



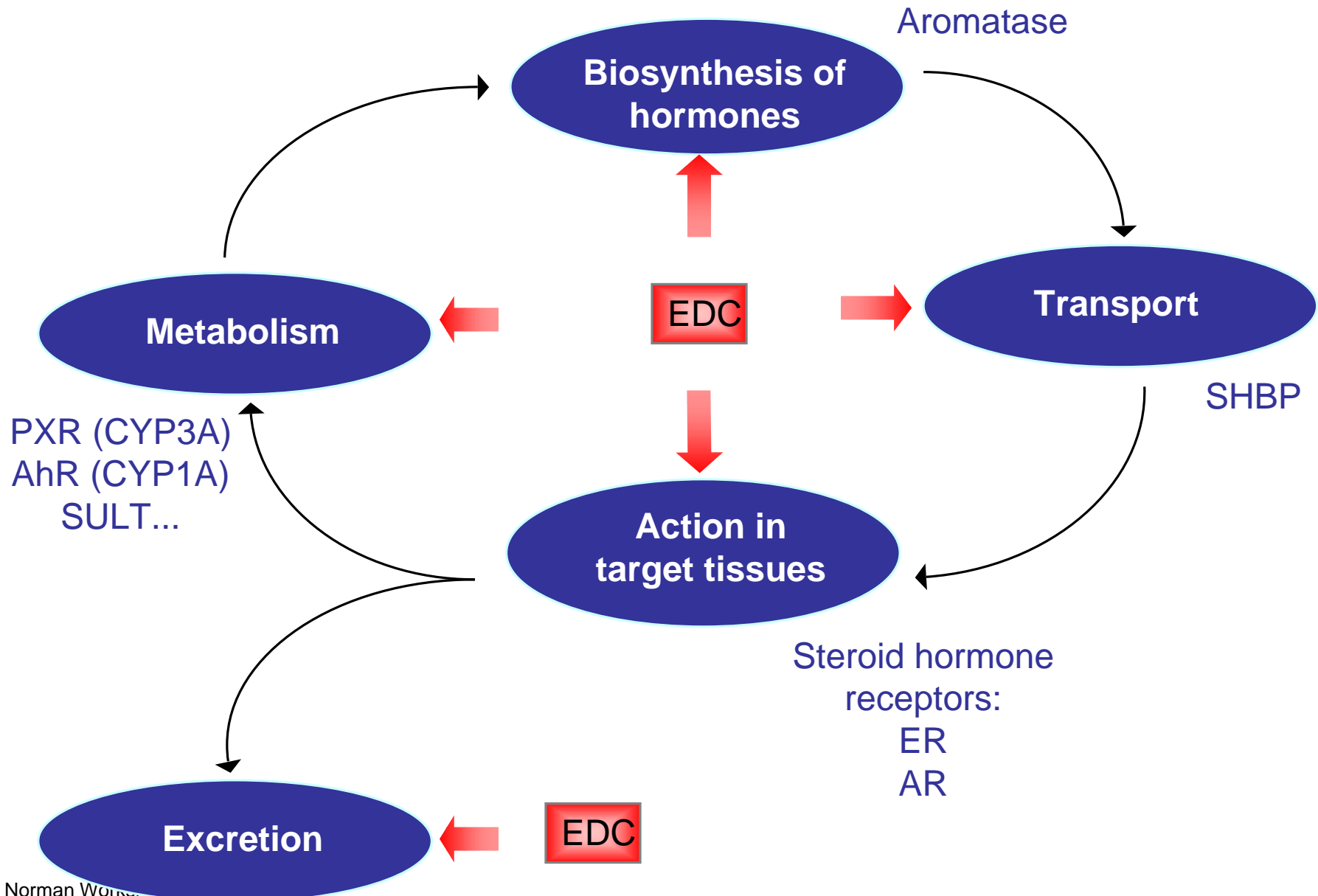
Multiple endocrine disrupting activities in French river sediments as assessed by the combined use of *in vitro* bioassays and chemical analyses

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Unit of Ecotoxicological Risk Assessment

INERIS

Modes of action of EDCs





Context

Environmental endocrine disrupting chemicals (EDCs) :

