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for toxic compounds
in the environment

Ecotoxicology
Current issues in Research vs Regulation

Ludek Blaha + ecotox colleagues

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UNIVERSITAS MASARYKIANA BRUNENSIS

Take home messages from this presentation

- Approaches and intentions of ecotoxicology researchers (freedom) and ecotoxicity-results users = regulators (bound by laws) are completely different

- Examples of current hot topics and gaps that are slowly reaching sufficient coverage by regulation
  - Nanomaterials
  - Pharmaceuticals
  - Individual chemicals (limits) vs mixture effects
  - Complex contaminated matrices: Analyses of priority chemicals according to law – vs - Effects of mixtures determined in bioassays

When

Where

the assessment of toxicity is needed ?

What

to assess for toxicity ?

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When & where the toxicity assessment is needed?

View of the researcher

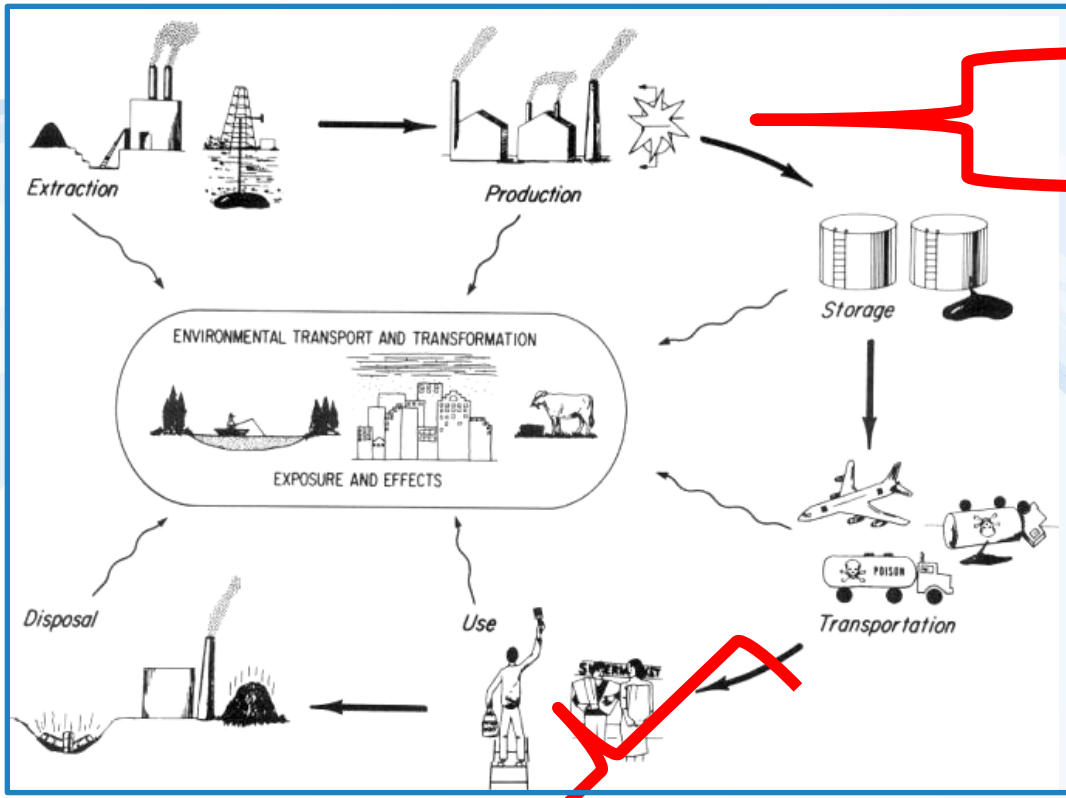
Anytime!

… depending on researcher's budget

View of the regulator

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

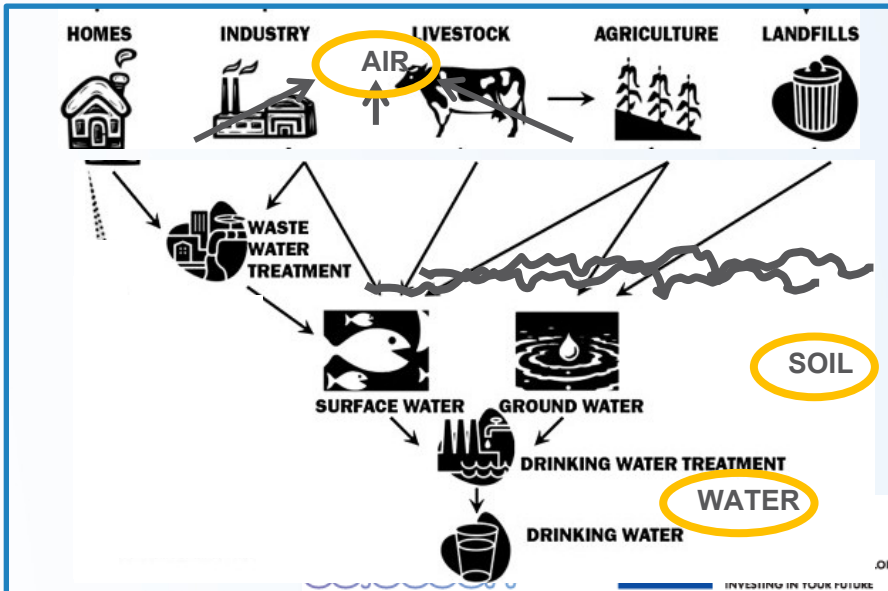
REACH (ECHA)

PPP (EFSA)

MPs (EMA)

Two approaches:

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



WFD – surface w.
GWD – ground w.

Air quality

Food and feed

Soil & Sediments

Wastes

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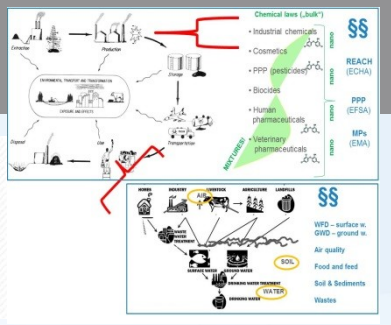
What to assess for toxicity?



	Current research topics	As required by law
Individual chemicals (prospective)		
Mixtures (prospective)		
Contaminated samples (retrospective)		

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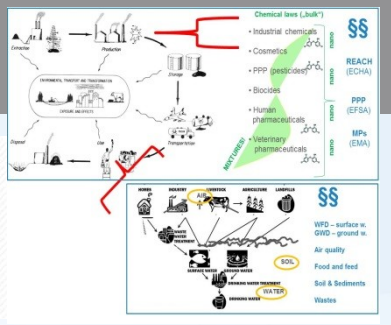
What to assess for toxicity?



