

# CALUX-Monitoring of Dutch waters

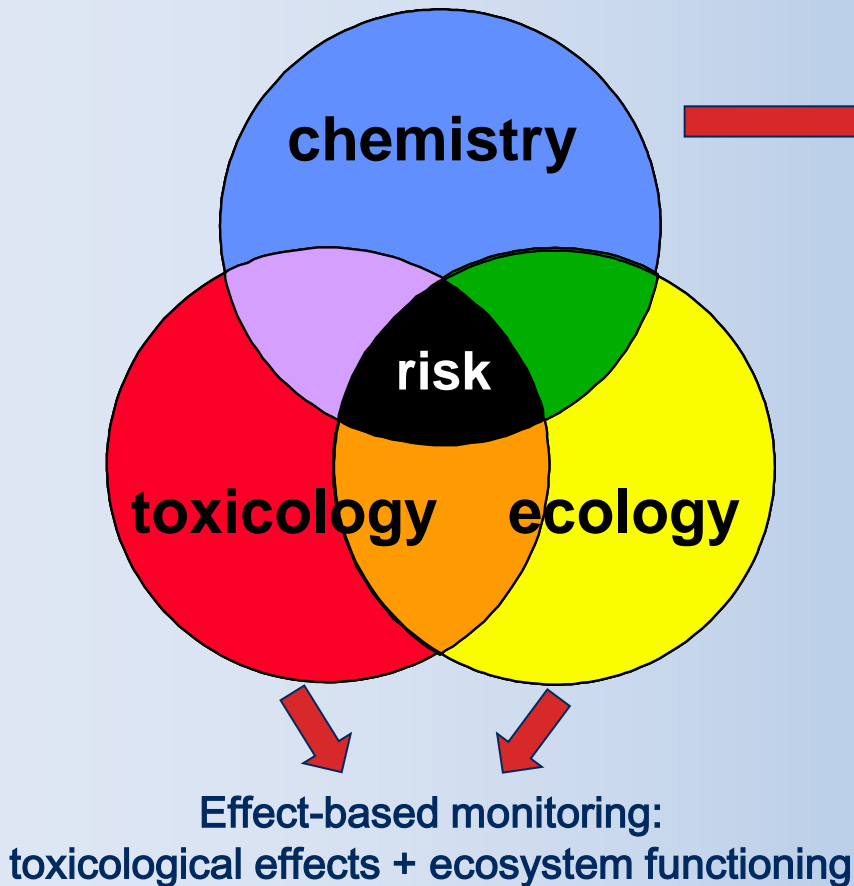


Thijs de Kort, Barry Pieters & Stefan Kools  
Team Ecology, Grontmij Amsterdam

# Introduction

- Emissions of micropollutants to Dutch surface waters
- Wastewater Treatment Plants (WTP) relevant point source
- Many type of pollutants: heavy metals, PAH, pesticides, pharmaceuticals
- Monitoring ecological and chemical based: Water Framework Directive (WFD)
- Compounds: monitoring mostly chemical based
- Effect-based monitoring as first screening tool not often used

# Environmental Risk Assessment: TRIAD approach



Chemical monitoring:  
compounds

Which step first?

# Differences between monitoring strategies

## ■ Chemical monitoring:

- 😊 □ Concentrations of target compounds known
- 😞 □ Concentrations unknown of compounds not analyzed
- 😞 □ Ecological effects not known
- 😞 □ Combined ecological effects of all compounds present not known

## ■ Effect-based monitoring:

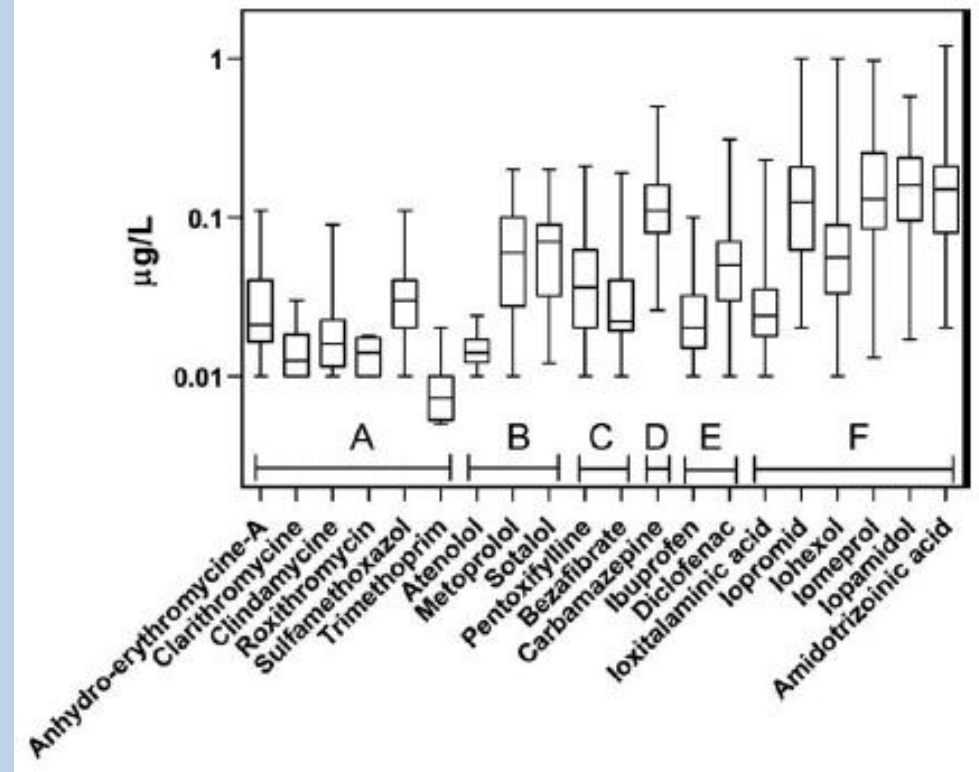
- 😊 □ Ecological effects known
- 😊 □ Ecological effects can be attributed to mechanisms of action of (groups of) compounds
- 😞 □ However, observed effects cannot be 100% attributed to single compounds
- 😊 □ Combined effects of all known and unknown compounds
- 😞 □ No information about concentrations of compounds

