



Endocrine disruptors in water

Minne Heringa
Merijn Schriks

Biodetectors 2009, 24 September 2009

KWR

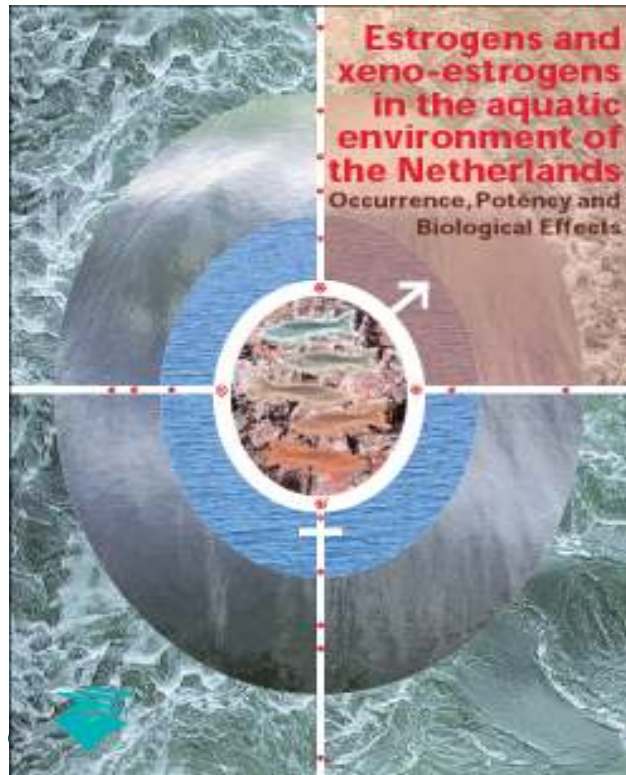
Watercycle Research Institute

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

Introduction - endocrine disruption

After “Our Stolen Future” (Theo Colborn *et al.*, 1996), a lot of attention towards estrogenic effects of compounds;



1999-2001; Dutch research into the presence of (xeno)estrogens in the aquatic environment

→ (xeno)estrogens in Dutch surface water

→ do they reach drinking water?

Estrogenic compounds in drinking water – recent scientific findings

SHORT COMMUNICATION

Screening of estrogen-like activity of mineral water stored in PET bottles

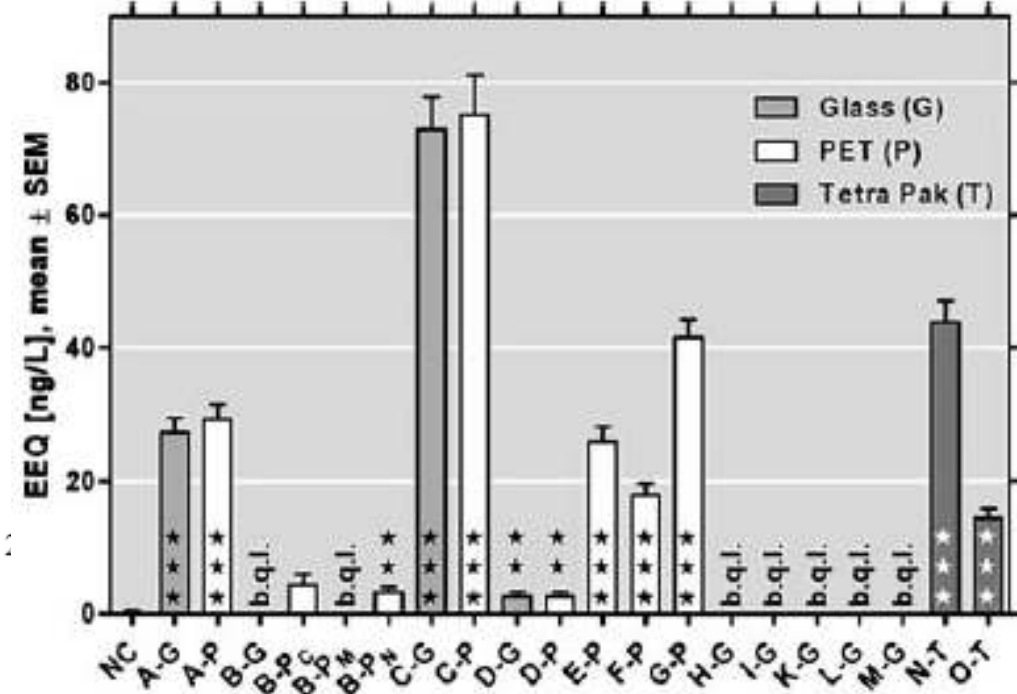
Barbara Pinto*, Daniela Reali

^aBrand ID number EEQs (ng/L)

1	17.6
2	13.9
3	8.41
4	4.1
5	3.5
6	23.1
7	0.9
8	4.8
9	11.5
TW-SuW	17.2
TW-GrW	15.1
TW-SpW	0.46
Blank	0

Endocrine disruptors in bottled mineral water: total estrogenic burden and migration from plastic bottles

Martin Wagner • Jörg Oehlmann Environ Sci Pollut Res March 2009



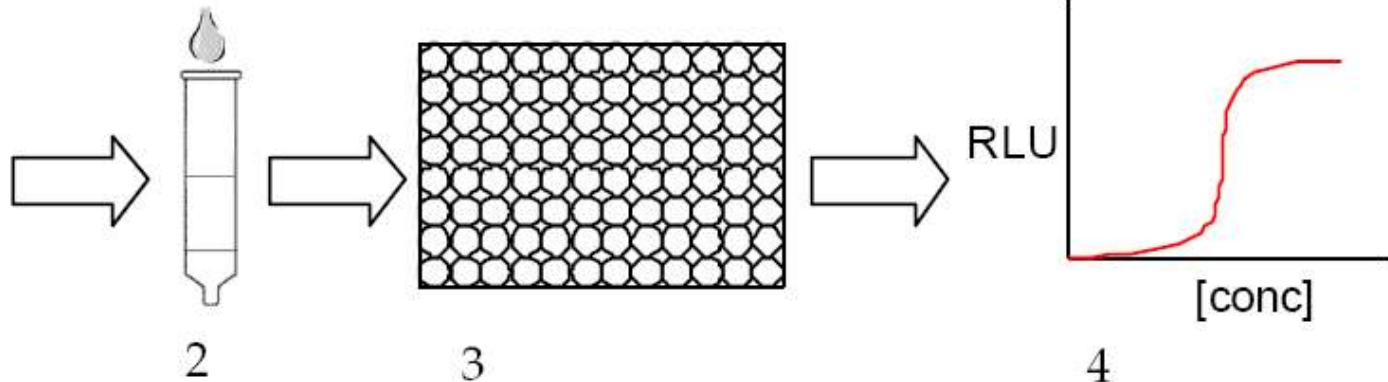
B. Pinto, D. Reali / Int. J. Hyg. Environ. Health 212 (2009) :

