



Research centre  
for toxic compounds  
in the environment

# Ecotoxicology

## Current issues in Research vs Regulation

**Ludek Blaha + ecotox colleagues**

ceToCoEn



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OP Research and  
Development for Innovation



# 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



# When & where the toxicity assessment is needed?

View of the researcher



**Anytime!**

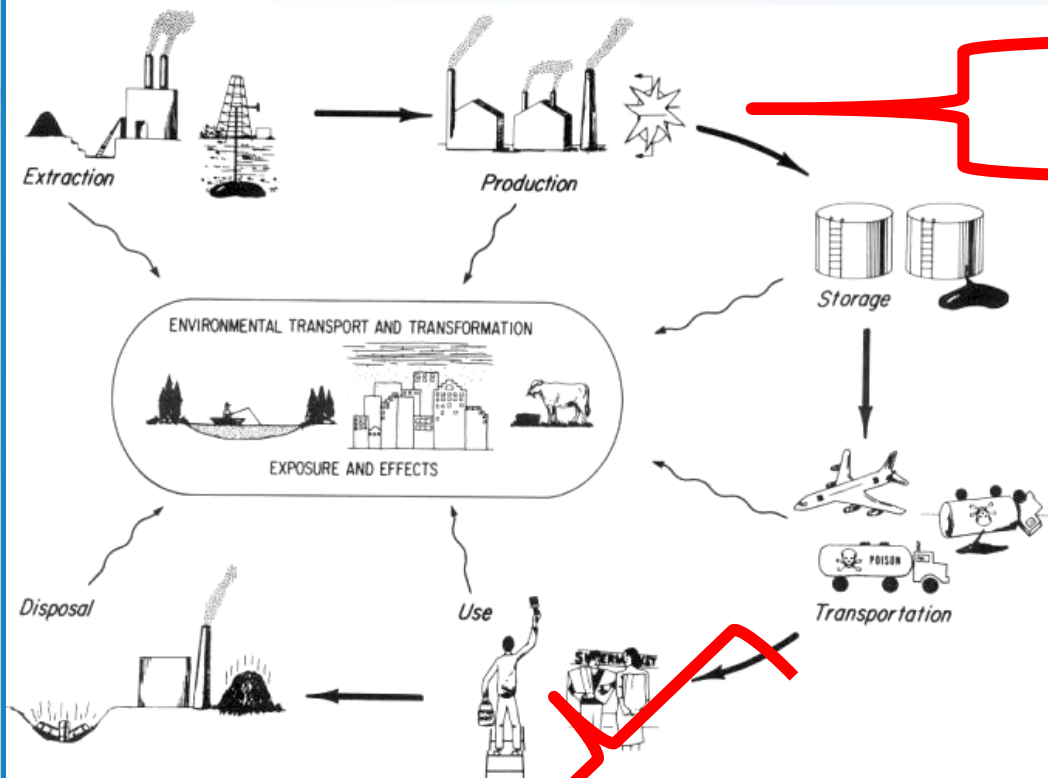
... depending on  
researcher's  
budget

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

nano  
nano  
nano  
nano

**REACH**  
(ECHA)

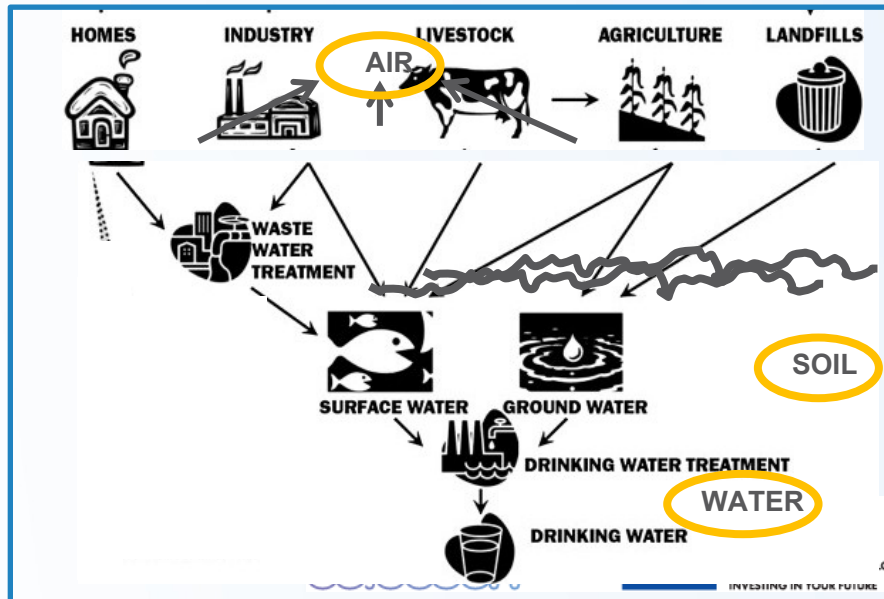
**PPP**  
(EFSA)

**MPs**  
(EMA)

**MIXTURES!**

**Two approaches:**

- Prospective (chemicals...)
- Retrospective (mixtures ...)



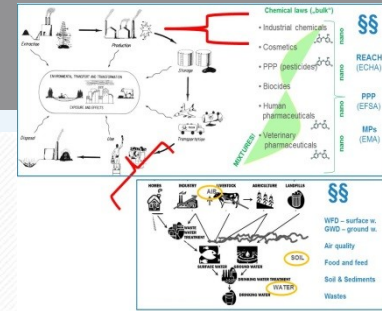
WFD – surface w.  
GWD – ground w.

Air quality

Food and feed

Soil & Sediments

# What to assess for toxicity?

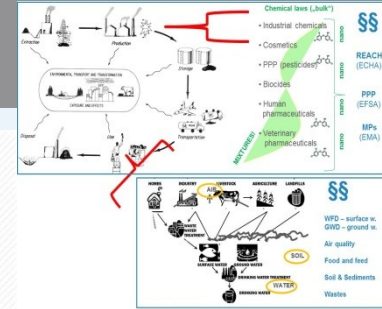


	Current research topics	As required by law
Individual chemicals (prospective)	<div style="border: 1px solid black; width: 100%; height: 100%;"></div>	
Mixtures (prospective)		
Contaminated samples (retrospective)		