# Effect-based monitoring for identification sources of pollutants

- ER-Calux assay
  - Measure for estrogenic activity
  - Luciferase activity of cells
  - Estrogenic pharmaceutical compounds: 17a-ethinylestradiol, 17a/b-estradiol, estrone, estriol
  - Expression: equivalent of 17b-estradiol (ng EEQ/l)
- GR-Calux assay
  - Measure for glucocorticoid activity
  - Luciferase activity of cells
  - Glucocorticoid pharmaceutical activity: cortisol, cortisone, dexamethasone, prednisolone, prednisone, triamcinolone acetonide
  - Expression: equivalent of dexamethasone (ng DEQ/l)

# Many pharmaceuticals detected in rivers Rhine and Meuse

T. ter Laak et al., *Environment International*, 2010



Including anti inflammatory and contraceptive pills...  
Effect-based screening with Calux assays useful tool

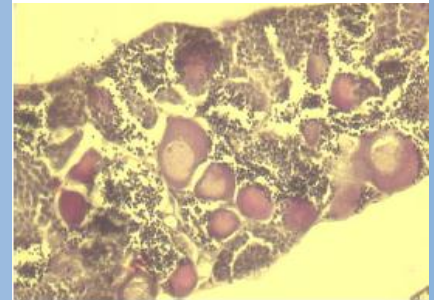


# Endocrine disruption contraceptive pills

## Effects fish of 17 $\beta$ -Estradiol (E2) and 17 $\alpha$ -Ethinylestradiol (EE2)

- Vitellogenine induction ( $\geq 5$  ng/L E2,  $\geq 1$  ng/L EE2)
- Reduction growth gonades
- Feminization male fish  
( $\geq 10$  ng/L E2,  $\geq 100$  ng/L EE2)
- Altered sex ratio
- Altered breeding behaviour
- Collapse of populations

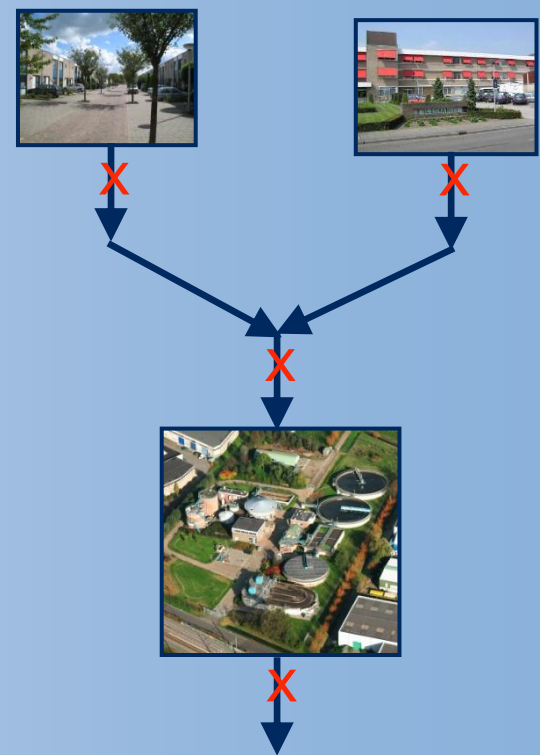
 Effect-based monitoring necessary!



# Case Study 1: ZORG project

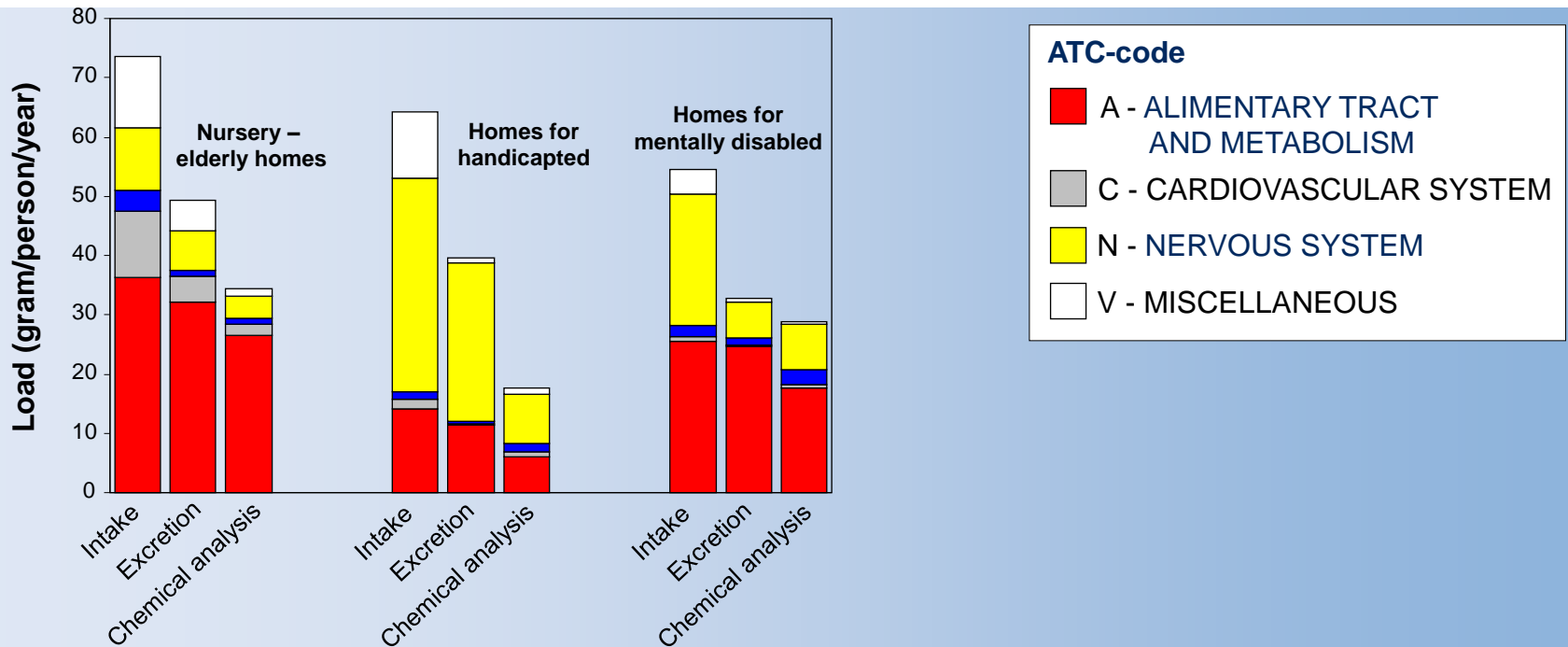
- Aim: quantify pharmaceutical emissions in wastewater
- Sources for monitoring:
  - effluent care institutions
  - effluent households
  - influent WTP
  - effluent WTP
- Chemical analysis: 53 compounds
- Information on intake and excretion
- Effect analysis wastewater:
  - ER-Calux assay: estrogen activity
  - GR-Calux assay: glucocorticoid activity