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Studies KWR - BDS



1



2

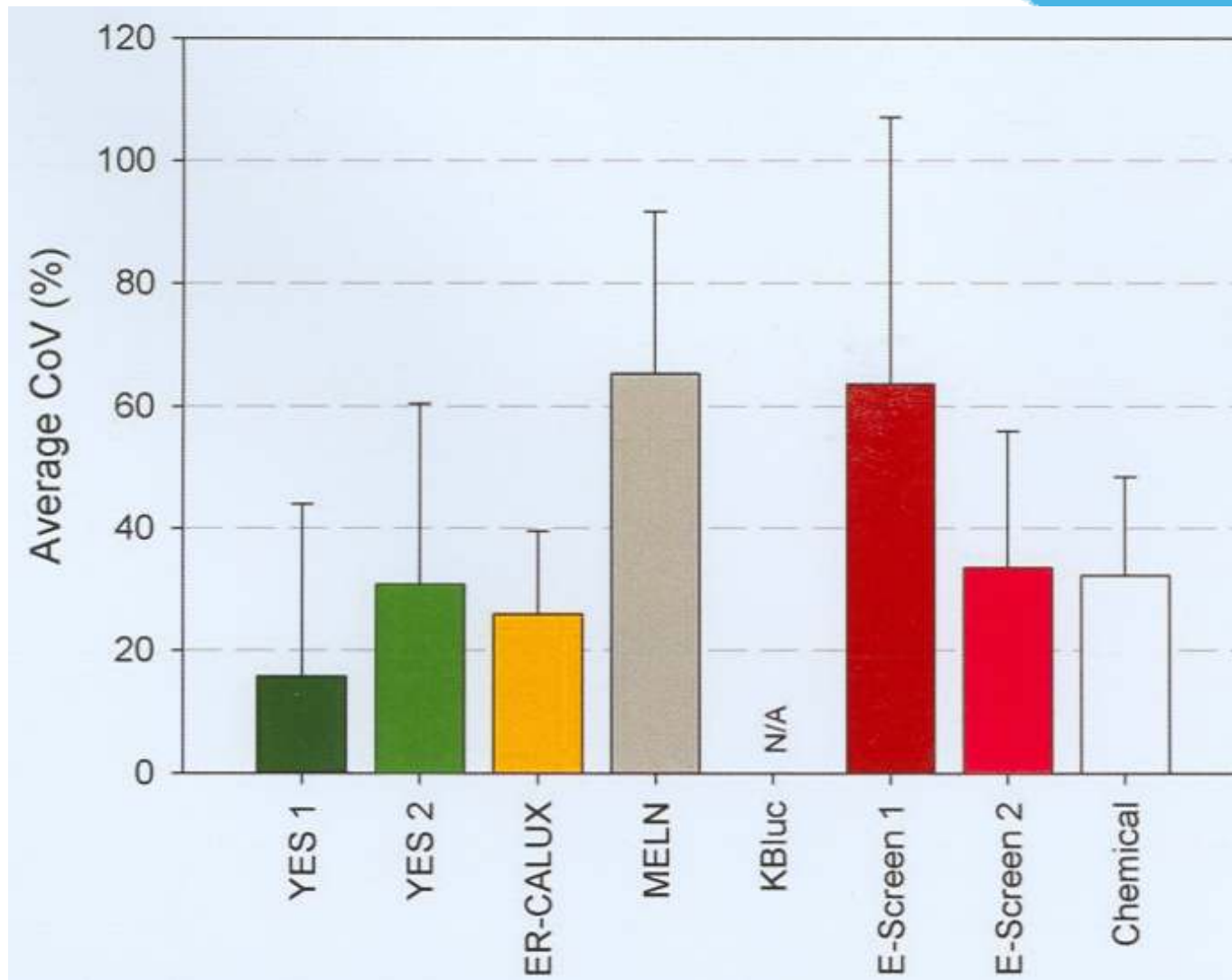
3

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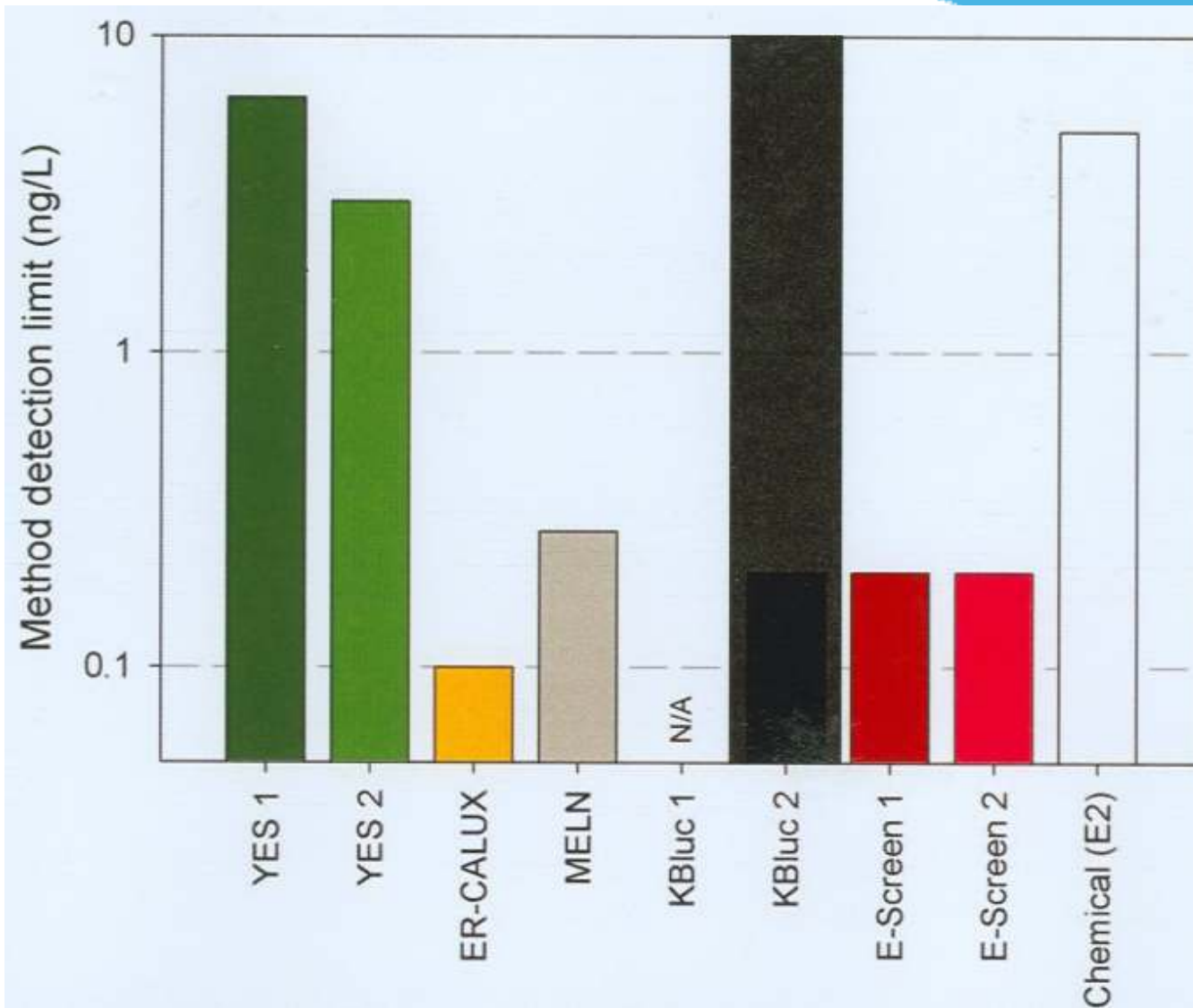
- KWR: sample collection-preparation-extraction;
- BDS: CALUX[®] bioassays

KWR

GWRC comparison of estrogenicity tests: coefficient of variation



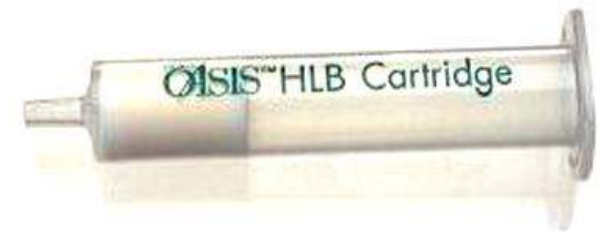
GWRC comparison of estrogenicity tests: detection limit



Methods

Sample collection

- Ultra-cleaned glass bottles
- Storage at 4°C and filtration within 24 hrs.



