



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!

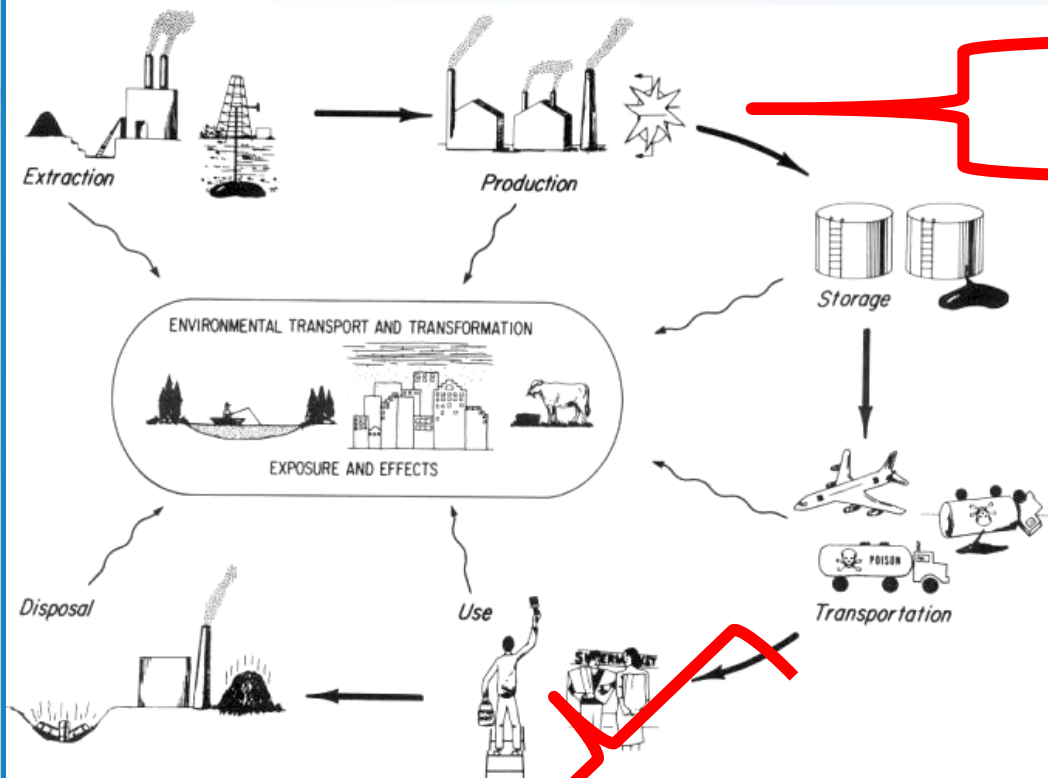
... depending on
researcher's
budget

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)

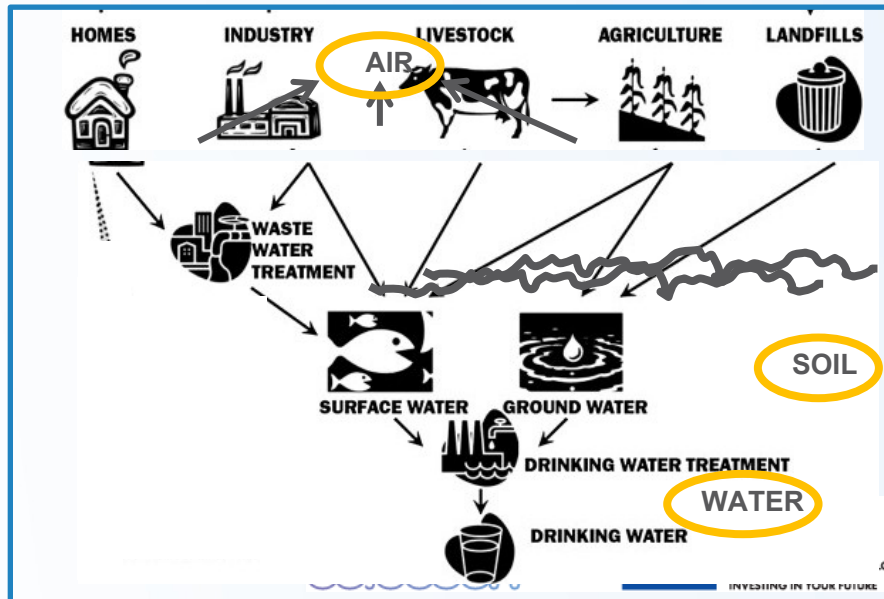
PPP
(EFSA)

MPs
(EMA)

MIXTURES!

Two approaches:

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



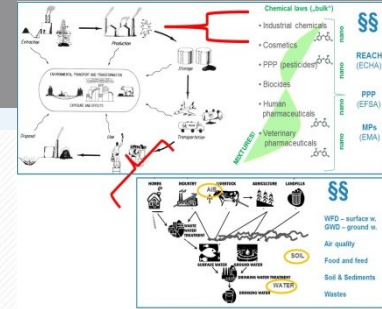
WFD – surface w.
GWD – ground w.

Air quality

Food and feed

Soil & Sediments

What to assess for toxicity?

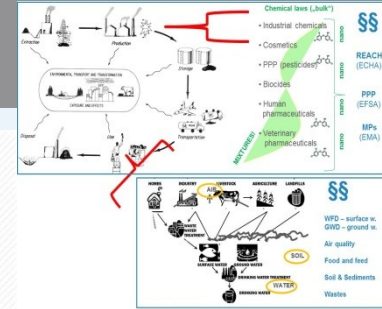


	Current research topics	As required by law
Individual chemicals (prospective)		
Mixtures (prospective)		
Contaminated samples (retrospective)		



