Market Uptake for New Technologies – Lessons from Practice

Innovative effect- and non-animal-based methods (EBM/NAM) – how to overcome barriers to market integration




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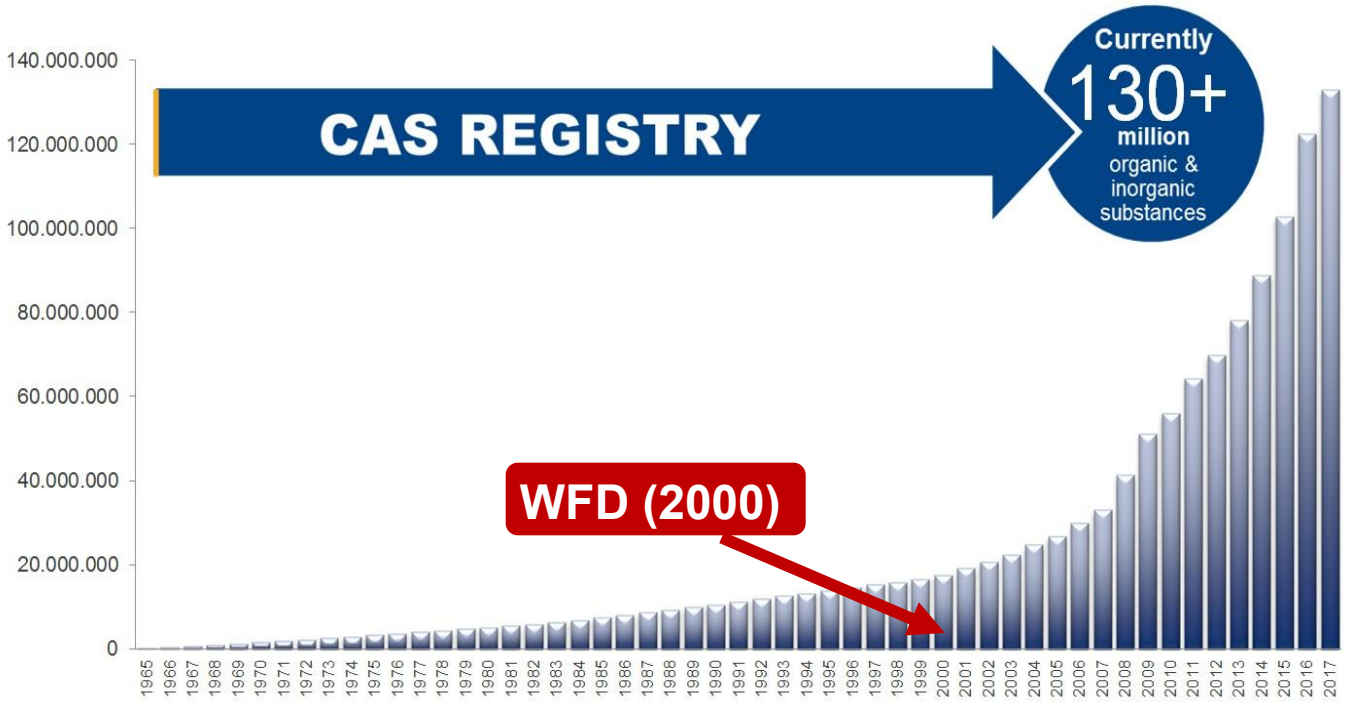
Funded by the European Union

Is the explosion of new chemicals in last decades covered by current EU guidance?

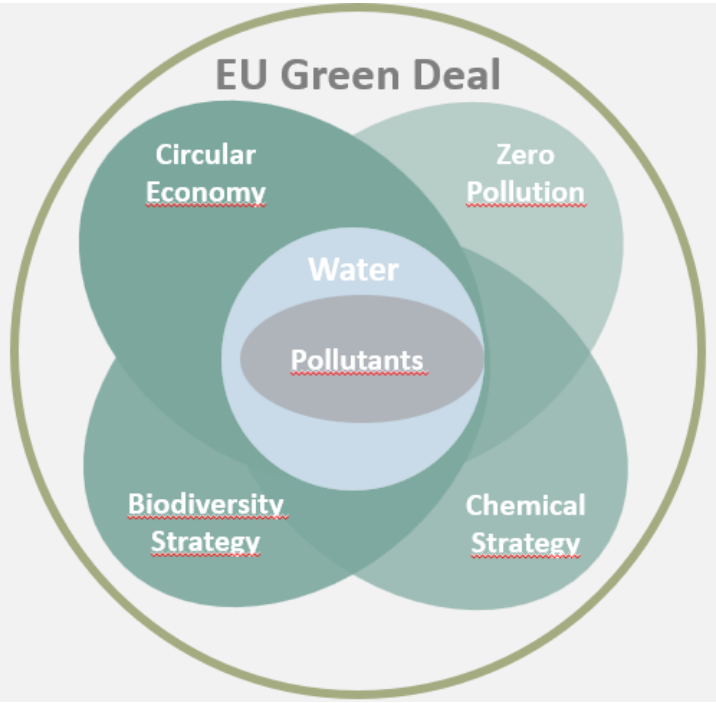


Increasing diversity of chemicals

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

Source: CAS

EU Green Deal – zero pollution by toxic-free testing



Zero Pollution Action Plan:
Achieve zero pollution in air, water, and soil by 2050.

Biodiversity Strategy 2030:
Protect and restore biodiversity, aiming for nature's recovery by 2030.

Circular Economy Action Plan:
Reduce resource use, minimize waste, and promote sustainable growth.

Chemical Strategy for Sustainability:
Ensure chemicals are safe and sustainable, protecting health and the environment.

Latest news about Effect-based methods (EBM)

Council of the EU | Press release |

23 September 2025 19:52

Water pollution: Council and Parliament reach provisional deal to update priority substances in surface and ground waters

Effect-based monitoring (EMB) will be introduced for surface waters, improving the ability to detect harmful chemical mixtures. EBM is an advanced approach used to assess water quality, focusing not just on specific chemicals but on the **overall impact** of pollutants on ecosystems and organisms in the water. Instead of just measuring individual chemicals, EBM evaluates how a combination of chemicals affects the environment. In particular, a limited and targeted use of EBM will be mandatory for estrogenic substances, during a 2-year period.

Safe Crew project a partner in ZP4Water Cluster

Water systems worldwide are confronted with a **complex mixture of thousands of known and unknown (unregulated) emerging compounds.**

Major challenge to deliver **safe and affordable water services** to a growing population.

The SafeCREW project catalyzes **innovations in several European water treatment sites.**

Room is now given in the **Drinking Water Directive** to develop an **effect-based monitoring program (EBM).**

Non-animal and effect-based methods (i.e. CALUX® NAM/EBM) to assess the impact of **disinfection by-products (DBPs)** in different waters and in contact with plastic materials.

