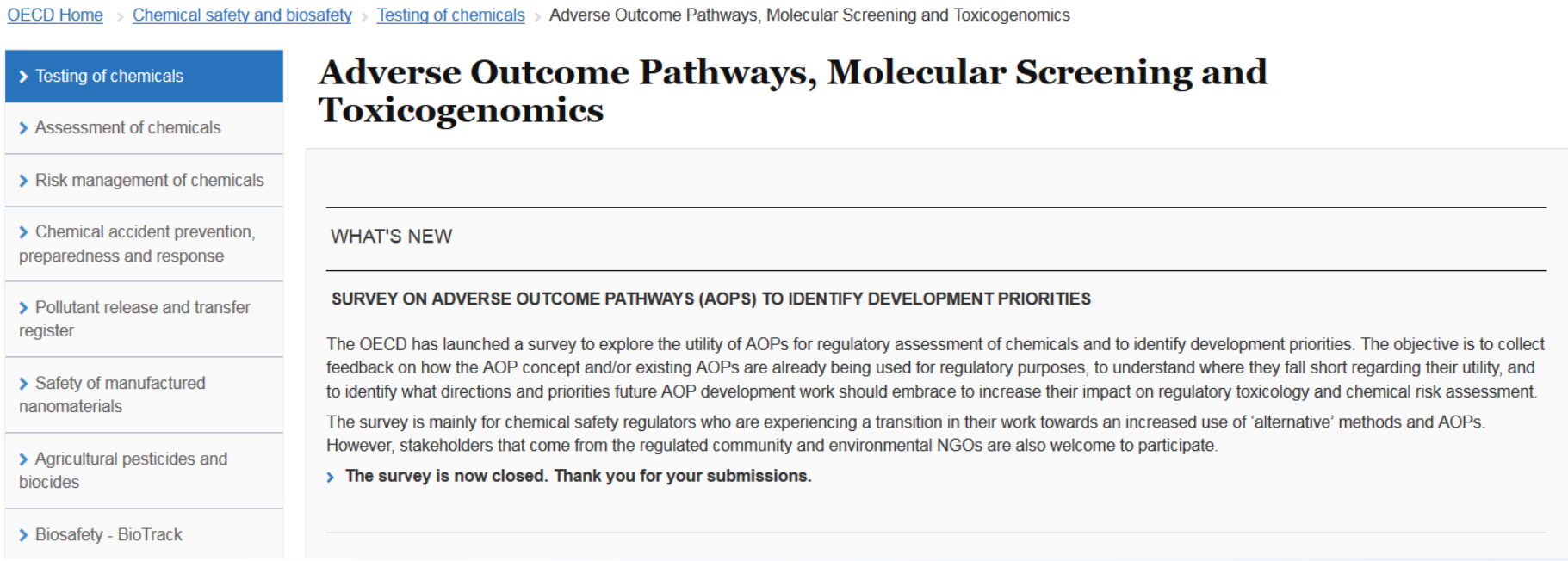


The **EXISTING KNOWLEDGE** is used **to link the** two anchor points: **Molecular Initiating Event** (MIE) and **Adverse Outcome** (AO) **via a series** of intermediate steps: **Key Events**

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.

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





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

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

What AOPs are now in AOP Wiki (May 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)