8 waterboards



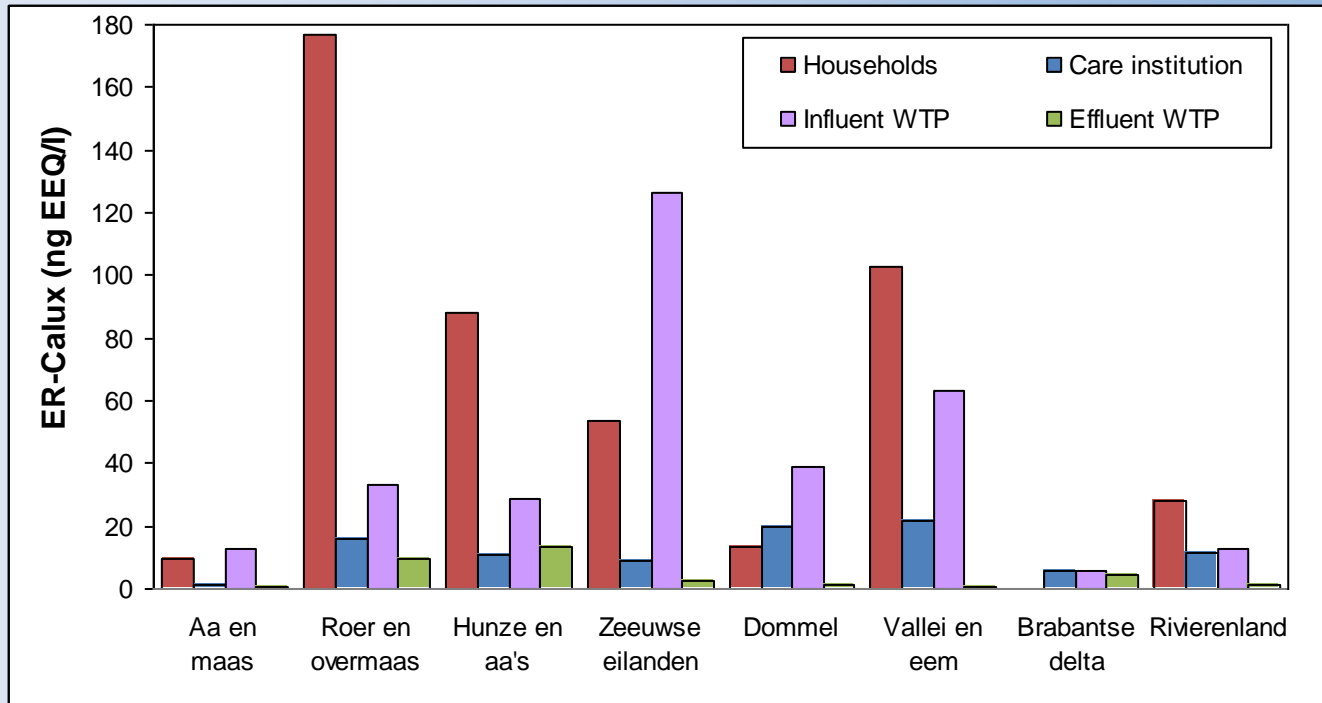


# Comparison pharmaceutical loads based on intake, excretion and chemical analysis



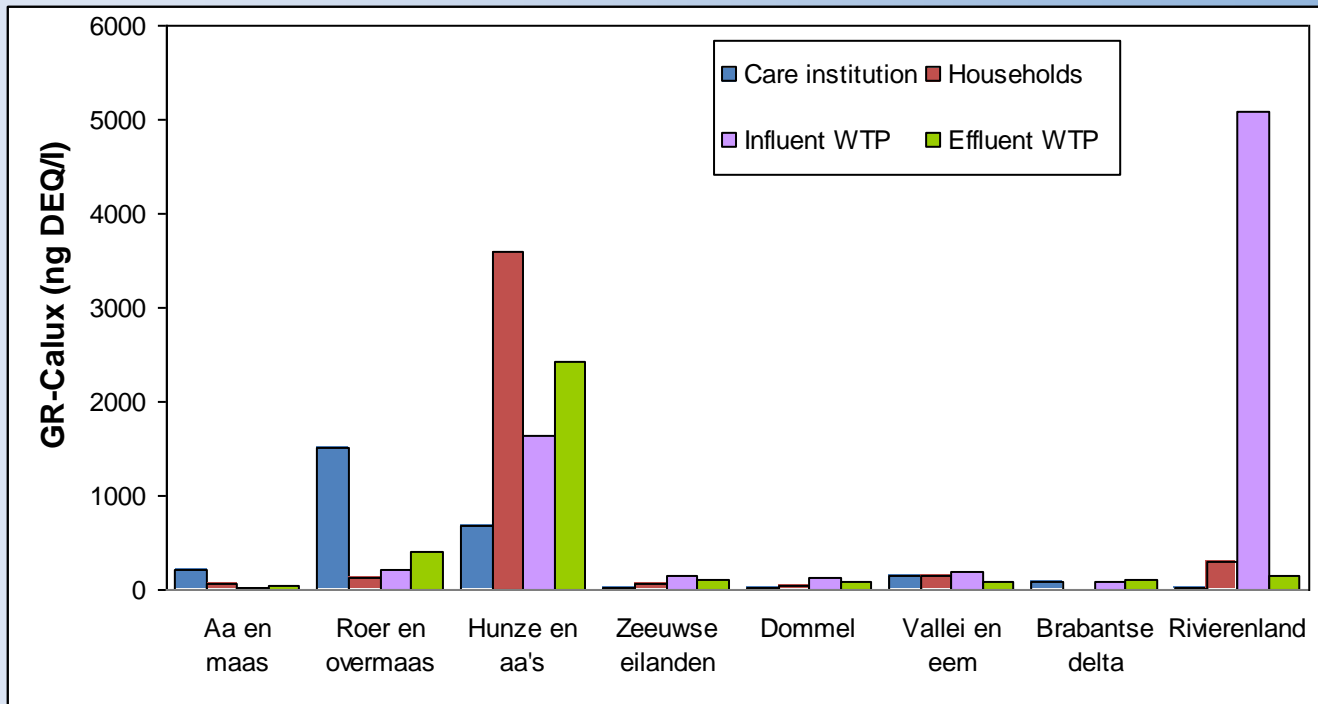
- Pharmaceutical groups A and N dominate, but use differ between care institutions
- Discrepancies loads due to limiting number of compounds chemically analyzed

# Results ER-Calux: estrogen activity



- high removal rates of estrogen activity in WTP
- care institutions: mostly lower estrogen activity than households (except ZE)
- households: RO, HA and VE high estrogen activity

# Results GR-Calux: glucocorticoid activity



- differing removal rates of glucocorticoid activity in WTP
- care institutions: RO and HA high glucocorticoid activity
- households: HA high glucocorticoid activity

# Additional research for identification compounds highest contribution to effects?

TABLE 2. Concentration of Hormones in Extracts of Various Wastewaters Collected in The Netherlands

sample location (sampling date)	GR CALUX (log dex EOs/L)	glucocorticoids	LC-MS/MS measured concentration* (ng/L)	relative potency (REP) <sup>b</sup>	predicted (total) dex EOs <sup>c</sup> (ng dex EOs/L)
Industry wastewater (12/06/2006; Van der Linden et al., 2008)	243 ± 32	cortisol	13 ± 1 (80%)	0.07 ± 0.08	0.92 ± 1.1
		cortisone	28 ± 3 (80%)	<0.0008 ± 0.00008	max. 0.02 ± 0.1
		dexamethasone	80 ± 9 (89%)	1 ± 0.05	90.0 ± 0.1
		prednisolone	247 ± 28 (73%)	0.2 ± 0.1	49.3 ± 0.5
		prednisone	N.D. (77%)	-	-
		triamcinolone acetonide	N.D. (74%)	-	-