Research  
for toxic  
in the er

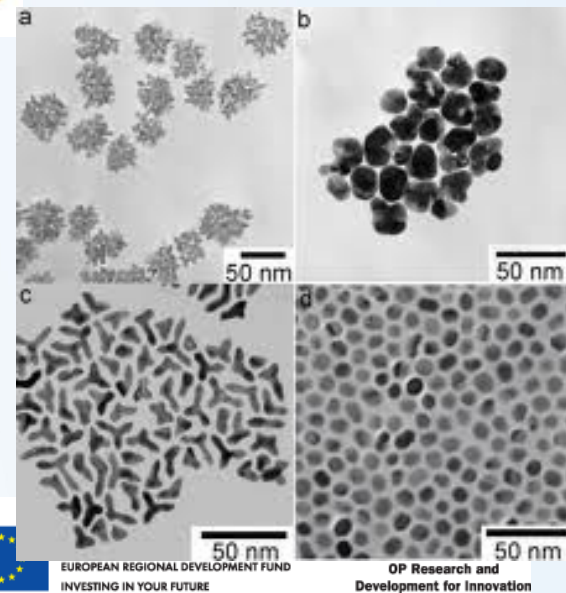
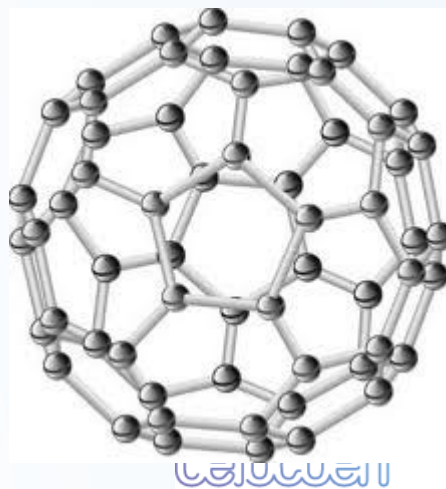
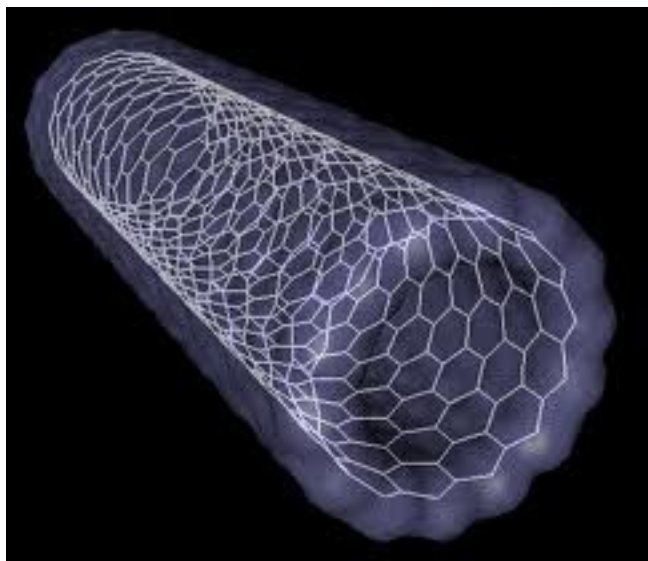
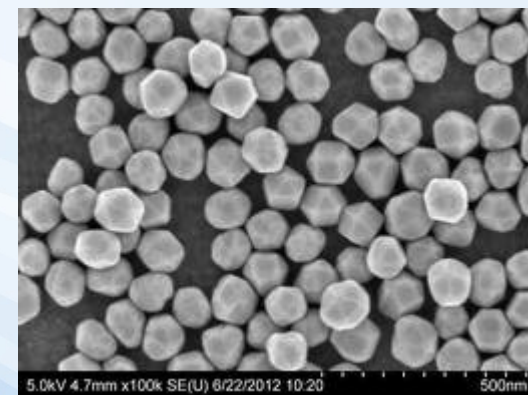
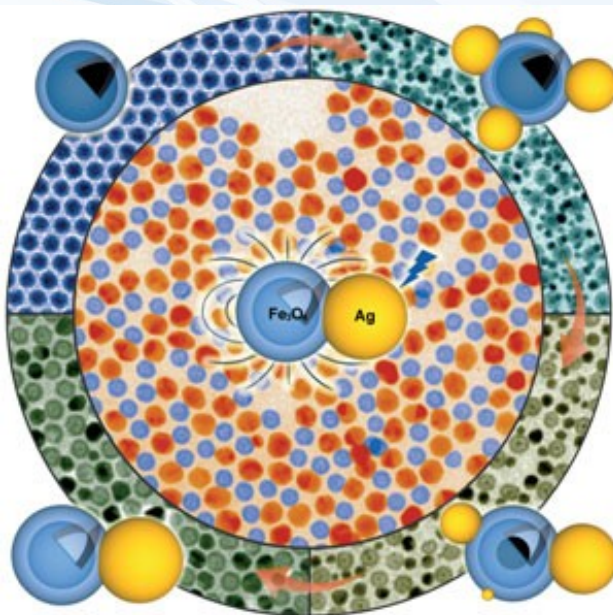
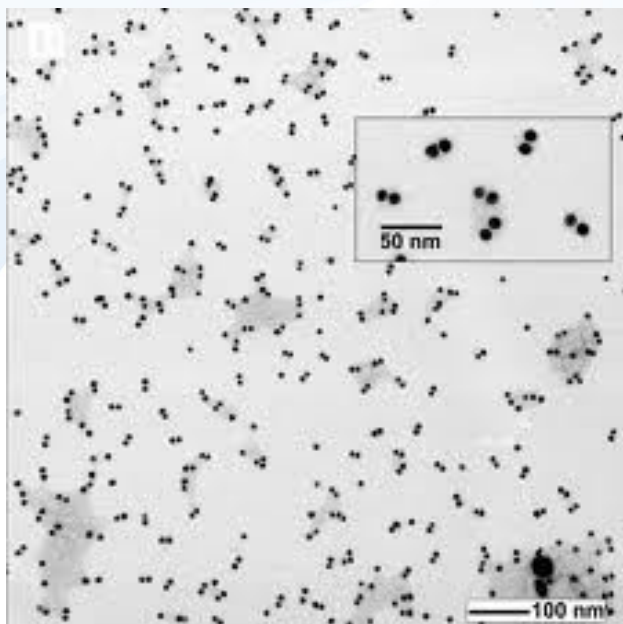
# What to assess for toxicity?