Sample extraction

- Pre-filtration with 0.45 µm cellulose nitrate filters (Sartorius)
- L/L extraction with ethyl acetate or SPE extraction (OASIS HLB)
- Evaporation under N₂, redissolving in DMSO
- Storage at -18°C until further analysis

Methods - sampling

2 (water) sampling campaigns:

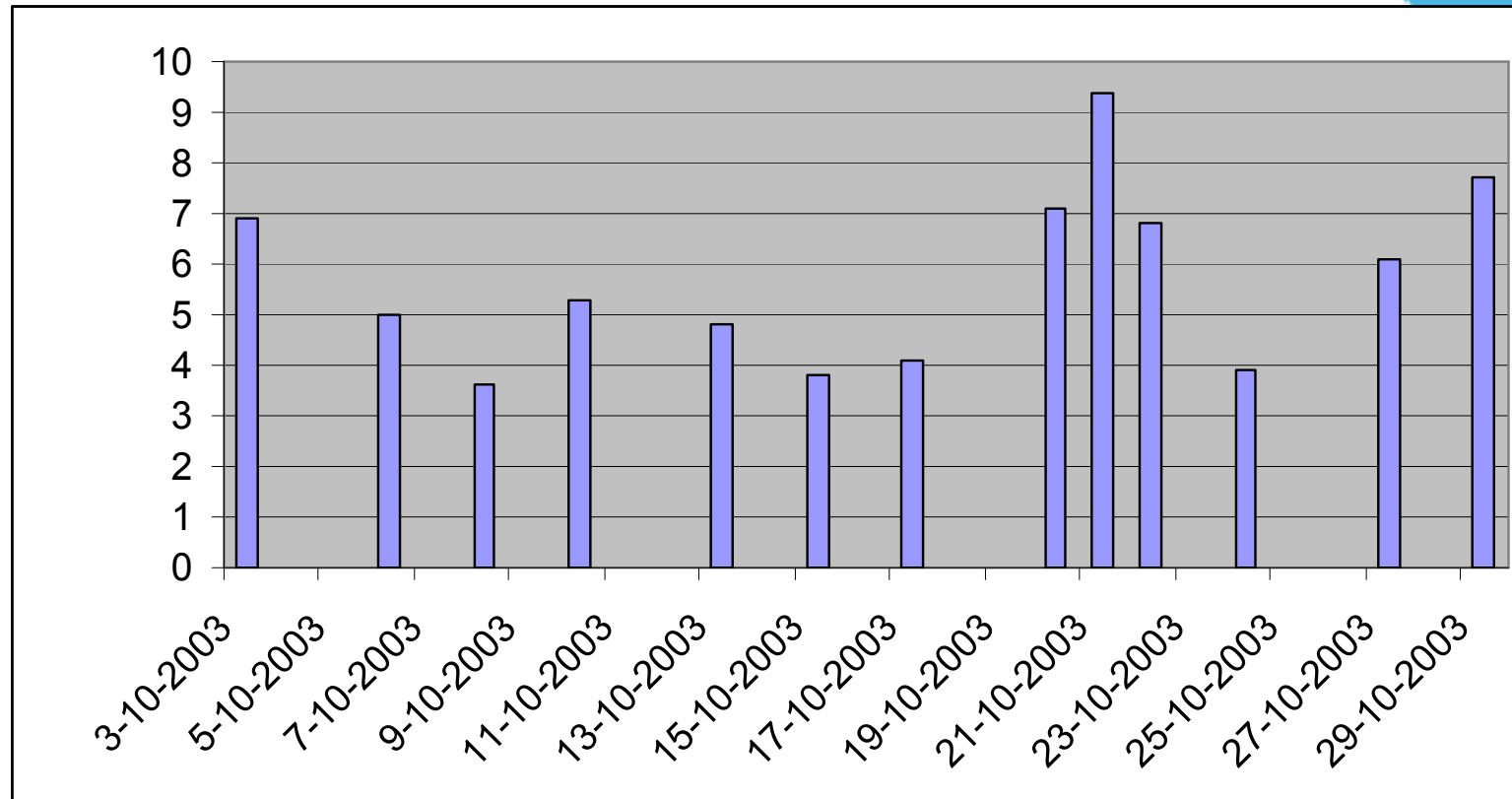
A) Various sources (9x) throughout the NL
(not hydrologically connected)
(Linden et al., 2008)

B) 2 sample locations in the NL,
hydrologically connected, aug 2007-aug
2008), bimonthly sampling

- Rhine (Lobith, entrance Rhine NL)
- Lekkanaal (Nieuwegein, major intake site for drinking water)
(Schriks et al., in prep)

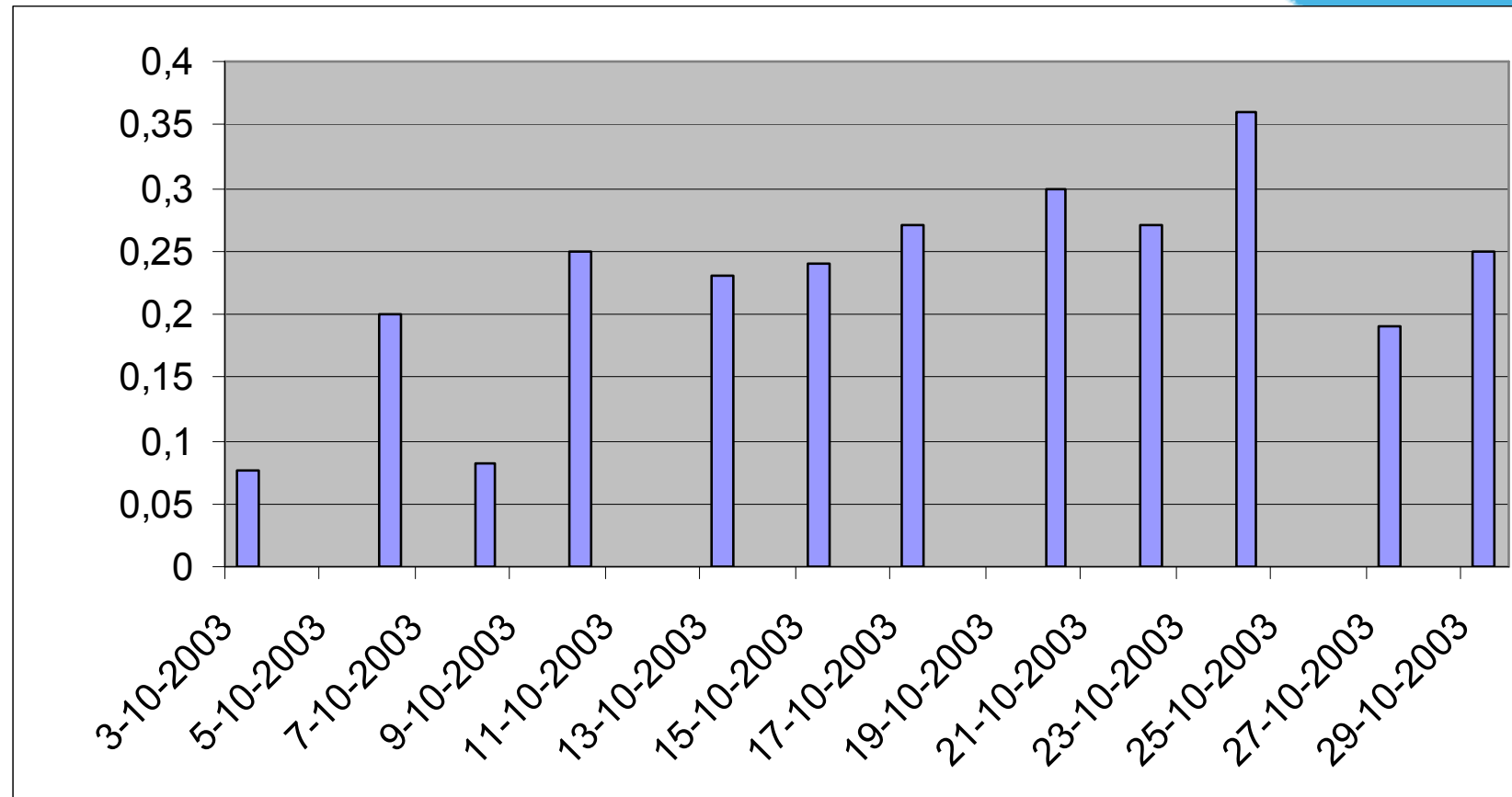


Estrogenic activity in Meuse, October 2003 (ER-CALUX, ng EEQ/L)



