Stadt Zürich
Wasserversorgung

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Zurich University
of Applied Sciences

zh aw

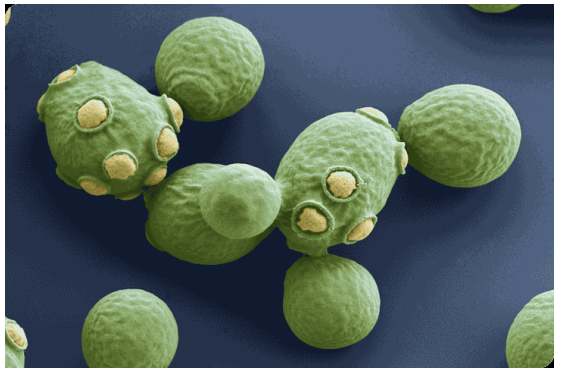
Life Sciences and
Facility Management

Institute of
Natural Resource Sciences

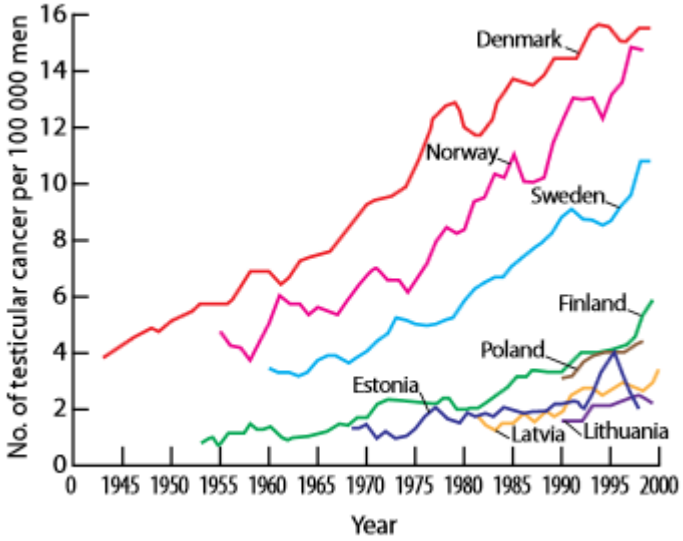
Screening untreated drinking water on estrogenic activity

using the planar-YES, a TLC-EDA tool

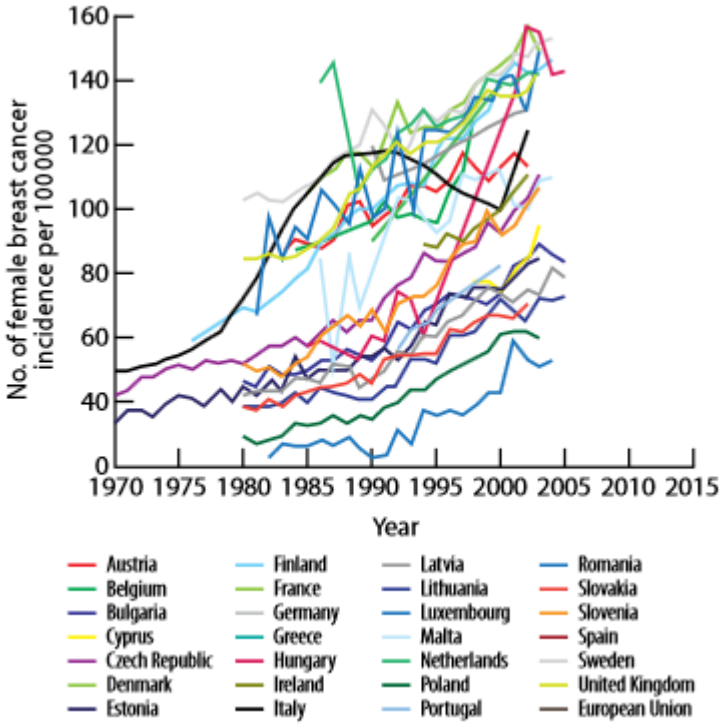
Andreas Schoenborn // July 3, 2014 // HPTLC 2014 Lyon



Testicular & breast cancer increase (WHO)