Research
for toxic
in the er

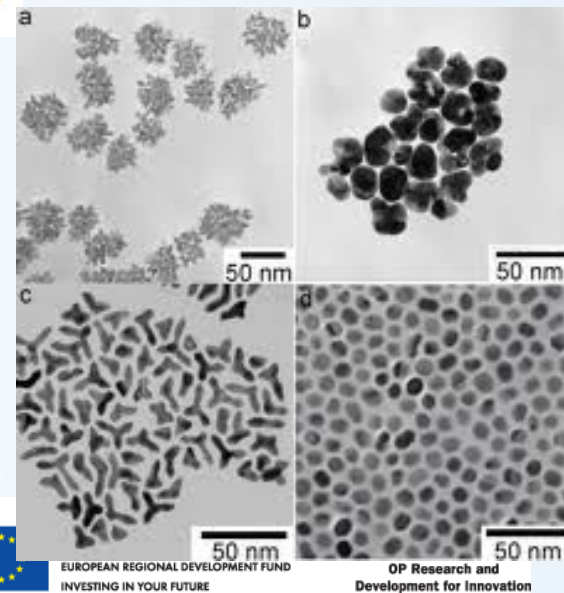
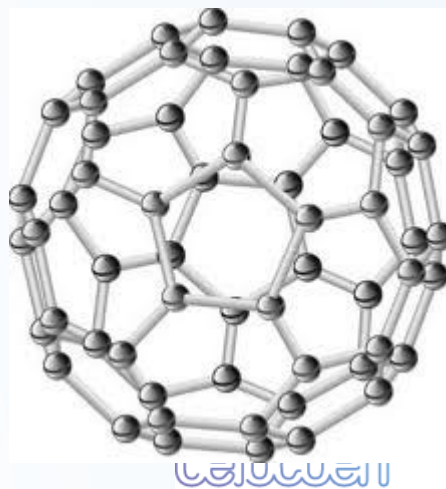
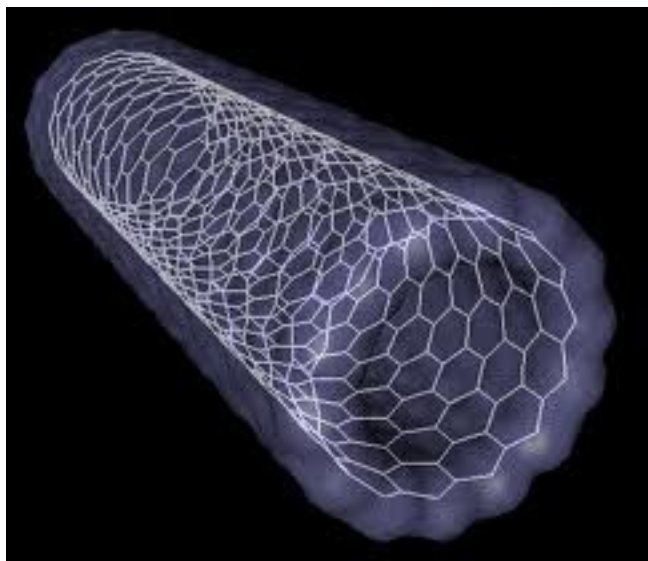
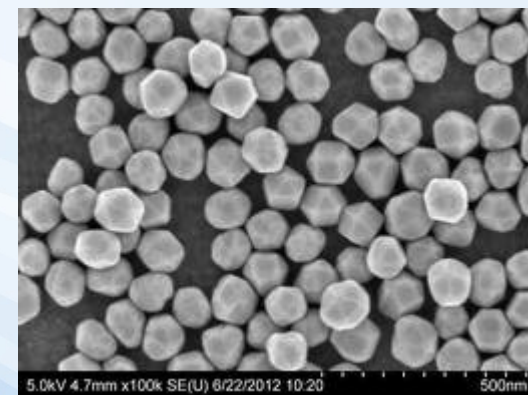
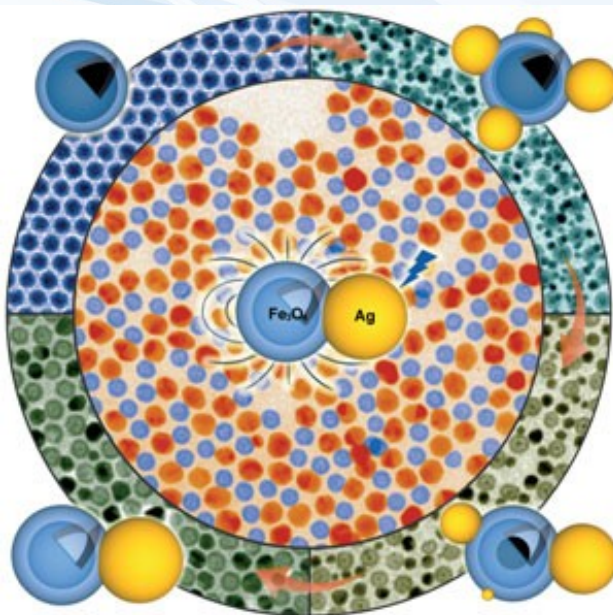
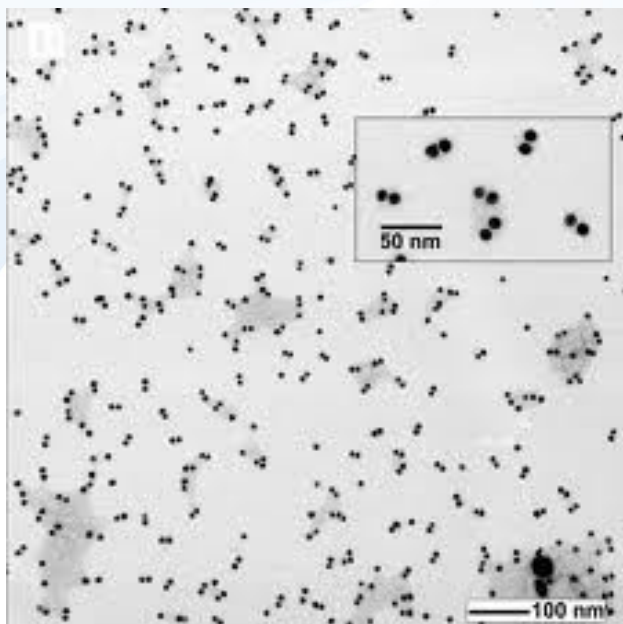
What to assess for toxicity?