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

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



Additional measurements in March 2004 at intake points along the Meuse

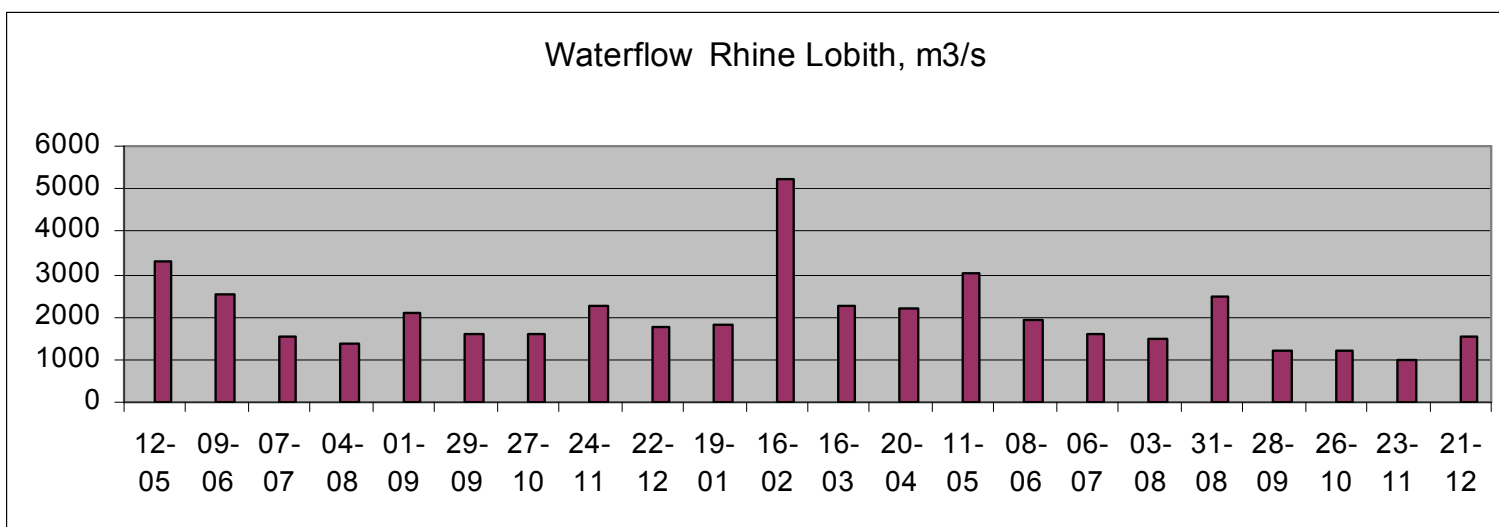
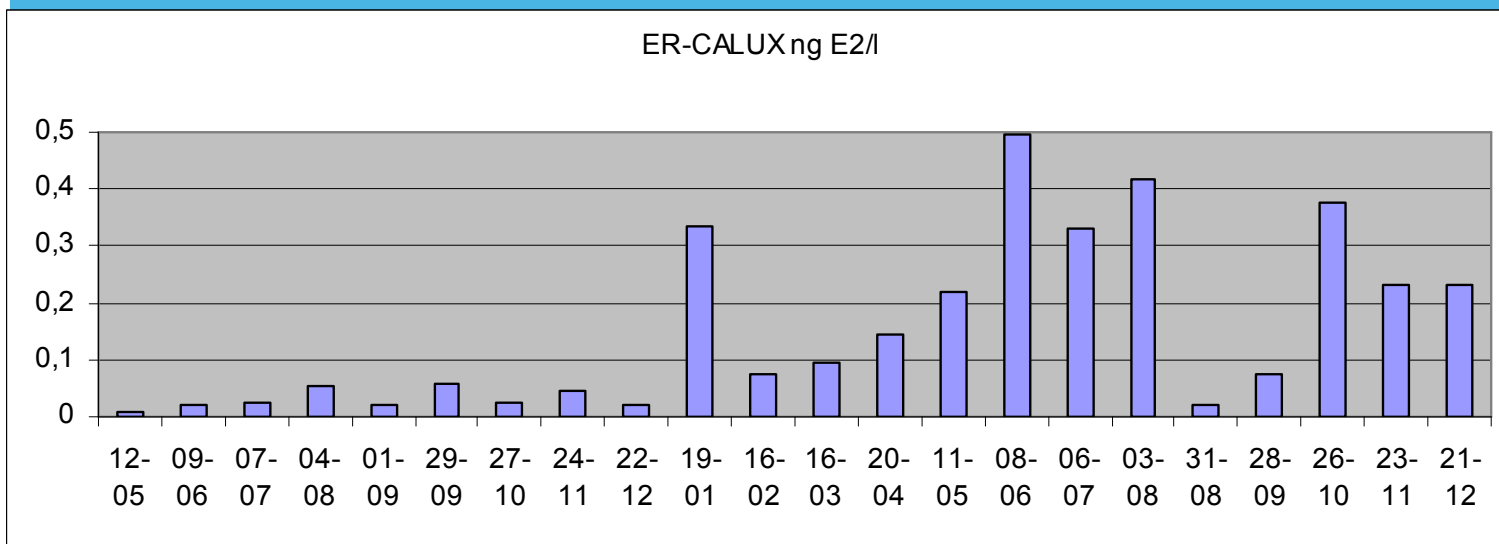
Raw water: ER-CALUX (ng EEQ/L)

- 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

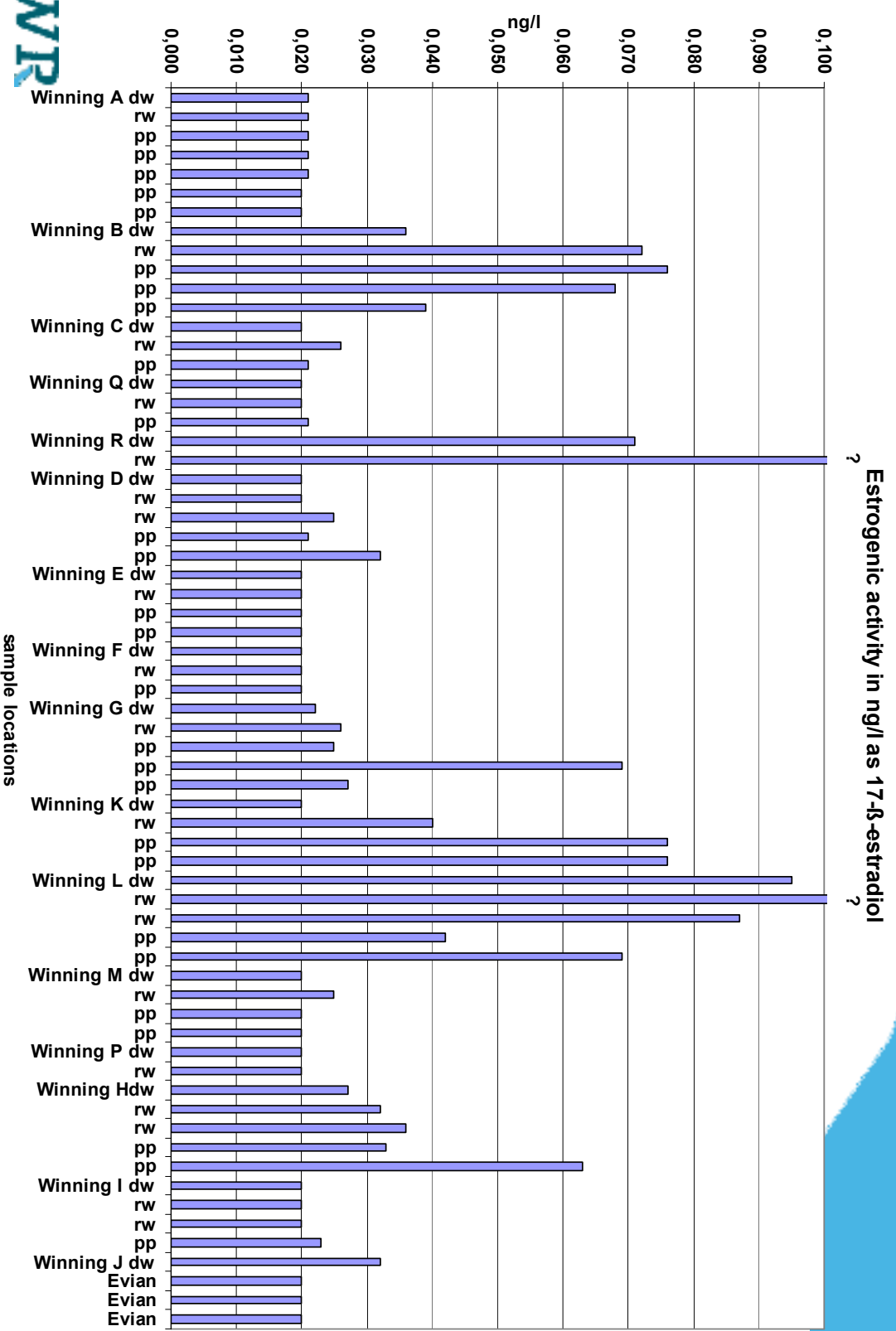
Drinking water:

