Lessen uit Watermonitoring: Effect-gebaseerd meten van opkomende verontreinigingen in bodem- en grondwater

CALUX bioassays

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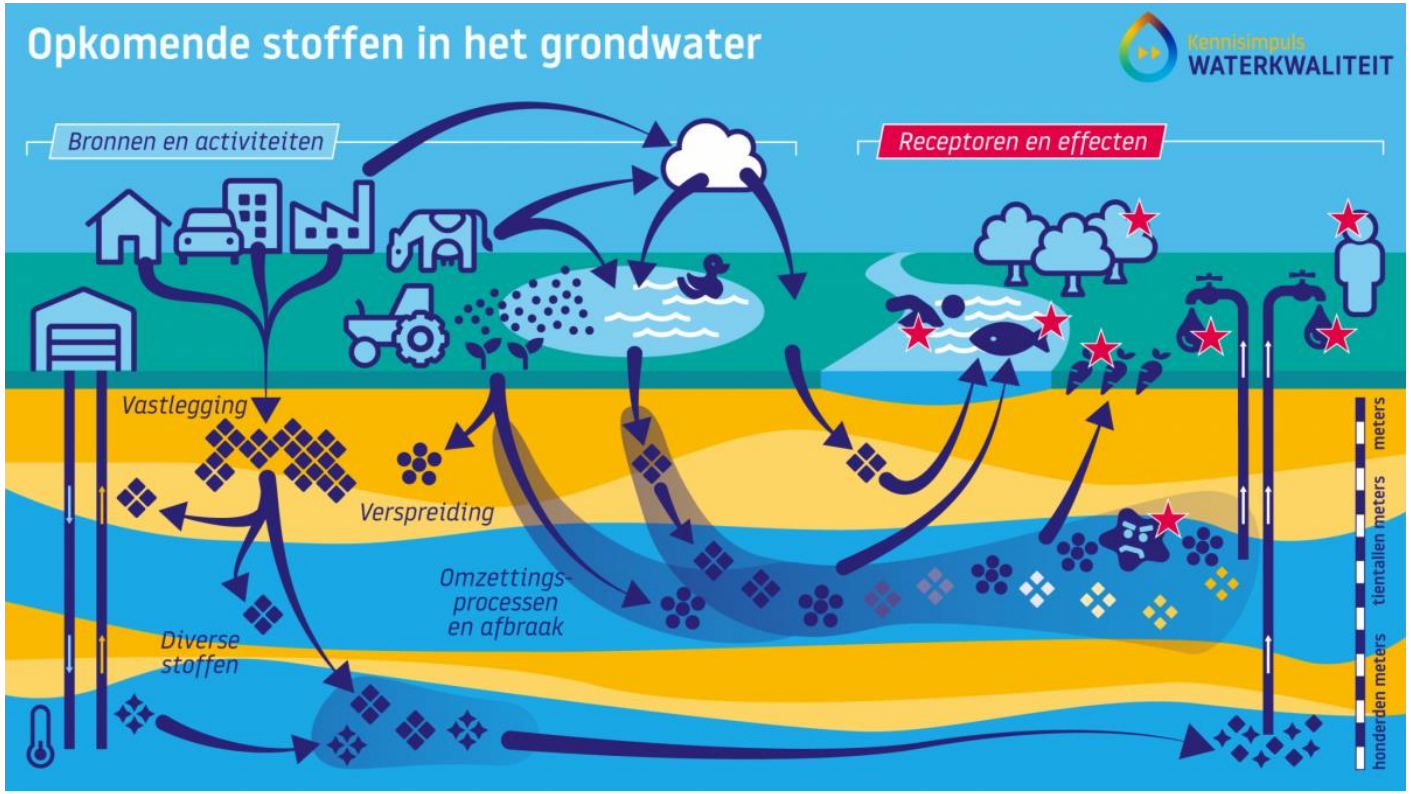
bioclear earth

BodemBreed FORUM

Verontreiniging van grondwater/bodem



(Opkomende) verontreinigingen in water:

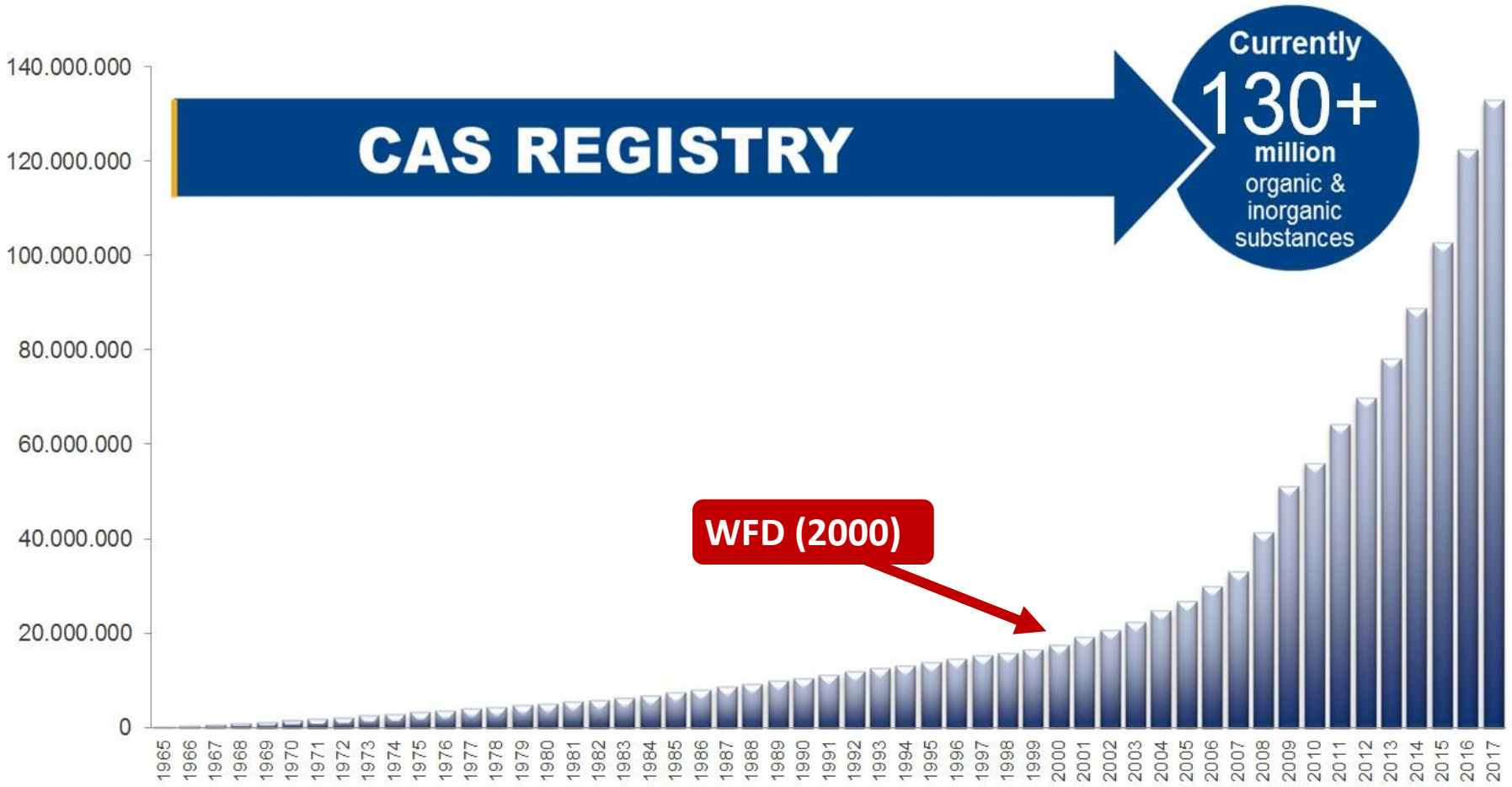
- nieuwe en relatief onbekende stoffen
- (vaak) niet genormeerd
- grote effecten op milieu en humane gezondheid

- PFAS
- persoonlijke verzorgingsproducten
- (dier-)geneesmiddelen
- industriele chemicalien
- consumentenproducten
- gewasbeschermingsmiddelen
- …….

