

Screening of oestrogen-like activity in tap and mineral drinking waters

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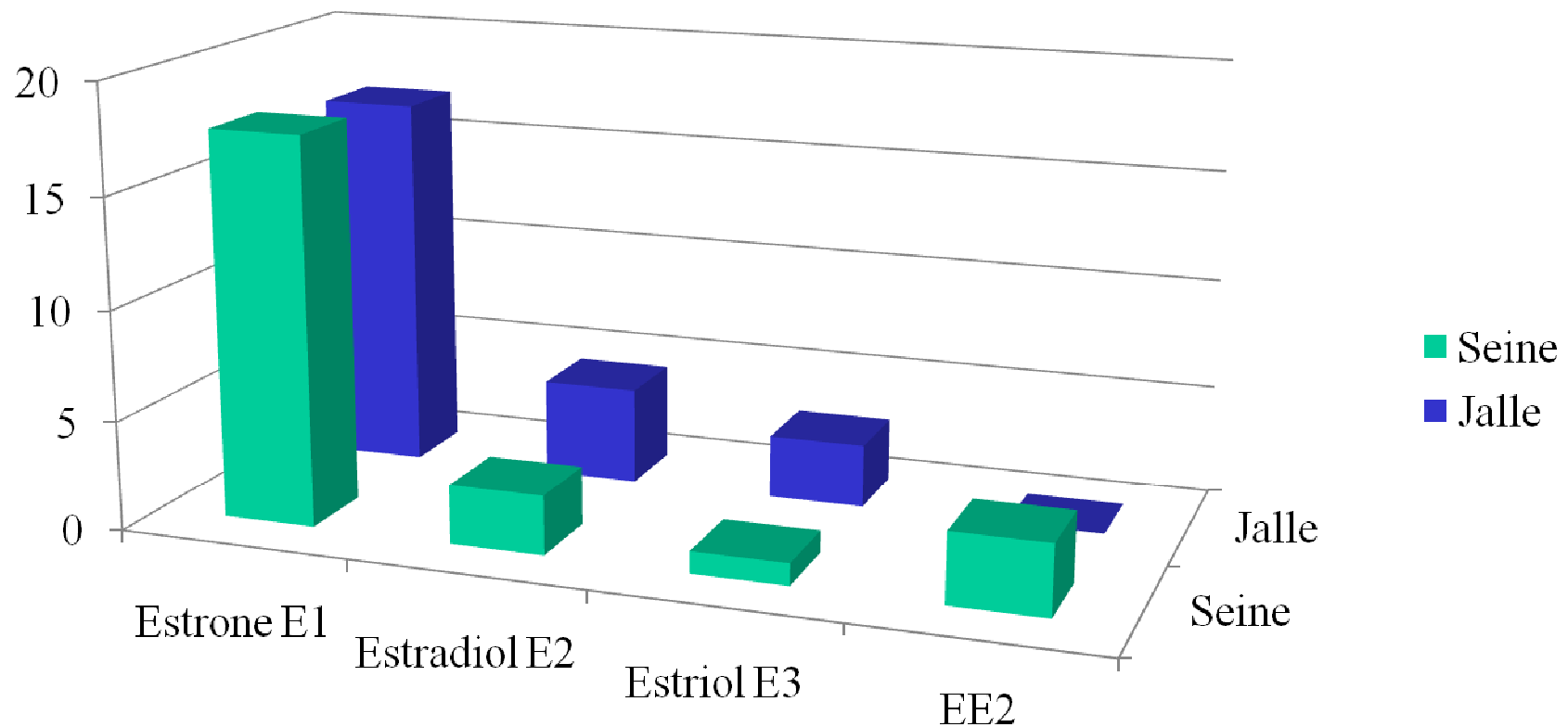
Background

- Estrogenic compounds in water
- Tools to detect estrogenic activities
- Theoretical Estrogenic potencies in waters
- Estrogenic potencies measured in waters

Concentrations of EDCs in surface water and drinking water ($\mu\text{g/l}$)

	Surface water	Drinking water
• <u>Hormones</u>		
– estron	1 - 10 ng/l	5 ng/l
– 17 β -estradiol	1 - 5 ng/l	< 0,3 - <1 ng/l
– 17 α -ethinylestradiol	1 - 5 ng/l	< 0,3 - <1 ng/l
• <u>Phthalates</u>		
– DEP	1 – 10 $\mu\text{g/l}$	1 - 2,1 $\mu\text{g/l}$
– DEHP	1 - 5	1,3 - 2,5
• <u>Alkylphenoethoxylates</u>		
– Nonylphenoethoxylates	0,1 - 4,6	0,5 - 2
– Octylphenoethoxylates	0,1 - 0,7	< 0,5
• Bisphenol a	0,01 - 1	0,01 - 0,1
• Pesticides	0,01 - 5	0,01 - 0,1
• PCBs	0,01 - 0,02	< 0,01
• PCAs	0,01 - 0,1	< 0,01

Assessment of river contamination by estrogenic compounds (ng/l), in Paris and Bordeaux areas