- 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

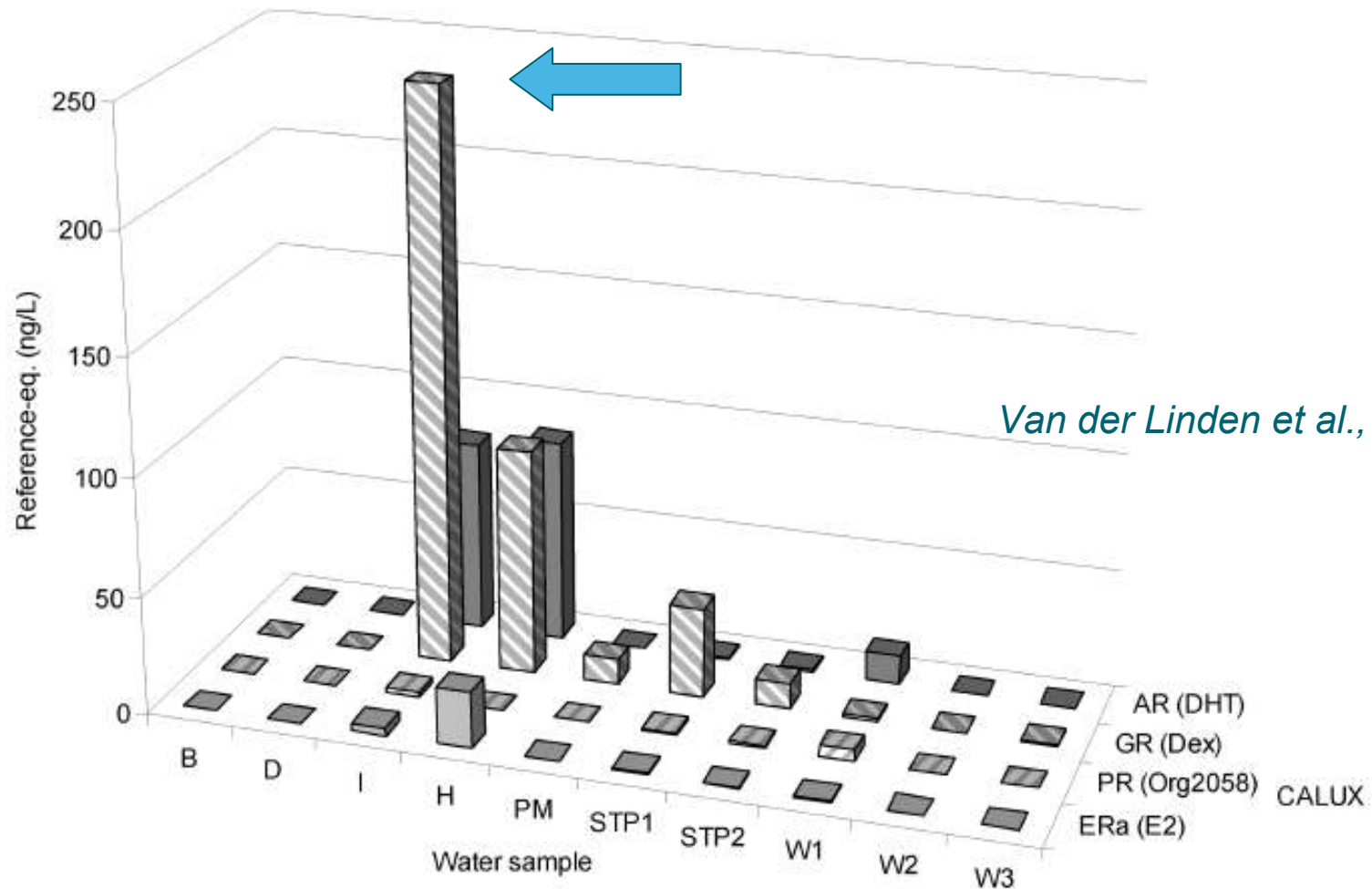
- Estrogenic levels in Meuse of concern
 - Ecotoxic effects (fish)
 - Trigger value for human risk reached
- Estrogenic levels in Rhine and in raw groundwaters do not give rise to concern
- Estrogenic levels in drinking water too low to give concern

→ But how about other hormones?

Five hormonal activities with CALUX-battery (BDS)

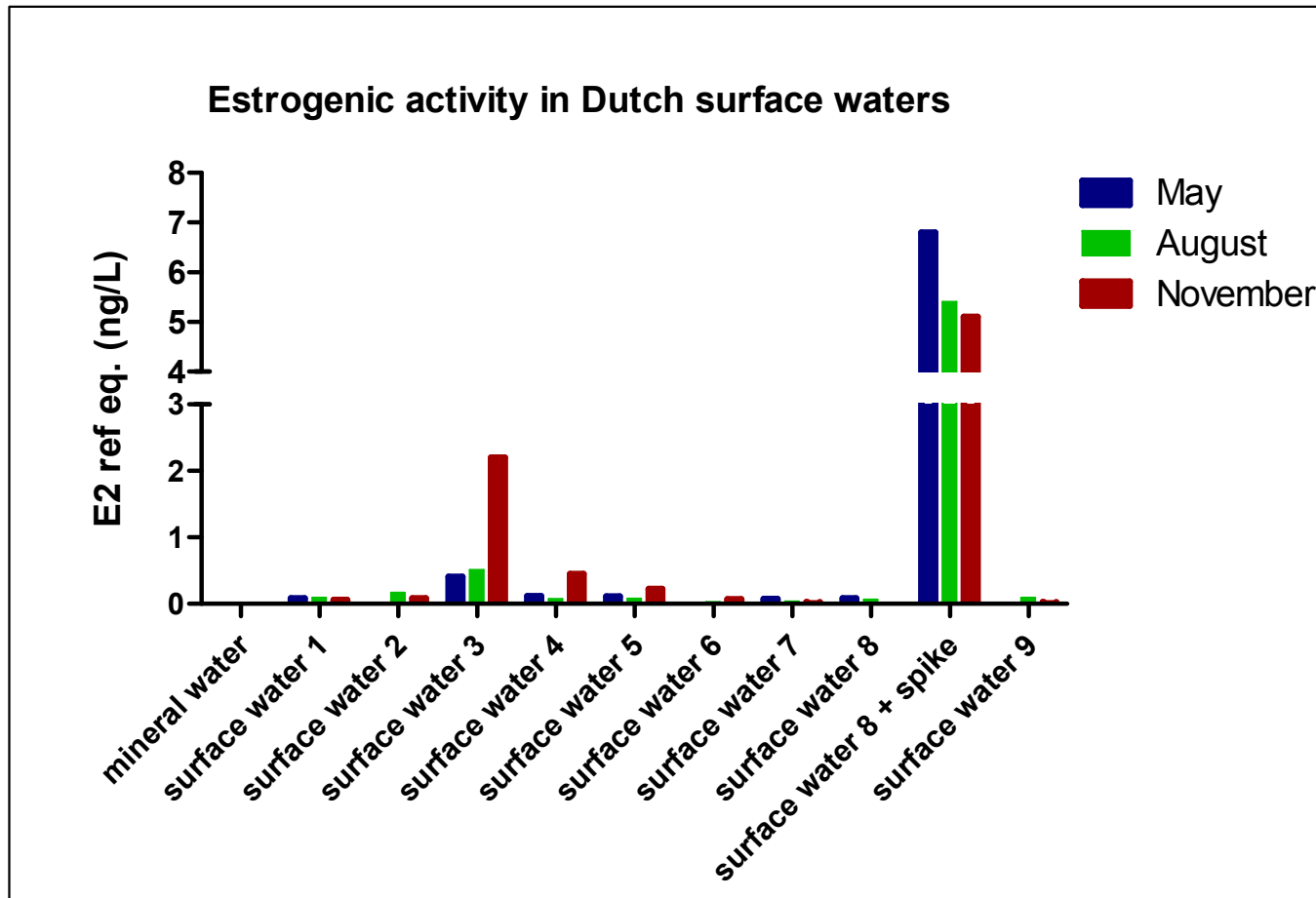
- ER-CALUX for estrogenicity
 - AR-CALUX for androgenicity
 - PR-CALUX for progestagenic activity
 - TR-CALUX for thyroid activity
 - GR-CALUX for glucocorticoid activity
-
- All in U2OS bone cell line
 - No natural hormone receptors
 - No interference from cross-talk between hormone systems

Multiple types of hormone activity (CALUX bioassays) in different water samples (2006-2007)