Kennisimpuls WATERKWALITEIT

Bron: Deltafact – Opkomende stoffen in grondwater

ZZS – Zeer Zorgwekkende Stoffen



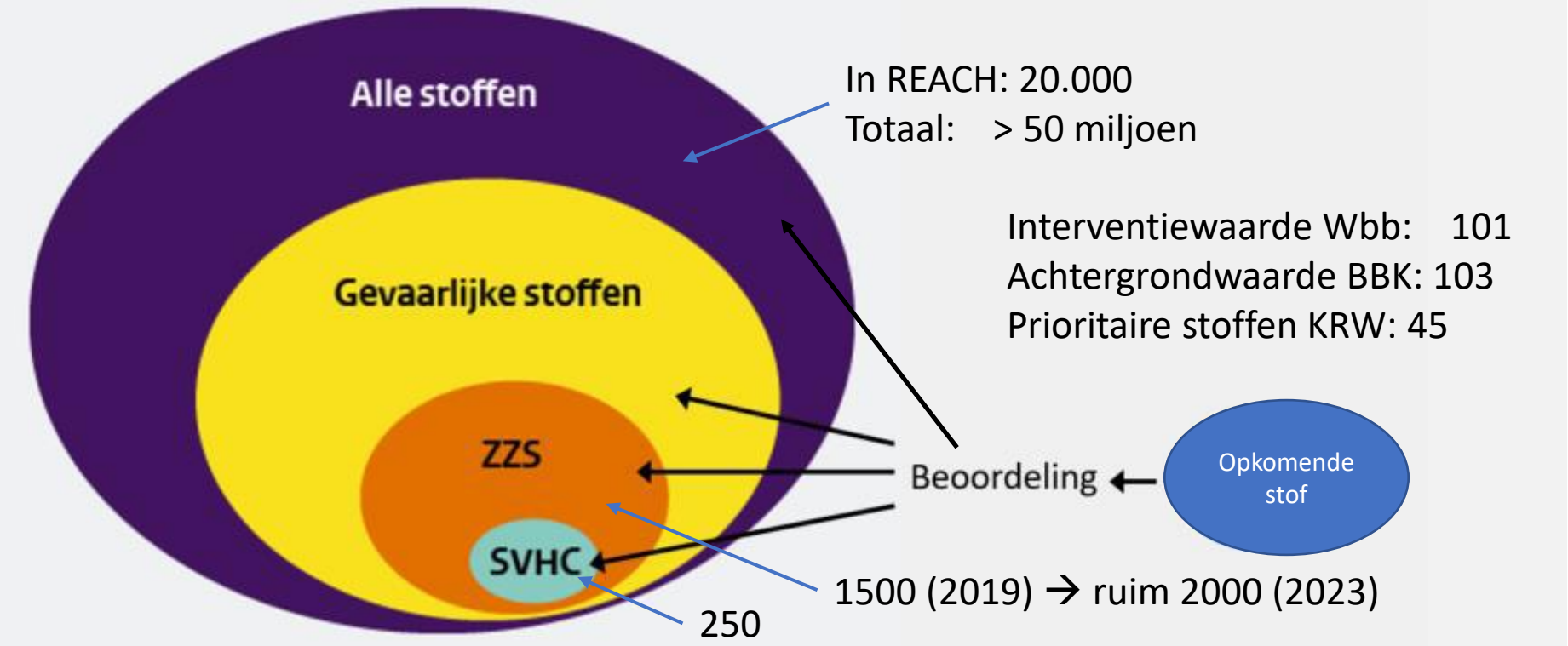
Increasing diversity of chemicals

> 140,000,000 substances on global market; 30,000 water relevant

Source: CAS

creating with the power of nature

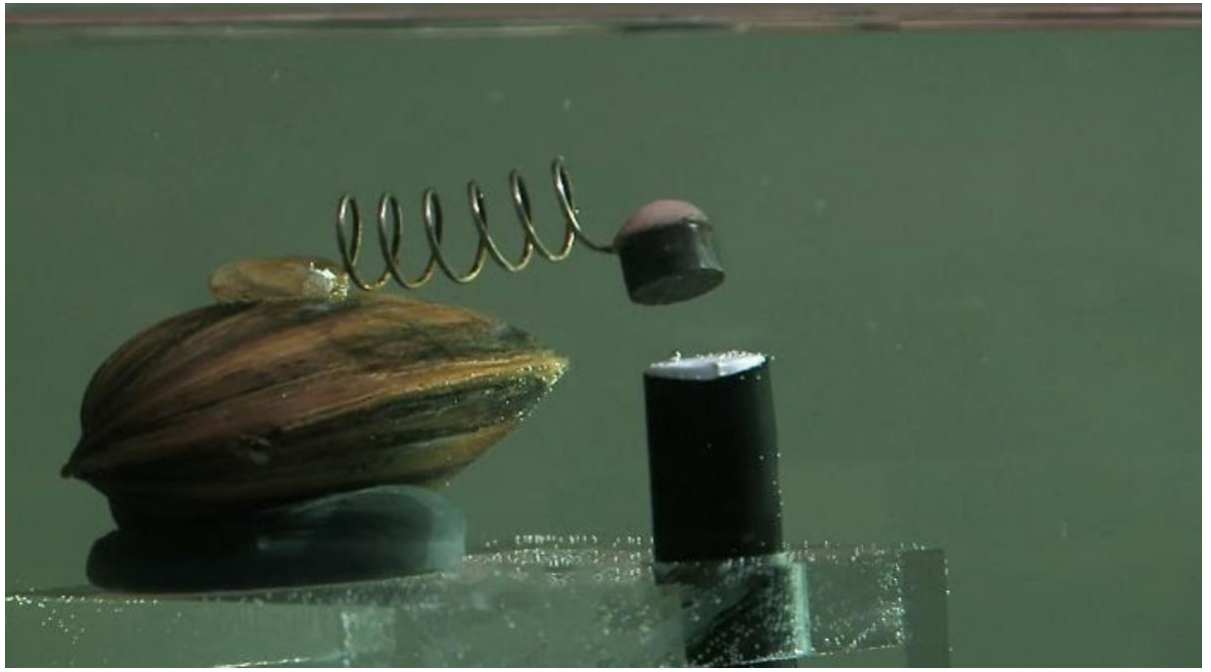
Waarom effectmetingen?



Bron: RIVM en Expertisecentrum PFAS

Effectmetingen als indicator

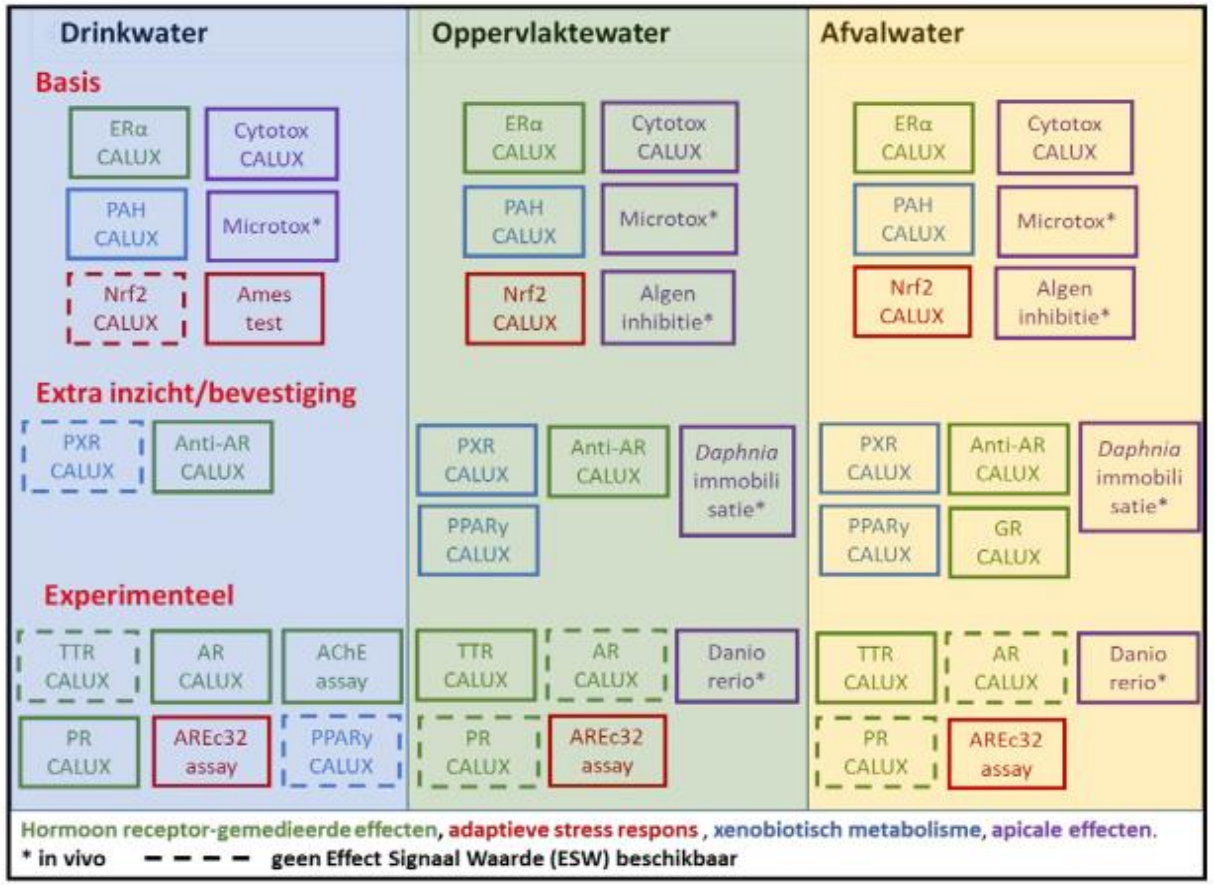
Toepassingen oppervlaktewater



- Watervlooien bij Lobith
- Mossels bij oppervlaktewaterinname voor drinkwater

Effectmetingen in water

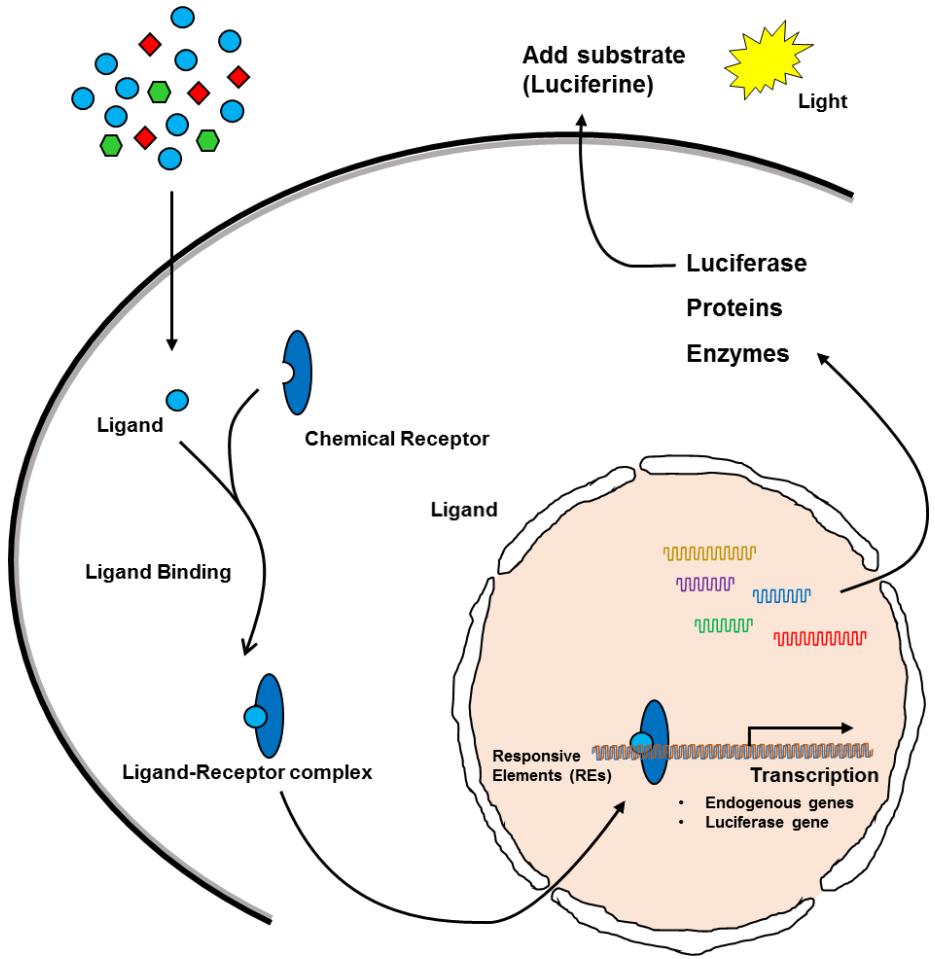
- Kennisimpuls Water
- Sleutelfactor Toxiciteit 2.0



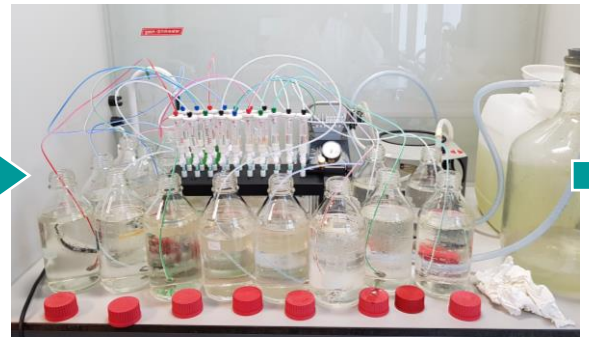
Figuur 2. Schematische weergave van de basis-set bioassays om toe te passen binnen Nederland. Voor de bioassays waar een gestippelde lijn te zien is, is op dit moment geen effect signaal waarde (ESW) beschikbaar waaraan getoetst kan worden of de bioassay respons een risico aangeeft.