https://aopwiki.org/aops

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

https://aopwiki.org/aops

AOP Example: MIE aromatase inhibition

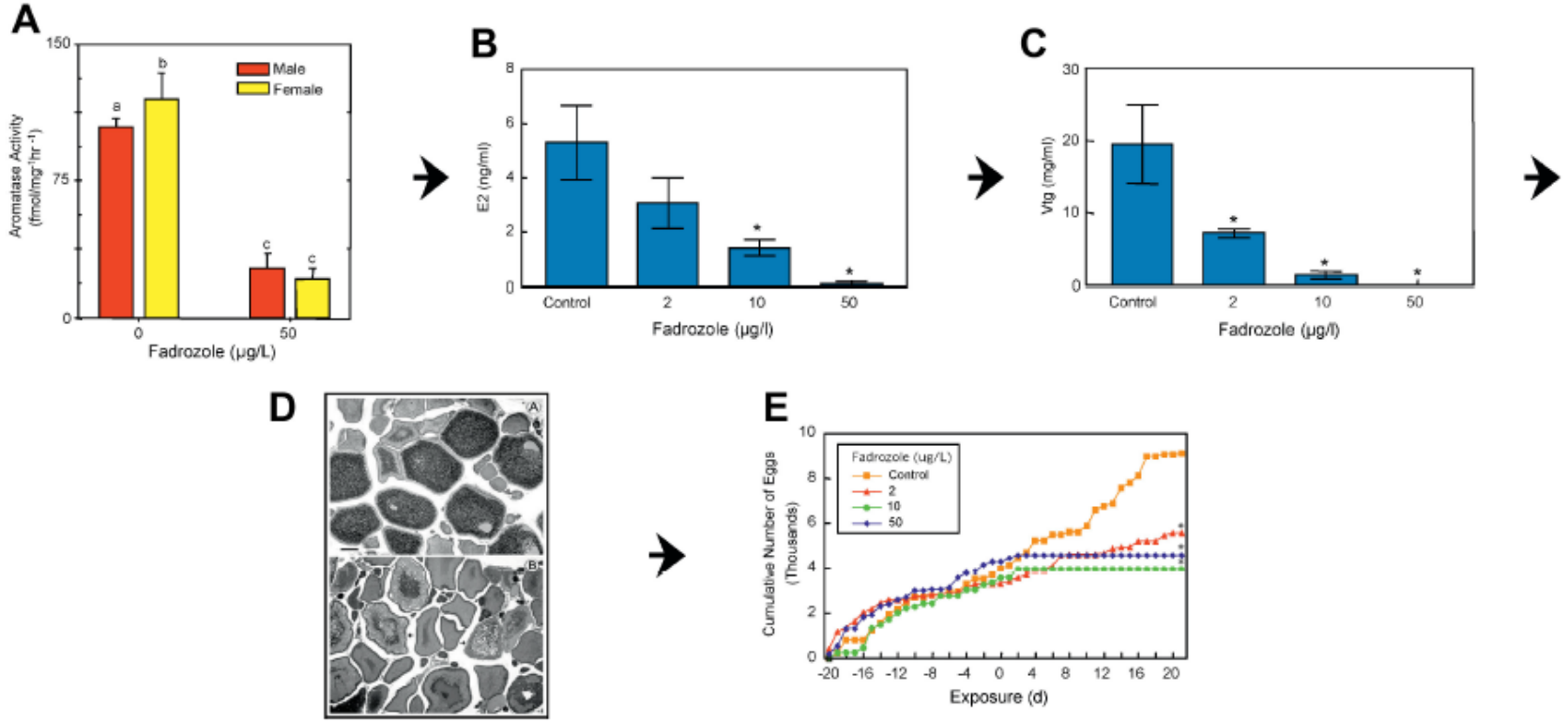
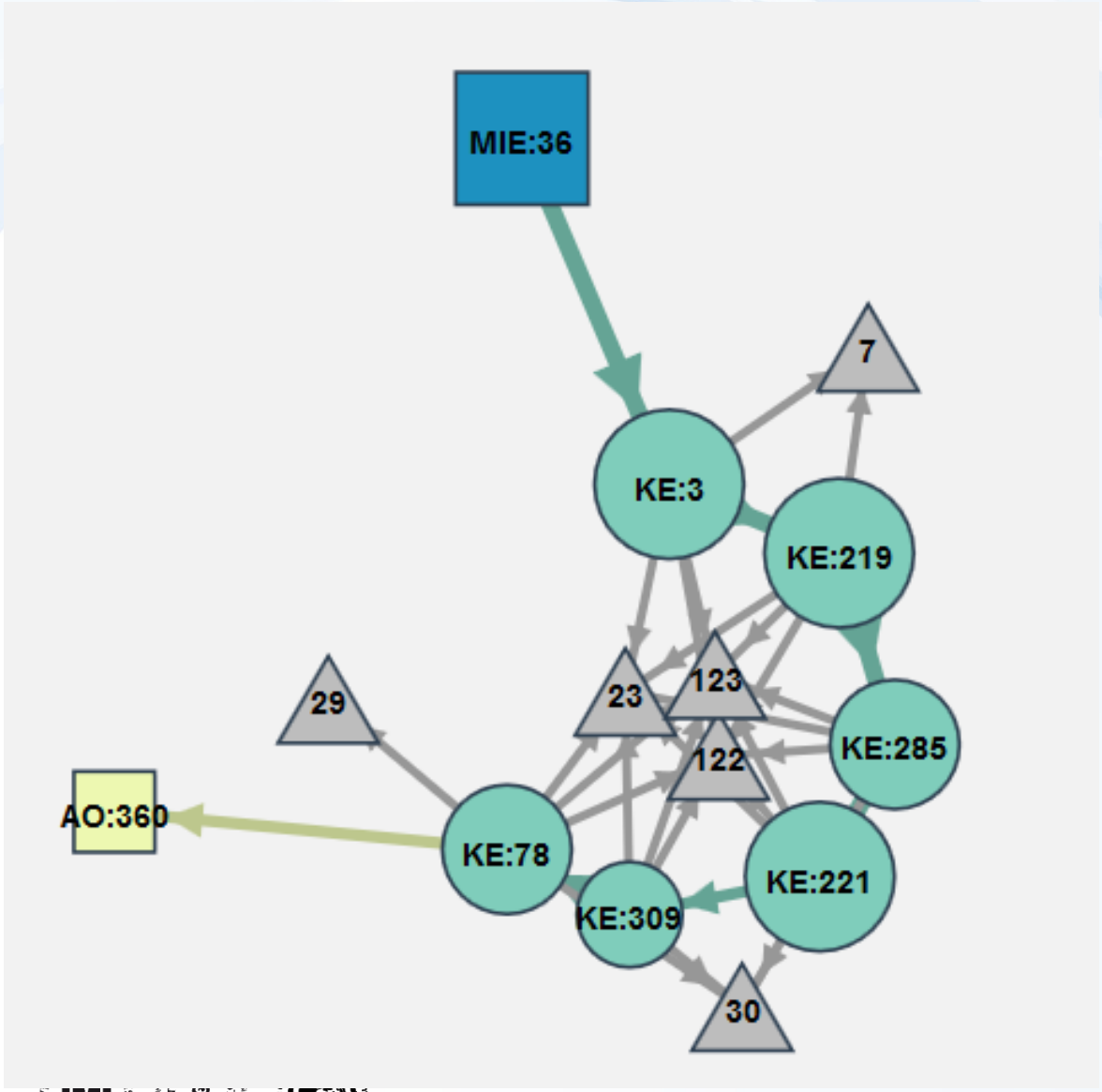


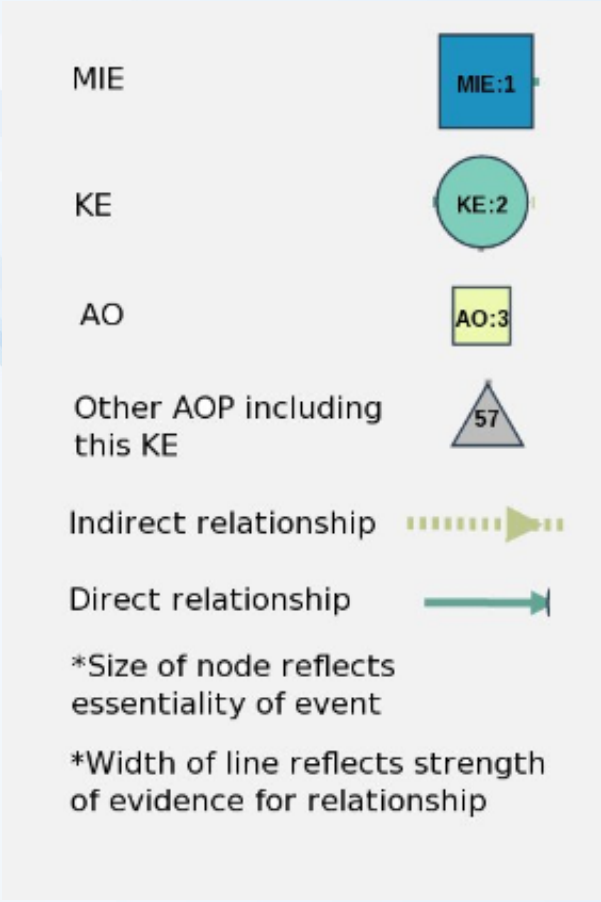
Fig. 3. An adverse outcome pathway in fish [2,50]. Aromatase inhibitor example. (**A**) Aromatase inhibition by fadrozole; (**B**) Reduction in circulating estradiol; (**C**) Reduction in circulating vitellogenin (Vtg); (**D**) Histopathology of ovarian tissue, top panel normal ovary, bottom panel fadrozole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