Richiardi et al., 2004



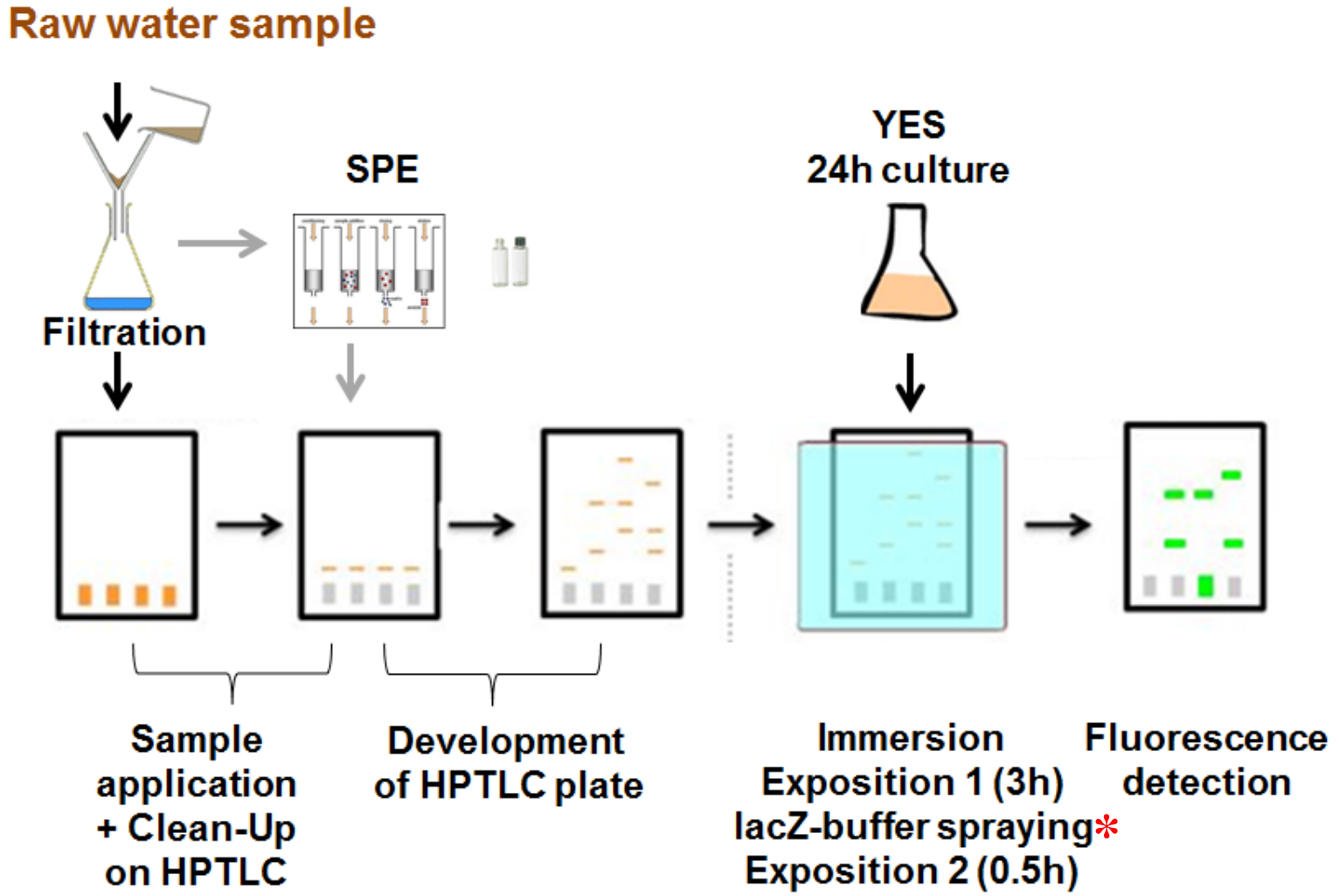
http://data.euro.who.int/hfadb/

Quelle: WHO 2013

Four study objectives

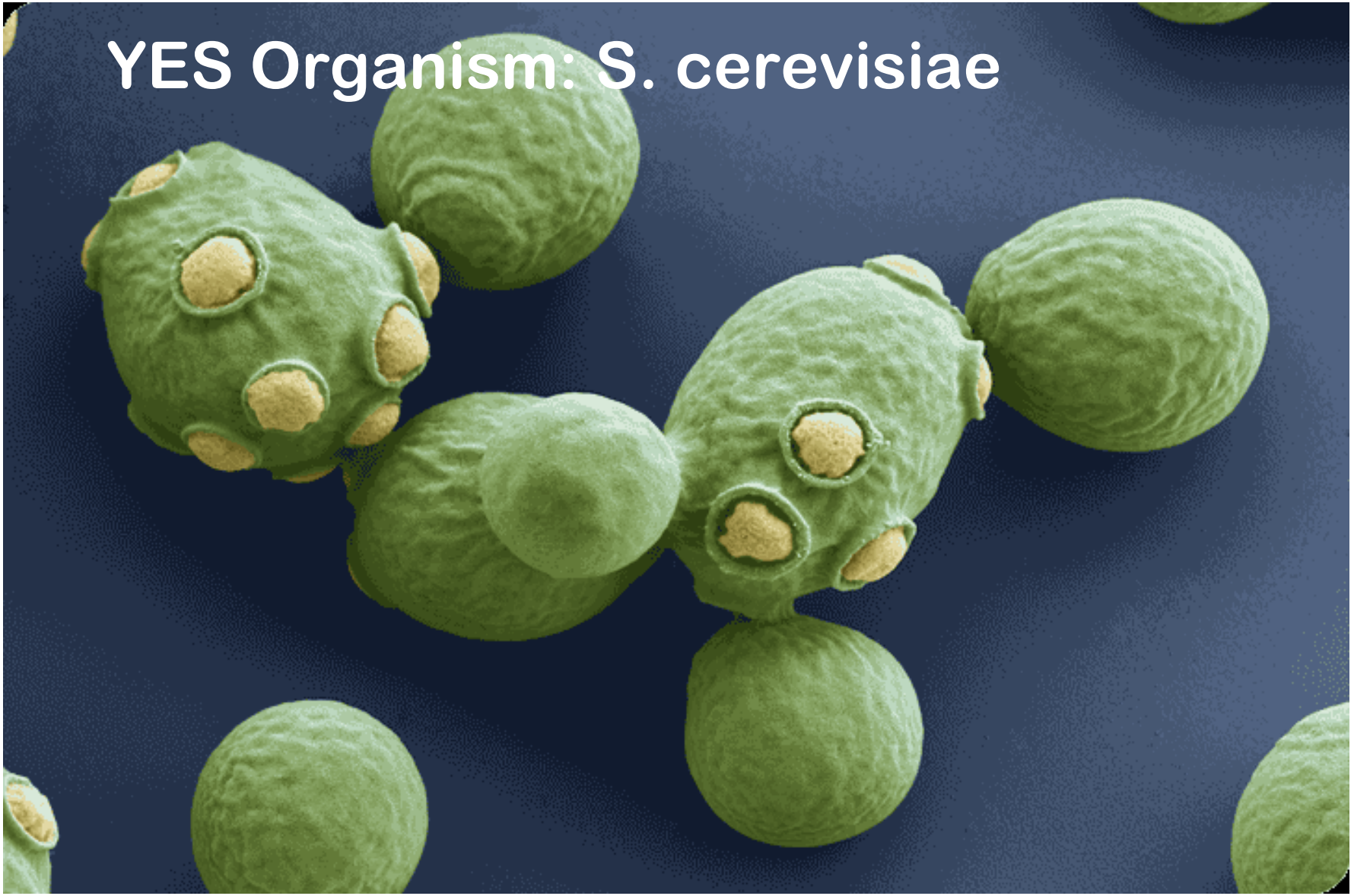
1. Estrogenic activity of selected untreated drinking water samples
2. Indications for the origin of estrogenic activity
3. Added value of undeveloped vs. developed plates
4. Proof-of-concept for direct processing of water samples on HPTLC plates (data not presented)

«planar-YES» - Workflow



* with fluorescent indicator MUG

YES Organism: S. cerevisiae



http://www.microbiologyonline.org.uk/themed/sgm/img/slideshows/3.1.4_fungi_2.png

Sample provenience

1. Evian-Water (negative control)
2. River bank infiltrate, Zurich
3. Sewage treatment plant outlet, Zurich
4. Limmat water, Zurich
5. Rhine water, Basel

- *Water sample divided in 2 parts*
- *Part 2 spiked with 7 estrogens and xenoestrogens to 10 ng/l each.*
- *Extraction 3 times with SPE on consecutive days => 6 vials per sample*

Standard mixture for spikes

Added to sample before extraktion;
10 ng/L of each substance:

- Bisphenol A (REP = 0.00005)
- E1, Estrone (REP = 0.47)
- E2, 17β-Estradiol (REP = 1)
- Mestranol (REP = 0.073)
- Norethisterone (REP = 0.04)
- EE2, 17α-Ethinylestradiol (REP = 0.88)
- Estriol (REP = 0.0076)

Expected EEQ in sample: app. 25 ng/l

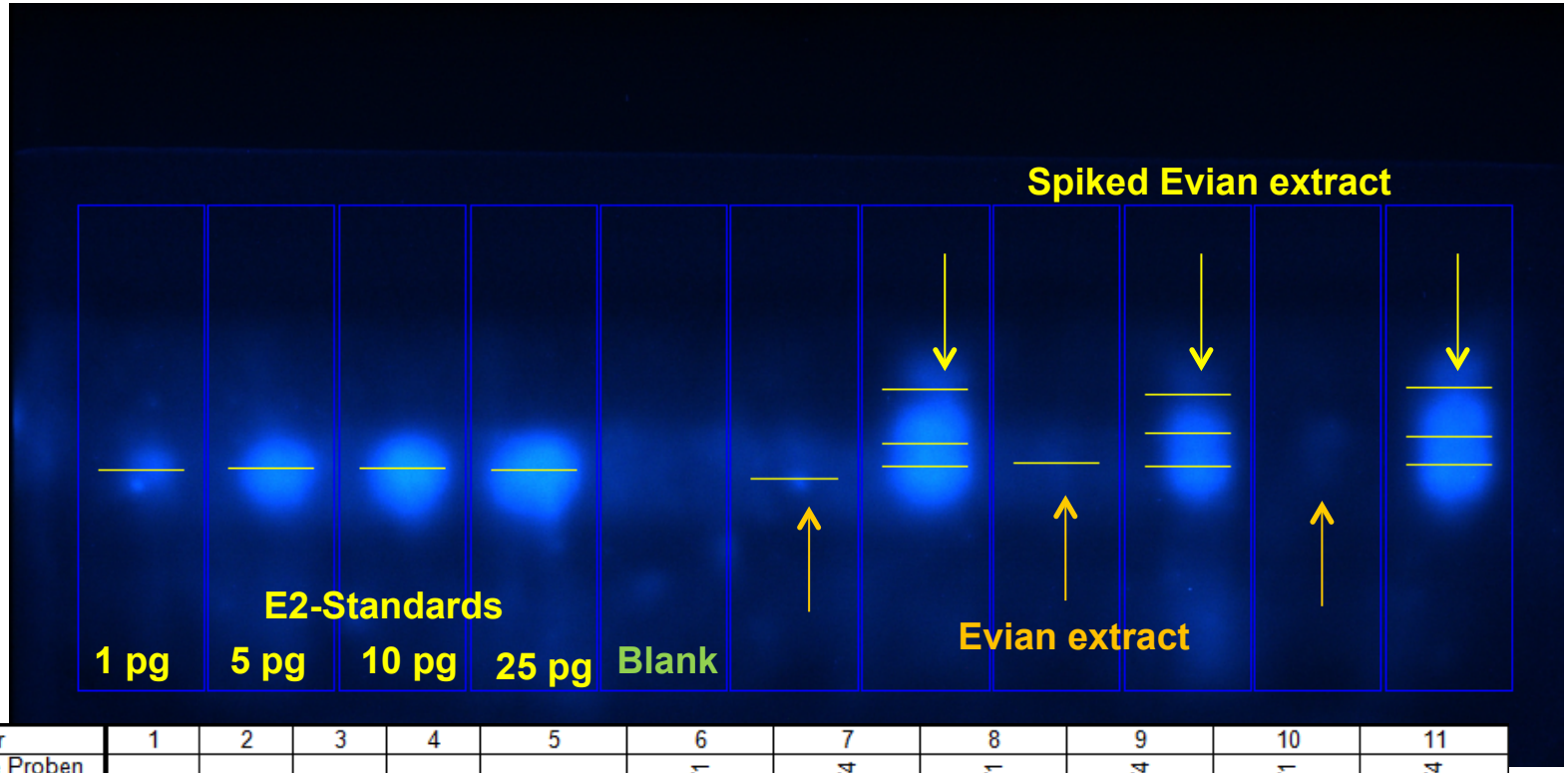
Source: Michael Koss, Wasserversorgung Zürich

SPE procedure (performed by WVZ)

- pH-Wert of sample adjusted to 7 (with conc. HCl)
- Pre-filtration with GF
- (50% of samples: addition of standard mixture)
- SPE with Oasis HLB 6cc on Caliper Autotrace
- Drying of solid phase with N_2, 60 min
- Elution with 4 mL acetone, 1 mL methanol
- Drying
- Take up in 1 mL ethanol

