# Endocrine activity and endocrine active compounds in Swiss drinking waters

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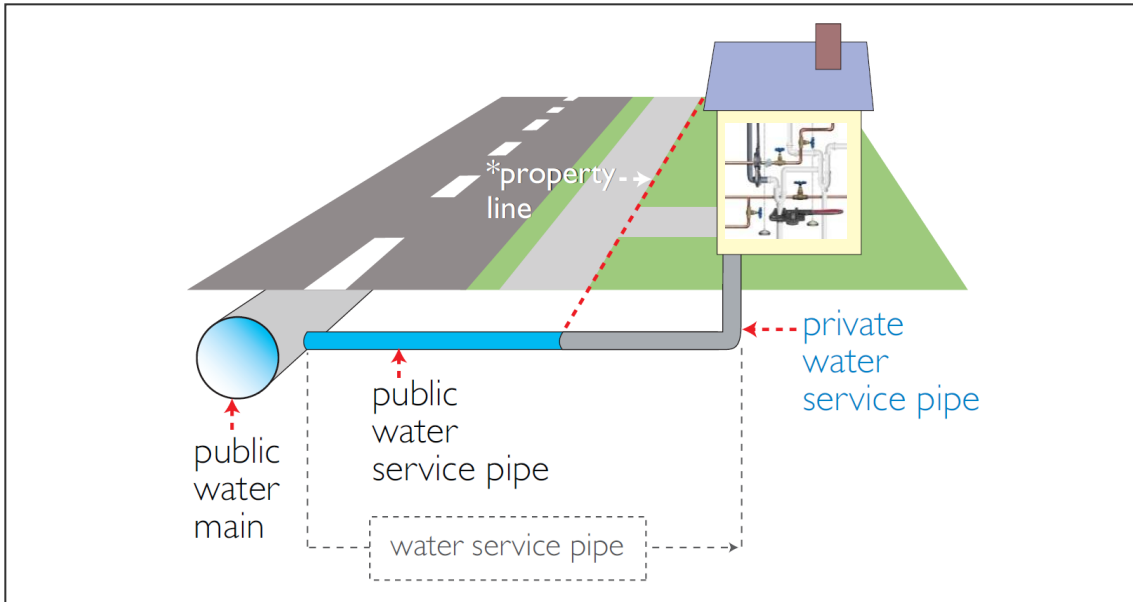
# Introduction

- Evidence of adverse effects in wildlife caused by EDCs
- Raising concern of potential adverse effects of EDCs for human health

  - e.g. estrogenic activity found in bottled water (PET bottles)

- Drinking water at the end of the distribution system can be in longer contact with plastic materials. Leaching of chemicals in the tap water?
- Do EDCs in drinking water pose a risk towards human health?



# Formation of premature thelarche: contribution of drinking water?

- From 2000 to 2010, 8-10 cases of premature thelarche of unknown etiology have been observed in the western part of Switzerland.

**Premature thelarche**

- (temporal) development of breast tissue prior to the normal age of pubertal onset in girls (before 8 years) without other signs of puberty.
- It is usually considered a benign condition that disappears without influencing statural growth or the timing of puberty.
- Premature thelarche is thought to be of endogenous origin, but exposure to substances with estrogenic activity (e.g. from drinking water or other sources) has to be considered as well

- It was hypothesized among pediatricians that there might be a link between the development of these cases of premature thelarche and the consumption of possibly contaminated tap water.
- The symptoms of premature thelarche apparently decreased or disappeared entirely after changing the consumption from tap water to bottled water within weeks and months.

# Investigation

- 2010-2012: screening of tapwater in households with/without cases of premature Thelarche:

  - estrogenicity (CALUX bioassay)
  - anti-androgenicity (CALUX bioassay)

  - target chemicals with estrogenic activity:
    - bisphenol A (also anti-AR)
    - nonylphenol (also anti-AR)
    - 17β-estradiol
    - 17α-ethinylestradiol
    - estrone

- Evaluation of the potential link between the consumption of tap water and the formation of premature thelarche:

  - literature data study
  - comparison of different exposure routes (e.g. diet)