- variety of chemical classes and origins
- multiple modes of biological action

French situation

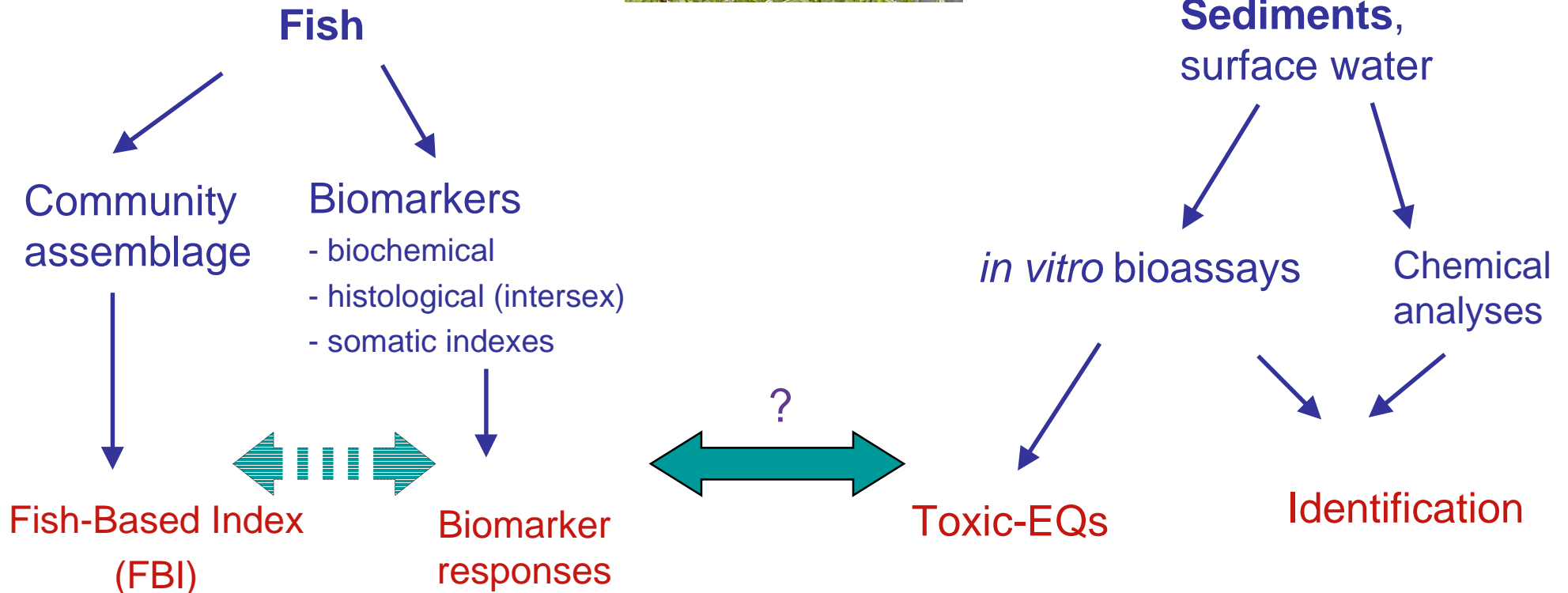
- estrogens in WWTP effluents (*Cargouet et al, 2004, Labadie and Budzinski 2005, Muller et al 2008*)
- limited data on contamination levels and biological impacts in freshwater systems (rivers)

Field monitoring of EDCs : overall approach

WFD Sites

IMPACTS

CONTAMINATION



Case study : small agricultural streams



Lézarde

Pressure : agriculture

Fish Index : 2 (reference site)



Réveillon

Urban and agriculture

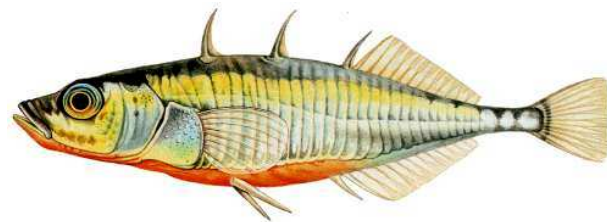
5 (bad quality)



Rhonelle

agriculture

3 (medium quality)



Three-spined stickleback
(*Gasterosteus aculeatus*)

Estrogenicity biomarker:

VITELLOGENIN in male fish

Androgenicity biomarker:

SPIGGING in female fish

Case study : small agricultural streams



Lézarde

Pressure : agriculture
Fish Index : 2 (reference site)



Réveillon

Urban and agriculture
5 (bad quality)



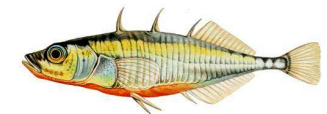
Rhonelle

agriculture
3 (medium quality)

Vitellogenin -

++

+



Spigging -

-

+

EROD +/-

++

+



Endocrine disruption in fish



Sediment contamination by EDCs ?

