Sources, fates and toxicological effects of endocrine disruptors and Pharmaceuticals in the aquatic environment

Claude Casellas,

Aurélie Escande, Hélène Fenet, Elena Gomez

UMR 5569, « Hydrosciences Montpellier »

Endocrine disruptors & pharmaceutical compounds: challenges and solutions for the water sector

Symposium Berlin 10 February

ERA Framework

Contaminants in the environment

In 2009, $50*10^6$ organic and inorganic substances possess CAS number (20 % increase in less then one year)

Long term actions, since 2004 ,

⇨ Adoption of the regulation concerning the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), formally adopted in December 2006,

⇨ European directive on environmental quality standards for priority substances under the water framework directive (2006)

Research needs:

⇨ Hazard assessment tools are in progress

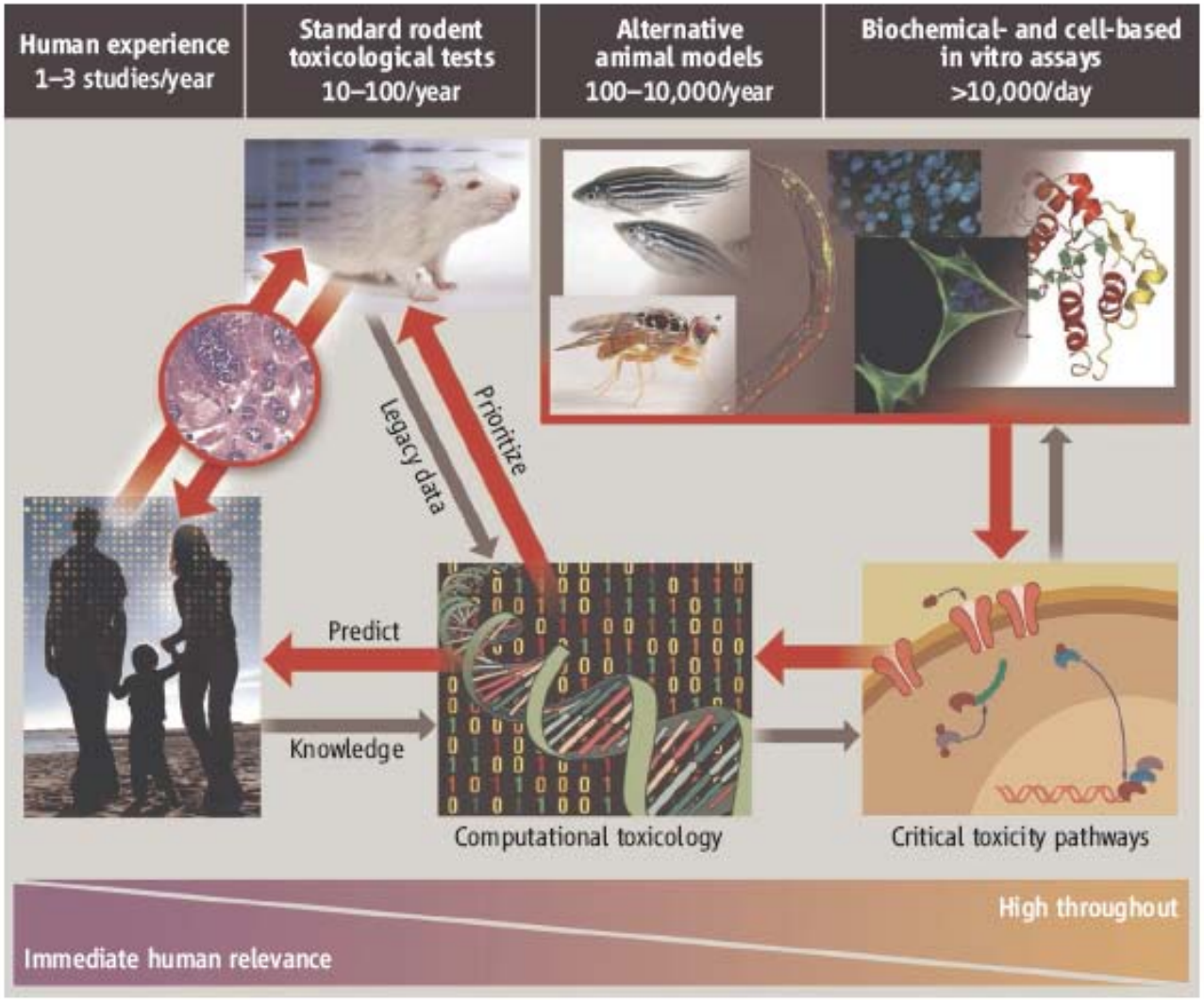
⇨ Complex mixture and regulation

Hazard Assessment

REACH: around 30000 substances are in a first priority list

US-EPA: Considering almost 10000 substances

Objectives: High throughput screening for the identification of toxicity pathways and prioritization



CREDIT: NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES, NATIONAL INSTITUTES OF HEALTH

Transforming toxicology. The studies we propose will test whether high-throughput and computational toxicology approaches can yield data predictive of results from animal toxicity studies, will allow prioritization of chemicals for further testing, and can assist in prediction of risk to humans.

Priority lists

•**Persistence**

stricto sensu : Stable chemical structure

Continuously released in the environment: pseudo-persistent (some pharmaceuticals)

•**Bioaccumulation**

Passive transport : lipophilicity

Active transport : « drug delivery », nanoparticules

•**Toxicity**

Direct

Indirect: nanoparticules (ratio surf/size, catalysis),

Delayed effects: ex: carcinogenicity

Hazard Assessment

Difficulties

- Vulnerability (developmental stage, genetic variability)
- Multi-exposure to contaminants with different modes of action
- Environmental complex matrix
- Emergence

What is emergent?