## Site selection and samples:

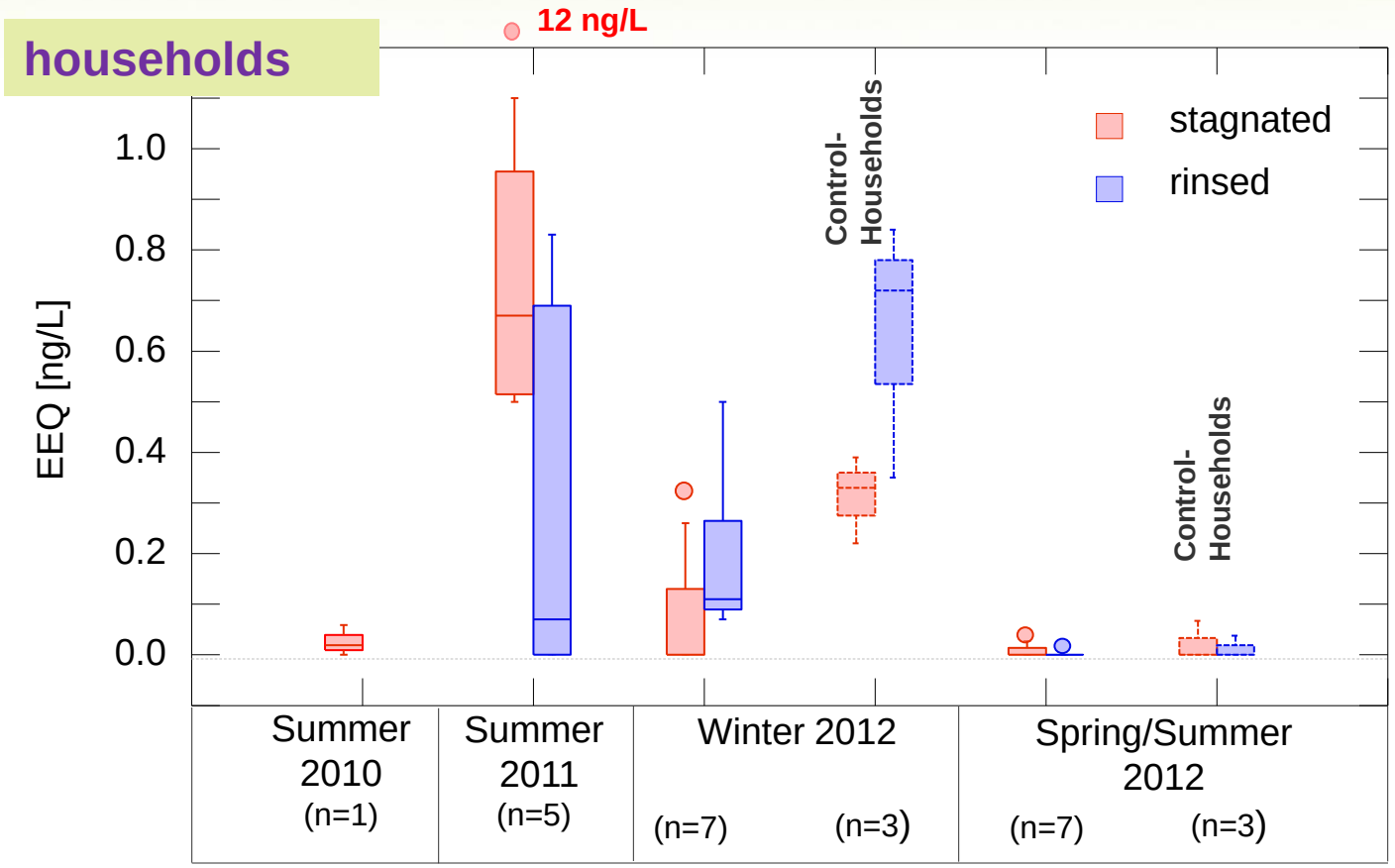
- 10 private households (7 with cases of premature thelarche): stagnant (~12 h) and rinsed tap water
- 2 drinking water suppliers – raw, processed and finished drinking water
- Different sampling campaigns: 2010 – 2012

household H1
household H2
household H3
household H4
household H5
household H6
household H7
household H8
household H9
household H10

drinking water supplier 1
- 69% Lake water: filt., ozone, $ClO_2$
- 22% Groundwater: partially UV
- 9% Spring water: chlorine

drinking water supplier 2
- 100% Lake water: ozone, act. carbon, sand filt.

# Results: estrogenic activity




- **30/54 samples > detection limit** of 0.02-0.03 ng/L EEQ
- **range 0-1 ng/L EEQ** (outlier 12 ng/L EEQ)
- no significant differences between households with/without cases of premature thelarche
- No clear trends between stagnated and rinsed water