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



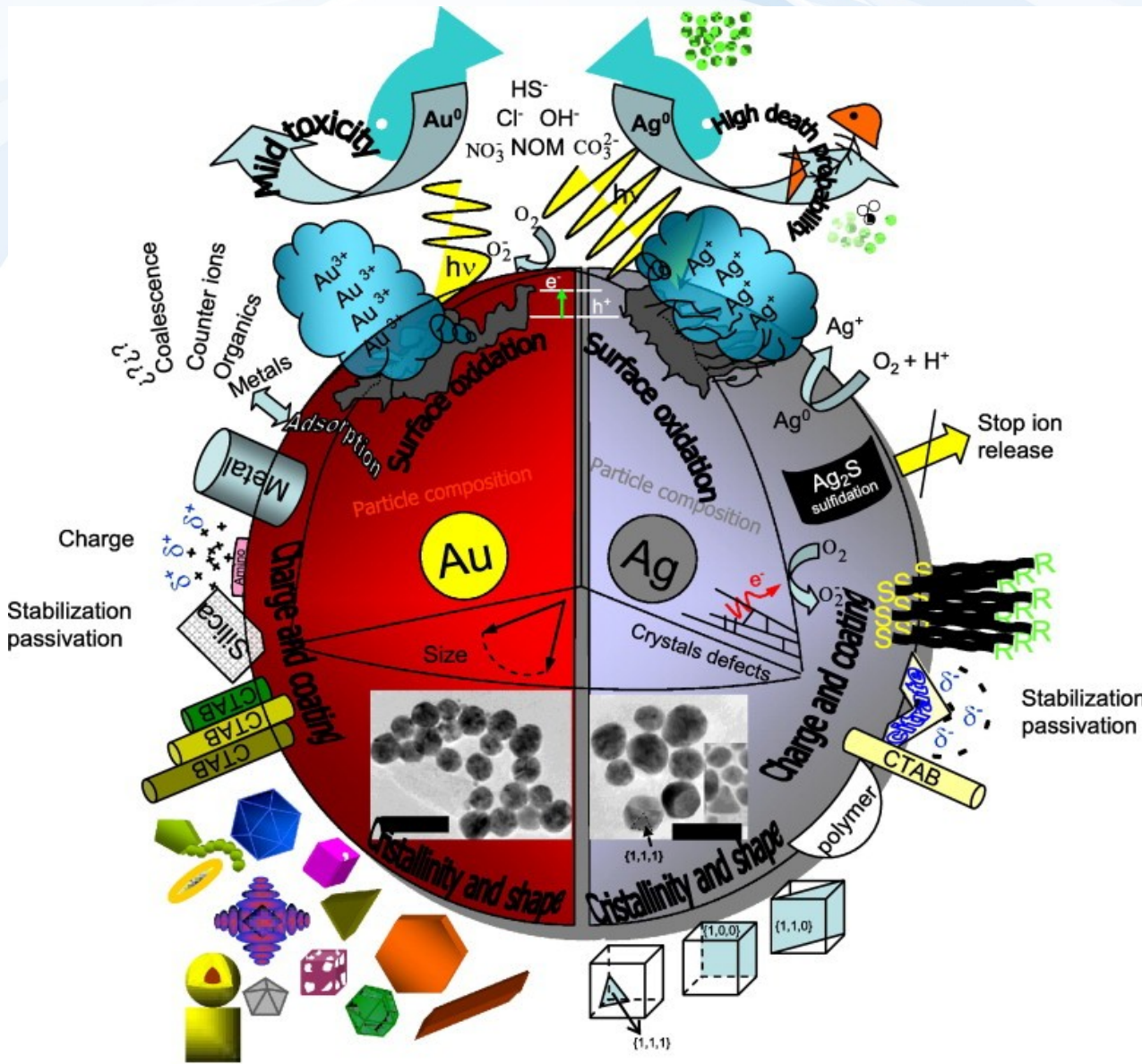
Nanoparticles - examples



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Toxicity of nanoparticles ...



(Mostly unknown)
Parameters may
Affect ecotoxicity

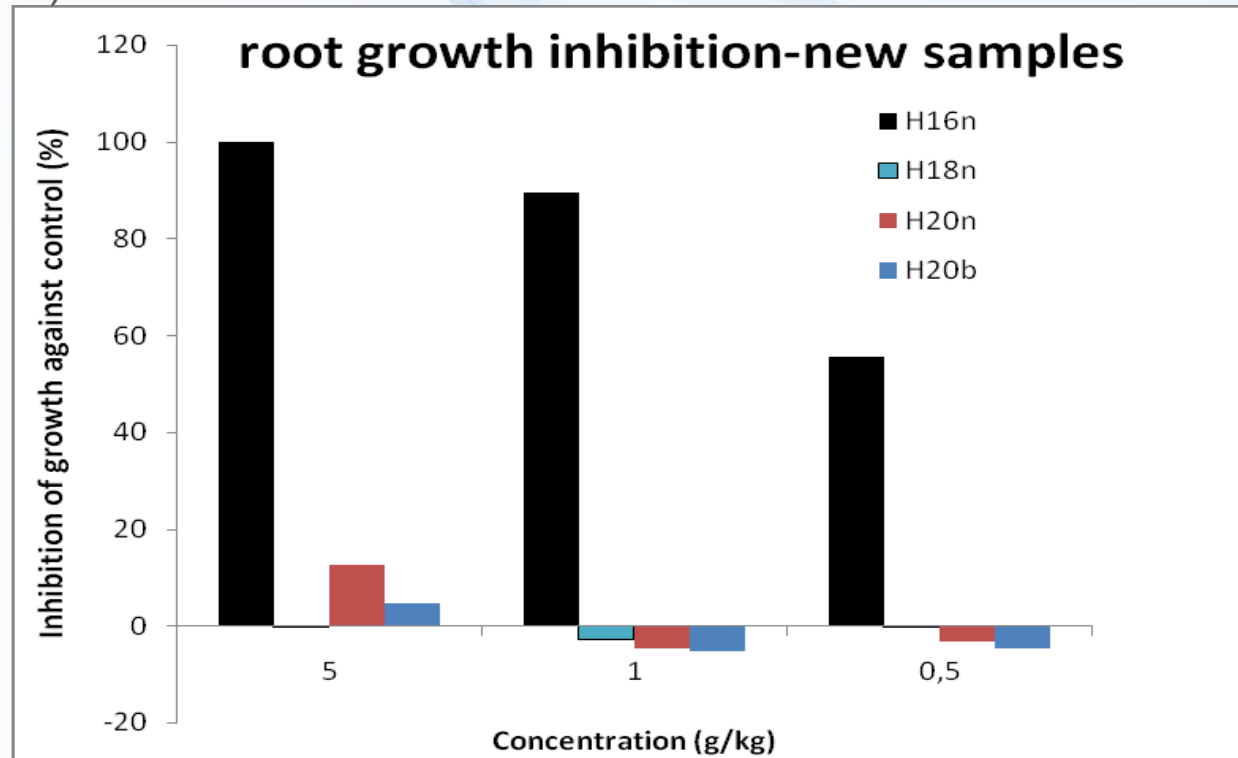
Composition (chemical)
Surface (size, area)
Charge
Reactivity
Interactions with ions,
other chemicals...