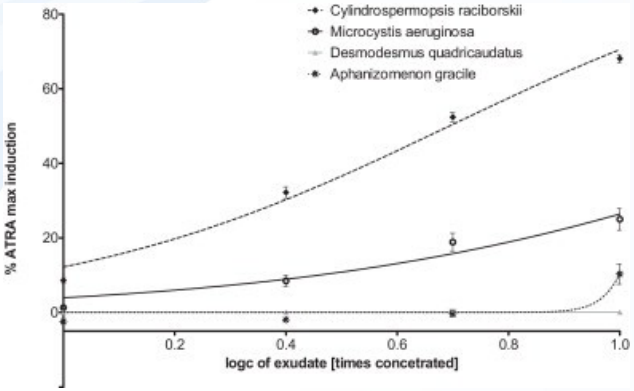
Aromatase inhibition leading to reproductive dysfunction (in fish)

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



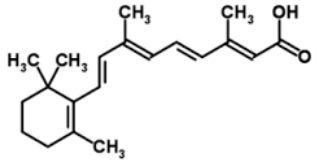


AOP Example from RECETOX: Modulation of RAR/RXR → developmental toxicity in fish

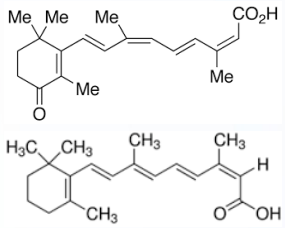


Activation of RAR/RXR

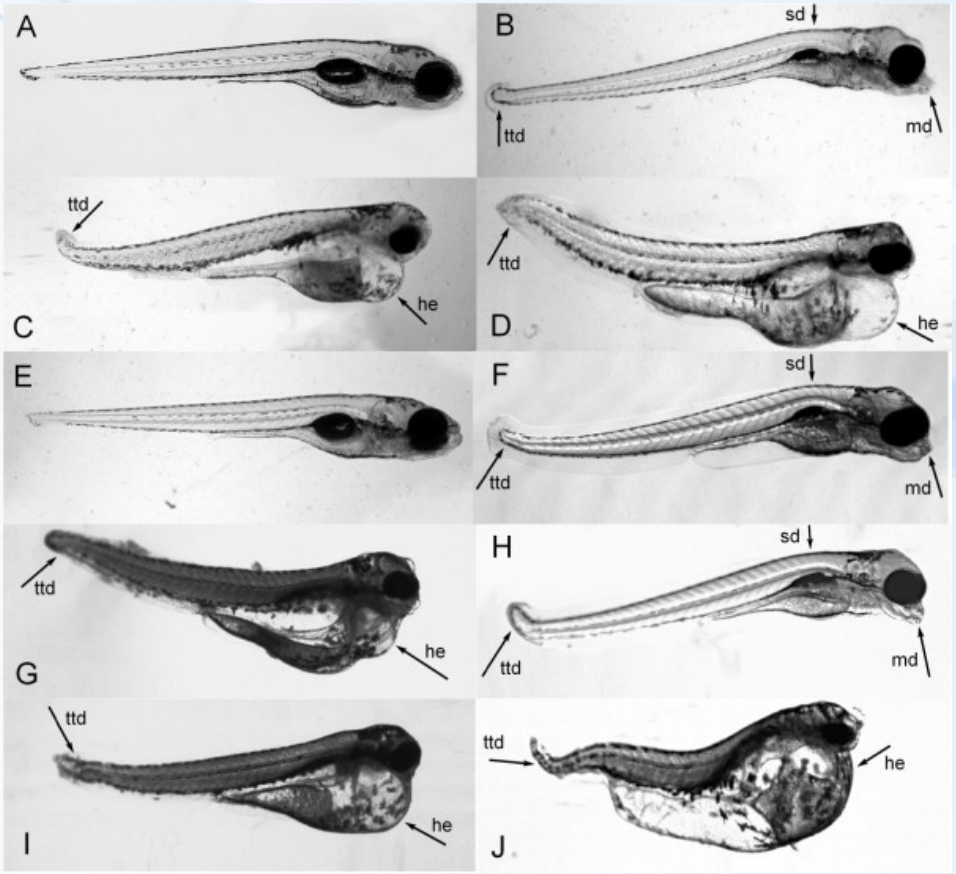
in P19/A15 cells by atRA and cyanobacterial metabolites



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

Summary on How

- **Toxicology is about doses**
  - The goal is LD(LC)50 or NOAEL/NOEC

- **Legislation defines**

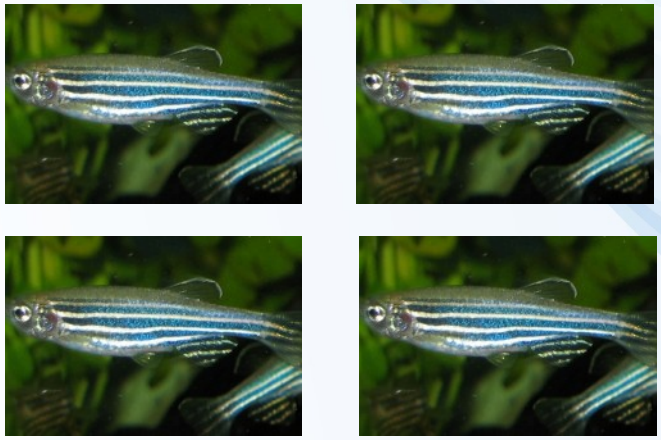
  … what assays and how to do them
  - About 30 assays
  - The most widely used standard - OECD Guidelines for Testing of Chemicals

- **Replacing „black box“ in traditional testing**
  - Synthesis of mechanistic and omics data
  - Adverse Outcome Pathways
  - Strategically supported by OECD, EU, USA

What if not

Do we need testing? Are there alternatives ?

3Rs



Replacement

Reduction

Refinement

Why doing replacement, reduction, refinement?

