



RÉPUBLIQUE
FRANÇAISE

*Liberté
Égalité
Fraternité*



JOURNÉE TECHNIQUE AQUAREF

Mesure de l'activité œstrogénique dans l'eau par bioessai *in vitro*

> 6 octobre 2026, LNE, Paris

Contexte et besoins réglementaires

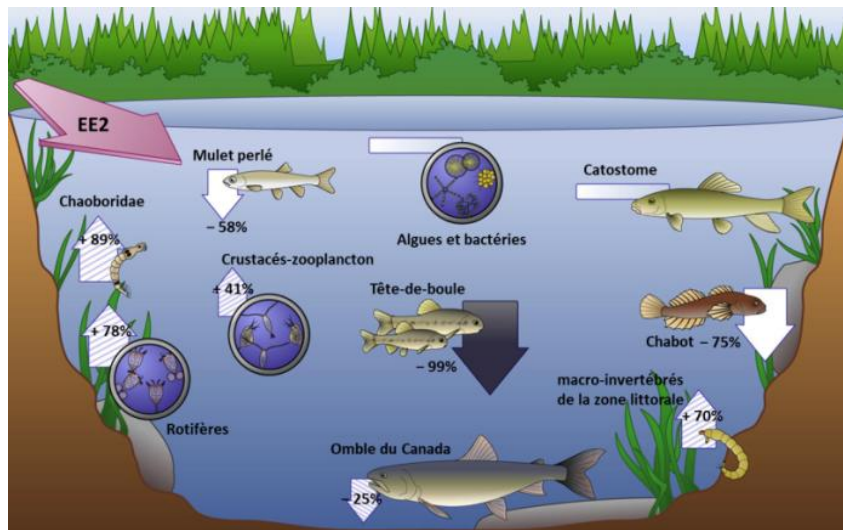
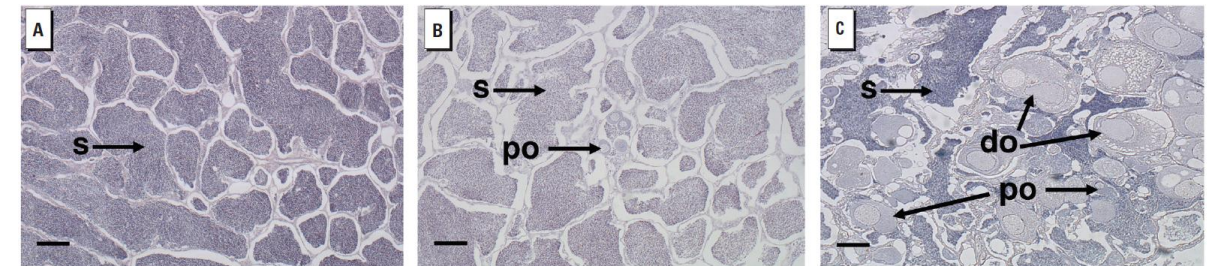
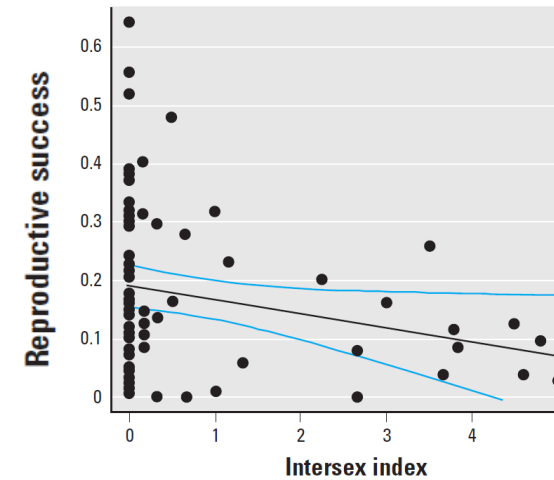
olivier.perceval@ofb.gouv.fr

POURQUOI S'INTÉRESSER À LA PRÉSENCE D'ŒSTROGÈNES STÉROÏDIENS DANS LES MILIEUX AQUATIQUES ?

Diminution de la fertilité et du succès reproducteur (nb de descendants par géniteur) chez certaines espèces de poissons en aval de rejets de STEU (Jobling *et al.*, 2002 ; Harris *et al.*, 2011)

Observation d'anomalies du tissu reproducteur, présence de cellules sexuelles femelles dans les gonades mâles (ovotestis)

Intersexualité : phénomène résultant de l'action combinée des œstrogènes stéroïdiens et des anti-androgènes présents dans les rejets de STEU (Jobling *et al.*, 2009)



Extinction de certaines populations de cyprinidés à la suite d'une exposition prolongée à l'EE2 à des concentrations réalistes d'un point de vue environnemental, i.e. 4-5 ng/L (Kidd *et al.*, 2007)

PREMIÈRES TENTATIVES D'ÉLABORATION DE NQE POUR EE2 ET E2

Environ. Sci. Technol. 2008, 42, 7049-7054

Derivation of an Aquatic Predicted No-Effect Concentration for the Synthetic Hormone, 17 α -Ethinyl Estradiol

DANIEL J. CALDWELL,^{*,1} FRANK MASTROCCO,¹
THOMAS H. HUTCHINSON,^{*,2} REINHARD LÄNGE,³
DAGOBERT HEITERICK,¹ COLIN JANSSEN,⁴
PAUL D. ANDERSON,⁵ AND JOHN P. SUMPTER¹
¹Johnson & Johnson, New Brunswick, New Jersey; Pfizer, New York, New York;
AstraZeneca, Brezham, United Kingdom; Bayer Health Care, Berlin, Germany;
EURASACARDIS, Gent, Belgium; University of Gent, Belgium; AMEC Earth
& Environmental, Westford, Massachusetts; Department of Geography and
Environment, Boston University, Boston, Massachusetts; Institute for the
Environment, Brunel University, Uxbridge, United Kingdom

Received March 3, 2008. Revised manuscript received June 26, 2008. Accepted July 15, 2008.

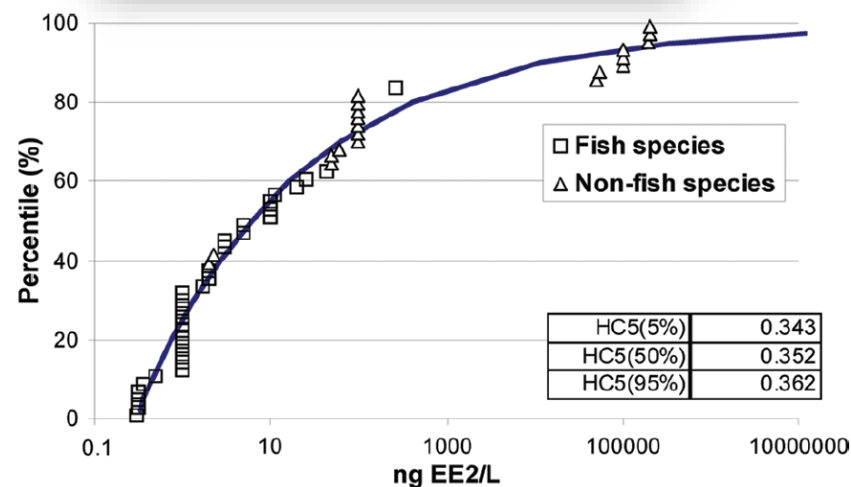
17 α -Ethinyl estradiol (EE2) is a synthetic estrogen widely used in combination with other steroid hormones in oral contraceptives and in the contraceptive patch. EE2 has been detected in sewage treatment plant effluents in the low nanogram-per-liter range and occasionally in surface waters in the U.S., U.K., Canada, Brazil, Germany, and elsewhere. The mode of action is receptor-mediated, and estrogen receptors exist in mammals and other vertebrates. A large number of studies on the effects of EE2 on aquatic organisms exist. One hundred English language studies published between 1984 and 2007, one as yet unpublished study, and findings published in conference proceedings (in German) were compared to published data quality criteria to identify the most relevant studies for deriving a predicted no-effect concentration (PNEC). Reproduction in fish was identified as the most sensitive end point in aquatic species. A species sensitivity distribution was constructed using no observed effect concentrations (NOECs) for reproductive effects from 29 papers in 26 species, resulting in a median hazardous concentration at which 5% of the species tested are affected (HC₅) of 0.25 ng/L. After comparing this HC₅ to all of the laboratory and field-derived toxicity information available for EE2, we recommend using 0.35 ng/L as the PNEC for EE2 in surface water. This PNEC is below 95% of the existing NOECs for effects on reproduction and is also below virtually all of the NOECs for vitellogenin induction in the key fish reproduction studies.