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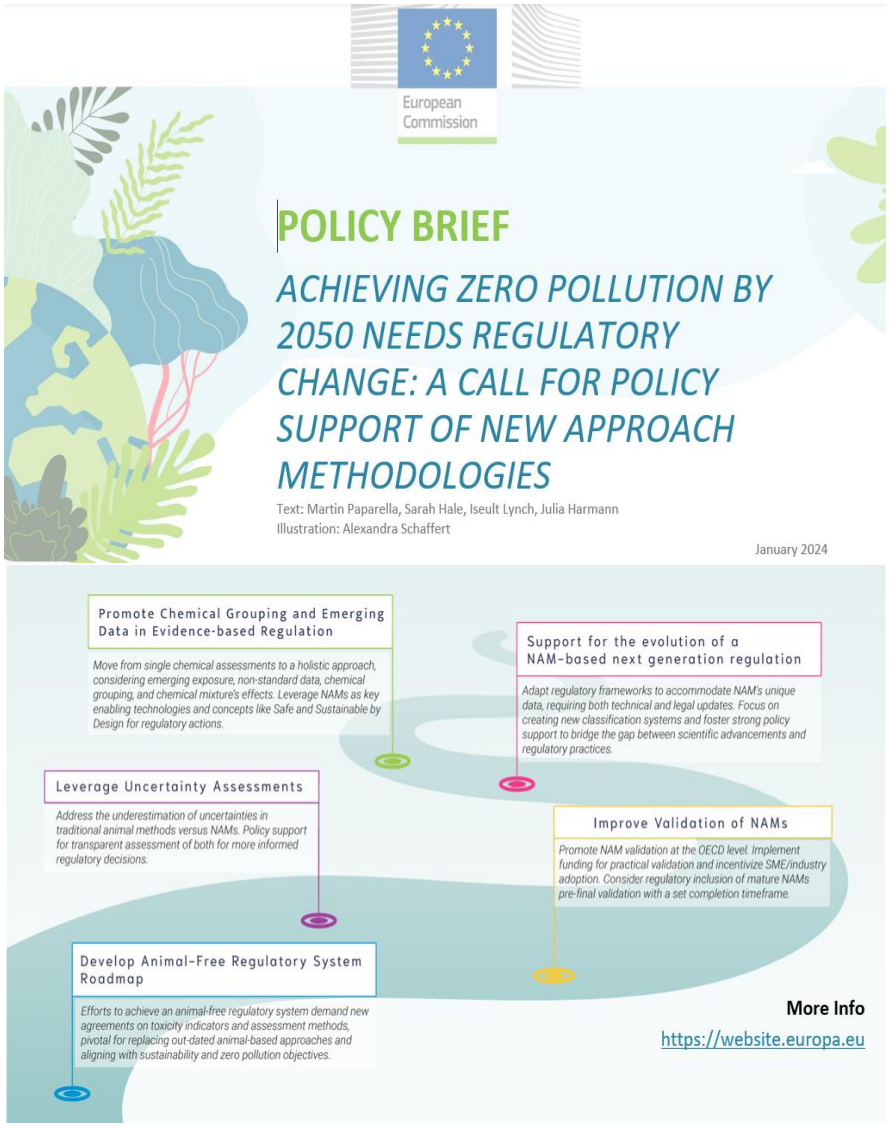
Policy briefs about Effect-based methods (EBM)



Policy brief for Toxic-free and Zero Pollutions aims of the EU Green Deal strategy

Non-animal- and effect-based methods (NAM/EBMs) for testing of single and complex mixtures of Disinfection-by-products (DBPs), their impact on plastics in contact with and drinking water case studies in several EU countries in the

Policy recommendations

- **Support for using NAM/EBM-based bioanalysis and chemical grouping for regulatory action**
- **Support for improving the validation and application process**
- **Change management by training**
- **NAM based next generation regulation (NGR) such as described in CEN WA 18201 and**
- **Support for the initiative to develop a European roadmap following the last decisions from the EU council[3].**

Funded by the European Union

EU technical report (2014): Effect-based methods (EBM/NAM)

ERα CALUX (agonistic/antagonistic)

The ERα Responsive (ERα) CALUX® comprises a human bone marrow cell line (U2OS), incorporating the firefly luciferase gene coupled to Estrogen Responsive Elements (EREs) as a reporter gene for the presence of estrogens and/or estrogen-like compounds. Following binding of estrogens or estrogen-like compounds to the cytosolic estrogen receptor, the ligand-receptor complex binds the ERE. Cells that are exposed to estrogens and/or estrogen-like compounds not only express proteins that are under normal circumstances associated to ERE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compounds 17β-estradiol. ERα CALUX bioassays report total 17β-estradiol equivalents for environmental matrices.

- **What is analysed (endpoint; unit):** pg 17β-estradiol equivalents/g sample processed
- **Test duration:** 24h
- **Method used**: Dutch Rijkswaterstaat RIKZ-Specie-08 guideline; Australian Water Commission; Ongoing evaluations at the ISO-TC 147 standardisation group led by BFG-Germany; EPA California; China National Water Quality Monitoring in Jinan.
- **Positive control used**: 17β-estradiol (E-2)
- **Matrices (sediment, water, tissue etc) that can be investigated:** Any type of sample.
- **Cells examined:** Human bone marrow cell line
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ) (see below).
- **What /type of/ substances does the assay respond to** Binding to the Estrogen receptor (alpha and beta for original ER CALUX and only alpha for ERalpha CALUX)
- **Sensitivity (LOD/Q):** The bioassays' LOQ is 35 pg 17β-estradiol equivalents per amount of material processed. For example, if 5 grams of dried soil/sediment or 1 liter of water is processed an LOQ of 7 pg 17β-estradiol equivalents per gram of soil/sediment or 35 pg 17β-estradiol equivalents per liter of water is obtained respectively. Original ER CALUX: 0.1 ng EEQ/l water (see e.g. Leusch, 2008).
- **Variability (e.g. CV for single substance tests) if known:** <20%
- **Influence by cytotoxicity/risk of false positives/negatives:** Depending on the SPE extraction/clean-up as well as type of water matrix.
- **Complexity/learning period:** 1 week of training
- **Costs:** Low[58]. Costs are generally not depending on matrix studied.
- **Commercial availability:** Commercial ISO 17025 accredited performers available
- **WFD relevance:** This bioassay analysis is more sensitive than most chemical analyses (lowest LOD reported by Loos 2012 is e.g. 0.1 ng/l for a chemical analysis of EE-2 and E-2, if using USEPA method 1698; in practice the LOQ that is possible to reach by regular laboratories is generally higher). The assay could therefore be very valuable on a screening level to identify water bodies at risk due to the combined exposure to a large number of estrogenic substances that could constitute RBSPs (see case studies "Laxsjön – investigating sediment contamination, using chemical and in vitro bioassay approach") and to lower the frequency of analytical high end monitoring in water bodies for E2. EE2 and E2 are also suggested to be included in 2008/105/EC. Because EE2 is significantly (about 10-25 times) more potent *in vivo* than E2, but only 3 times more potent in ER CALUX, this should be taken into account if evaluating data in an absolute manner (comparison with EQS), when considering the need for additional studies. In vivo studies of oestrogenic response, or using precautionary EE2 equivalents can be considered, if the presence of EE2 is likely, e.g. via high ratio of municipial waste water. The EU-EQS proposal for E2 is based on a SSD approach of the most sensitive aquatic organisms, and concludes that an

DR CALUX

The Dioxin Responsive (DR) CALUX® comprises rat hepatoma cell lines (H4IIE), incorporating the firefly luciferase gene coupled to Dioxin Responsive Elements (DREs) as a reporter gene for the presence of dioxins (PCDDs) and dioxin-like compounds (e.g. furans (PCDFs) and dioxin-like PCBs (dlPCBs)). Following binding of dioxins and/or dioxin-like compounds to the cytosolic Arylhydrocarbon receptor (AhR), the ligand-receptor complex binds the DRE. Cells that are exposed to dioxins or dioxin-like compounds not only express proteins that are under normal circumstances associated to DRE, but also luciferase. By addition of the appropriate substrate for luciferase, light is emitted. The amount of light produced is proportional to the amount of ligand-specific receptor binding, which is benchmarked against the relevant reference compounds (2,3,7,8-TCDD). DR CALUX bioassays report total 2,3,7,8-TCDD TEQs for environmental matrices and total BEQs for food/feed matrices.

