



## **Water testing**

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BioDetectors 2007

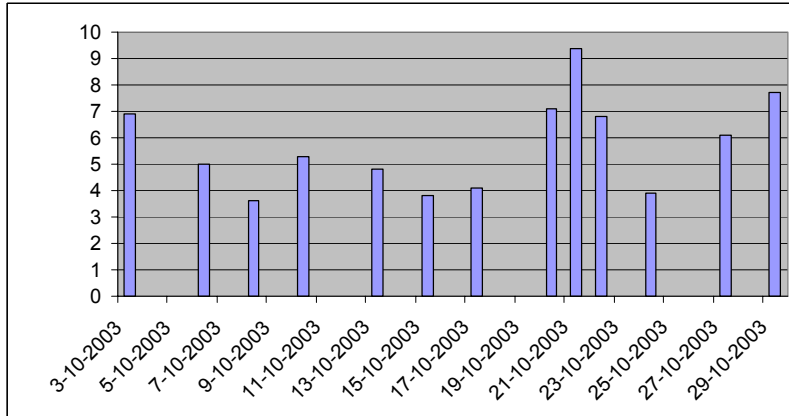
# Introduction



- Dutch drinking water prepared from ground water and surface water
- Present screening for chemical contaminants by chemical analysis and health effects by literature
  - Unknown compounds missed
  - Total effect of mixture unknown
- Research on applying toxicity tests
  - Genotoxicity
  - Endocrine disruption
- Application of CALUX assays to study water contamination in the Netherlands



## Estrogenic activity in Meuse, October 2003 (ER-CALUX, ng EEQ/L)

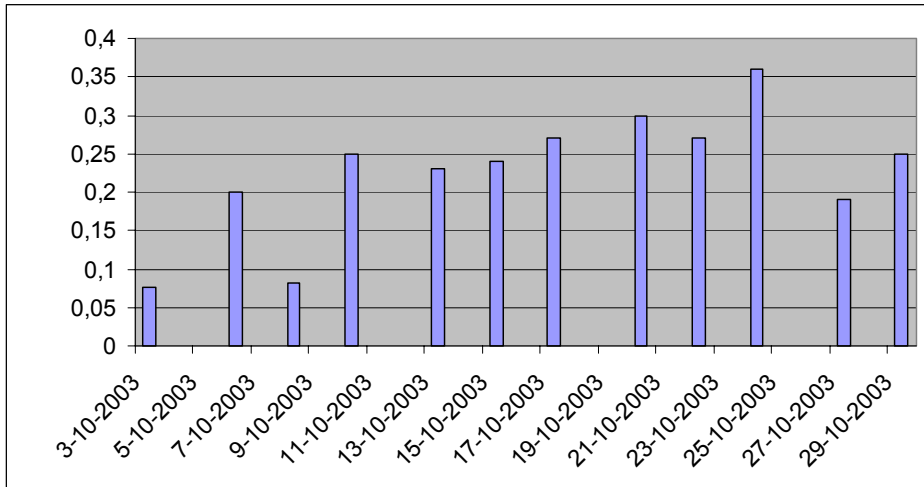


Trigger value human health: 7 ng EEQ/L (RIVM)

Bogers, R., et al. *Environ Int* 2007, 33, (3), 292-301.



# Estrogenic activity Rhine (Lekkanaal), October 2003 (ER CALUX, ng EEQ/L)



Bogers, R., et al. *Environ Int* 2007, 33, (3), 292-301.



