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Environmental monitoring of endocrine disrupting chemicals through the integration of bioassays and chemical analyses

Laetitia Six, OVAM, Policy Coordinator

belgium24.eu

Environmental monitoring of endocrine disrupting chemicals (EDC) through the integration of bioassays and chemical analyses

Partnership for the Assessment of the Risks from Chemicals

Laetitia Six (OVAM), on behalf of EDC PilotSurvey Project Team

Brussels, ENSOr workshop, 15 March 2024

PARC

SAMEN MAKEN WE MORGEN MOOIER
OVAM

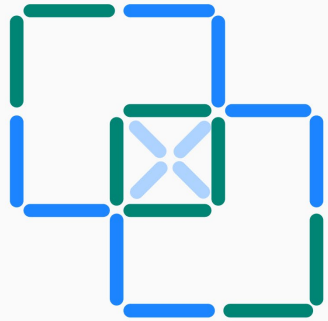
Outline of the presentation

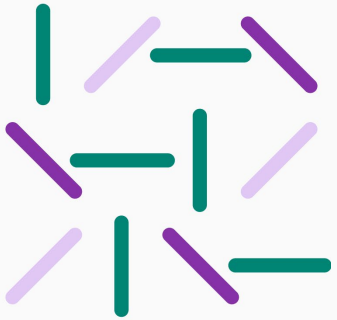
PARC in a nutshell

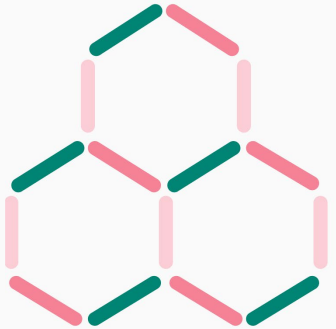
Why endocrine disrupting chemicals?

Monitoring strategy

Conclusions & take home messages







PARC in a Nutshell

PARC = Partnership for the Assessment of Risks from Chemicals

- A public-public **partnership** under Horizon Europe
- Co-fund budget
- Started 1st May 2022 ➔ duration of 7 years
- ≈ 200 partners from 29 countries
- Includes 3 European Agencies:

European Environment Agency, efsa, ECHA EUROPEAN CHEMICALS AGENCY

- 13 Belgian partners:

WE MAKE TOMORROW BEAUTIFUL OVAM, DEPARTMENT OF ENVIRONMENT & SPATIAL DEVELOPMENT, ISSeP Institut scientifique de service public, federal public service HEALTH, FOOD CHAIN SAFETY AND ENVIRONMENT, UHASSELT KNOWLEDGE IN ACTION