 ***in vitro* bioassays**

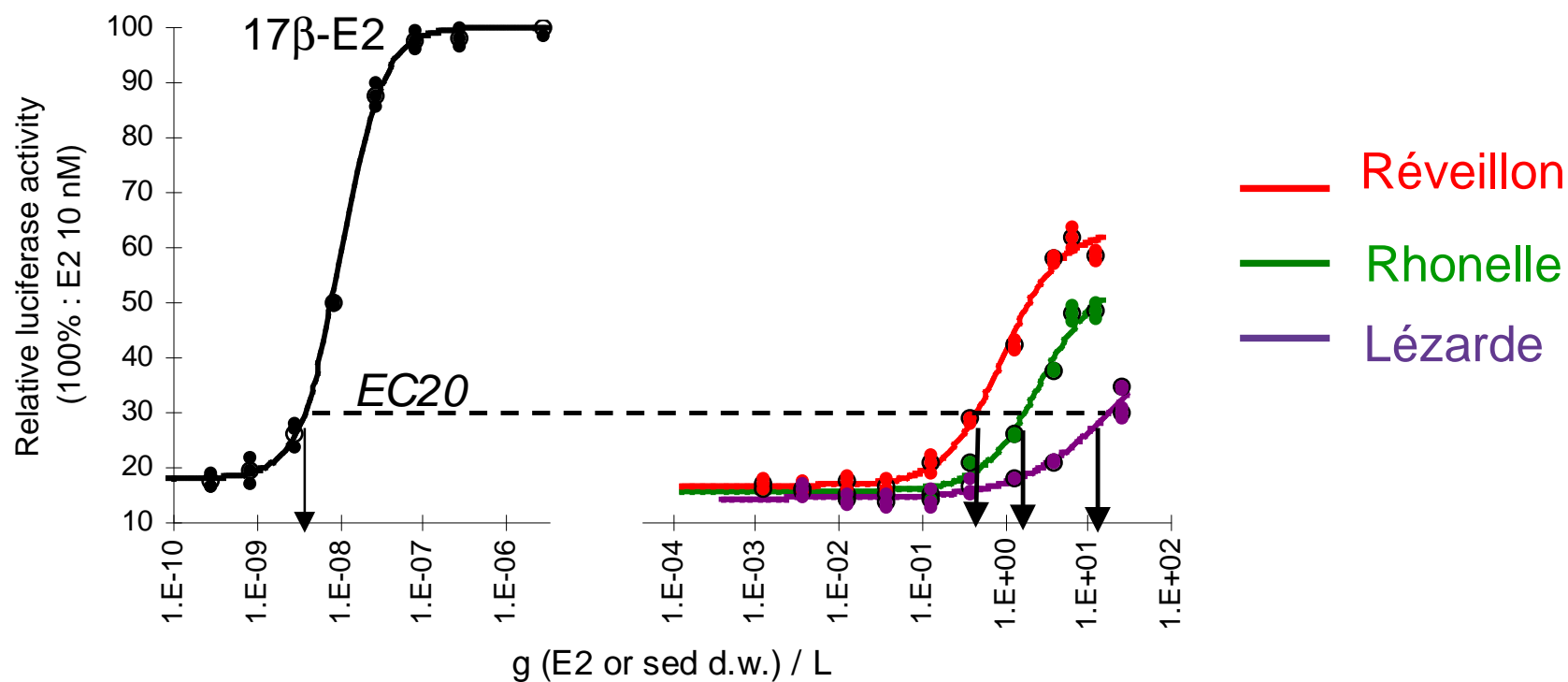
Reporter cell-based assays

	Target receptor	Cell lines	Endpoint	Reference ligands (EC ₅₀)
Hormonal activity	ER	MELN (MCF-7, ERE-LUC)	Luciferase	E2 (0.02 nM)
	AR anti-AR	MDA-kb2 (MDA-MD-453, MMTV-Luc)	Luciferase	DHT (0.1 nM) Flutamide (0.1 µM)
Xenobiotic Metabolism	AhR	PLHC-1	EROD 4 h EROD 24 h	BaP (5 nM) TCDD (0.1 nM)
