



Research centre
for toxic compounds
in the environment

Ecotoxicology

Current issues in Research vs Regulation

Ludek Blaha + ecotox colleagues

ceToCoEn



EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE



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!

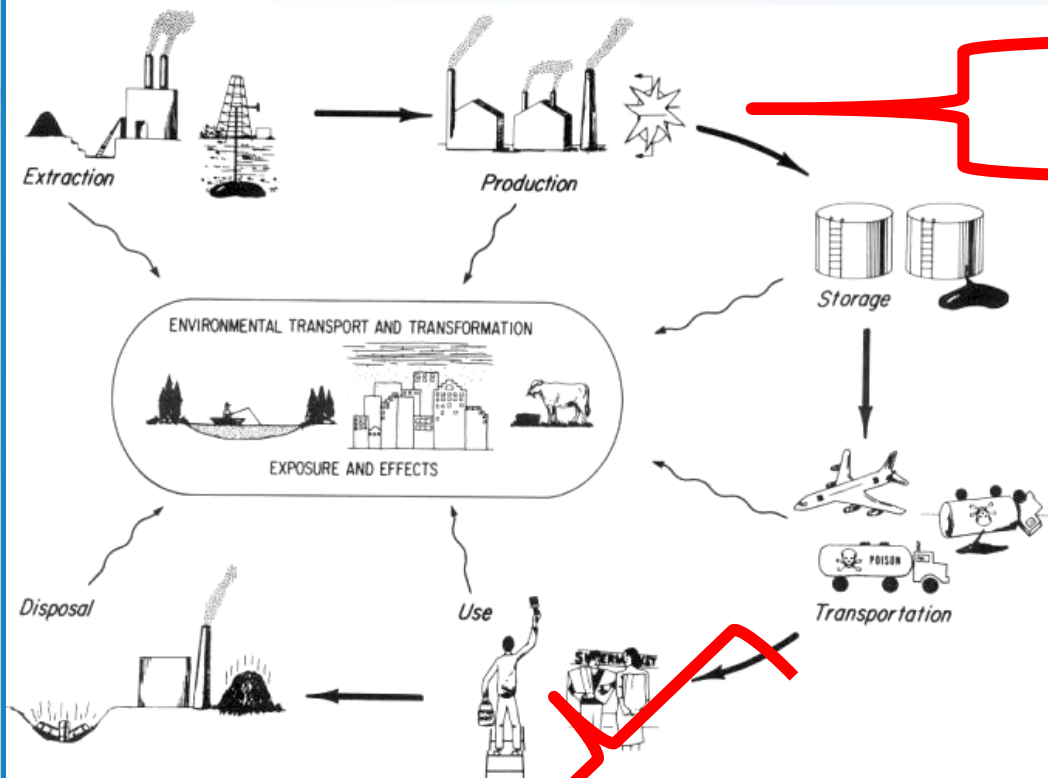
... depending on
researcher's
budget

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)

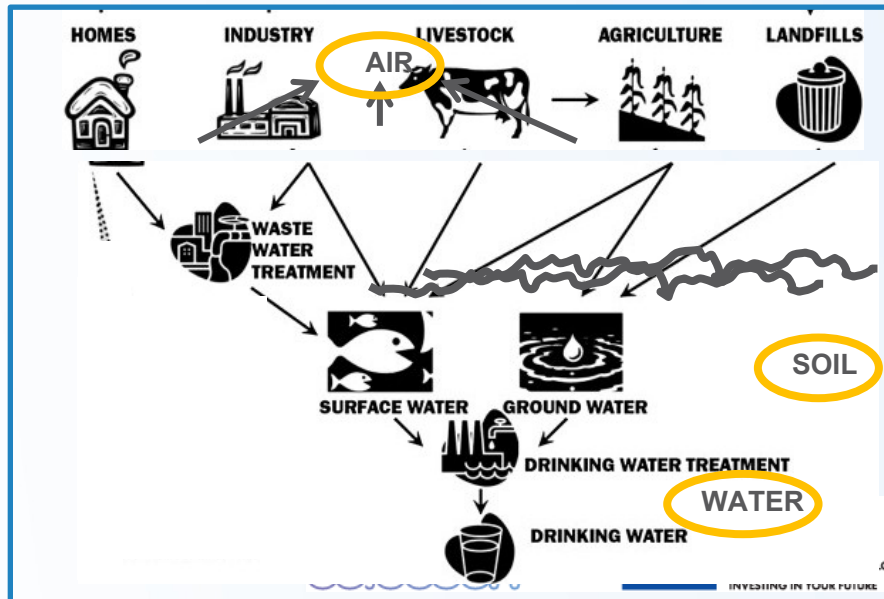
PPP
(EFSA)

MPs
(EMA)

MIXTURES!

Two approaches:

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



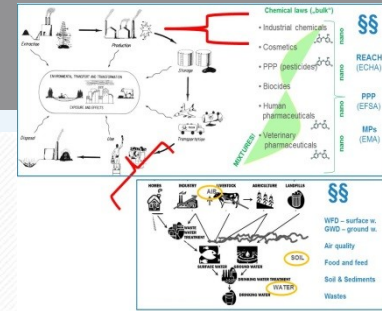
WFD – surface w.
GWD – ground w.

Air quality

Food and feed

Soil & Sediments

What to assess for toxicity?

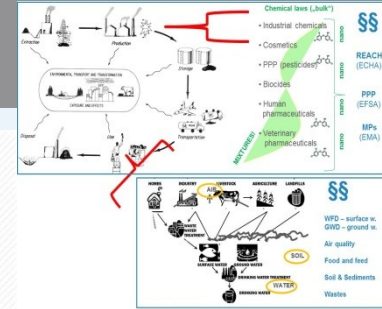


| | Current research topics | As required by law |
|--------------------------------------|-------------------------|--------------------|
| Individual chemicals (prospective) | | |
| Mixtures (prospective) | | |
| Contaminated samples (retrospective) | | |