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IWA affiliate

**Global Water
Research Coalition**

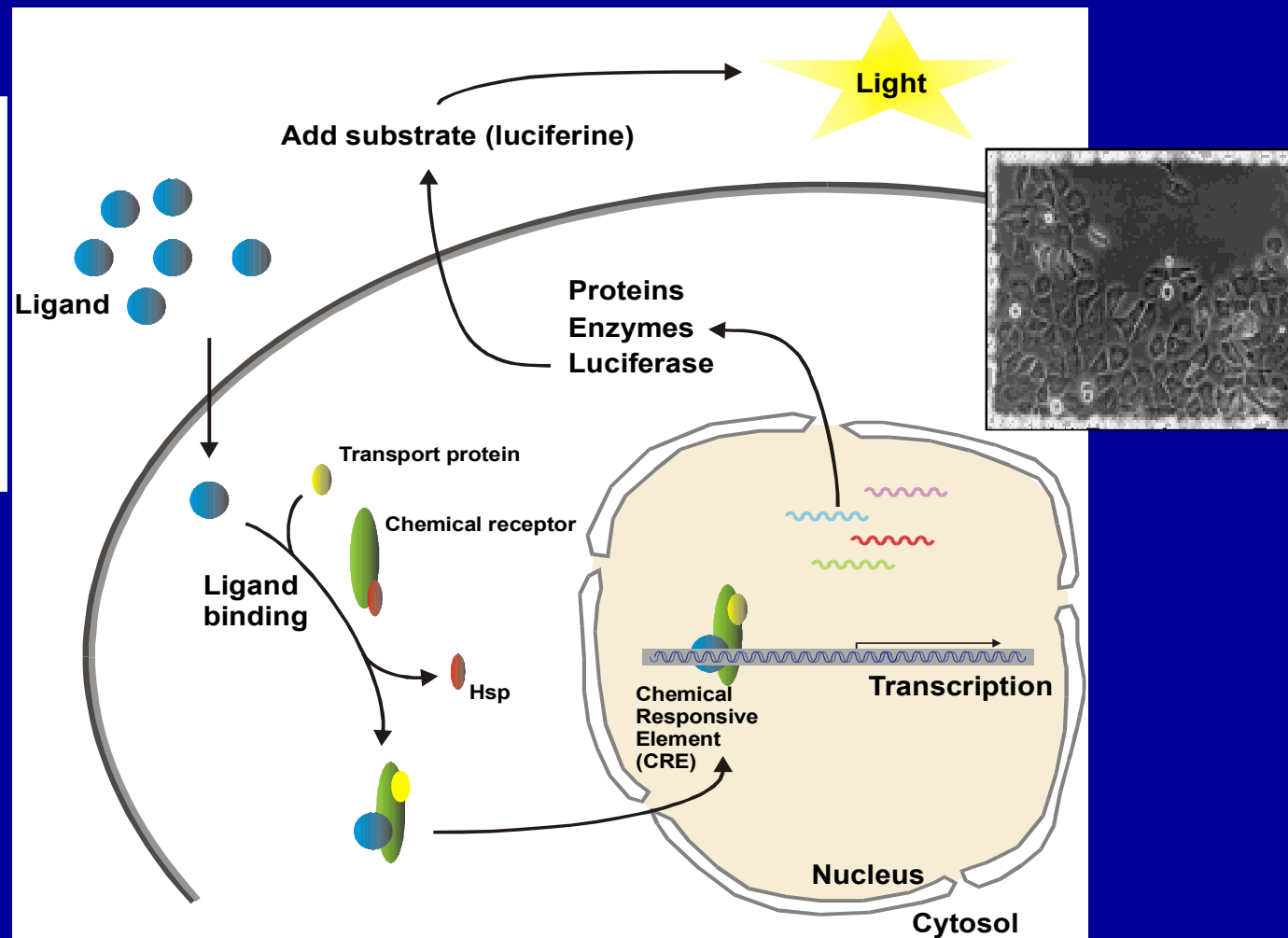
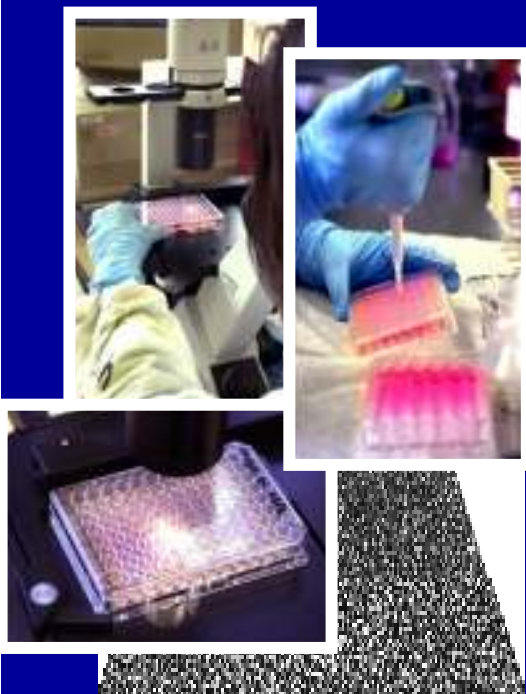
TOOLS TO DETECT ESTROGENIC ACTIVITY IN ENVIRONMENTAL WATERS