- Emergent contaminants ?

New molecules

Newly detectable molecules

Transformed molecules (pharmaceuticals, metabolites)

What is emergent?

•Emergent concern?
Known substances but recent knowledge on their fate, behaviour or effects (ex. acrylamide, environmental nitration)

•Emergent effects?
A chemical family that disrupt a function and leads to unpredictible effects (endocrine disruptors)

•Emergent risk?
Lack of knowledge on the occurrence and effects in the environment (nano-particules)

Exposure assessment

Chemical analysis in complex matrix – Transformation processes

- Analytical challenge
- Transformation products
- Distribution in environmental compartments (water, sediments..)
- Importance of interfaces (biofilms)
- High number of contaminants at low doses

Transformation products, metabolites

Pharmaceuticals are excreted at various levels of metabolization

Some pharmaceuticals have bioactive metabolites

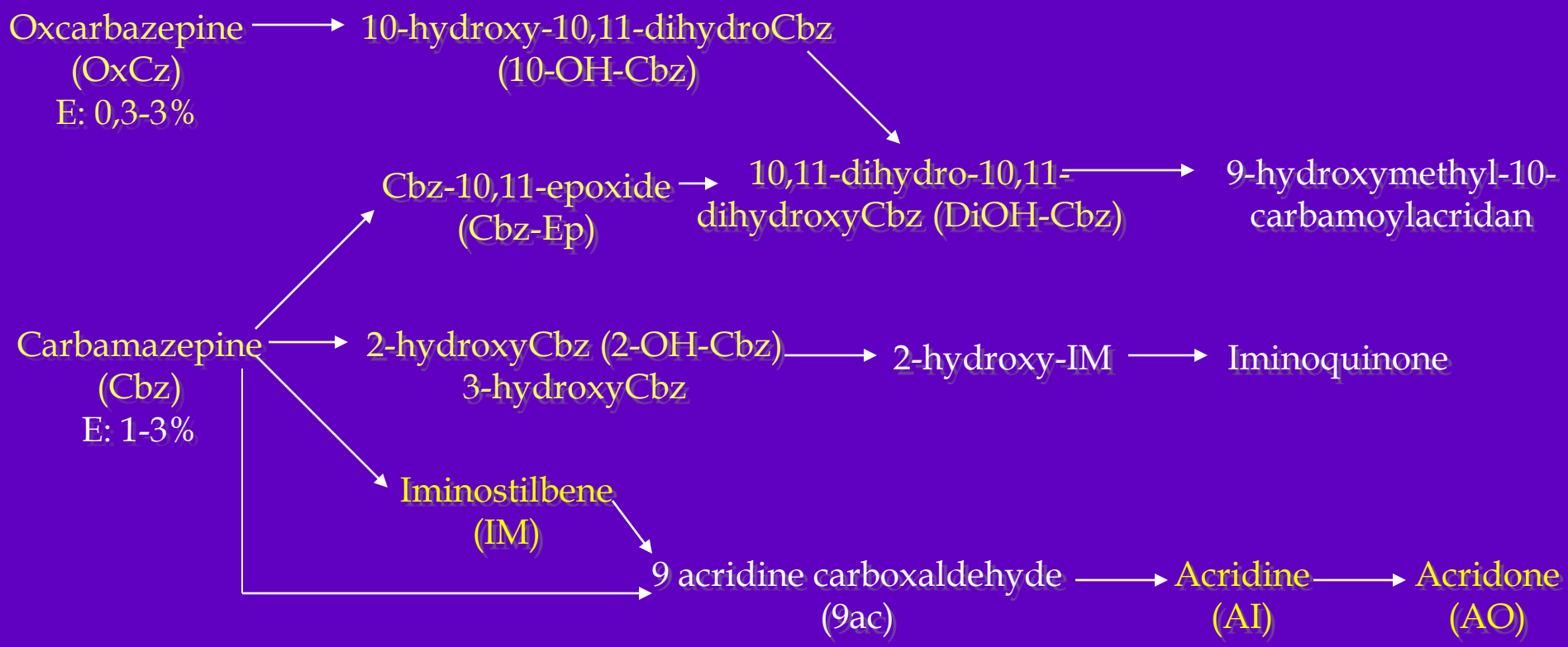
Pharmaceuticals are partly biodegraded in wastewater treatment plants