→ Effects on
environmental Fate
and toxicity



Ecotoxicity of nanoparticles – RECETOX example

Comparison of toxicity - 4 „appeared to be the same“ particles
(one producer – 4 different lots)
(zerovalent iron – ZVI – Fe⁰)



?? 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

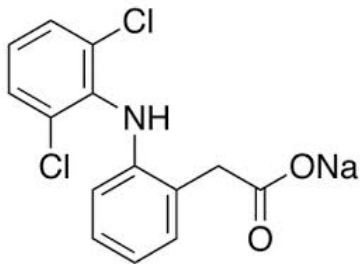
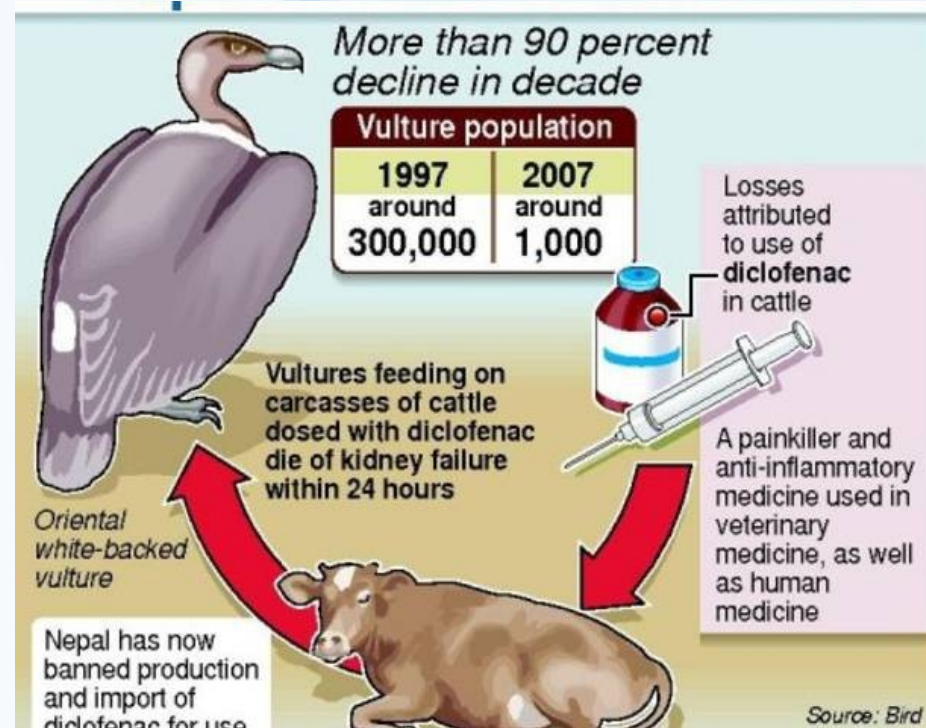
Manufacturing waste

Possible releases to the environment

Example 1 - DICLOFENAC

Unexpected effects at NON-TARGET species

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



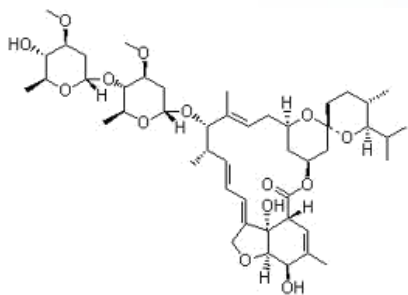
Example 2 – AVERMEKTIN-like antiparasitics

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)



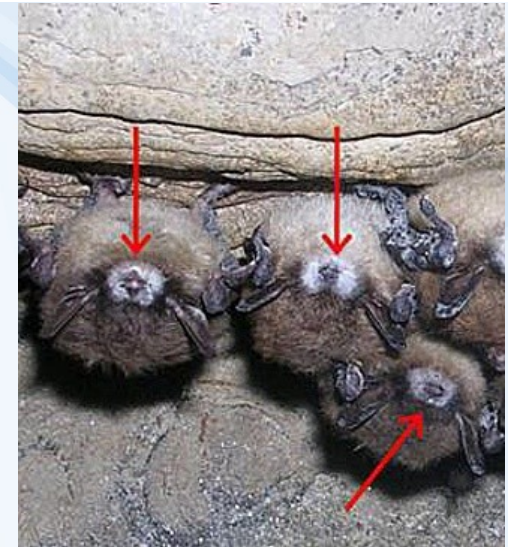
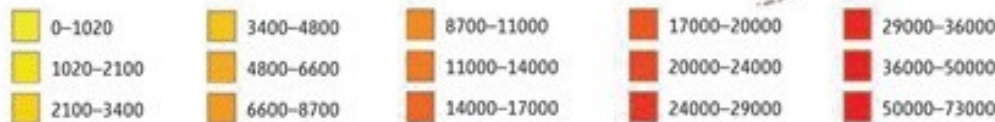
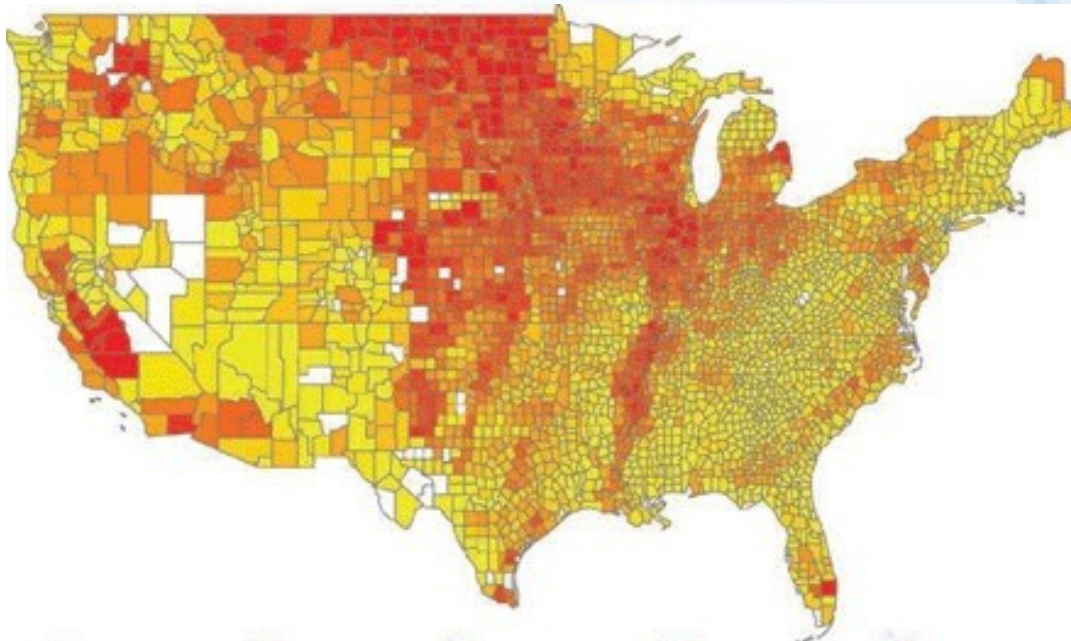