					Σ 140.2 ± 1.3
hospital wastewater 1 (12/11/2006; Van der Linden et al., 2008)	96 ± 13	cortisol	275 ± 27 (80%)	0.07 ± 0.08	19.2 ± 1.1
		cortisone	381 ± 44 (80%)	<0.0008 ± 0.00008	max. 0.03 ± 0.8
		dexamethasone	N.D. (89%)	-	-
		prednisolone	315 ± 30 (73%)	0.2 ± 0.1	63.0 ± 0.5
		prednisone	117 ± 12 (77%)	<0.002 ± 0.0004	Max. 0.2 ± 0.2
		triamcinolone acetonide	41 ± 4 (74%)	2.3 ± 0.04	93.2 ± 0.1
					Σ 176.7 ± 1.5
hospital wastewater 2 (8/19/2009)	609 ± 79	cortisol	301 ± 29 (80%)	0.07 ± 0.08	21.1 ± 1.1
		cortisone	472 ± 54 (80%)	<0.0008 ± 0.00008	max. 0.4 ± 0.1
		dexamethasone	N.D. (89%)	-	-
		prednisolone	1918 ± 182 (73%)	0.2 ± 0.1	383.6 ± 0.5
		prednisone	545 ± 58 (77%)	<0.002 ± 0.0004	1.1 ± 0.2
		triamcinolone acetonide	14 ± 1 (74%)	2.3 ± 0.04	31.1 ± 0.1
					Σ 437.2 ± 1.3
paper mill treated wastewater (01/15/2007; Van der Linden et al., 2008)	11 ± 2	cortisol	N.D. (80%)	-	-
		cortisone	N.D. (80%)	-	-
		dexamethasone	N.D. (89%)	-	-
		prednisolone	N.D. (73%)	-	-
		prednisone	N.D. (77%)	-	-
		triamcinolone acetonide	N.D. (74%)	-	-
					0
sewage treatment plant effluent (11/15/2006; Van der Linden et al., 2008)	38 ± 13	cortisol	N.D. (80%)	-	-
		cortisone	N.D. (80%)	-	-
		dexamethasone	N.D. (89%)	-	-
		prednisolone	N.D. (73%)	-	-
		prednisone	N.D. (77%)	-	-
		triamcinolone acetonide	14 ± 1 (74%)	2.3 ± 0.04	31.1 ± 0.1
					Σ 31.1 ± 0.1

## High-Resolution Mass Spectrometric Identification and Quantification of Glucocorticoid Compounds in Various Wastewaters in The Netherlands