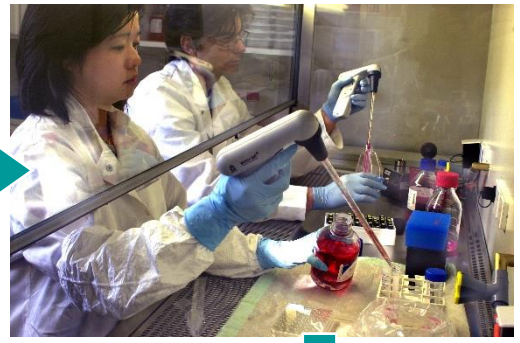
Principle CALUX effect-based reporter gene assays

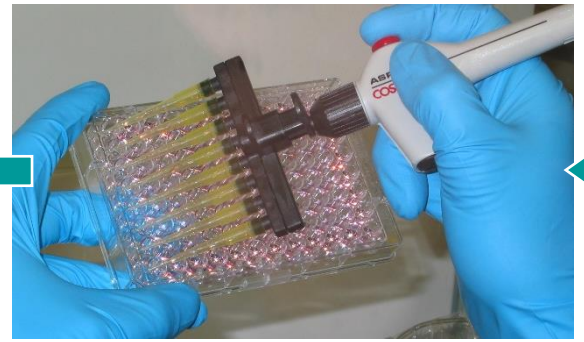
name	pathway	reference compound
DR CALUX	dioxin receptor activation	2,3,7,8-TCDD
PAH CALUX	dioxin receptor activation	benzo-a-pyrene
ER CALUX	estrogen receptor activation	17β-estradiol
ERalpha CALUX	estrogen receptor α activation	17β-estradiol
Anti-ERalpha CALUX	repression estrogen receptor α activation	tamoxifen
ERbeta CALUX	estrogen receptor β activation	17β-estradiol
Anti-ERbeta CALUX	repression estrogen receptor β activation	tamoxifen
AR CALUX	androgen receptor activation	dihydrotestosterone
Anti-AR CALUX	repression androgen receptor activation	flutamide
PR CALUX	progesterone receptor activation	progesterone
Anti-PR CALUX	repression progesterone receptor activation	RU486
GR CALUX	glucocorticoid receptor activation	dexamethasone
Anti-GR CALUX	repression glucocorticoid receptor activation	RU486
TRβ CALUX	thyroid receptor activation	T3
RAR CALUX	retinoic acid receptor activation	retinoic acid
PPARγ CALUX	PPARγ activation	rosiglitazone
PPARα CALUX	PPARα activation	GW7674
PPARδ CALUX	PPARδ activation	L165041
LXR CALUX	LXR activation	GW3965
kappaB CALUX	NFκB pathway activation	TPA
P21 CALUX	transcription of p21 inhibitor of cell cycle progression	actinomycin D
Nrf2 CALUX	activation of the Nrf2 pathway	curcumin
P53 CALUX	p53-dependent pathway activation	actinomycin D
genotox CALUX	p53-dependent pathway activation +/-S9	cyclophosphamide
TCF CALUX	wnt/TCF pathway activation	lithium chloride
AP1 CALUX	AP1 pathway activation	TPA
HIF1alpha CALUX	Hif1alpha pathway activation	cobaltous chloride
ER stress CALUX	ERSE activation leading to endoplasmic reticulum stress	tunicamycin

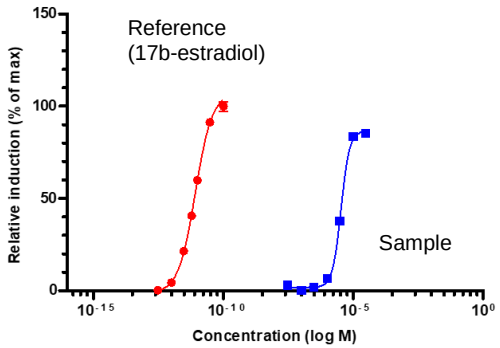


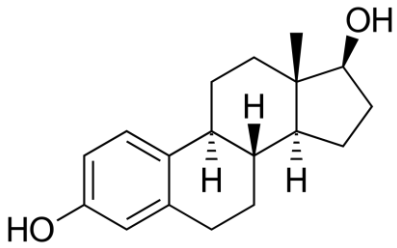
Bioanalysis of water samples







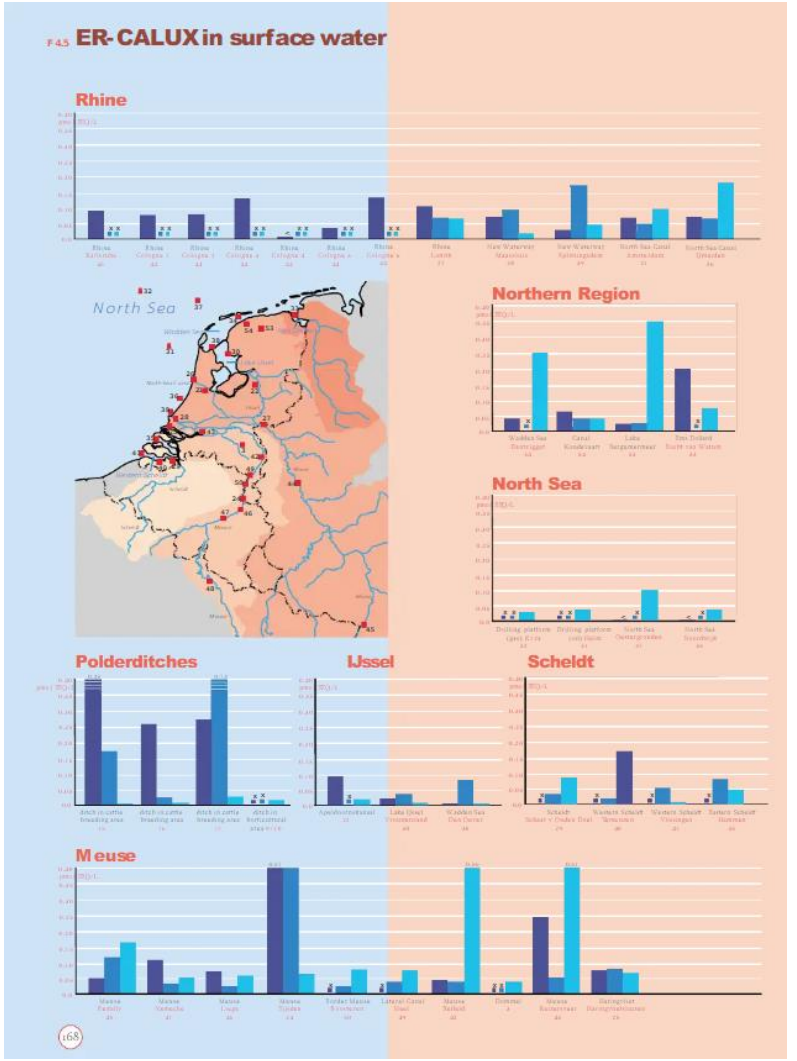




ng 17b-estradiol eq./l water

LOES, het Landelijk Onderzoek Oestrogene Stoffen

17b-Estradiol equivalents (pmol 17b-estyradiol eq./l)				
Compartment	***n***	**Range**	**(*n* > lod)**	**Median**
Industrial wastewater				
effluent	3	0.2 - 9.5	(3)	0.9
influent	5	5.8 - 560	(4)	317
Municipal wastewater				
effluent	10	<lod - 2.2	(9)	0.3
influnet	13	2.4 - 275	(13)	27
Surface water				
surface water	90	<lod - 0.61	(85)	0.07
polder ditchjes	11	0.003 - 0.74	(11)	0.03
rainwater	3	0.01 - 0.22	(3)	0.13



Dutch River water: Dommel highest ERα CALUX and intersex in bream

Vethaak et al. February 2002. RIZA/RIKZ - report no. 2002.001

From development to implementation

development

DR CALUX
1st CALUX (WUR) (**1991**)

Case/demonstration studies
Commercial/governmental partners
(e.g. RIZA/RIKZ – ER in Dutch water (**1993 - ongoing**)

Research projects
EU DEMEAU; EU TECHNEAU; EU TOXRISK; EU AQUANES;
EU PROMISCES; EU SAFECREW……(**2004 – ongoing**)

Smart Integrated Monitoring (SIMONI)
- selection of assays (**2020 – ongoing**)
- effect-based trigger values (**2013 – ongoing**)

Publications
Murk et al. **1996** (DR CALUX) ……. Behnisch et al. **2021**; de Schepper et al. **2023** (PFAS CALUX)

Validation - standardisation
ECVAM; OECD; ISO/DIN/DIS; NORMAN (**2016 – ongoing**)

Inclusion Regulatory Frameworks
UNEP Technical Guidelines (Basel Convention)
REACH
WFD (Implementation Strategy EBM)
…….

implementation

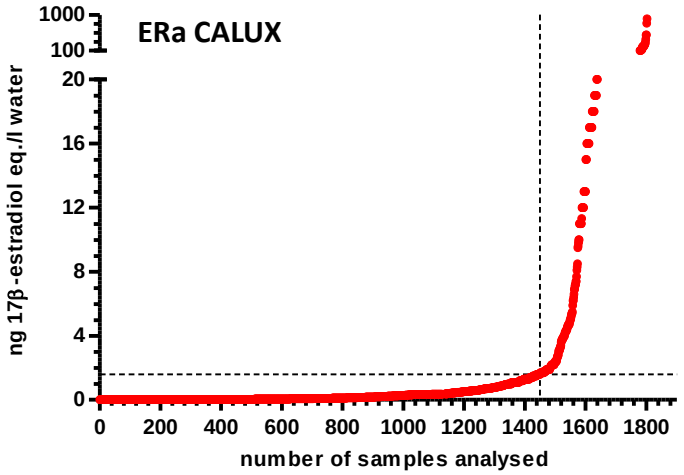
Implementation in regulatory frameworks for risk assessment

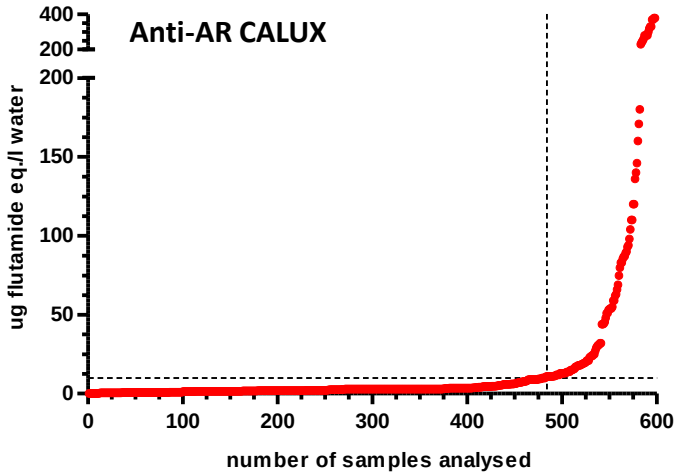
Wat is veilig?