Research
for toxic
in the er

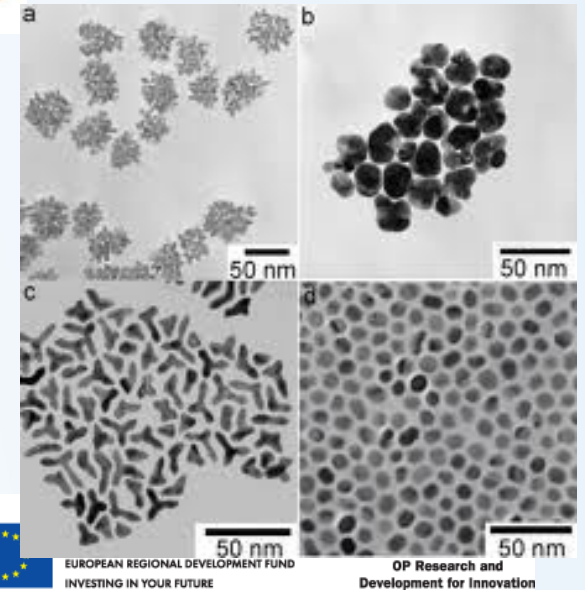
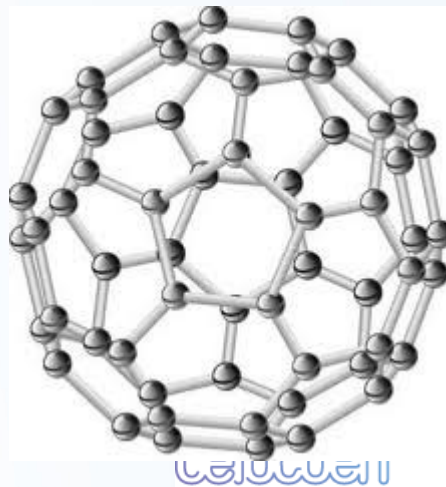
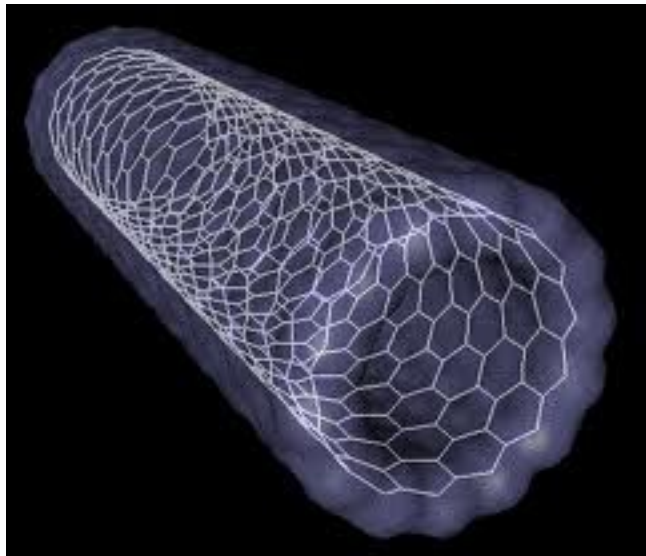
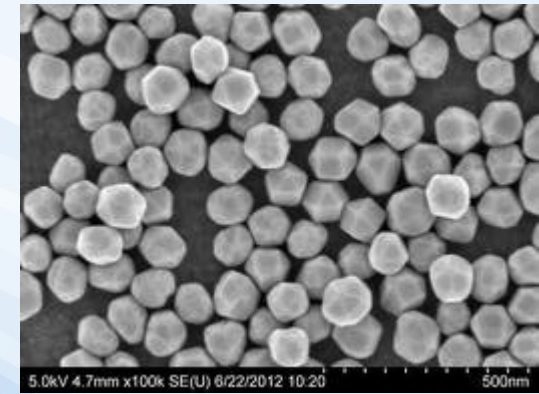
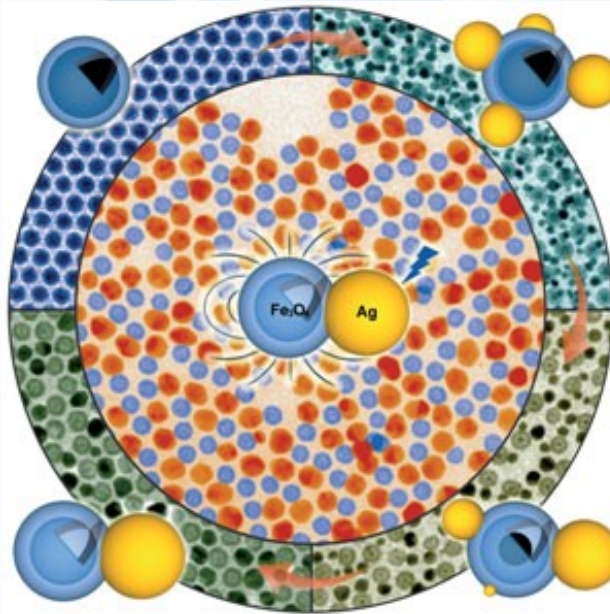
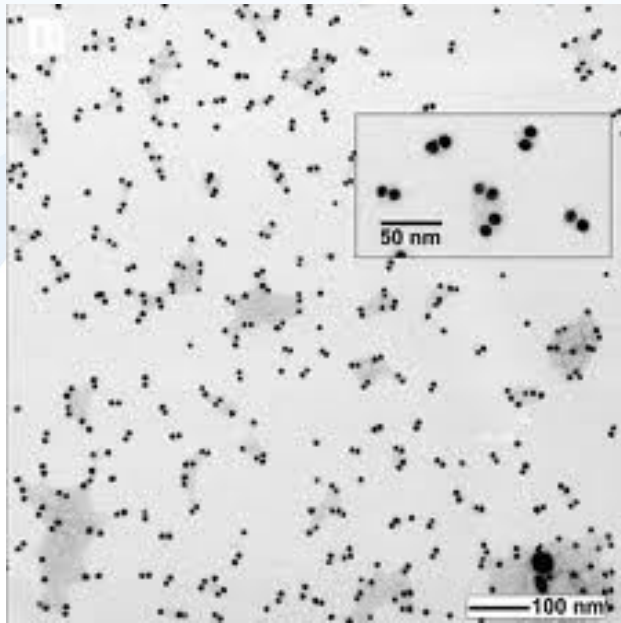
What to assess for toxicity?