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

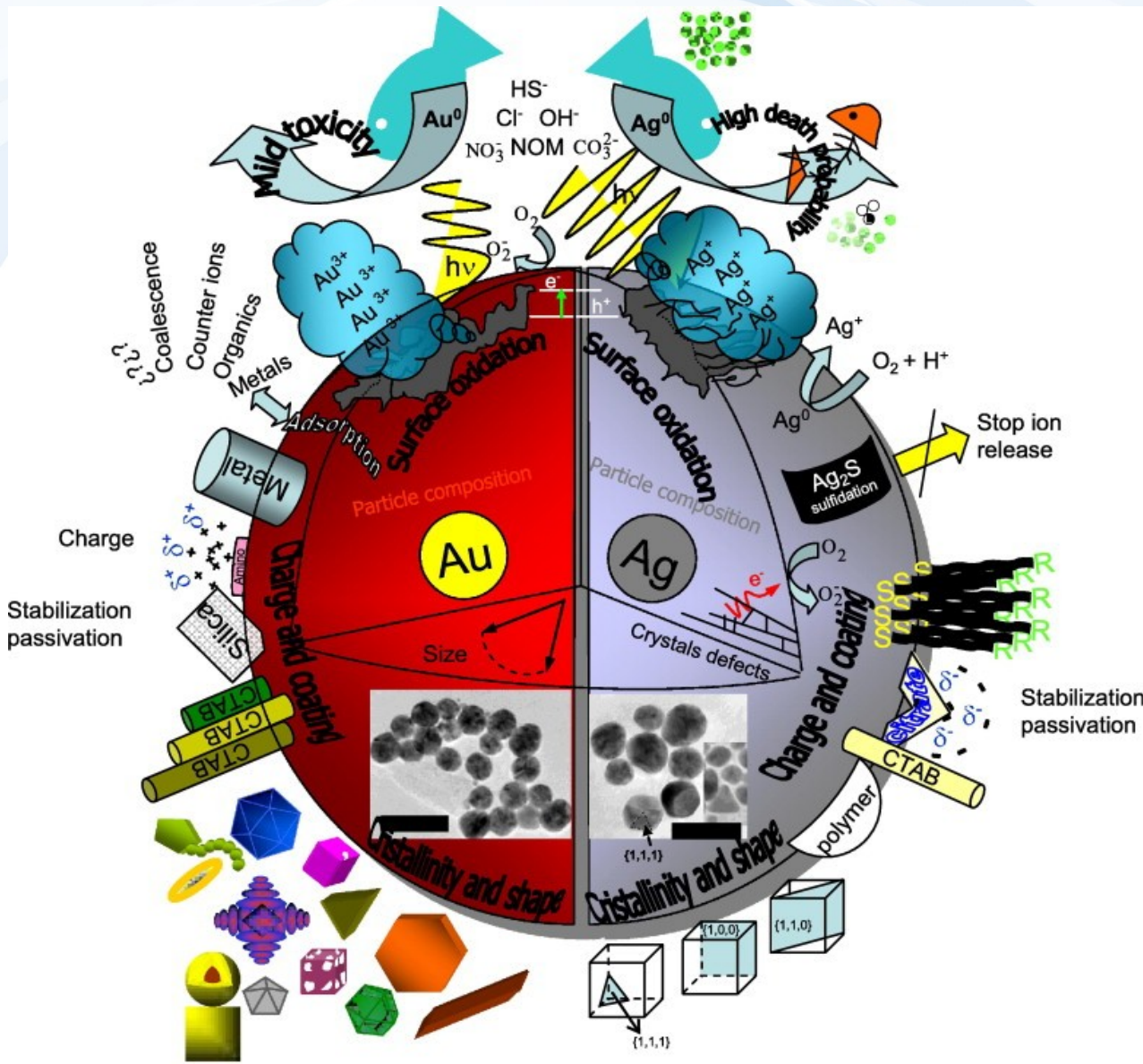


# Nanoparticles - examples





# Toxicity of nanoparticles ...



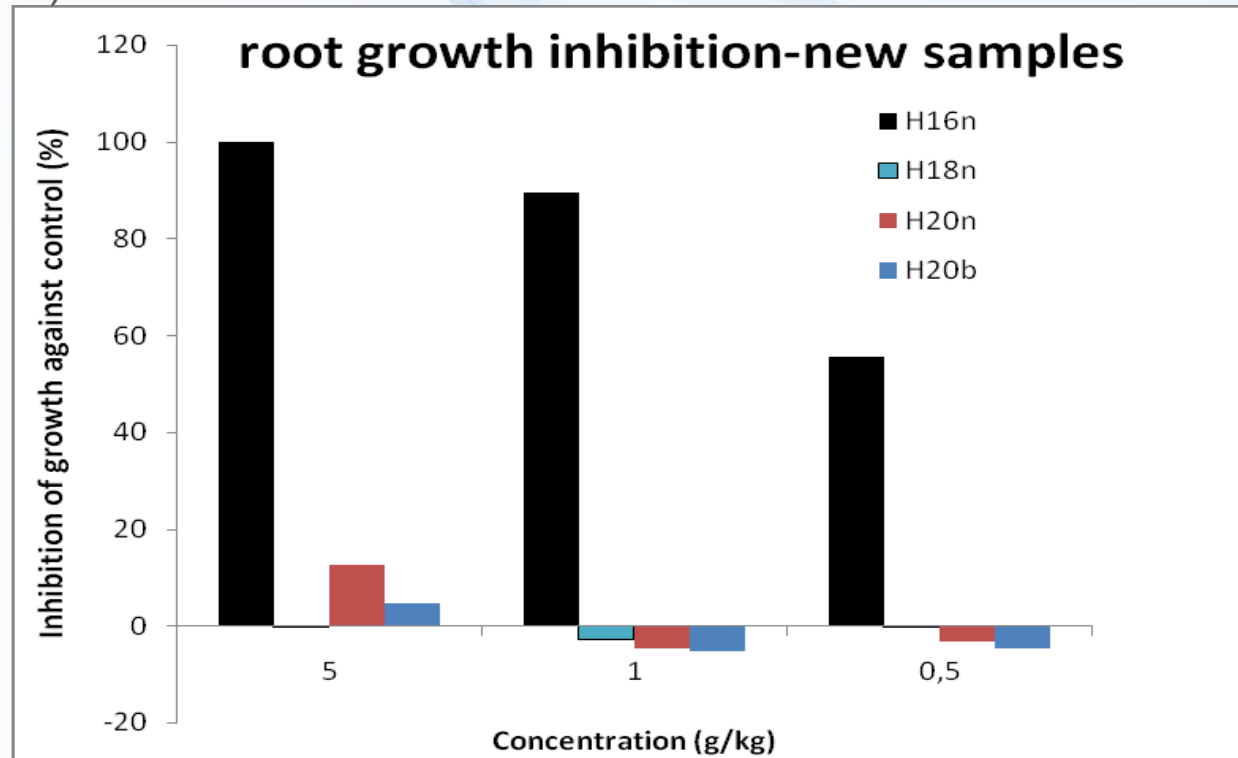
(Mostly unknown) Parameters may Affect ecotoxicity

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<sup>0</sup>)