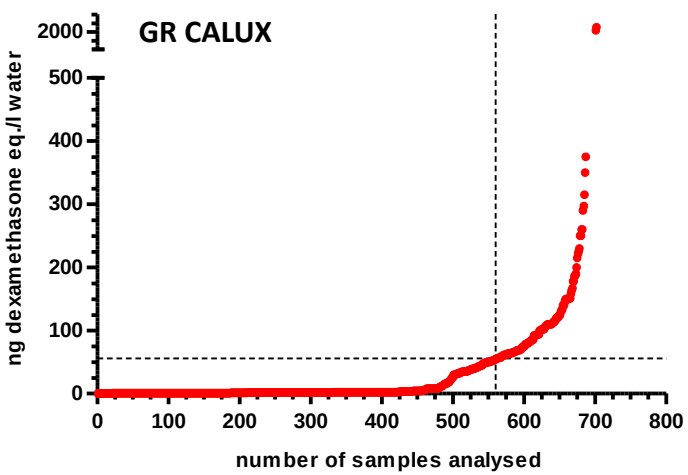
Bepalen effect-based trigger values

	Brand et al.	van der Oost et al.	Escher et al.	BDS
Estrogens (ng 17b-estradiol eq./l water)	3.8	0.5	0.1	1.6
Anti-androgens (ug flutamide eq./l water	n.d.	25	14	10
Glucocorticoids (ng dexamethasone eq./l water)	21	100	considered not relevant	56

$$\text{SIMONI score} = \frac{\sum \frac{\text{Effect bioassys}}{\text{ESW}} * \text{gewicht}}{0{,}5 * \text{Totaal gewicht bioassys}}$$

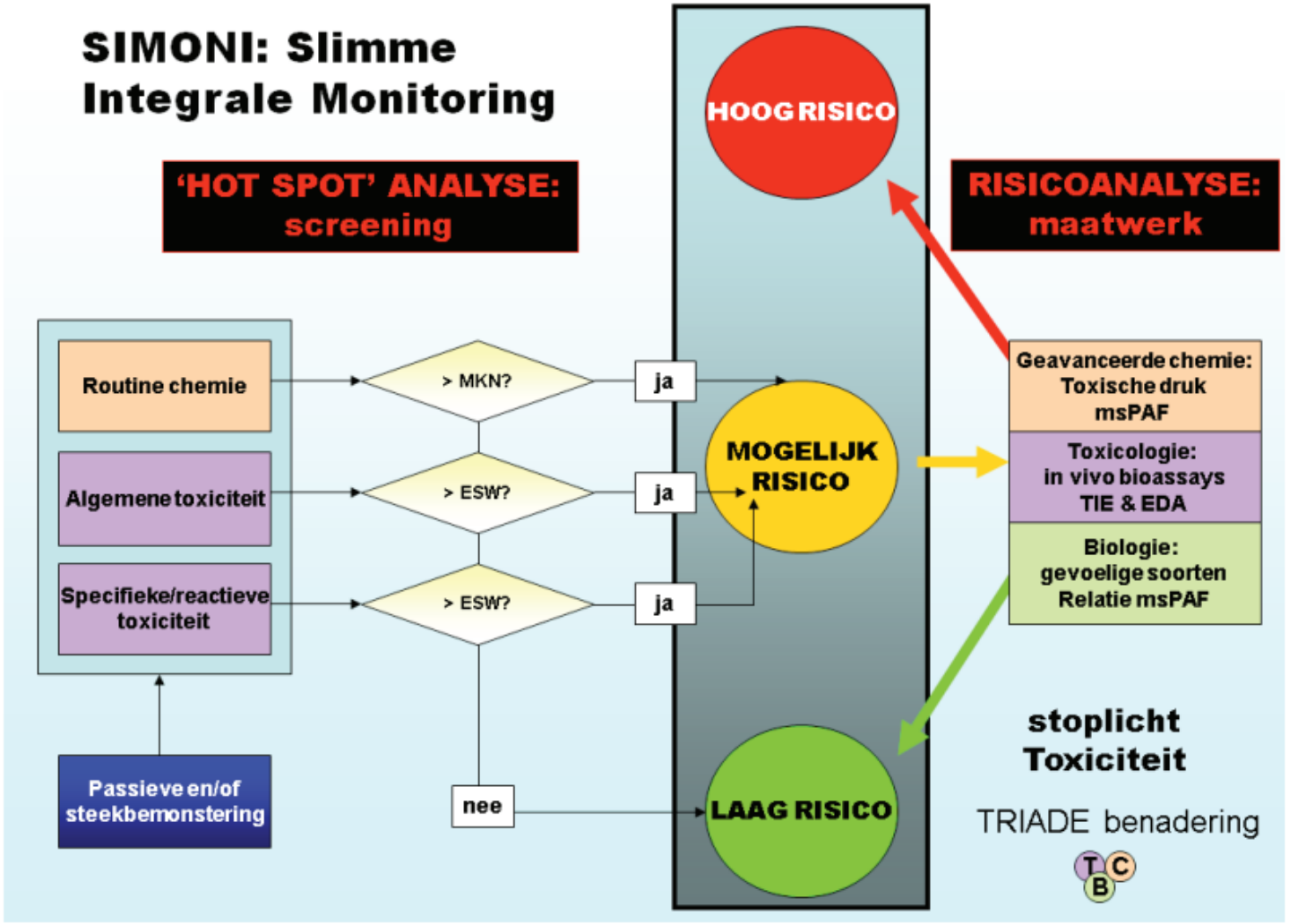






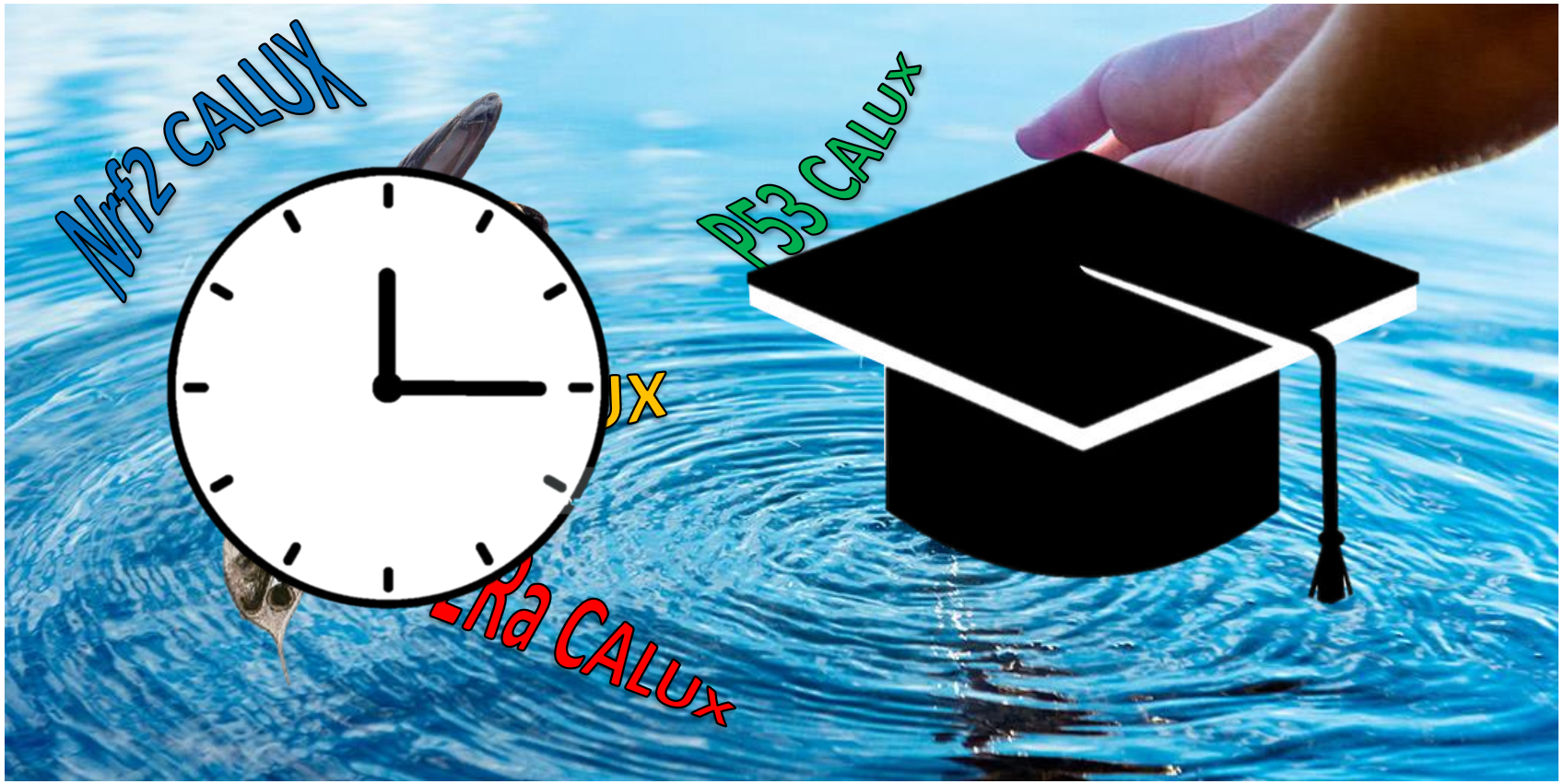
Brand et al. (2013) Environmental International, 55:109-118
Van der Oost et al. (2017) Environ Toxicol Chem. 36, 2385-2399
Escher et al (2018) Sci. Total Environm. 628-629:748-765