- Because activists put pressure to do so?
- Because animal welfare is a concern for EU citizen?
- Because animal testing is “bad” and “alternatives” are good?
- Because I will get “better” results?
- Because it is cutting edge technologies?
- Because I have to? E.g. EU law directive 2010/63/eu, ban on animal testing for cosmetics

- 3Rs are driven by EU laws, little by Member States.
- Scientific agenda is not driven enough by scientists itself…
- Academia is in general more reactive than proactive e.g. stop vivisection’s ECI

European Policies on 3Rs

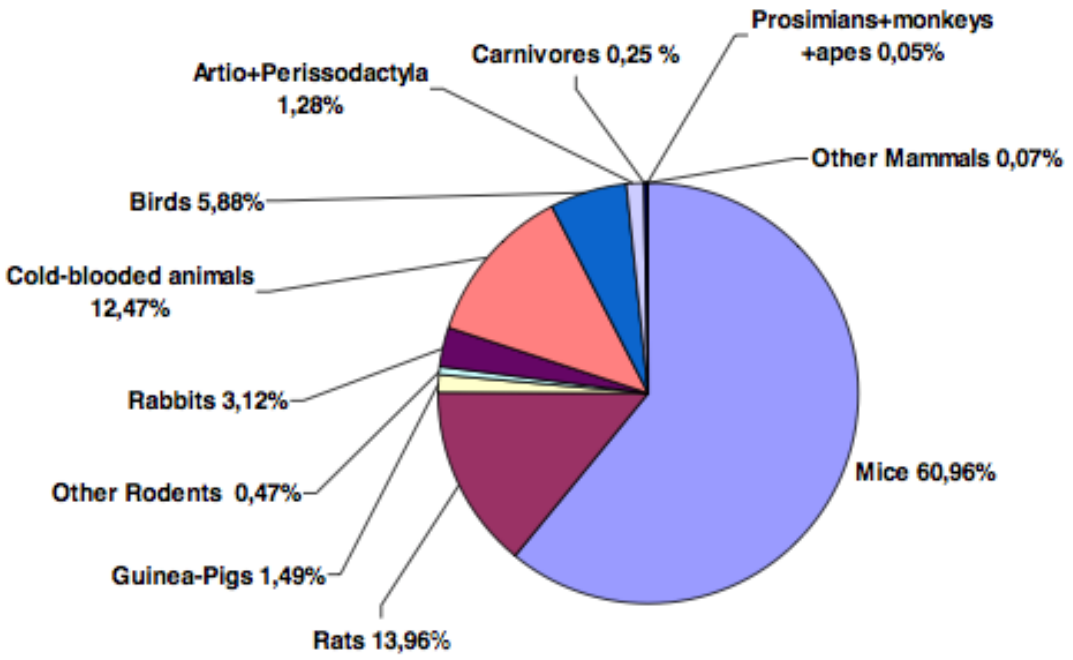
ENVIRONMENT

DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 22 September 2010

on the protection of animals used for scientific purposes

Figure 1.1
Percentages of animals used by classes in the Member States

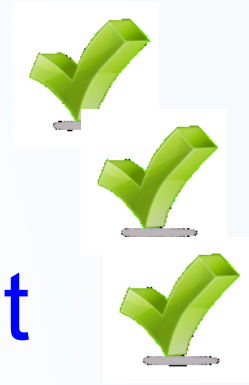


Use of animals in EU (2011)

Table 1.0: Changes in species number and proportion between 2008 and 2011

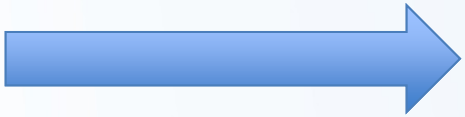
Species		Number of animals in EU 27 2008	Number of animals in EU 27 2011	Change since 2008	% change by species
1.a	Mice (Mus musculus)	7122188	6999312	-122876	-1,73
1.b	Rats (Rattus norvegicus)	2121727	1602969	-518758	-24,45
1.c	Guinea-Pigs (Cavia porcellus)	220985	171584	-49401	-22,35
1.d	Hamsters (Mesocricetus)	32739	25251	-7488	-22,87
1.e	Other Rodents (other Rodentia)	39506	28465	-11041	-27,95
1.f	Rabbits (Oryctolagus cuniculus)	333213	358213	25000	7,50
1.g	Cats (Felis catus)	4088	3713	-375	-9,17
1.h	Dogs (Canis familiaris)	21315	17896	-3419	-16,04
1.i	Ferrets (Mustela putorius furo)	3208	2540	-668	-20,82
1.j	Other Carnivores	2853	4982	2129	74,62
1.k	Horses, donkeys and cross-breds (Equidae)	5976	6686	710	11,88
1.l	Pigs (Sus)	92813	77280	-15533	-16,74
1.m	Goats (Capra)	3840	2907	-933	-24,30
1.n	Sheep (Ovis)	30190	28892	-1298	-4,30
1.o	Cattle (Bos)	33952	30914	-3038	-8,95
1.p	Prosimians (Prosimia)	1261	83	-1178	-93,42
1.q	New World Monkeys (Ceboidea)	904	700	-204	-22,57
1.r	Old World Monkeys (Cercopithecoidea)	7404	5312	-2092	-28,25
1.s	Apes (Hominoidea)	0	0	0	0,00
1.t	Other Mammals (other Mammalia)	5704	7888	2184	38,29
1.u	Quail (Coturnix coturnix)	9626	5614	-4012	-41,68
1.v	Other birds (other Aves)	754485	669451	-85034	-11,27
1.w	Reptiles (Reptilia)	4101	3824	-277	-6,75
1.x	Amphibians (Amphibia)	61789	29583	-32206	-52,12
1.y	Fish (Pisces)	1087155	1397462	310307	28,54
1.z	TOTAL	12001022	11481521	-519501	-4,33