*?? 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

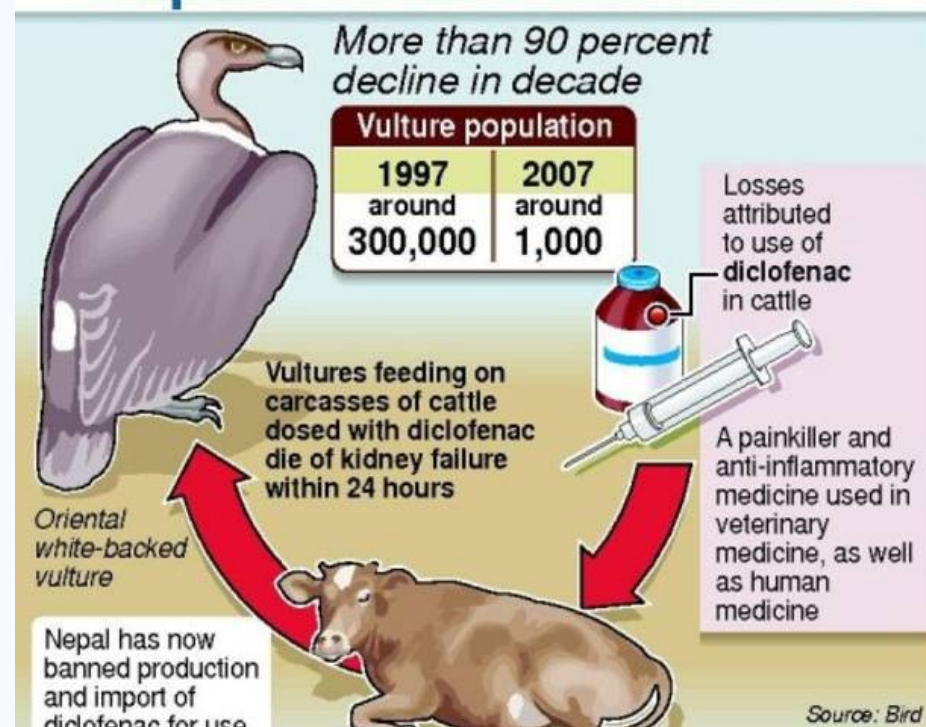
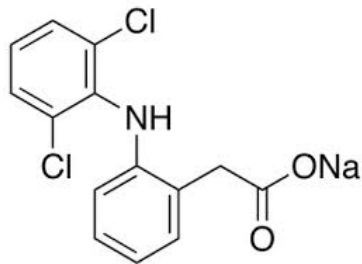
Manufacturing waste

Possible releases to the environment

# Example 1 - DICLOFENAC

## Unexpected effects at NON-TARGET species

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



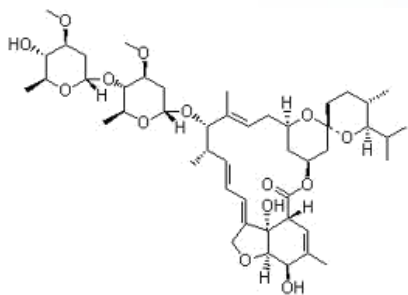
## Example 2 – AVERMEKTIN-like antiparasitics

**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)



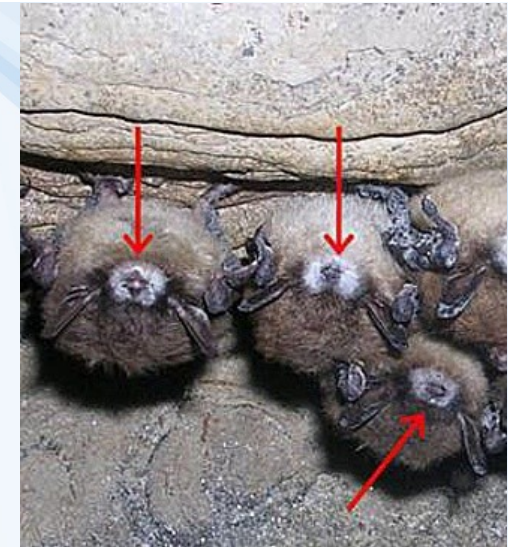
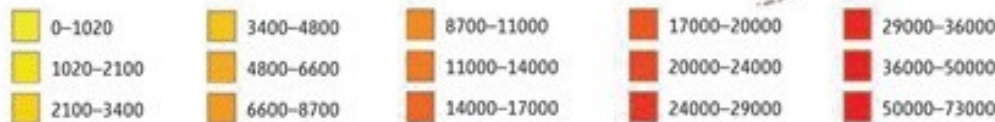
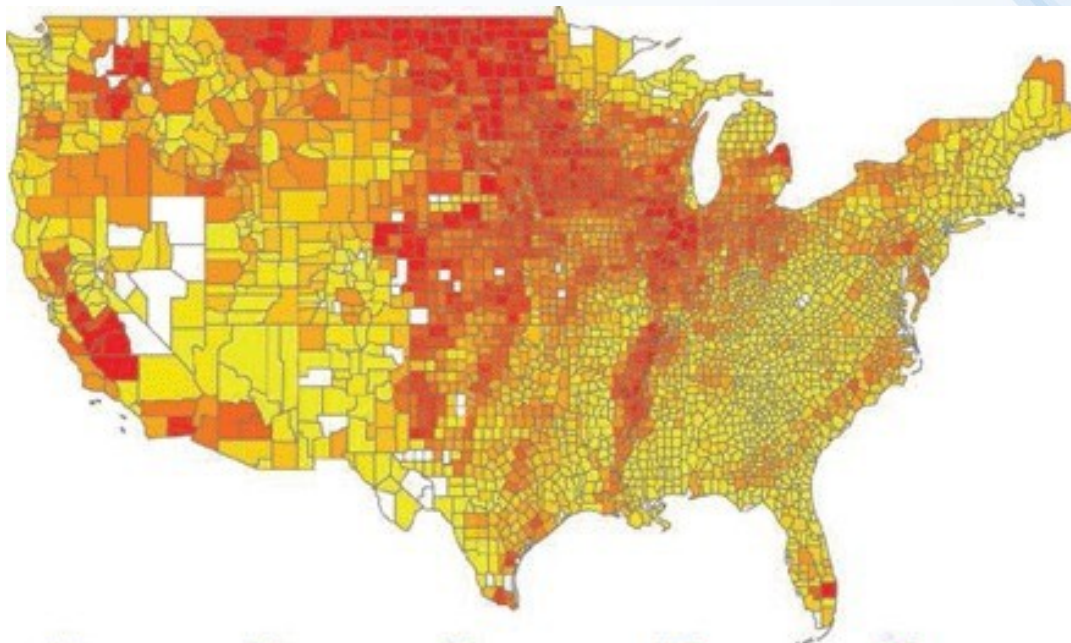


CONSERVATION

# Economic Importance of Bats in Agriculture

Justin G. Boyles,<sup>1\*</sup> Paul M. Cryan,<sup>2</sup> Gary F. McCracken,<sup>3</sup> Thomas H. Kunz<sup>4</sup>

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



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



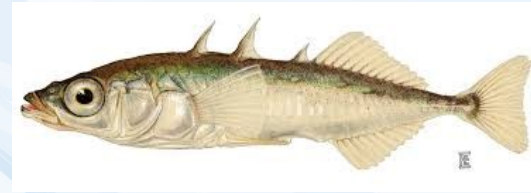
Daniel P. Roche, Katie E. McGhee\*  
and Alison M. Bell

*School of Integrative Biology, University of Illinois, Urbana, IL 61801, USA*

\*Author for correspondence (*kemcghee@illinois.edu*).