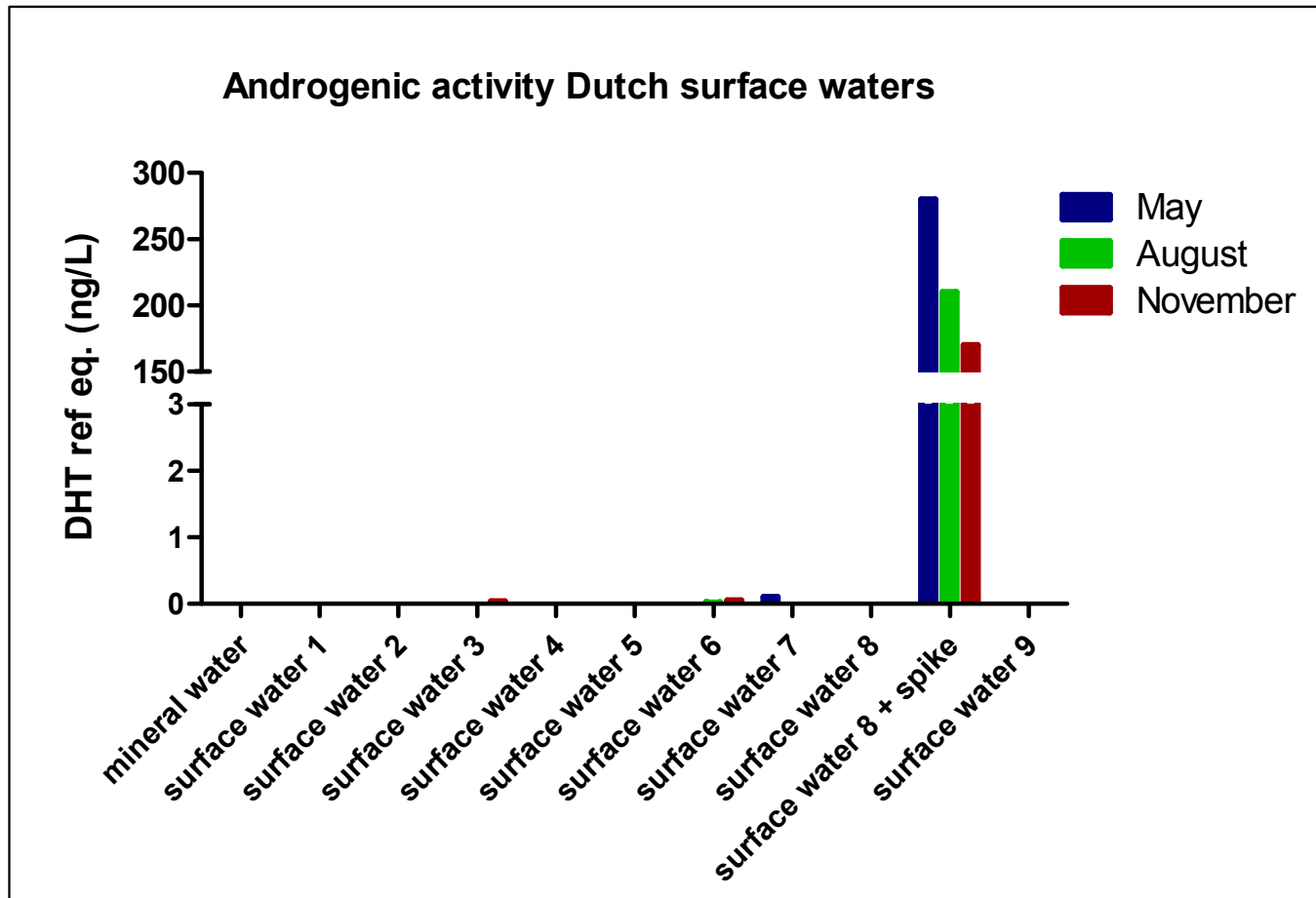


Van der Linden et al., ES&T 2008

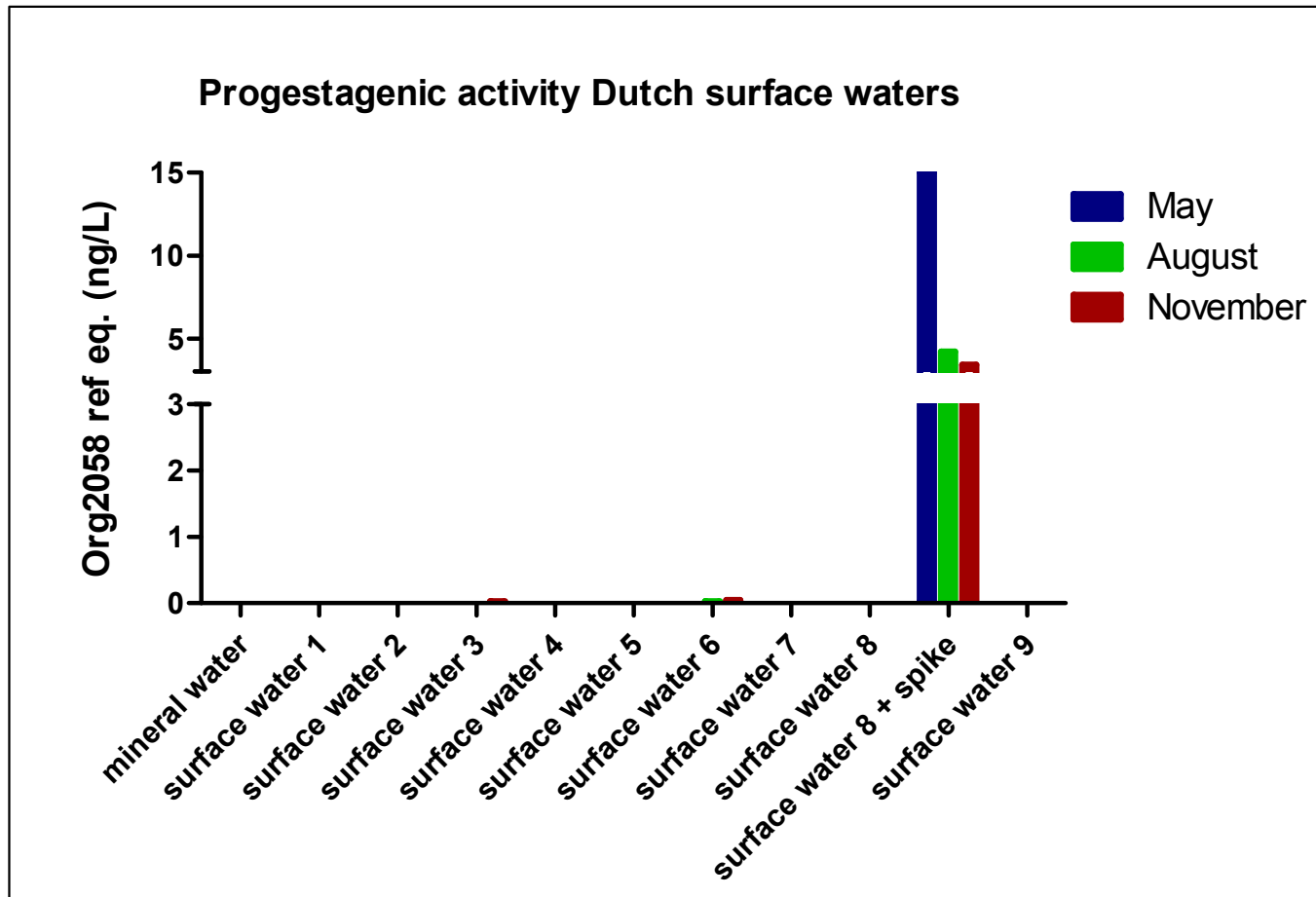
Analysis 9 inlets of Dutch surface water for drinking water production in three seasons of 2007