INTRODUCTION

17 α -Ethinyl estradiol (EE2) is a synthetic estrogen used in combination with other steroid hormones in oral contraceptives and in the contraceptive patch. EE2 is only partially metabolized and incompletely removed by sewage treatment plants (STPs) and, therefore not surprisingly, has been detected in sewage treatment plant effluents in the low nanogram-per-liter range and occasionally in surface waters in the U.S., U.K., Canada, Brazil, Germany, and elsewhere (1–6). EE2 is not the only compound with estrogenic activity detected in STP effluents (7–11) although available data suggest natural and synthetic hormones represent the majority of the estrogenic activity in many effluents (12, 13). Its relatively high relative biological activity (14–18) has led to a great deal of research on EE2, providing a far greater body of toxicity data than is available for most compounds. Estrogens in Whole Effluents: Effects in Fish Populations. Purdom et al. (19) are often cited as one of the first papers to show that effluents can be estrogenic to fish by inducing the synthesis of vitellogenin (VTG). Now many papers report that a number of fish species respond to nanogram per liter (ng/L) concentrations of EE2 by synthesizing VTG (19–23). Although laboratory studies exist in which exposure to EE2 causes an increase in VTG in male (and also female) fish and coincides with observed adverse reproductive effects, concluding that EE2 in natural settings might also be causing reproductive effects is not possible because, as noted above, other compounds in effluents also have estrogenic activity. Indeed, determining the environmental relevance of effects from trace levels of EE2 in effluents requires an aquatic risk assessment that focuses specifically on the potential effects of EE2. Key to the conduct of such an assessment is determining the allowable concentration of EE2 in surface water, also referred to as the predicted no-effect concentration (PNEC).

Why the Usual Approaches To Derive a PNEC Are Not Viable for EE2. A variety of methods have been proposed to derive PNECs. Many of these assume that limited toxicity data are available for a compound and derive a PNEC using various types of uncertainty or assessment factors. For example, if only acute data are available, the European

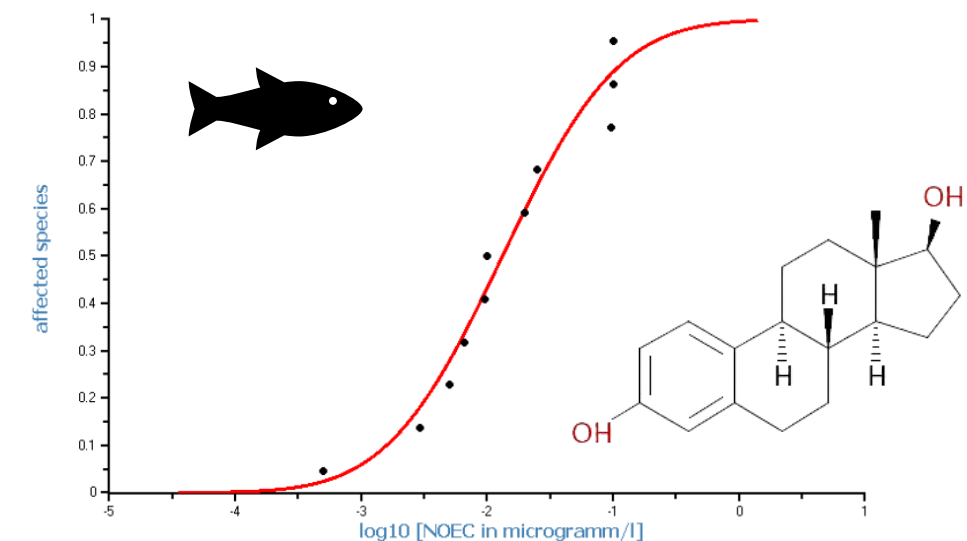
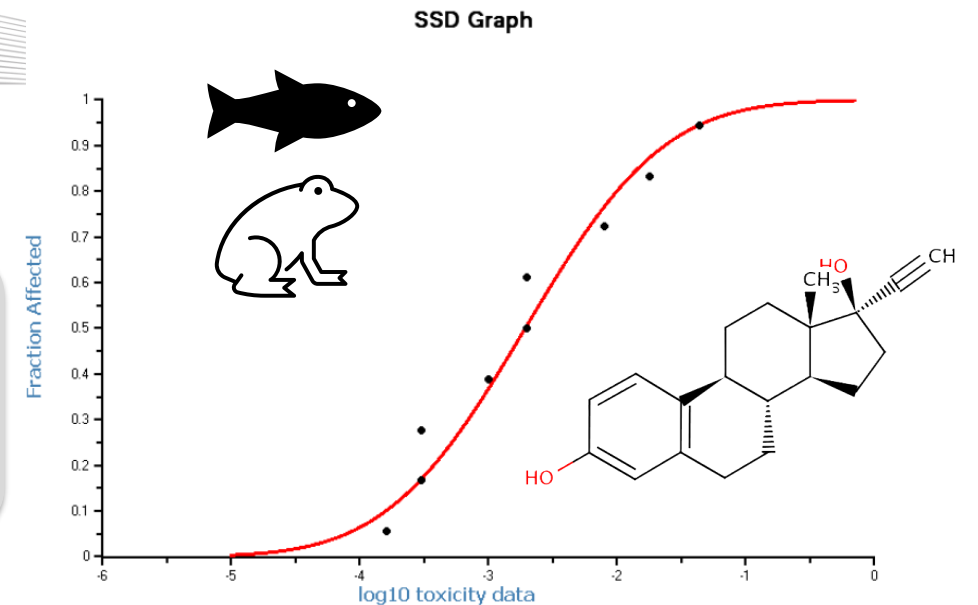
* Corresponding author address: 410 George Street, New Brunswick, New Jersey 08901; phone: 732-524-1088; fax: 732-524-3296; e-mail: dcaldwell@jnj.com.
¹ Johnson & Johnson.
² Pfizer.
³ Present address: Plymouth Marine Laboratory, Plymouth, U.K.
⁴ AstraZeneca.
⁵ Bayer Health Care.
⁶ EURASACARDIS.
⁷ University of Gent.
⁸ AMEC Earth & Environmental and Boston University.
⁹ Brunel University.