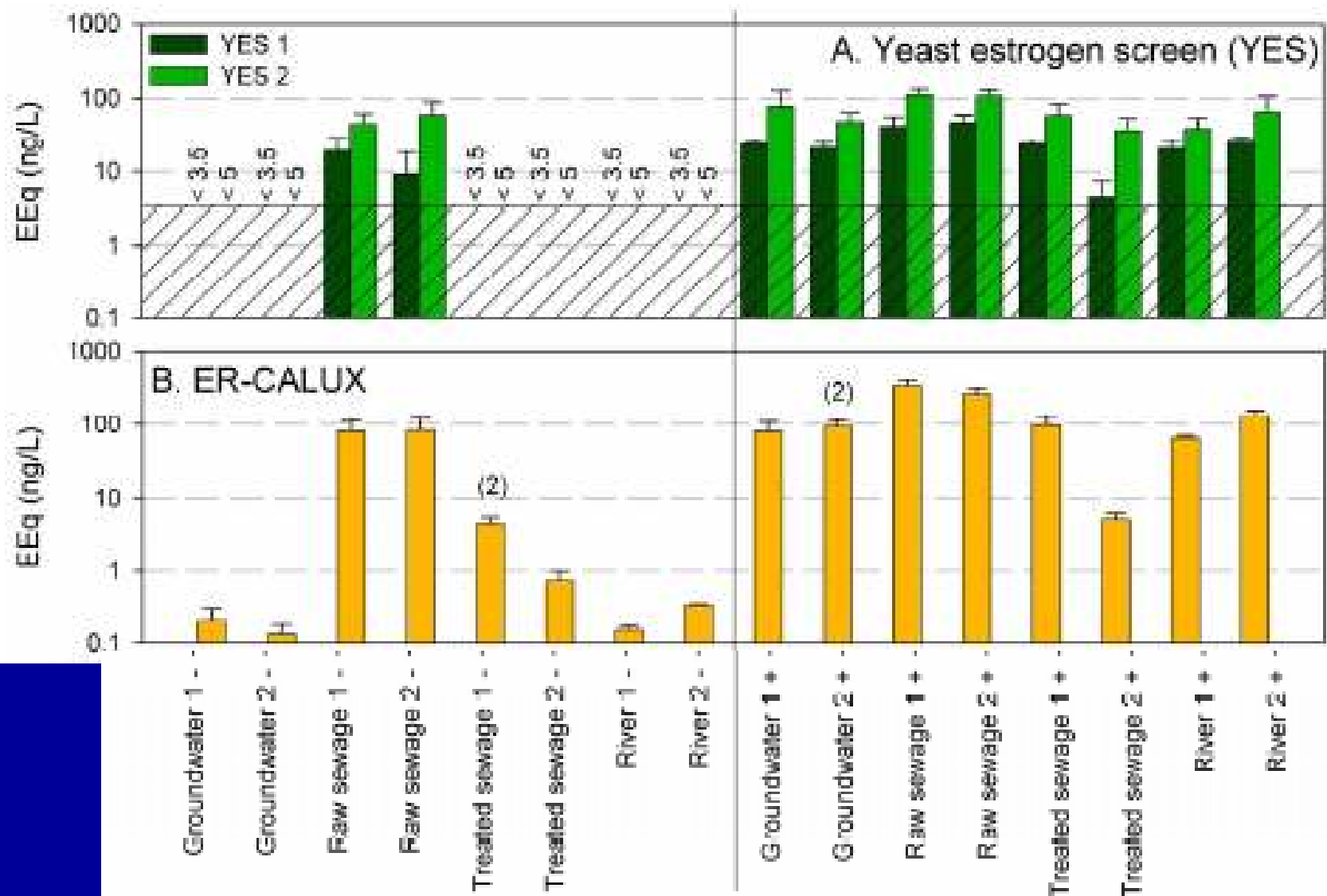
by:

Frederic DL Leusch, Ph.D.
CRC Water Quality and Treatment

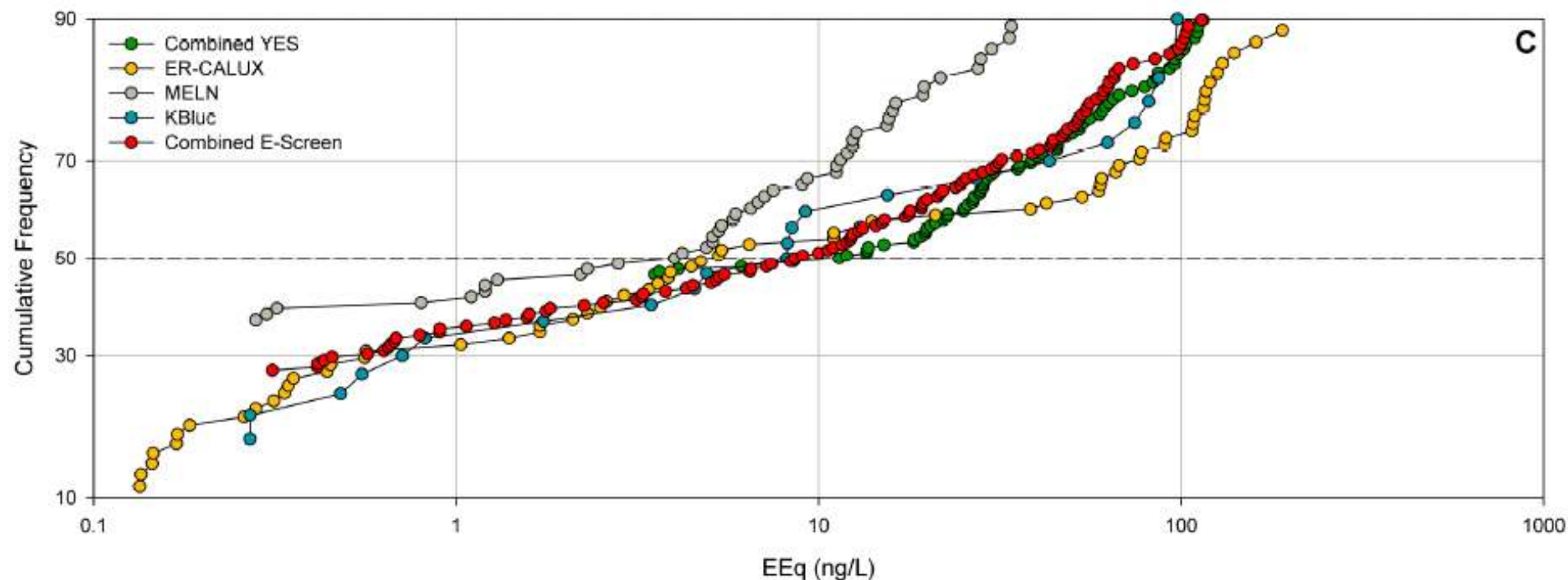
2008

ER-CALUX[®] method to screen ER active compounds (T47D)





Estrogenicity (Expressed as Estradiol Equivalents, EEQ) in the Environmental Samples as Determined by Bioassay.



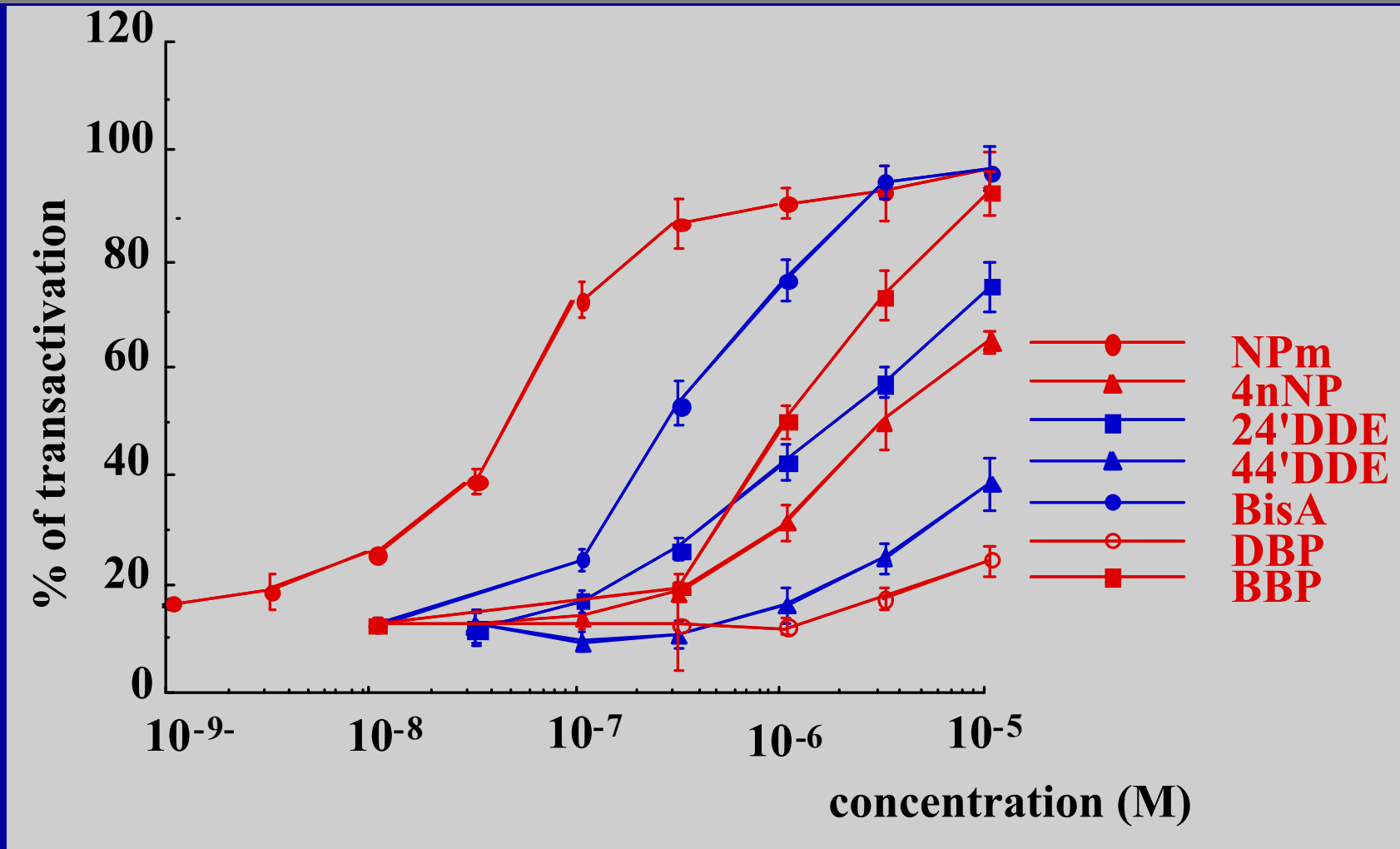
Kaplan-Meier Descriptives for Bioassay Data

Assay	Median EEq (ng/L)	Censored data ^(a) (n ₀)	Uncensored data (n ₁)	Sample size (n)
YES 1	5.52	39	45	84
YES 2	14.8	38	46	84
Combined YES	11.4	77	91	168
ER-CALUX	5.30	8	73	81
MELN	4.10	30	54	84
KBluc	8.18	4	26	30
E-Screen 1	7.28	22	62	84
E-Screen 2	12.4	23	61	84
Combined E-Screen	8.57	45	123	168

Notes: Nondetects included in the survival analysis as “non-events”.