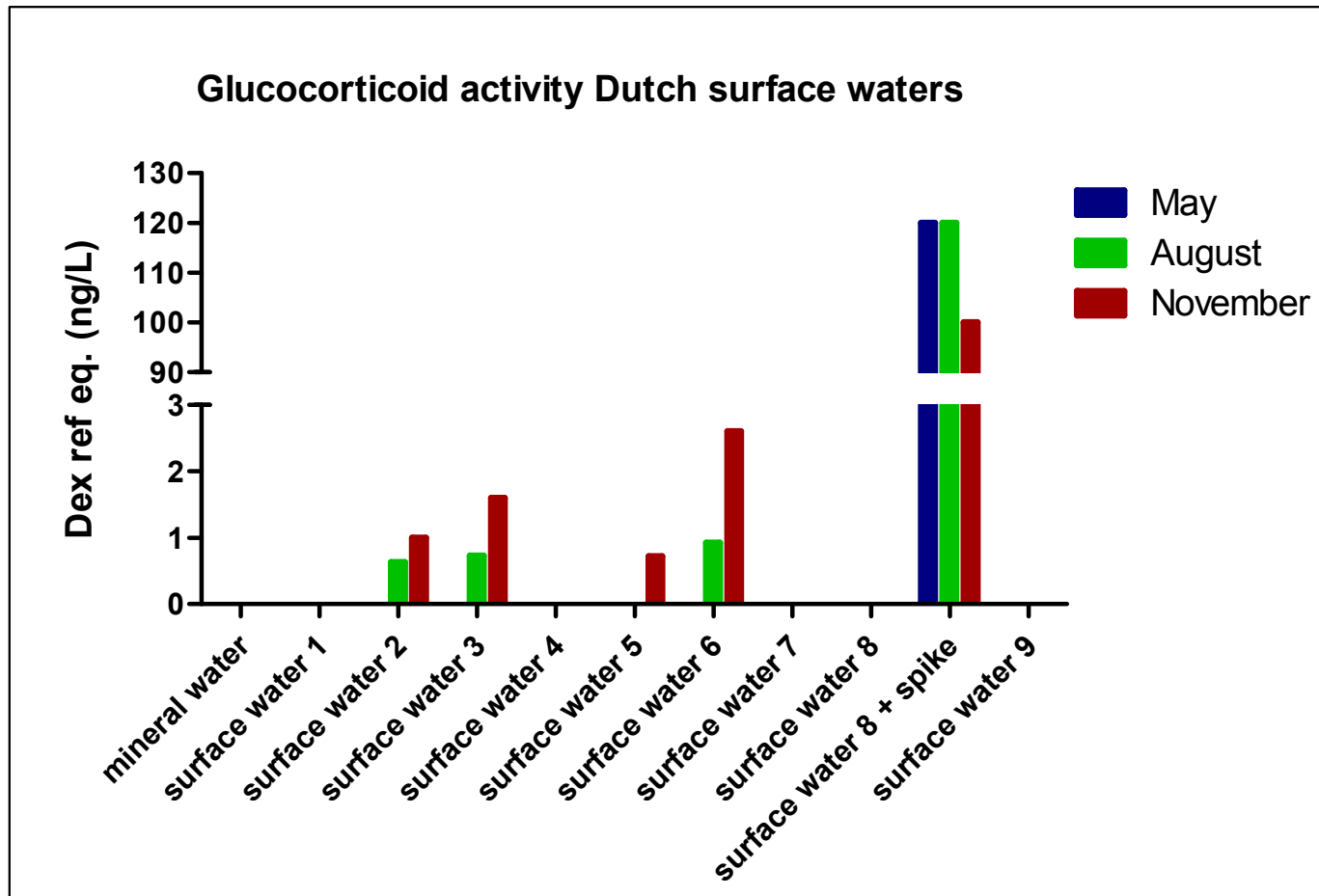
Analysis 9 inlets of Dutch surface water for drinking water production in three seasons of 2007



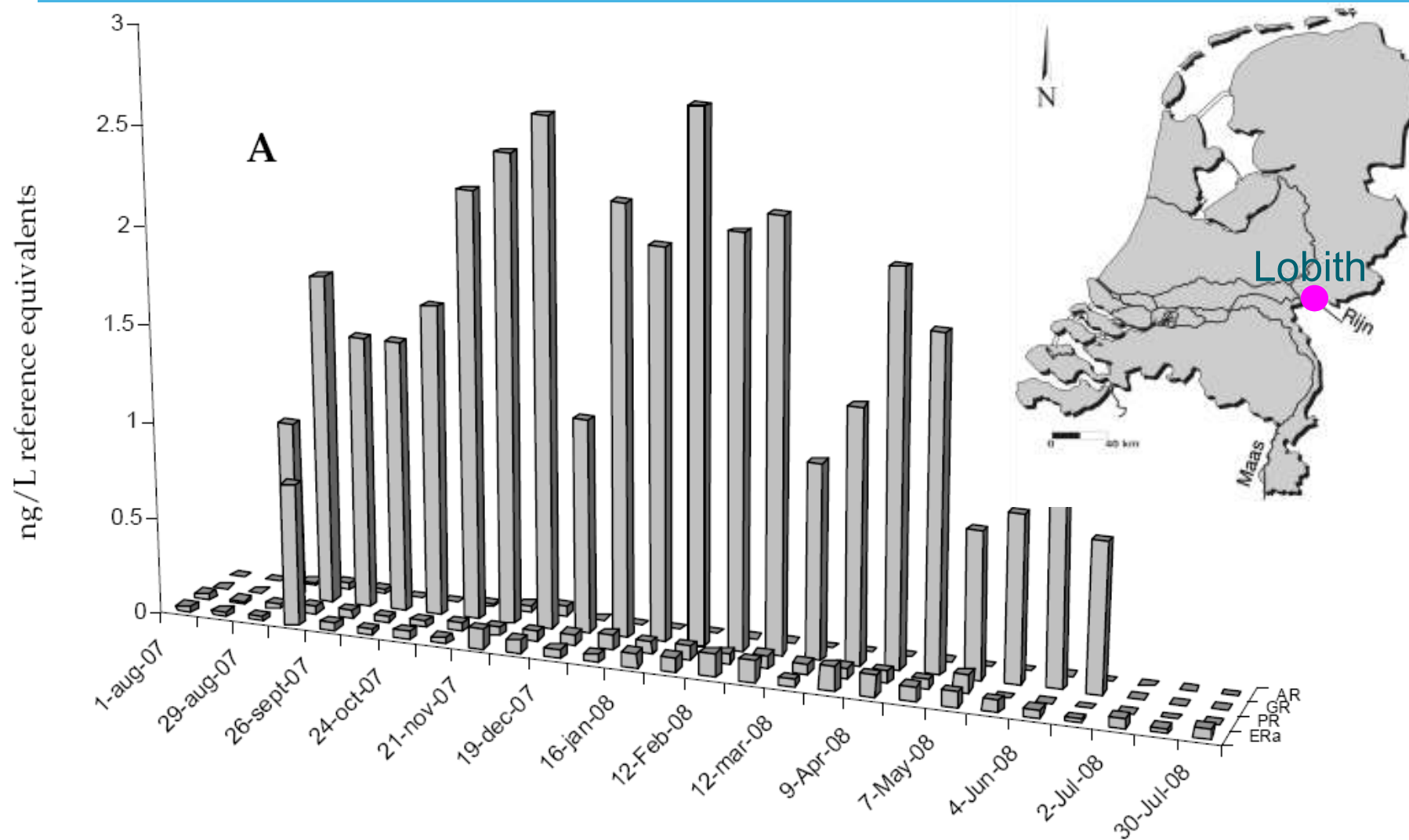
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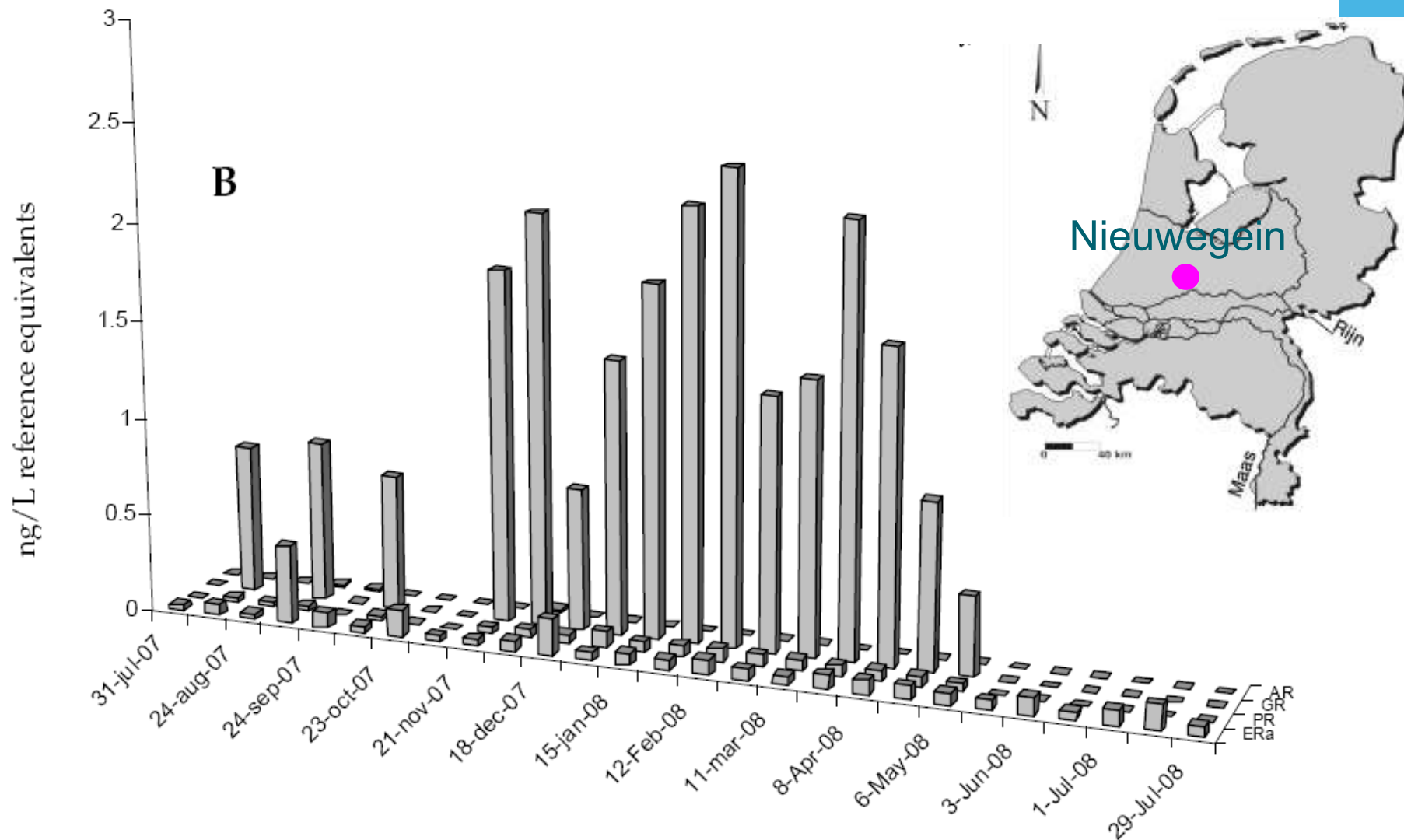
Analysis 9 inlets of Dutch surface water for drinking water production in three seasons of 2007