	PXR	HGPXR (GAL4RE-Luc/GAL4-hPXR)	Luciferase	SR12,813 (0.1 µM)

HG-PXR (Lemaire et al,2006), MELN (Balaguer et al, 1999), MDA-kb2 (Wilson et al 2002)

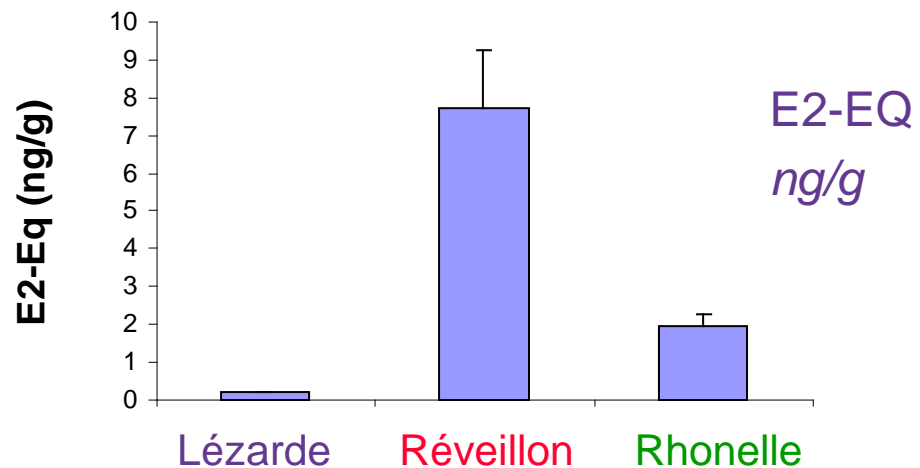
Estrogenic activities in sediment (crude organic extracts)

MELN cells

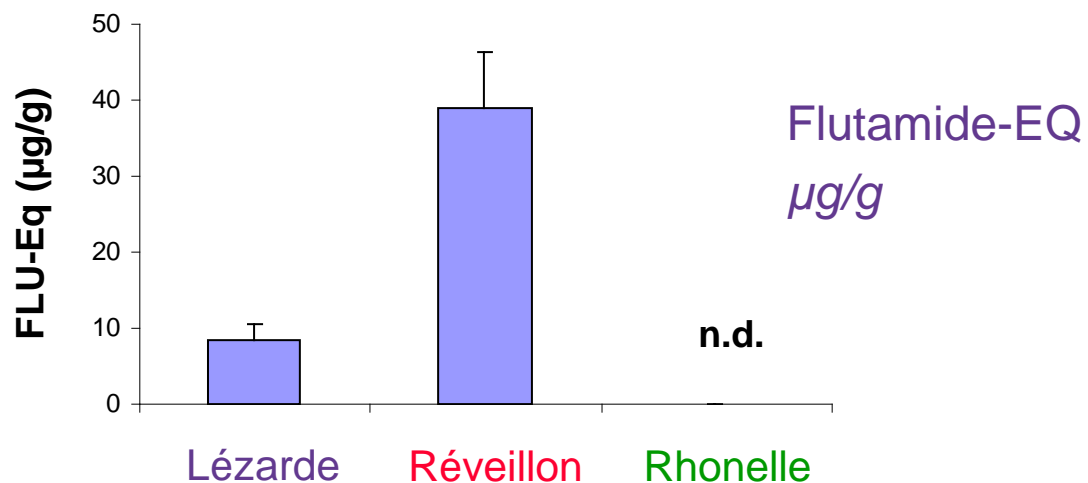


$$E-EQ = \frac{EC_{20} \text{ of } 17\beta\text{-E2}}{EC_{20} \text{ of sample}}$$