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

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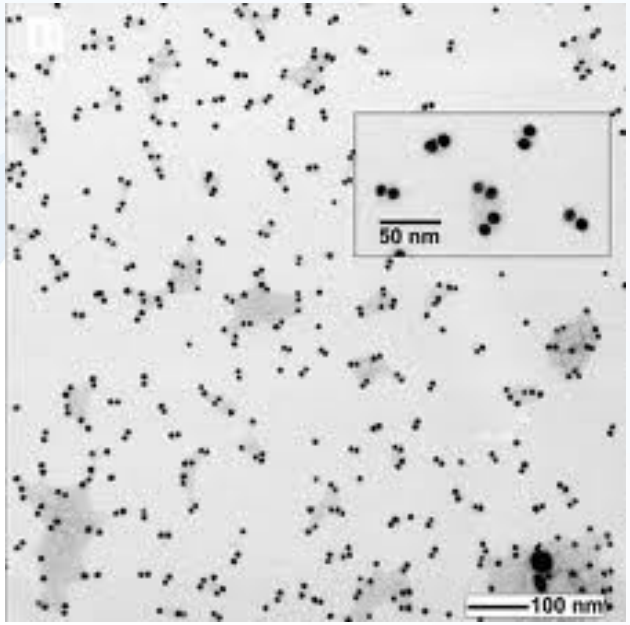
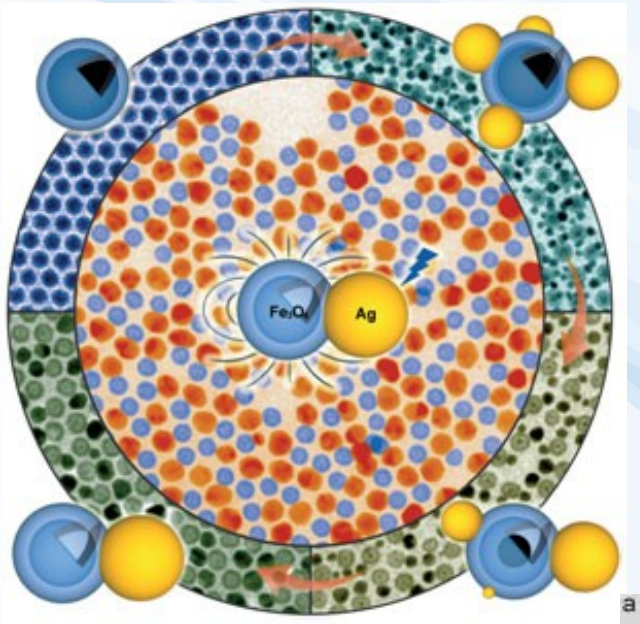
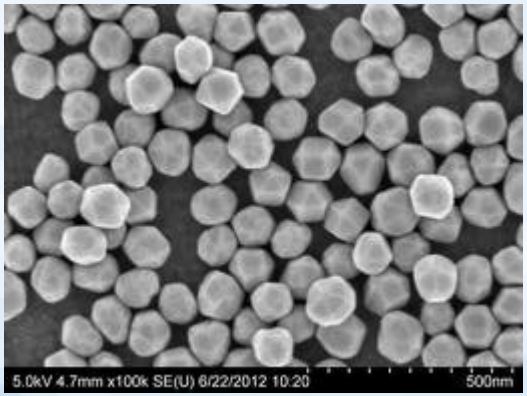
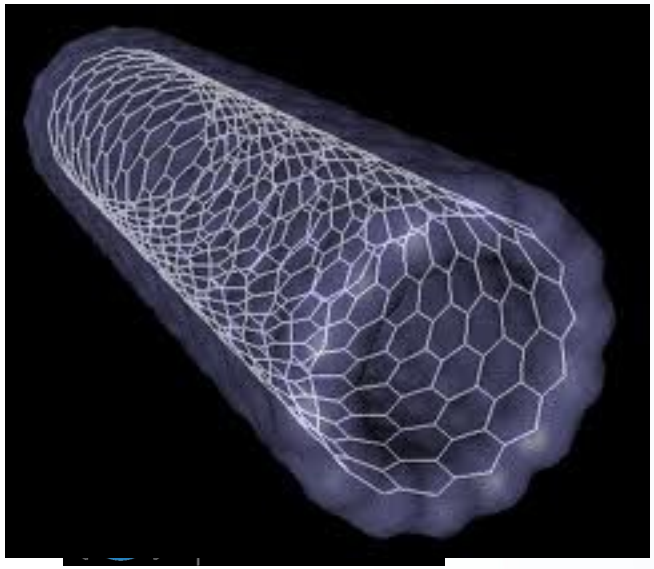
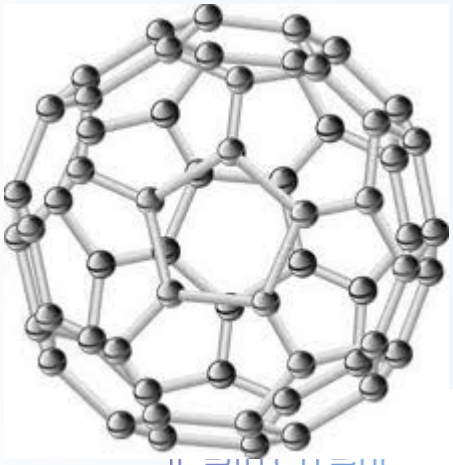
Nanoparticles - examples