# Results: anti-androgenic activity

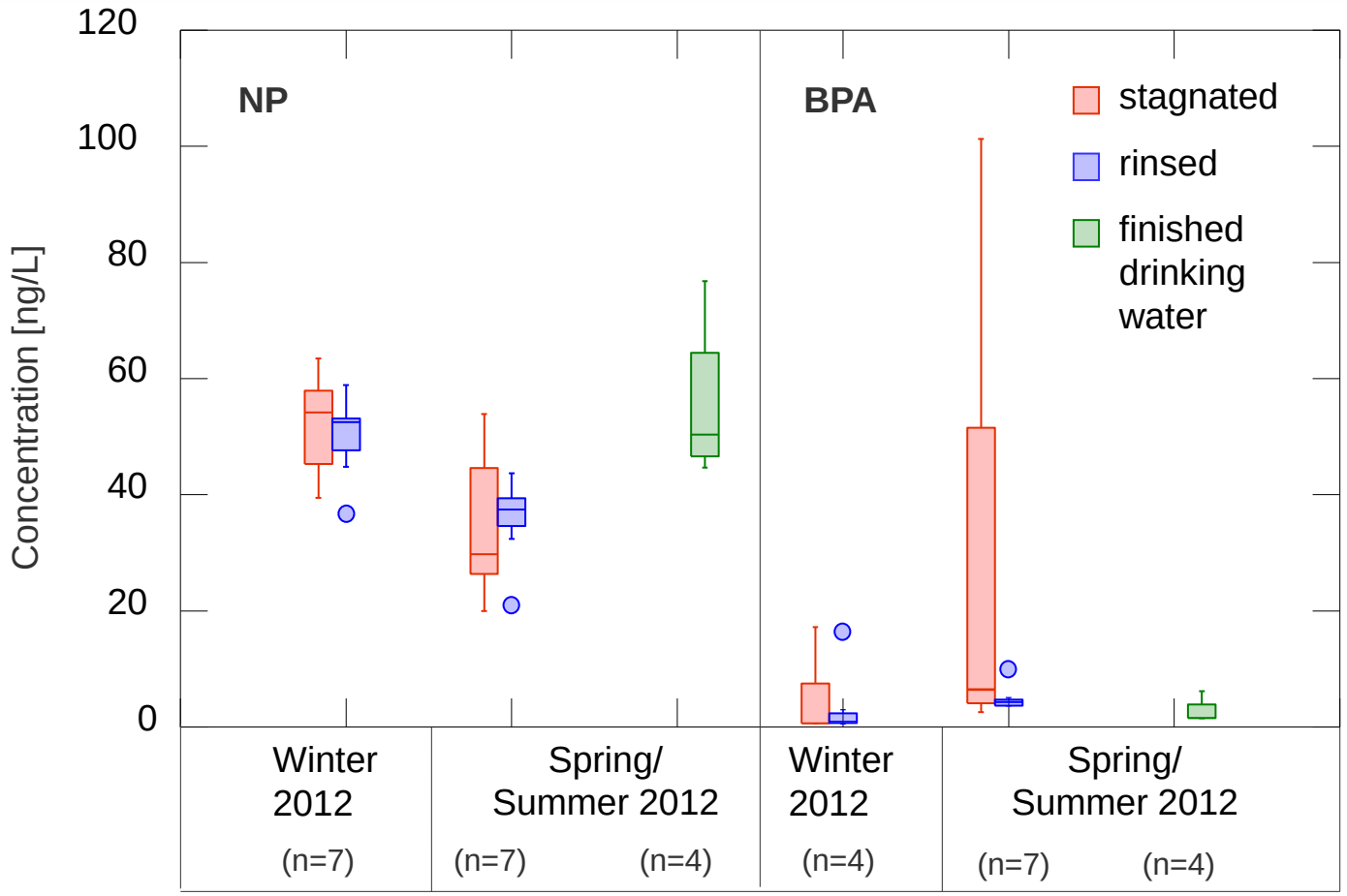
**households**

- **3/50 samples > detection limit** of 3.1-3.5 μg/L FEQ
- **range 0-7.7 μg/L FEQ**

**finished drinking water and raw water**
(at the water production site)

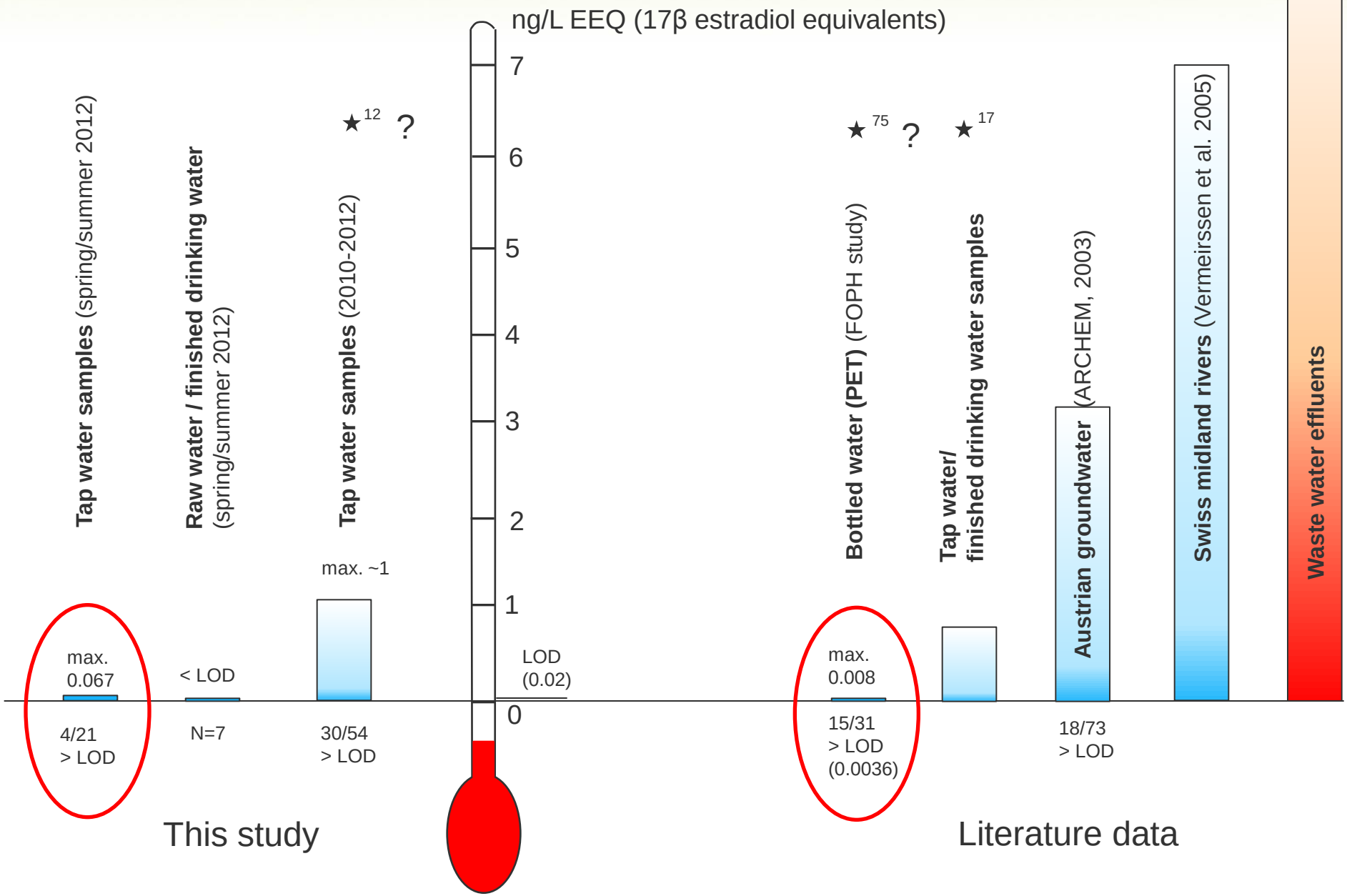
- **no** detectable ER or anti-AR activity