## ***In vivo* test with male Fathead minnow; flow-through systems**





# Fathead minnow (*Pimephales promelas*, Teleostei, Cyprinidae)



 **NOTOX**

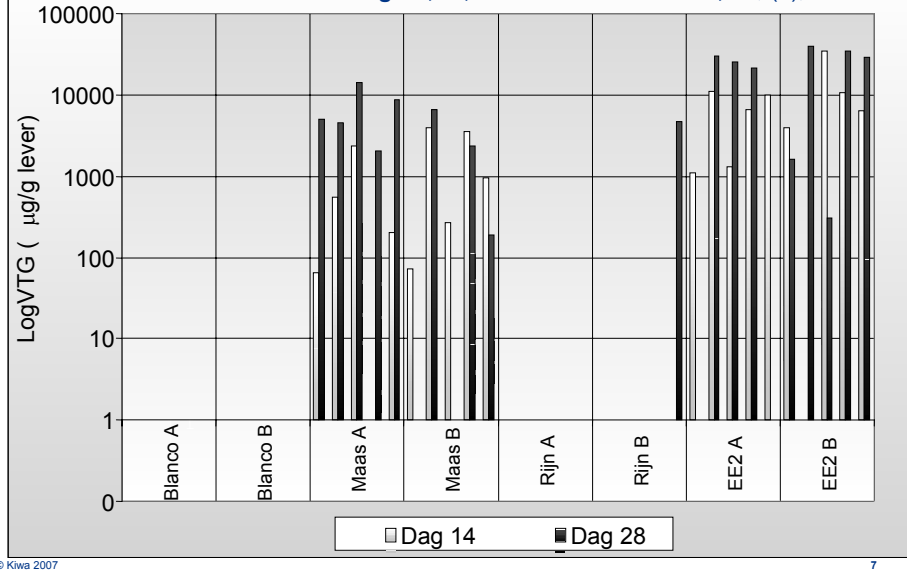
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## Vitellogenin concentration in male fish (Fathead Minnow)

Bogers, R., et al. *Environ Int* 2007, 33, (3), 292-301.



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- Deze in vivo test is een van de eerste pogingen om direct oestrogene effecten in oppervlaktewater te meten met een standaard laboratorium testopzet.

## Additional measurements in March 2004 at intake points along the Meuse



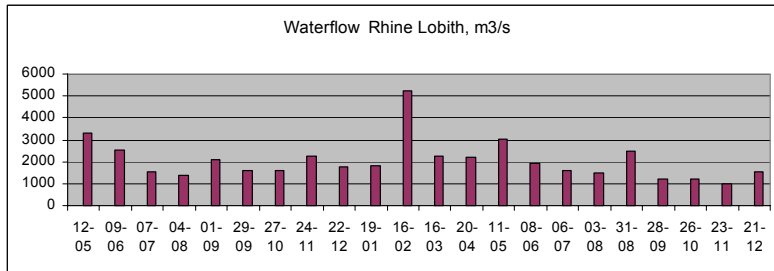
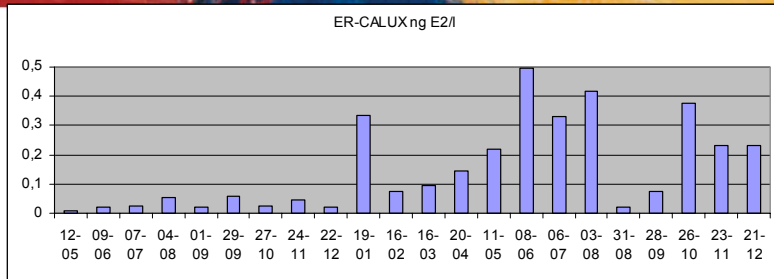
<b>Raw water:</b>	<b>ER-CALUX (ng EEQ/L)</b>
■ Berenplaat	1,1 - 1,7
■ Braakman	1,1 - 1,4
■ Andelse Maas	0,6 - 0,8
■ Heel	0,7 - 1,0
■ Roosteren	< 0,014
■ Albert Kanaal	0,074 - 0,076
■ Tailfer	0,18 - 0,53