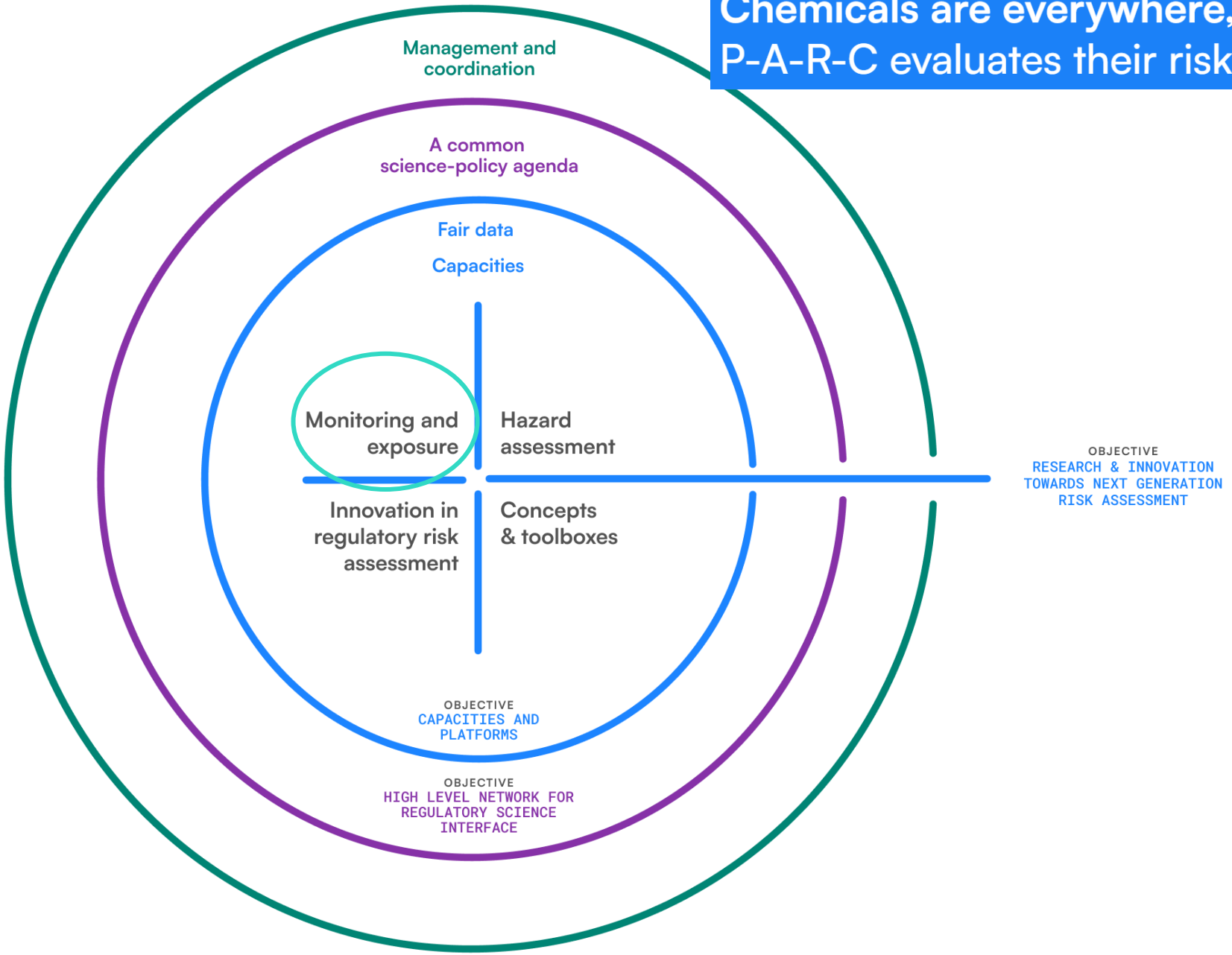
sciensano, VUB VRIJE UNIVERSITEIT BRUSSEL, KU LEUVEN, ILVO Flanders Research Institute for Agriculture, Fisheries and Food, GHENT UNIVERSITY, Provincie Antwerpen, University of Antwerp, vito

PARC Structure

Global Objective

Consolidate and **strengthen** the EU's **R&I** capacity for **chemical risk assessment** to protect **human health** and the **environment** and contribute to a non-toxic environment and a circular economy.

Chemicals are everywhere, P-A-R-C evaluates their risks



WP4: Monitoring and Exposure

4.1 Human Biomonitoring

Consolidate and further develop the **human biomonitoring platform**, generating and analysis of HBM data, and develop the network of qualified laboratories for biomarkers analysis

4.2 Environmental & Multisource Monitoring

Understand the **presence of chemicals in the environment**, their exposure to humans, considering multiple sources (e.g. air, water food, consumer products)

4.3 Innovative tools and methods

Develop **innovative tools and methods** to improve human, food and environmental monitoring schemes, contribute to an early warning detection of chemicals of emerging concern.