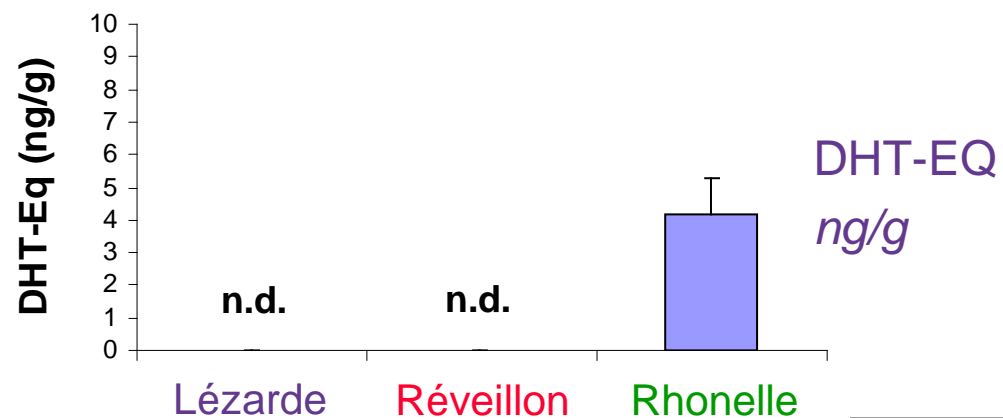
Estrogenic activities



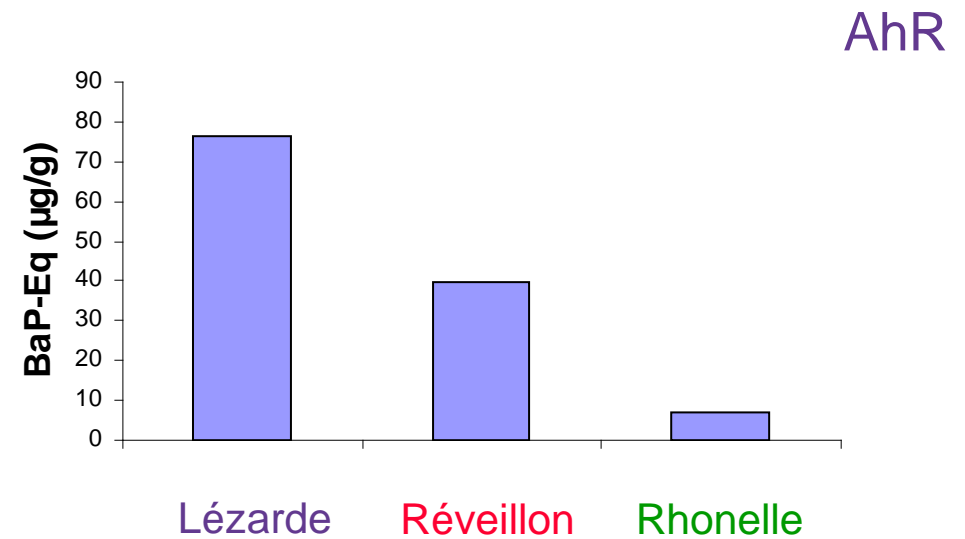
Anti-androgenic activities



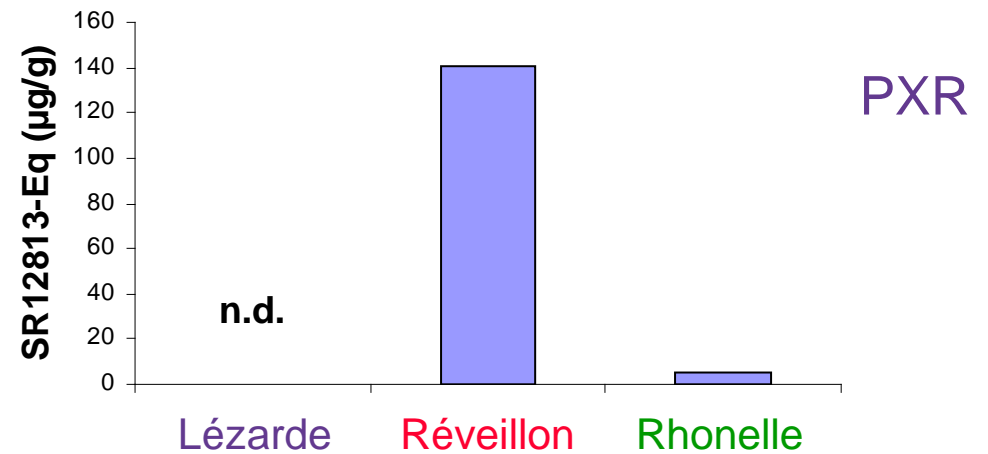
Androgenic activities



PAH-like activities (BaP-Eq)



PXR-mediated activities (SR12813-Eq)



Nature of PXR activators ?
Xeno-estrogens ?



Endocrine disruption in fish



Sediment contamination by EDCs ?

👉 **cell bioassays**



Nature of endocrine active chemicals ?

👉 **Target chemical analyses**

(Xeno-)estrogens (ng/g sed. d.w.)

	Lézarde	Réveillon	Rhonelle
DES	<0.1*	<0.1	<0.1
17 α -E2	<0.4	3.11	<0.4
17 β -E2	<0.3	1.38	0.81
Estrone	0.65	1.27	0.88
MeEE2	<0.3	<0.3	0.39
EE2	<1.5	<1.5	<1.5
4-NP	<0.19	<0.19	<0.19
4-OP	<0.18	778.0	<0.18
BPA	<0.82	828.3	130.0
(α + β)Endosulfan	193.1	<24	<24
E2-EQs-chem	0.13	2.12	0.99
E2-EQs-bio	0.20	7.85	1.96

E2-Eq and EEF are based on EC₂₀ values

* quantification limit

PAHs and PCBs (ng/g sed. d.w.)