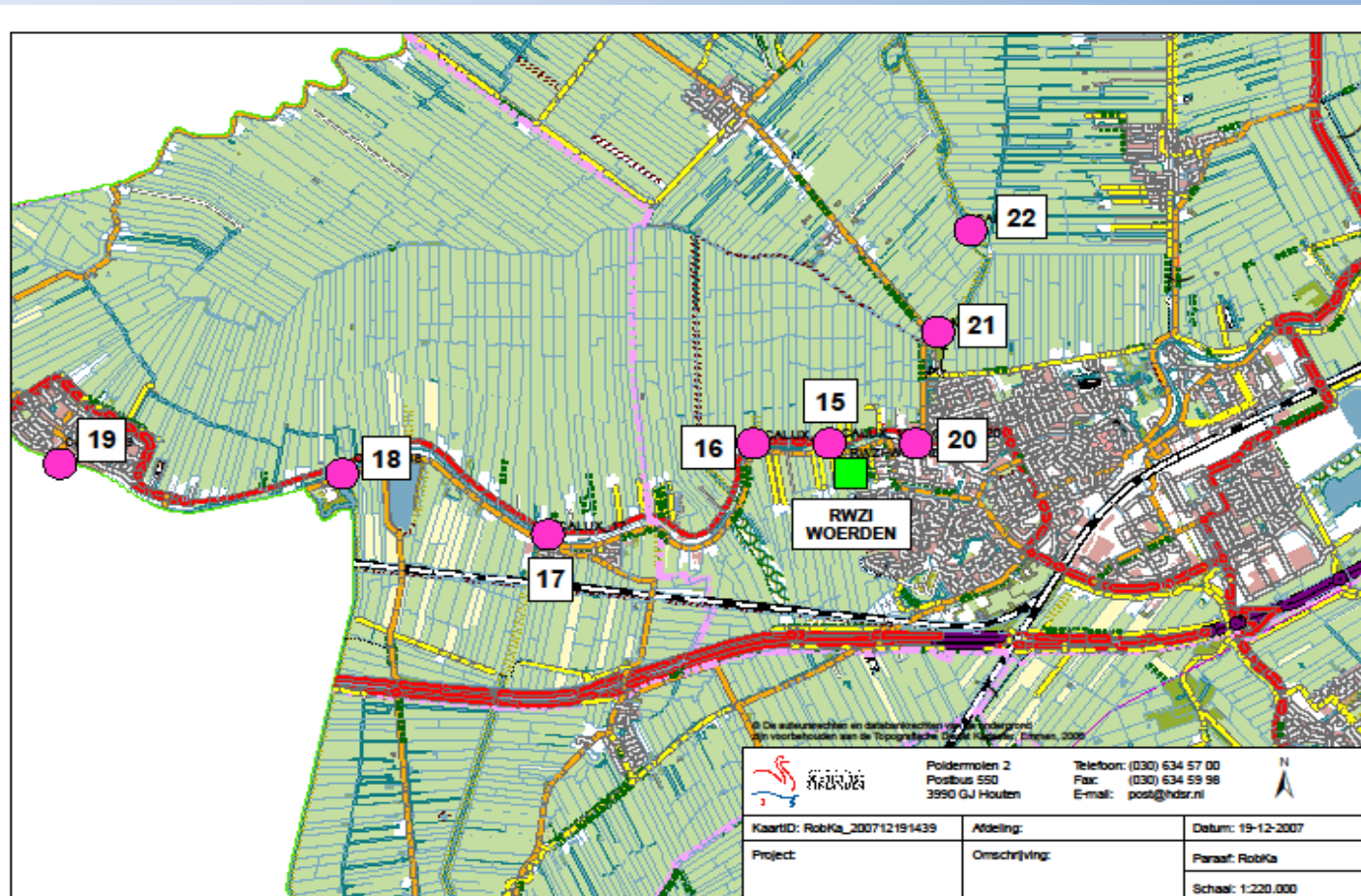
MERIJN SCHRIKS,<sup>\*,†</sup> JAN A. VAN LEERDAM,<sup>†</sup> SANDER C. VAN DER LINDEN,<sup>†</sup> BART VAN DER BURG,<sup>‡</sup> ANNEMARIE P. VAN WEZEL,<sup>†</sup> AND PIM DE VOOGT<sup>†,§</sup>

*KWR Watercycle Research Institute, Nieuwegein, The Netherlands, BioDetection Systems B.V., Amsterdam, The Netherlands, and Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands*

*Received January 4, 2010. Revised manuscript received May 3, 2010. Accepted May 11, 2010.*