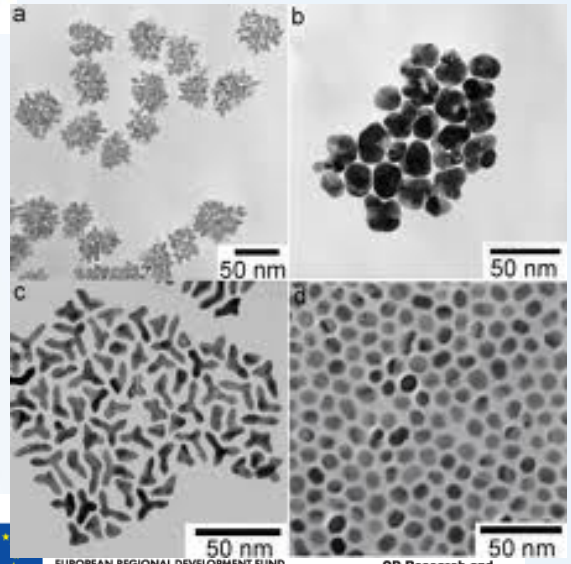












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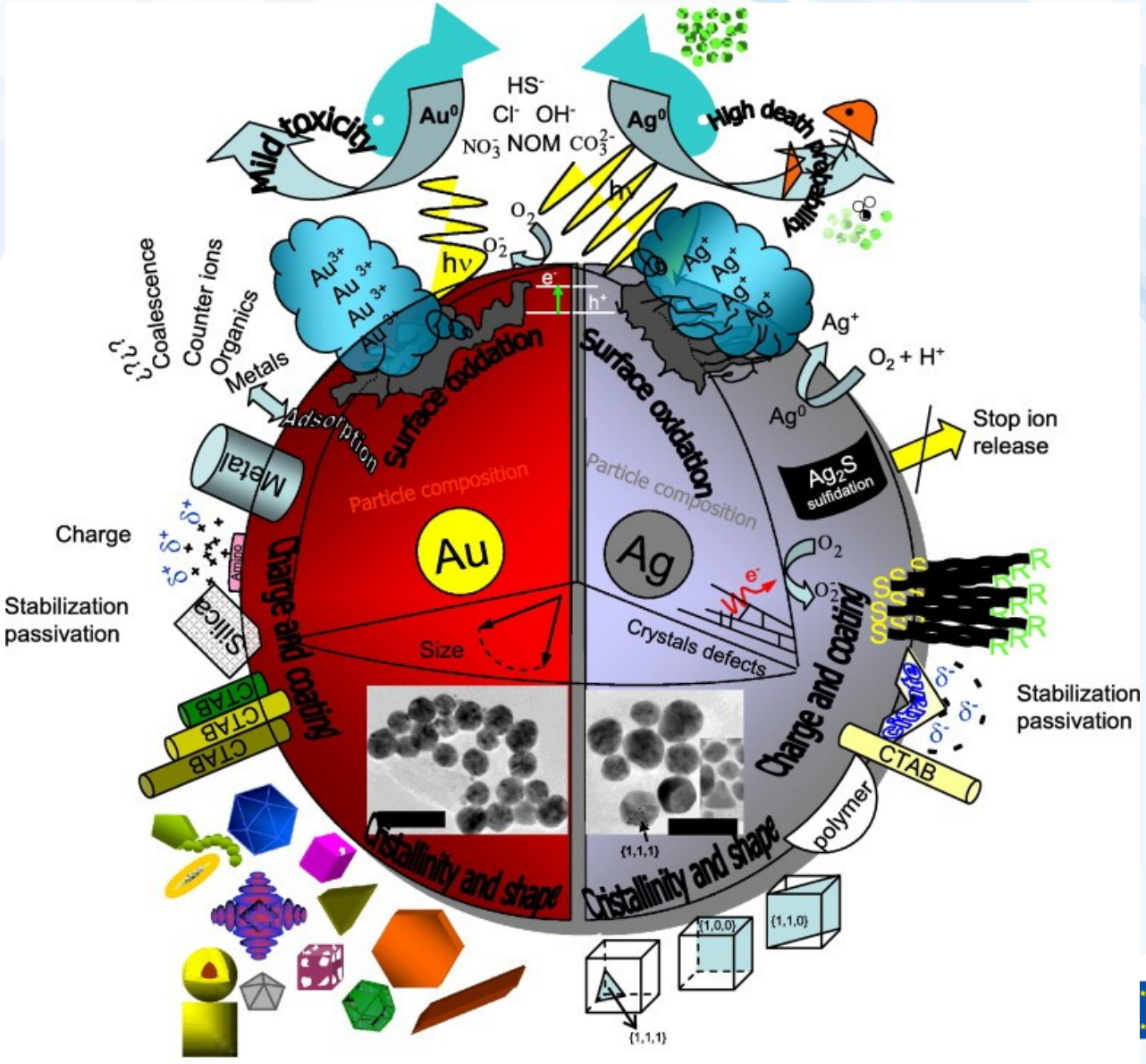
Toxicity of nanoparticles …



(Mostly unknown) Parameters may Affect ecotoxicity

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

➔ Effects on environmental Fate and toxicity

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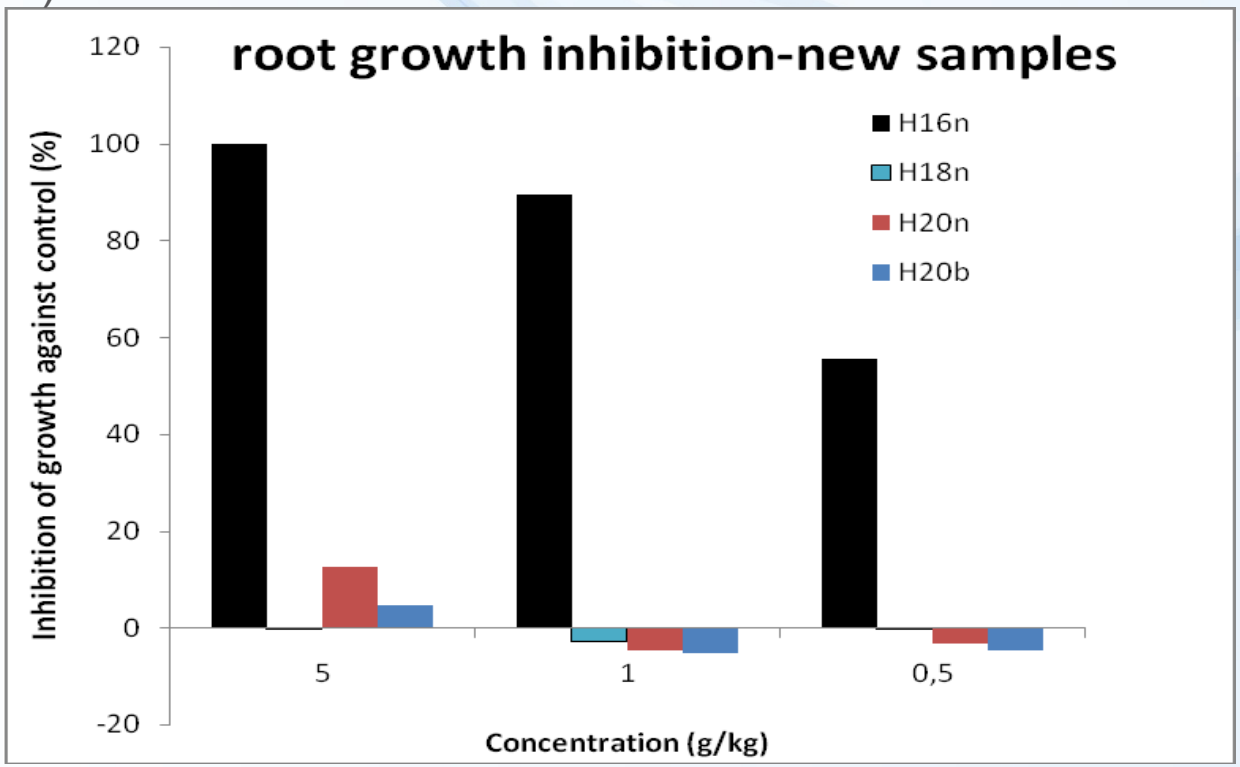
Ecotoxicity of nanoparticles – RECETOX example