- **What is analysed (endpoint; unit):** ng 2,3,7,8-TCDD equivalents/kg sample processed
- **Test duration:** 24h
- **Method used**: Marine Quality Assurance procedures available in the future through between particular independent laboratories (Davies & Vethaak 2012)
- **Positive control used**: 2,3,7,8-TCDD
- **Matrices (sediment, water, tissue etc) that can be investigated:** Any type of sample, but the substances that the assay responds to are in the aquatic environment primarily found accumulated in e.g. sediments and biota (tissues).
- **Cells examined:** Rat liver cell line
- **Sample volume or mass needed for different matrices:** Depending on type of material analysed and required Limit of Quantitation (LOQ) (see below).
- **What /type of/ substances does the assay respond to:** Ah receptor active compounds, e.g. Polyhalogenated dioxins/furans, dioxin like PCBs, and if using other pretreatment of samples also PAHs (see PAH CALUX).
- **Sensitivity (LOD/Q):** . The bioassays' LOQ is 1 pg 2,3,7,8-TCDD equivalents per amount of material processed. For example, if 5 grams of dried soil/sediment or 1 liter of water is processed, an LOQ of 0.2 ng 2,3,7,8-TCDD equivalents per gram of soil/sediment or 1 ng 2,3,7,8-TCDD equivalents per liter of water is obtained respectively.
- **Variability (e.g. CV for single substance tests) if known:** <20%
- **Influence by cytotoxicity/risk of false positives/negatives:** As the sample is cleaned up by a sulphuric acid treatment and afterwards with an additional step to separate dl-PCBs from PCDD/Fs, cytotoxicity is rarely occurring. In case of false positive/false negative guided levels has to be established to compare it with. In case of the EC project HORIZONTAL no false positive or false negative samples occurred. For such methods usually a false positive and negative ratio of 5% is reasonable.
- **Complexity/learning period:** 2 weeks of training
- **Costs:** Low[56], especially compared to chemical analysis of dioxins and dioxin-like compounds. Generally not depending on matrix studied.
- **Commercial availability:** Commercial ISO 17025 accredited performers are available

Funded by the European Union

EU strategy for EBMs under WFD

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)

October 2021

Conclusion

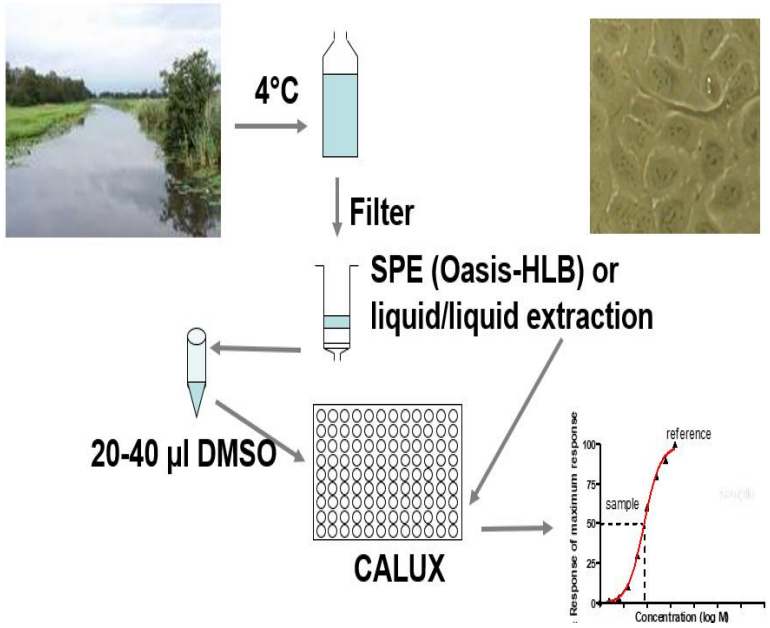
The Technical Report concluded that the main use of effect-based monitoring tools in the current WFD context would be:

- As screening tools, as part of the pressures and impacts assessment to aid in the prioritisation of water bodies to study further;
- To establish early warning systems, to prioritise further studies in areas that are not concluded to be at risk because they are located far from known local sources;
- To take the effects from mixtures of pollutants or not routinely analysed chemicals ("unknowns") into account (e.g. to support investigative monitoring where causes of a decline of specific species are unknown);
- To provide additional support in water and sediment quality assessment, though not as a replacement for conventional chemical and ecological monitoring under the WFD.

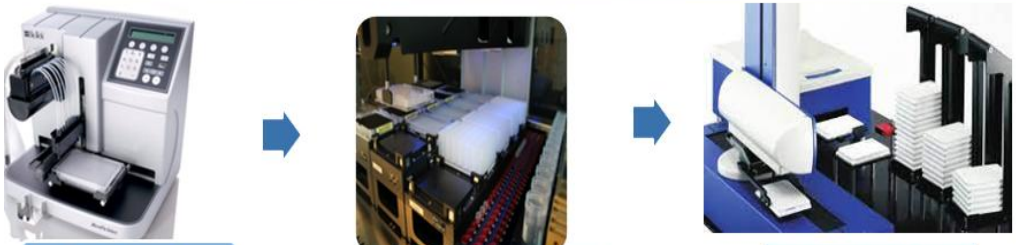
It was also concluded that EBMs are at the moment particularly suitable as part of investigative monitoring programmes, for which the regulatory requirements are less formally determined.

Effect-based methods (EBM/NAM)

Water sampling & extraction



Robotic CALUX analysis



SEEDING

Ready-to-seed cells in 96/384-well format

EXPOSURE

Dilution series (TCDD and sample)

DETECTION

Luminescence detection

Advantages:

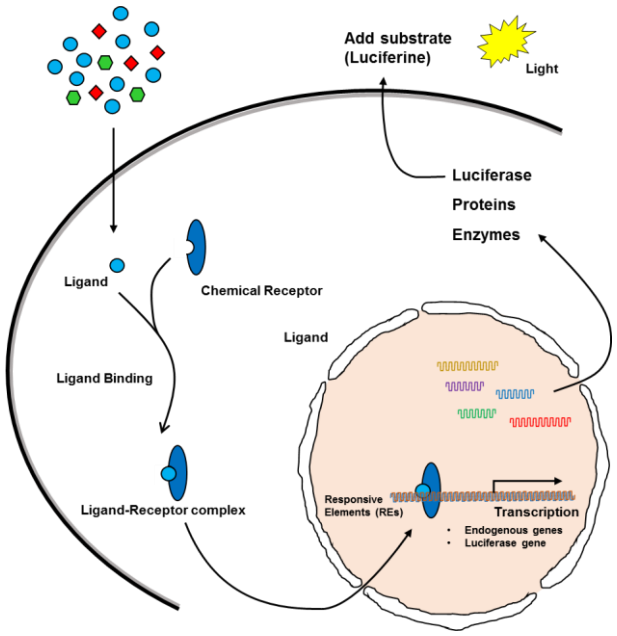
High-through-put

Many different chemicals (e.g. Dioxins, PFAS, plastic)