Bioassays: Smart Integrated Monitoring

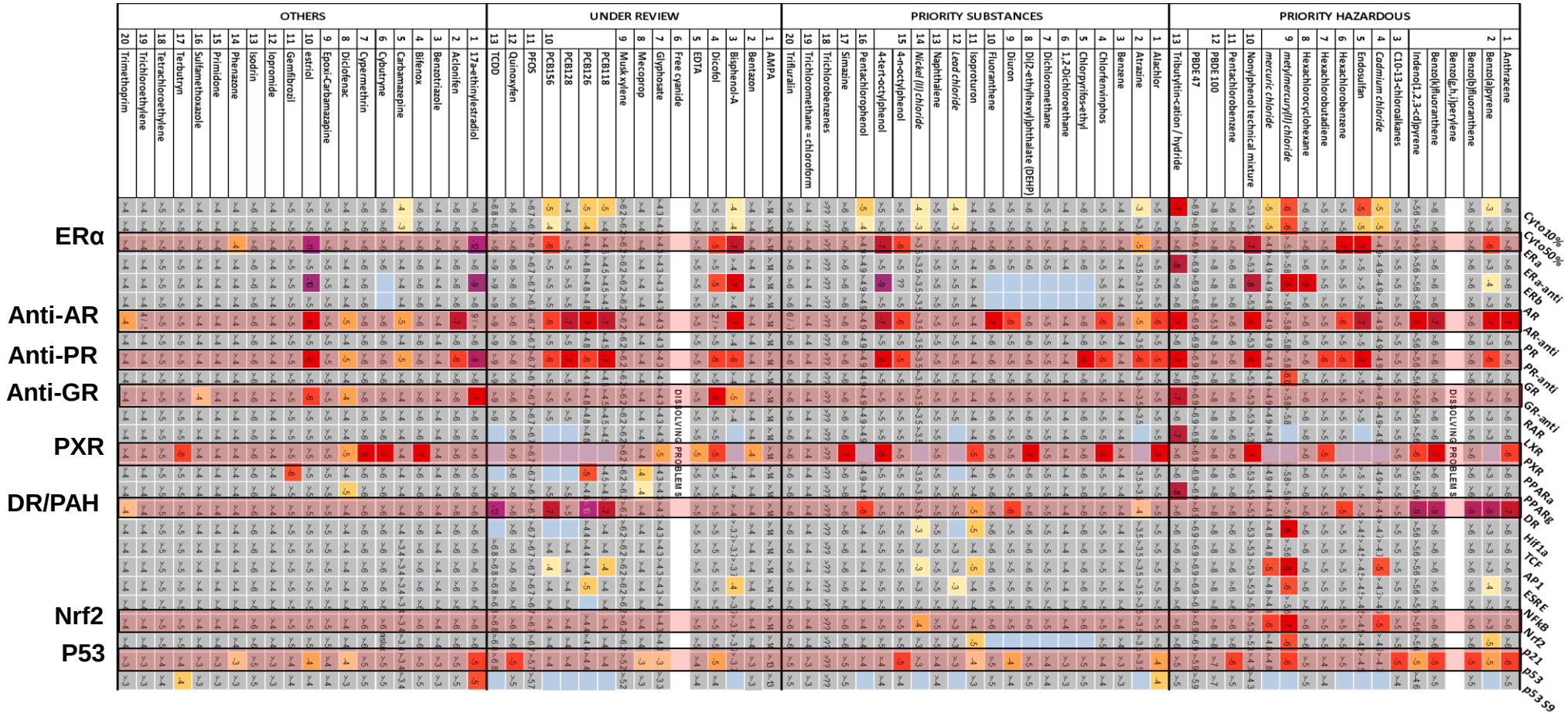


Van der Oost et al. (2017). Environm. Toxicol. Chem. *36*, 2385-2399

Welke bioassays?



Wide-panel screening WFD priority compounds



Bioassay selectie

European strategy for EBMs using CALUX assays

Technical Proposal for Effect-Based Monitoring and Assessment under the Water Framework Directive

Report to the Common Implementation Strategy (CIS) Working Group Chemicals on the outcome of the work performed in the subgroup on Effect-Based Methods (EBM)

MANDATE 2016-2018

October 2021

Main use of effect-based monitoring tools in current WFD context:

- screening tools (to prioritise water bodies for further studies)
- early warning systems
- effect of mixtures of pollutants / “unknowns”
- support in water and sediment quality assessment

Measured endpoint or molecular target	Effect-based method	Role in Adverse Outcome Pathway	Reference compound	EBT (ng/l)
activation of estrogen recepotor (ER)	ERa CALUX	hormone receptor regulation	17β-estradiol	0.40
activation of aryl hydrocarbon receptor (AhR)	PAH CALUX	Toxicokentics	Benzo[a]pyrene	50
induction of oxidative stress response	Nrf2 CALUX	adaptive stress response	Dichlorvos	0.60
activation of pregnan x receptor (PXR)	PXR CALUX	Toxicokentics	di(2-ethylhexyl)-phthalate	1300
activation of peroxisome proliferator activated receptor (PPARy)	PPARy CALUX	Toxicokentics	Rosiglitazone	10*
antagonistic activity on the androgen receptor (AR)	anti-AR CALUX	hormone receptor regulation	Flutamide	25*
antagonistic activity on the progestonic receptor (PR)	anti-PR CALUX	hormone receptor regulation	Endosulfan	5.0

* van der Oost et al. (2017) Environm. Toxicol. Chem. 36. 2385–2399

KiWK strategie

Water monitoring
matrix-specifieke selectie van bioassays (**Base-set**) ter beoordeling waterkwlaiteit / toepassing van EBTs

Specific chemical groups
Selectie van bioassay gebaseerd specifieke stof / groep stiffen (**Custom-set**)

Sleutelfactor Toxiciteit

Kalibratie van de risicogrenzen voor interpretatie van bioassays

Auteurs:
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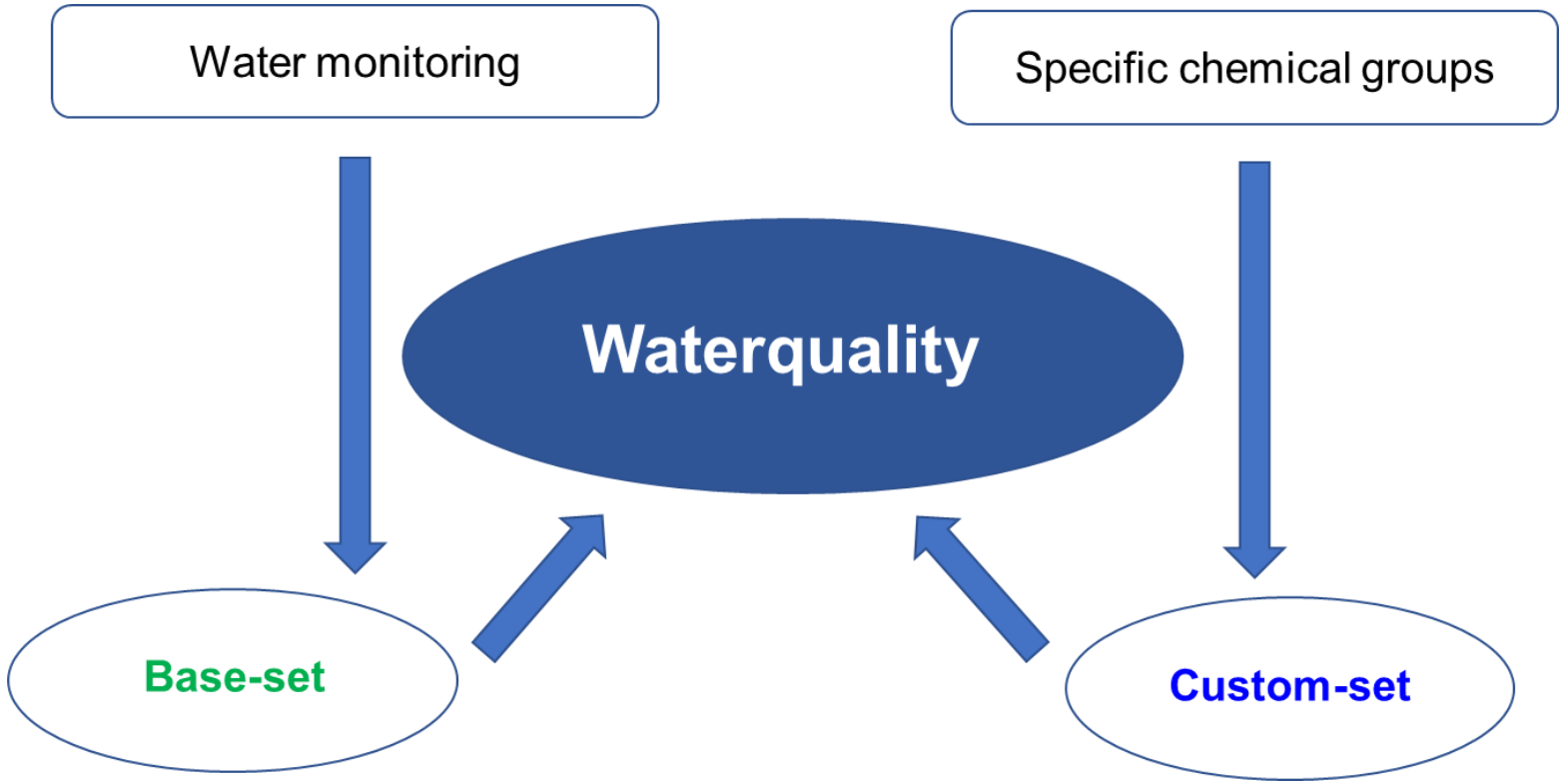
T. E. Pronk (KWR water)
S.J.P. van den Berg (WEnR)
R. van der Oost (Waternet)