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

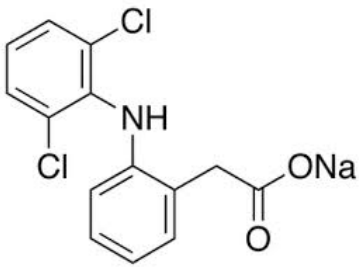
Manufacturing waste

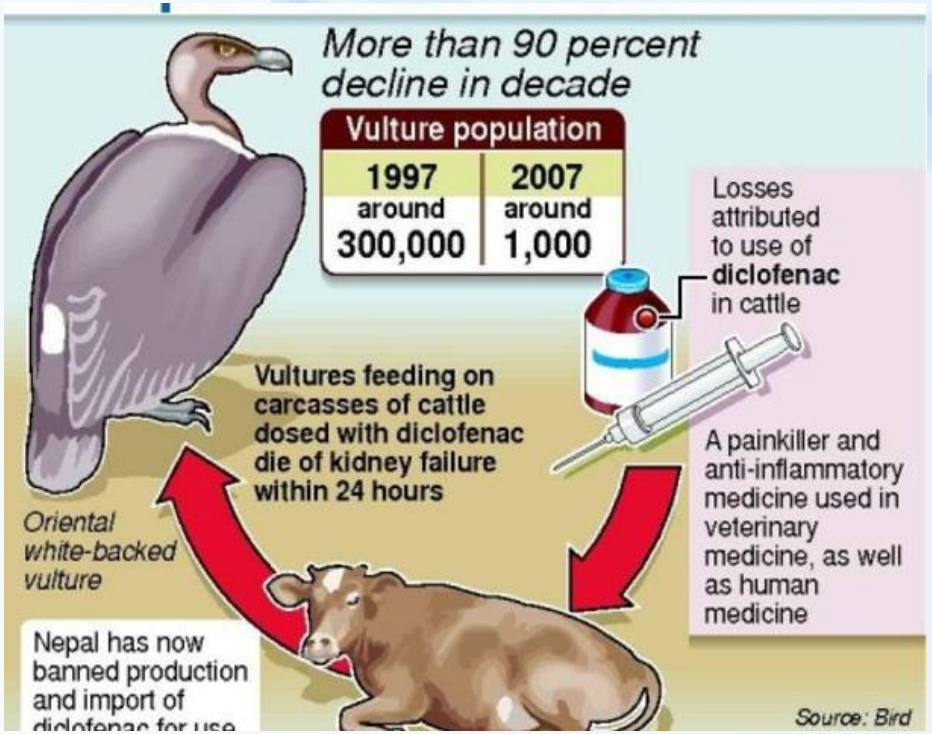
Possible releases to the environment

Example 1 - DICLOFENAC

Unexpected effects at NON-TARGET species

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





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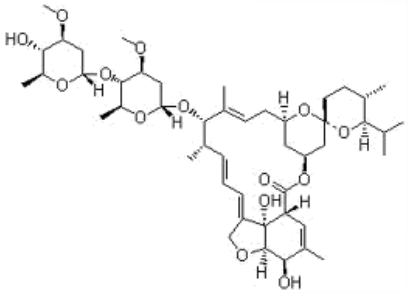
Example 2 – AVERMEKTIN-like antiparasitics

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)



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POLICYFORUM

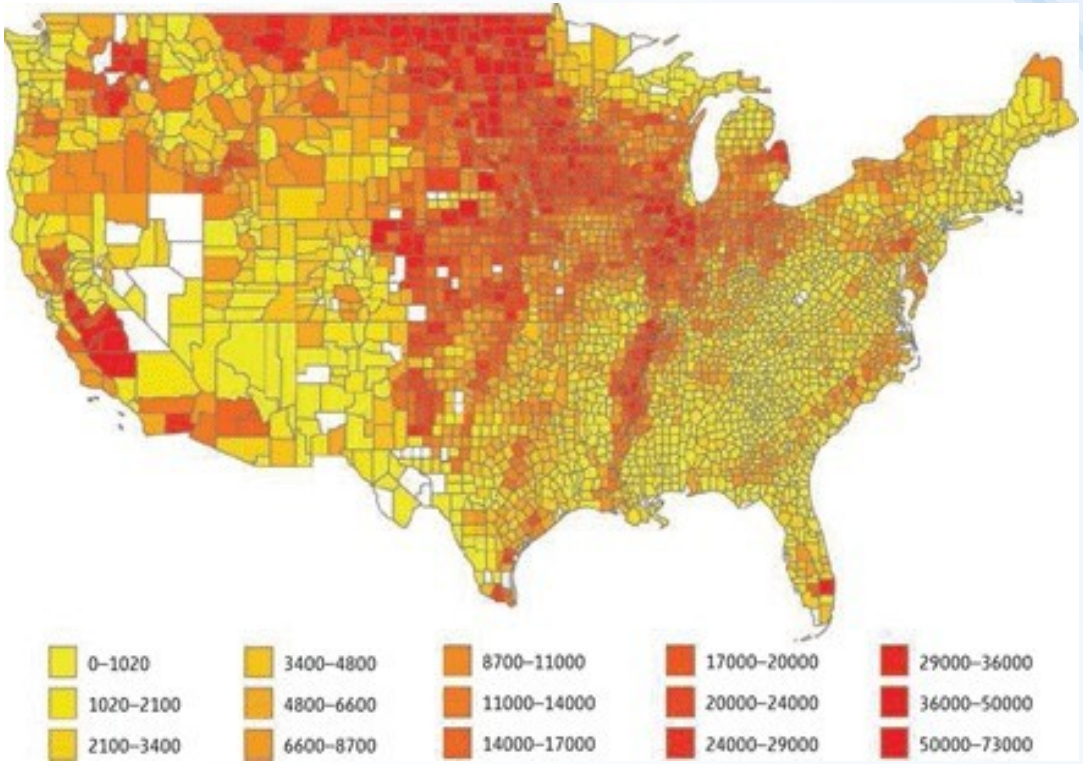
Science
AAAS

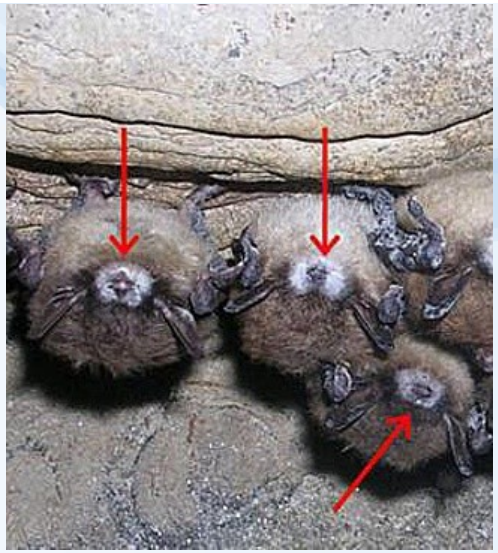
CONSERVATION

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 (6025) 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

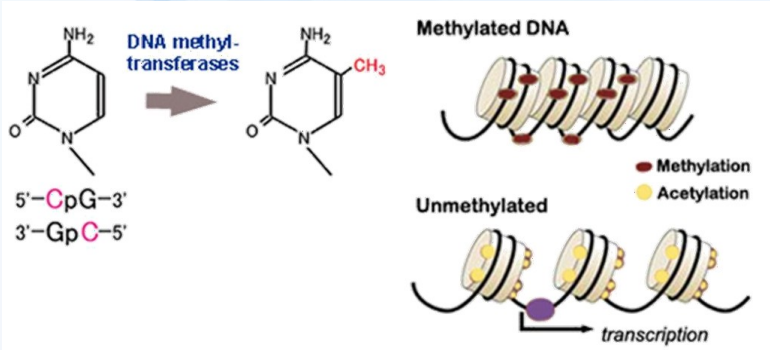


Table 1. Behaviours (mean ± s.e.) of the offspring from the maternal treatments.