- VALIDATION
  - MoA
  - Reliable
  - Relevant



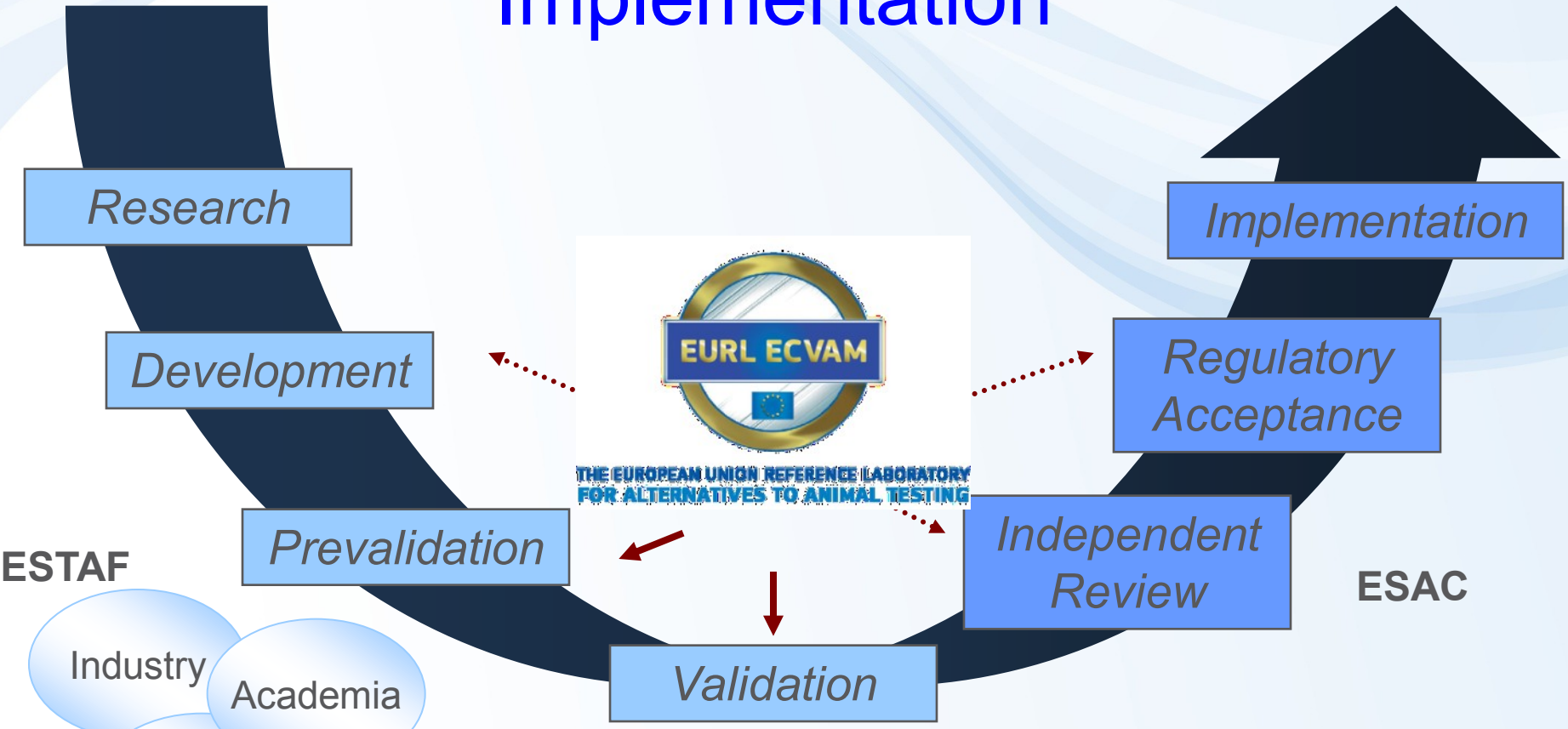
EURL ECVAM
THE EUROPEAN UNION REFERENCE LABORATORY FOR ALTERNATIVES TO ANIMAL TESTING

Substance Tested



e.g. endocrine disrupters receptor binding

Alternative Methods – R&D to Implementation



7-8 YEARS

JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection (IHCP)

European Commission > JRC > IHCP > TSAR

TSAR : Tracking System for Alternative test methods Review, Validation and Approval in the Context of EU Regulations on Chemicals

The Process
+ Review and Validation
+ Regulatory Approval

Validation of Methods

Approval of Methods
− Skin Corrosion
TER
EpiSkin™
EpiDerm™
SkinEthic™ RHE
EST-1000™
CORROSITEX
+ Skin Irritation
− Eye Irritation
BCOP
ICE
IRE
HET-CAM
CM
FL
LVET
+ Skin Sensitisation
+ Mutagenicity
+ Acute Systemic Toxicity
+ Repeated Dose Toxicity
+ Reproductive Toxicity
+ Other
+ Acute Toxicity to Fish

TSAR is a tool to provide a transparent view on the status of **alternative methods** as they progress from purely scientific protocols submitted for pre-validation to being actively used in a regulatory context.

This tracking system intends to cover all steps, from the initial submission for pre-validation until final adoption by inclusion in the EU legislation and/or related Guidance Documents, when appropriate. It is worth mentioning that not all alternative methods will or need to be included in the Test Methods regulation (TMR, Commission Regulation (EC) No 440/2008 of 30 May 2008), as this Regulation only contains relevant methods for the assessment of properties of chemicals that fall directly under its remit (see below some links to relevant legislation that contains data requirements). In addition to TMR, a number of methods are used on a day to day basis in a regulatory context through other product related guidance, as part of intelligent testing strategies or as pre-screening methods. Regardless of the way of implementation, they all contribute to the replacement, reduction and refinement of the use of animals in scientific procedures.

The process of validation and regulatory approval has been broken down into a number of steps. Although this is a continuous process that may, sometimes, also involve some iterations, for practical reasons it has been broken down in two parts:
A) **Review and Validation.**
B) **Regulatory Approval** (see simplified scheme for alternative methods).
These have, on its turn, been broken down into several stages. An explanation of each stage can be found by clicking on the submenus of the **"The Process"** menu on the left side of the screen.

The methods whose status of validation or regulatory acceptance are tracked here have been grouped by the relevant endpoint they cover, as can be seen in the left side menus.

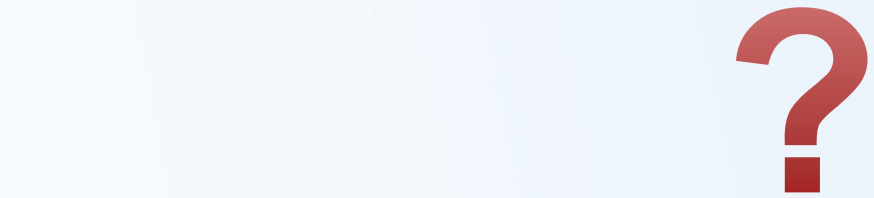
However, currently, the system only contains information tracking specific alternative methods in terms of the regulatory approval part from the stage "Validation statement" onwards. The remaining parts of the TSAR web site dealing with the other stages in the process of validation and regulatory approval are under construction and it is foreseen that they will be added in the near future. Some other utilities as site searching capabilities will also be added in future.

The drop-down menus on the left hand side of the screen allow the user to display the information on individual alternative methods by just clicking on them.

The test methods have been classified according to a simple colour code:
Green: Already in the EU legislation or other regulatory use.
Orange: Undergoing process to be incorporated in the EU regulatory context.
Purple: No regulatory use identified.