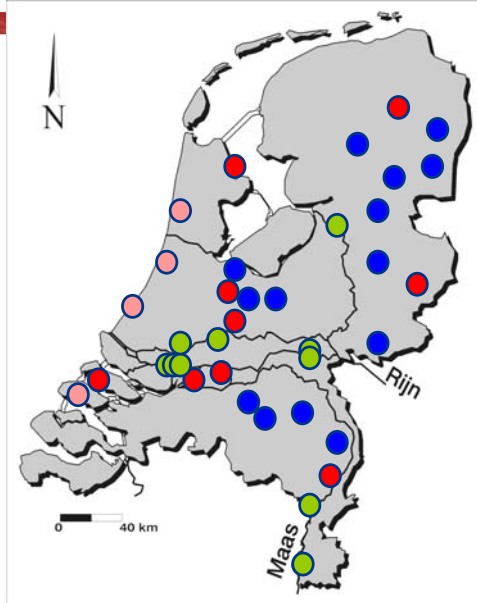
<b>Drinking water:</b>	
■ All locations	< 0,014 (LOES: 0,021- 0,022)



## Seasonal pattern: estrogenic activity in the Rhine (Lobith) measured with ER-CALUX® in 2004 - 2005 (RIWA)



# Estrogenic activity in groundwaters





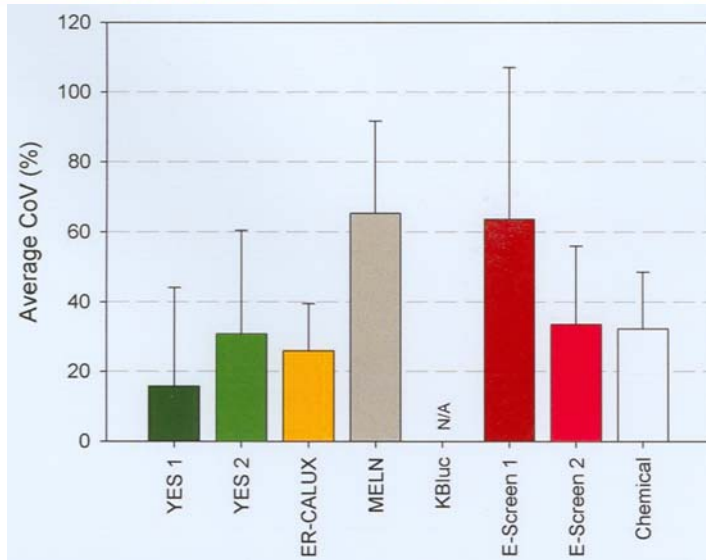
## Conclusions - 1



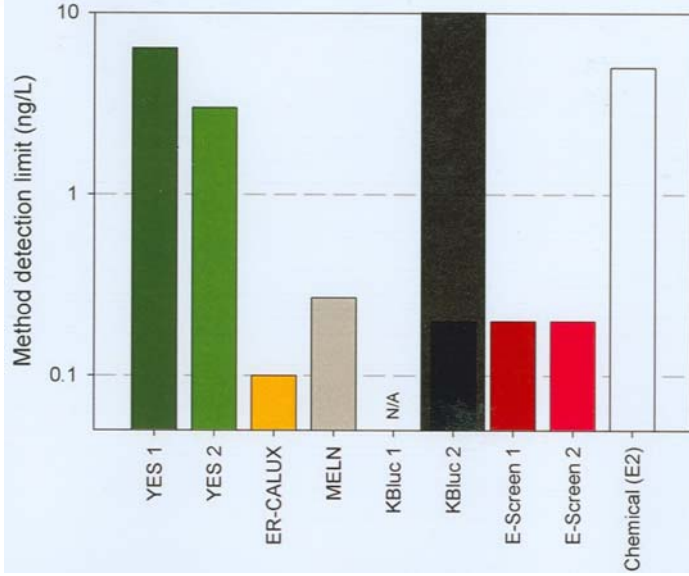
- **Estrogenic levels in Meuse of concern**
  - Ecotoxic effects (fish)
  - Trigger value for human risk reached
- **Estrogenic levels in Rhine and in raw groundwaters do not give rise to concern**
- **Estrogenic levels in drinking water too low to give concern**