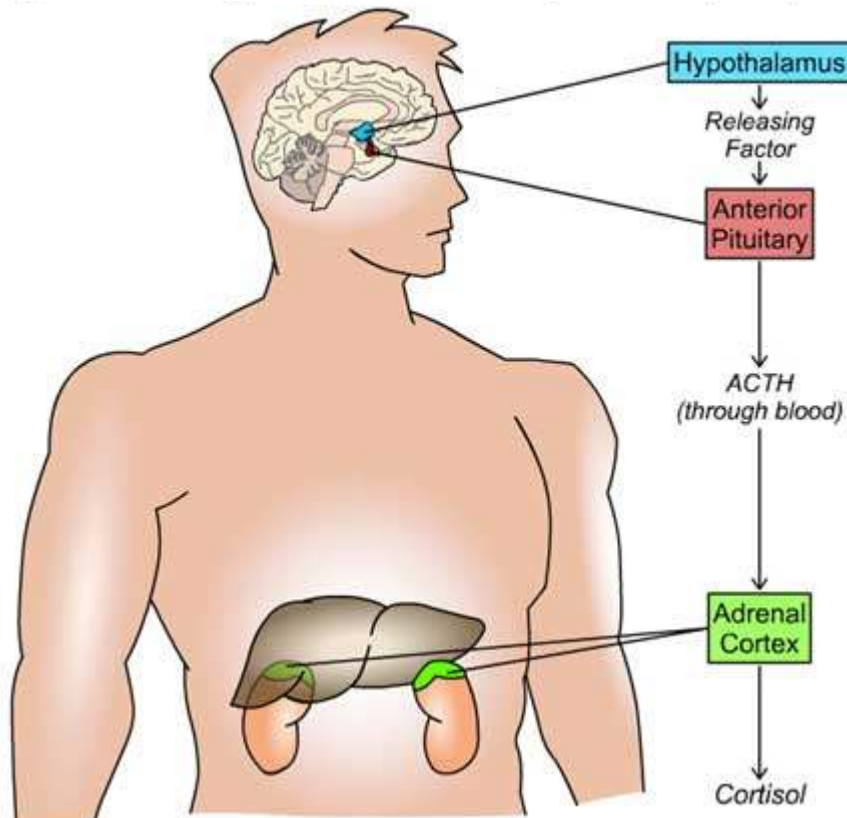
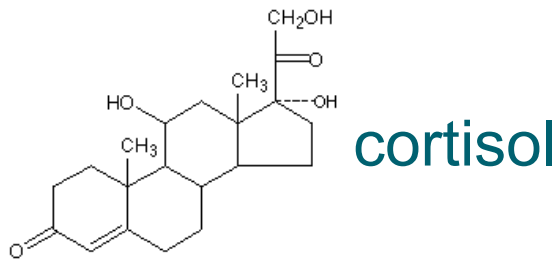
Two-weekly analysis of the Rhine at Lobith (2007-2008)



Two-weekly analysis of the Rhine at Nieuwegein (2007-2008)



Role of glucocorticoids in physiology/development



Glucocorticoids

- Released under stress-conditions
- Many effects on metabolism
 - Gluconeogenesis;
 - Inhibition of glucose utilization;
 - Promotion of lipolysis;
- Major medical application (suppression of immuno/allergic reactions).
- Examples: dexamethasone, prednisone

Glucocorticoid drugs

Drug (Source US RX-list top 200 rating, 2009)	Compound	Human disease
Nasonex (29)	Mometasone furoate monohydrate	Hay fever (adults, children > 2 yrs)
Nasacort AQ (86)	Triamcinolone acetonide	Hay fever (adults, children > 2 yrs)
Rhinocort Aqua (111)	Budesonide	Hay fever (adults, children > 6 yrs)
Pulmicort Respules (122)	Budesonide	Asthma
Tobradex (131)	Dexamethasone	Infection of the eyes
Ciprodex Otic (145)	Dexamethasone	Infection of the ears

Prescribed compounds in the NL (Source RIVM)	Amount (kg)
Cortisol	447
Prednisolone	316
Prednisone	54
Triamcinolone acetonide	37
Cortisone	27
Dexamethasone	7



Identification of responsible compounds

- Chemical analysis with LC-DAD-LTQ-FT Orbitrap MS (KWR)



Confirmation criteria:

1. Retention time;
2. Accurate mass;
3. Product ion masses.

- Individual compounds tested on GR-CALUX (BDS) → potency
- Currently ongoing...

Conclusions - 1

CALUX bioassays

- 1) Very low androgenic and progestagenic activity seen in Dutch surface waters
- 2) Thyroid activity is difficult to analyse as T3 is not extracted well
- 3) Glucocorticogenic activity present in several Dutch surface waters during the sampling campaign
- 3) The significance for human health needs to be determined
→development of a guideline value;
- 4) The ecological consequences of exposure to glucocorticogenic compounds are unknown;
- 5) The origin of glucocorticogenic compounds is unknown, but possibly delivered by STPs from municipalities and industries.

Conclusions - 2

Identification

- Various glucocorticoids present in wastewaters (low ug/L range).
 - Glucocorticoids are most probably from human and/or veterinary origin.
 - Measured activity can not completely be explained by the identified compounds.
- 4) Further research necessary on removal of glucocorticoids during drinking water treatment

Acknowledgements

- KWR**
- Ton van Leerdam, Dennis Vughs
 - Rene van Doorn
 - Merijn Schriks

- BDS**
- Sander van der Linden, Bart van der Burg

This research was funded by the Joint Research Programme of the Dutch Water Utilities (BTO) and RIWA-Rhine

Thank you for your attention!