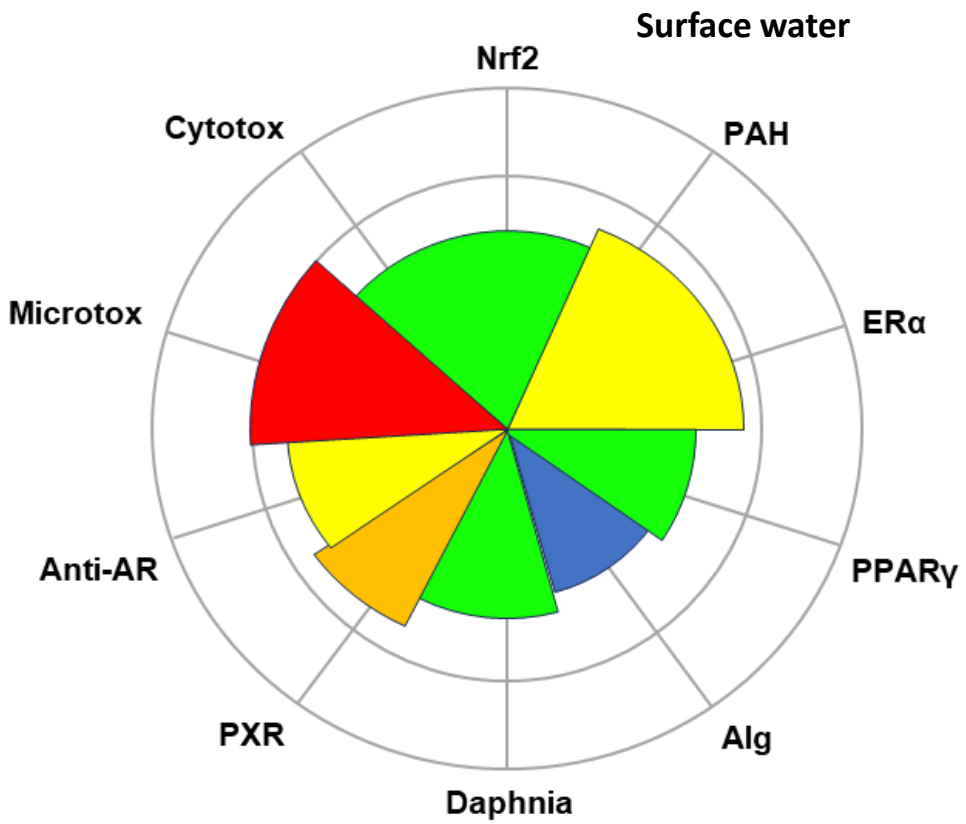
Contact: tessa.pronk@kwrwater.nl

Datum: 21 december 2021

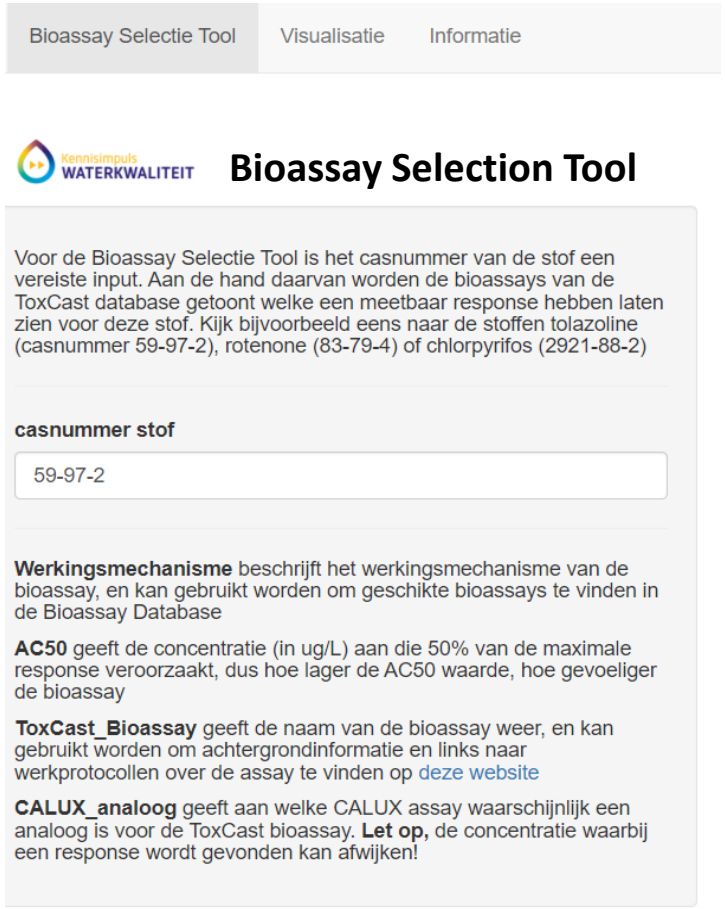


Bioassay selectie

Basis-set selection



Custom-set selection

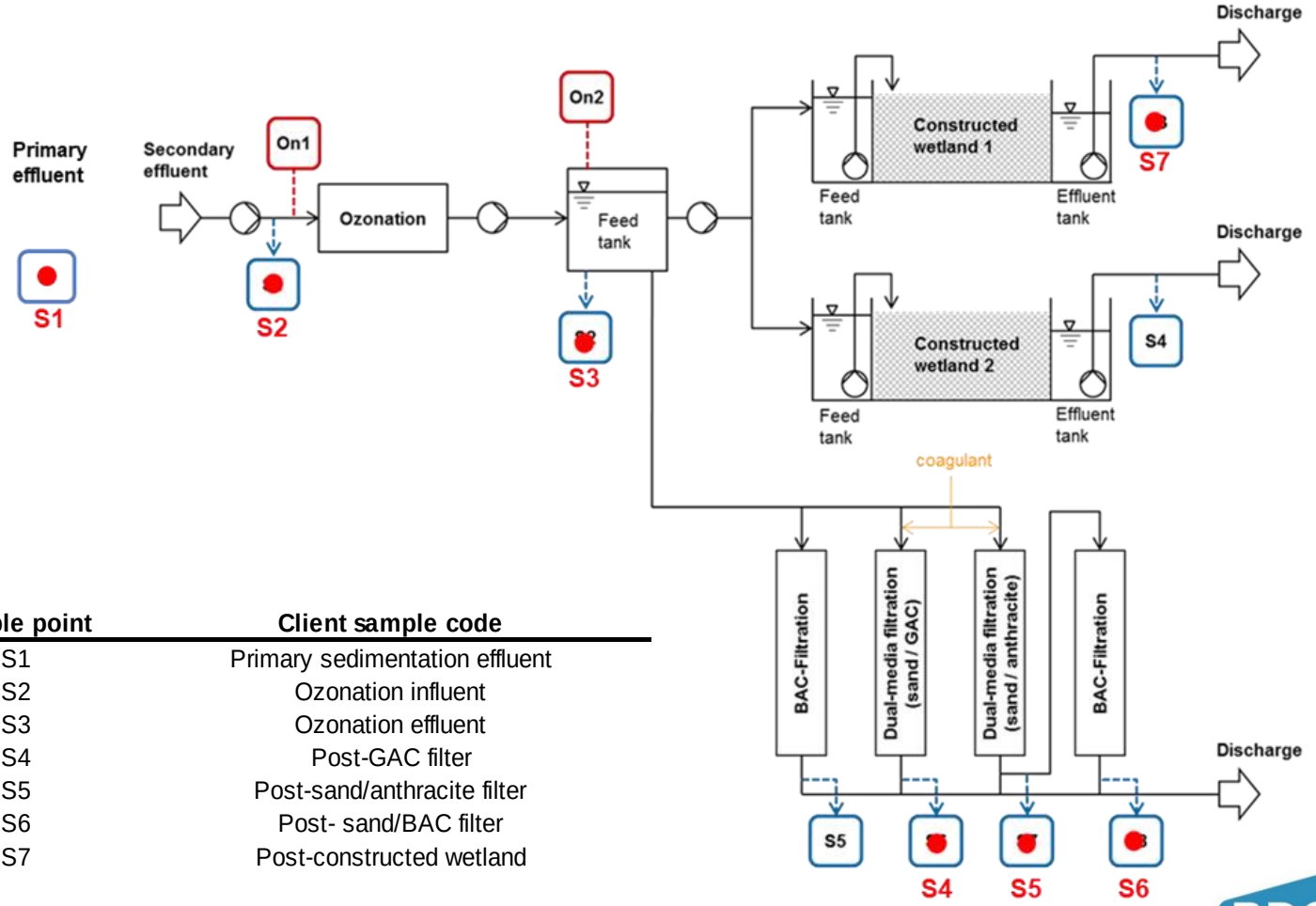


Assessment of water treatment efficiency

23/01/2018	S1	S2	S3	S4	S5	S6	S7
Cytotox CALUX	LOQ	1.8	LOQ		LOQ	LOQ	LOQ
AR CALUX	155	LOQ	LOQ		LOQ	LOQ	LOQ
anti-AR CALUX	LOQ	4.6	LOQ		LOQ	LOQ	LOQ
ERa CALUX	36	1.8	0.1		0.4	0.3	0.1
GR CALUX	110	210	71		87	21	25
anti-PR CALUX	40	LOQ	LOQ		LOQ	LOQ	LOQ
PPARa2 CALUX	400	LOQ	LOQ		LOQ	LOQ	LOQ
PPARg2 CALUX	1300	LOQ	LOQ		LOQ	LOQ	LOQ
PXR CALUX	LOQ	25	12		19	LOQ	8
Nrf2 CALUX	760	180	110		77	79	51
P53 CALUX (+S9)	LOQ	10000	LOQ		LOQ	LOQ	LOQ

17/04/2018	S1	S2	S3	S4	S5	S6	S7
Cytotox CALUX	53.0	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
AR CALUX	430	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	LOQ	1.1	2.6	LOQ	LOQ	1.8	LOQ
ERa CALUX	45	1	LOQ	LOQ	LOQ	LOQ	LOQ
GR CALUX	15	160.0	LOQ	LOQ	42.0	LOQ	50.0
anti-PR CALUX	0	LOQ	LOQ	LOQ	0.0	LOQ	LOQ
PPARa2 CALUX	140	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	LOQ	81	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	80	100	93	LOQ	37	35	11
Nrf2 CALUX	810	320	130	LOQ	190	110	140
P53 CALUX (+S9)	2200	570	LOQ	LOQ	LOQ	LOQ	LOQ

16/07/2018	S1	S2	S3	S4	S5	S6	S7
Cytotox CALUX	25	2.8	0.7	LOQ	LOQ	LOQ	LOQ
AR CALUX	130	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
anti-AR CALUX	21	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
ERa CALUX	51	1.0	0.1	LOQ	LOQ	LOQ	LOQ
GR CALUX	130	110	48	LOQ	41	24	22
anti-PR CALUX	40	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PPARa2 CALUX	420	LOQ	20	LOQ	LOQ	LOQ	LOQ
PPARg2 CALUX	1100	LOQ	LOQ	LOQ	LOQ	LOQ	LOQ
PXR CALUX	100	72	48	8.3	30.0	33.0	31.0
Nrf2 CALUX	740	200	170	LOQ	190	82	110
P53 CALUX (+S9)	25000	LOQ	LOQ	LOQ	1500	LOQ	LOQ



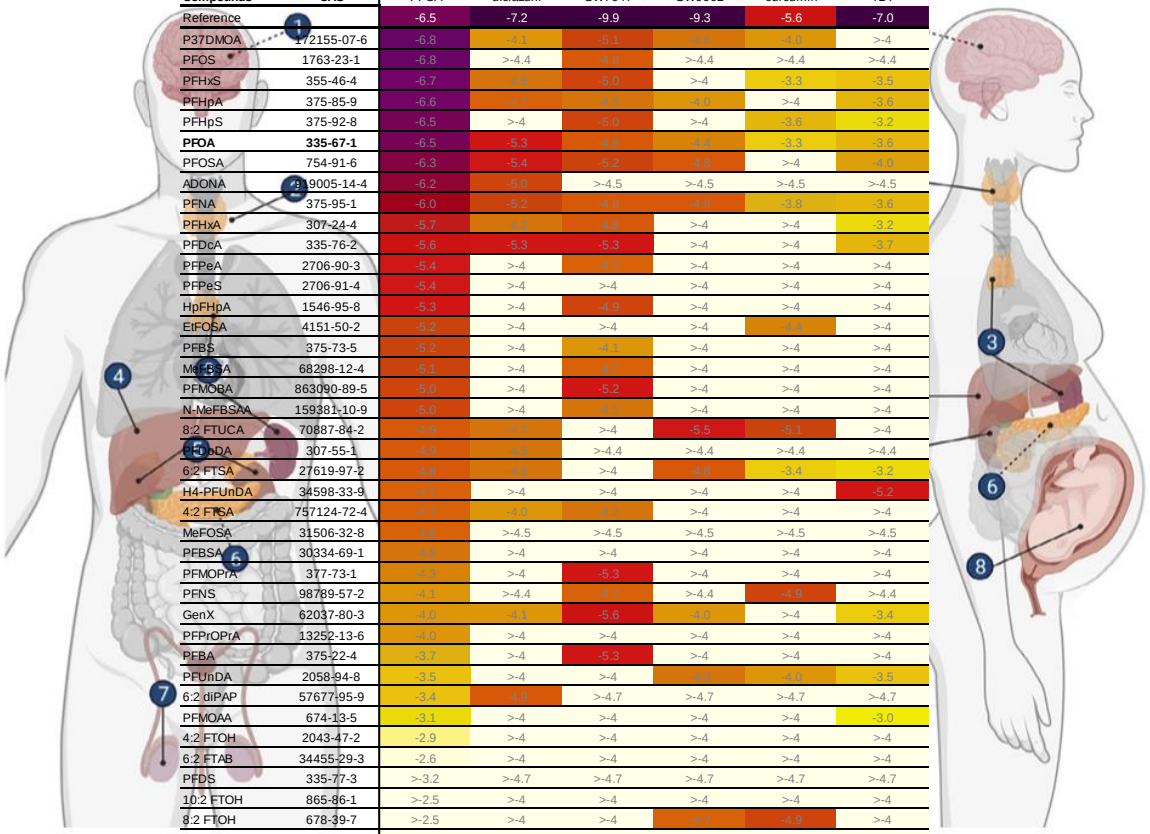
sample point	Client sample code
S1	Primary sedimentation effluent
S2	Ozonation influent
S3	Ozonation effluent
S4	Post-GAC filter
S5	Post-sand/anthracite filter
S6	Post- sand/BAC filter
S7	Post-constructed wetland