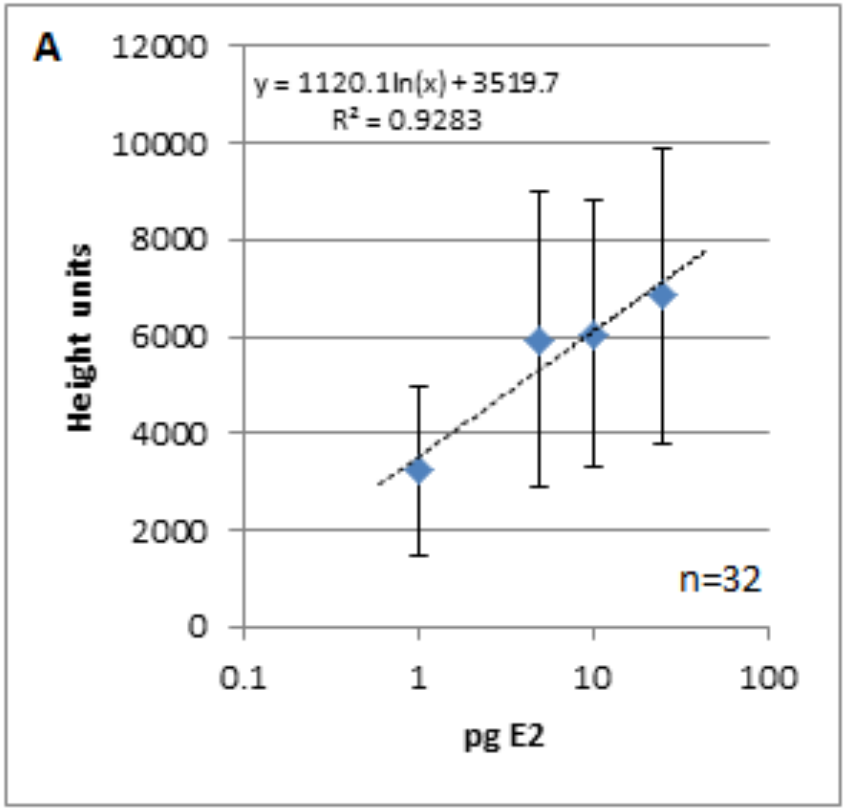
Concentration factor
931 ± 5%

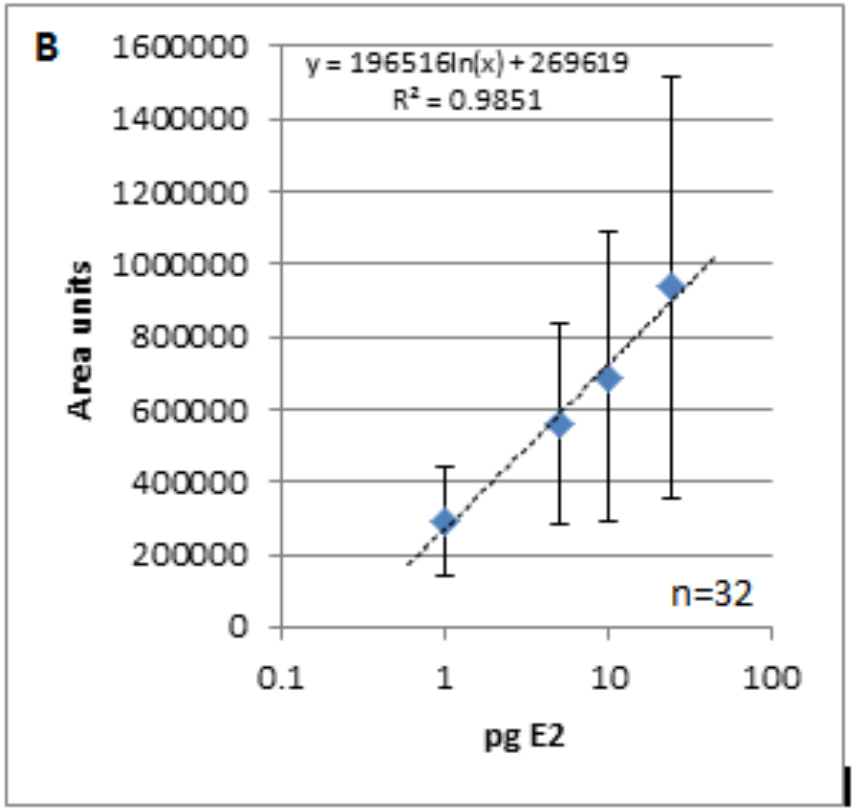
Evian water (negative control)



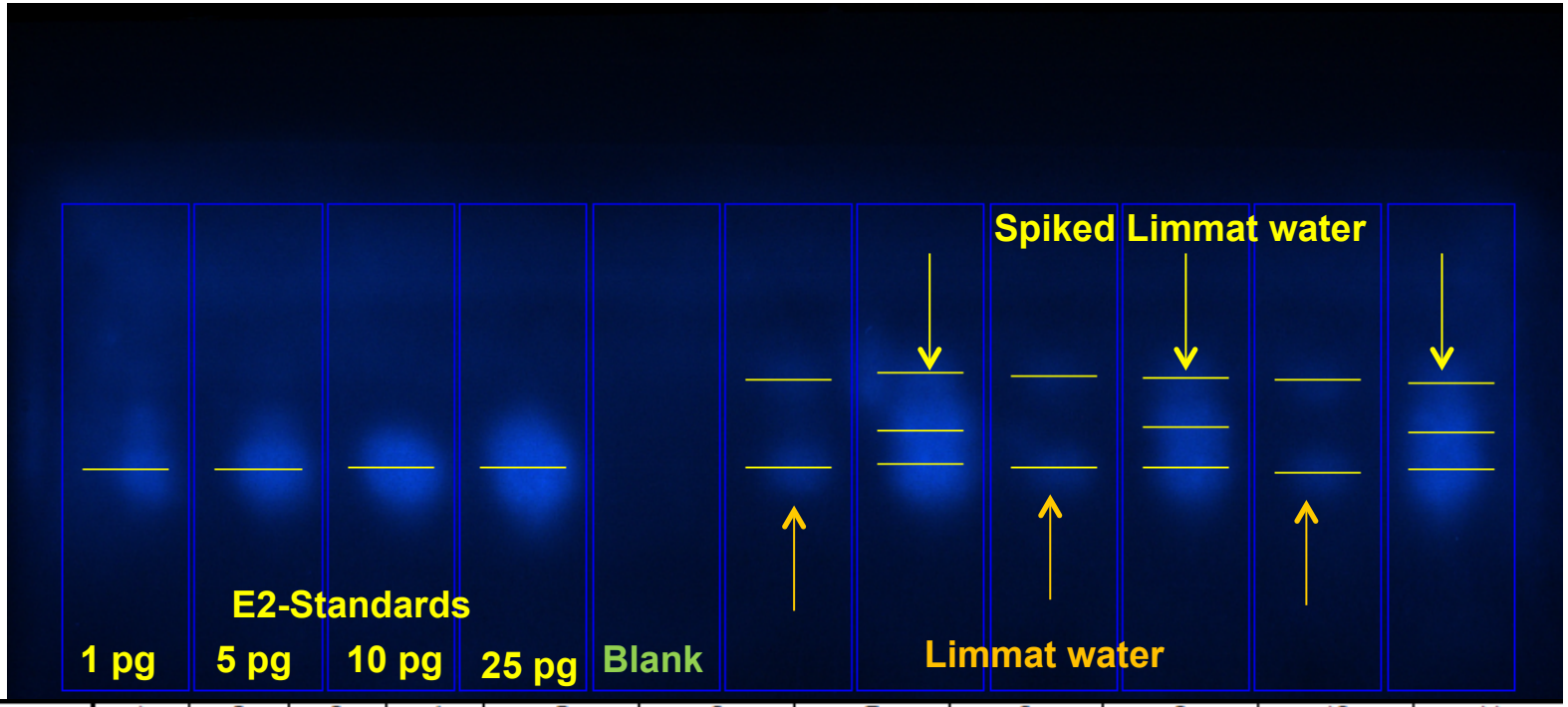
Spot-Nummer	1	2	3	4	5	6	7	8	9	10	11
Aufgetragene Proben	**1 pg E2** (0.2 µg/l)	**5 pg E2** (1.0µg/l)	**10 pg E2** (2.0µg/l)	**25pg E2** (5.0 µg/l)	**P5 Lösemittel-kontrolle** WVZ: P5	**Kontrolle: Evian** WVZ: K/R1 1/1 nativ (05.08.13)	**Kontrolle: Evian** WVZ: K/R1 1/4 aufgestockt (05.08.13)	**Kontrolle: Evian** WVZ: K/R2 2/1 nativ (06.08.13)	**Kontrolle: Evian** WVZ: K/R2 2/4 aufgestockt (06.08.13)	**Kontrolle: Evian** WVZ: K/R3 3/1 nativ (08.08.13)	**Kontrolle: Evian** WVZ: K/R3 3/4 aufgestockt (08.08.13)
Aufgetragenes Probenvolumen	5 µl	5 µl	5 µl	5 µl	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl
Hormonlösung	L6-E2	L4-E2	L5-E2	L7-E2	P5 (16.09.13)	K/R1 1/1 (05.08.13)	K/R1 1/4 (05.08.13)	K/R2 2/1 (06.08.13)	K/R2 2/4 (06.08.13)	K/R3 3/1 (08.08.13)	K/R3 3/4 (08.08.13)