	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

MIXTURE TOXICITY EU interlaboratory test

Testing comparability of existing and innovative bioassays for water quality assessment

Main questions:

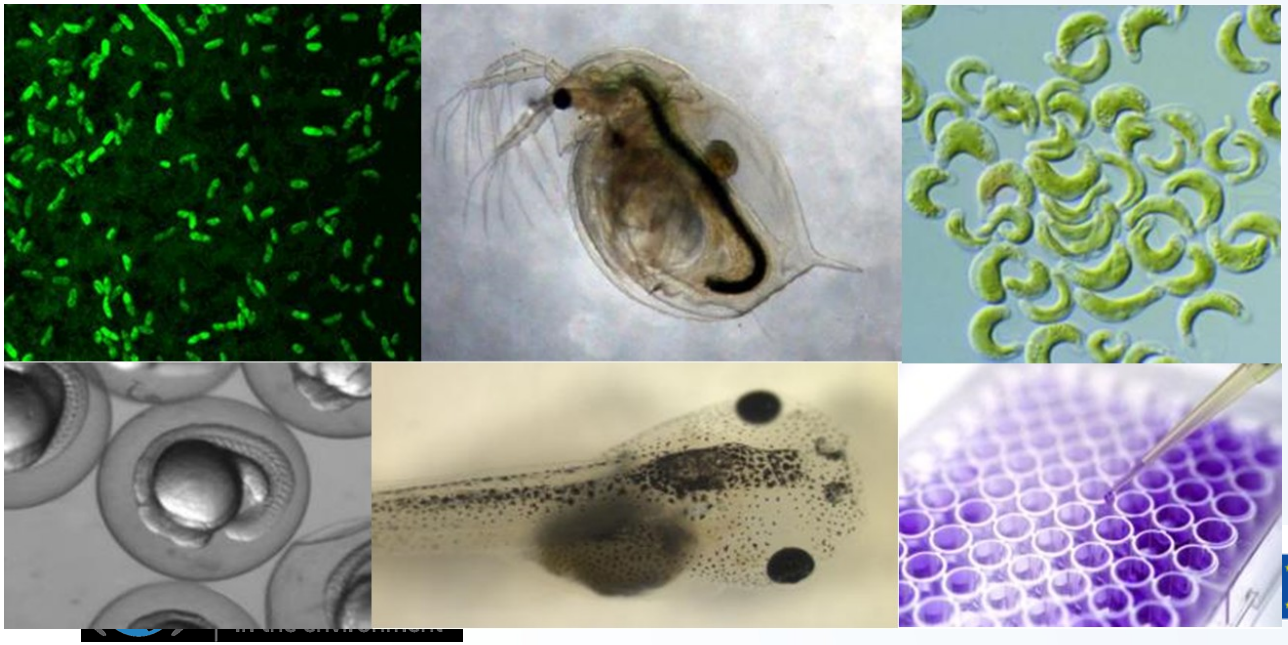
Are current limits (for individual compounds) safe?

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**

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MIXTURE TOXICITY EU interlaboratory test

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

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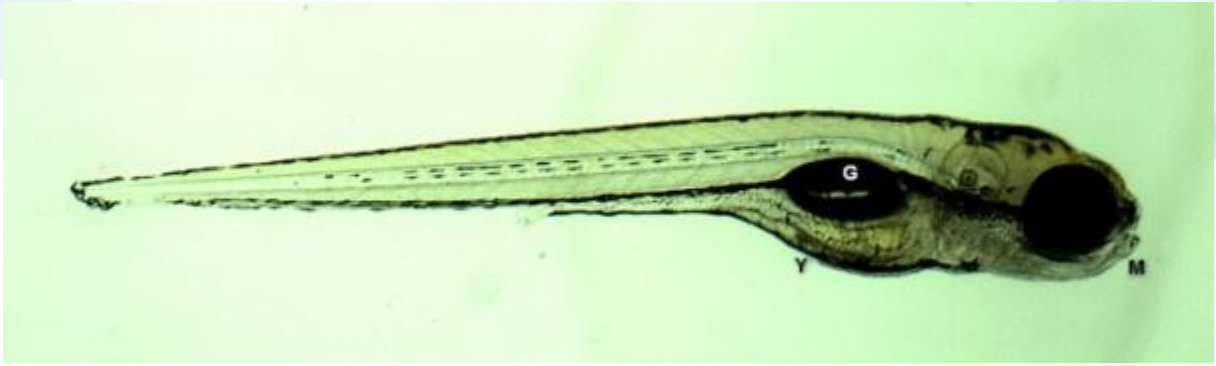
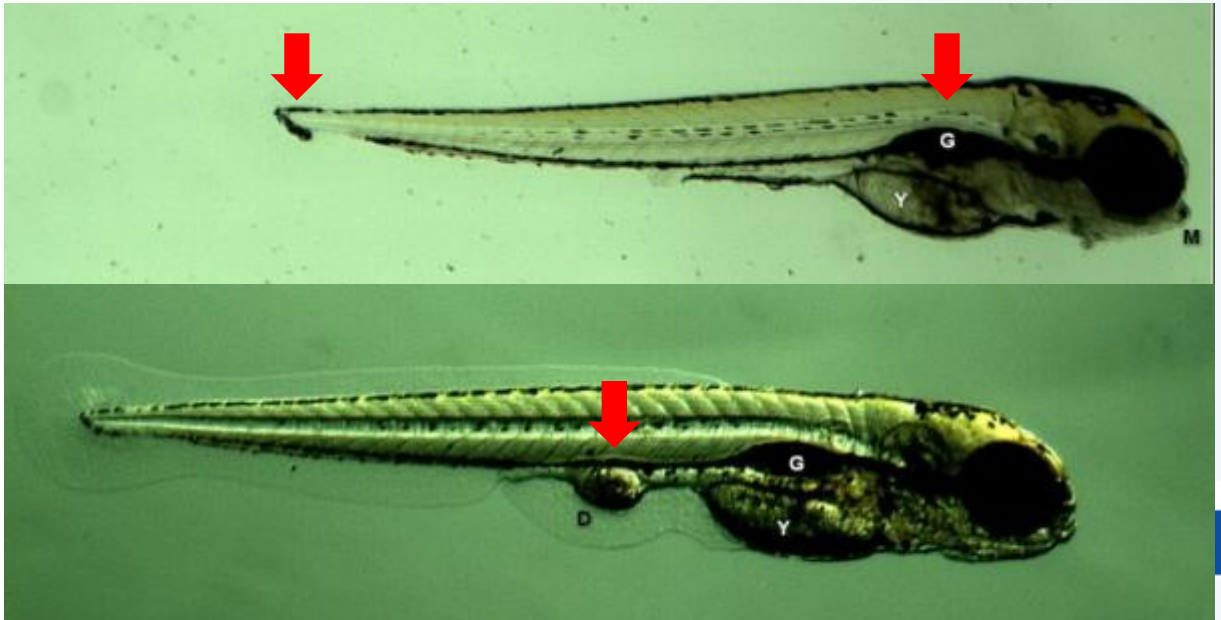
MIXTURE TOXICITY EU interlaboratory test