**Stress**

→ multigeneration effects



**Epigenetics**

→ DNA methylations

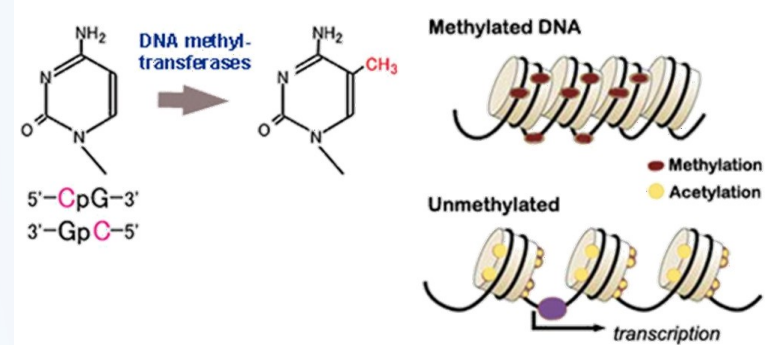


Table 1. Behaviours (mean  $\pm$  s.e.) of the offspring from the maternal treatments.

	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

2x difference

# MIXTURE TOXICITY EU interlaboratory test

Testing comparability of existing and innovative bioassays for water quality assessment

## Main questions:

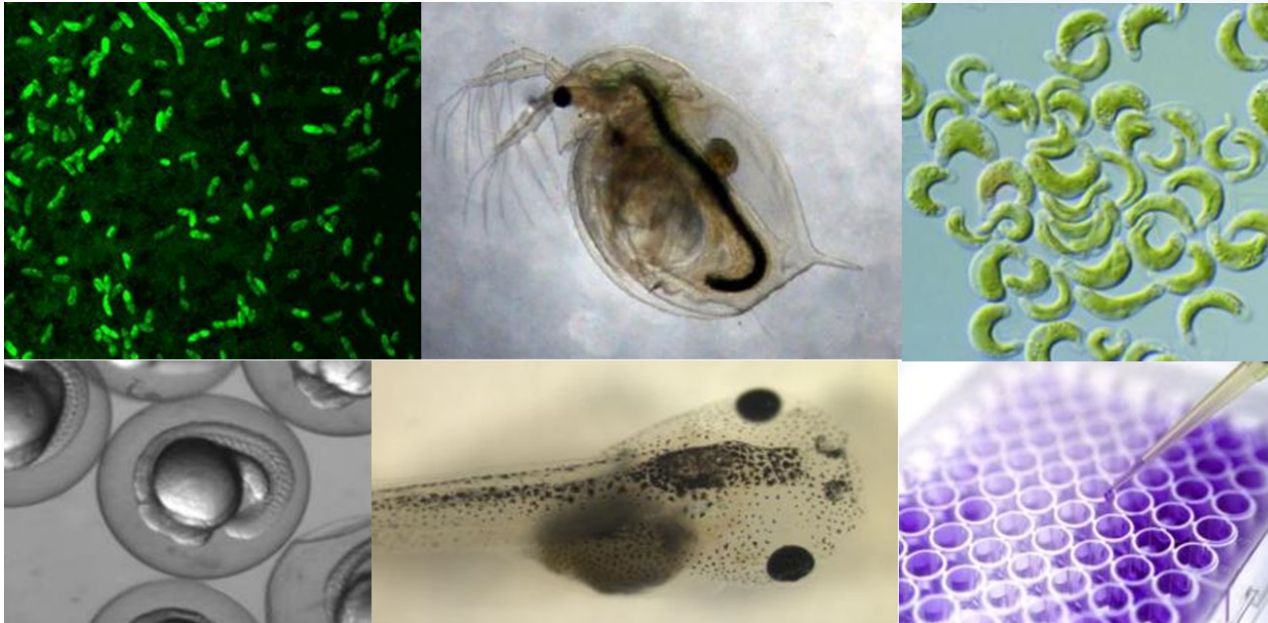
Are current limits (for individual compounds) safe?

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

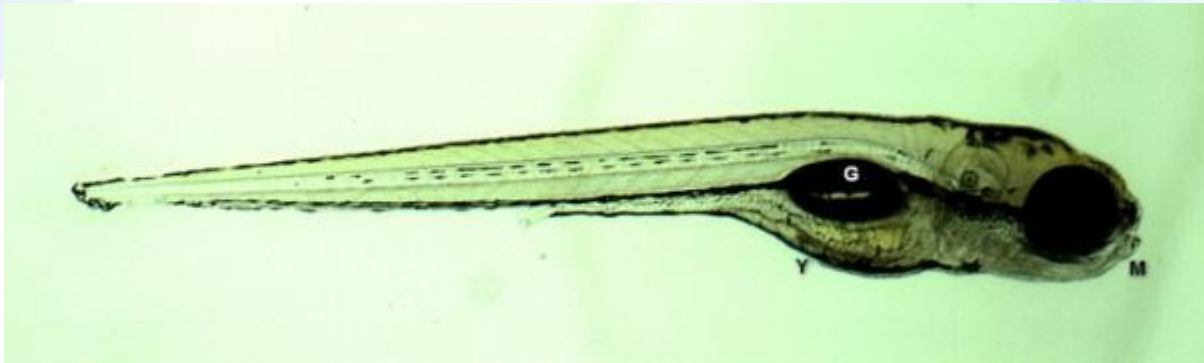


Research  
for the  
Environment

# MIXTURE TOXICITY EU interlaboratory test

Testing comparability of existing and innovative bioassays for water quality assessment

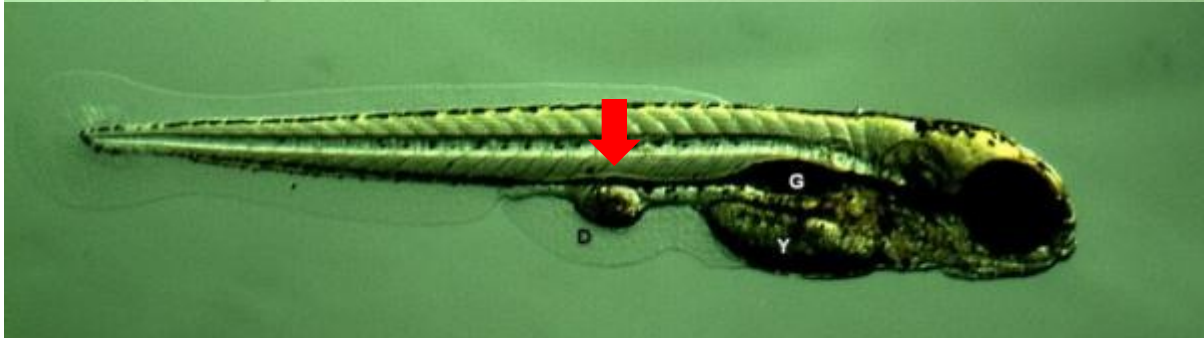
Example: Effects of mixtures on *D. rerio* fish embryos