Calibration with E2 standards



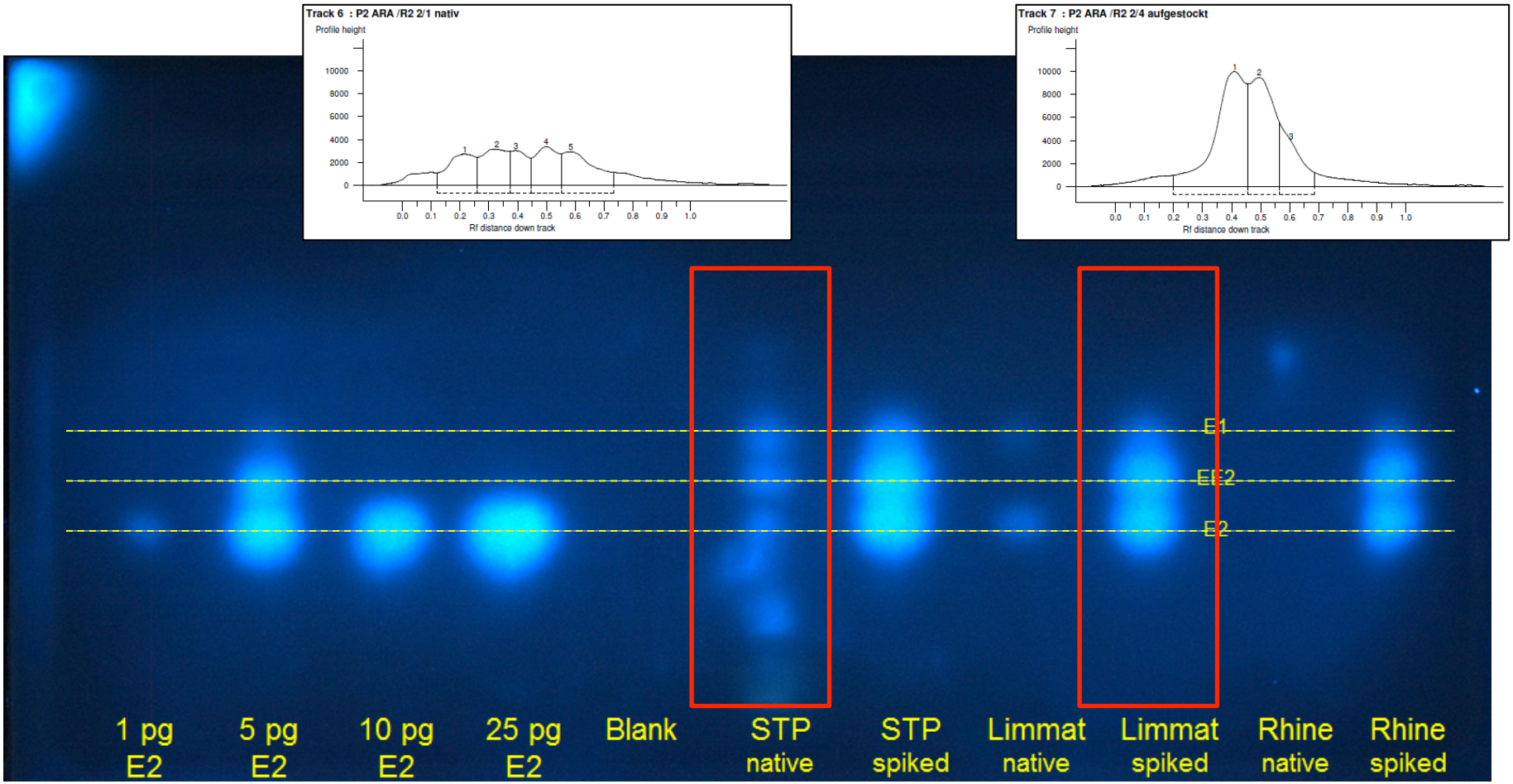


Limmat water (objective 1)

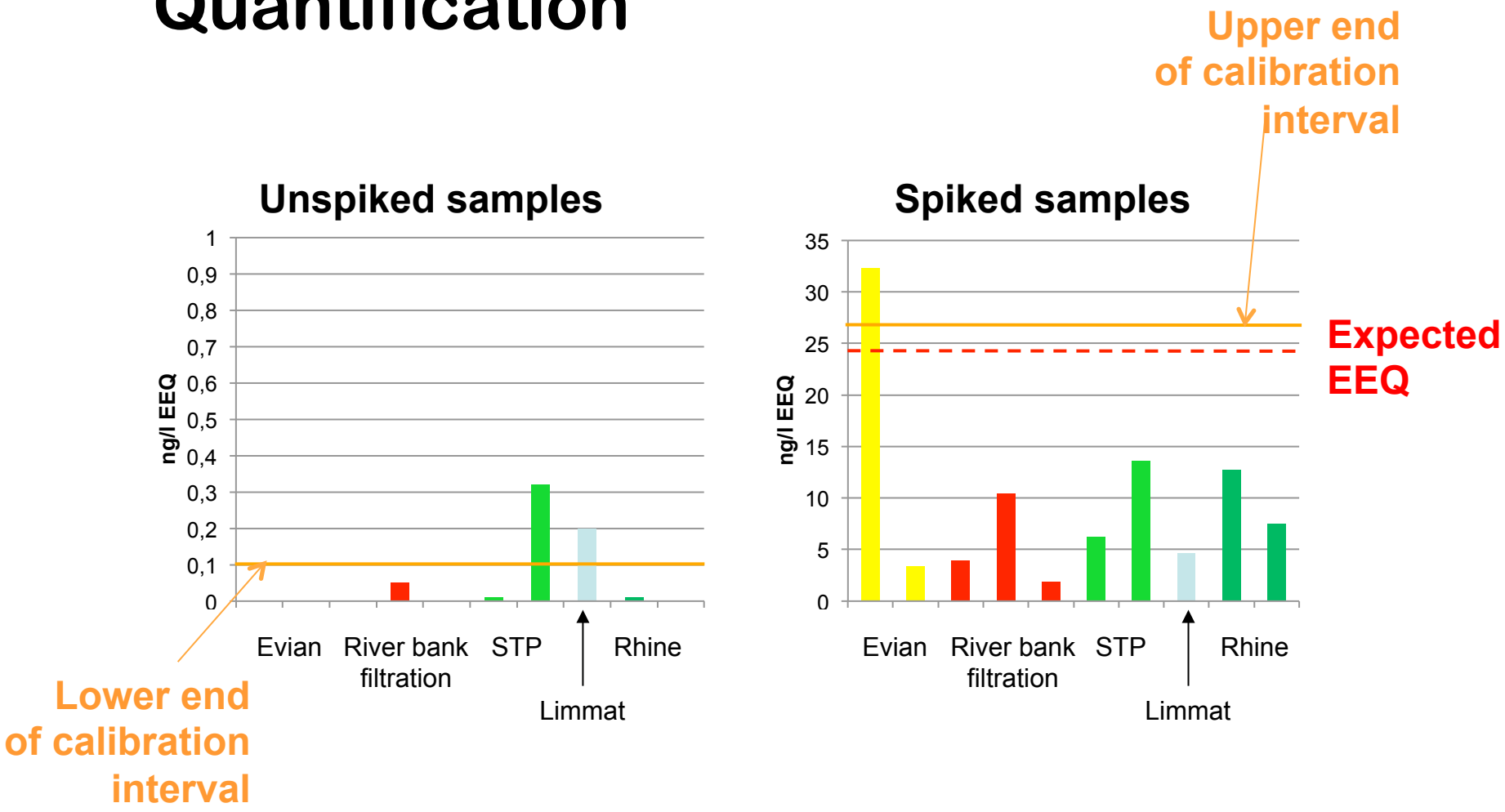


Spot-Nummer	1	2	3	4	5	6	7	8	9	10	11
Aufgetragene Proben	**1 pg E2** (0.2 µg/l)	**5 pg E2** (1.0µg/l)	**10 pg E2** (2.0µg/l)	**25pg E2** (5.0 µg/l)	**Lösemittel-kontrolle ZHAW**	**P3: LIM** WVZ: P3/R1 1/1 nativ (21.08.13)	**P3: LIM** WVZ: P3/R1 1/4 aufgestockt (21.08.13)	**P3: LIM** WVZ: P3/R2 2/1 nativ (21.08.13)	**P3: LIM** WVZ: P3/R2 2/4 aufgestockt (21.08.13)	**P3: LIM** WVZ: P3/R3 3/1 nativ (23.08.13)	**P3: LIM** WVZ: P3/R3 3/4 aufgestockt (23.08.13)
Aufgetragenes Probenvolumen	5 µl	5 µl	5 µl	5 µl	1 µl	10 µl	1 µl	10 µl	1 µl	10 µl	1 µl
Hormonlösung	L6-E2	L4-E2	L5-E2	L7-E2	EtOh ZHAW	P3/R1 1/1 (21.08.13)	P3/R1 1/4 (21.08.13)	P3/R2 2/1 (21.08.13)	P3/R2 2/4 (21.08.13)	P3/R3 3/1 (23.08.13)	P3/R3 3/4 (23.08.13)

Origin of estrogenic activity (objective 2)