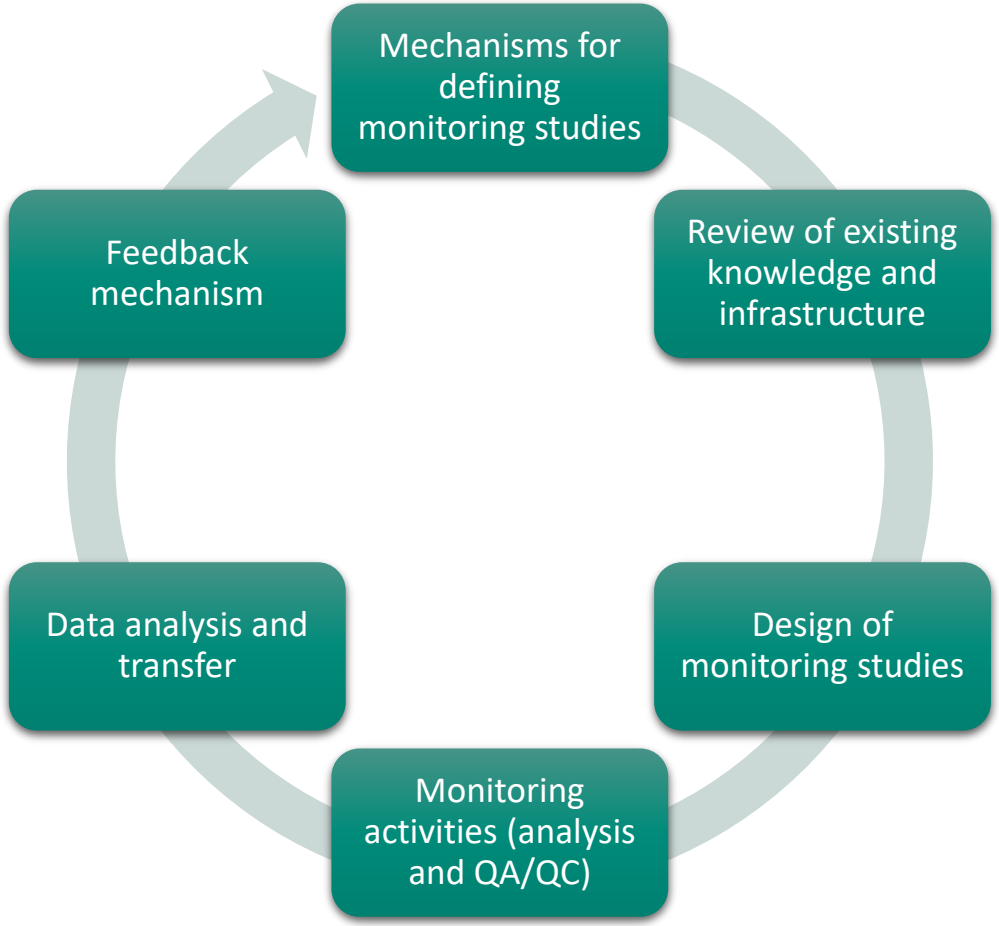
Task 4.2 Environmental & Multisource Monitoring

Requirements:

- Respond to regulatory needs
- Build on existing information and infrastructure

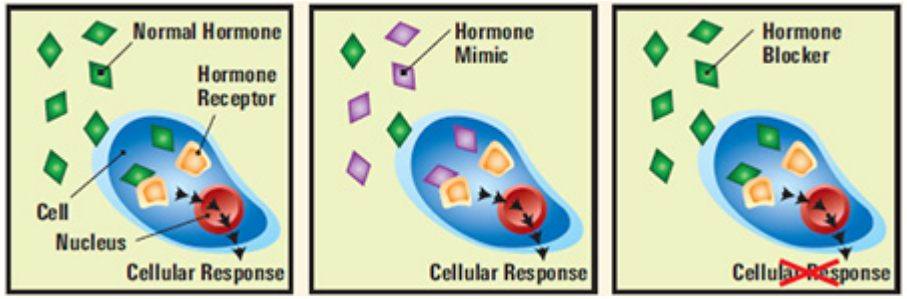
First cycle of projects (2022 – 2025):

Pilot study on **PFAS** and **endocrine disrupting chemicals (EDCs)** to establish the overall process of **environmental monitoring**

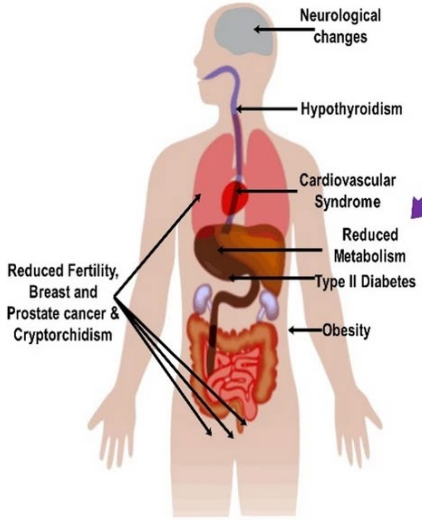


What are EDCs?