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

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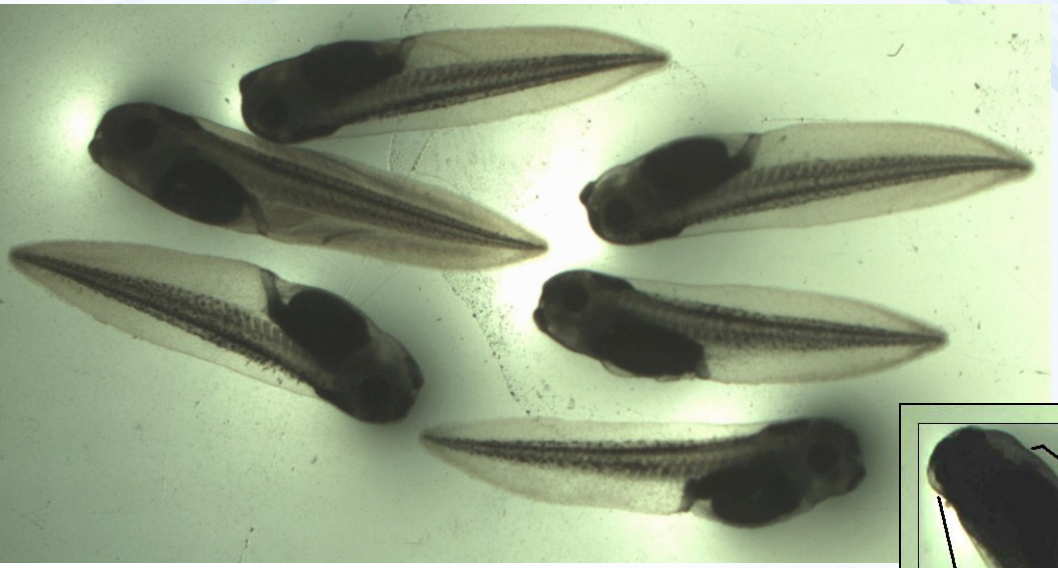
MIXTURE TOXICITY EU interlaboratory test

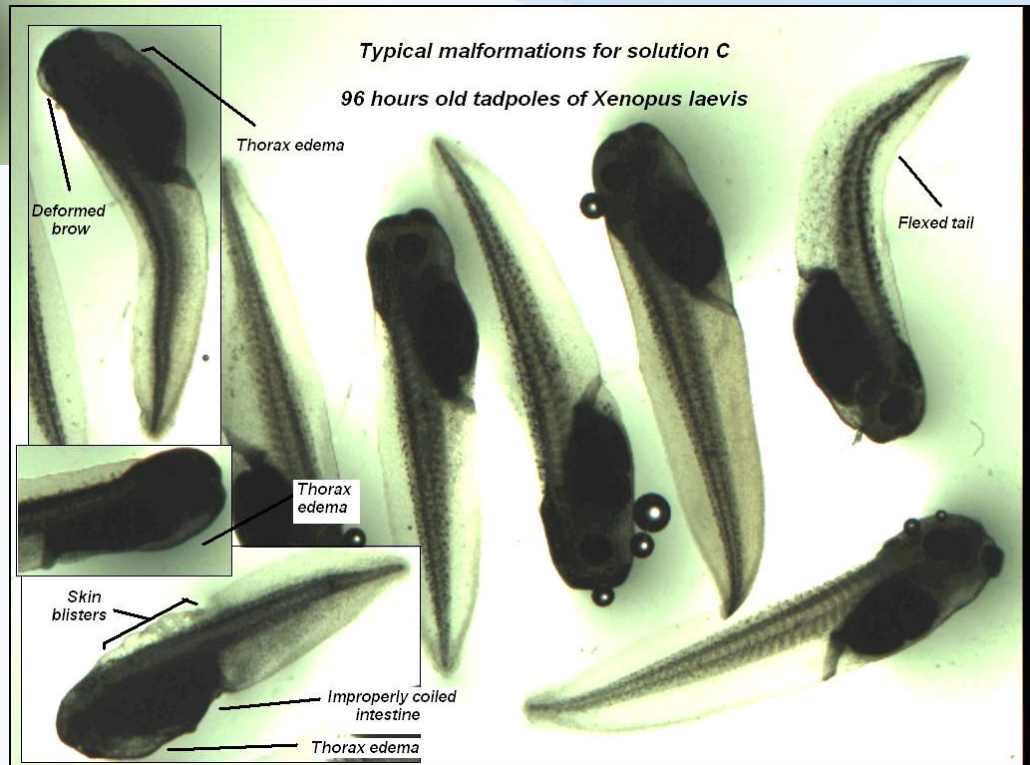
Testing comparability of existing and innovative bioassays for water quality assessment

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



Controls

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



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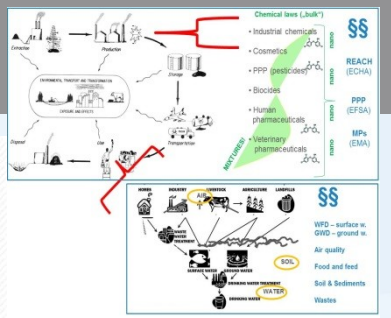
Biotest	A	B	C
Microtox	26 and 36% stimulation of luminescence in 15 and 30 mins of exposure, respectively	18 and 35% stimulation of luminescence in 15 and 30 mins of exposure, respectively	22 and 39% stimulation of luminescence in 15 and 30 mins of exposure, respectively
Algae growth inhibition test 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

What to assess for toxicity?



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Individual chemicals (prospective)	Engineered **nanomaterials**/particles **Ecological effects** (e.g. of pharmaceuticals) **Endocrine** disruption & **chronic** diseases	Industry & biocides (REACH) PPPs = pesticides Pharmaceuticals Cosmetics
Mixtures (prospective)	**Multistressors** +T°C, salinity, pathogens, irradiation, food **Exposome**	
Contaminated samples (retrospective)		

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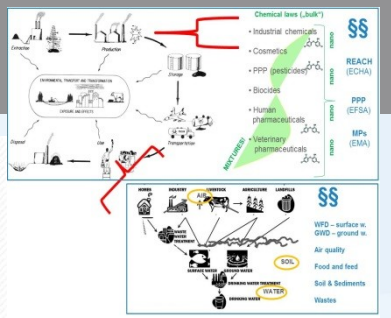
What to assess for toxicity?