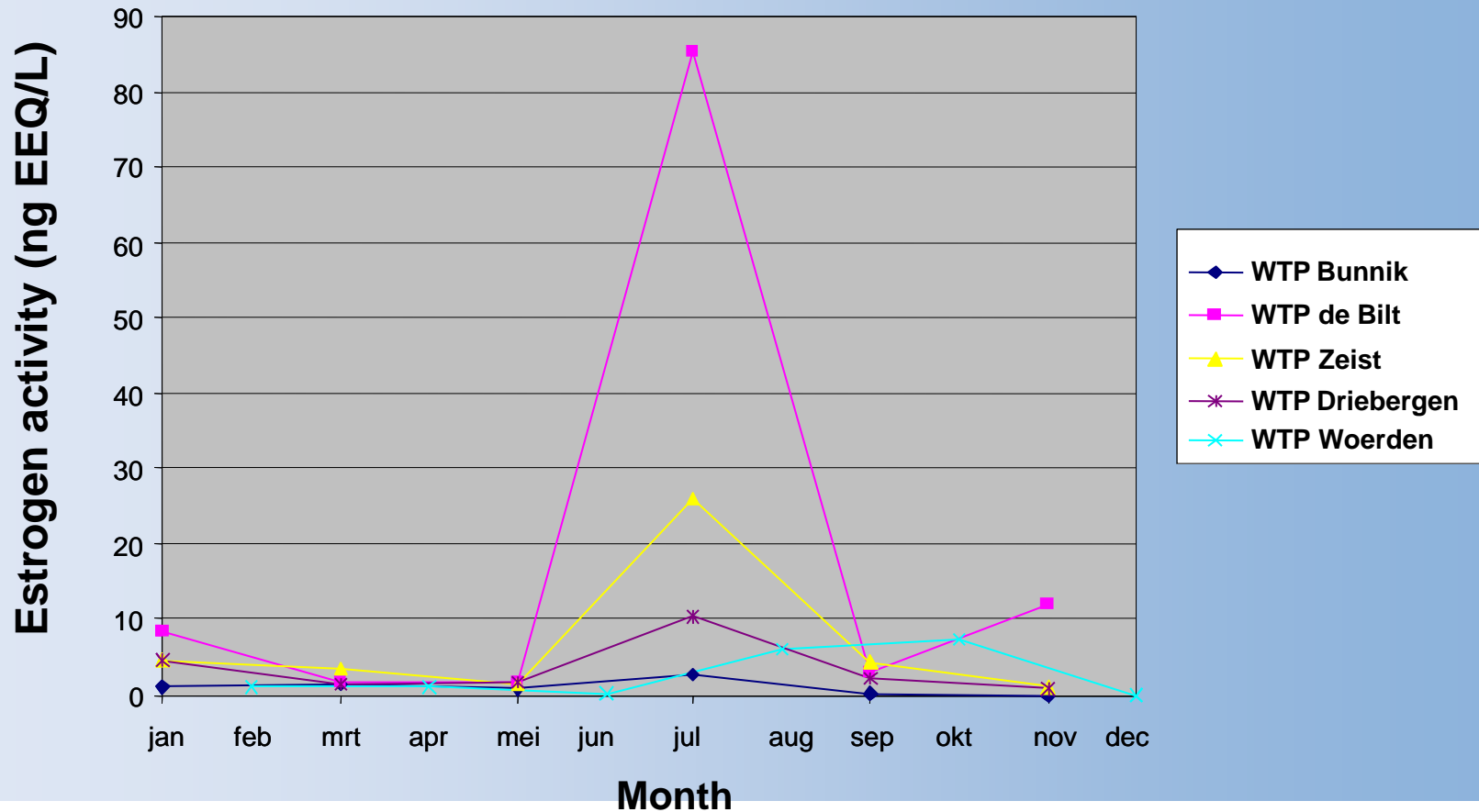
# Case Study 2: Monitoring of estrogen activity of Oude and Kromme Rijn

- Aim: characterization endocrine activity released from WTP's to surface waters
- ER-Calux monitoring WTP effluents, up- and downstream river