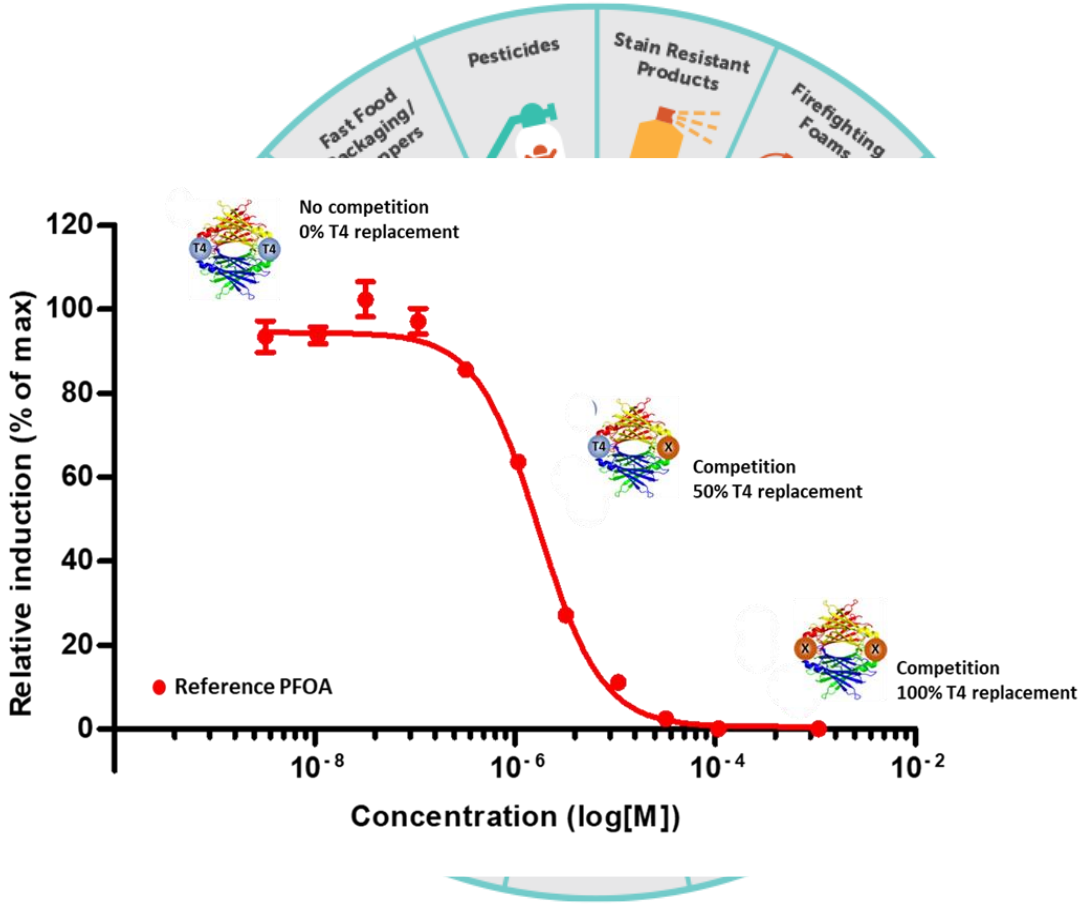
BioDetection Systems

Nieuwe toepassingen: analyse van PFAS

(Poly- en perfluoralkylstoffen)

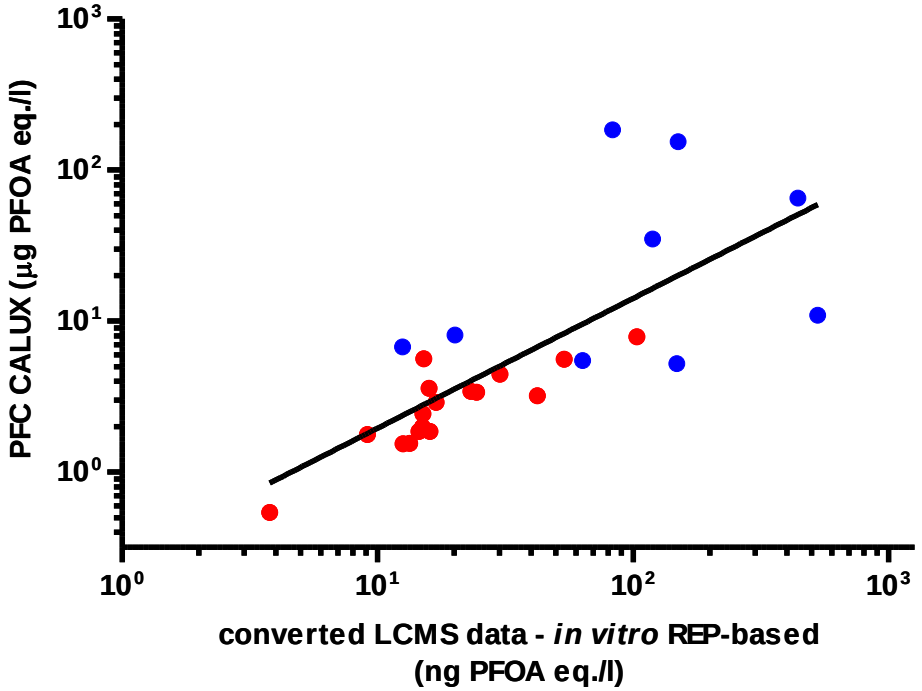
Compounds	CAS	PFAS	anti-TRb	PPARa	anti-PPARg	Nrf2	cytotox
		PFOA	diclazuril	GW7647	GW9662	curcumin	TBT
Reference		-6.5	-7.2	-9.9	-9.3	-5.6	-7.0
P37DMOA	172155-07-6	-6.8	-4.1	-5.1	-4.5	-4.0	>-4
PFOS	1763-23-1	-6.8	>-4.4	-4.8	>-4.4	>-4.4	>-4.4
PFHxS	355-46-4	-6.7	-4.5	-5.0	>-4	-3.3	-3.5
PFHpA	375-85-9	-6.6	-4.5	-4.9	-4.0	>-4	-3.6
PFHpS	375-92-8	-6.5	>-4	-5.0	>-4	-3.6	-3.2
PFOA	**335-67-1**	-6.5	-5.3	-4.8	-4.4	-3.3	-3.6
PFOSA	754-91-6	-6.3	-5.4	-5.2	-4.8	>-4	-4.0
ADONA	919005-14-4	-6.2	-5.0	>-4.5	>-4.5	>-4.5	>-4.5
PFNA	375-95-1	-6.0	-5.2	-4.8	-4.8	-3.8	-3.6
PFHxA	307-24-4	-5.7	-4.5	-4.8	>-4	>-4	-3.2
PFDcA	335-76-2	-5.6	-5.3	-5.3	>-4	>-4	-3.7
PFPeA	2706-90-3	-5.4	>-4	-4.5	>-4	>-4	>-4
PFPeS	2706-91-4	-5.4	>-4	>-4	>-4	>-4	>-4
HpFHpA	1546-95-8	-5.3	>-4	-4.9	>-4	>-4	>-4
EtFOSA	4151-50-2	-5.2	>-4	>-4	>-4	-4.4	>-4
PFBS	375-73-5	-5.2	>-4	-4.1	>-4	>-4	>-4
MeFOSA	68298-12-4	-5.1	>-4	-4.7	>-4	>-4	>-4
PFMOBA	863090-89-5	-5.0	>-4	-5.2	>-4	>-4	>-4
N-MeFBSAA	159381-10-9	-5.0	>-4	-4.5	>-4	>-4	>-4
8:2 FTUCA	70887-84-2	-4.9	-4.2	>-4	-5.5	-5.1	>-4
CFDoDA	307-55-1	-4.9	-4.5	>-4.4	>-4.4	>-4.4	>-4.4
6:2 FTSA	27619-97-2	-4.8	-4.5	>-4	-4.8	-3.4	-3.2
H4-PFUnDA	34598-33-9	-4.7	>-4	>-4	>-4	>-4	-5.2
4:2 FTSA	757124-72-4	-4.7	-4.0	-4.2	>-4	>-4	>-4
MeFOSA	31506-32-8	-4.6	>-4.5	>-4.5	>-4.5	>-4.5	>-4.5
PFBSA	30334-69-1	-4.5	>-4	>-4	>-4	>-4	>-4
PFMOPrA	377-73-1	-4.5	>-4	-5.3	>-4	>-4	>-4
PFNS	98789-57-2	-4.1	>-4.4	-4.7	>-4.4	-4.9	>-4.4
GenX	62037-80-3	-4.0	-4.1	-5.6	-4.6	>-4	-3.4
PFPrOPrA	13252-13-6	-4.0	>-4	>-4	>-4	>-4	>-4
PFBA	375-22-4	-3.7	>-4	-5.3	>-4	>-4	>-4
PFUnDA	2058-94-8	-3.5	>-4	>-4	-4.7	-4.0	-3.5
6:2 diPAP	57677-95-9	-3.4	-4.9	>-4.7	>-4.7	>-4.7	>-4.7
PFMOAA	674-13-5	-3.1	>-4	>-4	>-4	>-4	-3.0
4:2 FTOH	2043-47-2	-2.9	>-4	>-4	>-4	>-4	>-4
6:2 FTAB	34455-29-3	-2.6	>-4	>-4	>-4	>-4	>-4
PFDS	335-77-3	>-3.2	>-4.7	>-4.7	>-4.7	>-4.7	>-4.7
10:2 FTOH	865-86-1	>-2.5	>-4	>-4	>-4	>-4	>-4
8:2 FTOH	678-39-7	>-2.5	>-4	>-4	-4.7	-4.9	>-4
6:2 FTOH	647-42-7	>-2.5	>-4	>-4	>-4	>-4	>-4
2:2 FTOH	54949-74-5	>-2.5	>-4	>-4	>-4	>-4	>-4
6:2 FTSAM	80475-32-7	>-2.9	>-4.4	>-4.4	>-4.4	>-4.4	>-4.4
MeFOSAA	2355-31-9	>-3	>-4.6	>-4.6	>-4.6	>-4.6	>-4.6
FOSAA	2806-24-8	>-3	>-4.5	>-4.5	>-4.5	>-4.5	>-4.5
EtFOSAA	2991-50-6	>-3	-4.6	>-4.5	>-4.5	>-4.5	>-4.5
Tech-mix 1	Tech Mix	>0.5	>-1	>-1	>-1	>-1	>-1
Tech-mix 2	Tech Mix	>0.5	>-1	>-1	>-1	>-1	>-1