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

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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

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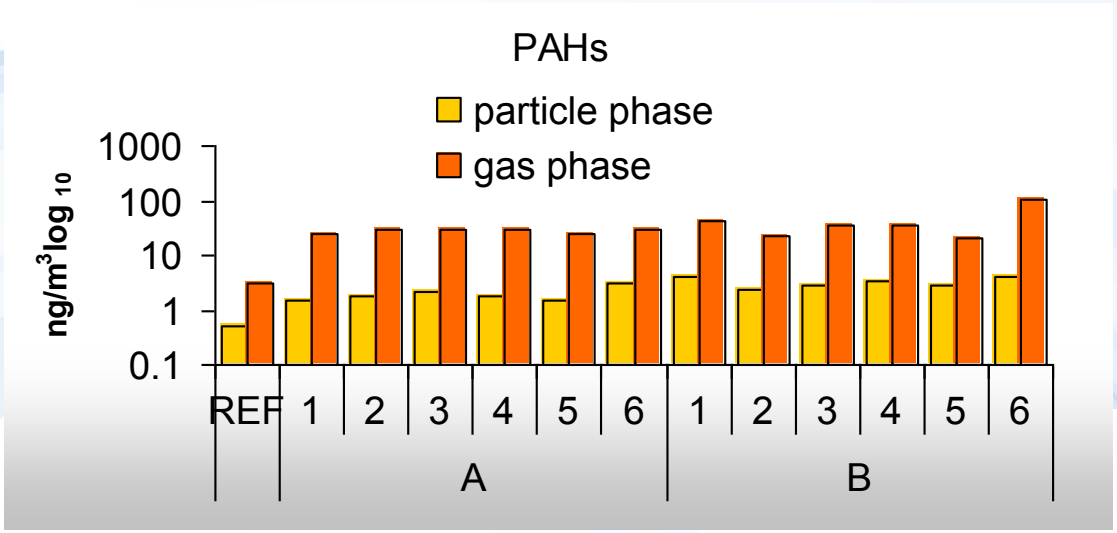
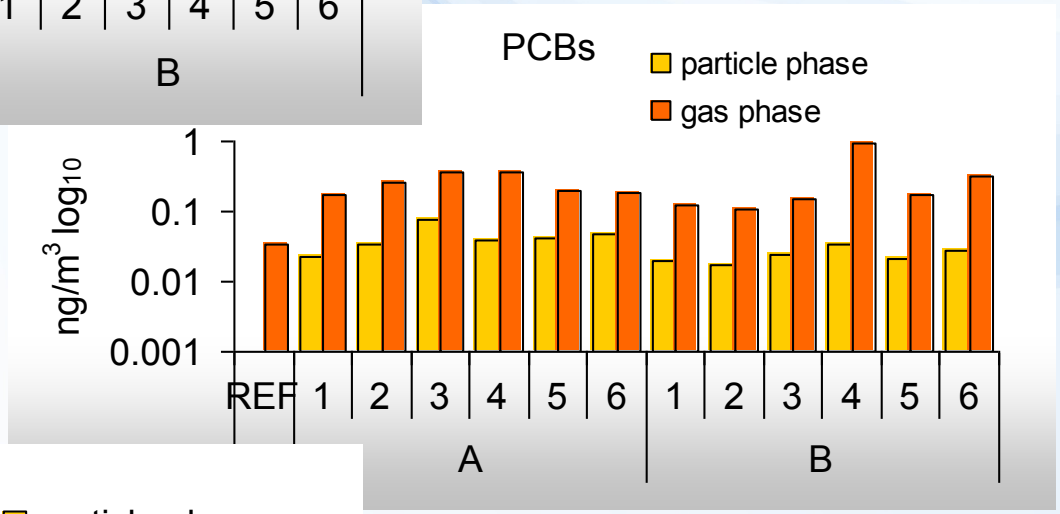
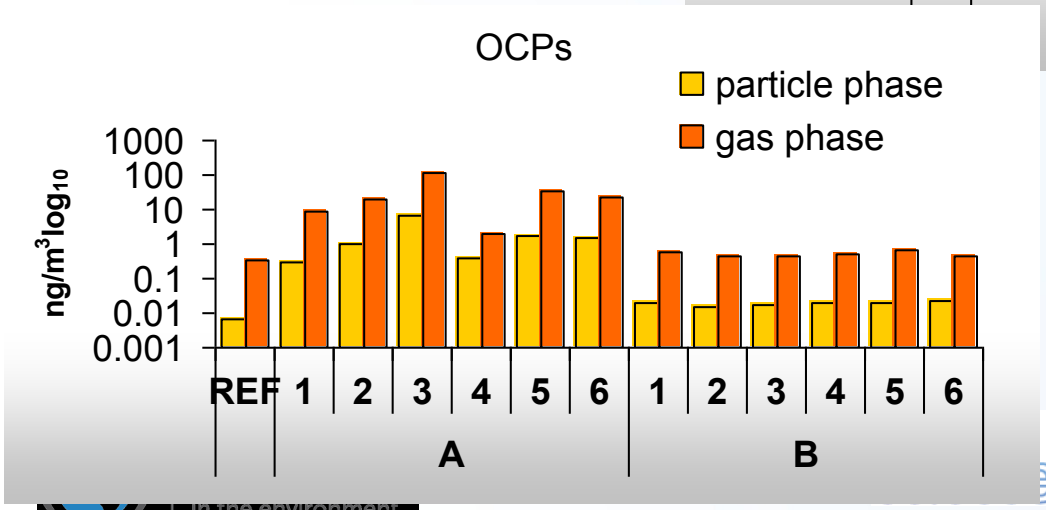
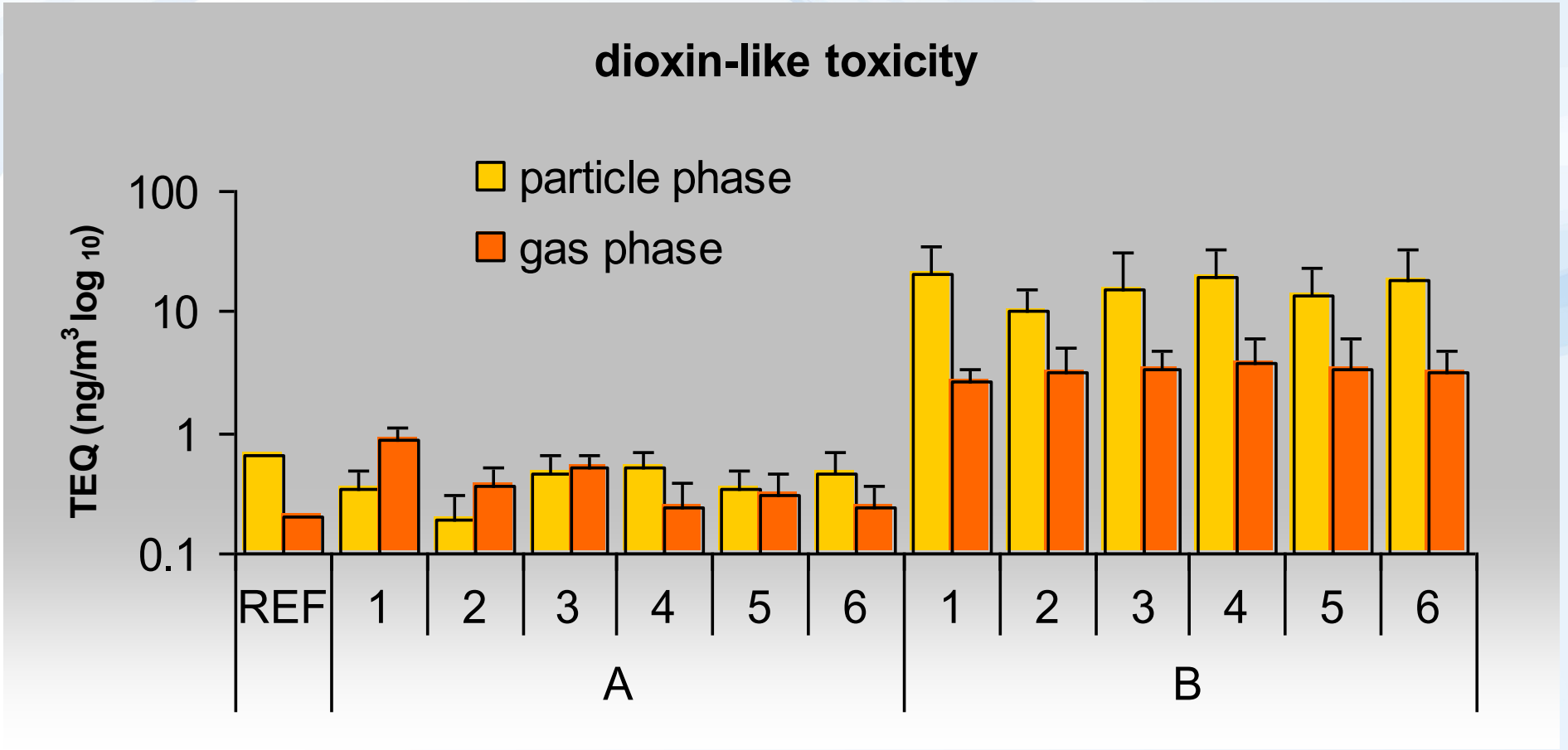
Chemical analyses