# Results: target compounds

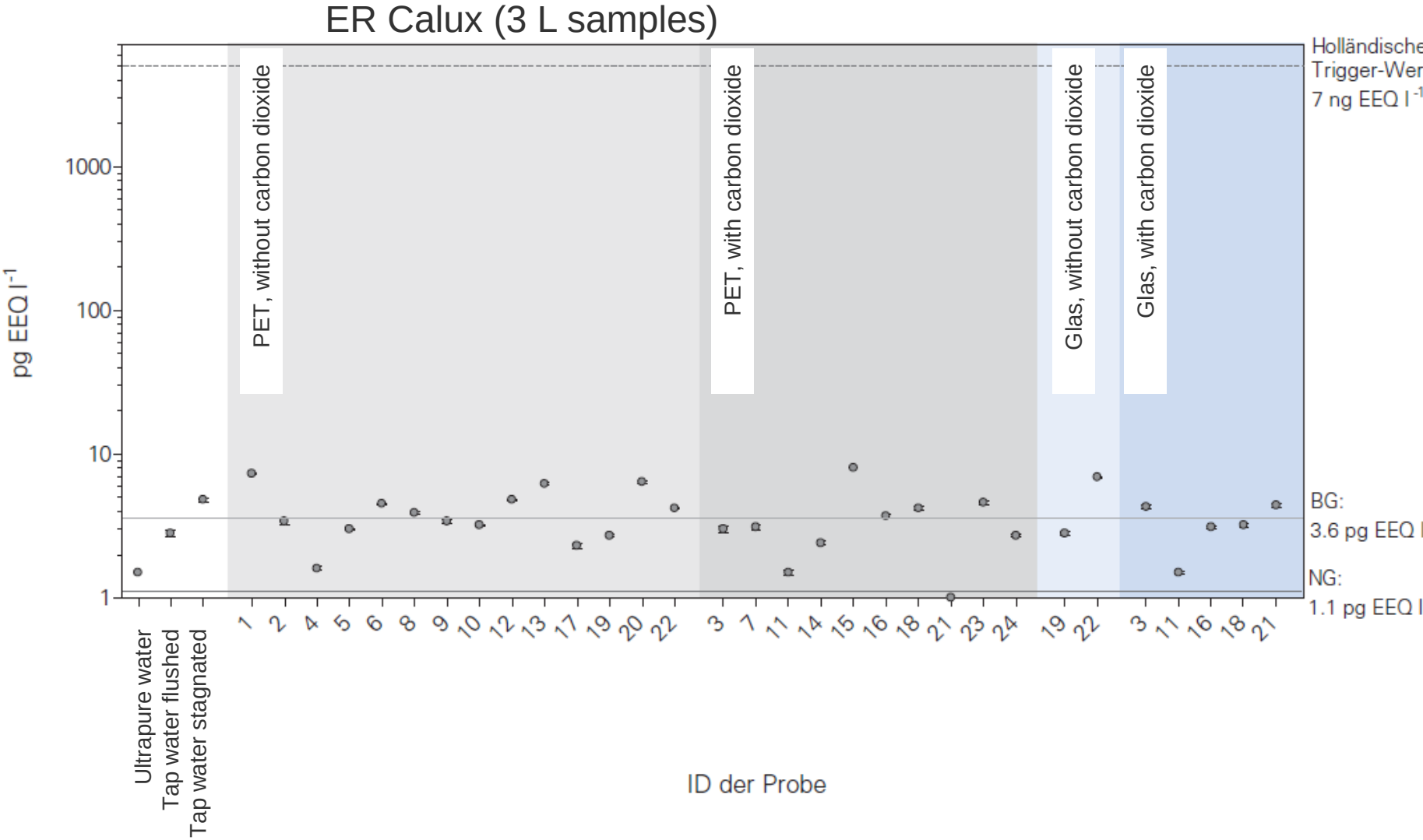



- NP in all water samples, no differences between water samples of different origin
- BPA: only elevated concentrations in two stagnated water samples
- **no E1,E2 and EE2** detected in drinking water samples