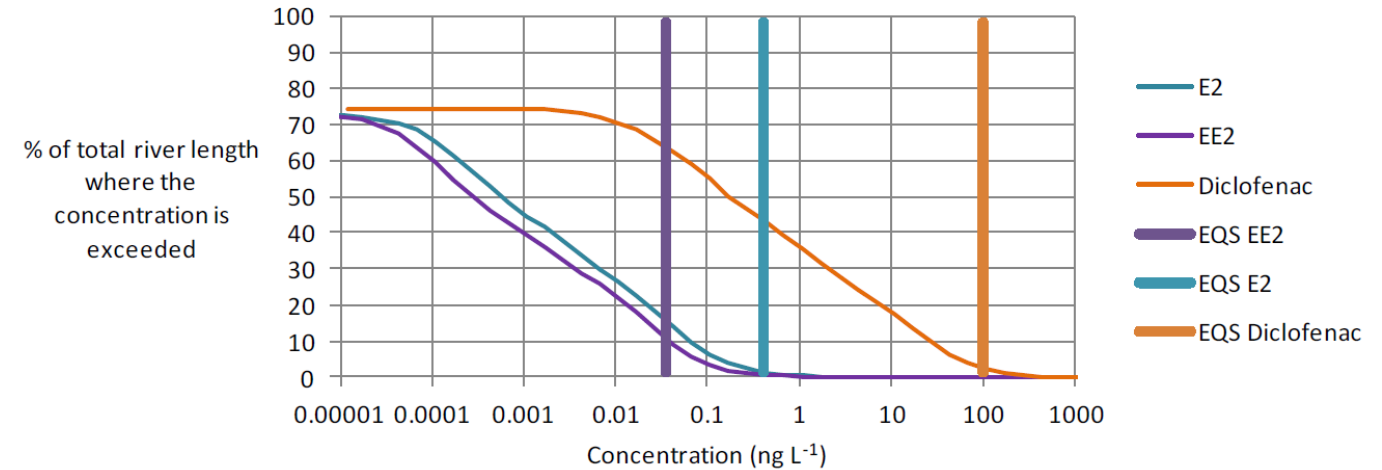
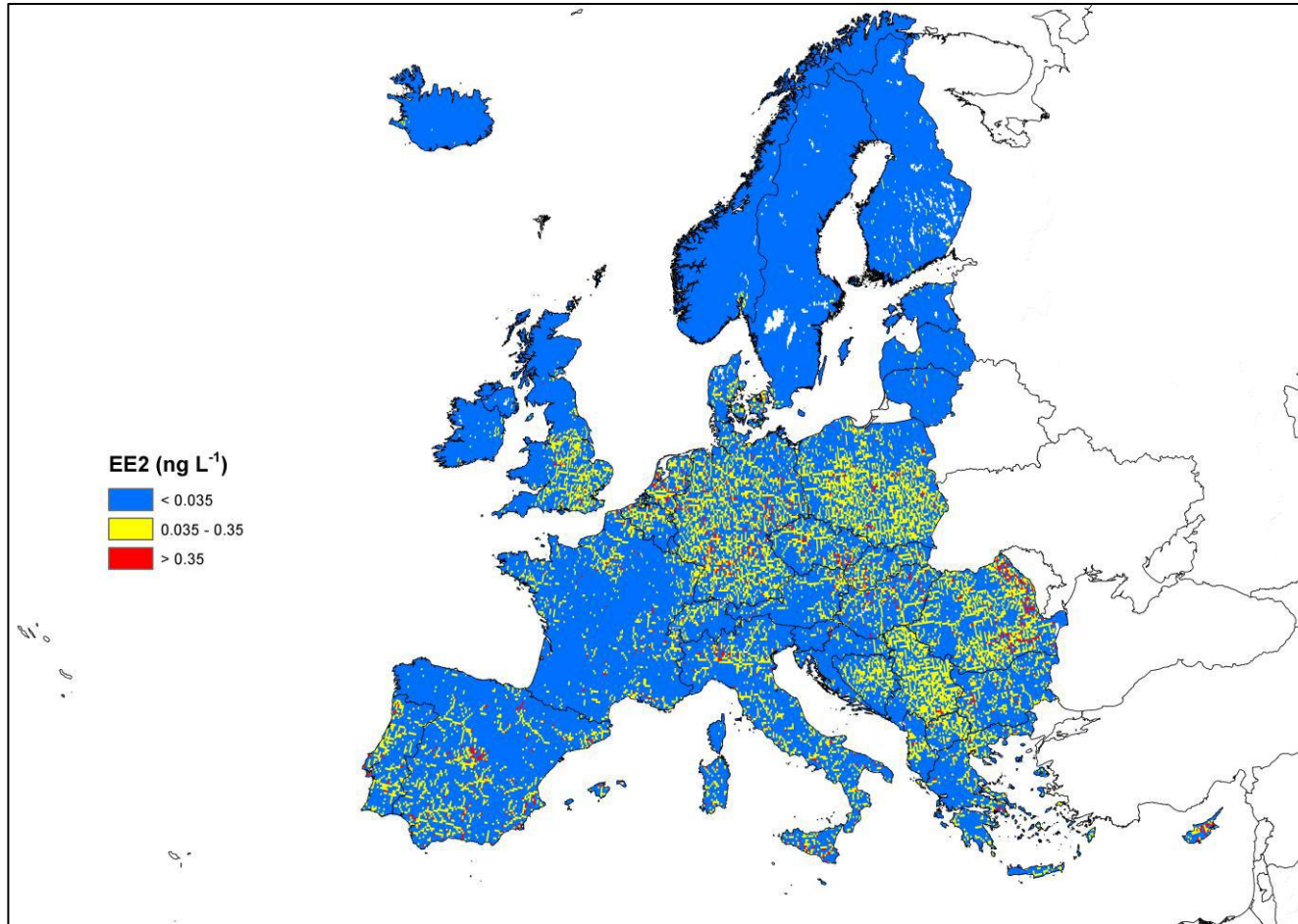
Caldwell et al. (2008)
PNEC chronique EE2 = 0,35 ng/L
(SSD HC5, AF = 1)

UE (2011)
NQE-MA EE2 = 0,035 ng/L
(SDD HC5, AF = 2)
NQE eaux littorales = 0,007 ng/L
(AF additionnel = 5)

UE (2011)
NQE-MA E2 = 0,4 ng/L
(SDD HC5, AF = 2)
NQE eaux littorales = 0,08 ng/L
(AF additionnel = 5)



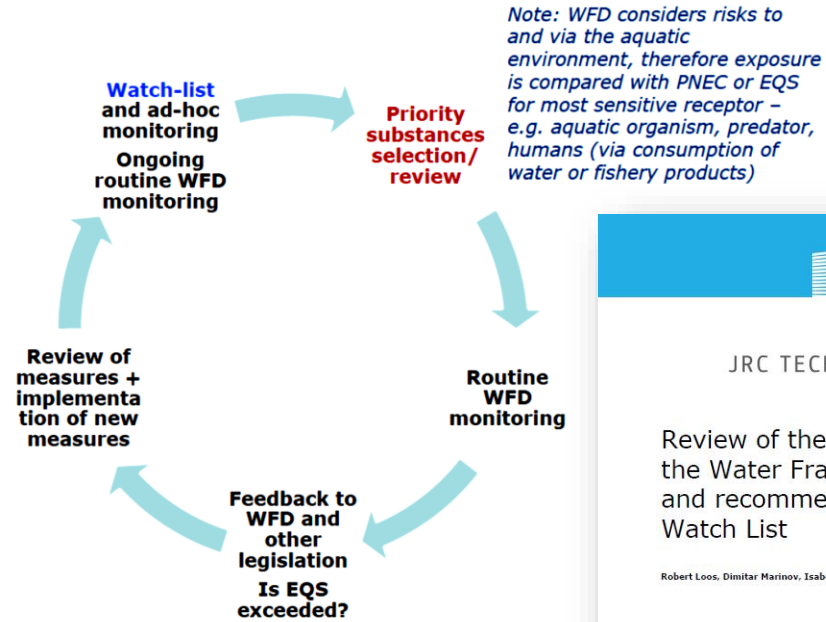
QUEL NIVEAU DE RISQUE DANS LES EAUX CONTINENTALES DE SURFACE EN EUROPE ?



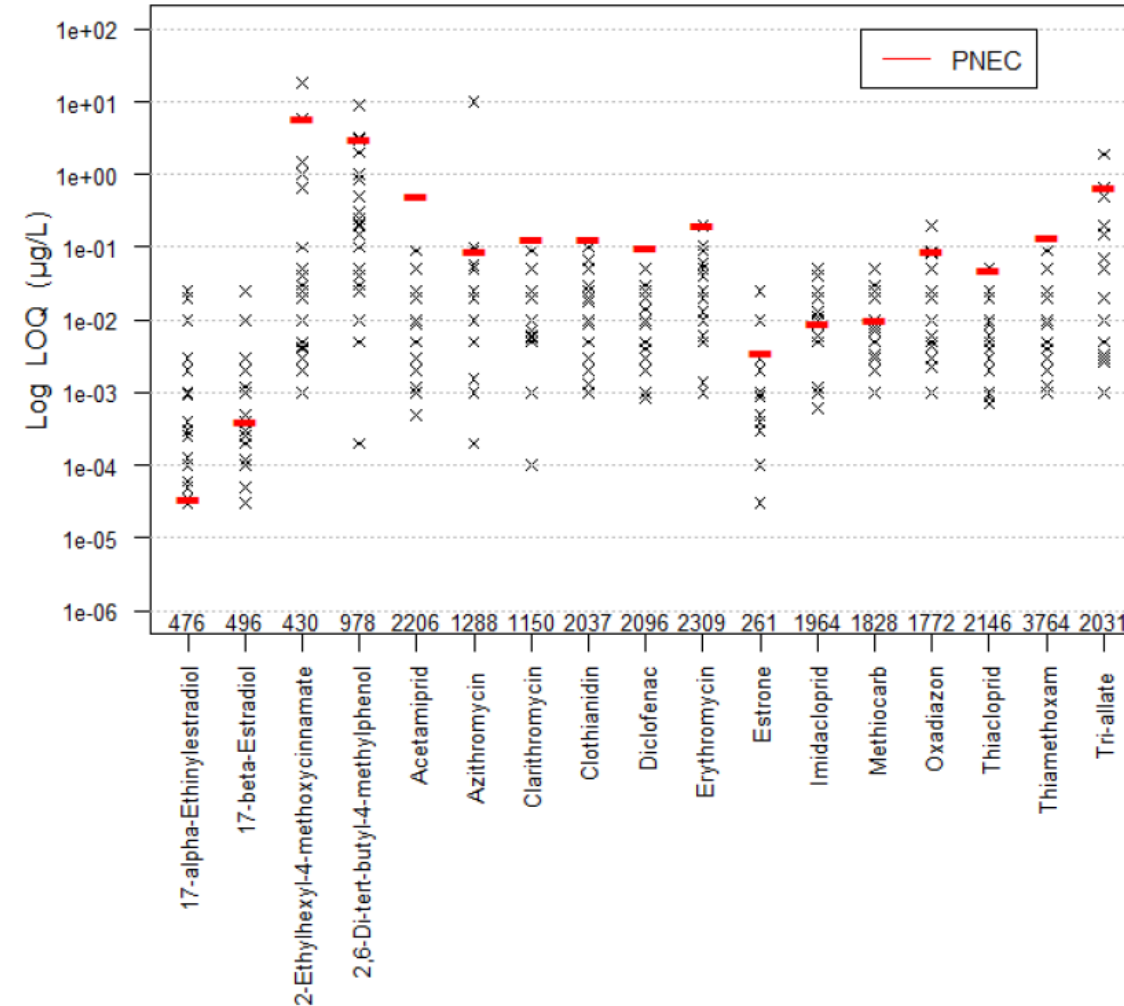
% de linéaire de cours d'eau pour lesquels les NQE-MA de l'EE2 (0,035 ng/L) et de l'E2 (0,4 ng/L) sont dépassées
→ modélisation basée sur les données de consommation de EE2 et E2, le taux d'abattement en sortie de STEU et le pouvoir de dilution du milieu récepteur