| | 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) | | |



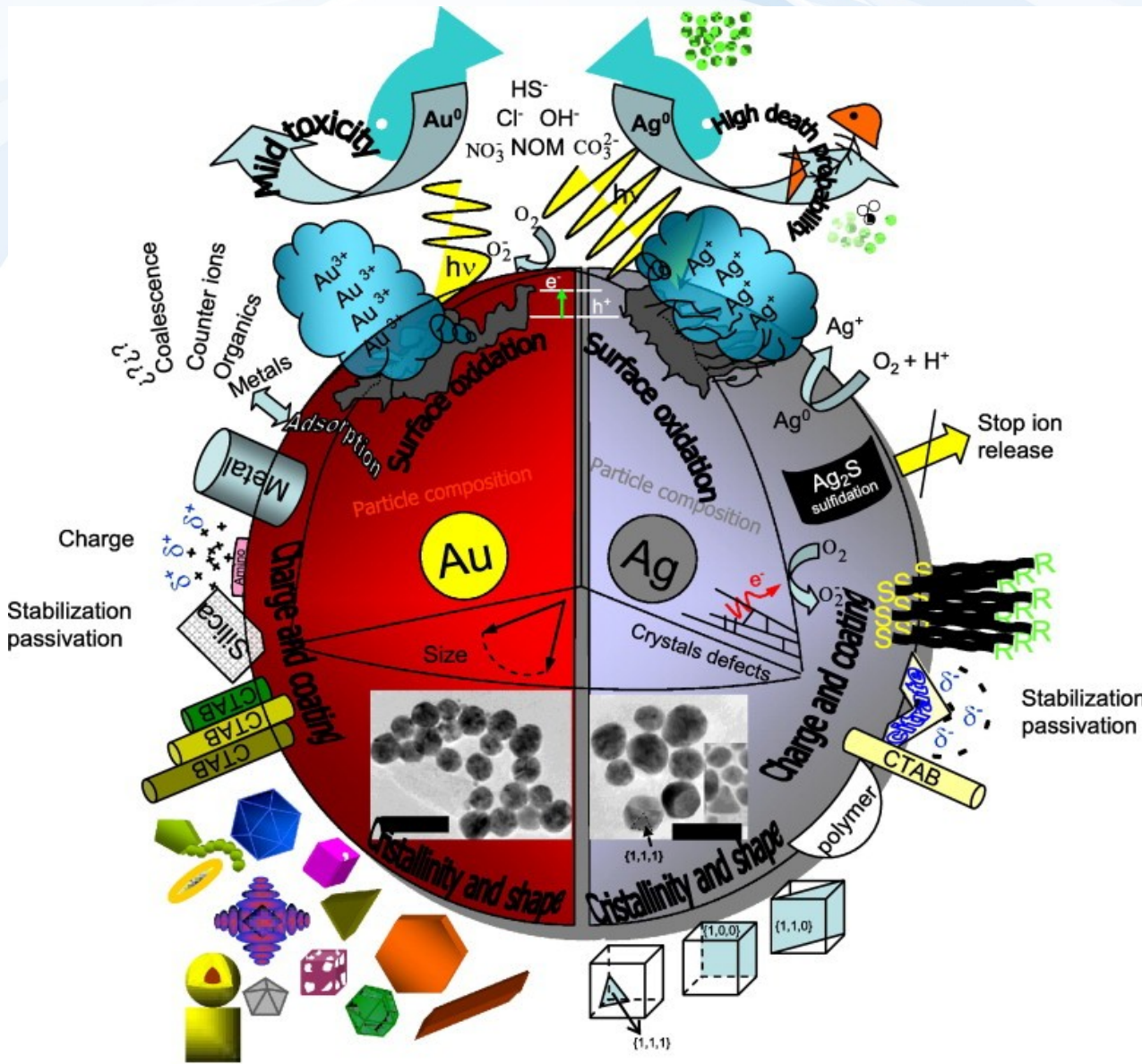
Nanoparticles - examples



EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE

OP Research and
Development for Innovation

Toxicity of nanoparticles ...



(Mostly unknown) Parameters may Affect ecotoxicity

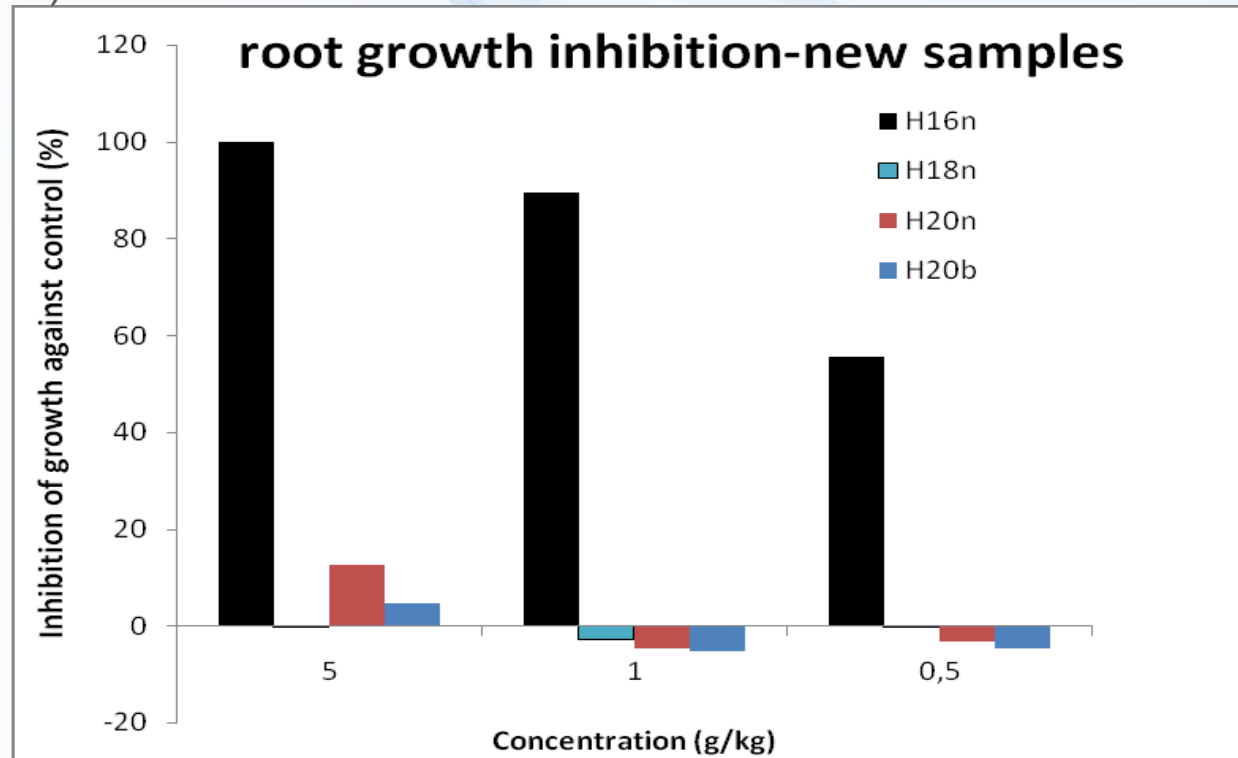
Composition (chemical)
 Surface (size, area)
 Charge
 Reactivity
 Interactions with ions, other chemicals...