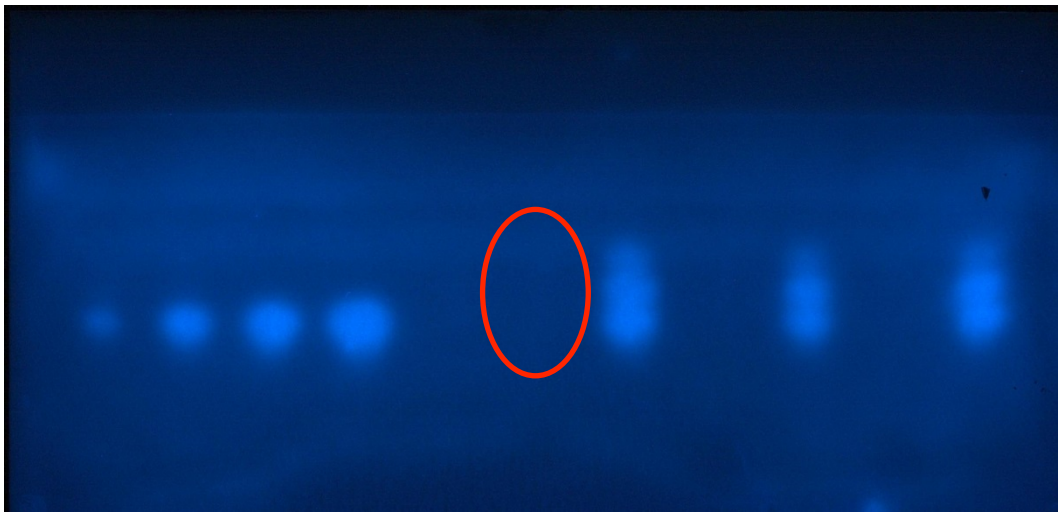


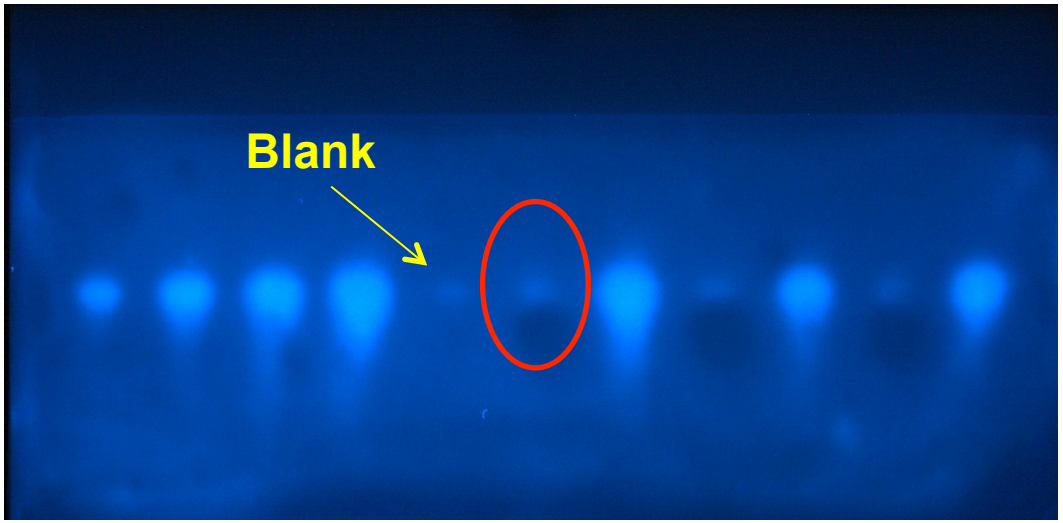
Quantification



Comparison developed / undeveloped

(objective 3)





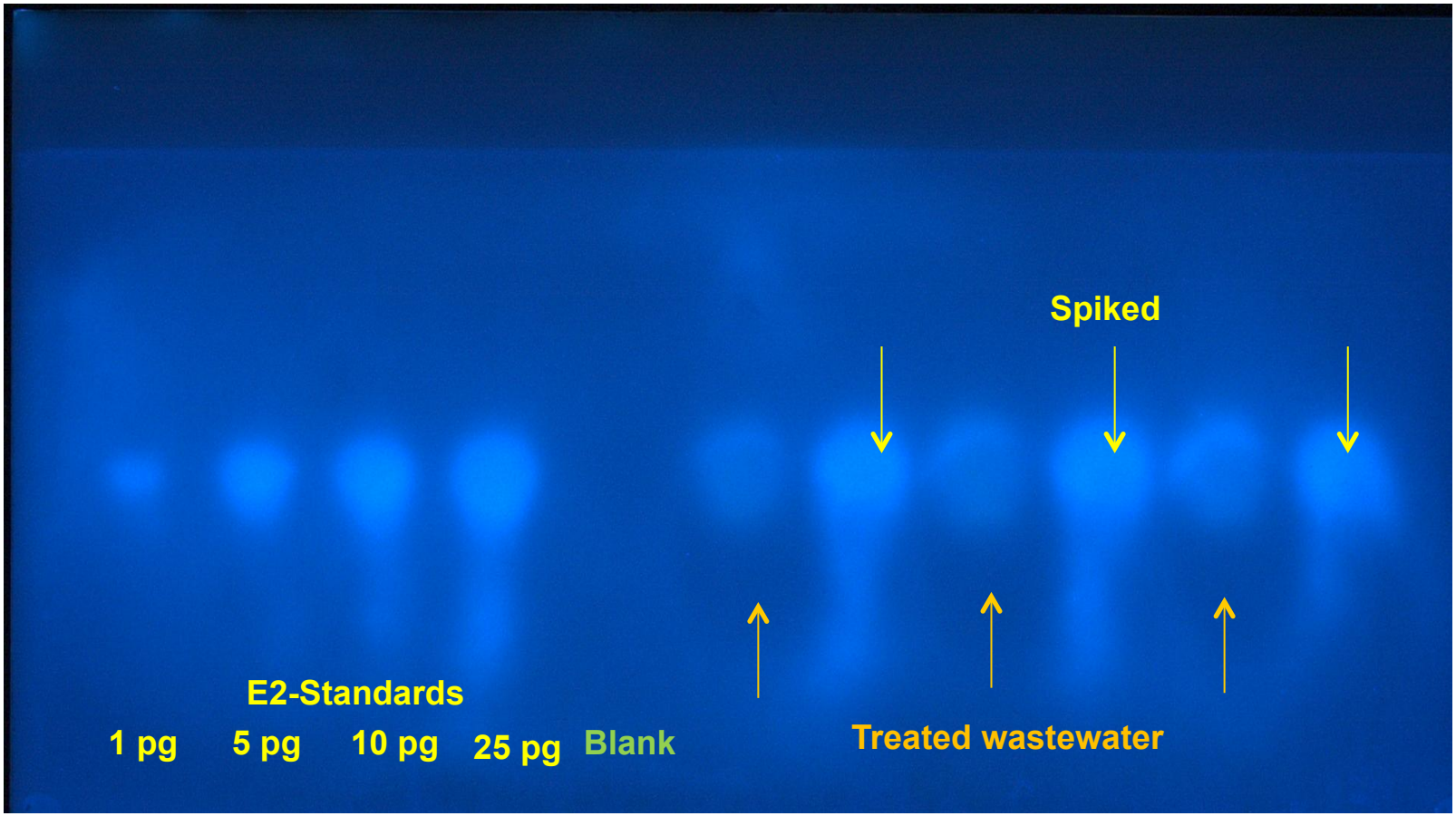
Rhine water

Developed:
EEQ not detectable

Undeveloped:
EEQ detectable

Total-EEQ:
<< 1 pg / 10µl extract?

Added value 2 (Treated wastewater)



Dimmed center of spot indicates inhibition!!!

Conclusion

- Net time demand: 1 day from sampling to result
- Robust, reproducible, 6-9 parallel samples
- LOQ < 1 pg/band EEQ (0.1 pg/band within reach)
- Potential to detect 1 ng/l EEQ in native water samples

Outlook

- Further refinement of procedure needed!
- Link to analytical chemistry difficult (low masses)
- Application to other matrices (cosmetics, exudates from plastics…)
- ISO certification of planar-YES (?)

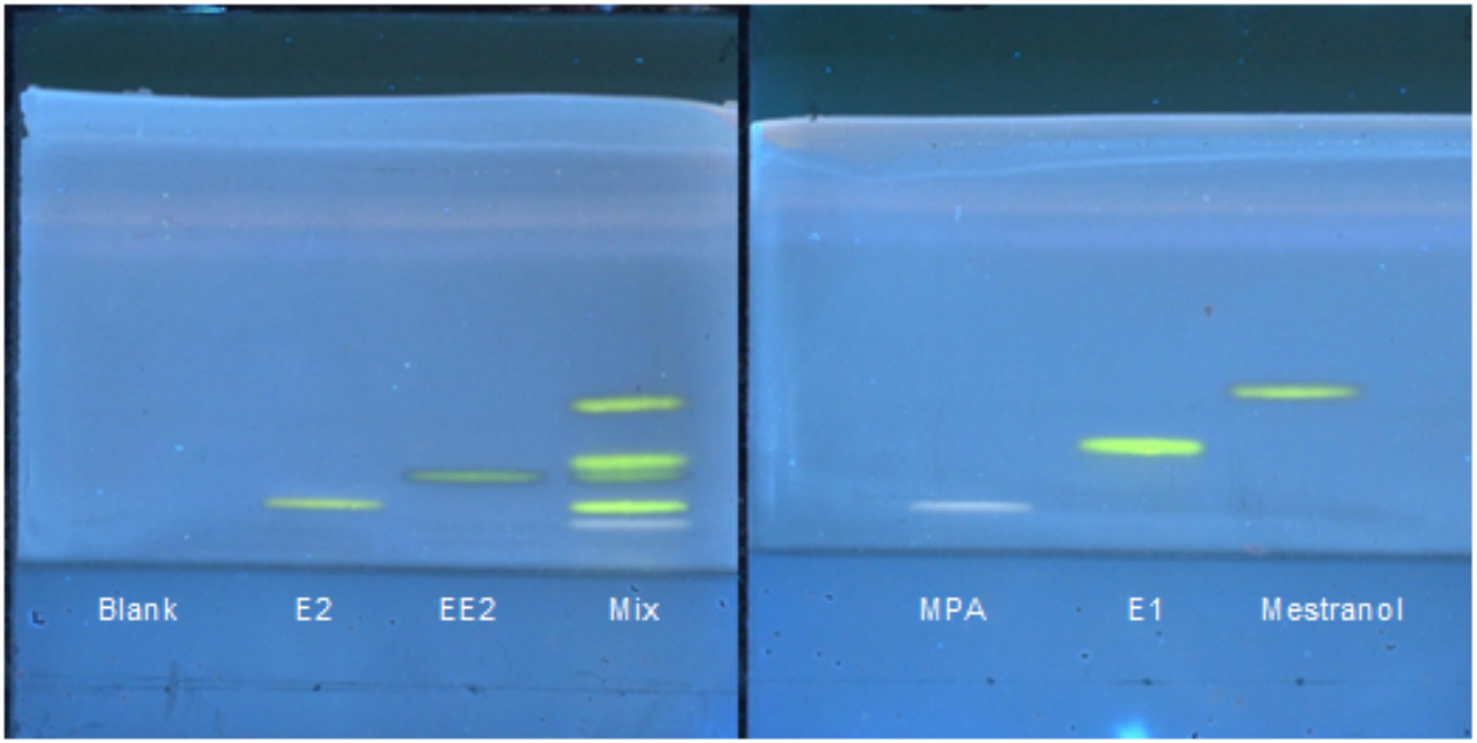
Thank you!