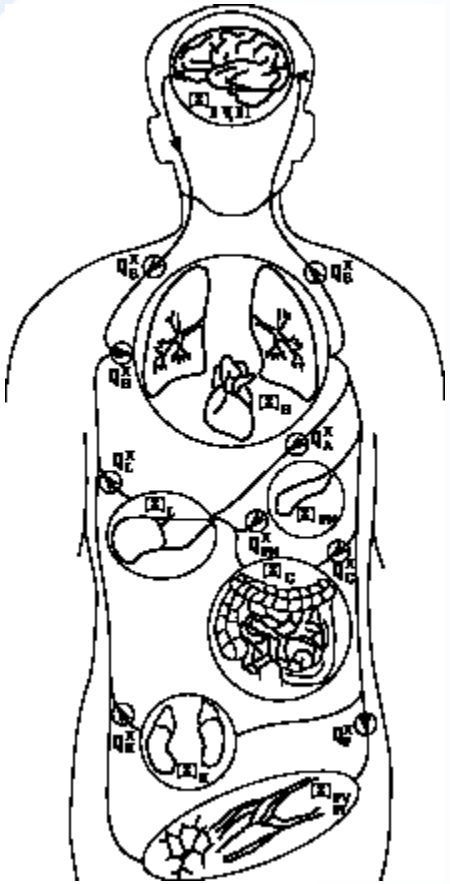
- >60 3Rs Tests submitted to ECVAM since 2008 (update 01/2015)
- 10 validated or ongoing validation => Prioritisation!

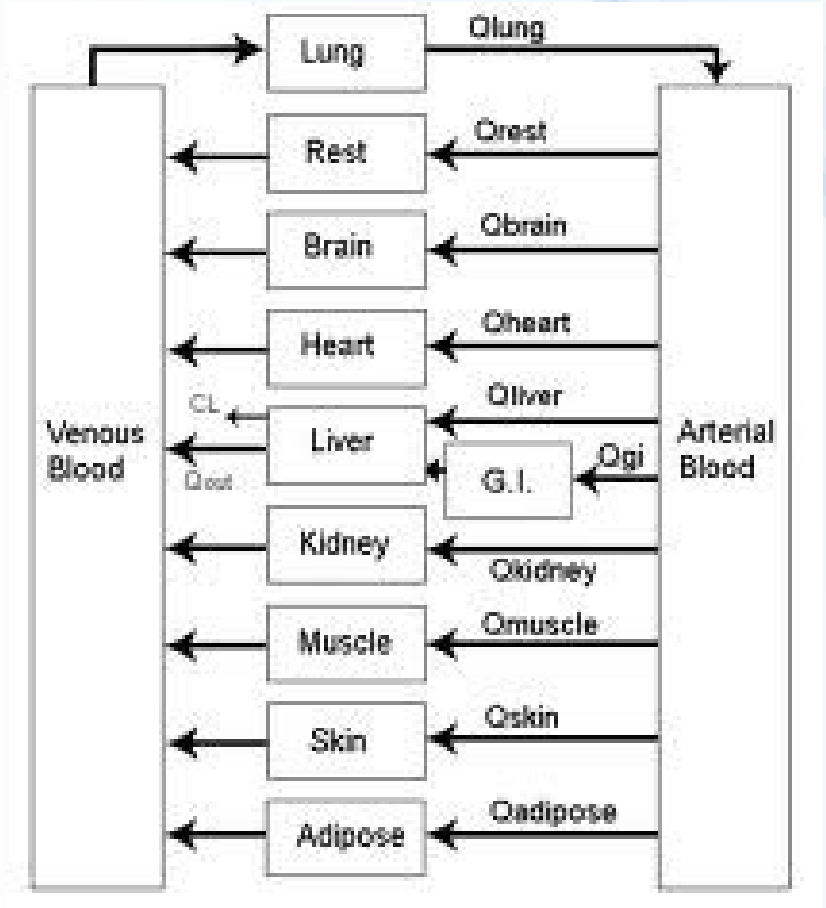
COMPUTATIONAL (ECO)TOXICOLOGY



PBPK models

PBPK (PBTK)
Physiologically based pharmacokinetic (toxicokinetic) models





Fragmentation of a complex systém to „boxes“

→ All Processes described by arrows (mathematical equations)

Example – computational toxicology for EDCs

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

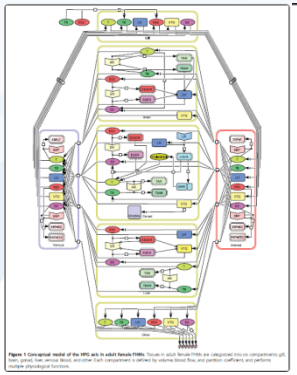
BMC Systems Biology

RESEARCH ARTICLE Open Access

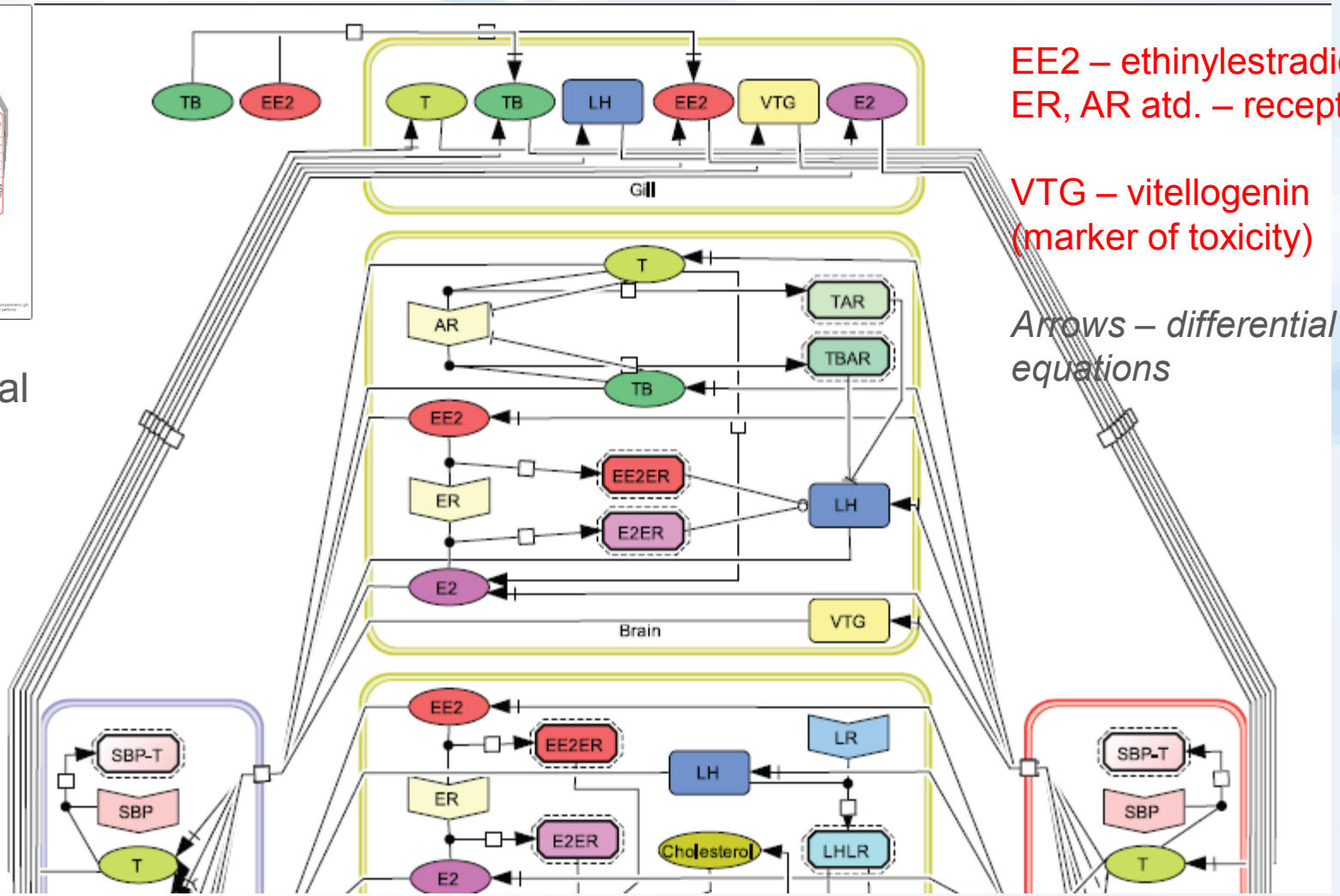
A computational model of the hypothalamic - pituitary - gonadal axis in female fathead minnows (*Pimephales promelas*) exposed to 17α-ethynylestradiol and 17β-trenbolone