Source: Johnson *et al.* (2013). Do Concentrations of Ethinylestradiol, Estradiol, and Diclofenac in European Rivers Exceed Proposed EU Environmental Quality Standards ? *Environmental Science and Technology*, 47 (21). 12297-12304. 10.1021/es4030035

MÉCANISME DE LISTE DE VIGILANCE : DES DIFFICULTÉS ANALYTIQUES RENCONTRÉES AU NIVEAU EUROPÉEN



Non-Quantified samples



- **EE2** : résultats de 25 pays membres de l'UE (558 échantillons), FQ = 14,7%, majorité des LQ > PNEC
- **E2** : résultats de 25 pays membres de l'UE (597 échantillons), FQ = 16,9%, moitié des LQ > PNEC
- **E1** : résultats de 23 pays membres de l'UE (574 échantillons), FQ = 54,7%, majorité des LQ < PNEC

FOCUS SUR LES DONNÉES ACQUISES AU NIVEAU NATIONAL

SYNTHÈSE DES RESULTATS DES CAMPAGNES NATIONALES « LISTE DE VIGILANCE »

Anne Togola, Sophie Lardy-Fontan

Avril 2019

Avec la contribution de François Lestremou

Document final

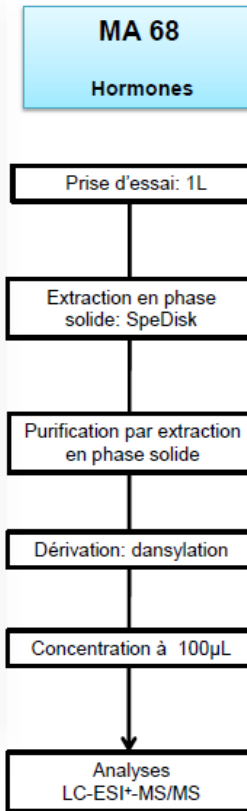
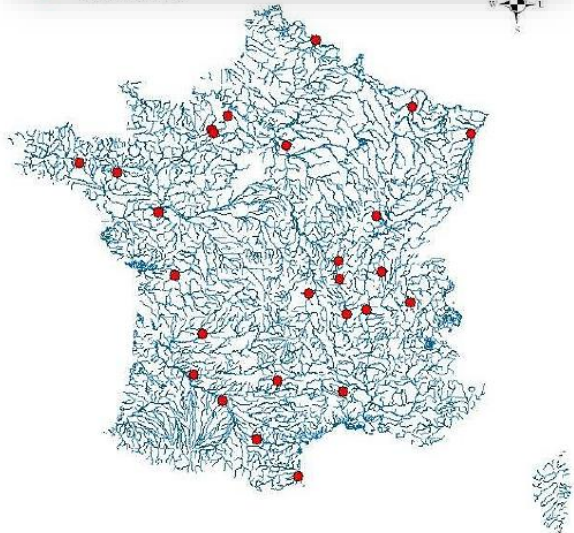
INERIS

améliorer la qualité
pour un développement durable

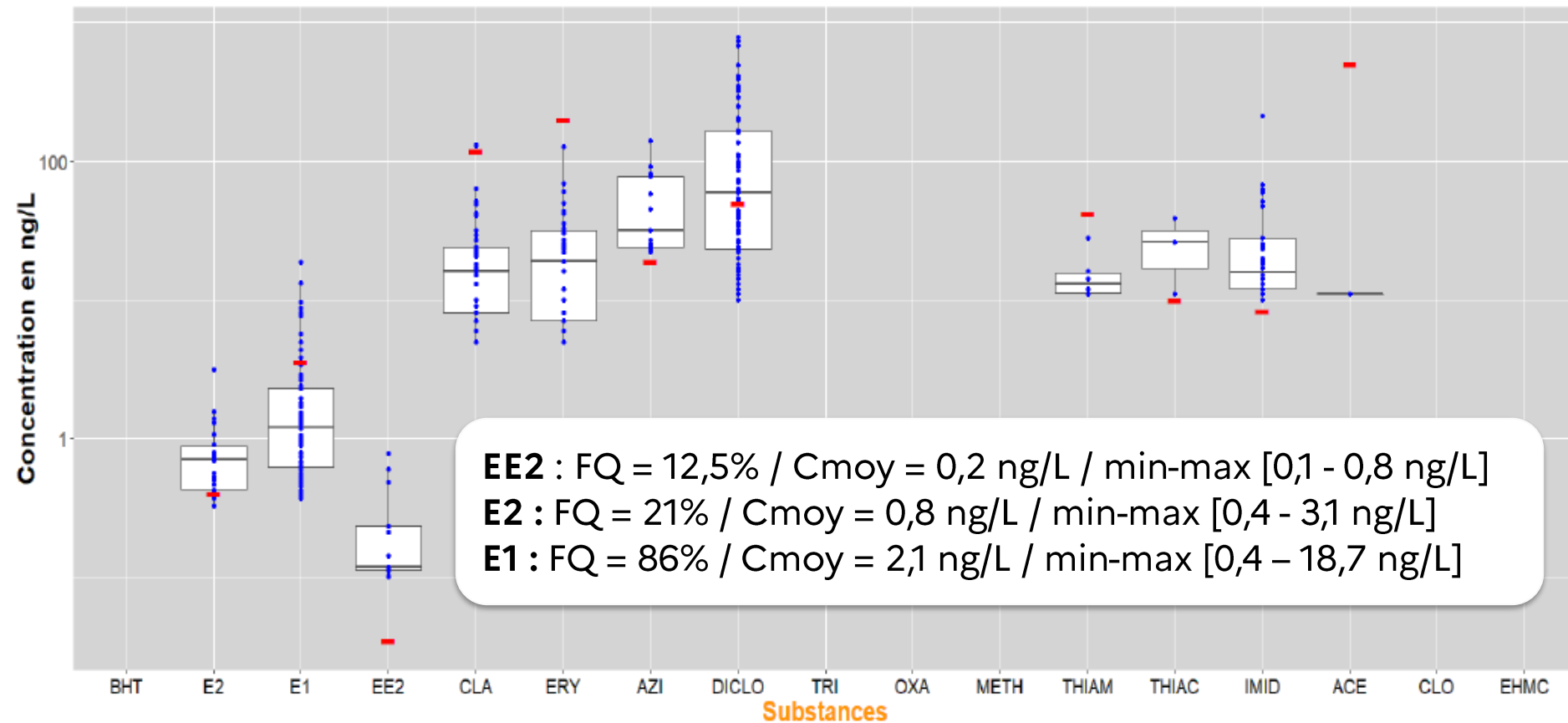
Agence Française
pour la Biodiversité

Ministère de l'Environnement
et du Climat

Sites WL FR



Substance - Acronyme	SANDRE	Limite de détection maximale acceptable (µg/L)	Analyse eau totale [3]	LOD (µg/L)	LOQ (µg/L)	Incertitude élargie à la LOQ (%) (k=2)
EE2	2629	0.000035	Jusqu'à 120mg/L de MES	0.000035	0,0001	50
E2	5397	0.0004	Jusqu'à 120mg/L de MES	0.0001	0.0004	35
E1	5396	0.0004	Jusqu'à 120mg/L de MES	0.0001	0.0004	35



INTÉRÊT DES MÉTHODES DE SURVEILLANCE BASÉES SUR LES EFFETS BIOLOGIQUES (EBM)

2014



2017



Recommendations for the use of effect-based methods for monitoring of estrogens in surface waters – Outcomes of the Dübendorf Workshop (21 June 2017)

In January 2012 the European Commission proposed *inter alia* that the highly potent estrogens Ethinylestradiol (EE2) and Estradiol (E2) should be prioritised for reduction measures in continental and coastal surface waters. This led to the decision of the Commission to include Ethinylestradiol (EE2), Estradiol (E2) and Estrone (E1) in the first EU Watch List for Union-wide monitoring (Commission Decision 2015/495) [1].