	Lézarde	Réveillon	Rhonelle
16 PAHs	19 466	10 278	2 518
PCB138	<LOQ	6.9	<LOQ
PCB153	<LOQ	6.7	<LOQ
BaP-EQchem	15 036	8 740	1 978
BaP-EQbiol	76 409	39 939	7 123
<i>Ratio</i>	19%	21%	27%

 *Partial contribution of the priority PAHs*

BaP-EQs are based on EC₂₀ values

IEF4h were determined for individual PAH (Louiz et al, submitted)



Endocrine disruption in fish



Site contamination by EDCs ?

☞ **cell bioassays**



Nature of endocrine active chemicals ?

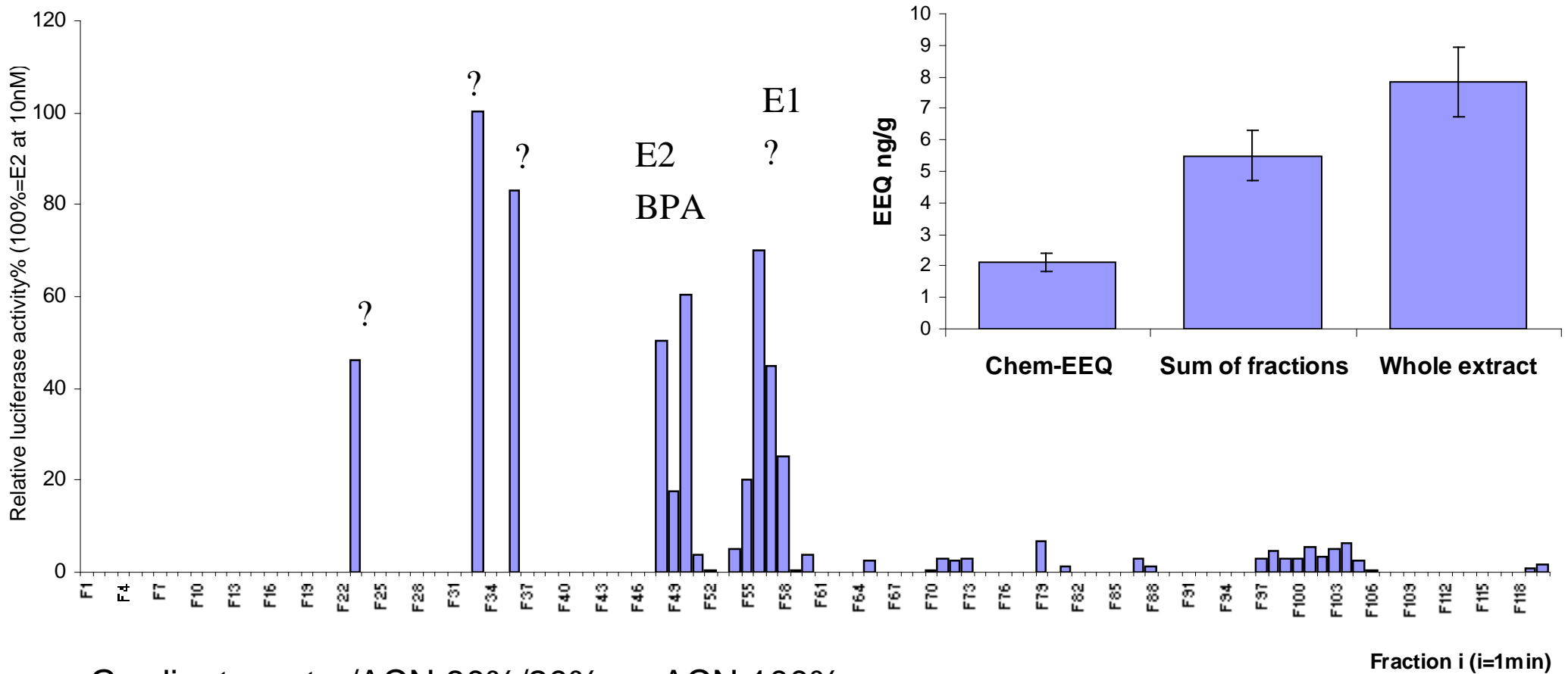
☞ **Target chemical analyses**



☞ **sample fractionation : Réveillon (prel. data)**

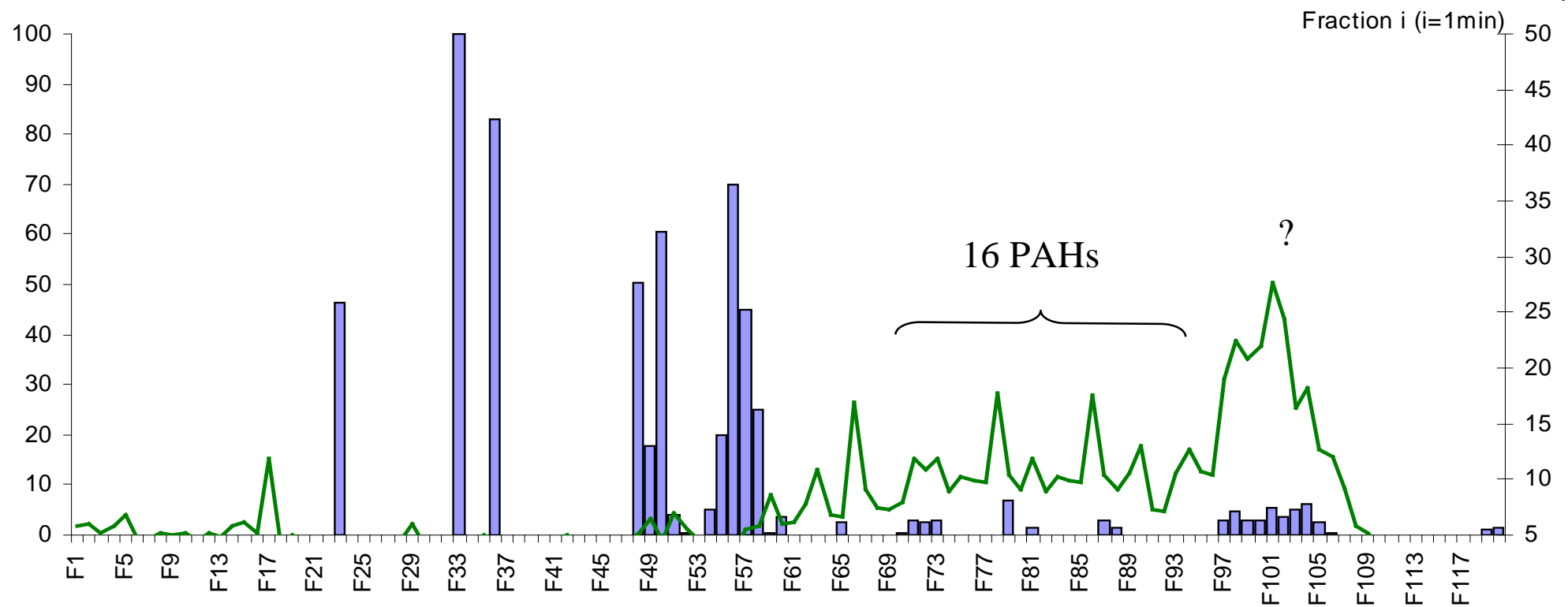
- Polarity : RP-HPLC
- Receptor affinity : ER-columns