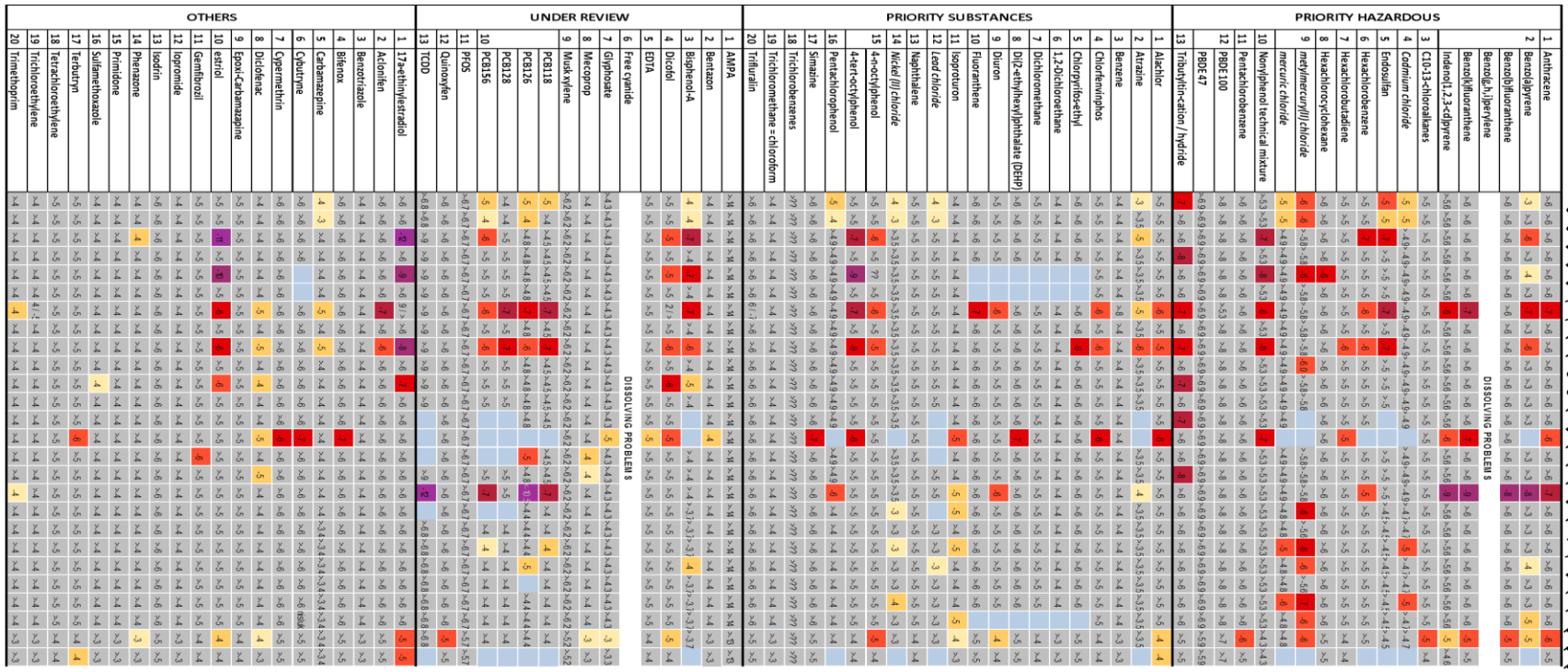
Cheaper, faster, easier as chemical single chemicals testing

Effect-based CALUX bioassays

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



WFD relevant compounds & CALUX EBM in vitro toxicity profiling



Most relevant in vitro toxic key events in water:

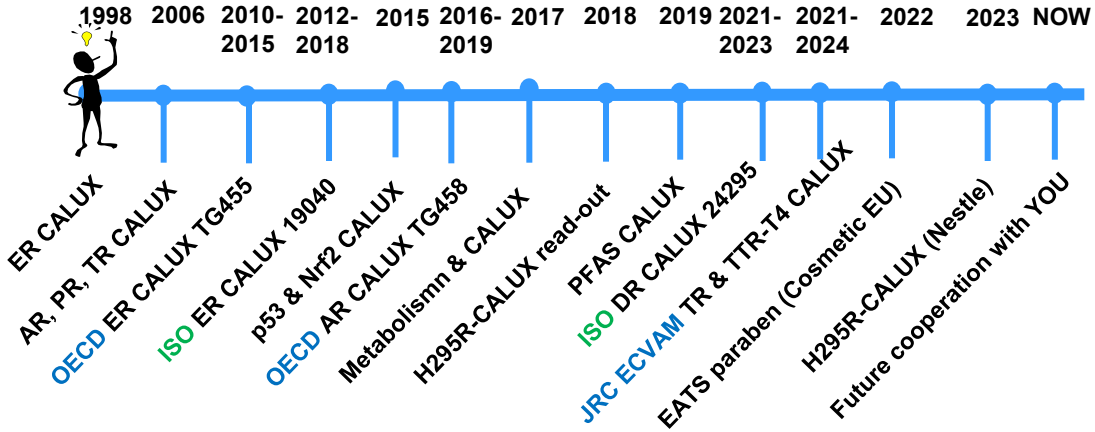
ER, anti-AR, anti-PR, anti-GR, PXR, AhR, Nrf2 and p53 CALUX

CEN WA 18201 (2025) Panel of effect-based ultra-trace bioanalysis tools for complex mixture of water pollutants incl. trigger values (EBT)

Bioassay	Σ PFAS [ng PFOA/L]	Σ Dioxin-like [ng TCDD/L]	Σ Estrogen-like [ng Estradiol/L]	Σ anti-AR-like [ng Flutamide/L]	Σ Early warning PXR [µg Nicardipine/L]
PFAS CALUX[1]	3000	—	—	—	—
DR CALUX[2]	—	0.05	—	—	—
ERα CALUX[3]	—	—	0.1		—
anti-AR CALUX[4]	—	—	—	14	—
PXR CALUX	—	—	—	—	3.0