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



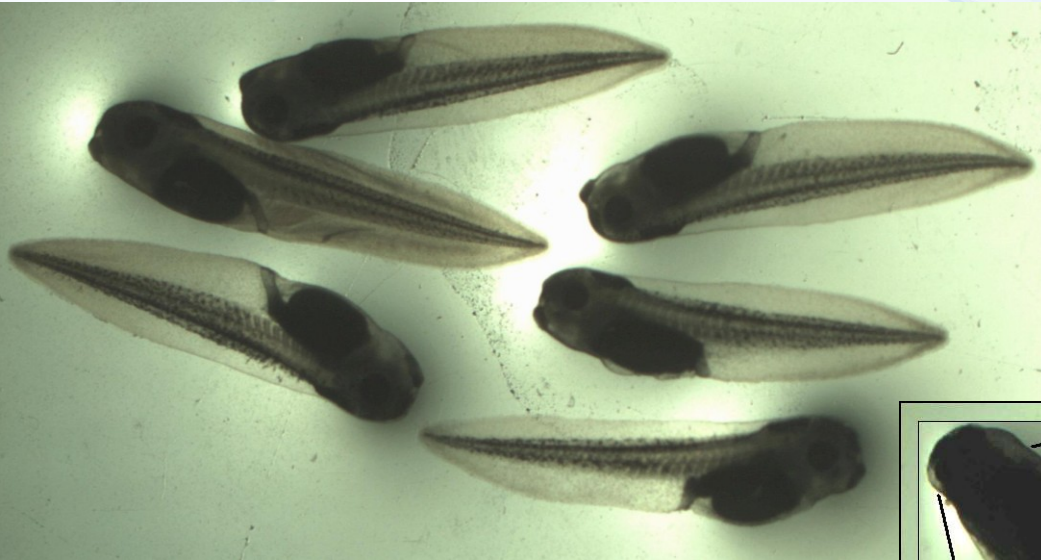
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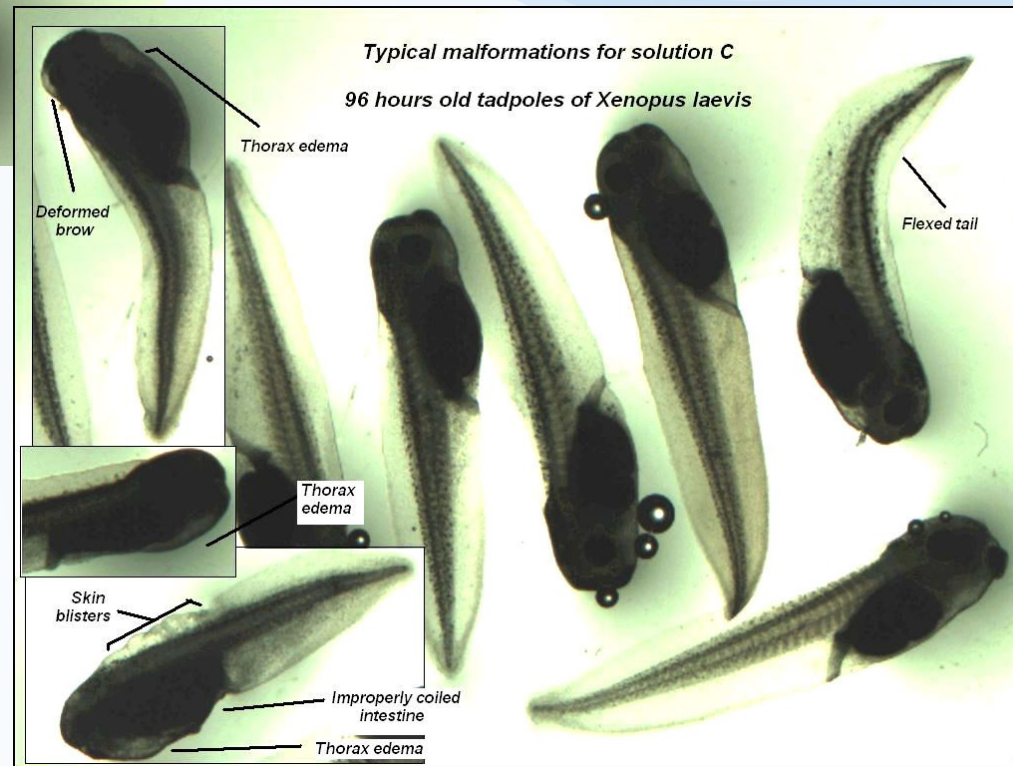
## Example: Effects of mixtures on *X. laevis* frog embryos







### Controls

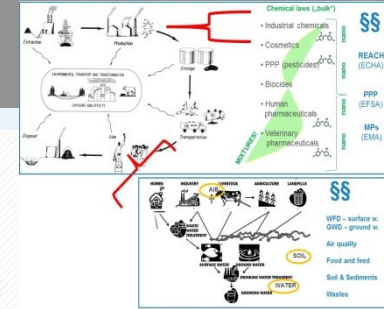
Carvalho, R. et al. (2014) Mixtures of chemical pollutants at European legislation safety concentrations: how safe are they?  
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Effects of RM 3 (i.e. safe) mixtures



<b>Biotest</b>	<b>A</b>	<b>B</b>	<b>C</b>
<b>Microtox</b>	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
<b>Algae growth inhibition test 96-h exposure</b> 	31% inhibition of growth compared to solvent control	20% inhibition of growth compared to solvent control	16% inhibition of growth compared to solvent control
<b>Acute immobilization test with <i>D. magna</i></b>	90% immobilization after 48 hours of exposure; 25% immobilization occurred in 50% concentration - not statistically significant	no effect observed	no effect observed
<b>Reproduction test with <i>D. magna</i> (21-d exposure)</b>	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
<b>FETAX (96-h exposure)</b> 	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
<b>FET (120-h exposure)</b>	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 
<b>In vitro - cytotoxicity</b>	no effect observed compared to solvent control	no effect observed compared to solvent control	no effect observed compared to solvent control
<b>In vitro - estrogenicity</b>	effect under LOQ	effect under LOQ	effect under LOQ
<b>In vitro - dioxin-like toxicity</b>	effect under LOQ	effect under LOQ	effect under LOQ
<b>In vitro - androgenicity</b>	effect under LOQ	effect under LOQ	effect under LOQ
<b>In vitro - antiandrogenicity</b>	effect under LOQ	effect under LOQ	effect under LOQ

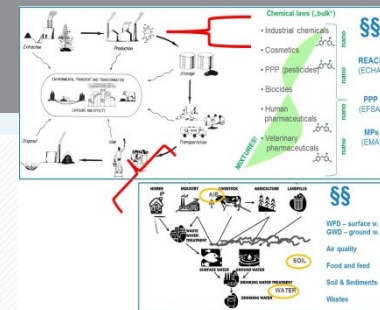
# What to assess for toxicity?