(a) Censored data = number of non-detects.

ER α cells enable the detection of estrogenic activity of chemicals



Potency of natural, phyto- and synthetic estrogens relative to estradiol in the ER-CALUX assay (Murk et al 2002)

Compound	Relative potency	Compound	Relative potency
Natural Estrogens:		Synthetic estrogens:	
17 β -Estradiol	1	Ethynyl-estradiol	1.2
17 α -Estradiol	5.6×10^{-2}	Diethylstilbestrol	0.1
Estrone	1.6×10^{-2}		
Estriol	1.0	Alkylphenols:	
Estradiol 3 β -D-glucuronide	n.c.*	4-nonylphenol (NP)	2.3×10^{-5}
Phyto-estrogens:		4-octylphenol (OP)	1.4×10^{-6}
Genistin	2.6×10^{-4}	4-tert-pentylphenol	2.3×10^{-5}
Diadzein	1.3×10^{-4}	NP1EO#	3.8×10^{-6}
Formononetin	1.1×10^{-4}	NP2EO#	1.1×10^{-6}
Biochanin A	5.3×10^{-4}	NP4EO#	1.1×10^{-7}
Genistein	6.0×10^{-5}	NP10EO#	n.c.*
Pesticides:		NP1EC#	n.c.*
o,p'-DDT	9.1×10^{-6}	NP2EC#	n.c.*
o,p'-DDE	2.3×10^{-6}	OP8/9EO#	n.c.*
Methoxychlor	1.0×10^{-6}	Phthalates:	
Dieldrin	2.4×10^{-7}	dimethylphthalate	n.c.*
Endosulfan	1.0×10^{-6}	diethylphthalate	3.2×10^{-8}
Chlordane	9.6×10^{-7}	dibutylphthalate	1.8×10^{-8}
Simazine	n.c.*	butylbenzylphthalate	1.4×10^{-6}
Atrazine	n.c.*	di 2-ethylhexy phthalate	n.c.
Desethylatrazine	n.c.*	dioctylphthalate	n.c.*
Deisopropylatrazine	n.c.*	bisphenol A	7.8×10^{-6}
Kepone	5.2×10^{-6}	Benzo (a) pyrene	1.9×10^{-6}
Lindane	n.c.*		

Background

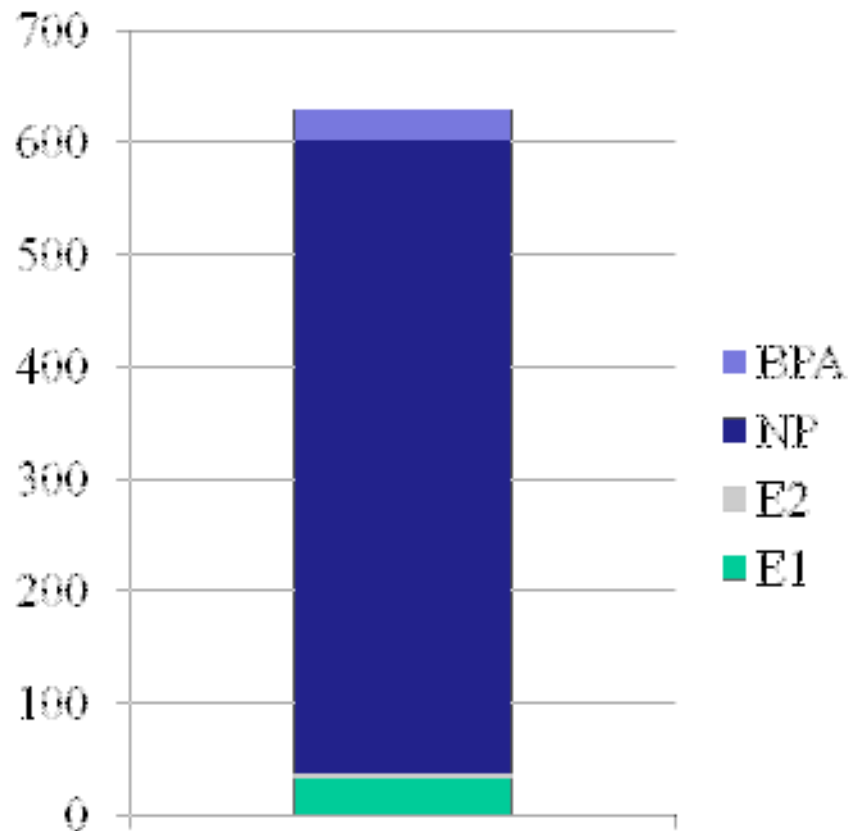
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Theoretical Estrogenic potencies in waters