[1]see Behnisch et al. (2023); [2]ISO 24295; [3]ISO/DIS 19040-3; [4]OECD TG 458

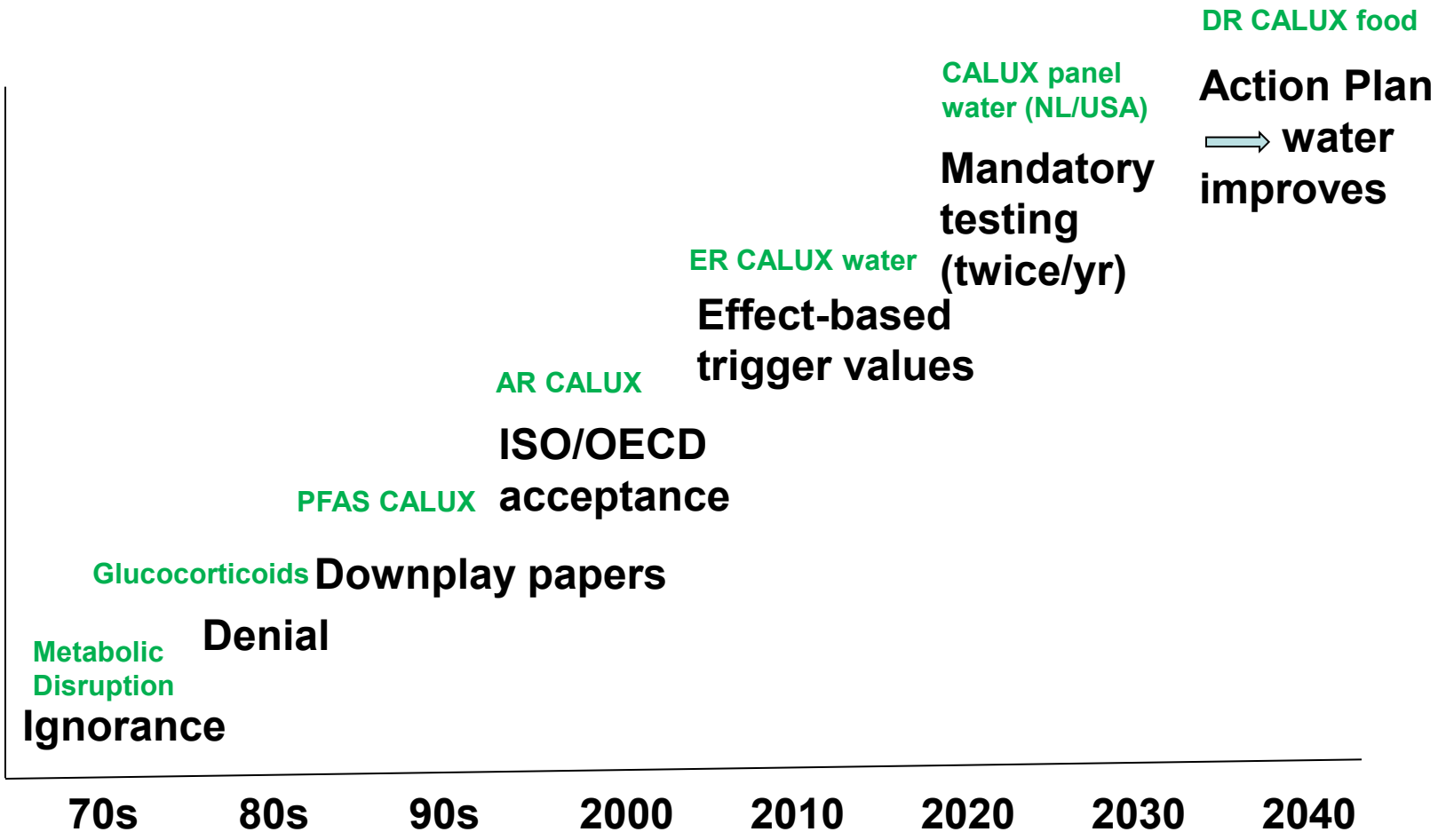
Barriers – slowly regulation framework



- list of pollutants get larger & larger
- list of relevant in vitro toxicity endpoints get longer & longer
- list of the be tested materials get larger & larger

standardization process for EDCs gets faster &faster (high-through-put)!

Barriers & evolution of different kinds of EBM/NAM



EU Projects related to Toxic-Free water

Driving force with local show-cases

PROMISCES	→	**PFAS-45 in water, soil, sediments**
safe CREW	→	**Disinfection products and by-products** **(Monitoring DWTP in Milano, IT and Tarragona, ESP)**
RISK HUNT3R	→	**Non-animal testing for a more sustainable & safer design of chemicals**
One Blue	→	**Contaminants of Emerging concern in Marine Water**
German UBA: Construction materials & EDCs	→	**Safer & Sustainable construction materials**

Strategic Targets

Main Steps to realise Strategic Objectives

Focus on International R&D leaders from Uni, Industry and Governments

Find “push buttons”: Pricing, TAT and Client Specialities

Follow the winners: why the came to us, what do we offer they need and which similar needs are on the market

Focus on Development of Countries in need of Clean Blue Water

Market Development: Research (Uni) pushes Industry which pushed Government

Strategic Initiatives in Detail

Flexible Pricing: Project and Client orientated

International Promotion: Grow faster by using Star-experts and Champion Labs

EU Authorities: New EU guidelines for WFD

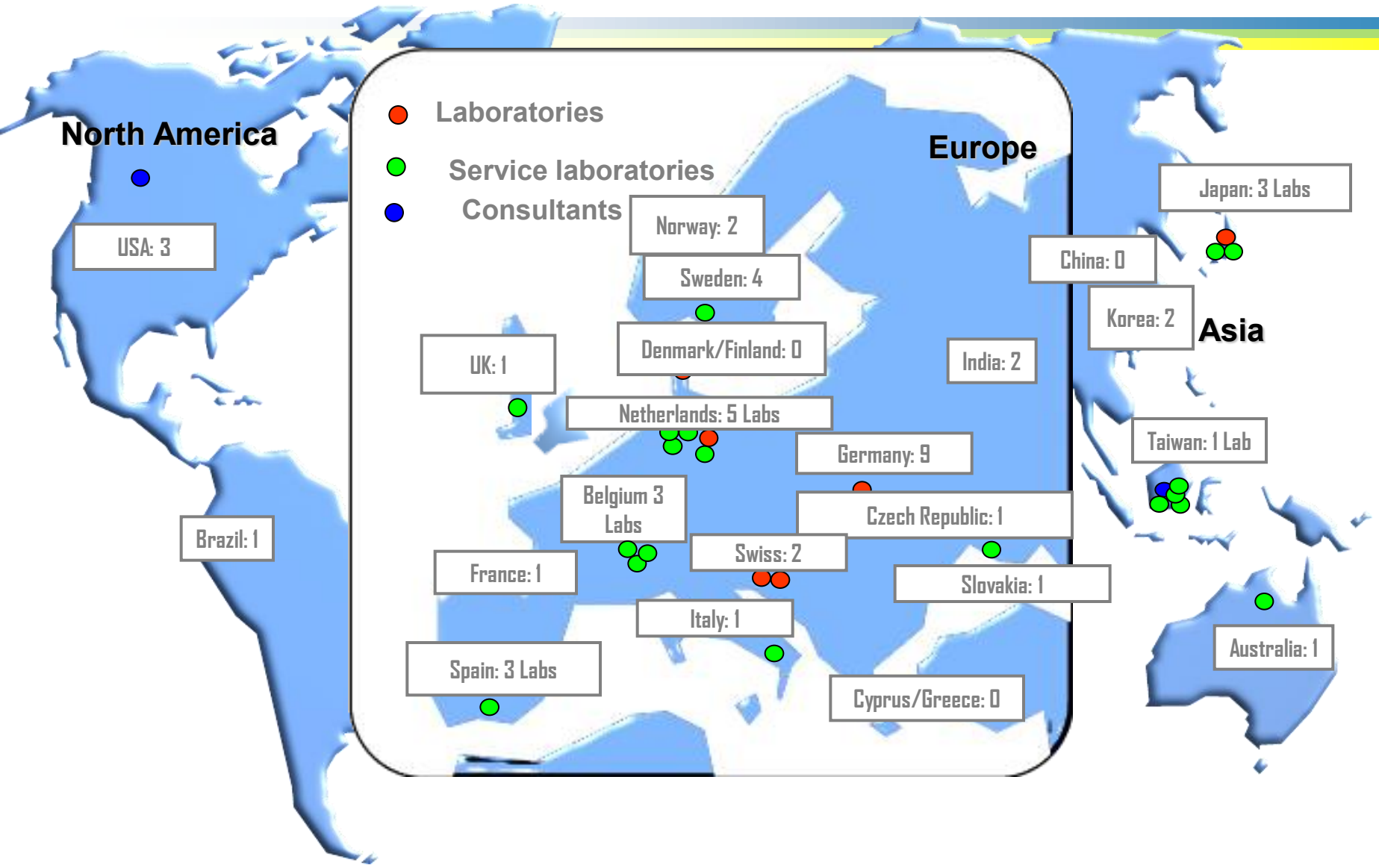
Continuosly New Market products: 1995 Dioxins, 2001 EDCs, 2010 Metabolic disrupters, 2020: Thyroid hormones axis (e.g. PFAS, plastic additives)