- ZHAW:
  - Andrea Grimmer
  - Dr. Evelyn Wolfram
- Wasserversorgung Zürich:
  - Dr. Michael Kost
  - Dr. Oliver Köster
- Bundesamt für Gesundheit:
  - Dr. Beat Brüschweiler

SPE vs. Clean-Up on HPTLC (objective 4)

Method	Samp-ling	Extraction	Appli-cation	Drying	Focus-sing	Drying	Plate development
State of art	X	With SPE	X (SPE extract)	X	---	---	X
A. Clean-Up with focussing	X	---	X (water sample)	X	X (with CH_3OH)	X	X
B. Clean-Up on HPTLC plate with concentration zone	X	---	X (water sample)	X	---	---	X
Time demand	---	ca. 1 d / 1-6 samples	ca. 1,5 h / plate		ca. 0,5 h / plate		ca. 1,5 h / plate

Focussing on HPTLC-K plates



Eintauchgrenze f. Derivatisierung

Entwicklungshöhe

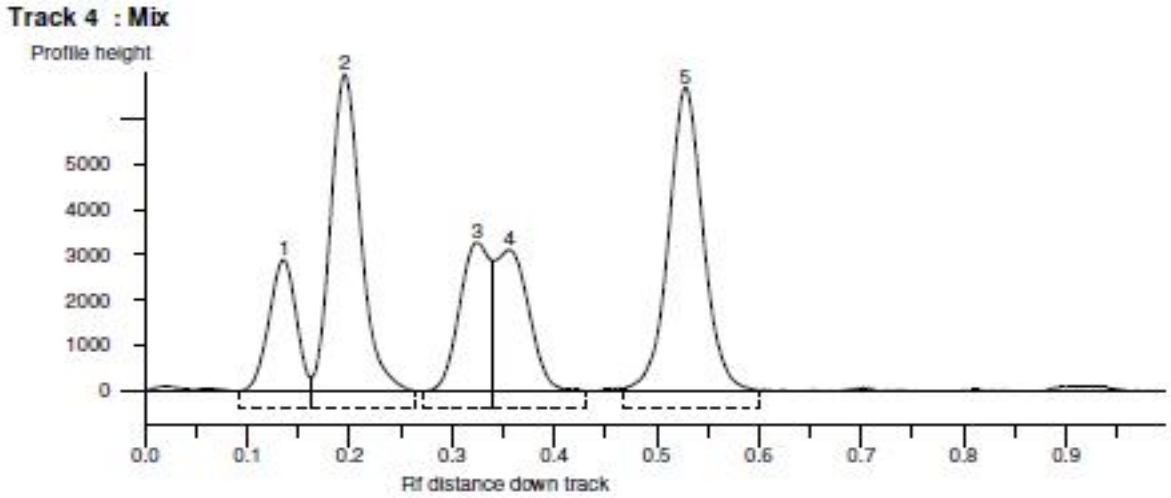
Phasengrenze Konzentrierungszone

Auftragehöhe

275 ng/Spot, dissolved in 5 µl ethanol (manual application). Height: 1 cm (in concentration zone). Development to 7,5 cm with ADC2 (Camag). Mobile phase: n-Hexan 4 / Ethylacetate 1,5.
Derivatization with mit sulfuric acid reactant. Light: 366 nm. Exposure time 3665.97ms, Gain 1.

P. Hofmann, 2013

Separation on HPTLC-K plates



Track 4

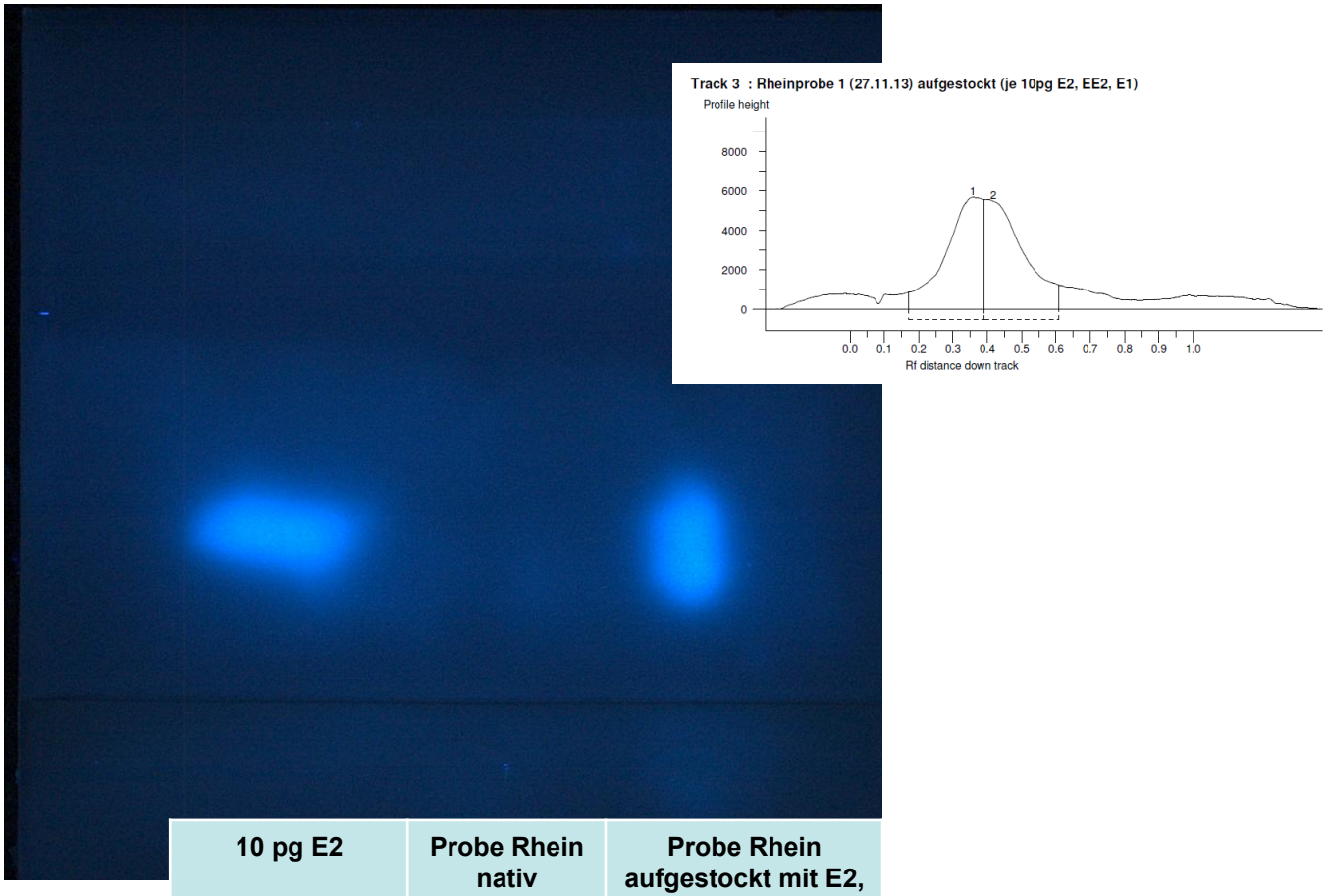
Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.091	0.0	0.136	2893.6	12.60	0.163	299.7	39316.6	10.89	
2	0.163	299.7	0.195	6975.1	30.38	0.263	7.7	104191.8	28.87	
3	0.272	0.0	0.324	3275.3	14.27	0.340	2864.2	49708.4	13.77	
4	0.340	2864.2	0.356	3117.2	13.58	0.431	9.2	52129.0	14.44	
5	0.467	75.8	0.528	6697.9	29.17	0.601	25.9	115575.7	32.02	

Total Height 22959.1 Total Area : 360922

275 ng/Spot, dissolved in 5 µl ethanol (manual application). Height: 1 cm (in concentration zone). Development to 7,5 cm with ADC2 (Camag). Mobile phase: n-Hexan 4 / Ethylacetate 1,5.
Derivatization with mit sulfuric acid reactant. Light: 366 nm. Exposure time 3665.97ms, Gain 1.

P. Hofmann, 2013

Proof-of-concept: Clean-Up on HPTLC-K



10 pg E2	Probe Rhein nativ	Probe Rhein aufgestockt mit E2, EE2, E1
5 µl (2.0 µg/l)	100 µl	100 µl

Potential endocrine disruptors (WHO)