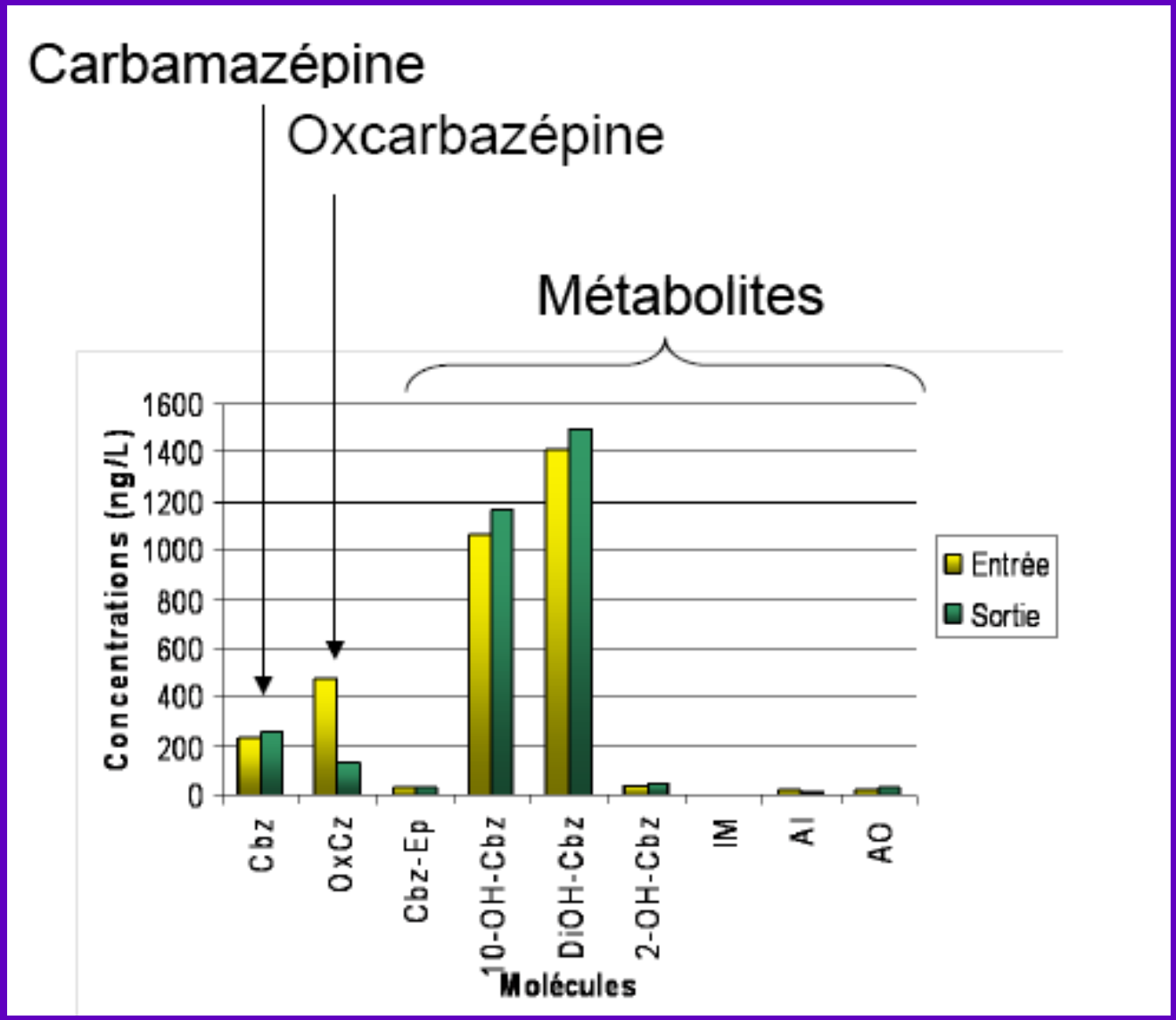
Some pharmaceuticals are deconjugated in wastewater treatment plants

Example of carbamazepine

Metabolization pathways of Carbamazepine - Oxcarbazepine

in vivo metabolization





Leclerc *et al.* 2009

Transformation products with UV radiation are acridine and acridone (Kosjek et al. *Environ. Sci. Technol.* ***2009, 43, 6256–6261)***

Occurrence in aquatic environment

- Concentrations in surface water:
  - Cbz: ~ hundred of ng/L
  - Metabolite DiOH-Cbz: ~ ten ng/L
- Photo-degradation of Cbz:
  - Half-life>50 h
  - Photolysis byproducts: AI et AO
- Adsorption of Cbz on suspended solids is limited
  - Occurrence in groundwater (~ 1 µg/l)

- Cbz was detected in drinking water at a maximum value of 30ng/L

Example of Endocrine disruptors

What is the endocrine system?

The endocrine system is a complex network of glands, hormones and receptors.

Endocrine disruption is not a toxicological endpoint *per se*, but it is a class of many mechanisms of action that may lead in different species to various types of effects which may result in adverse consequences on humans and ecosystems

What is the endocrine disruption?

EDCs interfere with the functioning of the endocrine system in at least 3 possible ways :

- By mimicking the action of a naturally-produced hormone,
- By blocking the receptors in cells receiving the hormones (hormone receptors), preventing the action of normal hormones;
- By affecting the synthesis, transport, metabolism and excretion of hormones, altering the concentrations of natural hormones.

Example of Endocrine diruptors

Some target receptors and their ligands

ERs : Estrogen receptors alpha and beta

natural or synthetic hormones, Alkylphenols, Pesticides, Phytoestrogenes, Cosmetics, …