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

Li (2011) BMC Systems Biology



Conceptual model



EE2 – ethinylestradiol
ER, AR atd. – receptors

VTG – vitellogenin (marker of toxicity)

Arrows – differential equations

Li (2011) BMC Systems Biology

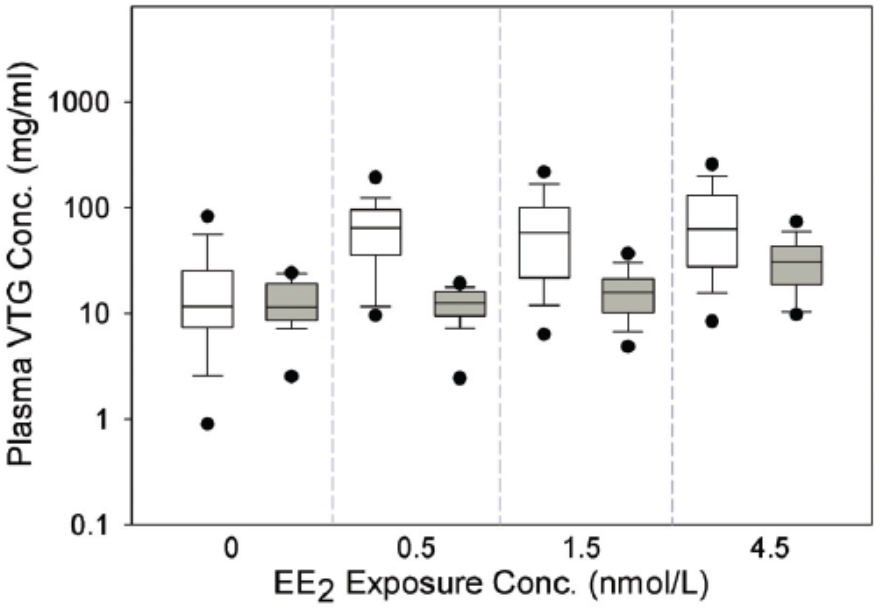


Figure 6 Comparison of model predictions with measured data in female FHMs exposed to EE_2. $n = 28$ at each sampling time. White boxes represent model predictions, and grey boxes represent measured data [42]. The x-axis represents EE_2 concentrations in ng/L. The solid line within the box marks the 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.

Results:

MODELLED (white)
Vs
MEASURED (grey)

…good comparable

Wrap-up

- **Eco/Toxicology matters**
  - Relevant especially for „chemicals“
  - … but also for „mixtures“ and contaminated samples
    - **Effect based tools in monitoring**
  - Important results improving lives
  - Exciting with many open questions

- **Regulatory and Science worlds are different … but are getting closer and closer**
  - Mechanistic knowledge and utilization of „omics“ data
  - Development of AOPs
  - In vitro (alternative) models
  - Quantitative computational toxicology

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Environmental Toxicology and Chemistry, Vol. 32, No. 1, pp. 32–48, 2013

Printed in the USA
DOI: 10.1002/etc.2043

Global Climate Change

INTERACTIONS BETWEEN CHEMICAL AND CLIMATE STRESSORS: A ROLE FOR MECHANISTIC TOXICOLOGY IN ASSESSING CLIMATE CHANGE RISKS

MICHAEL J. HOOPER,*† GERALD T. ANKLEY,‡ DANIEL A. CRISTOL,§ LINDLEY A. MARYOUNG,‖ PAMELA D. NOYES,# and KENT E. PINKERTON††

†U.S. Geological Survey, Columbia Environmental Research Center, Columbia, Missouri