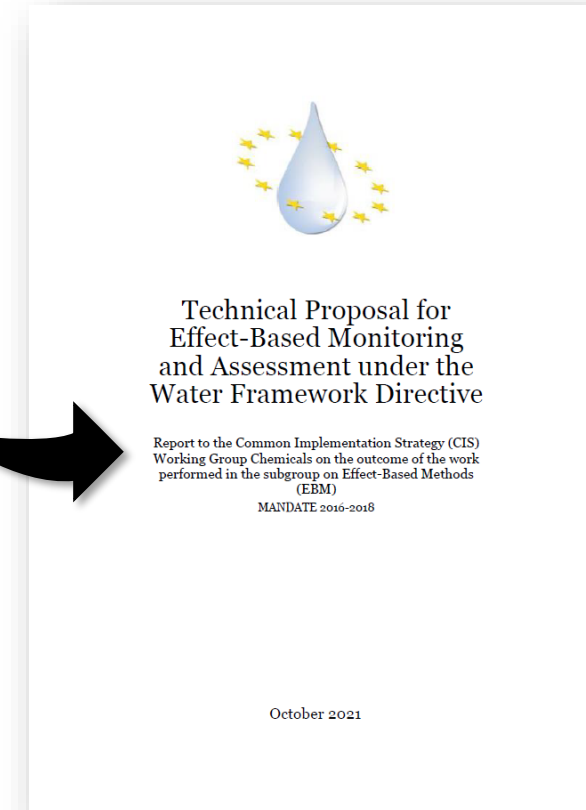
To address the analytical difficulties in monitoring these substances at reasonable cost, at the required threshold values (PNEC), two workshops were organised in February and in March 2013 and a first recommendation paper [2] was published, setting the basis for the actions needed for improved monitoring of estrogens and stressing the need to test the applicability of effect-based methods. The text is included in the annex of the EU technical report on effect-based tools [3].

The recommendation paper triggered the launch of the Science to Policy Interface (SPI) Estrogen monitoring project in 2014, as a voluntary initiative of 12 countries and 24 organisations in Europe under the leadership of Robert Kase and Mario Carere (<http://www.ecotoxcentre.ch/projects/aquatic-ecotoxicology/monitoring-of-steroidal-estrogens/>). The project demonstrated the feasibility of the use of *in vitro* effect-based methods for the measurement of estrogenic activity in water bodies.

Three years later, thanks to progress made in the estrogen monitoring project and in other research programmes, a new scenario has developed. The EU Water Directors welcomed and supported the proposal by the Commission to consider a more holistic approach for regulation of chemicals in the aquatic environment in view of the WFD review [4]. An Effect-Based Methods (EBM) Activity has been launched as part of the CIS-WFD programme [5]. And finally, the EBM results of the Estrogen monitoring project now provide concrete demonstration data about the performance of the tested effect-based methods (see upcoming publication Könnemann et al. 2017 [6] and Kase et al. 2017 [7]).

A further "Estrogen Monitoring recommendation workshop" was organised by Ecotox Centre at Eawag on 21st June 2017 in Dübendorf (Switzerland) to update, in the light of the new results of the estrogen monitoring project, the list of recommendations to be addressed to DG ENV as an input to the EBM Activity group for operational implementation of EBM in the regulatory framework as tools for assessment of estrogenic activity in surface water bodies. The workshop was moderated by Valeria Dulio and many EBM Activity participants as well as NORMAN and SOLUTIONS experts contributed to this outcome.

2021



Technical Proposal for Effect-Based Monitoring and Assessment under the Water Framework Directive

Report to the Common Implementation Strategy (CIS) Working Group Chemicals on the outcome of the work performed in the subgroup on Effect-Based Methods (EBM)

MANDATE 2016-2018

October 2021

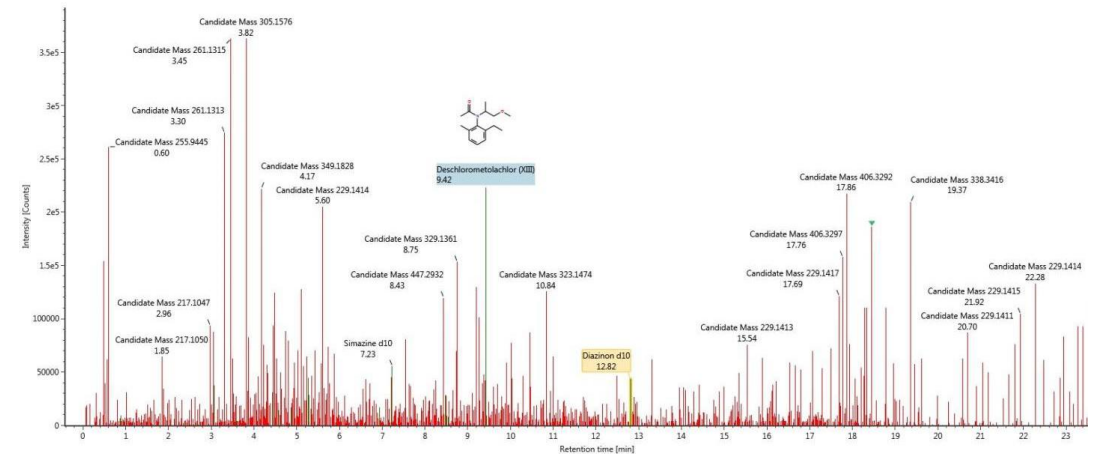
"In the WFD review, a more holistic approach, taking into account the presence of mixtures of chemicals acting together (for example through the use of effect-based tools in addition to group EQSs), could be considered, to provide a more accurate assessment of risks and a more appropriate targeting of monitoring and measures" (from discussion document later endorsed by the water directors)

INTÉRÊT DES MÉTHODES DE SURVEILLANCE BASÉES SUR LES EFFETS BIOLOGIQUES (EBM)

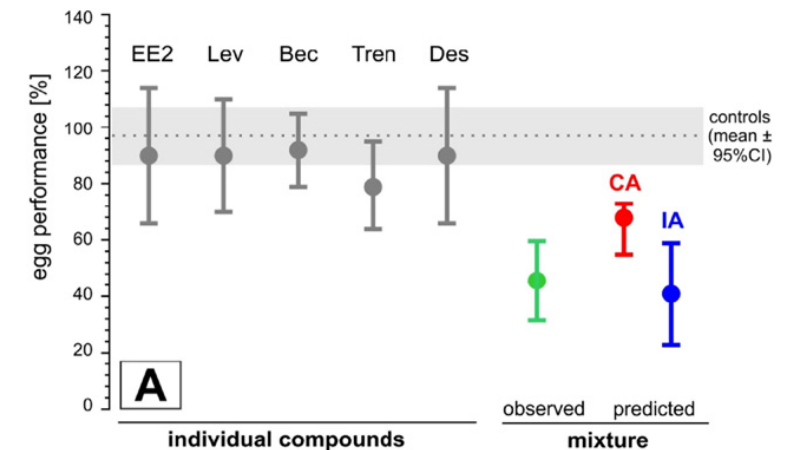
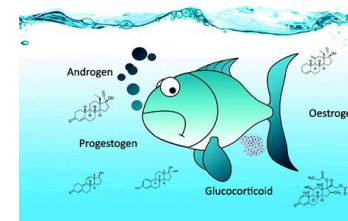
Les approches actuelles d'évaluation de la qualité des eaux (liste finie de substances, approche substance par substance) ne rendent que partiellement compte du risque « écotoxicologique » réel

- Présence de milliers de substances chimiques dans les milieux aquatiques dont on ne connaît souvent pas l'identité, ni la toxicité
- Dans les mélanges, les substances chimiques à leur niveau de concentration sans effet (NOEC) sont capables d'exercer une toxicité