→ 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

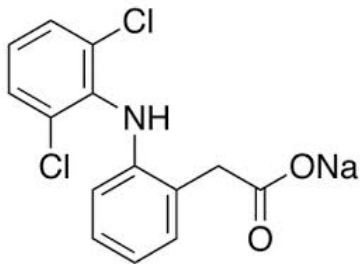
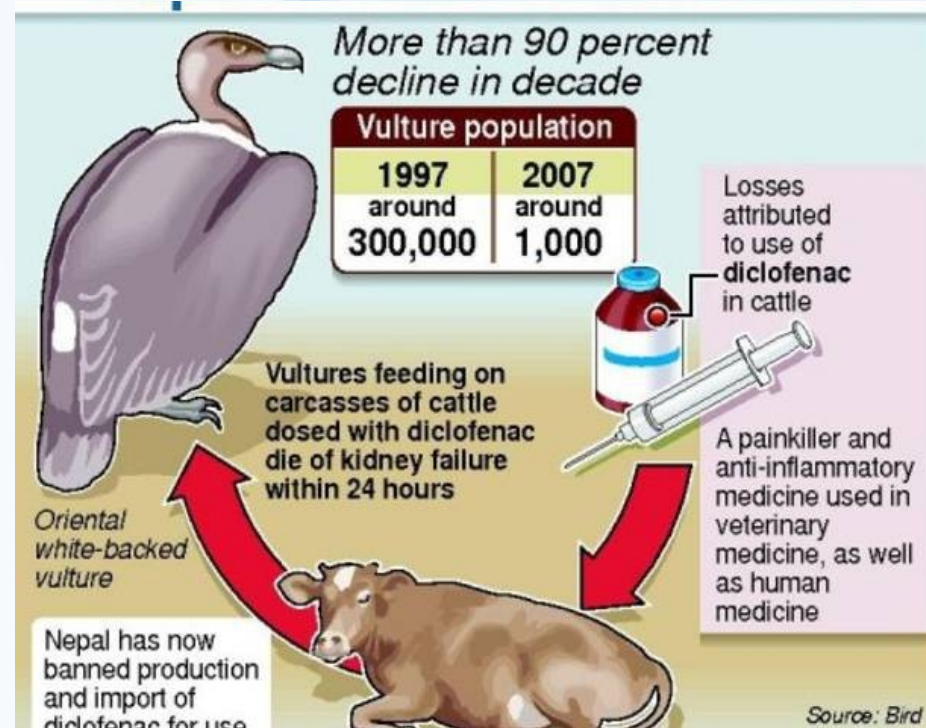
Manufacturing waste

Possible releases to the environment

Example 1 - DICLOFENAC

Unexpected effects at NON-TARGET species

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



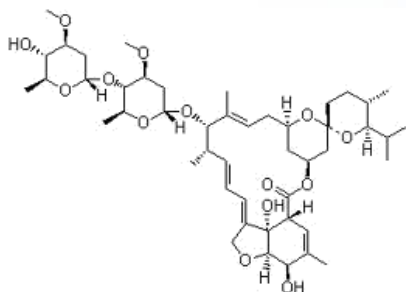
Example 2 – AVERMEKTIN-like antiparasitics

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)



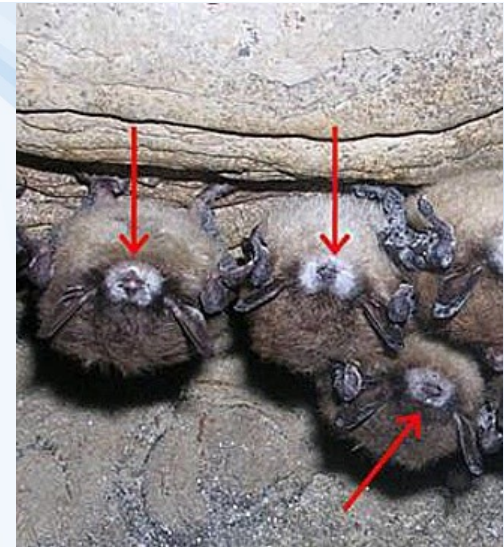
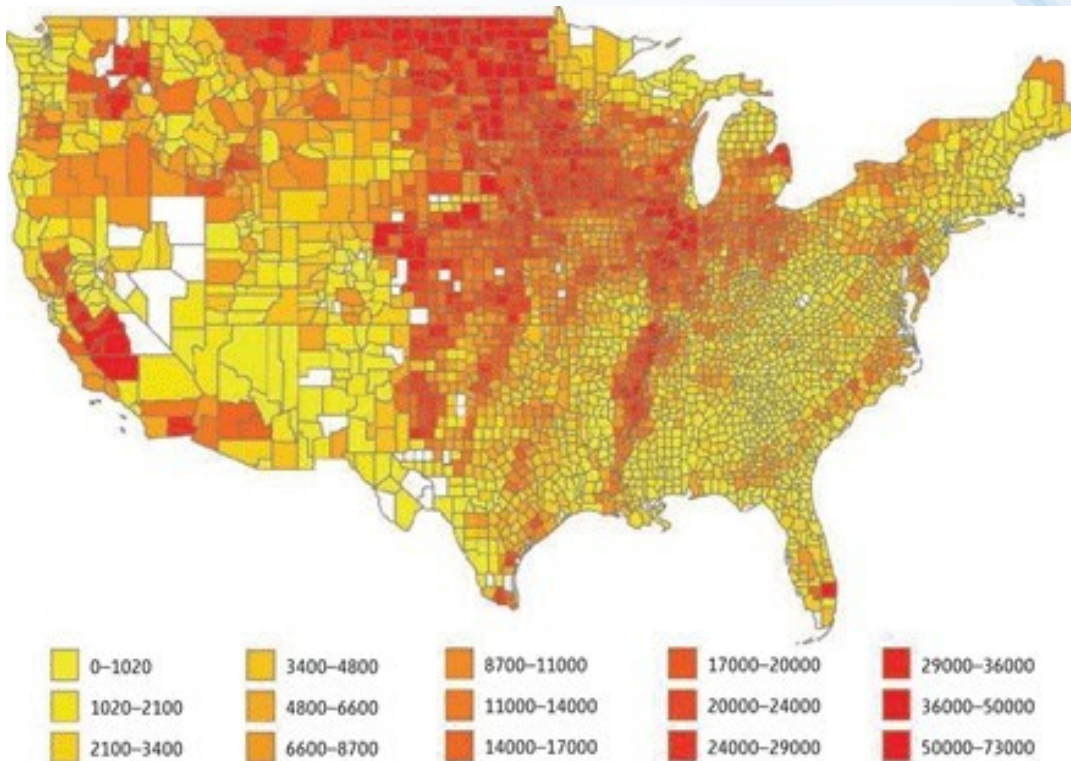


CONSERVATION

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

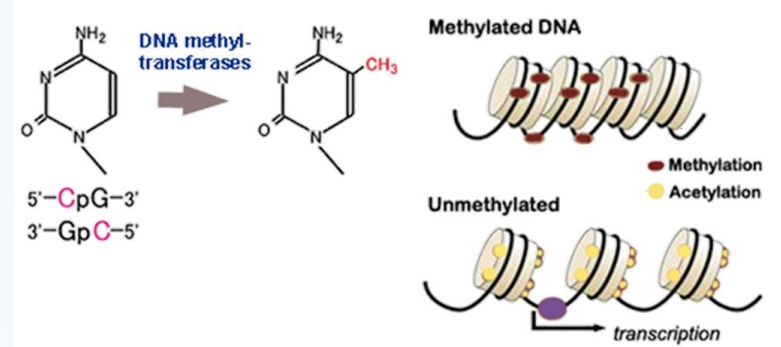


Table 1. Behaviours (mean \pm s.e.) of the offspring from the maternal treatments.

| | 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:

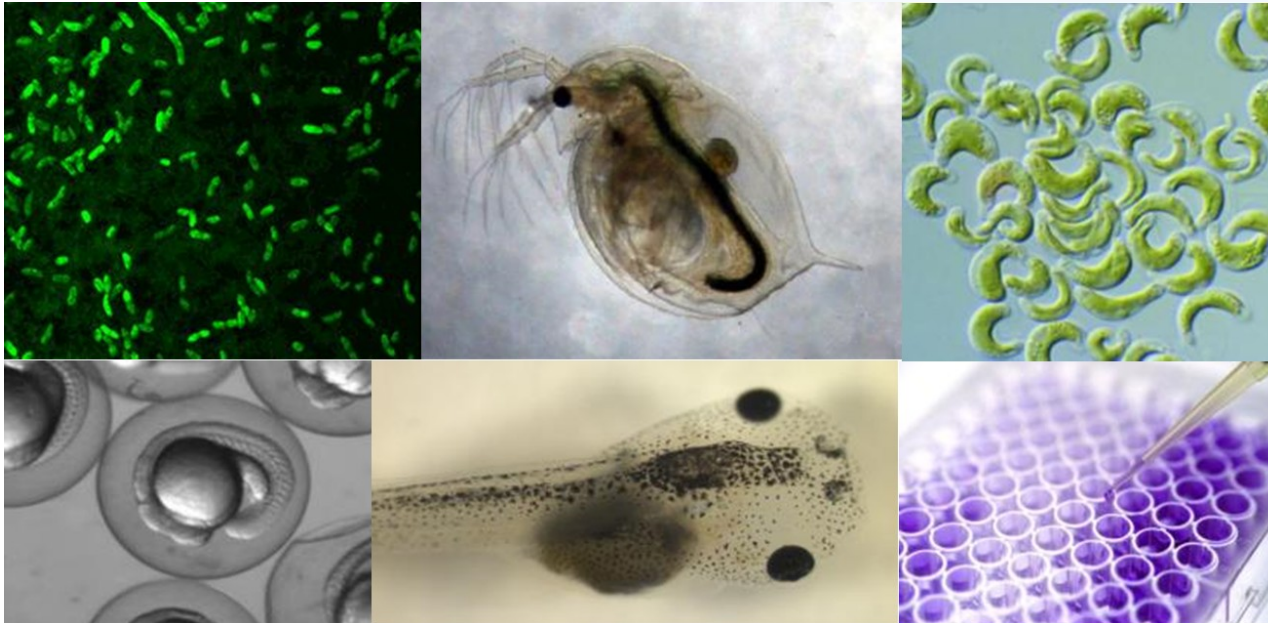
Are current limits (for individual compounds) safe?

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



EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE



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 |

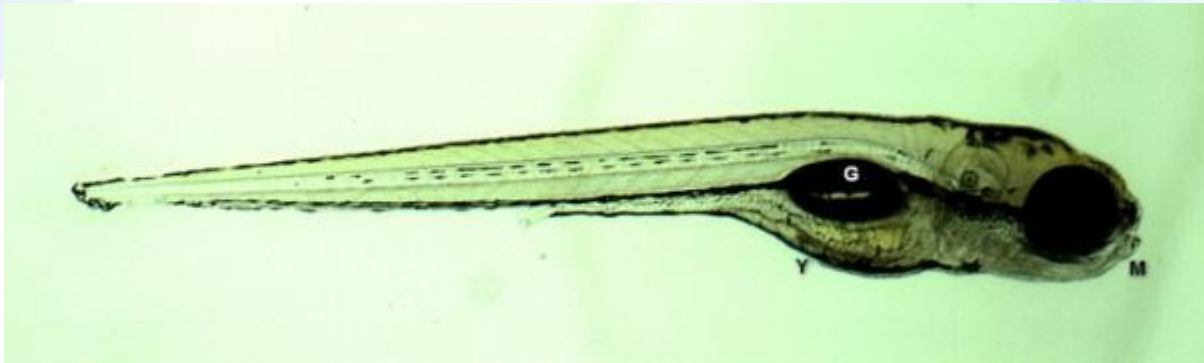


Research
for the
Environment

MIXTURE TOXICITY EU interlaboratory test

Testing comparability of existing and innovative bioassays for water quality assessment

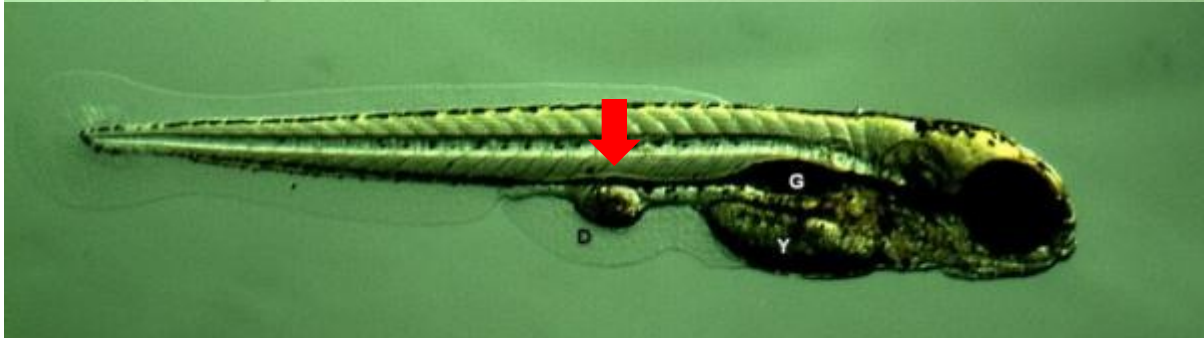
Example: Effects of mixtures on *D. rerio* fish embryos



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



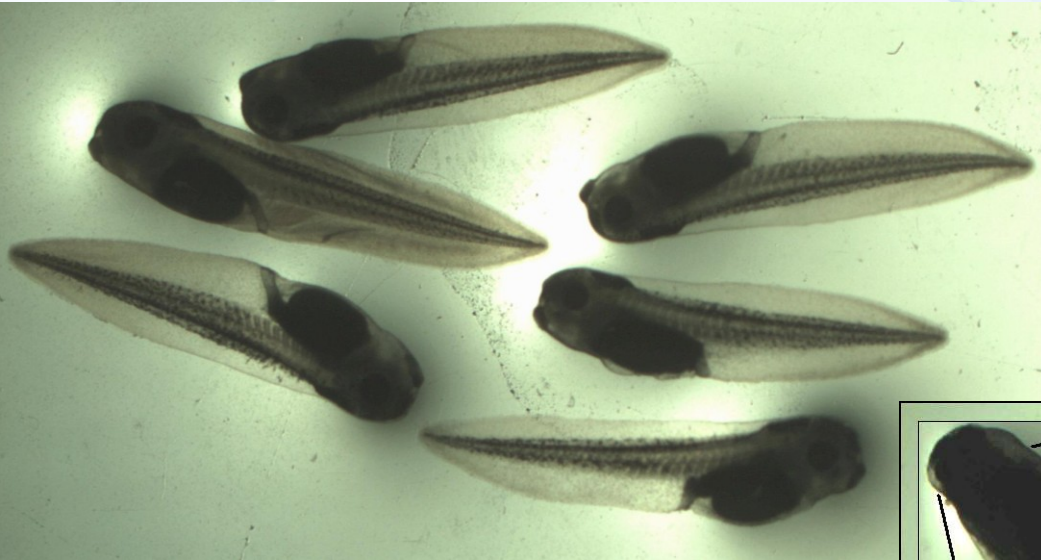
EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE

OP Research and
Development for Innovation

MIXTURE TOXICITY EU interlaboratory test

Testing comparability of existing and innovative bioassays for water quality assessment

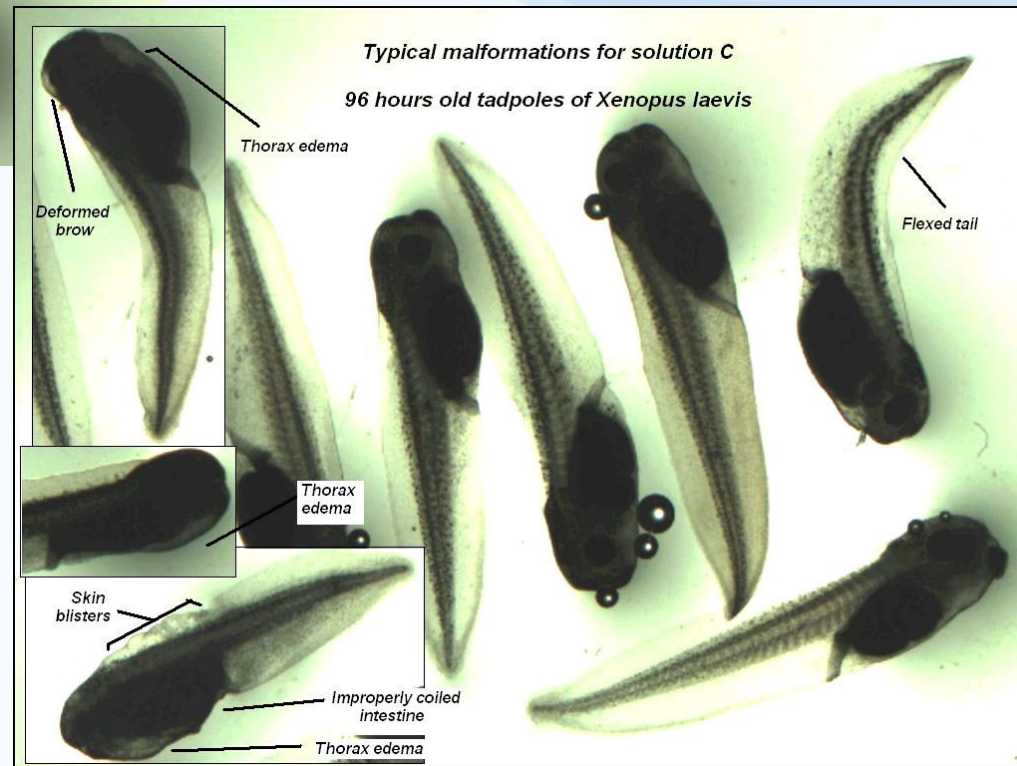
Example: Effects of mixtures on *X. laevis* frog embryos







Controls

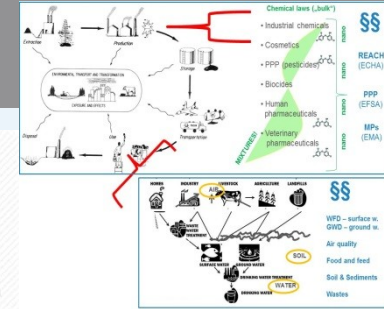
Carvalho, R. et al. (2014) Mixtures of chemical pollutants at European legislation safety concentrations: how safe are they?
Toxicol Sci 141(1): 218-233


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



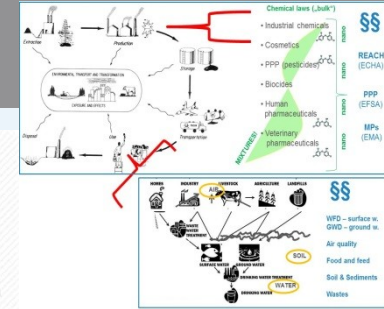
| Biotest | A | B | C |
|---|--|--|---|
| Microtox | 26 and 36% stimulation of luminescence in 15 and 30 mins of exposure, respectively | 18 and 35% stimulation of luminescence in 15 and 30 mins of exposure, respectively | 22 and 39% stimulation of luminescence in 15 and 30 mins of exposure, respectively |
| 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?



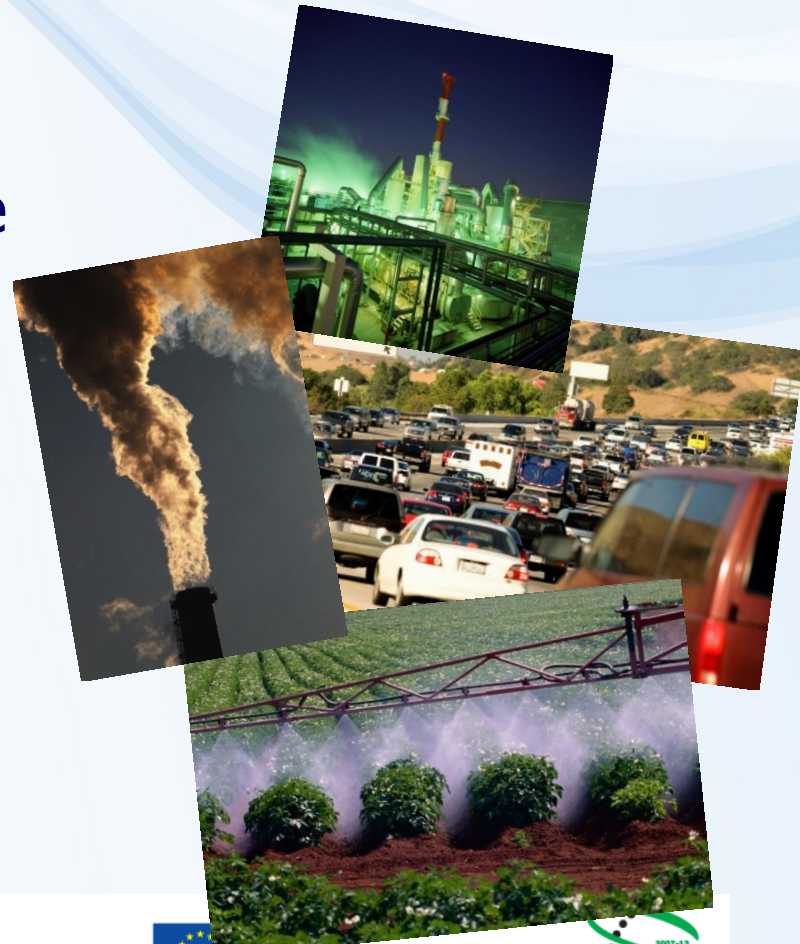
| | Current research topics | As required by law |
|--------------------------------------|--|---|
| Individual chemicals (prospective) | Engineered nanomaterials /particles Ecological effects (e.g. of pharmaceuticals) Endocrine disruption & chronic diseases | Industry & biocides (REACH) PPPs = pesticides 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”