Dioxin-like effects



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

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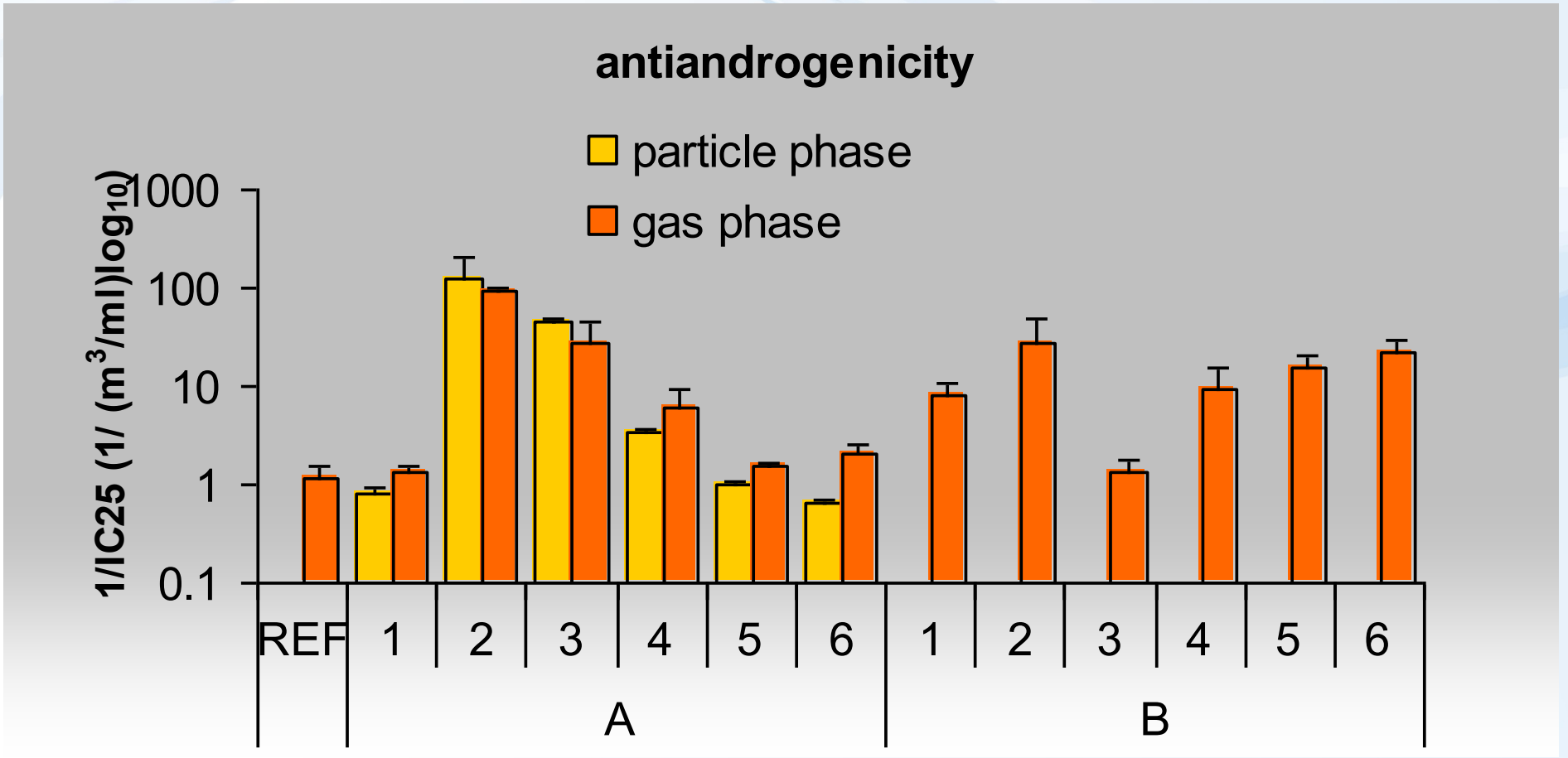
Antiandrogenic effects



- 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

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REACH

Contents lists available at ScienceDirect

Environment International

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

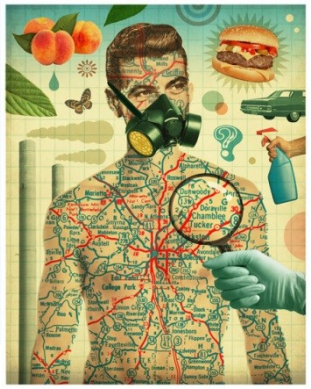
ELSEVIER

Review

What level of estrogenic activity determined by *in vitro* assays in municipal waste waters can be considered as safe?

Barbora Jarošová [a], Luděk Bláha [a], John P. Giesy [b], Klára Hilscherová [a,*]

[a] Masaryk University, Faculty of Science, RECETOX, Kamenice 5, CZ-62500 Brno, Czech Republic
[b] Department of Biomedical Veterinary Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada



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