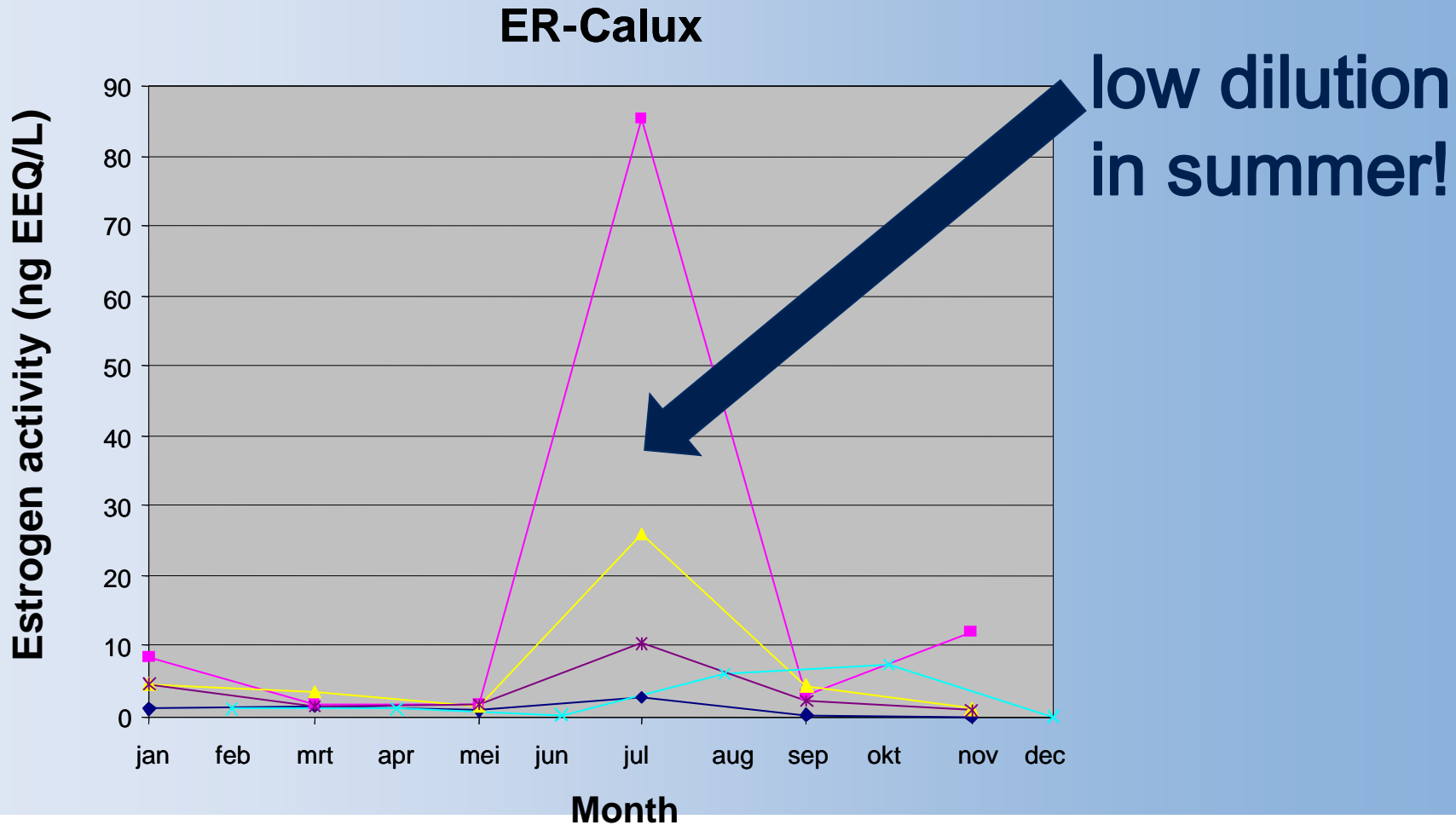
# Estrogen activity effluents WTP's

## ER-Calux



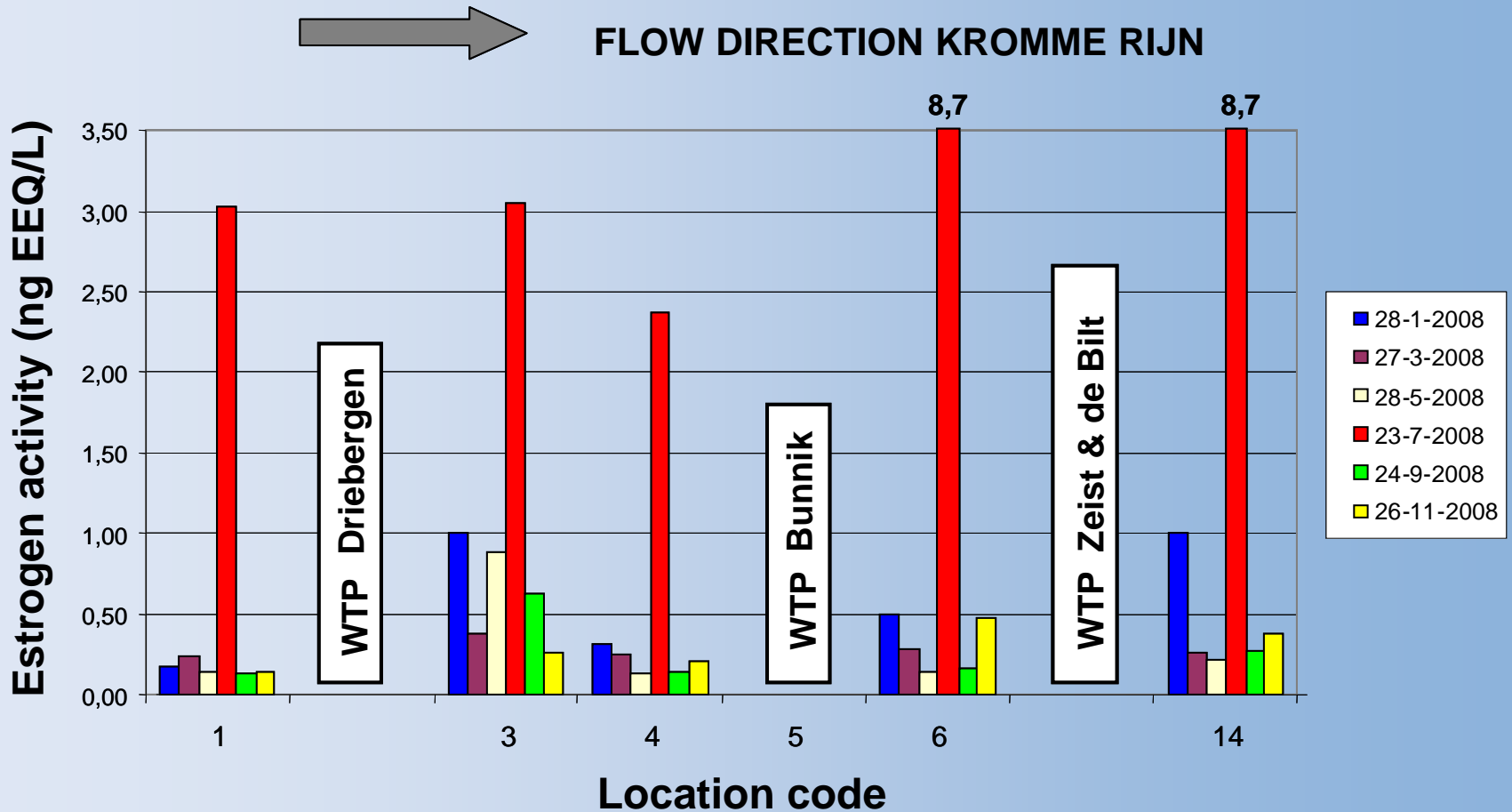


# Estrogen activity effluents WTP's



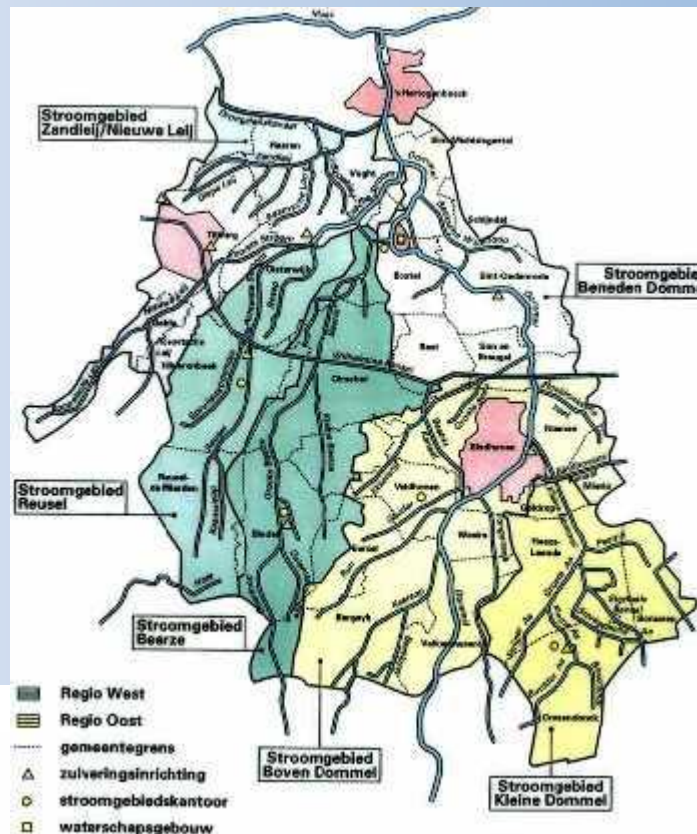
# Highest activity in summer

## Generally increased activity downstream



# Case Study 2: Monitoring of estrogen activity of river Dommel

- Aim: characterization endocrine activity released from WTP's to river Dommel
- ER-Calux monitoring WTP effluents, up- and downstream river