AhR: Receptor Aryl Hydrocarbon

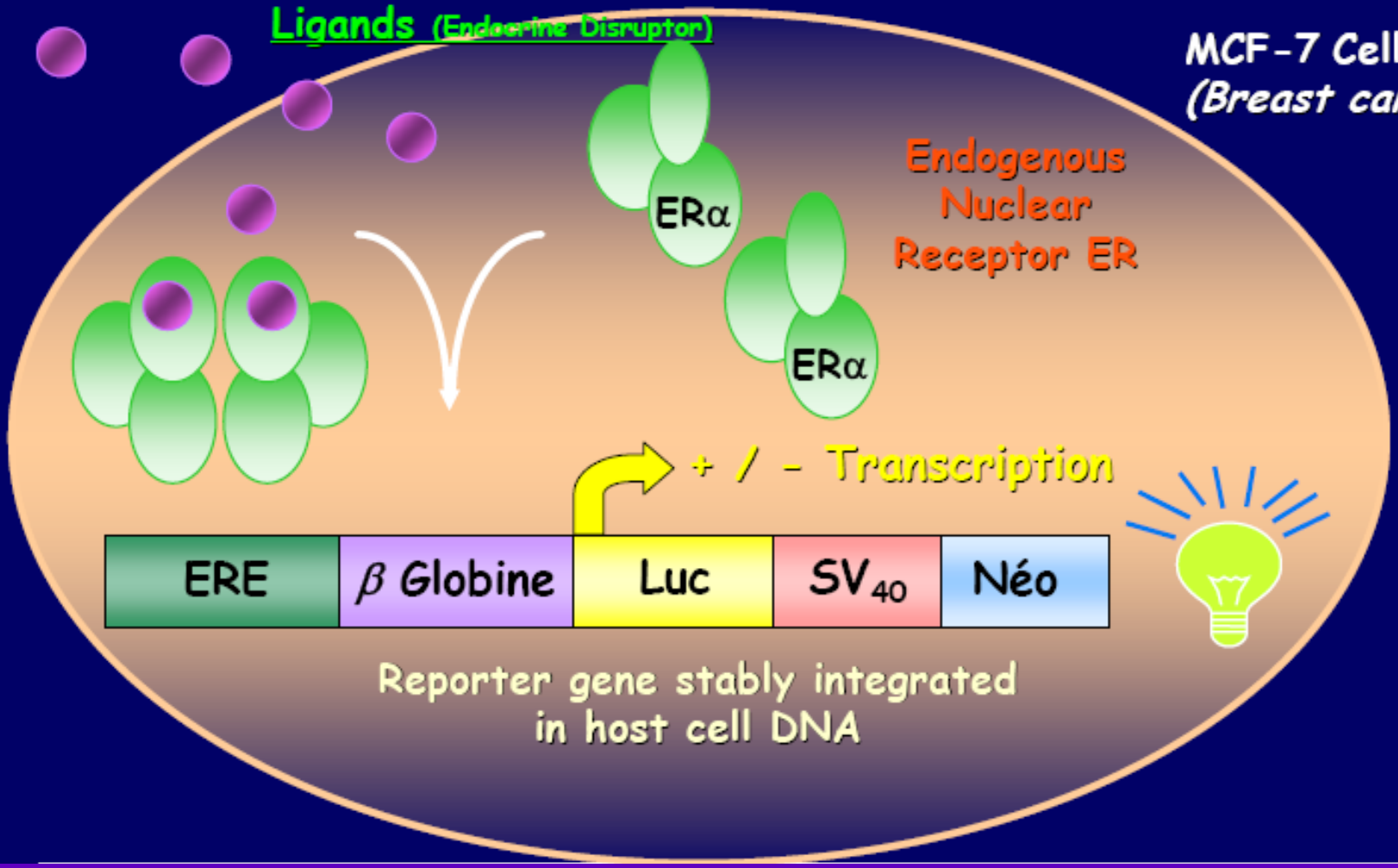
Polycyclic aromatic hydrocarbon (PAH), Poly-Chloro Biphenyl (PCBs)

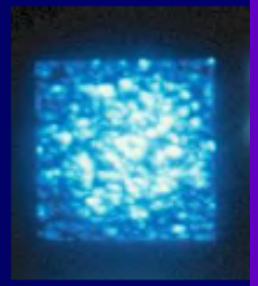
PXR-RXR : Pregnan Receptor X

natural or synthetic hormones, Alkylphénols

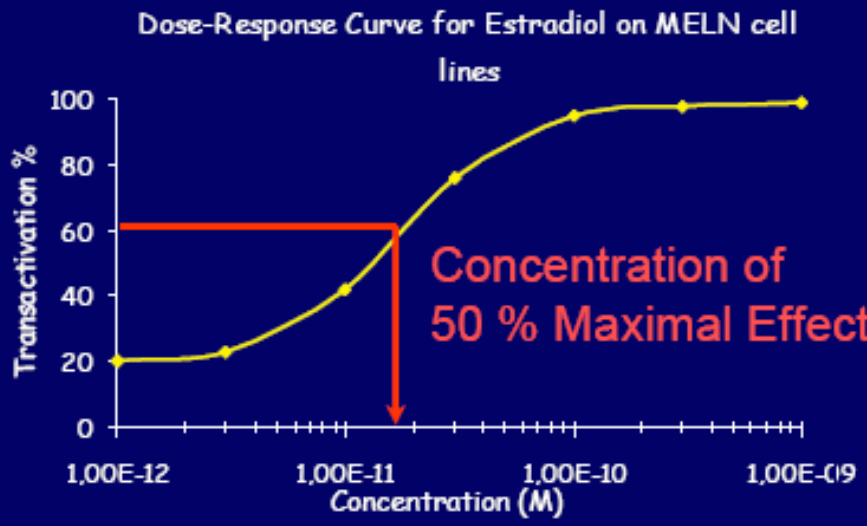
Pesticides, PCBs, brominates flame retardant, Phthalates, Cosmetics