CONSERVATION

Economic Importance of Bats in Agriculture

Justin G. Boyles,^{1*} Paul M. Cryan,² Gary F. McCracken,³ Thomas H. Kunz⁴

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

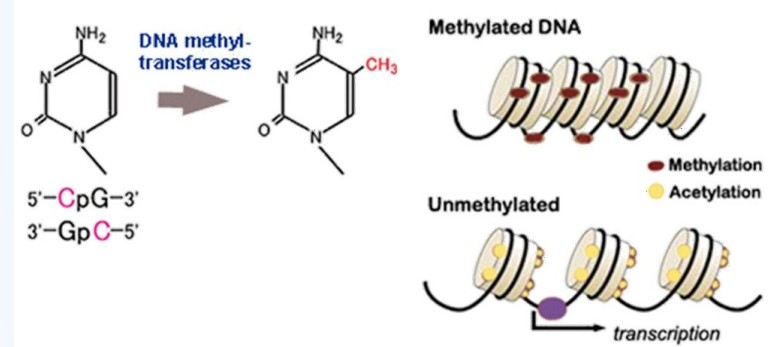


Table 1. Behaviours (mean \pm s.e.) of the offspring from the maternal treatments.

	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:

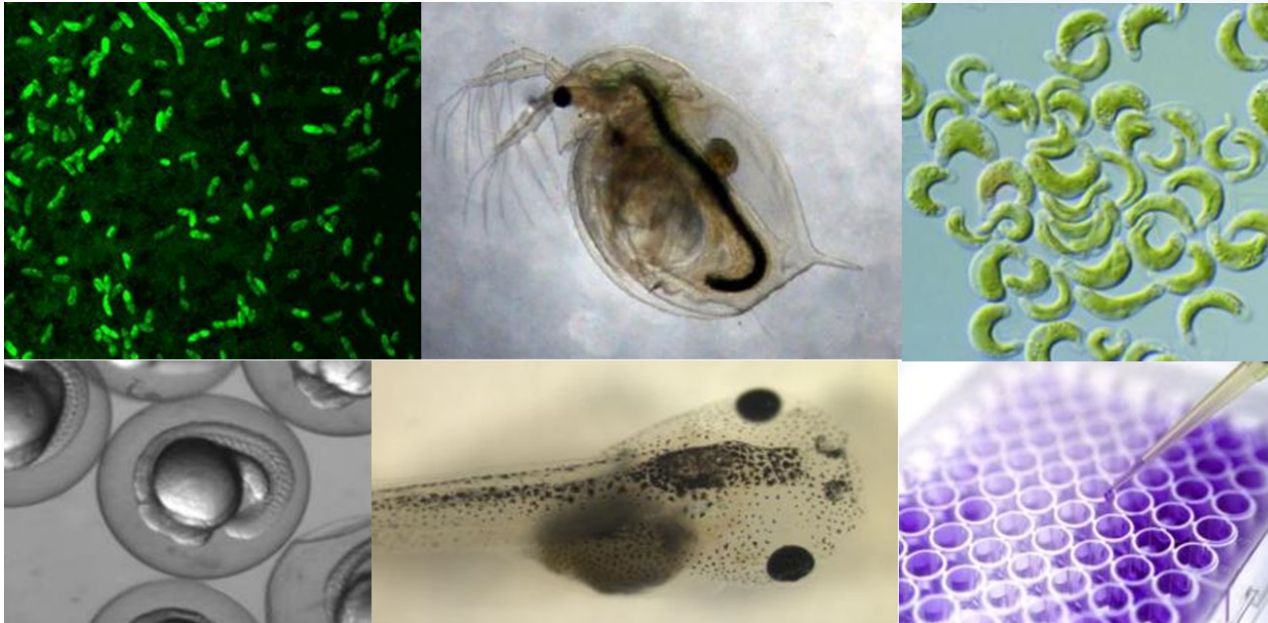
Are current limits (for individual compounds) safe?

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 ^a	RM 2 ^a	RM 3 ^a
<i>Priority substances</i> 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

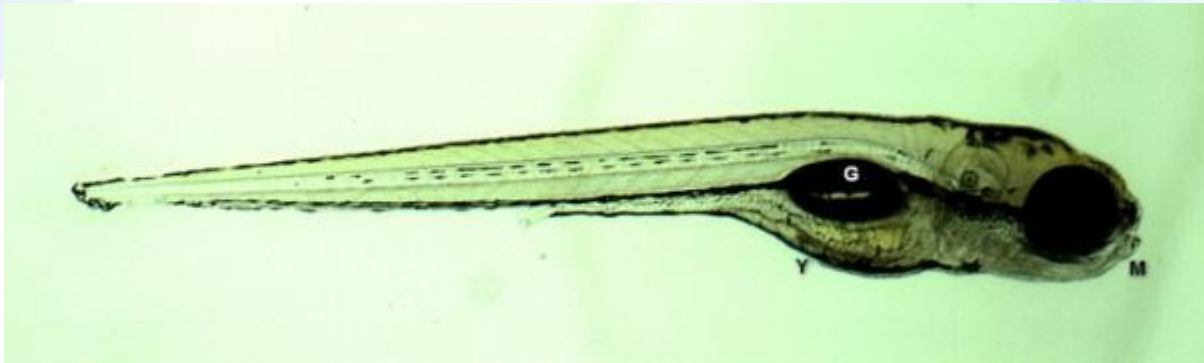


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for the
Environment

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Testing comparability of existing and innovative bioassays for water quality assessment

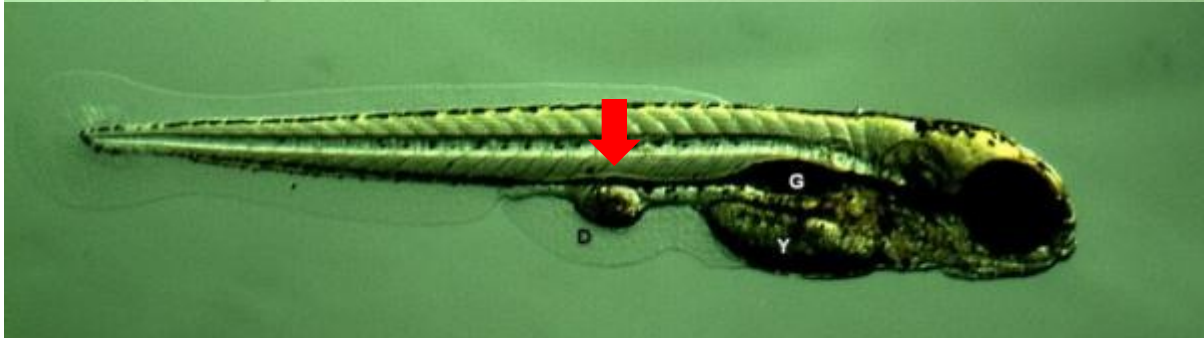
Example: Effects of mixtures on *D. rerio* fish embryos



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?