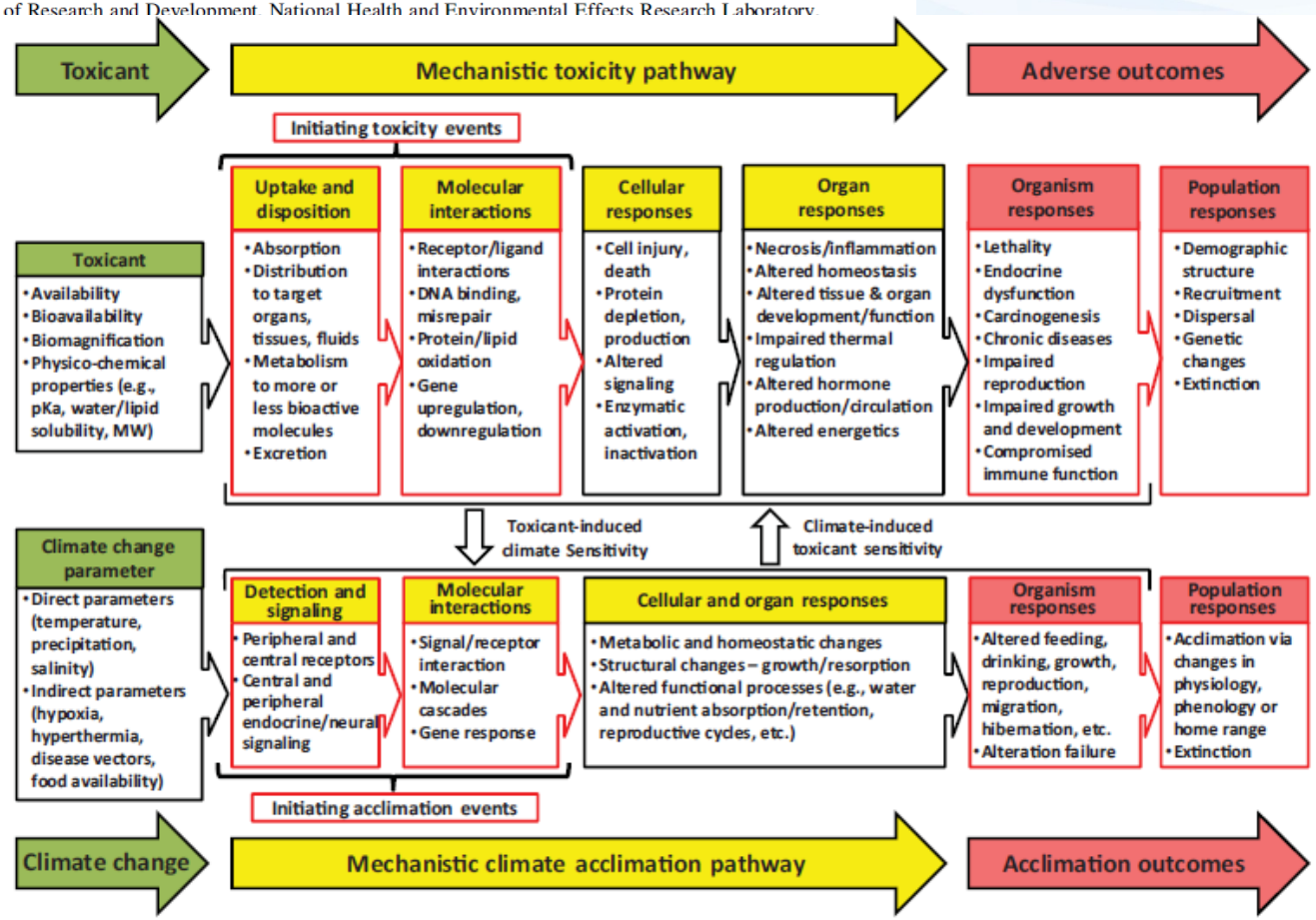
‡U.S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory

§Institute for Integrative Bird Behavic

‖Department of

#Nicholas Sc

††Center for Health



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§Institute for Integrative Bird Behavior Studies, Department of Biology, The College of William and Mary, Williamsburg, Virginia, USA
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#Nicholas School of the Environment, Duke University, Durham, North Carolina, USA
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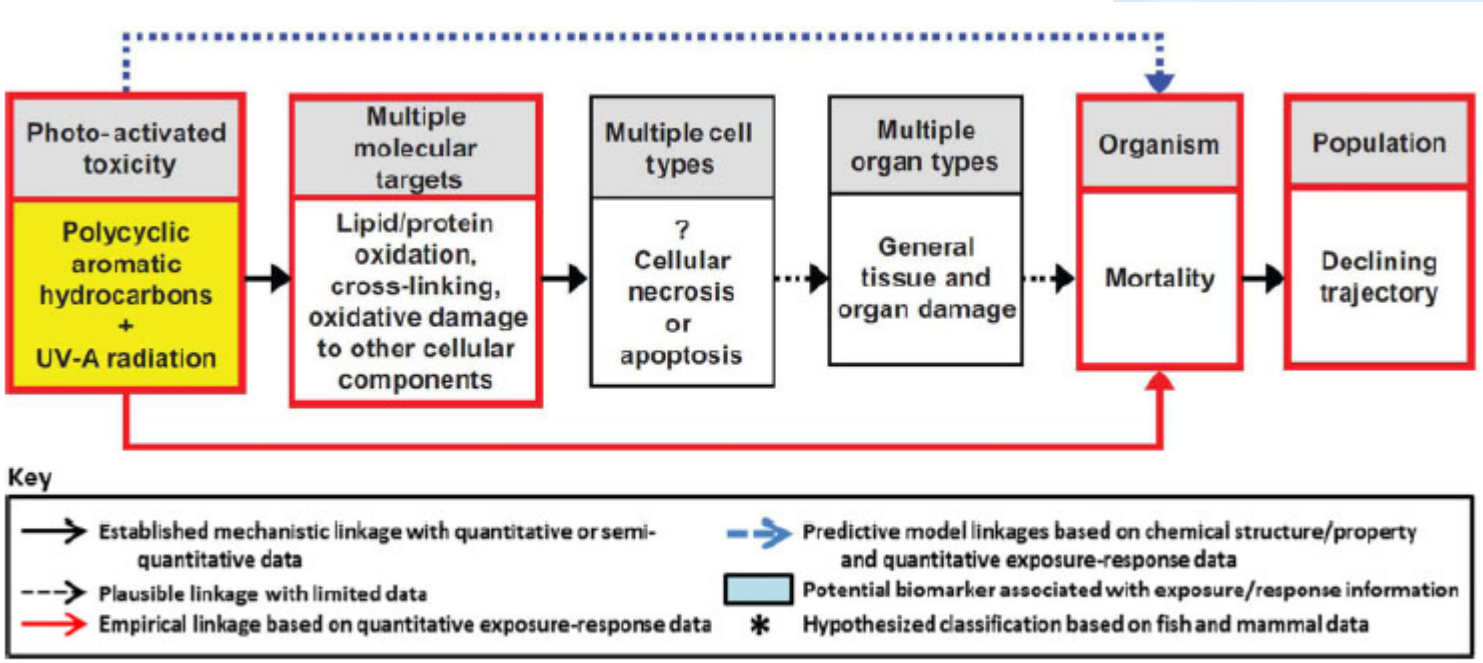

Fig. 2. Adverse outcome pathway of the interaction of ultraviolet radiation with polycyclic aromatic hydrocarbons. With permission from Ankley et al. [14]. [Color figure can be seen in the online version of this article, available at wileyonlinelibrary.com.]

Thank you for your attention

blaha@recetox.muni.cz