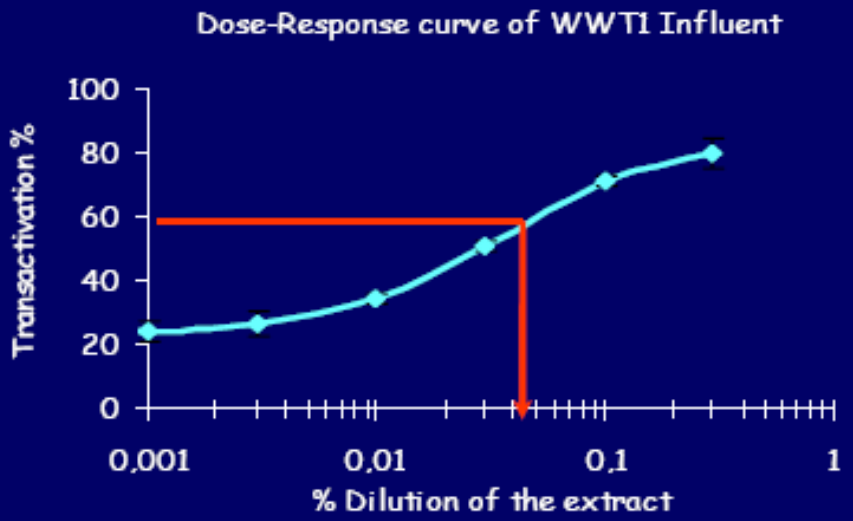
Luciferase ER reporter cell line





Estradiol Equivalent



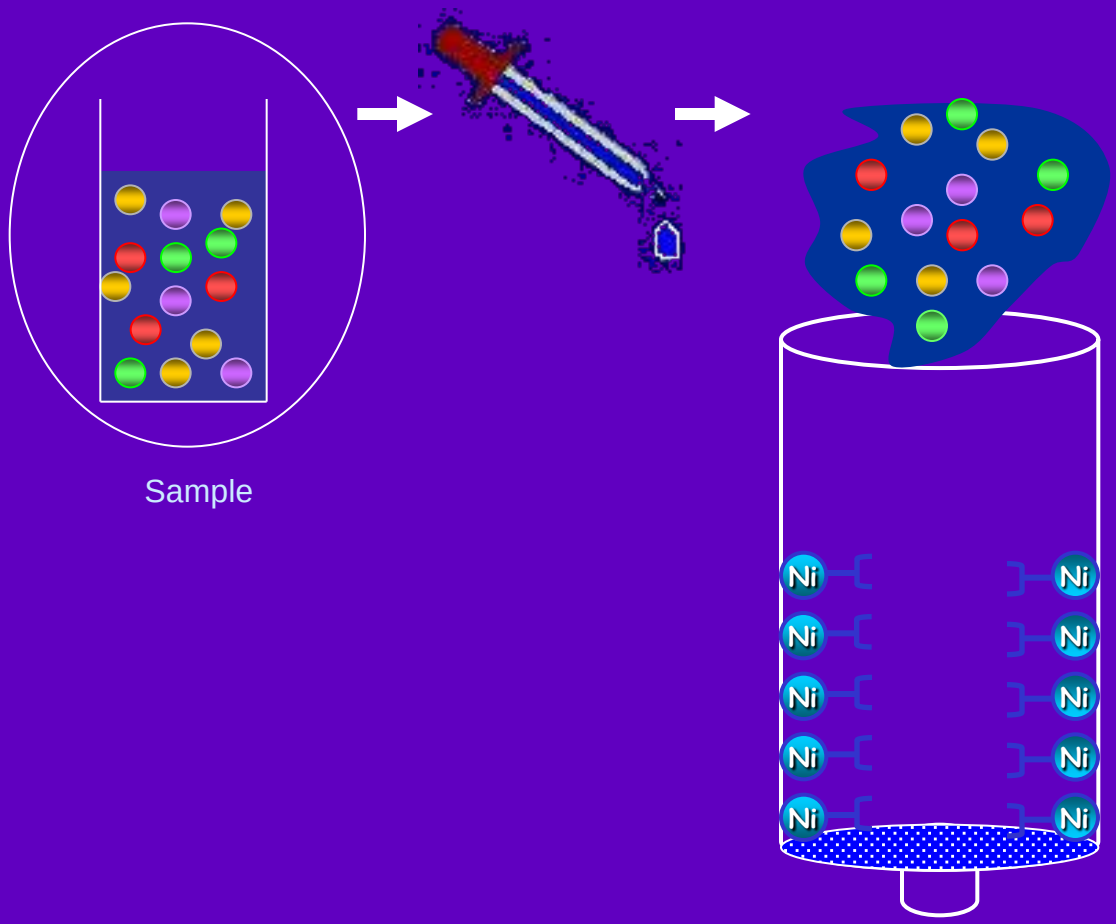


17,6 pM Estradiol ⟷ 0,04 % Water extract

Estradiol Equivalent concentration in influent

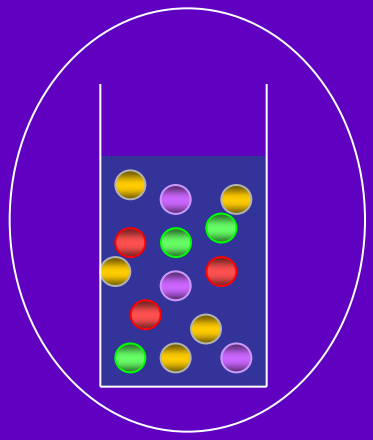
38,8 $ng.L^{-1}$

Purification of environnemental EDs on Sépharose Ni-Receptor