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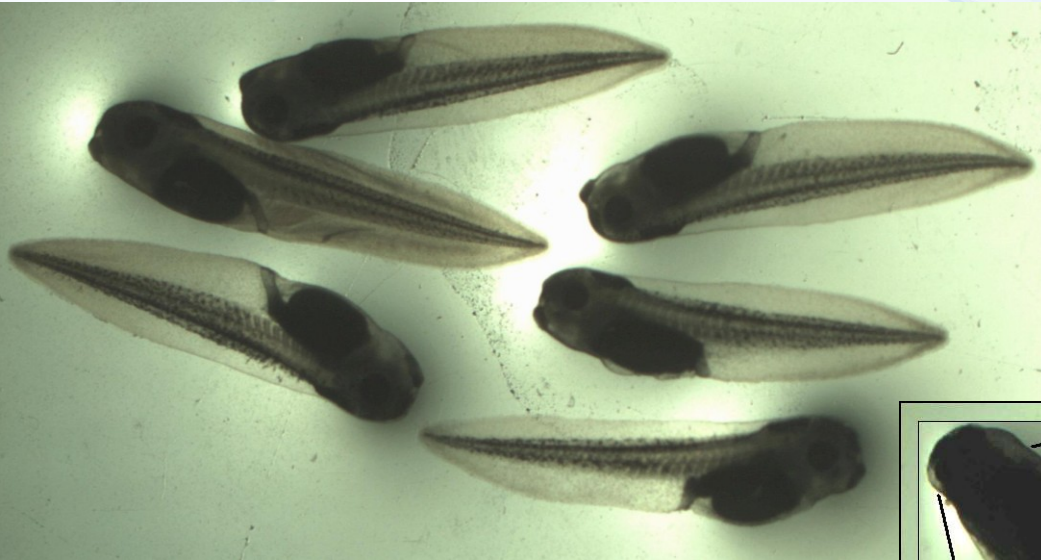
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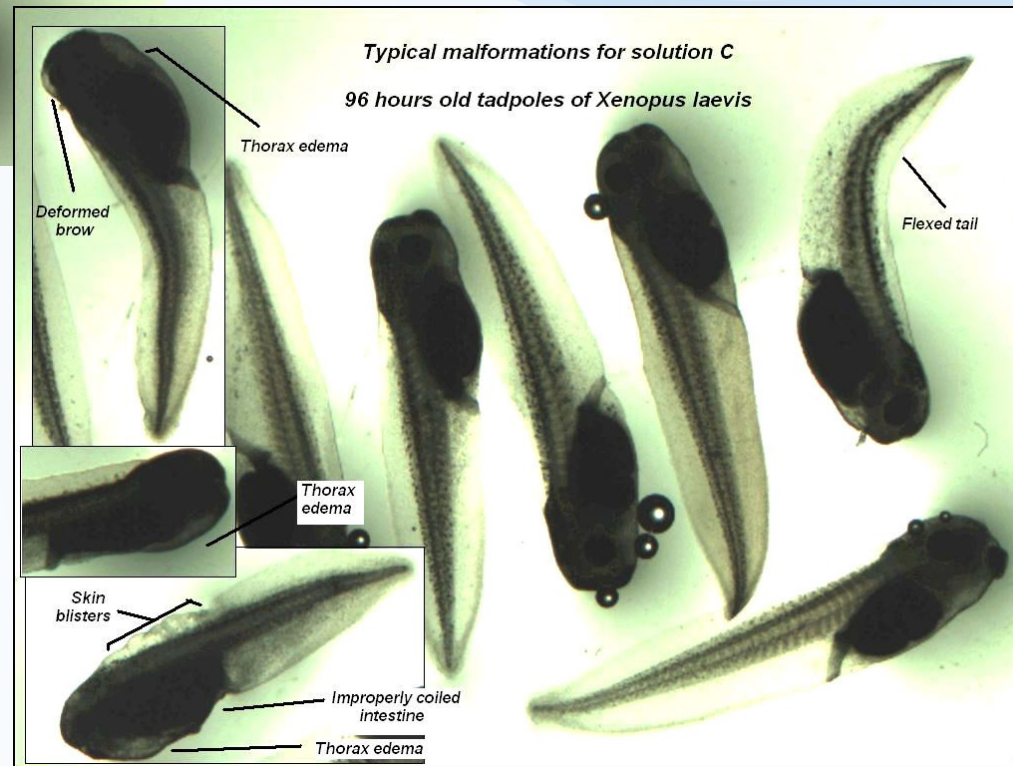
Example: Effects of mixtures on *X. laevis* frog embryos







Controls

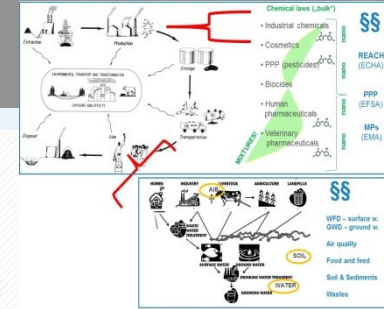
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Effects of RM 3 (i.e. safe) mixtures



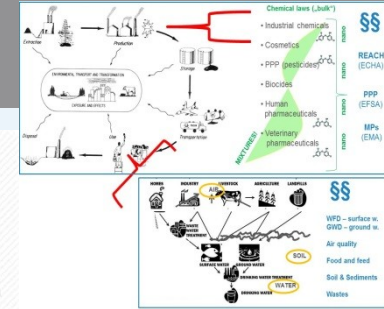
Biotest	A	B	C
Microtox	26 and 36% stimulation of luminescence in 15 and 30 mins of exposure, respectively	18 and 35% stimulation of luminescence in 15 and 30 mins of exposure, respectively	22 and 39% stimulation of luminescence in 15 and 30 mins of exposure, respectively
Algae growth inhibition test 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 <i>D. magna</i>	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 <i>D. magna</i> (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?