	Current research topics	As required by law
Individual chemicals (prospective)	Engineered <b>nanomaterials</b> /particles <b>Ecological effects</b> (e.g. of pharmaceuticals) <b>Endocrine</b> disruption & <b>chronic</b> diseases	Industry & biocides (REACH) PPPs = pesticides Pharmaceuticals Cosmetics
Mixtures (prospective)	<b>Multistressors</b> +T°C, salinity, pathogens, irradiation, food <b>Exposome</b>	 <b>LOADING</b>
Contaminated samples (retrospective)		



# What to assess for toxicity?



	Current research topics	As required by law
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Mixtures (prospective)	<b>Multistressors</b> +T°C, salinity, pathogens, irradiation, food <b>Exposome</b>	
Contaminated samples (retrospective)	Can analyzed chemicals explain observed effects ?	<b>Chemical analyses &amp; limits</b> (see lectures: RISK ASSESSMENT part) <b>Effect testing rare:</b> Remediation, dredged sediments (CZ), effluents  

# Contaminated samples? Case study “air”

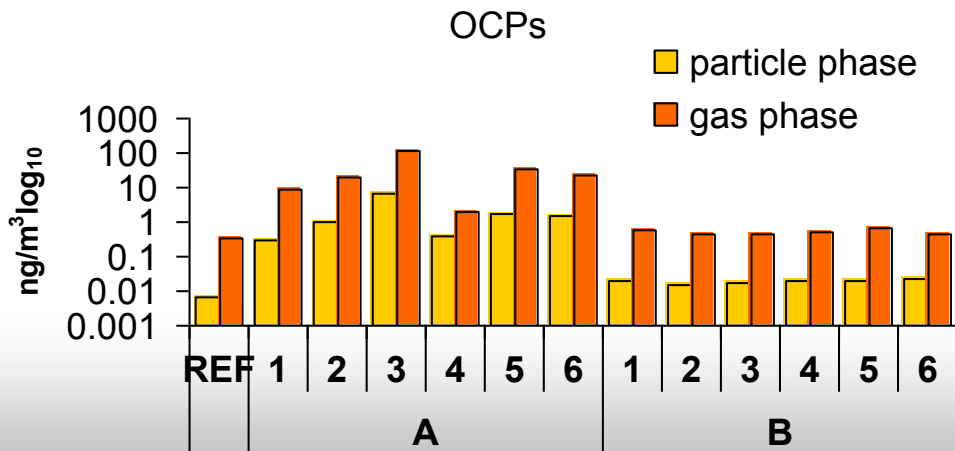
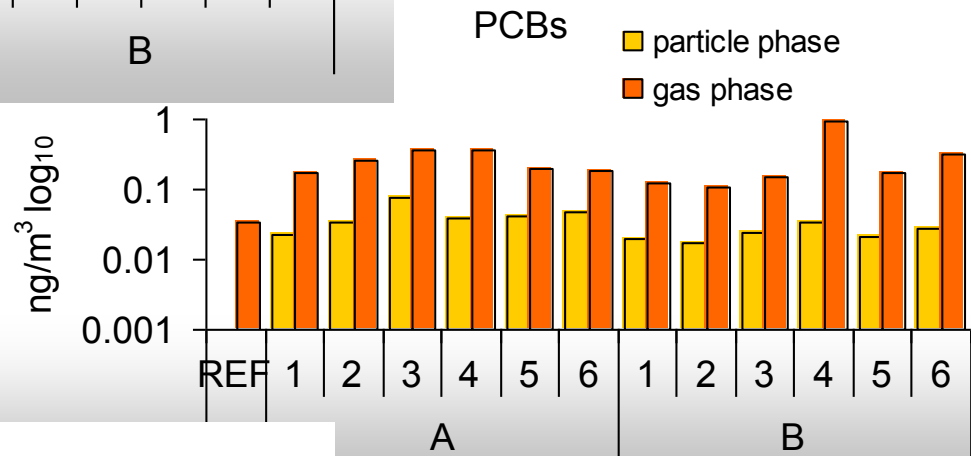
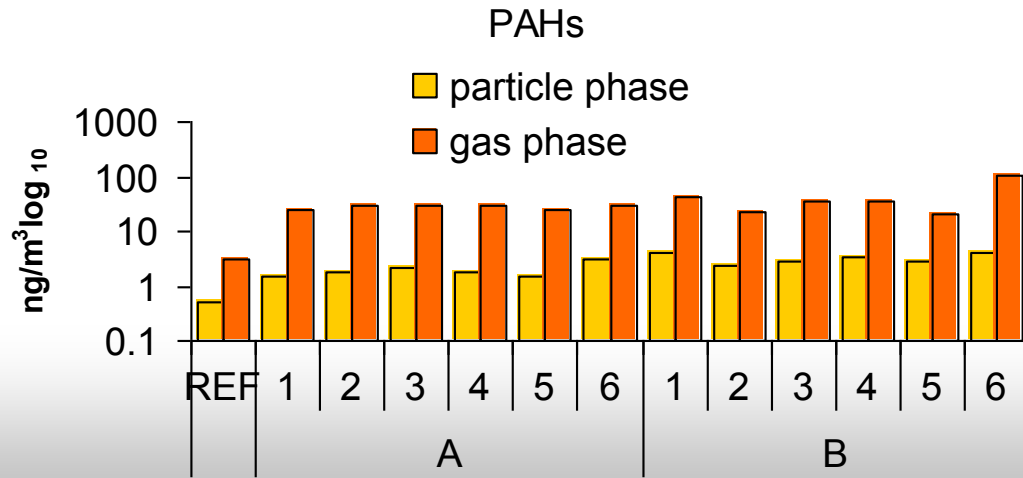
## Active sampling particles *vs* gaseous phase