Milestones and Timelines

Overview of the Strategy Implementation

Next Steps: Action plan

Global partners to overcome barriers



Main Steps to achieve the Strategy

Main strategic initiatives to reach our goals

Toxic-free water
CALUX panel

- Adopt the EU guidelines for WFD
- Appoint R&D Projects and key-experts in the field
- Contact innovative researcher in the field with EU project proposals

PFAS

- EU projects as demo cases
- Bridge existing chemical analysis (TEQ) with our effect-based solution (BEQ)
- Talks to European Key Experts

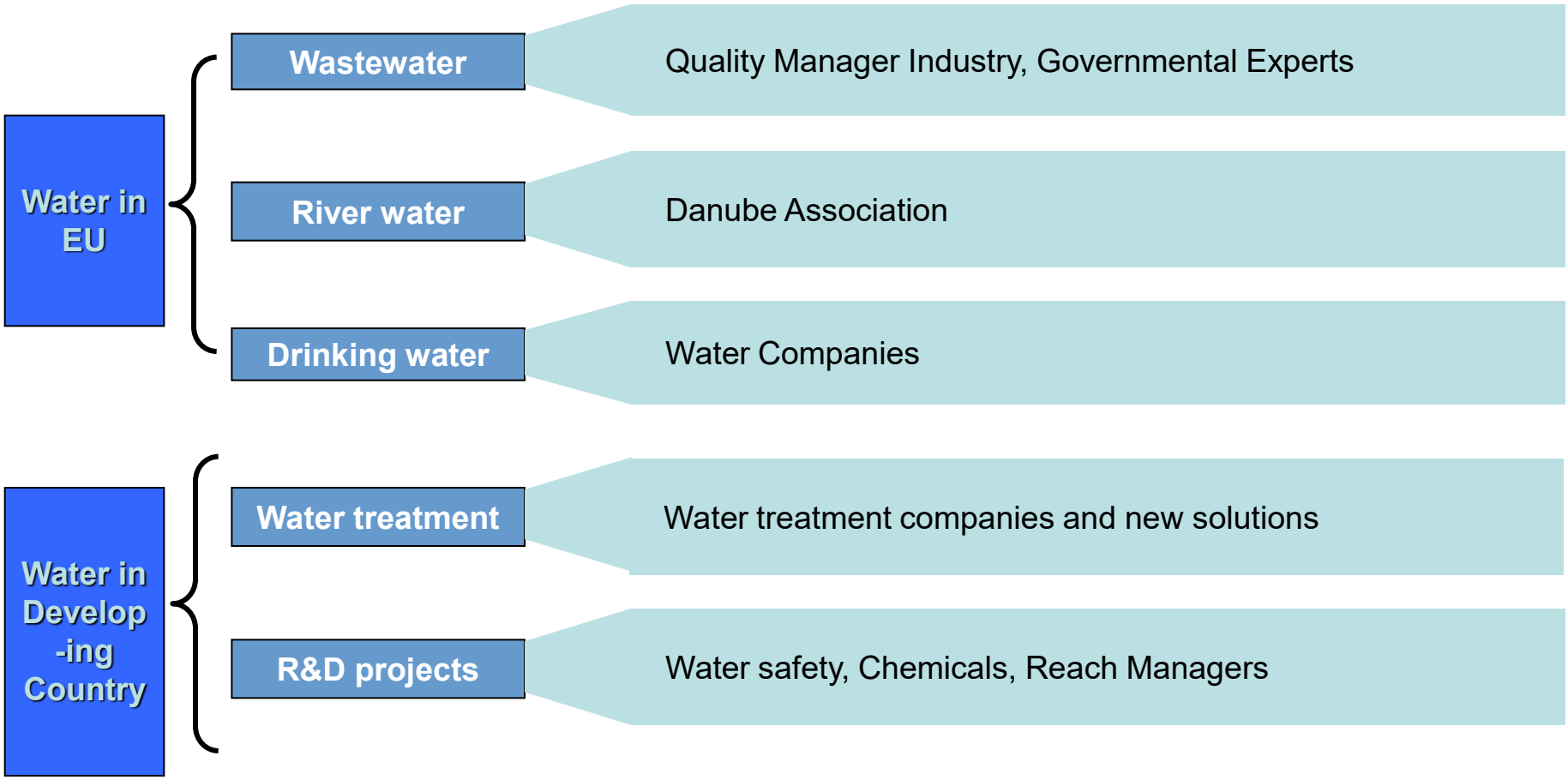
EU projects

- Market research new water guidelines
- Test the market potential with a co-operation partner
- Expand from surface water to waste & ground/drinkwater
- Expand international activities

Others

- Expert start-up bonus
- Start-up projects booth
- Activities for sport doping?
- Yearly BioDetectors Conferences