RP-HPLC fractionation : ER activities in Réveillon



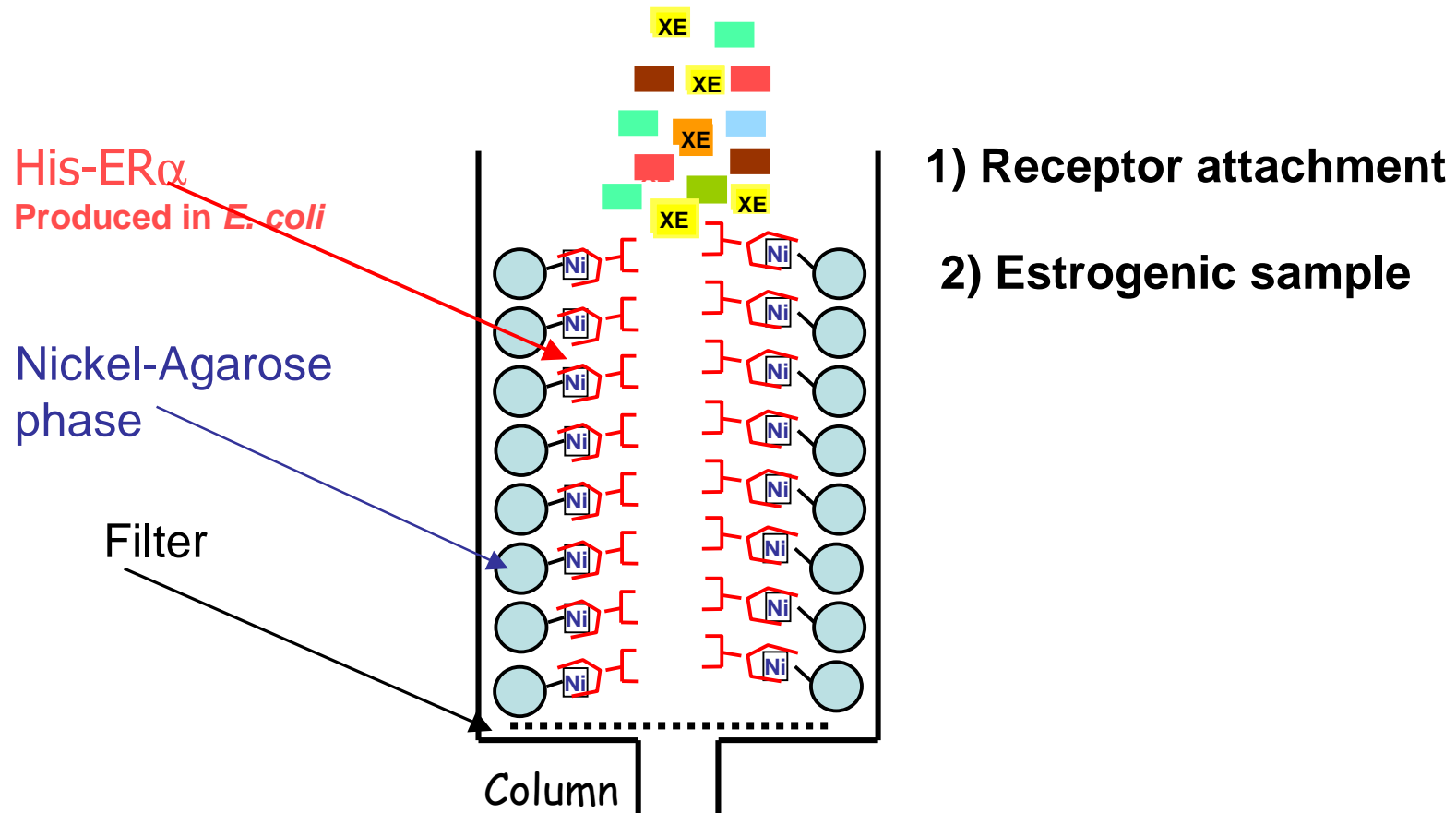
Gradient : water/ACN 80%/20% → ACN 100%

RP-HPLC fractionation : AhR activities in Réveillon



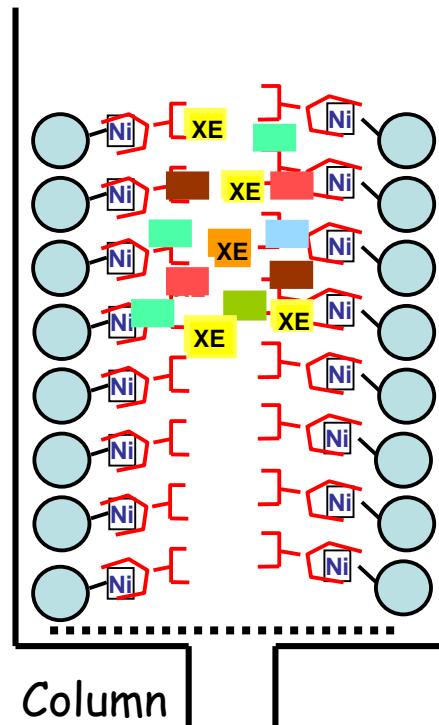
 *Identification of active fractions : to be done !*

Purification on hER α affinity column



Pillon *et al.*, *Environ. Health Persp.* (2005) 113, 278-84

Purification on hER α affinity column



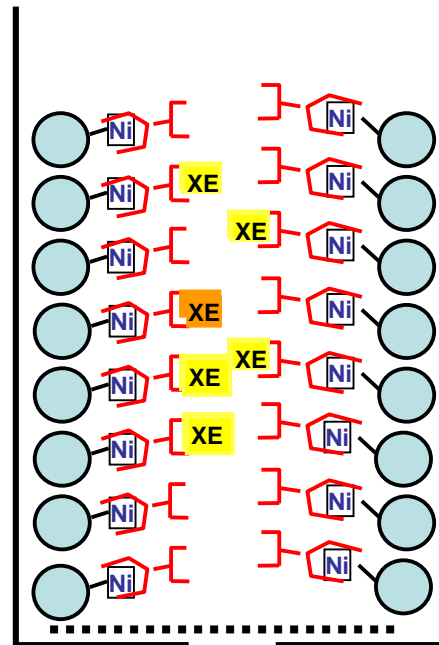
1) Receptor attachment

2) Estrogenic sample

3) Ligand binding

Pillon *et al.*, *Environ. Health Persp.* (2005) 113, 278-84

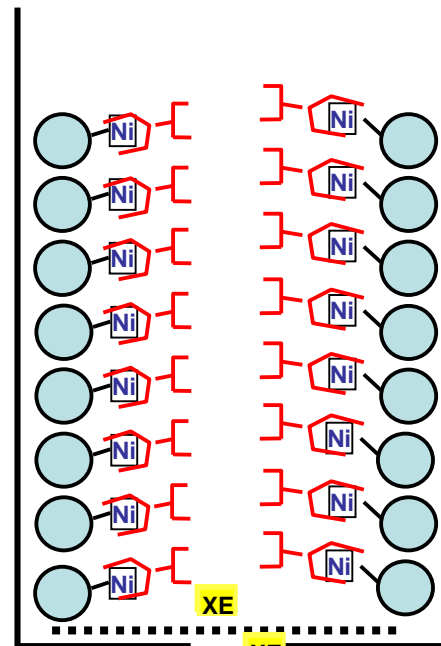
Purification on hER α affinity column



- 1) Receptor attachment
- 2) Estrogenic sample
- 3) Ligand binding
- 4) Washing step