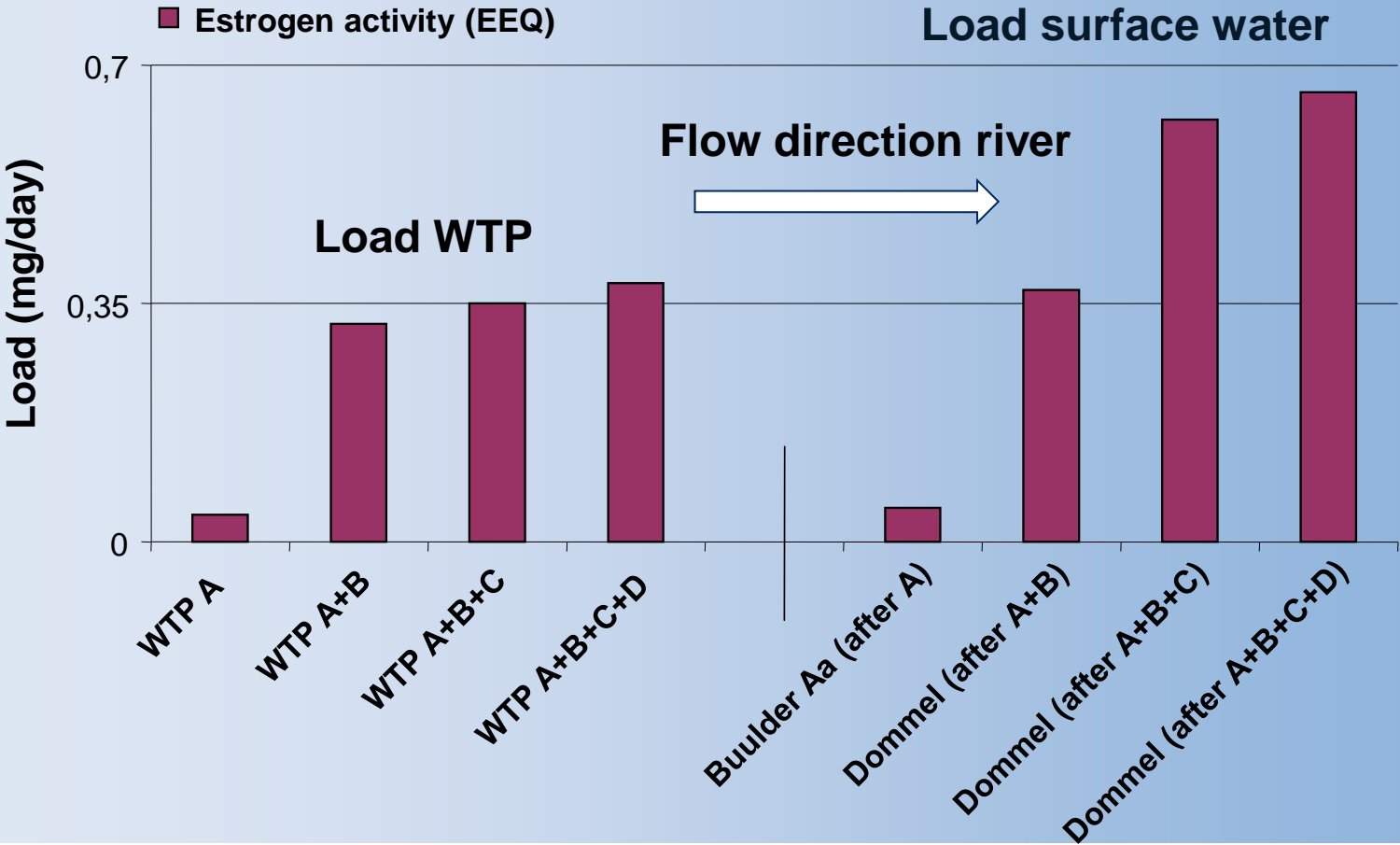
# Endocrine activity effluents WTP's

	Effluent WTP Tilburg-Noord	Effluent WTP Biest-Houtakker	Effluent WTP Haaren	Effluent WTP Soerendonk	Effluent WTP Eindhoven	Effluent WTP Sint-Oedenrode	Effluent WTP Boxtel	Effluent WTP Hapert
ER-Calux assay (ng EEQ/l)	3,17	3,39	2,68	8,67	2,68	2,79	2,77	2,93

# Endocrine activity surface water

	Zandleij Tilburg-Noord	Reusel Biest-Houtakker	Essche Stroom Haaren	Buulder Aa Soerendonk	Dommel Eindhoven	Dommel Sint-Oedenrode	Dommel Boxtel	Grote Beerze Hapert
ER-Calux assay (ng EEQ/l)	3,62	1,24	0,93	2,63	1,07	1,19	1,19	2,58

# Distribution of estrogenic activity





# Conclusions

- Effect-based monitoring may be **useful screening tool**
- Effect assessment with assays such as Calux can **identify hot spots** for emission of pollutants
- Effect-based monitoring often **much cheaper** than chemical monitoring
- Effect-based monitoring can **link** occurrence of compounds to biological effects
- However, cannot identify (concentrations of) **single compounds responsible** for effects
- Only assessment of toxicology, chemistry and ecology **combined** provides insight in actual ecological risks

# Questions?