- Persistente und bioakkumulierende Halogenide: Langlebige organische Schadstoffe (z. B. Polychlorierte Dibenzodioxine und Dibenzofurane, Polychlorierte Biphenyle, Hexachlorbenzol, Perfluoroctansulfonat, Polybromierte Diphenylether, Polybromierte Biphenyle, Chlordan, Mirex, Toxaphen, Dichlordiphenyltrichlorethan, Lindan, Endosulfan); andere persistente und bioakkumulative Stoffe (z. B. Hexabromcyclododecan, Chlorparaffine, Perfluorierte Carbonsäuren, Octachlorostyrene, PCB Methyl Sulfone)
- weniger persistente und weniger bioakkumulative Stoffe: Weichmacher und Additive (z. B. Phthalsäureester, Triphenylphosphat, Diethylhexyladipat, Butylbenzole, Triclocarban, Butylhydroxyanisol); Polycyclische aromatische Kohlenwasserstoffe (z. B. Benzopyren, Benz(a)anthracen, Pyren, Anthracen); Halogenphenole (z. B. 2,4-Dichlorphenol, Pentachlorophenol, Hydroxy-PCB, Hydroxy-PBDE, Tetrabrombisphenol A, 2,4,6-Tribromphenol, Triclosan); nicht-Halogenphenole (z. B. Bisphenol A, Bisphenol F, Bisphenol S, Nonylphenol, Octylphenol, Resorcinol)
- Pestizide, Arzneimittel und Kosmetika: gegenwärtig genutzte Pflanzenschutzmittel (z. B. 2,4-Dichlorphenoxyessigsäure, Atrazin, Carbaryl, Malathion, Mancozeb, Vinclozolin, Prochloraz, Procymidon, Chlorpyrifos, Fenitrothion, Linuron); Arzneimittel, Wachstumshormone und Kosmetika (z. B. Diethylstilbestrol, Ethinylestradiol, Tamoxifen, Levonorgestrel, Fluoxetin, Flutamid, 4-Methylbenzyliden-campher, Octylmethoxycinnamat, Parabene, Octamethylcyclotetrasiloxan, Decamethylcyclopentasiloxan, Dodecamethylcyclohexasiloxan, Galaxolid, 3-Benzyliden-Campher)
- Andere Chemikalien: Metalle und Organometalle (z. B. Arsen, Cadmium, Blei, Quecksilber, Methylquecksilber, Tributylzinnhydrid, Triphenylzinn); natürliche Hormone (z. B. 17-beta-Estradiol, Estron, Testosteron); Phytoöstrogene (z. B. Isoflavone, Coumestan, Mykotoxine, Prenylflavonoide)

Quelle: WHO 2013

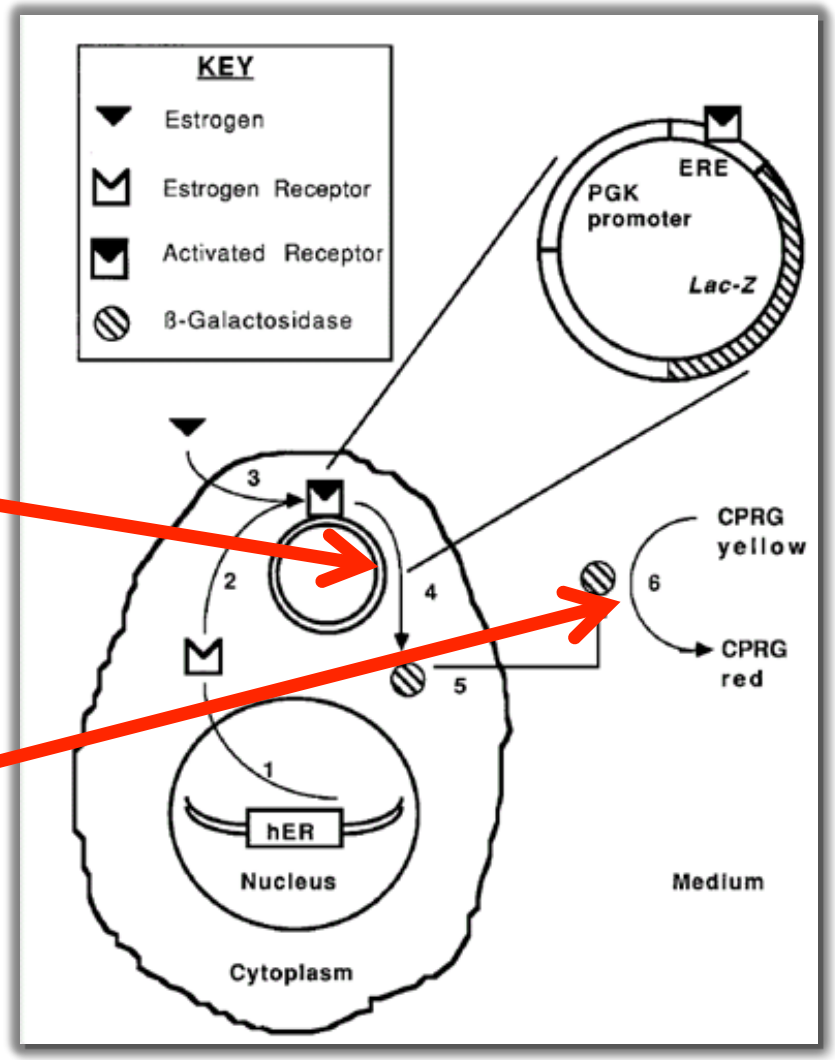
Zahlen zu den Rohwasser-Messungen

- 6 Messdurchgänge (davon 2 x als Vorversuch)
- 20 auswertbare HPTLC Platten (10 x 20 cm, 10 x 10 cm)
- 206 aufgebrachte Tracks, davon:
  - 112 Extrakte (mit und ohne DC)
  - 74 E2-Standards (mit und ohne DC)
  - 20 Lösungsmittelblanks (mit und ohne DC)
- 271 auswertbare Datensätze
  - 99 gut auswertbare Peaks (37%)
  - 101 mit Vorbehalt auswertbare Peaks (37%)
  - 34 Tracks ohne detektierbare Peaks (13%)
  - 37 nicht auswertbare Tracks (14%)