Washing fraction

Purification on hER α affinity column



1) Receptor attachment

2) Estrogenic sample

3) Ligand binding

4) Washing step

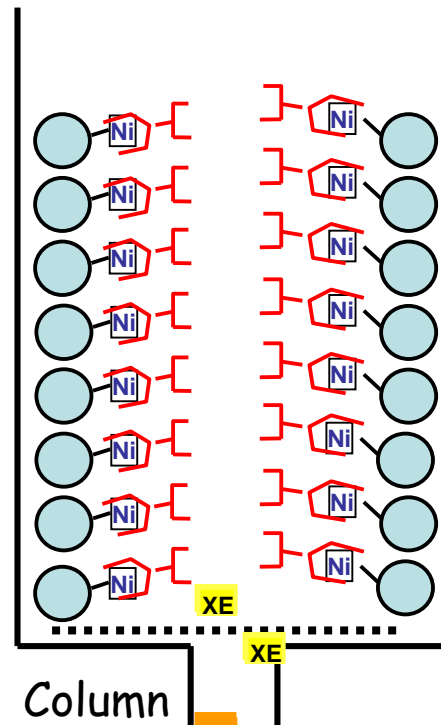
5) Elution

Washing fraction



Elution fraction

Purification on hER α affinity column



1) Receptor attachment

2) Estrogenic sample

3) Ligand binding

4) Washing step

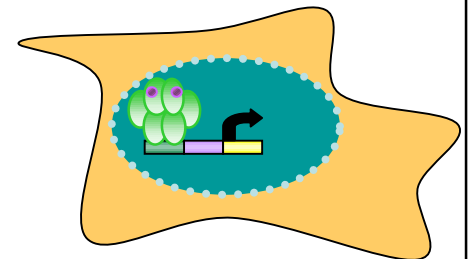
5) Elution

6) Testing for ER, AhR, PXR activities in bioassays

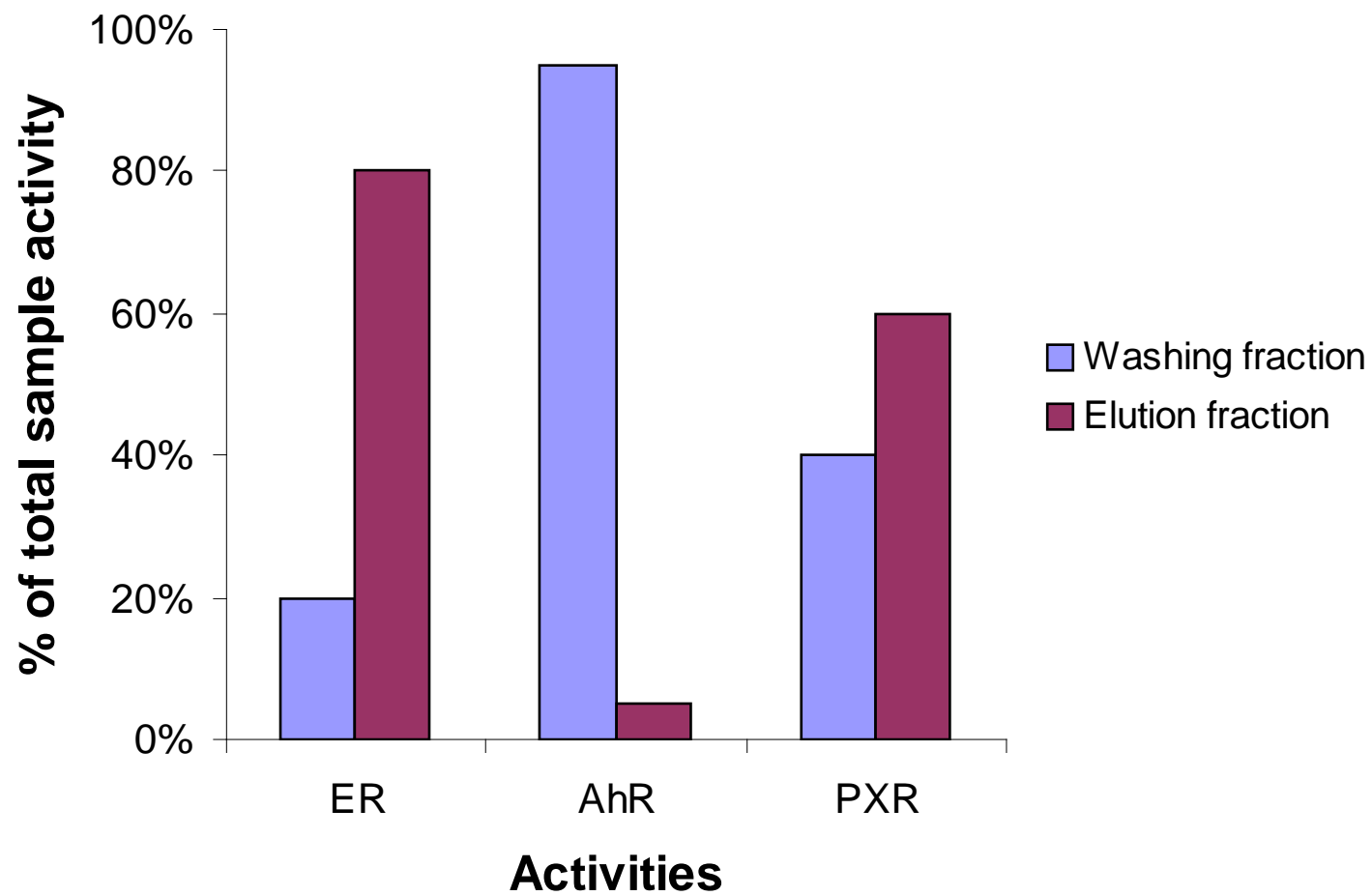
Washing fraction



Elution fraction



Preliminary results with Réveillon sediment





Summary

Occurrence of endocrine disruption in wild stickleback

- agricultural sites, without influence of major WWTP

Occurrence of ED activities in sediments ?

- Profiling : ER, anti-AR, AR, PXR, AhR

Nature of active compounds ?

- E2, E1 and PAHs as main contributors...
- ...but only partially
- Unknown : polar ER fractions
- Unknown : AR and PXR agonists



Future work

Identification of isolated fractions

- full scan MS, qTOF, IR, UV
- confirmation in bioassays

To extend the approach to sites with **androgenic** responses in sticklebacks

SURVAQUA project

- National Research Programme on EDs (PNRPE 2007-2009)
- INERIS (JM Porcher), CEMAGREF, Bordeaux Univ, Montpellier Univ., Le Havre Univ., UCO
- 13 sites

Thanks to collaborators

INERIS

Biomarkers in fish, in vitro bioassays

François BRION, Wilfried SANCHEZ, Nicolas CREUSOT, Jean-Marc PORCHER



Chemical analyses

Saïd KINANI, Stéphane BOUCHONNET



Recombinant receptor columns, in vitro models

Sonia DAGNINO, Patrick BALAGUER



Field surveys

Jean-Maxence DITCHE

Financial support : Ministère de l'Écologie et du Développement Durable (BCRD 2006-2009, PNRPE)