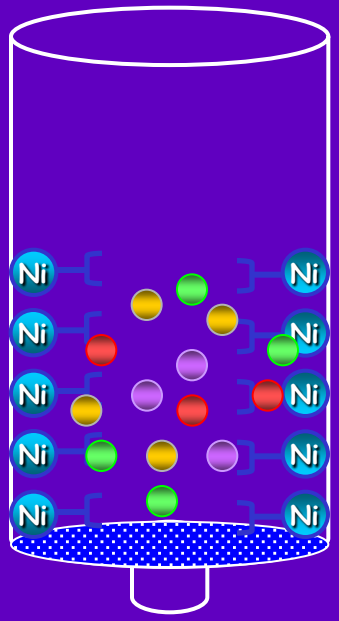


1. Sepharose-Nickel
2. Receptor binding
3. Sample deposition

Purification of environmental EDs on Sépharose Ni-Receptor

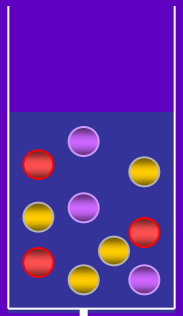


Sample

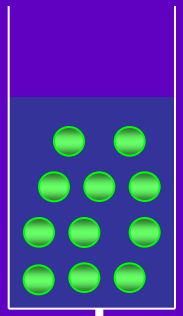


1. Sepharose Nickel
2. Receptor binding
3. Sample deposition