	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)	Multistressors +T°C, salinity, pathogens, irradiation, food Exposome	
Contaminated samples (retrospective)		

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)	Multistressors +T°C, salinity, pathogens, irradiation, food Exposome	
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  

Contaminated samples? Case study “air”

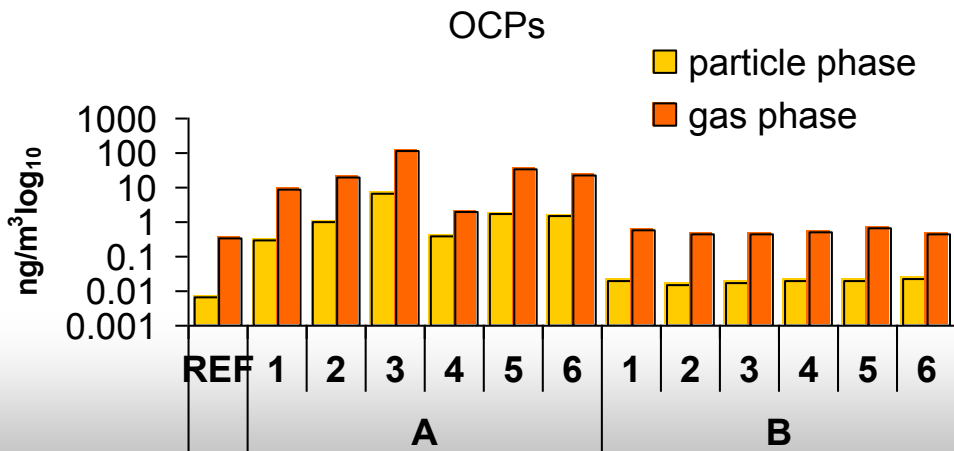
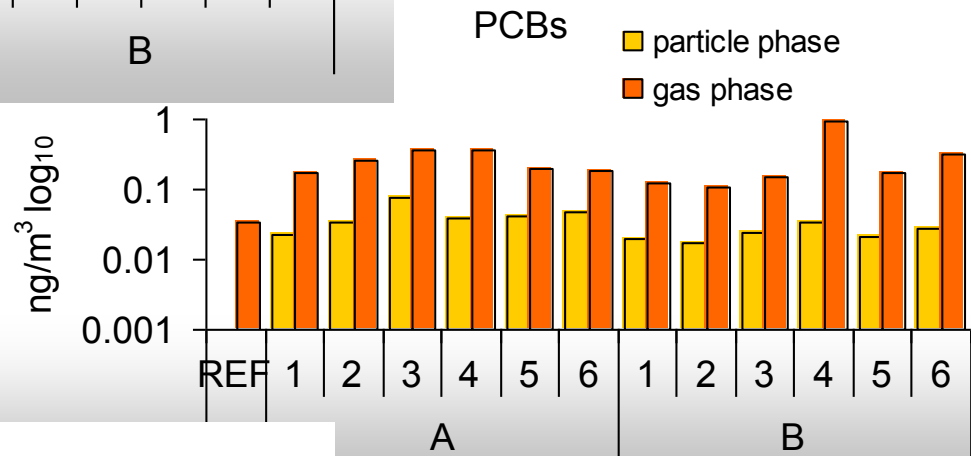
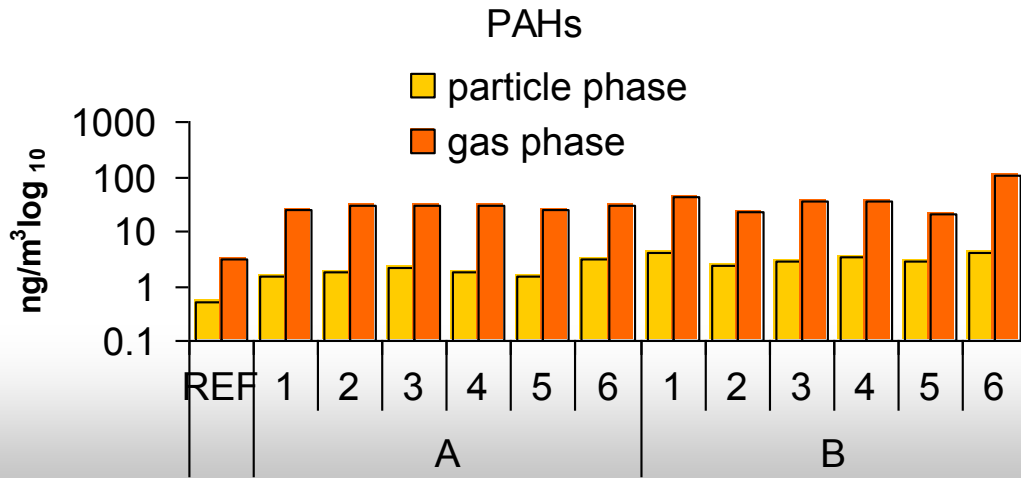
Active sampling particles *vs* gaseous phase