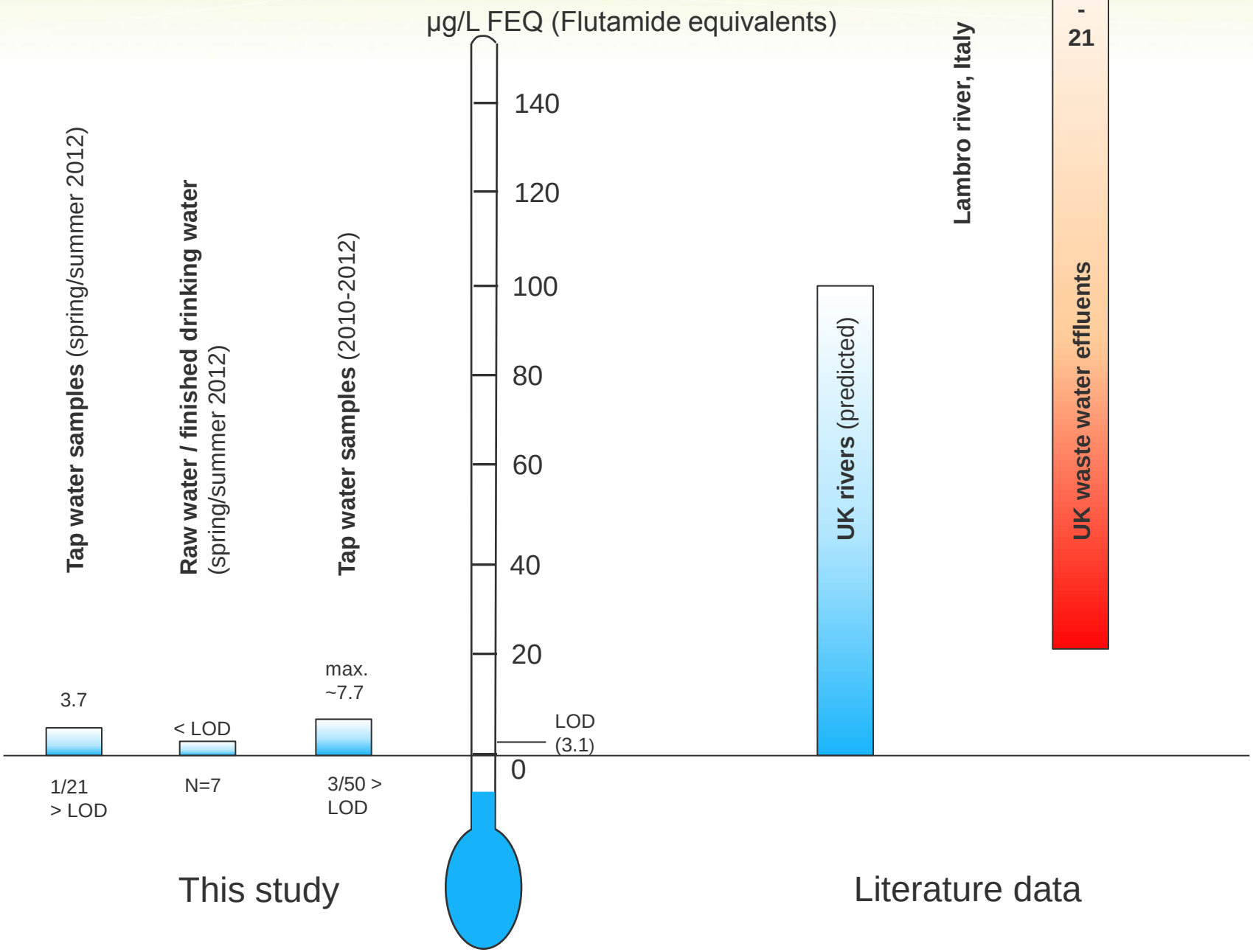
# Comparision: estrogenic Activity



# Bottled water study (Swiss Federal Office for Public Health, 2011)

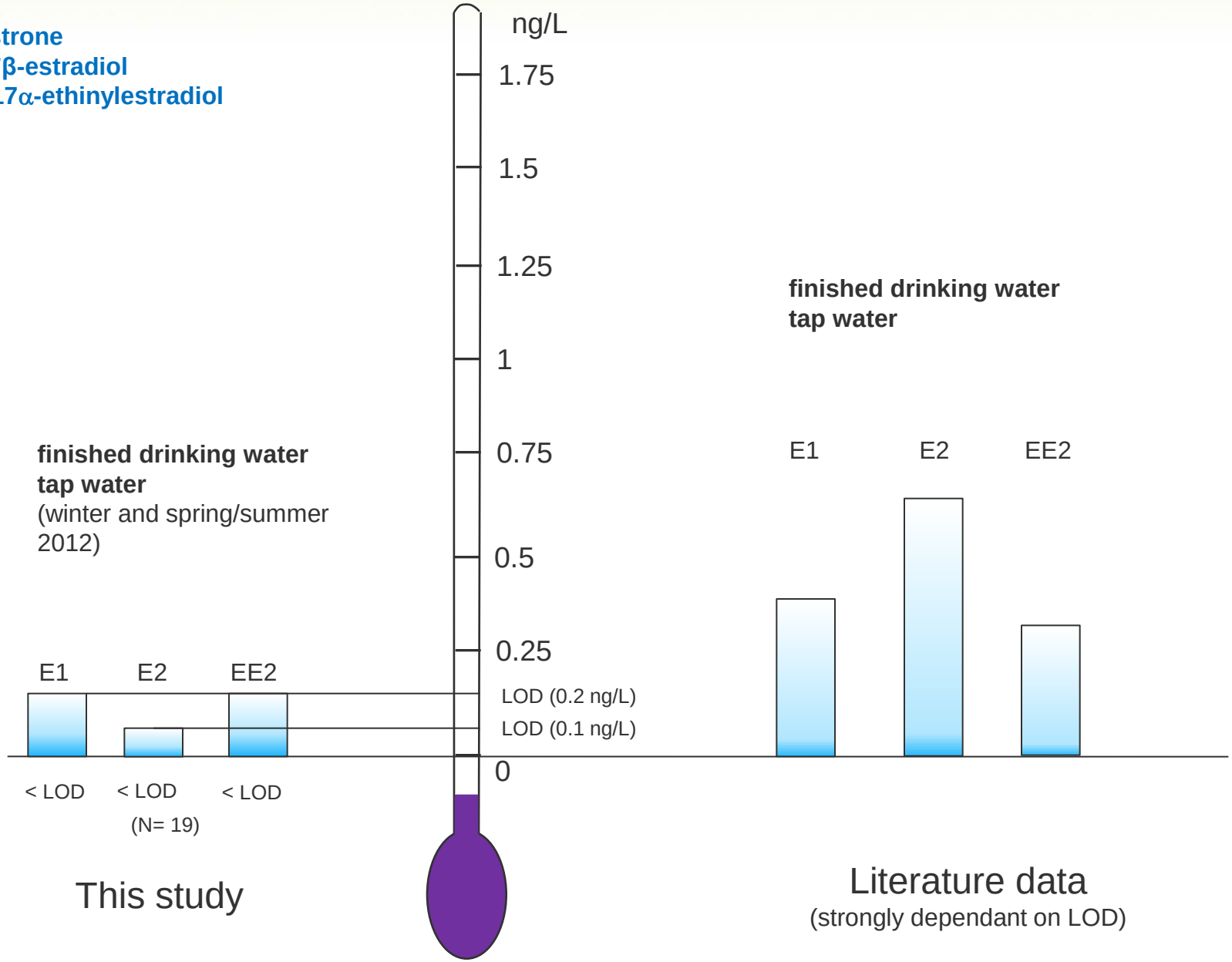


# Comparison: anti-androgenic Activity



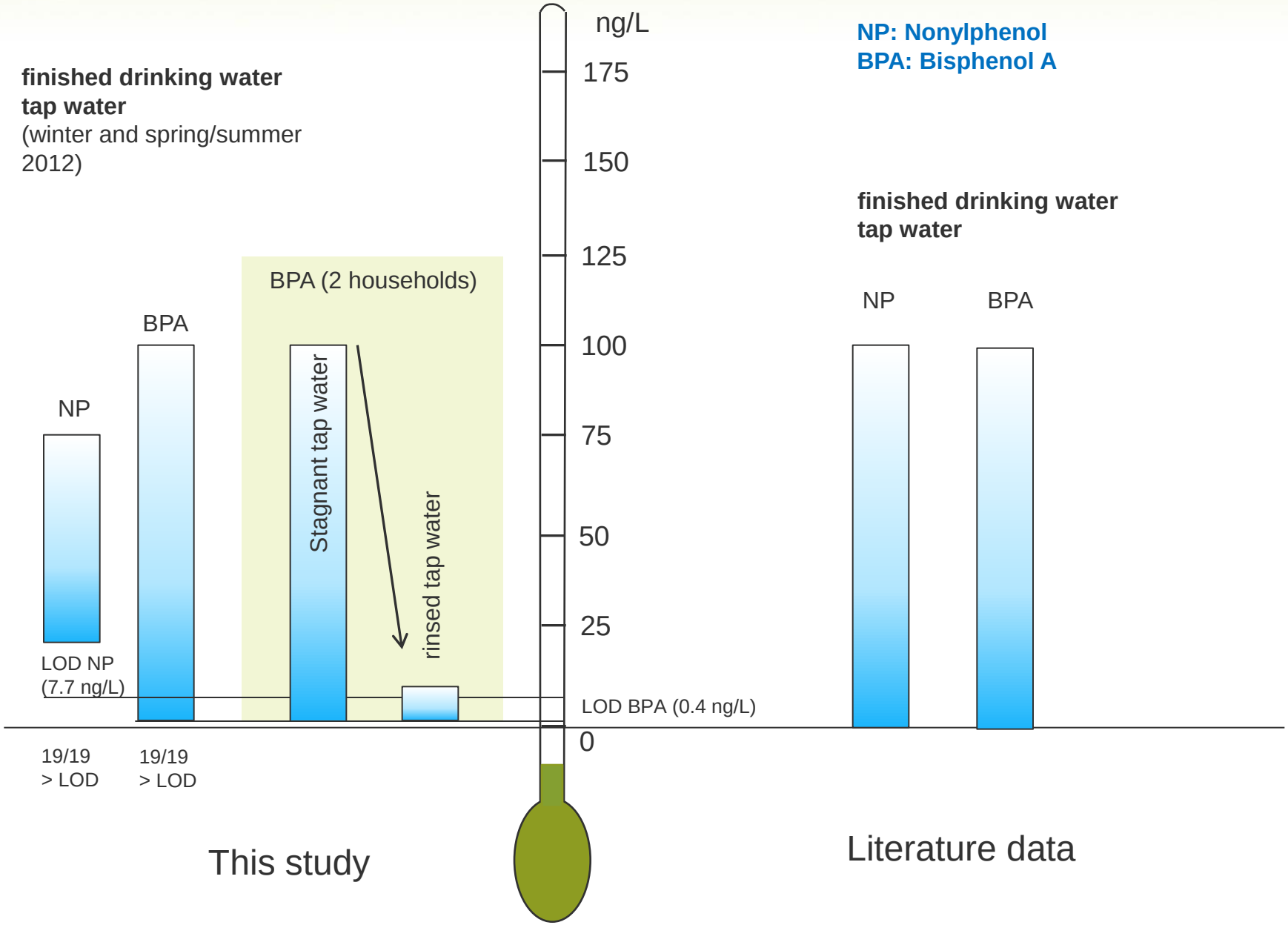
# Comparison: E1, E2, EE2

**E1: estrone**
**E2: 17β-estradiol**
**EE2: 17α-ethinylestradiol**



# Comparison: NP, BPA

**finished drinking water**
**tap water**
(winter and spring/summer 2012)



# Comparison of measured and calculated activity

- **Max. 5%** of estrogenic activity in positive samples is explained by target compounds
- **Max. 1%** of anti-androgenic activity in positive samples is explained by target compounds

| | Relative estrogenic potency (in EEQ) |
|---|---|
| E2 | 1 |
| E1 | 0.056 |
| EE2 | 1.2 |
| NP | 2.3E-5 |
| BPA | 7.8E-6 |

$$EEQ = \sum_i conc_i * REP_i$$

EEQ= 17β-estradiol equivalents (ng/L)