Description of Market Segments



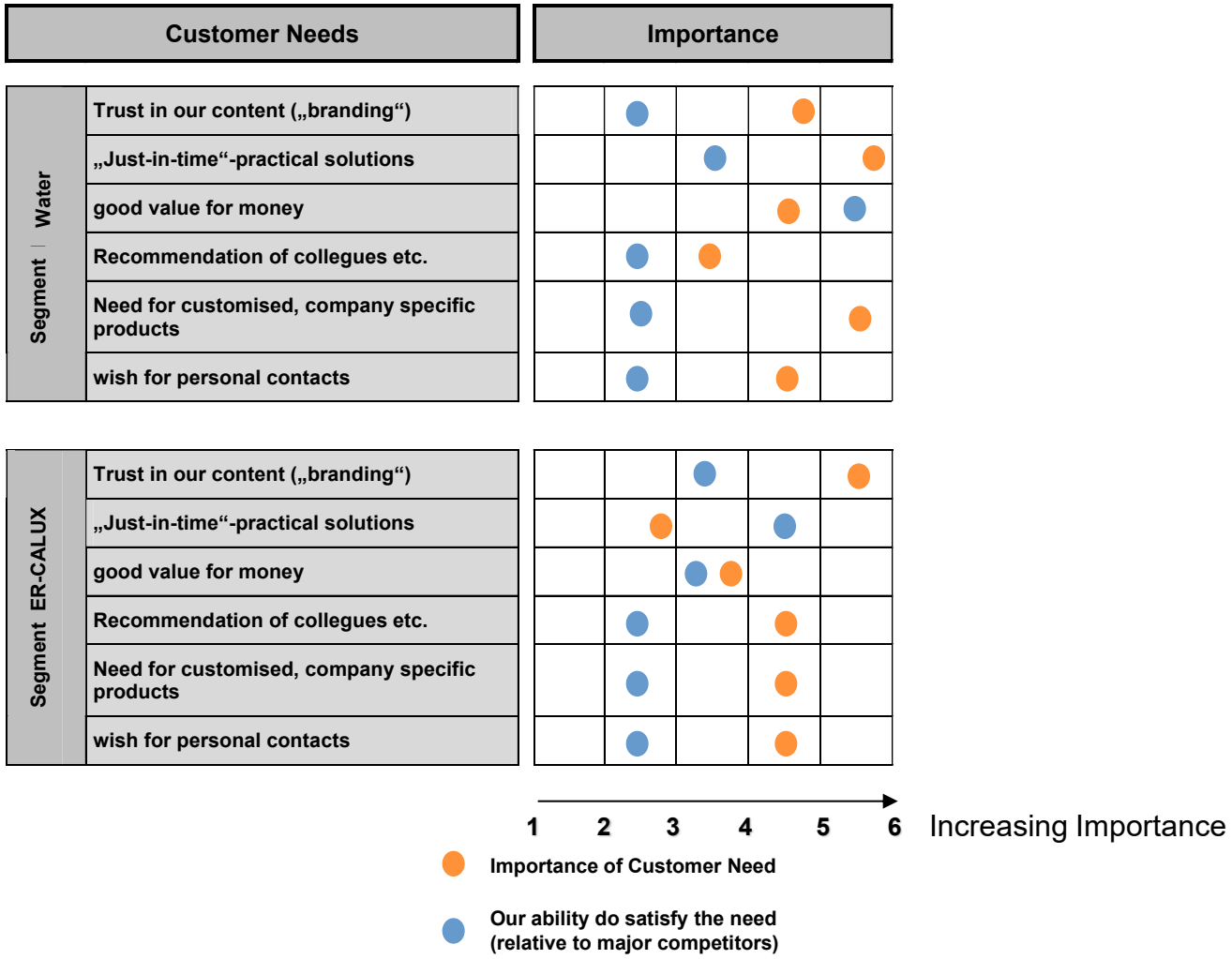
Market Trends

Market trends and the consequences in our relevant markets

Trends	Consequences
One stop shopping effect-based methods (EBM)	**Application of panel of non-animal based testing for cytotoxicity, EDCs, PAHs, PFAS, POPs, oxidative stress, metabolic disruptors & others**
Price dependent on water crisis and client	**Flexible price system + "Push button" Analyses**
Price war on environmental samples	**Developing of "simple" method for shipment of water samples** **Push European guidelines for other environmental matrices (sediment, soil)**

Analysis of Customer Needs

The major customer needs for each market segment were analysed



SWOT Analysis in Segment Water

Strengths

- Faster and cheaper than non-target analysis
- ER and DR CALUX now ISO approved
- BDS much higher market share in EU and globally compared to other competitrs due to increasing marketing promotions
- Intensive global network
- Successful management of several crisis situation
- Strong team of experts for reportergene assays

Weaknesses

- CALUX panel is only a screening method
- Low customer and local government acceptance
- Need of more training for decision makers
- Not enough key speaker/champion potential; not enough „Star- experts“ with a high level of recognition

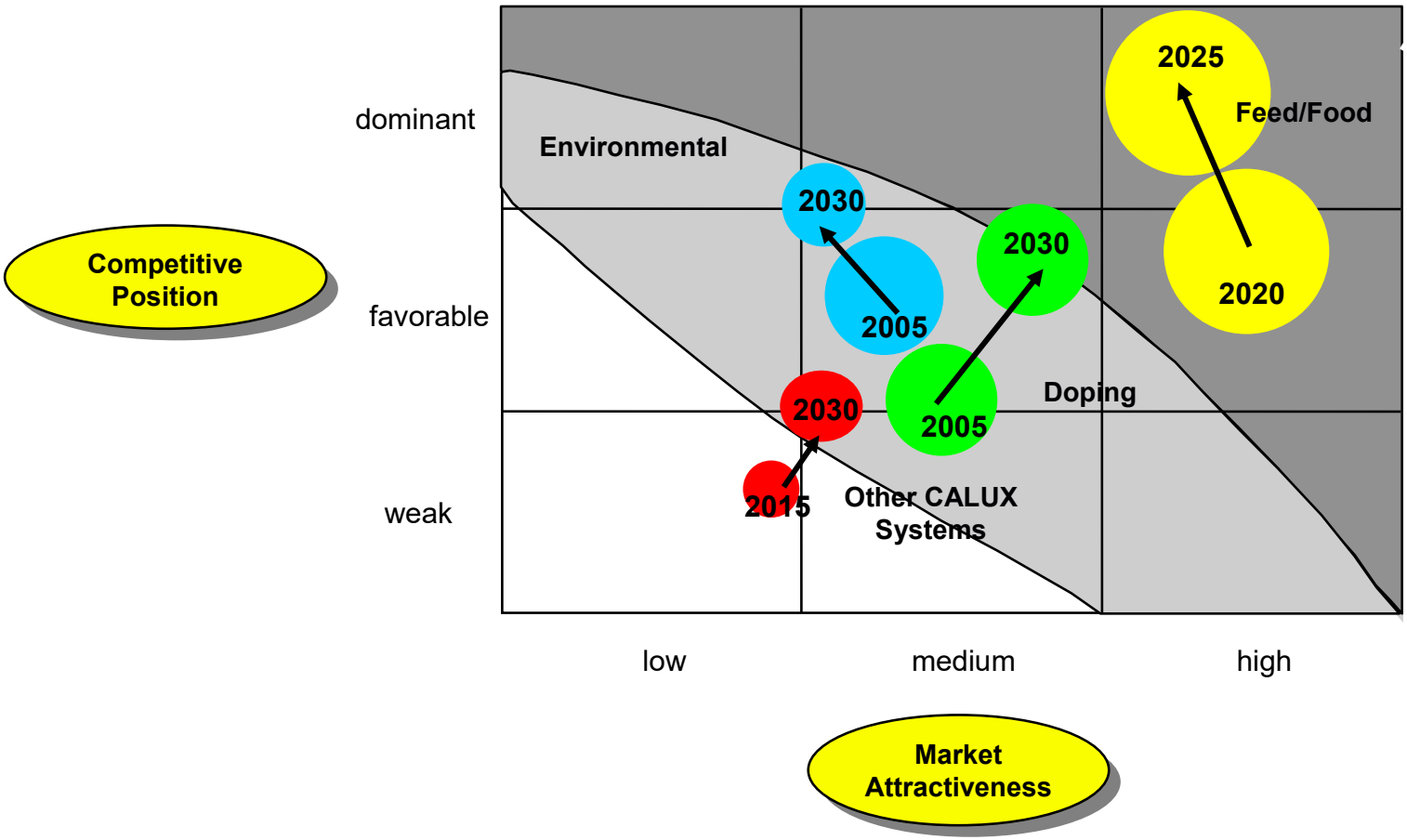
Opportunities

- To expand our contacts to the drinking water industry
- To expand the recognition of local authorities and key speakers
- Adjusting our products on other markets

Threats

- The CALUX competitors will increase their global activities
- Toxic-free National water strategies using our EBM/NAM-based innovations will decrease (e.g. USA reduces environmental protection)