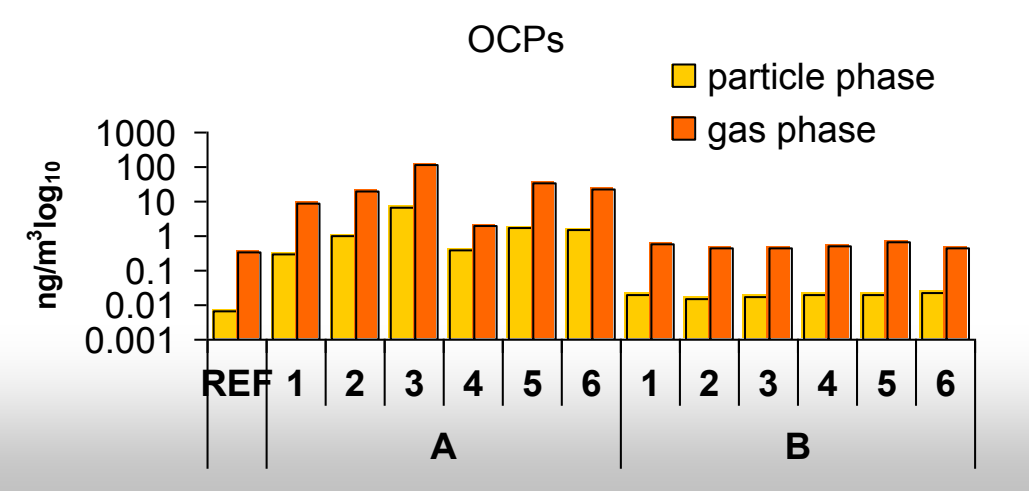
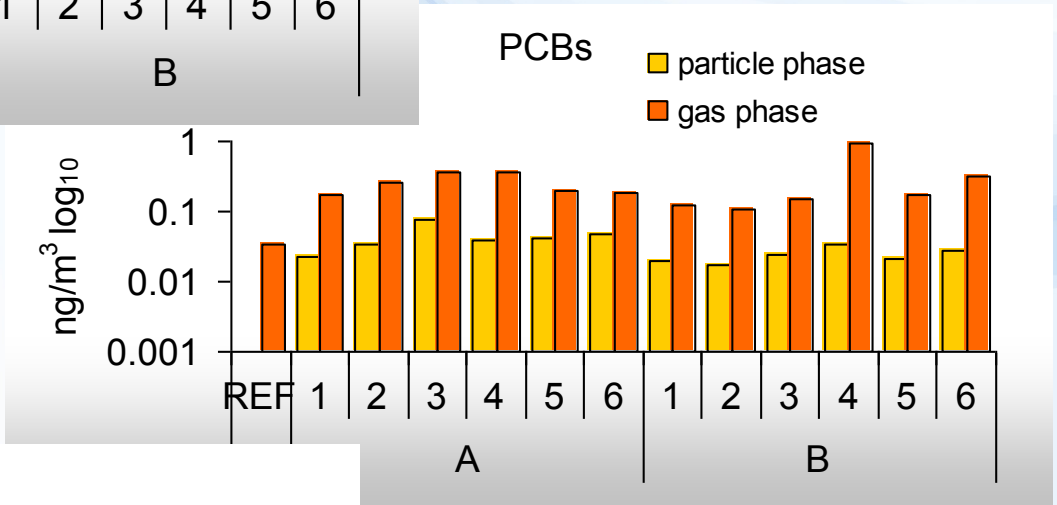
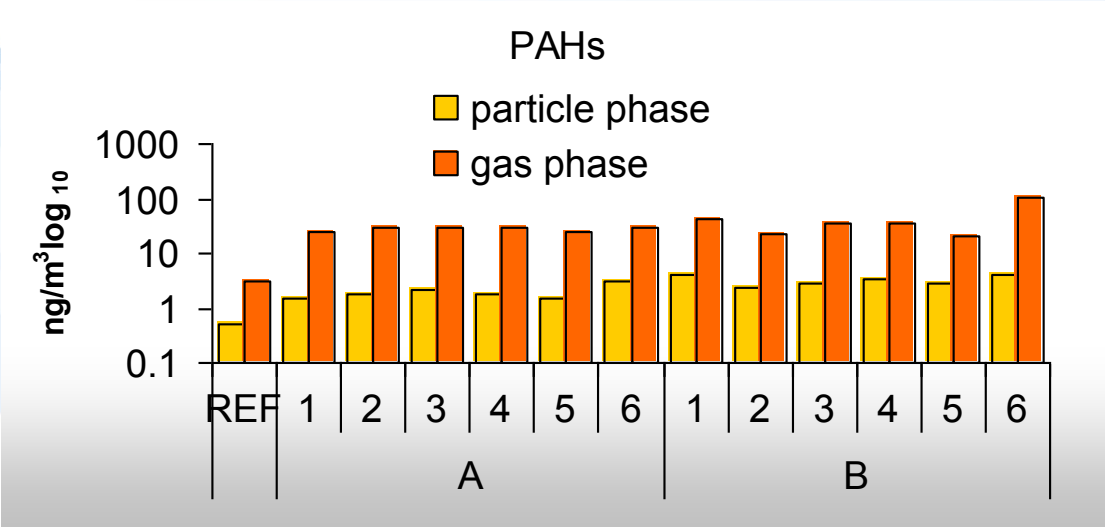
Active sampling particles *vs* gaseous phase