## Comparison of estrogenicity tests: coefficient of variation



## Comparison of estrogenicity tests: detection limit



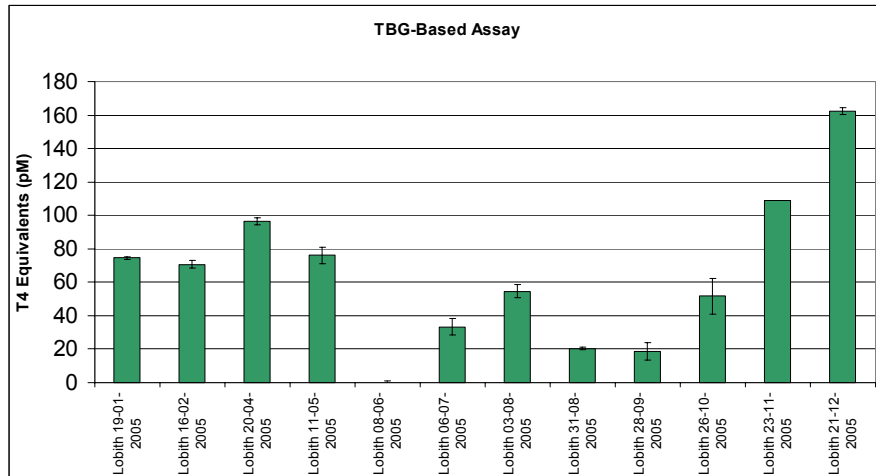


## Different types of hormones, natural and synthetic

- **Estrogens**
  - 17 $\alpha$ -estradiol, estrone, estriol, 17 $\alpha$ -estradiol
- **Androgens**
  - testosterone, dihydro-testosterone, trenbolone
- **Progestogens**
  - progesterone, norethynodrel, levonorgestrel
- **Glucocorticoids**
  - Cortisol, dexamethasone, prednison, prednisolon
- **Thyroid hormones**
  - Tri-iodo-thyroine (T3), tetra-iodo-thyroine (T4)



## Thyroid hormone activity based on affinity for T3/T4 hormone binding protein (TBG) in Rhine (Lobith) in 2005 (RIWA)

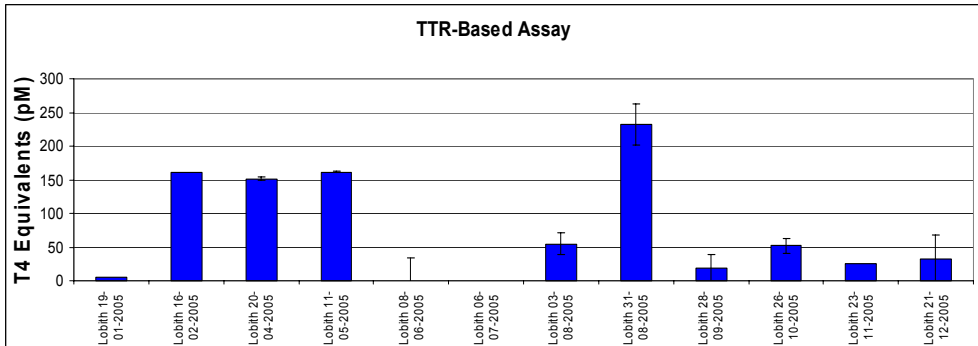


Meulenberg and Marchesini. Report RIWA Rhine Water Works, 2006.

- Two main components of the thyroid system in blood are tri- and tetra-iodo-thyronine, produced in the thyroid gland.
- Equilibrium between bound and free hormones defines thyroidal status.