Competitive Positioning for each Water Market Segment

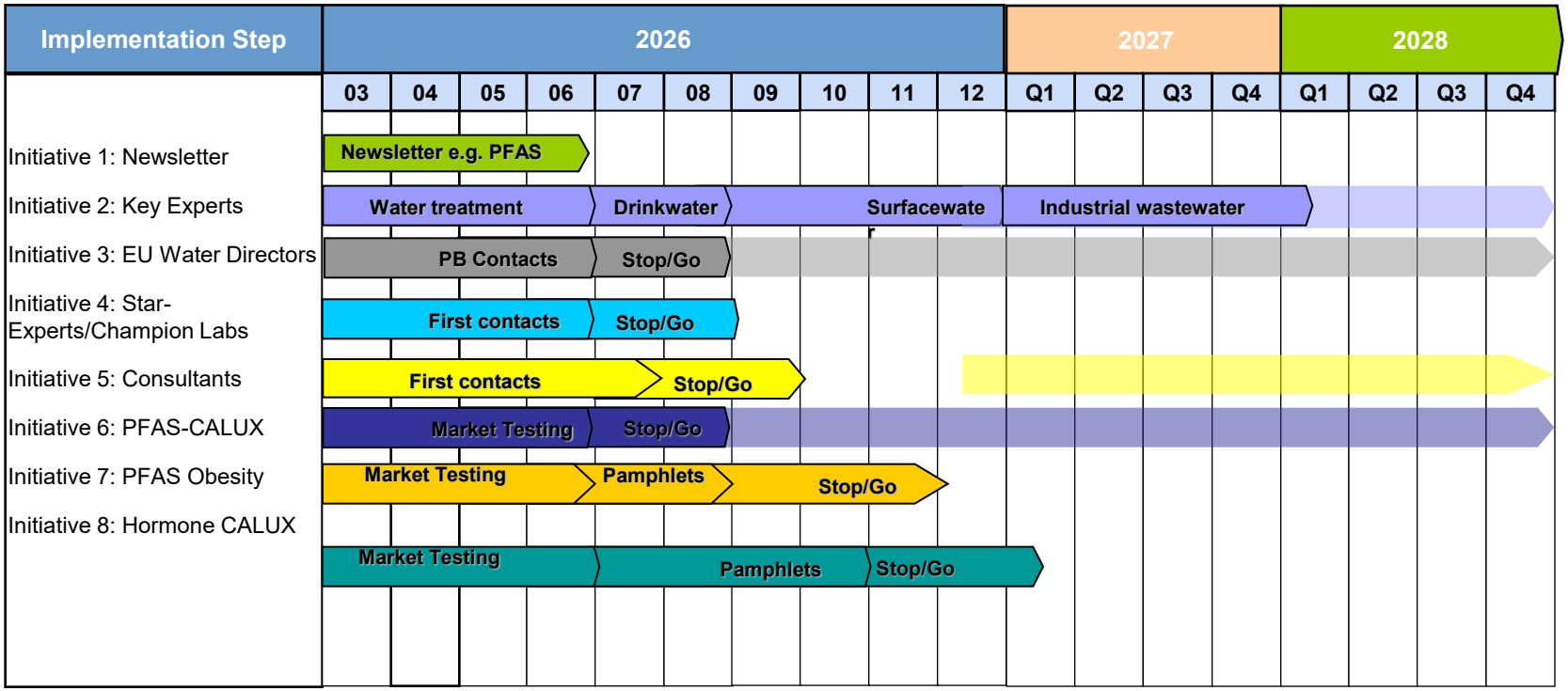


Strategic Initiatives in Detail

Strategic Initiative	CALUX panel for toxic-free water	Nr.	1

Description	Timeline and Resources
Content: Increase global perspectives of CALUX panel **Design/Intonation**: Targeted at Industrial water clients, water Laboratories and increase EU demand for other matrices (sediments) **Objectives**: • Contact all old/new addresses of water clients •Contact all water laboratories/Convince experts •Increase EU guidelines using bioassays **Conclusion:** Ensuring Market Bioassay LEADERSHIP	**Milestones:** • Adopt Business plan (2026) • Adopt expert list (01/2026) •EU New soi/sediment guideline Promotion **Resources, short term:** • Personnel: BDS, international kep experts • Content: Newsletter, new Marketing material, Mini-Pamphlets
Responsibilities	**Requirements/Preconditions**
Responsible: Marketing BDS **Team**: Key experts water such as Water Europe	• Update clients list and decide CRM • Building an European Star team • Marketing pamphlet professionals for newsletter set-up, pamphlet and mini-pamphlets •Decide booth and conference participitation

Overview of the Strategy Implementation

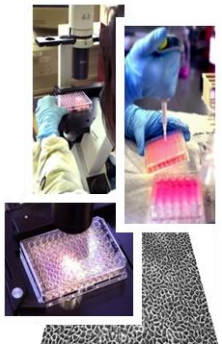


Necessary Water Decision Makers Next Steps

The following decisions need to be taken

Necessary Decision	Recommendation
1 Toxic-free Water Panel Marketing	▪ If we have a clear focus on quality managers in the feed and food segment it is our core business (b to b)
2 Extension of the market leadership by promoting champion labs and star-experts	▪ We will try it harder ...
3 16th BioDetectors Conference in Stockholm June 2026	▪ Introduction new lab, new presentation and contact persons
4 CRM: expand from global leaders in a few countries to their affiliations	▪ Please discuss..
5 Focus Industry, EU Candidate, POP Labs UNEP, Asia	▪ Please discuss..

How to overcome barriers with innovative bioanalytical solutions



- Toxic-free testing by covering complex mixtures of known and yet unknown chemicals
- Detection new compounds & water items = safer umbrella approach
- Develop safe & healthy new alternatives = Green Toxicology
- Apply NAM-based bioassays to non-animal in vitro safety assessment of chemicals in the water chain
- Innovative through wide-range of applications and attractive for international projects

QUESTIONS ?

Learn more about bio-analysis of PFAS……

PROMISCES - Biological PFAS detection systems video

www.youtube.com

Harrie Besselink of BioDetection Systems explains how PFAS can be detected using biological cells in this Voices from Field video of the course on Persistent Micropollutants - PFAS.

The material was developed in a coproduction between the EU project PROMISCES.eu and the Water Management Department of TU Delft.

https://www.youtube.com/watch?v=mNmtfraqtNE

The PROMISCES project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 101036449

Welcome at 16th BioDetectors Conference in Stockholm in June 2026

You are warmly invited to join the 16th BioDetectors Conference 2026.

With this Conference we want to gather industry experts, regulatory authorities and scientists to explore the latest advances in chemical management and environmental risk assessment, with a special focus on effect- and non-animal based methods (EBM/NAM) for chemicals (e.g. PFAS)/pharmaceuticals/plastics (e.g. EDCs)/cosmetics/others in the environment, industry and public health

25–26 June 2026

Stockholm, Sweden
AFRY, Frösundaleden 2°, 169 75 Solna

How to get there:
Stockholm Arlanda Airport (ARN). Taxi, train or bus from the Airport

Commuter train, metro or Taxi from Stockholm Central station.

Registration:
info@bds.nl or
Behnisch@bds.nl or
pia.engwall@ambiotox.com

Registration fee (excl. VAT):
€375 professionals
€225 students and post-docs
(includes 2 days conference and social event)

Organizing committee:
Pia Engwall, Magnus Engwall, Peter Behnisch