REP = relative estrogenic potential

| | Relative anti-androgenic potency (in FEQ) |
|---|---|
| NP | 0.3 |
| BPA | 0.6 |

$$FEQ = \sum_i conc_i * RP_i$$

FEQ= Flutamide equivalents (µg/L)
RP = relative anti-androgenic potency

# Risk evaluation

- Comparison of the daily intake with tolerable daily intakes (TDI)

| | TDI (per kg weight) | TDI | | Max. daily intake with 2 L/day (adult) 1.2 L/day (Child) | | TDI/ Max. daily intake by drinking water | |
|---|---|---|---|---|---|---|---|
| | | Adult (60 kg) | Child (25 kg) | Adult | Child | Adult | Child |
| **E1** | 0.86 ng/kg bw /day [1] | 51.6 ng/day | 21.5 ng/day | < 0.4 ng/day | < 0.24 ng/day | >129 | > 90 |
| **E2** | 50 ng/kg bw/day [2] | 3 µg/day | 1.25 µg/day | < 0.2 ng/day | < 0.12 ng/day | > 15‘000 | > 10‘400 |
| **EE2** | 0.043 ng/kg bw/day [1] | 2.6 ng/day | 1 ng/day | < 0.4 ng/day | < 0.24 ng/day | > 6.5 | > 4.5 |
| **NP** | 5 µg/kg bw/day [3] | 0.3 mg/day | 0.125 mg/day | < 150 ng/day | < 90 ng/day | > 2‘000 | > 1‘400 |
| **BPA** | 50 µg/kg bw/day [4] | 3 mg/day | 1.25 mg/day | < 200 ng/day | < 120 ng/day | > 15‘000 | >10‘400 |

[1] Australien Guidelines for recycled water , [2] WHO, [3] Danish EPA, [4] EFSA

- **Trigger value**: defines a level above which human health risk cannot be waived *a priori* and additional examination of specific endocrine activity may be warranted.

| | Reference | Max. measured activity in drinking water | **Trigger value** | Margin of safety |
|---|---|---|---|---|
| ER-activity | Brand et al. (2013) | < 1 ng/L (2010-2012)<br><br>< 0.067 ng/L (spring/summer campaign 2012) | 3.8 ng/L | > 3.8<br><br>> 56 |
| Anti-AR activity | - | 7.7 µg/L | n.a. | n.a |

Environ Int. 2013 May;55:109-18. doi: 10.1016/j.envint.2013.02.003. Epub 2013 Mar 28.