4. Ligands binding to receptors
5. Washing
6. Elution

Fraction released



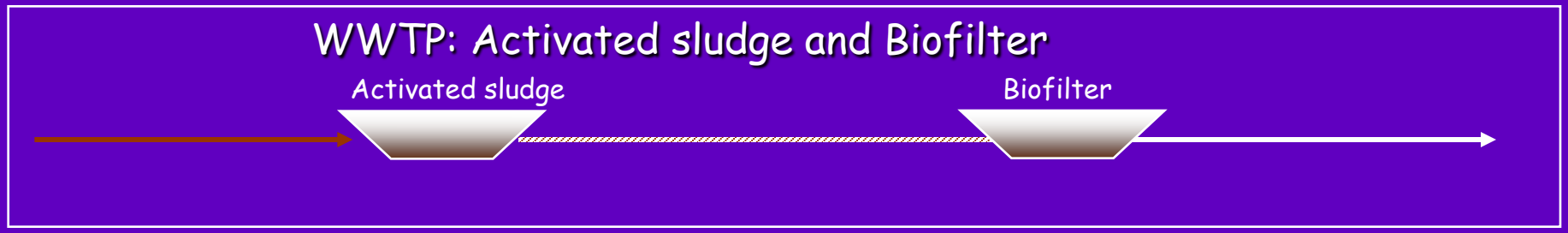
Fixed fraction

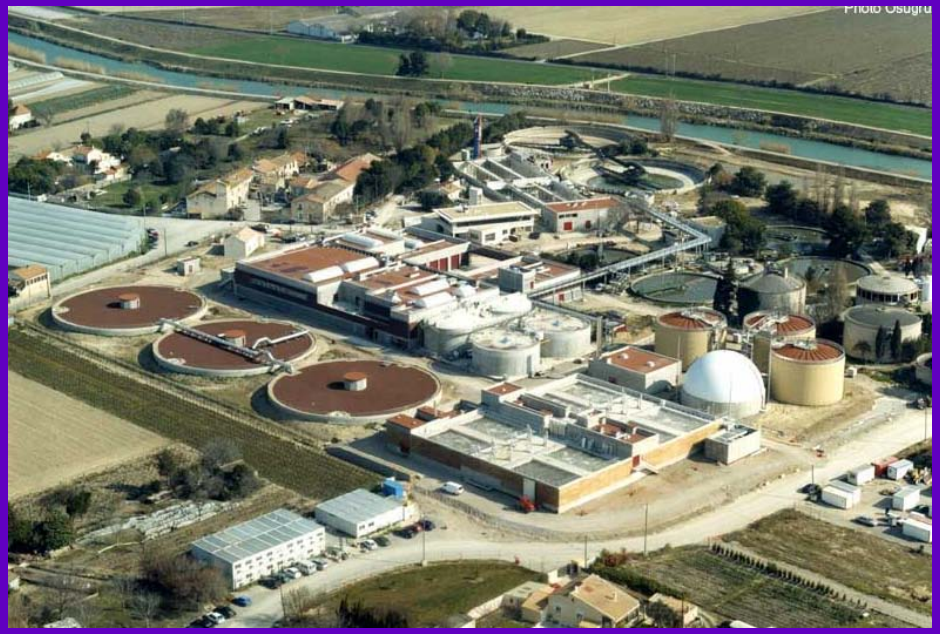


7. Activity measurement

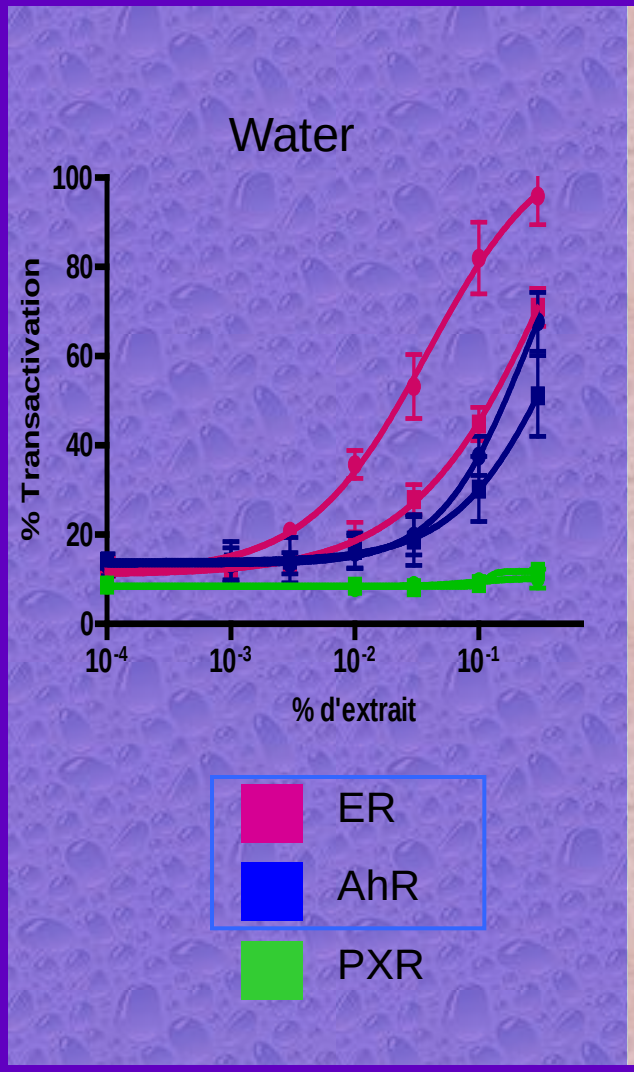
Application to wastewater

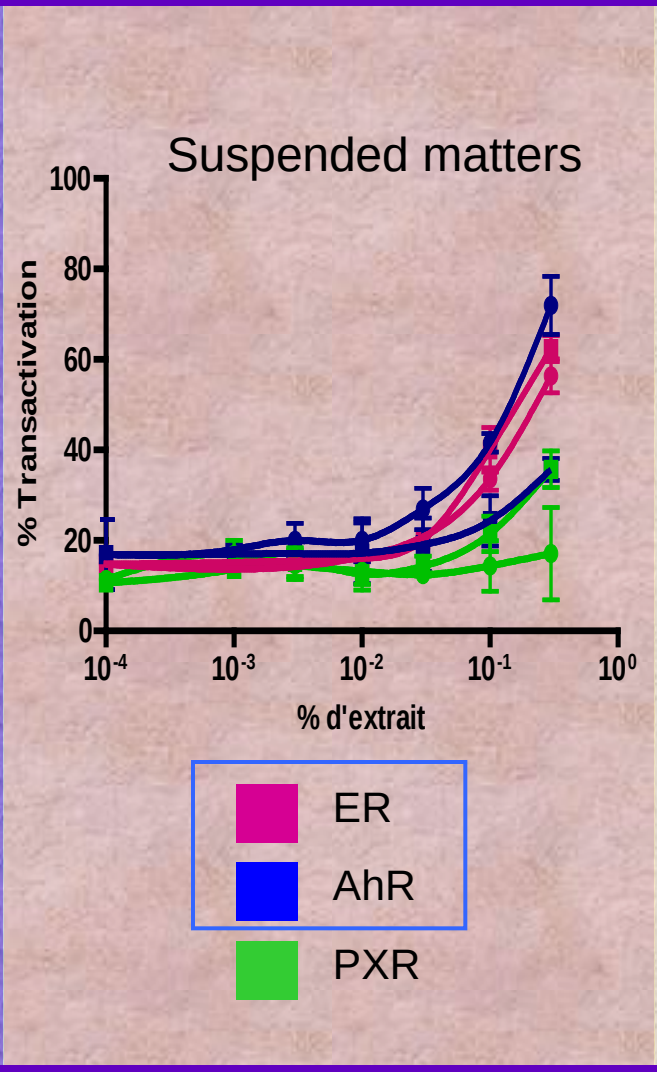
- Characterization of ERα, AhR and PXR activators