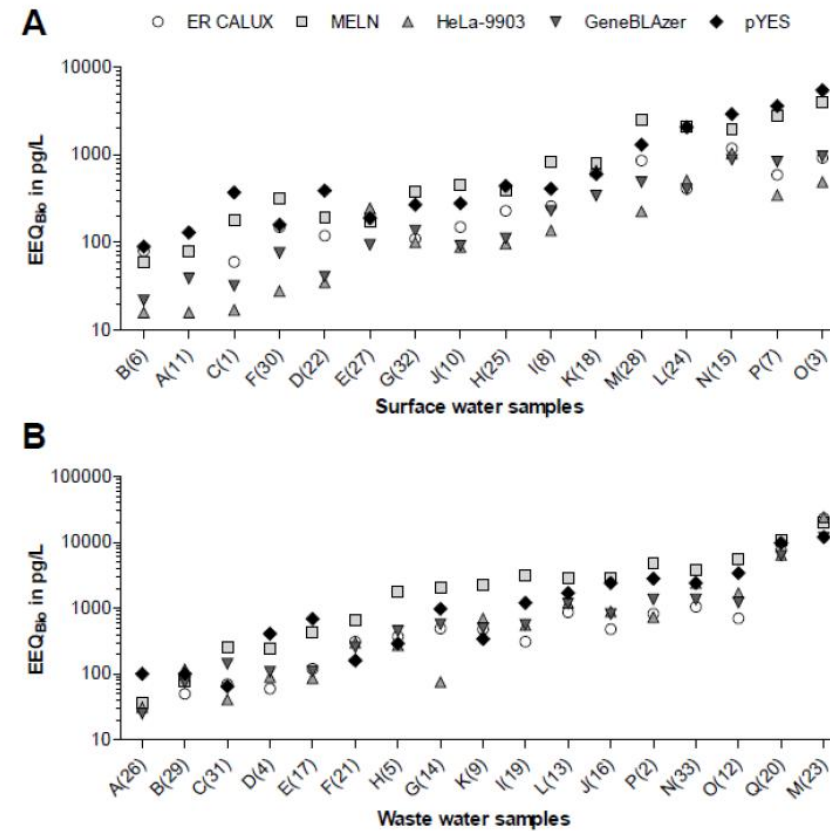
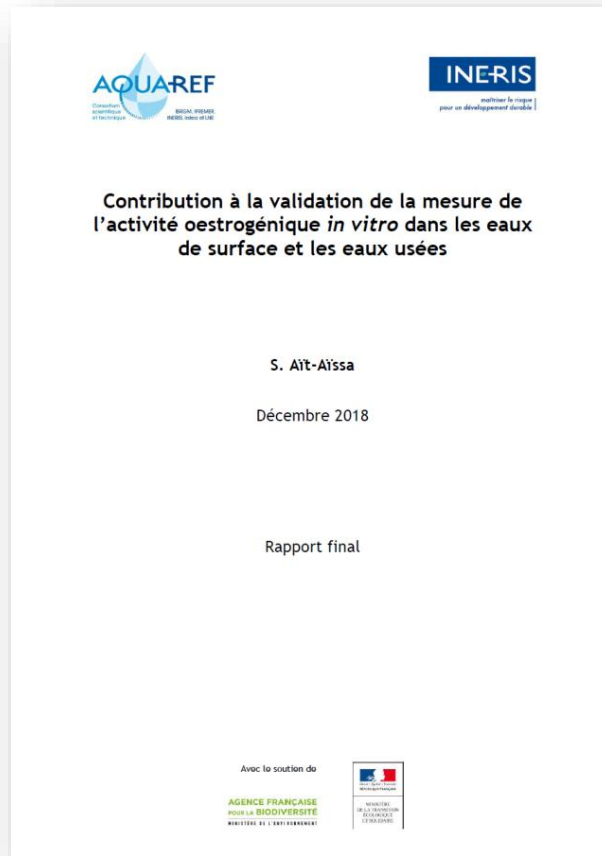
Bio-analyse = permet prendre en compte l'ensemble des substances présentes dans le milieu (y/c produits de transformation) et les effets de l'interaction de ces substances entre elles (synergie, antagonisme)



Source: Togola A, Lardy-Fontan S, Lestremau F, Soulier C. (2015), rapport BRGM/RP-65420-FR 75

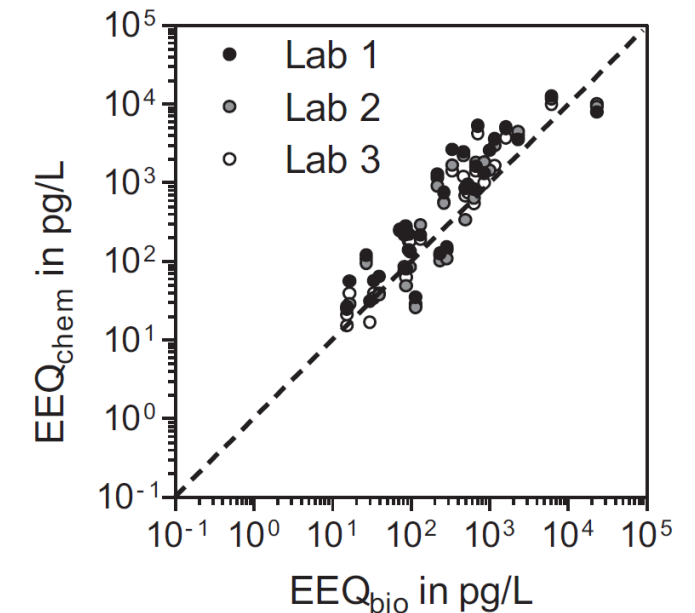
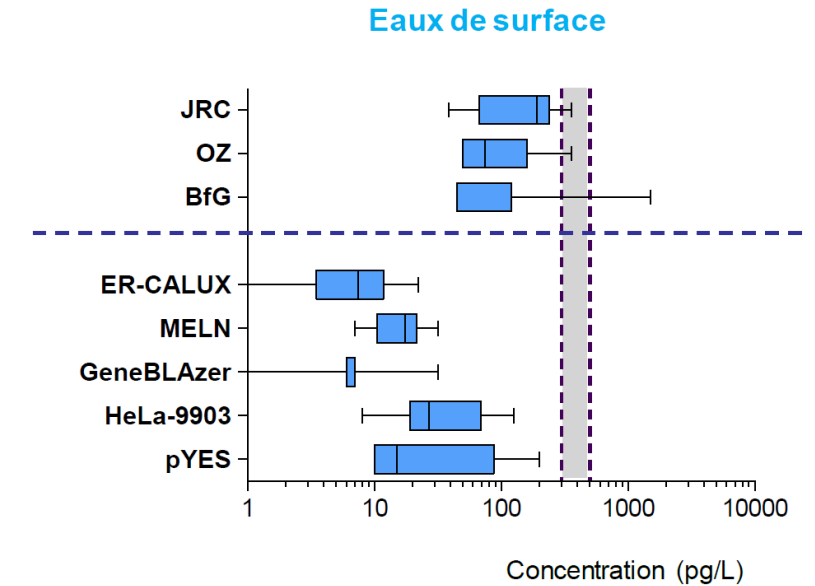


Source: Thrupp et al. (2018), Science of the Total Environment 619-620: 1482-1492



Analyse
HR-MS

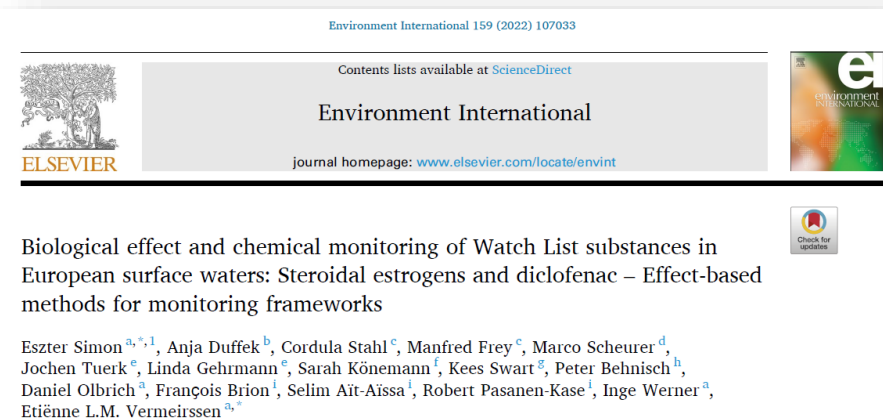
Bioessais



Etudes inter-laboratoires à l'échelle UE pour évaluer les performances (intra- et inter-essais) de 5 bioessais en comparaison avec les analyses chimiques ciblées de E1, E2 et EE2, pour des mélanges artificiels, des eaux de surface et des rejets d'eau usées

→ bonnes performances (répétabilité, reproductibilité, sensibilité et spécificité) des méthodes évaluées permettant de répondre aux exigences analytiques de surveillance des hormones œstrogéniques dans ces matrices environnementales

RÉSULTATS CONFORTÉS PAR DES CAMPAGNES UE COMPARANT MESURES PAR BIO-ANALYSE ET ANALYSES CHIMIQUES CIBLÉES



- >70 surface waters and 10 field blanks
- from 14 European countries
- collected between fall 2017 and spring 2018



Proposal, coordination and reporting

oekotoxzentrum
centre ecotox

Umwelt
Bundesamt

Sampling coordination,
sample processing and
distribution

BDS

Steroidal estrogens

oekotoxzentrum
centre ecotox

INE-RIS

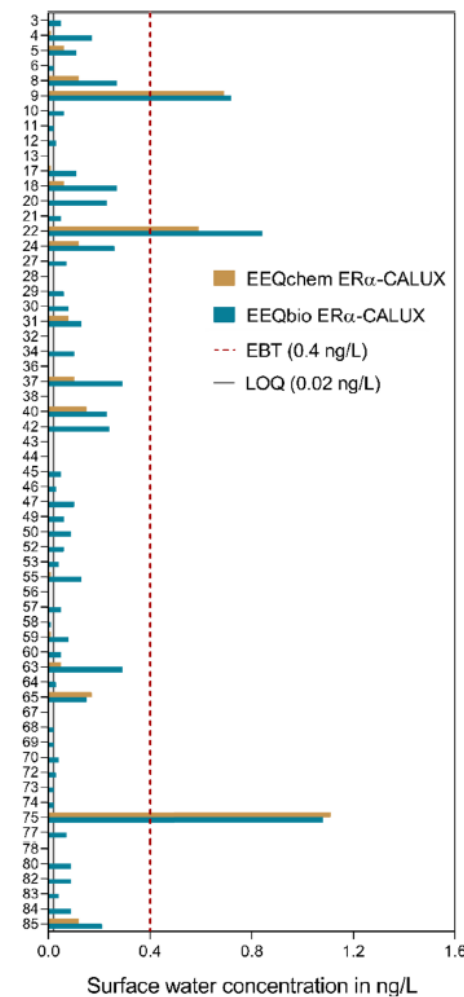
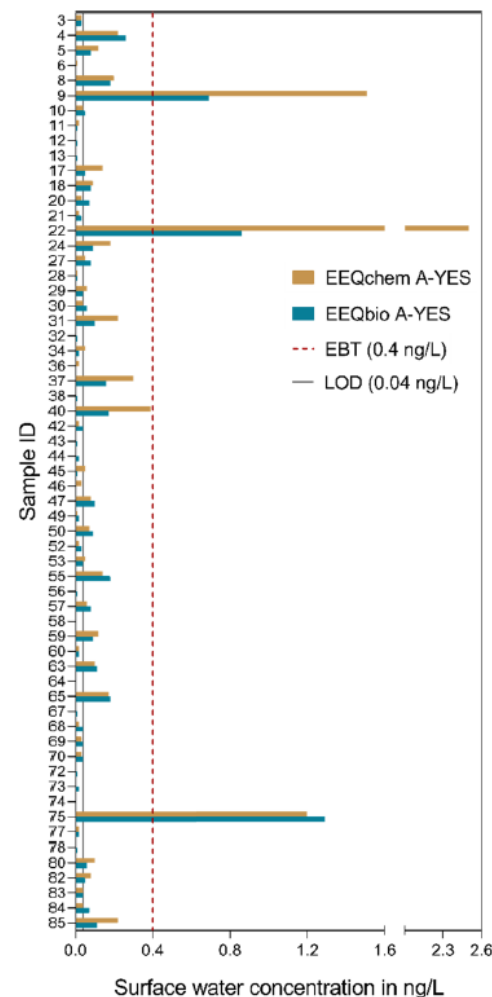
iuta

BDS

COX-inhibitors

TZW
Technologiezentrum
Wasser

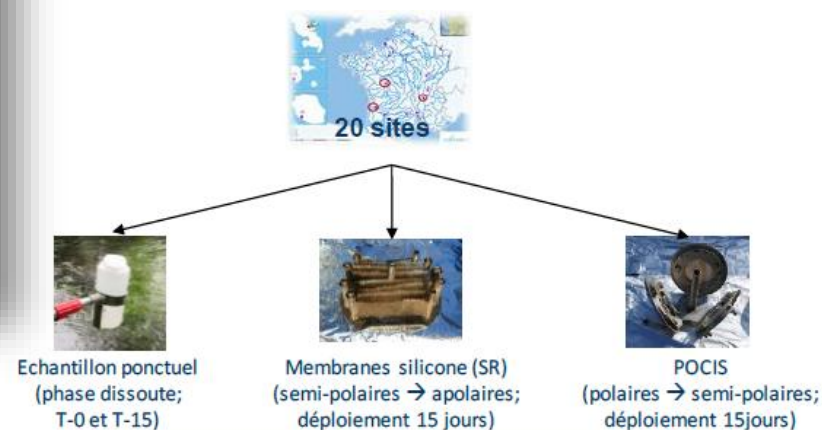
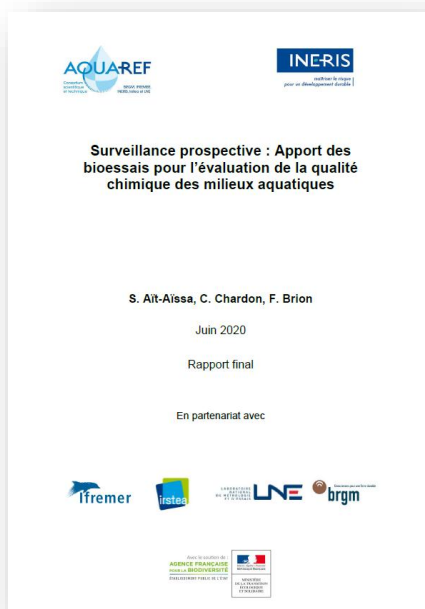
Steinbeis



71 échantillons d'eau de surface analysés

- Concentrations en E2-EQ comprises entre 0,04 ng/L et 1,3 ng/L (A-YES) et entre 0,02 ng et 1,1 ng/L (ER-CALUX)
- Davantage de mesures non quantifiées avec les analyses chimiques
- Seulement 3 échantillons dont les concentrations mesurées à l'aide des bioessais dépassent l'EBT

TEST DE L'UTILISATION DES BIOESSAIS *IN VITRO* (CONTEXTE DCE) → CHOIX DES STRATÉGIES D'ÉCHANTILLONNAGE



Profils toxicologiques *in vitro*

- Bioessais *in vitro* : perturbateurs endocriniens (ER, AR, GR) et dioxin-like (AhR)
- Comparaison ponctuel vs. EIP
- Priorisation de sites

Impact à l'échelle de l'organisme

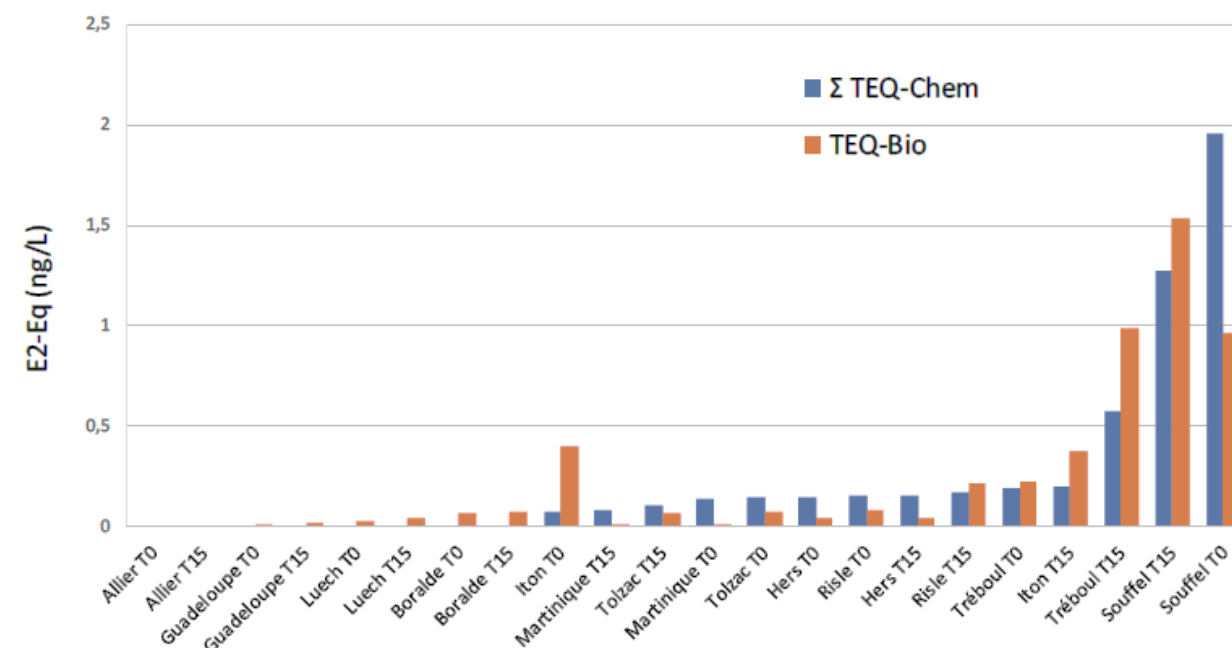
- Bioessai *in vivo* d'activité estrogénique
- Evaluation de valeurs seuils *in vitro*



Comparaison avec la chimie ciblée

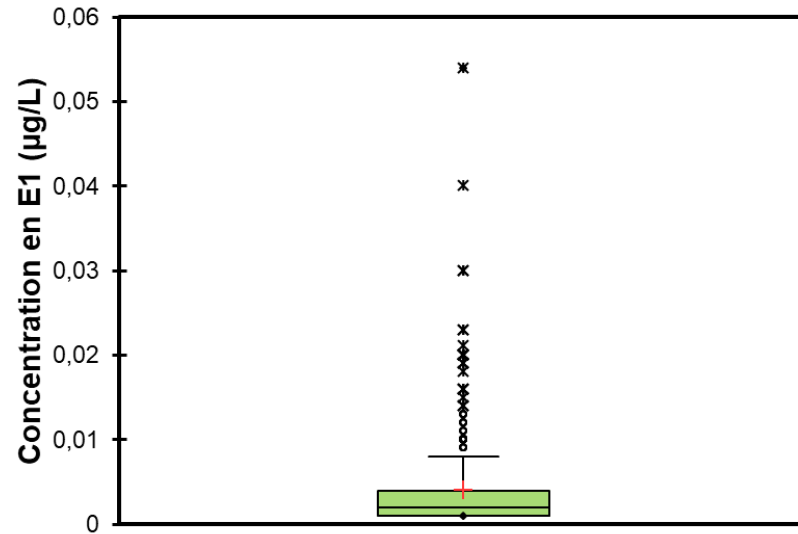
- Contribution de substances réglementaires (hormones)

	EAUX						POCIS						SR					
	ER	GR	AR	aAR	HAPL	DL	ER	GR	AR	aAR	HAPL	DL	ER	GR	AR	aAR	HAPL	DL
Allier (ref)		<17,6	<0,8	<1,3	16	<0,4			<0,03	<0,1	n.c.	n.c.	<0,002	<0,19	<0,01	<0,03	28	<0,009
Borlatte (ref)	<0,06	<14,2	<0,2	<1,3	50	<0,6	0,13	<1,0	<0,04	<0,1	n.c.	n.c.	<0,001	<0,08	<0,004	<0,01	65	0,02
Luech (ref)		<16,2	<0,7	<1,5	11	<0,4	0,06	<0,9	<0,04	<0,1	n.c.	n.c.	<0,009	<1,10	<0,04	<0,2	263	<0,034
Antifer	0,22	<13,2	<0,2	<1,2	<11	<0,4	0,29	3	<0,03	n.c.	n.c.	n.c.	0,04	<0,64	<0,02	<0,03	29	<0,015
Clain	1,28	<10,0	<0,3	<1,4	84	<0,2	0,04	<0,9	<0,11	<0,2	n.c.	n.c.	0,005	<0,17	<0,01	0,17	19	<0,009
Escaut	<0,04	<10,6	<0,2	<1,0	27	<0,3	3,36	<0,6	<0,03	<0,1	n.c.	n.c.	0,01	<0,26	<0,01	<0,01	57	0,06
Gier	8,78	115	n.c.	n.c.	73	<0,2	0,85	<1,1	0,64	n.c.	n.c.	n.c.	<0,002	<0,31	<0,01	<0,01	124	<0,024
Hers mort	<0,04	<8,6	<0,1	<0,7	30	<0,2	0,69	<2,8	<0,09	<0,1	n.c.	n.c.	<0,000	<0,02	<0,001	<0,002	4	0,01
Iton	0,38	870	n.c.	n.c.	105	<0,2	0,05	224	n.c.	n.c.	n.c.	n.c.	<0,001	6,87	n.c.	n.c.	14	0,02
Jalle	1,63	43	n.c.	n.c.	140	0,29	0,16	<0,5	<0,07	<0,1	n.c.	n.c.	<0,001	n.c.	0,08	n.c.	111	0,02
Lazaret	0,65	<12,9	<0,2	<1,0	13	<0,5	0,19	<0,4	<0,06	<0,1	n.c.	n.c.	<0,001	<0,26	<0,01	<0,02	27	<0,009
Risle	0,04	<13,9	<0,3	<1,3	41	<0,2	0,21	<0,9	<0,04	<0,1	n.c.	n.c.						
Rosselle	2,92	24	n.c.	n.c.	205	0,26	10,56	n.c.	1,57	n.c.	n.c.	n.c.	0,04	n.c.	0,85	n.c.	309	0,15
Souffel	1,25	59	n.c.	n.c.	99	<0,2	4,05	n.c.	1,03	n.c.	n.c.	n.c.	0,03	n.c.	0,48	n.c.	48	0,05
Tolzac	<0,06	<16,3	<0,2	<1,5	46	<0,7	0,25	<1,4	<0,07	<0,2	n.c.	n.c.	<0,002	<0,12	<0,01	<0,01	12	<0,009
Tréboul	0,60	<12,0	<0,2	<1,1	26	<0,3	0,33	n.c.	0,09	n.c.	n.c.	n.c.	<0,004	<0,41	<0,02	<0,04	39	<0,021
Vilaine	3,20	98	n.c.	n.c.	298	<0,3	0,77	n.c.	0,29	n.c.	n.c.	n.c.	<0,010	n.c.	0,56	n.c.	1182	0,24
Etang du Gol	<0,05	<10,9	<0,2	<0,8	54	<0,3	<0,05	<3,2	<0,15	<0,4	n.c.	n.c.	<0,001	<0,31	<0,01	<0,01	24	0,01
Grand Galion	<0,01	<13,3	<0,2	<0,5	57	<0,2	<0,01	<0,9	<0,04	<0,1	n.c.	n.c.	<0,001	<0,20	<0,01	<0,01	32	0,01
Pérou-pères	<0,01	<14,2	<0,3	<0,5	49	<0,2	0,12	<1,7	<0,18	<0,5	n.c.	n.c.	<0,001	<0,10	<0,004	<0,003	42	0,01



ANALYSE DES DONNÉES BANCARISÉES DANS NAÏADES (2022-2026)

Paramètre 5396



Statistique	RsAna
Nb. d'observations	506
Minimum	0,001
Maximum	0,054
1er Quartile	0,001
Médiane	0,002
3ème Quartile	0,004
Moyenne	0,004
Moyenne géométrique	0,003

E1 (eau brute et phase aqueuse)

- 506 mesures quantifiées sur plus de 21 000 analyses
- 100% mesures quantifiées > valeur NQE (0,36 ng/L)
- 100% des LQ des mesures non quantifiées > valeur de la NQE (LQ min = 1 ng/L)

Paramètre 8512



E2-EQ (eau brute)

- 132 analyses, aucune mesure quantifiée
- LQ varient entre 0,005 et 0,1 µg/L

Paramètre 5397



E2 (eau brute et phase aqueuse)

- 8 mesures quantifiées sur 18 843 analyses
- 100% mesures quantifiées > valeur NQE (0,18 ng/L)
- 100% des LQ des mesures non quantifiées > valeur de la NQE (LQ min = 1 ng/L)

**Merci de votre
attention**