Prinzip YES Test

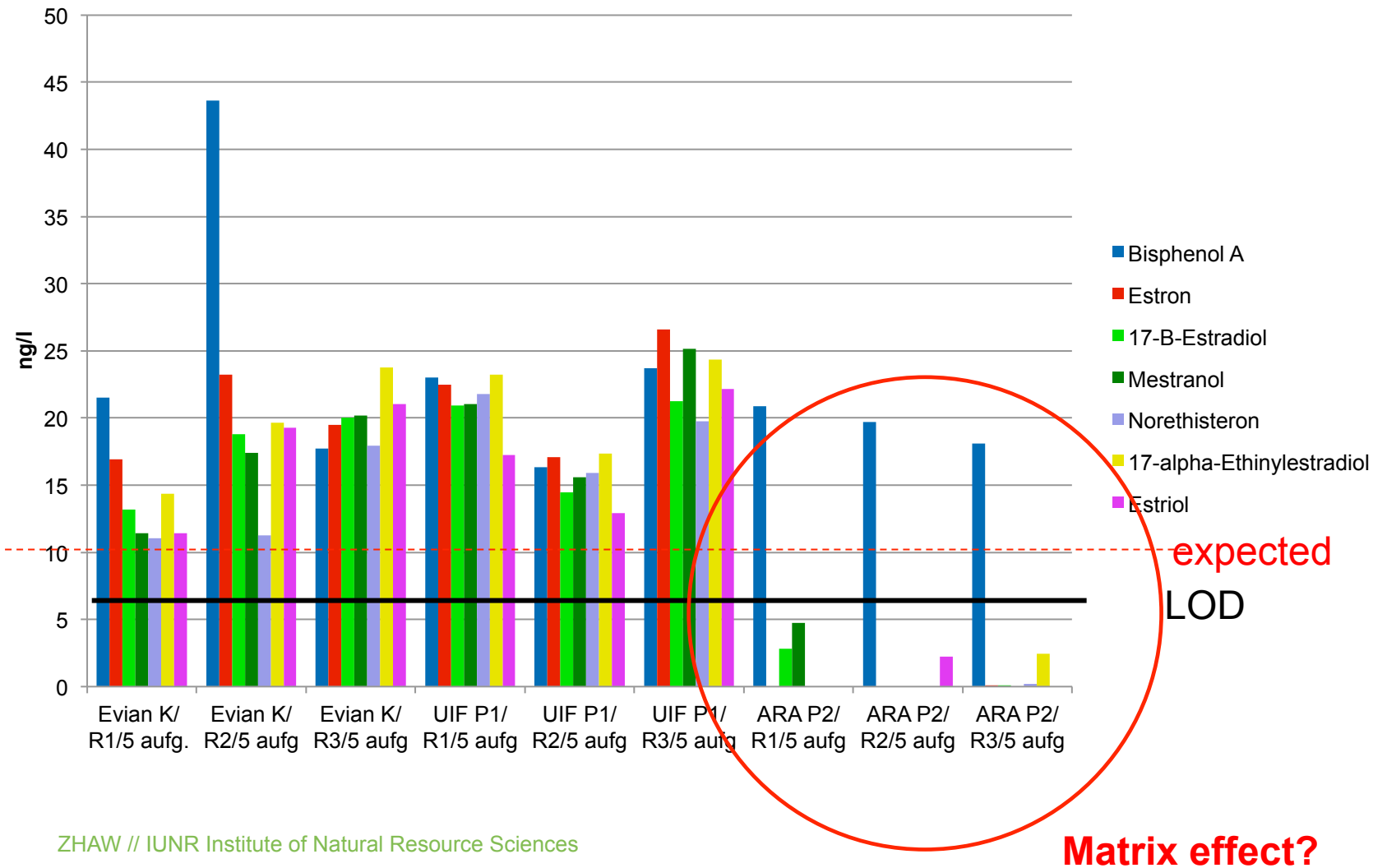
Amplifier 1

Amplifier 2



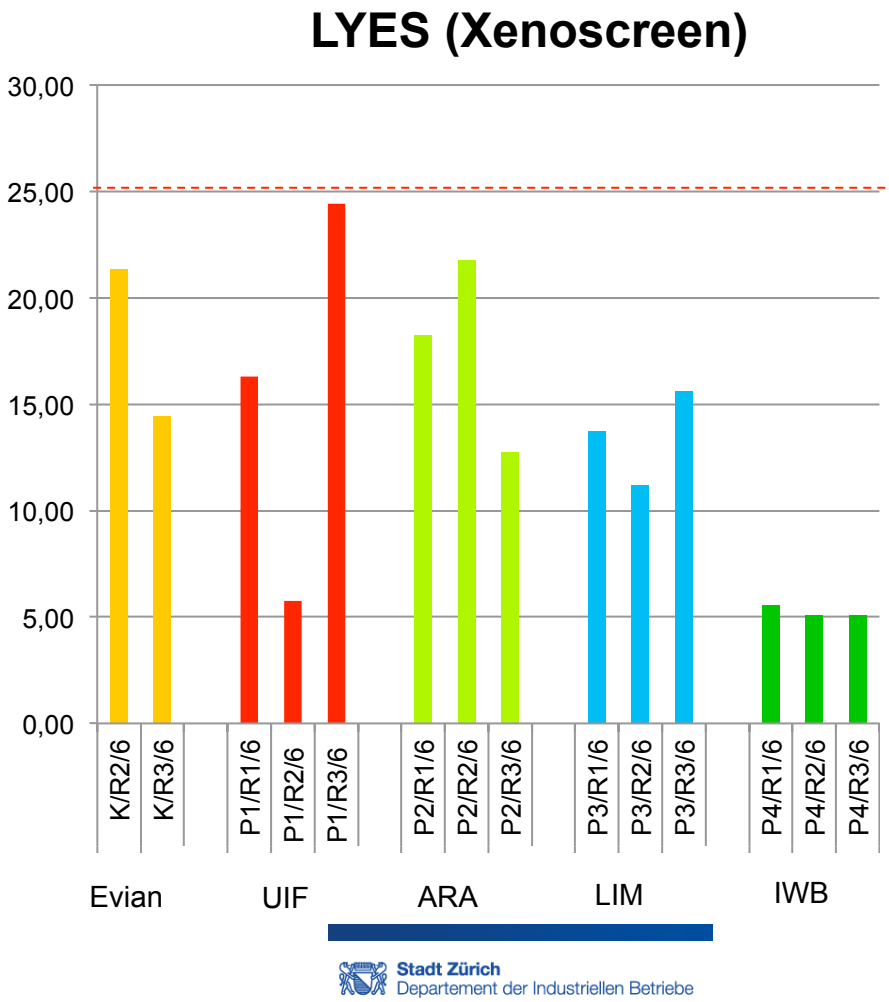
Routledge, E. J. & Sumpter, J. P., 1996, Env. Toxic. Chem., 15(3), 241 - 248

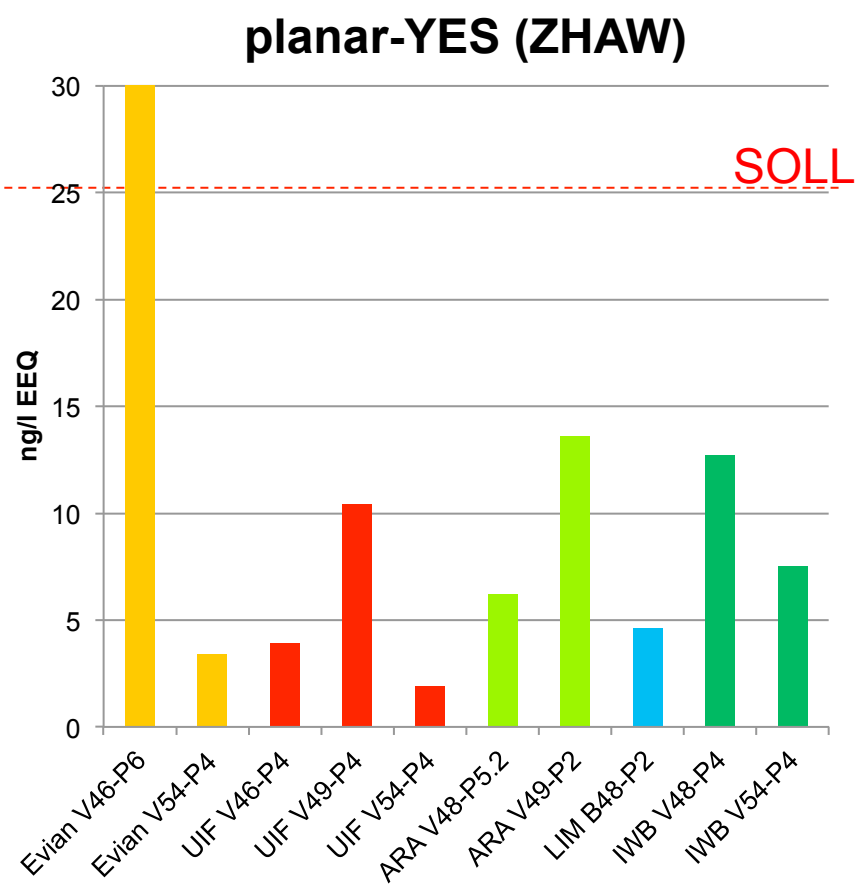
Target analytics of spiked samples



Matrix effect?

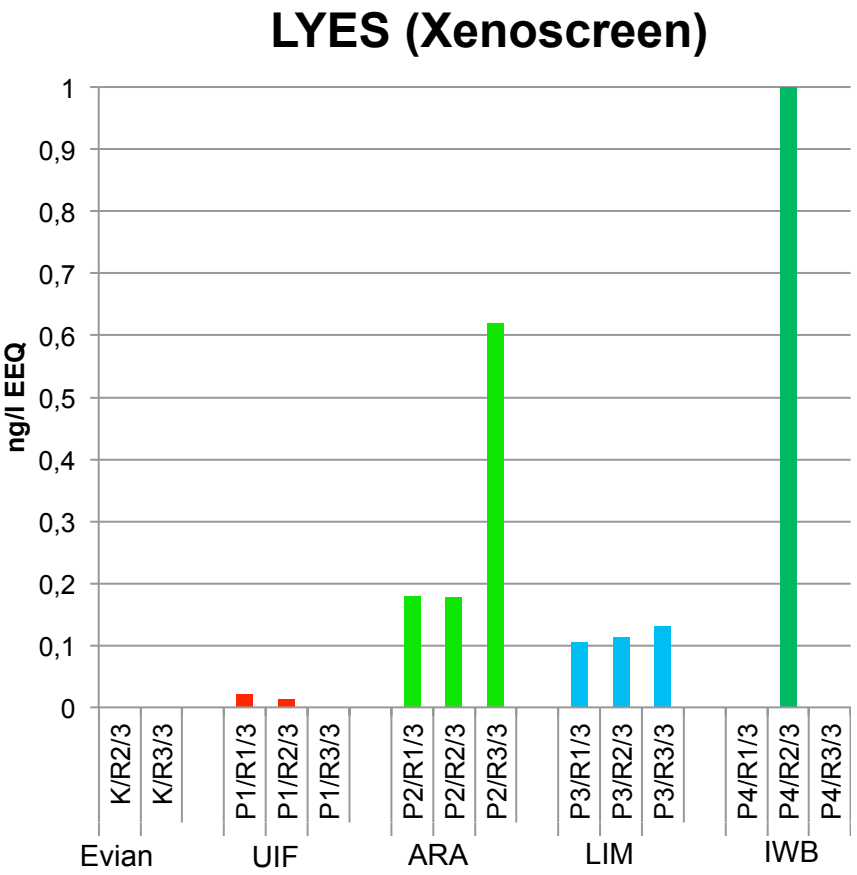
Spiked samples EEQ (ng/l)

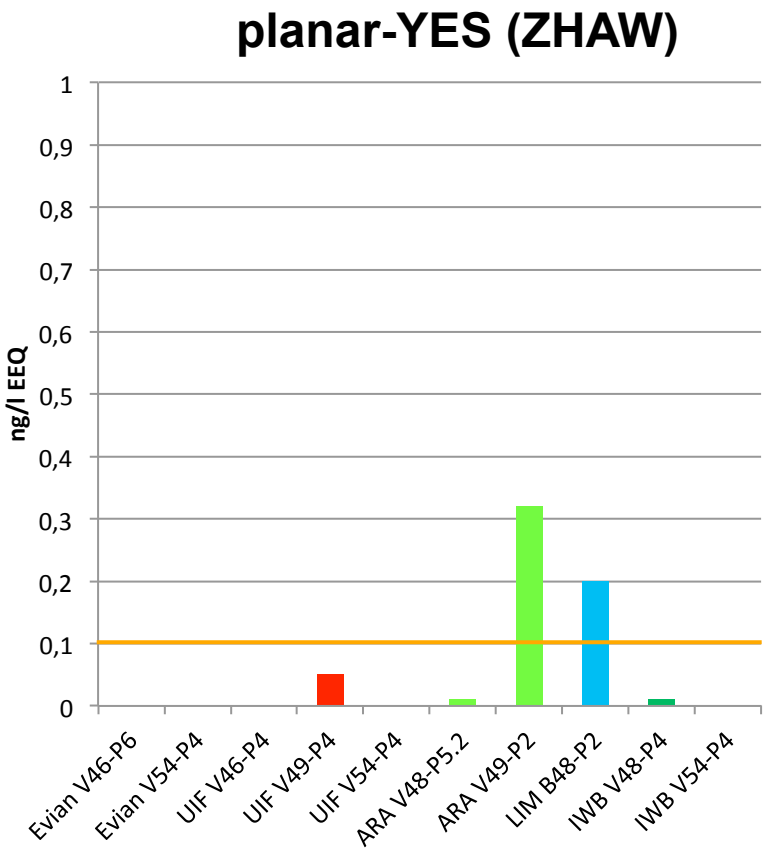




Stadt Zürich
Departement der Industriellen Betriebe

Native Proben EEQ (ng/l)





Stadt Zürich
Departement der Industriellen Betriebe

Why estrogens?