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

Novák et al. (2009) Environment International

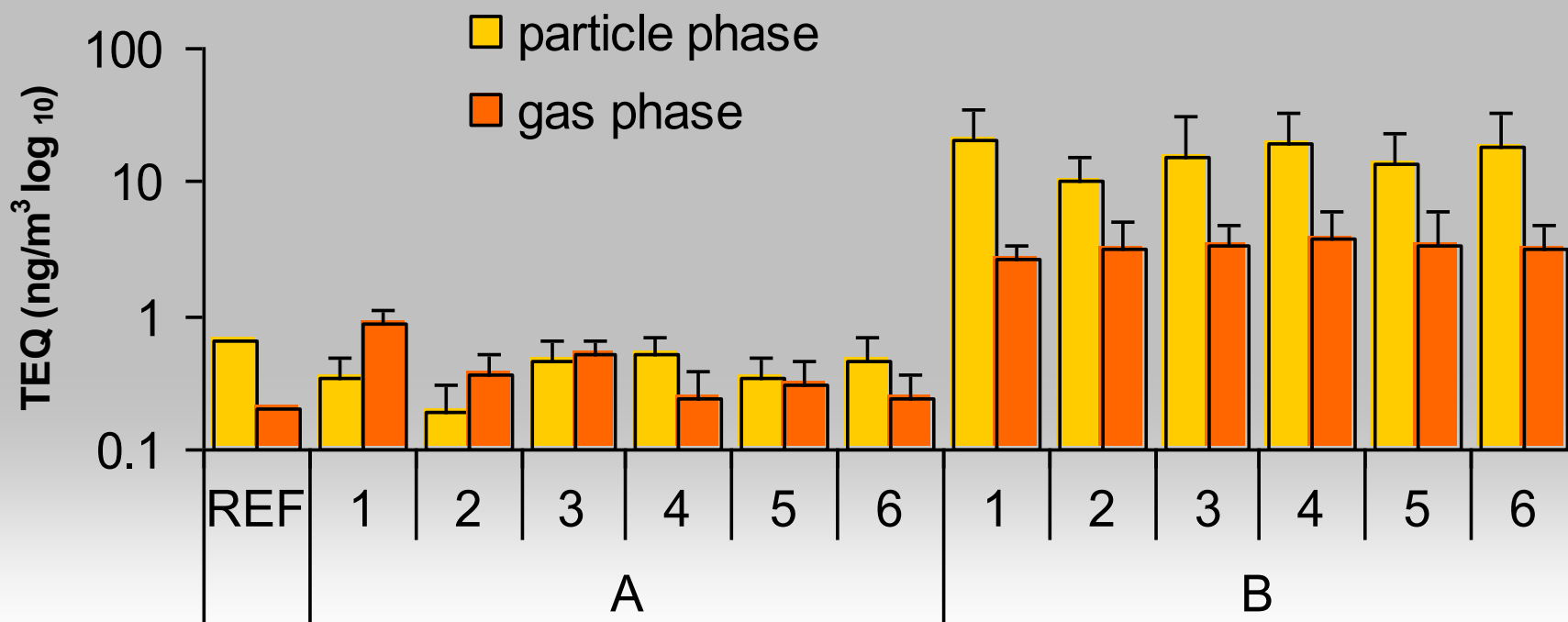


Chemical analyses



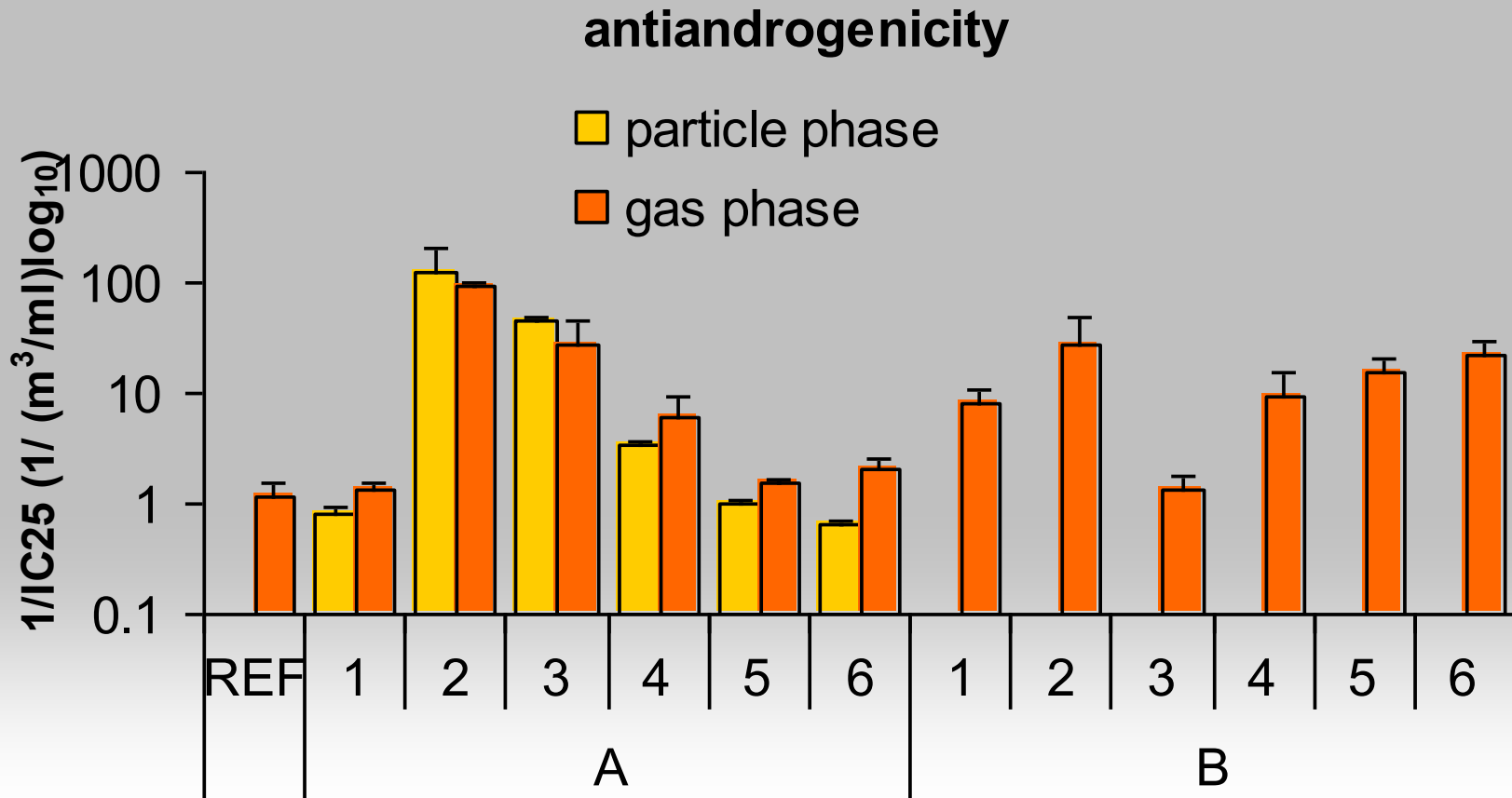
Dioxin-like effects

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“?**



Contents lists available at [ScienceDirect](#)

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?

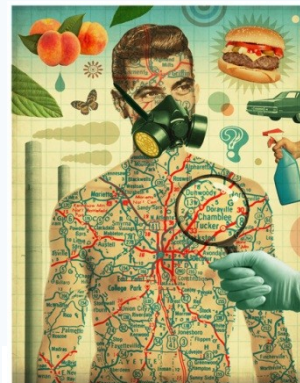
Barbora Jarošová ^a, Luděk Bláha ^a, John P. Giesy ^b, Klára Hilscherová ^{a,*}

^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

- **Research issues and questions**

- Nanomaterials, Pharmaceuticals, EDCs
- Mixtures!
- Exposome



cecoen



EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE