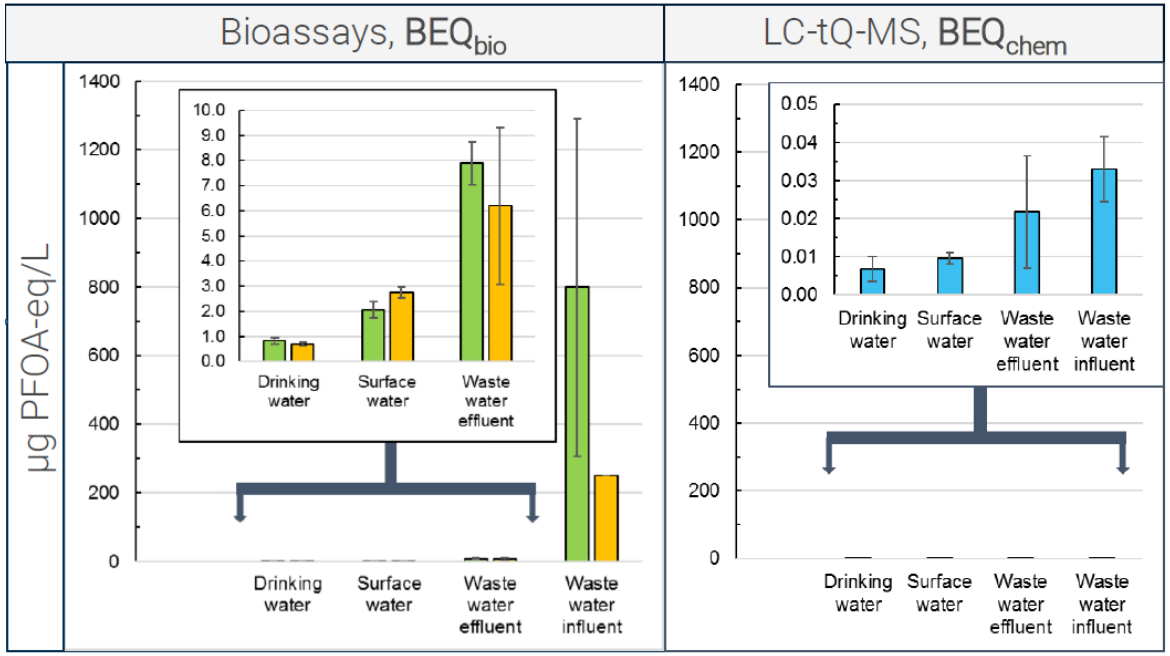


Per- en polygefluoreerde alkylstoffen (PFAS) water

- Surface water
- WWTP influent / effluent



Field study of TTR-T4 displacing activity in Dutch (drinking) water



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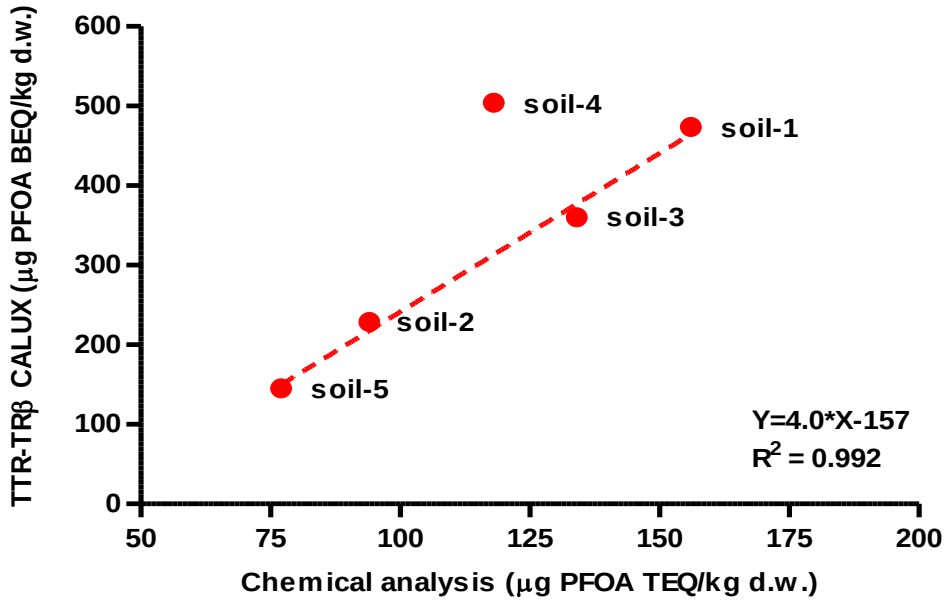
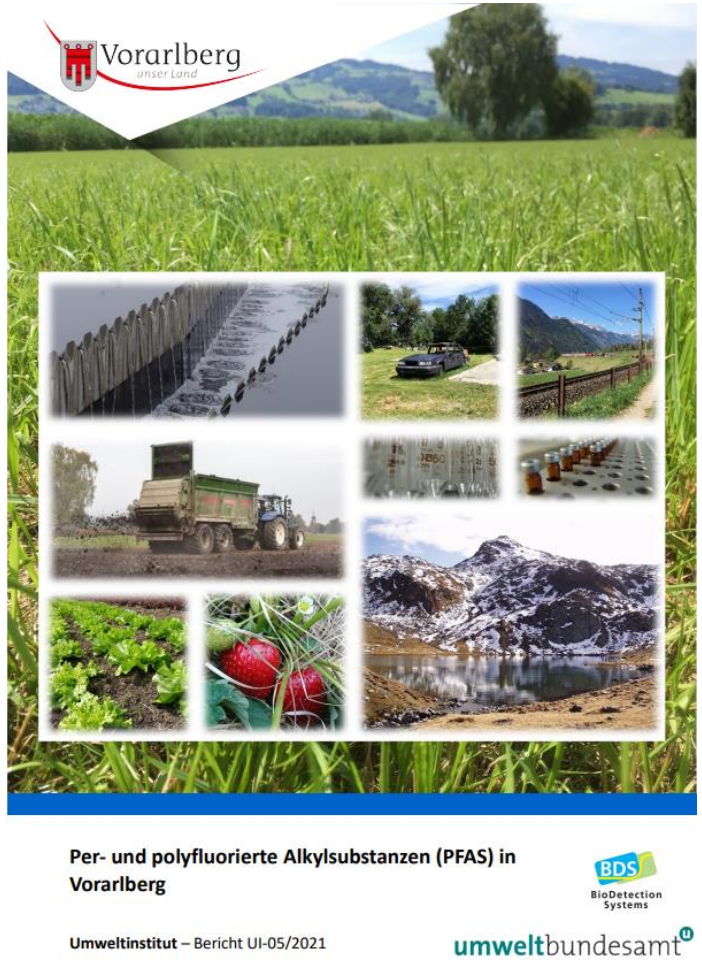
Testing the PFC CALUX® bioassay for monitoring the ...

De Schepper et al. (2023). Environm. Int. 181, 108256. DOI: 10.1016/j.envint.2023.108256

Per- en polygefluoreerde alkylstoffen (PFAS) bodem
(Vorarlberg; Austria)



Chemical analysis	REP		soil-1		soil-2		soil-3		soil-4		soil-5	
			PFAS	PFOA-EQ	PFAS	PFOA-TEQ	PFAS	PFOA-TEQ	PFAS	PFOA-TEQ	PFAS	PFOA-TEQ
PFBA	0.0018	(µg/kg d.w.)	0	0	0	0	1.6	0.0029	1.1	0.002	0	0
PFPeA	0.080	(µg/kg d.w.)	0	0	0	0	1.8	0.14	4.5	0.36	0	0
PFHxA	0.19	(µg/kg d.w.)	2	0.37	3.3	0.61	1.8	0.33	1.4	0.26	0.7	0.13
PFHpA	1.4	(µg/kg d.w.)	1.9	2.7	2.8	3.9	2.8	3.9	1.3	1.8	0	0
PFOA	1.0	(µg/kg d.w.)	10	10	5.7	5.7	15	15	5.7	5.7	2.8	2.8
PFNA	0.32	(µg/kg d.w.)	3.2	1	1.9	0.62	5.3	1.7	3.4	1.1	0.51	0.17
PFDcA	0.12	(µg/kg d.w.)	3.6	0.44	3.4	0.42	8.7	1.1	6.1	0.75	0.25	0.031
PFBS	0.052	(µg/kg d.w.)	0	0	0	0	0	0	0	0	0	0
PFHxS	1.6	(µg/kg d.w.)	0.91	1.4	1.5	2.3	1.1	1.7	1.3	2	0	0
PFHpS	1.0	(µg/kg d.w.)	0.25	0.24	0.69	0.67	0.25	0.24	0	0	0	0
PFOS	2.0	(µg/kg d.w.)	70	140	40	80	57	110	53	106	37	74
PFOSA	0.72	(µg/kg d.w.)	0	0	0	0	0.5	0.36	0	0	0	0
SUM TEQ (PFOA-TEQ)				156		94		134		118		77
Measured PFAS CALUX (PFOA-BEQ)				474		229		360		504		145



Bevestiging van chemie:

Voorbeeld Triade: Bevestig of ontkracht risico van grondwaterverontreiniging

- → Voorafgaand aan chemie:
- → Eerst effecten meten, dan koppelen aan stoffen, concentraties, maatregelen

Behoeften

- → Hoe erg is vergrijzing van grondwater
- → Meten van effecten bij ‘hotspots’, bijvoorbeeld stortplaatsen

Lopende projecten

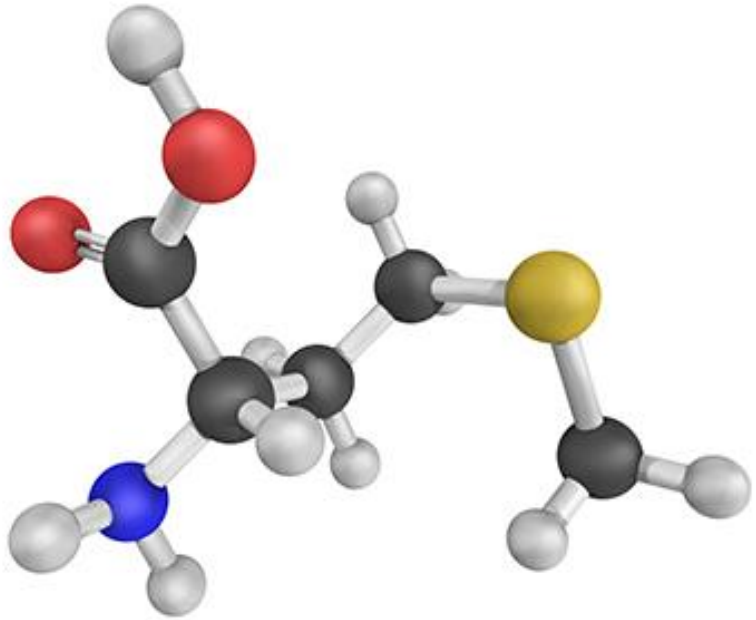
- Meerwaarde van bioassays ten opzichte van chemie?
- Kun je herkomst verontreiniging herleiden?

Lopende projecten

Eerst chemie → Groot aantal stoffen aanwezig

Vraagstelling: Meer onderzoek naar risico's nodig?

→ CALUX bioassays als eerste indicatie



Thank you for your attention

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bioclear earth

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