**Trigger values for investigation of hormonal activity in drinking water and its sources using CALUX bioassays.**

Brand W, de Jongh CM, van der Linden SC, Mennes W, Puijker LM, van Leeuwen CJ, van Wezel AP, Schriks M, Heringa MB.

KWR Watercycle Research Institute, Groningenhaven 7, 3433 PE Nieuwegein, The Netherlands. walter.brand@gmail.com

# Contribution of drinking water consumption to total exposure by diet

| | Total exposure via nutrition and beverages<br><br>(adults) | Exposure via drinking water<br><br>(adults) | Contribution of drinking water consumption to total exposure |
|---|---|---|---|
| NP | 7.5 µg NP/day [1] | < 150 ng NP/day | < 2% |
| BPA | 90 µg BPA/day [2,3] | < 200 ng BPA/day | < 0.22% |
| Estrogens | | | > 0.6% [5] |
| ER activity | Women: 27.5 ng EEQ/day [4]<br>Men: 34 ng EEQ/day [4] | < 2 ng EEQ/day | < 7% |
| Anti-AR activity | n.a. | - | - |

[1] Günther et al (2002), [2] EFSA (2006), [3] EFSA (2010), [4] Behr et al (2011), [5] Caldwell et al. (2010)

## Conclusions:

- target compounds and estrogenic activity found in household tap water, but

  - exposure to target compounds and estrogenic activity: diet far more important than consumption of drinking water.
  - target compounds: daily intake via drinking water << TDI
  - ER activity: << Trigger value of 3.8 ng/L EEQ

  → no risk for human health

- estrogenic activity in drinking water comparable to activity in bottled water

  → change from consumption of drinking to bottled water cannot explain decrease or disappearance of premature thelarche.

- anti-androgenic activity: rarely seen, slightly above LOD of CALUX

- leaching of chemicals (BPA, NP) from the tubing possible

# Questions?

| | Per Volume [ng/L EEQ] | | | | Per serving unit [ng/L EEQ] | | | |
|---|---|---|---|---|---|---|---|---|
| **Food/beverage** | **Median** | **Min.** | **Max.** | **Occurrence frequency (total samples)** | **Serving size [ml or g]** | **Activity/serving size (max.)** | **Bio assay** | **Ref.** |
| Apple juice | 0.74 | 0.73 | 0.79 | 3 (4) | 240 ml | 0.19 | YES | [22] |
| Green tea | 3 | 1.5 | 4.4 | 4 (4) | 240 ml | 1.1 | | |
| Infant formula | 0.79 | 0.73 | 0.85 | 2 (2) | 30 ml | 0.03 | | |
| Milk | 0.55 | 0.48 | 0.81 | 3 (3) | 240 ml | 0.19 | | |
| Soy infant formula | 1700 | 1500 | 1900 | 2 (2) | 30 ml | 57 | | |
| Soy milk | 4000 | 1900 | 4200 | 3 (3) | 240 ml | 1000 | | |
| Soy sauce | 220 | 28 | 510 | 4 (4) | 15 ml | 7.7 | | |
| Vegetable juice | 2.6 | 2.1 | 3.3 | 4 (4) | 240 ml | 0.79 | | |
| | | | | | | | | |
| Meat | - | - | - | - | 42 g [1] | 0.00 (0.091) [1] | YES | [85] |
| Cheese | - | - | - | - | 41 g [1] | 1.18 (1.75) [1] | | |
| Fish | - | - | - | - | 13 g [1] | 0.138 (0.187) [1] | | |
| Sweets | - | - | - | - | 21 g [1] | 2.09 (5.24) [1] | | |
| Bread | - | - | - | - | 134 g [1] | 21.2 (81.6) [1] | | |
| Soy | - | - | - | - | 3 [1] | 2.87 (5.84) [1] | | |

[1] Case for female adult: all products (including soy containing products)

[22] Stanford et al. (2010), *Journal American Water Works Association*
[85] Behr et al. (2011), *Food and Chemical Toxicology*

# Methods:

## In-vitro bioassay

max. 500 ml samples
SPE pre-concentration (x 10'000)

## LC-MS chemical analysis

1 Liter samples
SPE pre-concentration (x 5'000)

## Detection limits

**ER:** 20-30 pg/L 17β-estradiol equivalents (EEQ)

**Anti-AR**: 3.1-3.5 μg/L flutamide equivalents (FEQ)

- bisphenol A: 0.4 ng/L
- nonylphenol: 7.7 ng/L
- 17β-estradiol: 0.1 ng/L
- 17α-ethinylestradiol: 0.2 ng/L
- estrone: 0.2 ng/L