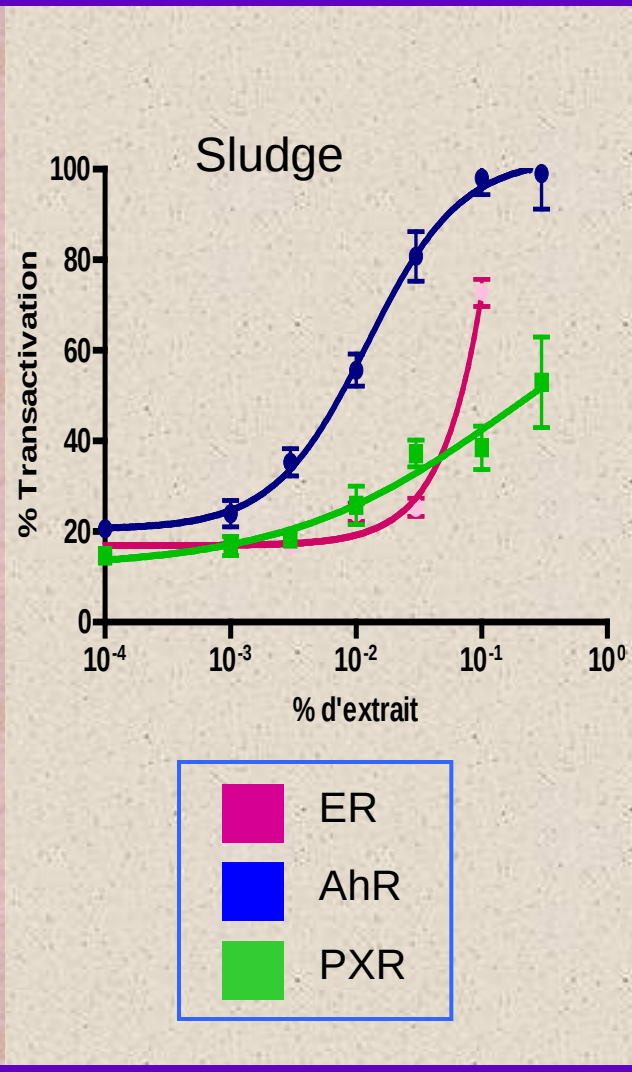




Results

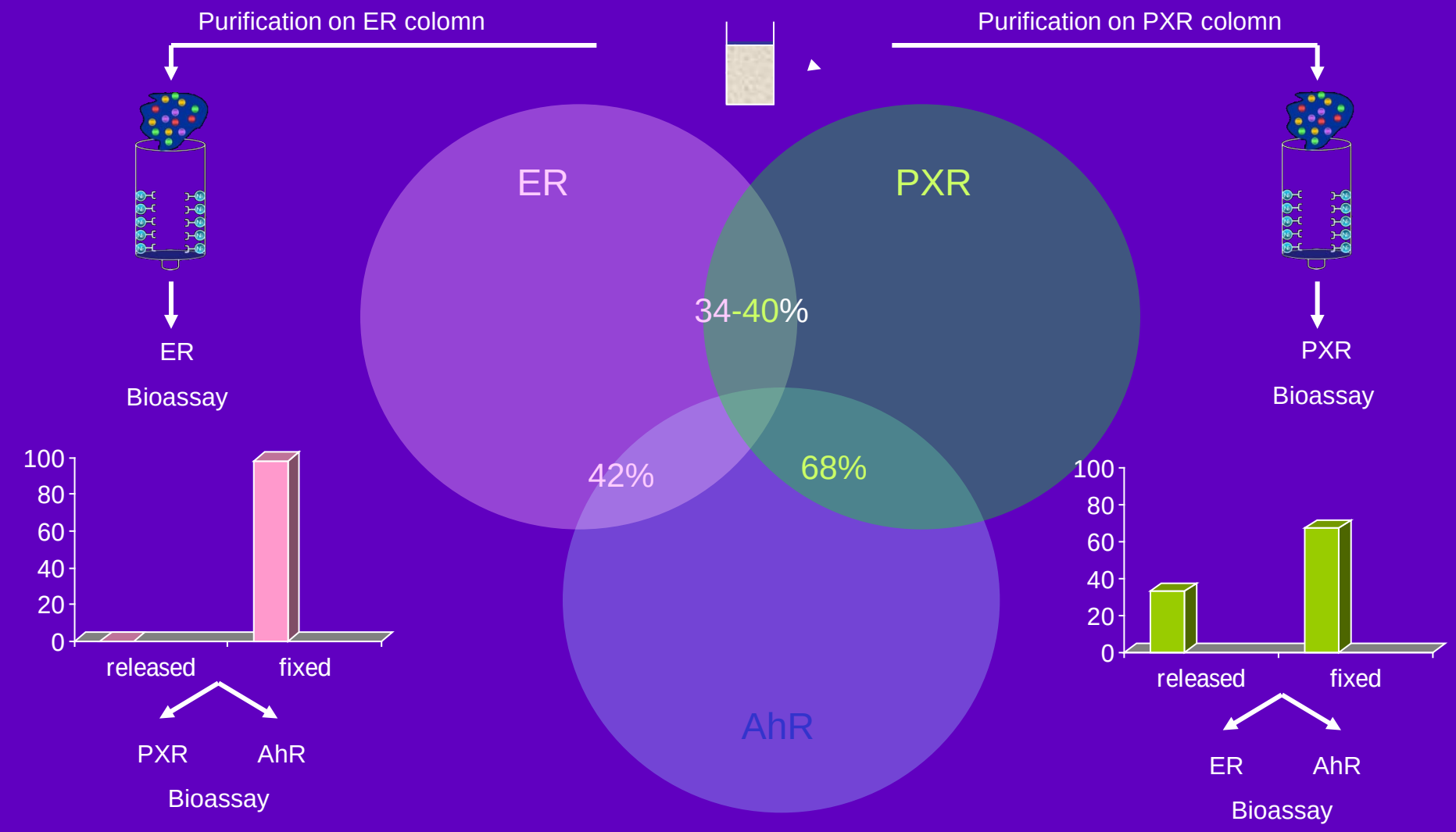

Water
% Transactivation
100
80
60
40
20
0
10-4
10-3
10-2
10-1
% d'extrait
ER
AhR
PXR


Suspended matters
% Transactivation
100
80
60
40
20
0
10-4
10-3
10-2
10-1
100
% d'extrait
ER
AhR
PXR


Sludge
% Transactivation
100
80
60
40
20
0
10-4
10-3
10-2
10-1
100
% d'extrait
ER
AhR
PXR

Purification on Ni-NTA-Receptor colomns

Sludge



Emergent contaminants in water

Concluding remarks

We must consider

- Regional scale
- Water usage
- Ecosystem vulnerability
- Human vulnerability

Hot-spot, Hot-moment

- We usually study mean behavior, while spatial and temporal fate is heterogeneous
- Conceptual models needs to be confirmed by experimental and in situ studies.
- Climate change will modify the frequency and magnitude of extreme events and though hot-spot, hot-moment (ex : flood, temperatures…)

Many questions remain to be addressed

Do we have tools to predict and anticipate unknown consequences

Do we have sufficient knowledge for decision making for monitoring some substances or ignore others

Will computational toxicology allow the study of mixtures at low doses

Do we have the good approach for studying complex environmental matrixes