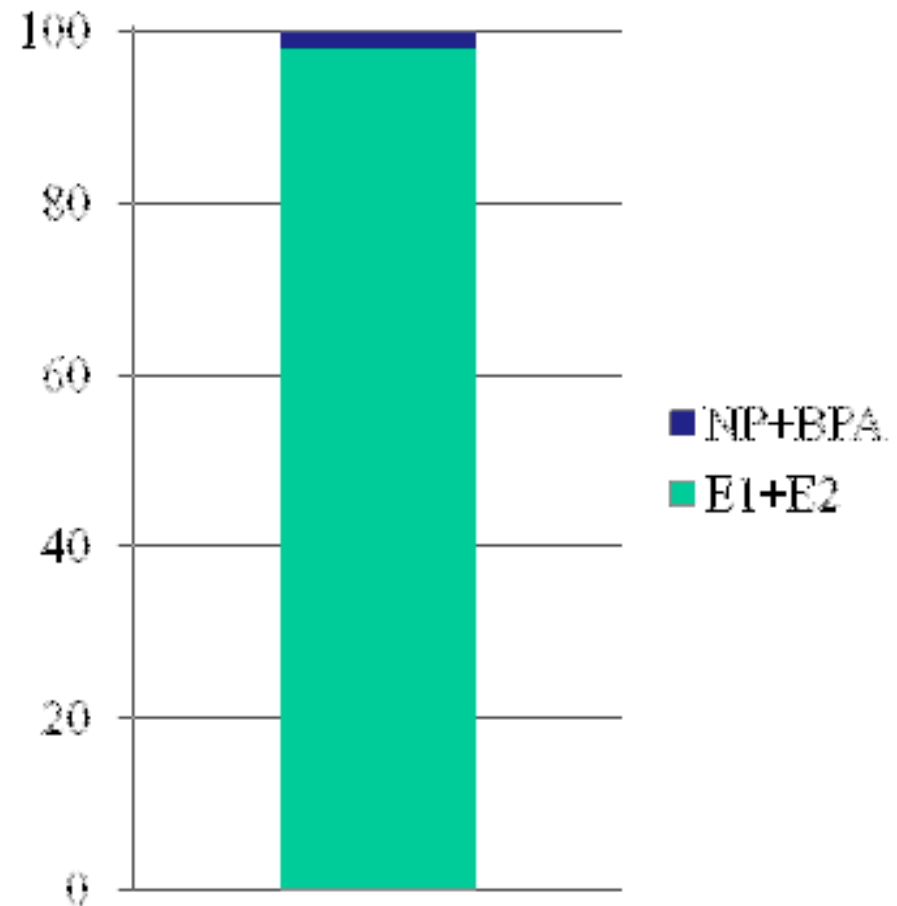
Compounds	EEFs	Mineral Waters		Tap Waters	
		Max value/l	EEQ/l	Max value/l	EEQ/l
ΣPhtalates	10 ⁻⁶	20 µg	20 pg	4 µg	4 pg
BPA	10 ⁻⁵	10 µg	100 pg	0.1 µg	10 pg
NP	2 x10 ⁻⁵	0.5 µg	10 pg	2 µg	40 pg
Pesticides	10 ⁻⁶			0.1 µg	0.1 pg
Σ organics			130 pg		55 pg
Antimony	1.5x10 ⁻³	2 µg	3 ng		
Estradiol	1			0.3 ng	0.3 ng
Estrone	1.6x10 ⁻²			5 ng	0.08 ng
Ethynyl-E	1.2			0.3	0.36
Σ EEQ			3.13 ng		0.8 ng

TTC: 1.5 µg/l

Identification of estrogenic compounds in wastewater effluent



Concentrations ng/L

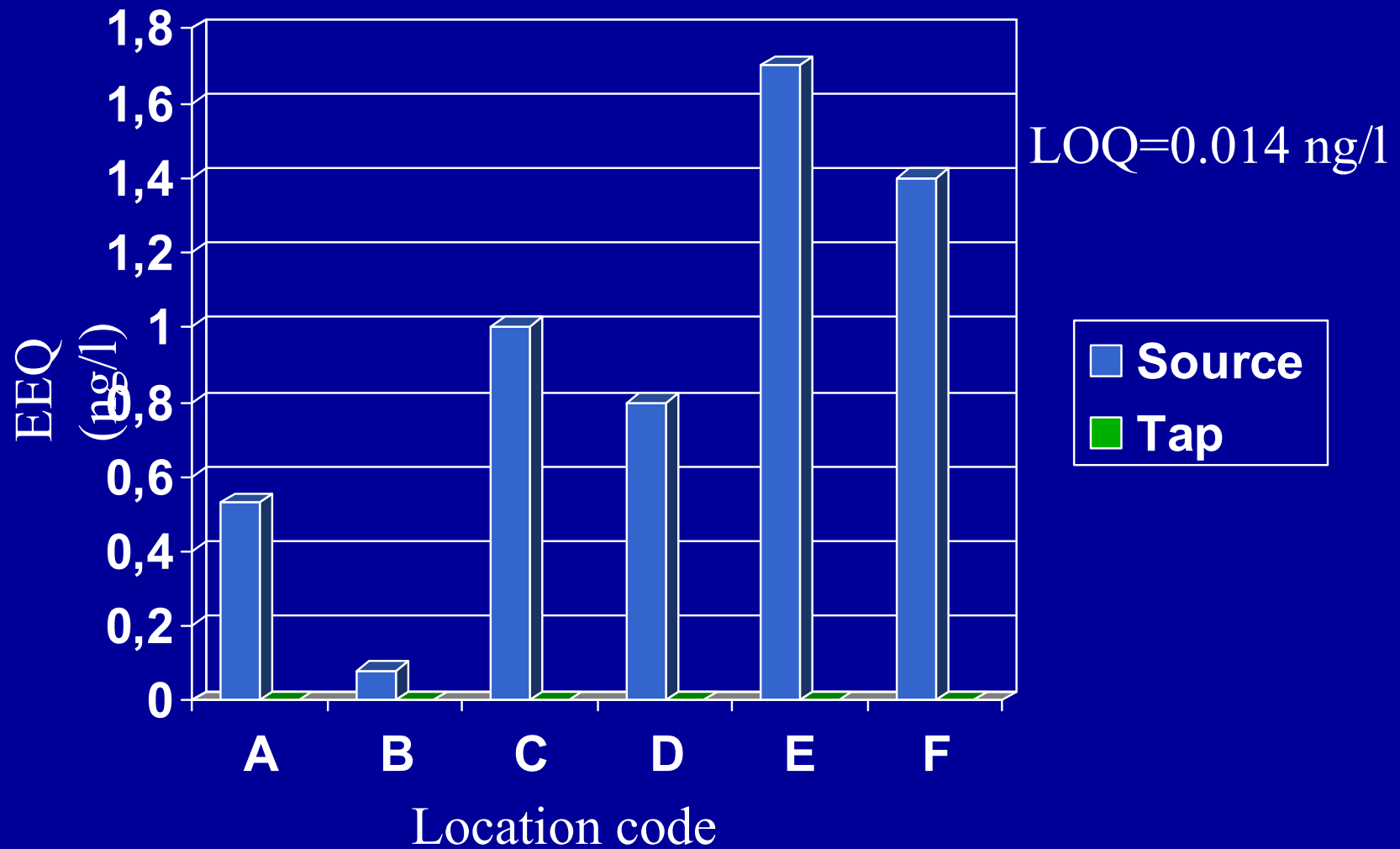


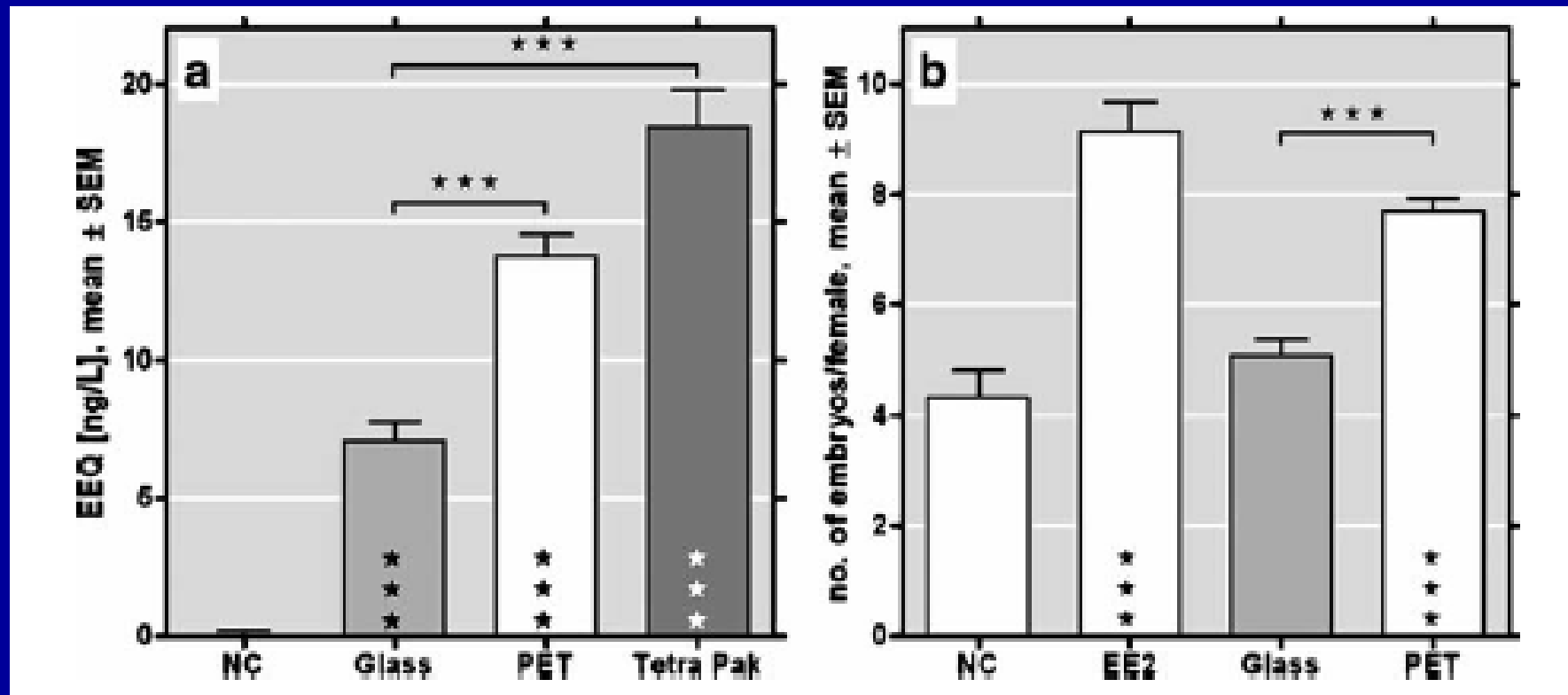
% EEQ (ER-CALUX)

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Estrogenic activity in drinking water before and after treatment measured with ER-CALUX





Estrogenic potencies of mineral water in vitro (a) and reproduction of *Potamopyrgus antipodarum* (b). Data were pooled according to packaging material. a Estrogenic potencies (EEQ) measured with the yeast estrogen screen. Negative control (NC), n=226; glass, n=610; PET, n=620; Tetra Pak, n=133. b Number of embryos produced by *Potamopyrgus antipodarum* after a 56-day period of culturing in glass and plastic bottles. Negative control (NC) and positive control (EE₂), n=60; glass, n=240; PET, n=360. Triple stars p<0.001 as determined by Mann-Whitney test

Discussion

- *The estrogenicity reported in this study might not arise only from plastic compounds (minors?) but from water source or process contamination (glass bottles) and/or unexpected compounds*
- Shotyk et al. (2006) found antimony in up to 30 times higher concentrations in mineral water from PET compared to glass bottles and confirmed its leaching from PET (Shotyk and Krachler 2007), in whose manufacturing antimony trioxide is used as catalyst.
- The maximum concentrations detected in mineral water (1–2 µg/l) have been shown to exhibit estrogenic activity in vitro (Choe et al. 2003)

Aim of the study

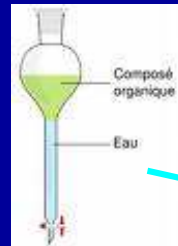
- The aim of this study was to assess the risk of exposure of the population to chemicals with estrogen-like activity through drinking waters
- **Comparison between**
 - Mineral and Tap waters (from surface water or ground water).
 - Glass and PET bottles
 - Storage time
 - Extraction protocols
- Six commercial brands of mineral water purchased from local markets and tap water were collected from French cities.

Materials and Methods 1

About 1000 ml water sample

Liquid-liquid extraction (Ethyl acetate)

Organic fraction ←



water fraction is discarded



Extract dissolved in 50 μ l of DMSO

Added to exposure medium



ER α + β CALUX[®] assay
24h incubation (0.1% DMSO)

Net concentration factor : 20



YES assay
3 to 5 days incubation
(Routledge & Sumpter, 1996)

Parameters 1 & 2: Glass/PET, ER-CALUX/YES

Brand ID number	Material	ER-CALUX®	YES Assay
A1	Glass	< LOD	< LOD
A2	PET	< LOD	< LOD
B1	Glass	< LOD	< LOD
B2	PET	< LOD	< LOD
C	PET	< LOD	< LOD
D	PET	< LOD	< LOD
E	PET	< LOD	n.m
F	PET	< LOD	n.m
TW-GW1		< LOD	n.m
TW-SW1		< LOD	n.m
TW-GW2		< LOD	n.m
TW-SW3		< LOD	n.m
Positive Control E2		71 ± 4 ng EEQ/l	68 ± 37ng EEQ/l

E2 : 200 µM

LOD ER α + β -CALUX ®= 0.008 ng EEQ/l

²²
LOD YES = 0.100 ng EEQ/l

Parameter 3: Time of storage

Brand ID number	Material	0 days	2 years
A1	Glass	< LOD	< LOD
A2	PET	< LOD	< LOD
B1	Glass	< LOD	< LOD
B2	PET	< LOD	< LOD
C	PET	< LOD	< LOD
D	PET	< LOD	< LOD
E	PET	< LOD	< LOD
F	PET	< LOD	< LOD

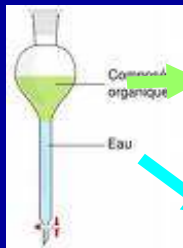
E2 : 200 μ M

LOD ER α + β -CALUX $\text{\textcircled{R}}$ = 0.008 ng EEQ/l

Materials and Methods 2

40 ml of water sample + 40ml of water sample

Liquid-liquid extraction
(Ethyl acetate)



Organic fraction is discarded

Water fraction

Freeze-dried

Redisolved in 2ml
Exposure medium

ER α CALUX®
ER β CALUX®

« **After extraction** »

Freeze-dried

Redisolved in 2ml
Exposure medium

ER α CALUX®
ER β CALUX®

« **Before extraction** »

Results of analysis 2

Brand ID number & Material	BEFORE EXTRACTION		AFTER EXTRACTION	
	ER α -CALUX [®] ng E2-eq./L	ER β -CALUX [®] ng E2-eq./L	ER α -CALUX [®] ng E2-eq./L	ER β -CALUX [®] ng E2-eq./L
MW1 Glass	<LOD	<LOD	<LOD	<LOD
MW2 Glass	<LOD	<LOD	<LOD	<LOD
MW3 Glass	<LOD	<LOD	<LOD	<LOD
MW4 Glass	<LOD	<LOD	< LOQ	< LOQ
MW1 PET	<LOQ	<LOD	<LOQ	<LOD
MW2 PET	<LOD	<LOD	<LOD	<LOD
MW3 PET	<LOD	<LOD	<LOQ	<LOD
MW4 PET	<LOD	<LOD	<LOQ	<LOD
MW5 PET	<LOD	<LOD	<LOD	<LOD
MW6 PET	<LOD	<LOD	<LOD	<LOD

LOD ER α -CALUX[®] = 0.01 ng E2-eq./L

LOD ER β -CALUX[®] = 0.1 ng E2-eq./L

LOQ ER α -CALUX[®] = 0.025 ng E2-eq./L₂₅

LOQ ER β -CALUX[®] = 0.3 ng E2-eq./L

RESULTS

- The quantification limit of the ER-CALUX was 25 times lower than measured for the YES test.
- The result shows that all the water samples did not exhibit any appreciable estrogenic activity (below the detection limit of 0.008 ng EEQ/l).
- No difference was found between PET and glass bottles mineral waters, and between mineral and tap waters.
- No effect of storage time
- No significant activity in organic and water soluble extracts
- These results are according with the migration limits and the drinking water regulations.