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

Novák et al. (2009) Environment International

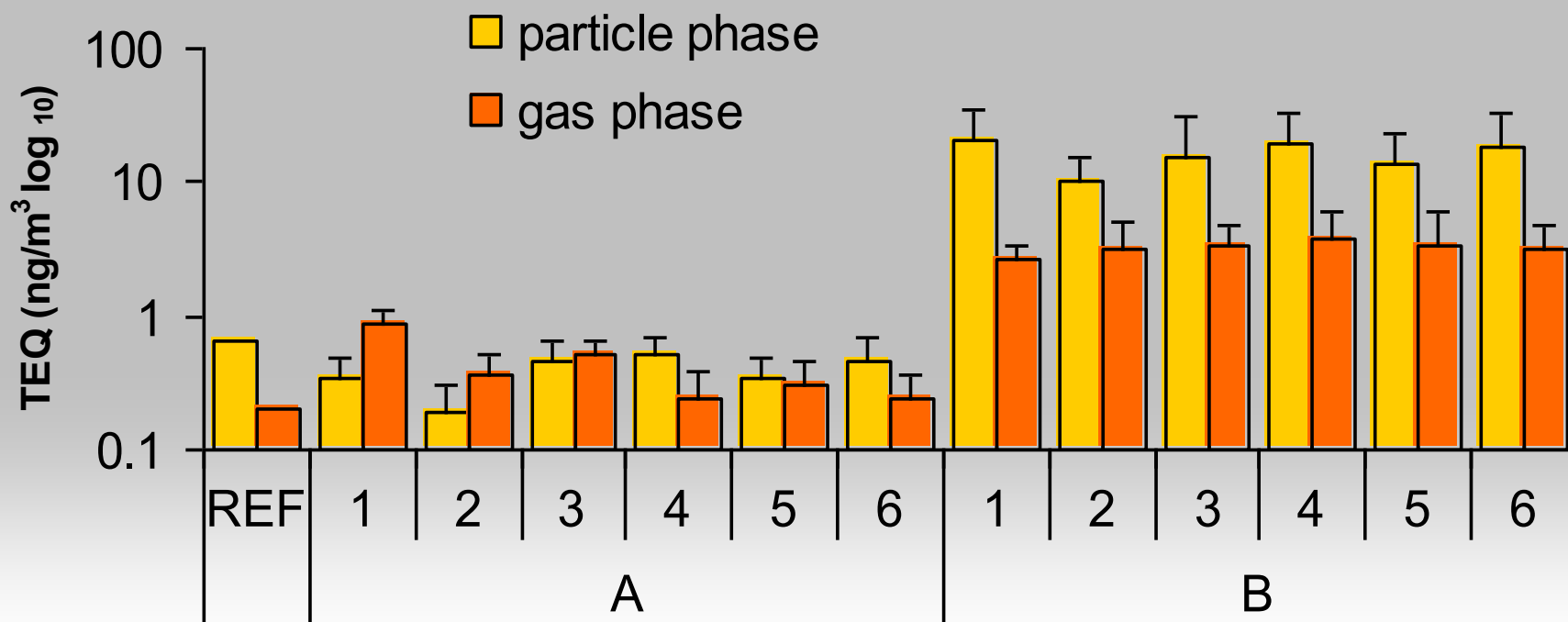


Chemical analyses



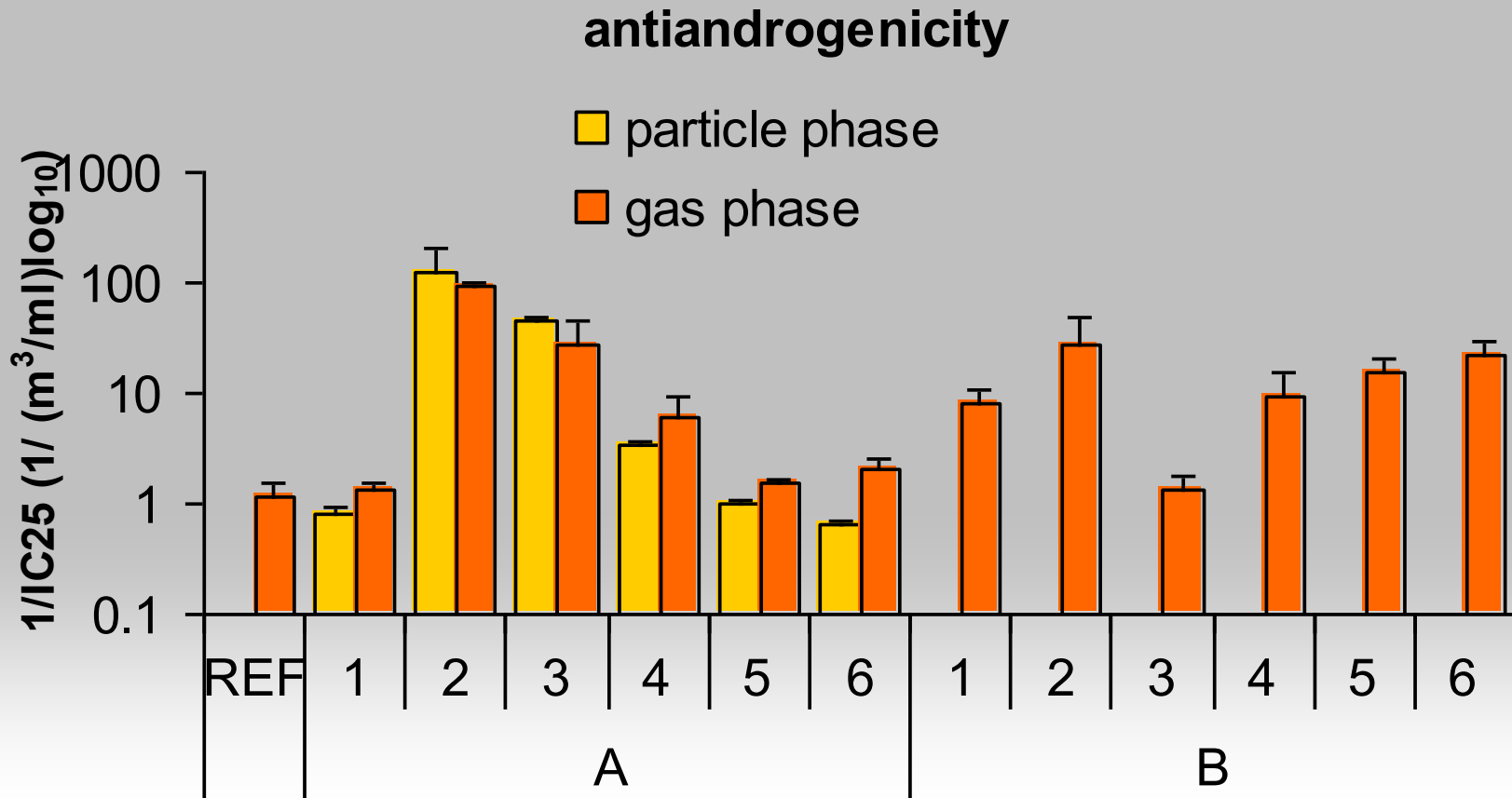
Dioxin-like effects

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“?**

- **Research issues and questions**

- Nanomaterials, Pharmaceuticals, EDCs
- Mixtures!
- Exposome



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Environment International

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

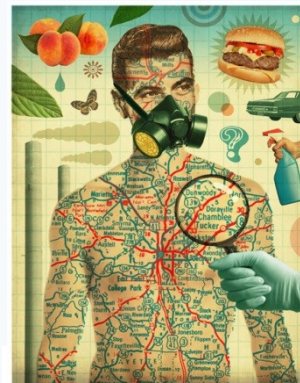
Review

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

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