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

Novák et al. (2009) Environment International



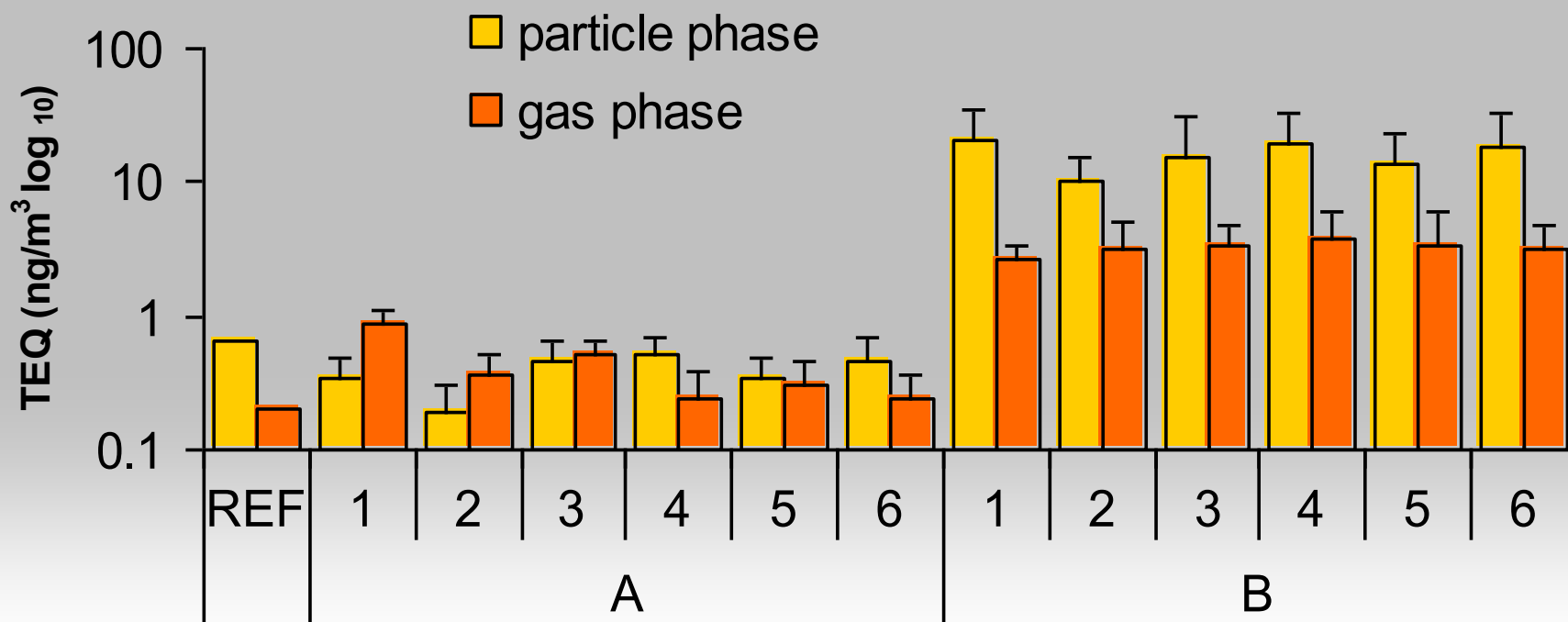
# Chemical analyses





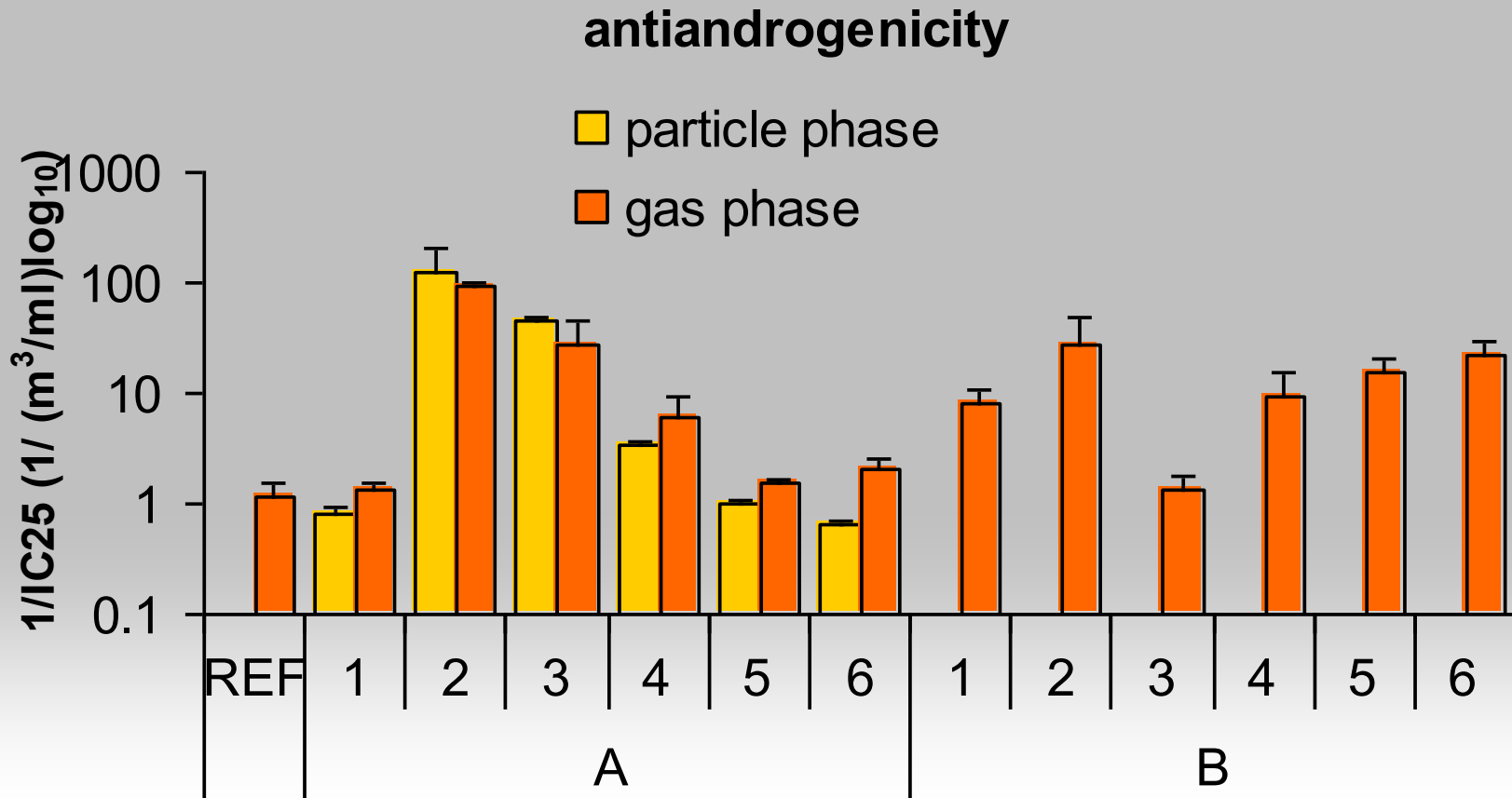
# Dioxin-like effects

## dioxin-like toxicity



- 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“?**



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Environment International

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

Review

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

Barbora Jarošová <sup>a</sup>, Luděk Bláha <sup>a</sup>, John P. Giesy <sup>b</sup>, Klára Hilscherová <sup>a,\*</sup>

<sup>a</sup> Masaryk University, Faculty of Science, RECETOX, Kamenice 5, CZ-62500 Brno, Czech Republic

<sup>b</sup> Department of Biomedical Veterinary Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

## Research issues and questions

- Nanomaterials, Pharmaceuticals, EDCs
- Mixtures!
- Exposome



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