## Thyroid hormone activity based on affinity for T3/T4 transthyretin protein (TTR) in Rhine (Lobith) in 2005 (RIWA)



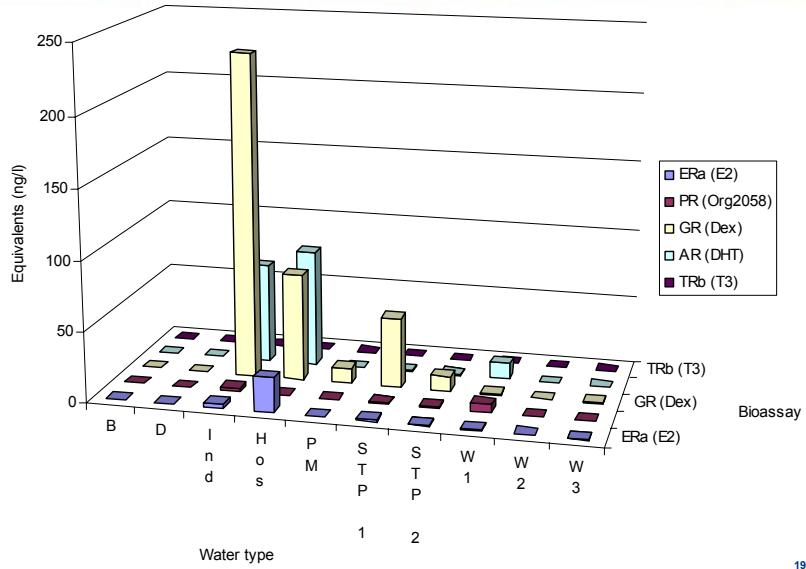
Meulenberg and Marchesini. Report RIWA Rhine Water Works, 2006.



## Five hormonal activities with CALUX-battery (BDS)

- ER-CALUX for estrogenicity
  - AR-CALUX for androgenicity
  - PR-CALUX for progestagenic activity
  - TR-CALUX for thyroid activity
  - GR-CALUX for glucocorticoid activity
- 
- All in U2OS bone cell line
  - No natural hormone receptors
  - No interference from cross-talk between hormone systems

# CALUX battery on different types of water



## Conclusions - 2



- **First results indicate considerable levels of progesterone, androgen and glucocorticoid activity in the water cycle**
  - further research on drinking water-risk necessary
  
- **Difference between results of thyroid receptor-based and transport protein-based need further study**

## Sample preparation



- **C18 SPE often used : polar compounds not well extracted**
- **We used LLE with ethylacetate: higher levels of estrogenicity**
- **Now tried SPE with OASIS HLB columns:**
  - better than LLE for polar compounds
  - Similar to LLE for non-polar compounds
  - Practical advantages

Minne Heringa, 10 October 2007



Partner for progress



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## Estrogens (ng/L) and xeno-estrogens (EDCs, µg/L) in drinking water (sources)



	Surface water	Drinking water
■ <b>Hormones</b>		
■ estrone		1 - 10 ng/L
■ 17β-estradiol	1 - 5	5 ng/L ?
■ 17α-ethinylestradiol	1 - 5	< 0,3 - <1
■ <b>Phtalates</b>		
■ DEP	1 - 10 µg/L	1 - 2,1 µg/L
■ DEHP	1 - 5	1,3 - 2,5
■ <b>Alkylphenoethoxylates</b>		
■ Nonylphenoethoxylates	0,1 - 4,6	0,5 - 2
■ Octylphenoethoxylates	0,1 - 0,7	< 0,5
■ <b>Bisphenol A</b>		0,01 - 1
■ <b>Pesticides</b>	0,01 - 5	0,01 - 0,1
■ <b>PCBs</b>	0,01 - 0,02	< 0,01
■ <b>PAHs</b>	0,01 - 0,1	< 0,01

- Geen complete lijst, onder meer ook nog organotinverbindingen, en sinds kort bekend ook geurstoffen.
- Lijst zal in de toekomst alleen maar groeien. Daarom effectmetingen met in vitro-bioassays. De ER-CALUX assay is zo'n test. Cellen afkomstig van menselijk weefsel, worden blootgesteld aan enkele microliters van een extract van een watermonster. Hormonen en xeno-oestrogenen aanwezig in dit extract zullen de celwand passeren, en aan de in de cel aanwezige oestrogeen receptor binden. Hierdoor wordt een reactie in gang gezet waarbij uiteindelijk het eiwit luciferace wordt gevormd. Dit wordt gemeten.
- Er zijn daarnaast meer endocrine effecten,