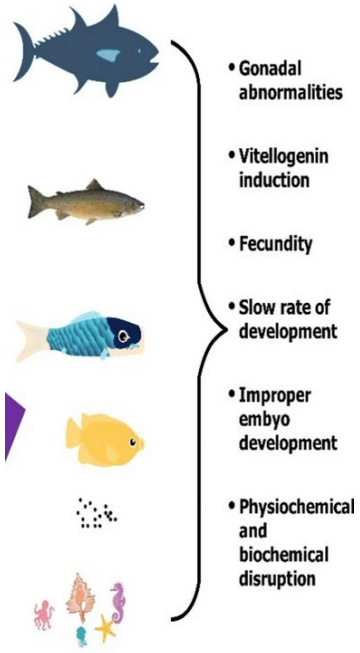
= natural or human-made chemicals that may mimic, block, or interfere with the body's hormones, which are part of the endocrine system.



https://www.niehs.nih.gov/health/topics/agents/endocrine



Adverse effect on Human



Adverse effect on Aquatic system

Puri et al.,Int. J. Environ. Sci. Technol. 20, 12875–12902 (2023)

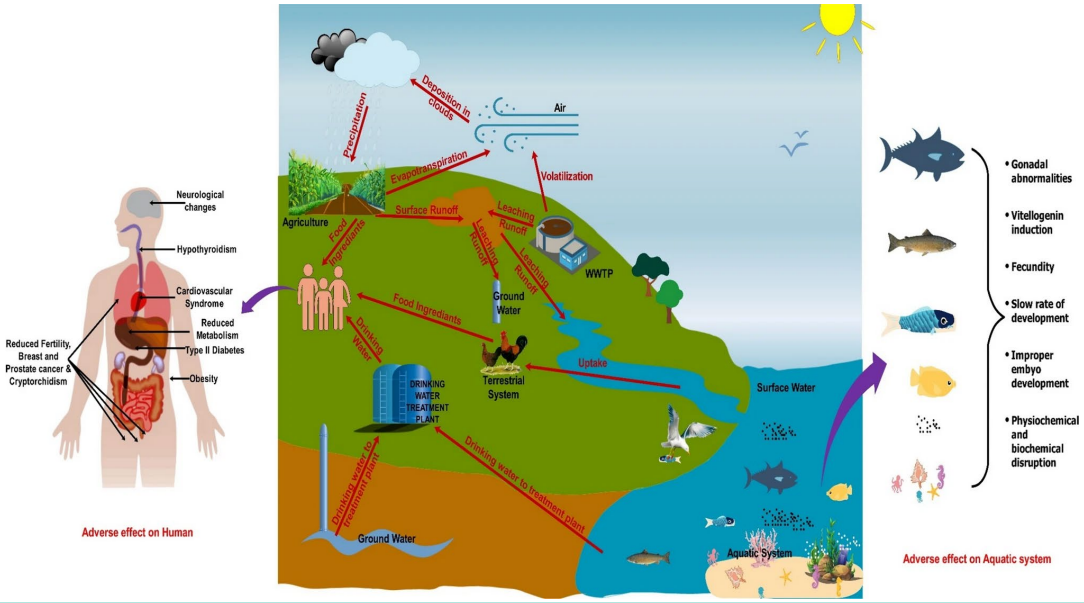
Why EDCs as a first case study in PARC?

- EDCs = a big versatile group of chemical classes, such as bisphenol A, dioxines, PFAS, phthalates, …
- They are a group due to their toxic endpoint rather than their chemical similarity (<> PFAS)
- Expected that there are still many unknown and less studied compounds

https://www.niehs.nih.gov/health/topics/agents/endocrine

Environmental fate?

- Diffuse pollution due to large number of sources and uses
- There is a need for knowledge on the sources, pathways and occurrence levels in Europe



Puri et al.,Int. J. Environ. Sci. Technol. 20, 12875–12902 (2023)

Longlist of EDCs as starting point

Candidate EDCs	No substances
Hormones	20
EDList1	107
EDList2	73
EDList3	10
PPP EFSA	9
ANSES/Deduct	906
ECHA-Bisphenols	158
Norman S20 (BP)	52
Norman S67 (BP)	77
Norman S87(BP)	92
VEGA predictions	6079
Total number of substances	**7327**

Regulated as EDCs (1034 substances)

- *Already identified (126 substances)*

  ED List I: Substances identified as endocrine disruptors at EU level

  ED List III: Substances considered, by the evaluating National Authority, to have endocrine disrupting properties

  PPP EFSA

- *Non regulated but under evaluation (908 substances)*

  ED List II + ANSES/Deduct: Substances under evaluation for endocrine disruption under an EU legislation

Hormones (20 substances)

Suspects from model predictions + other lists (6273 substances)

➔ Large group of EDCs potentially not on the radar yet!

Objectives of the EDC monitoring strategy

- **Determine a baseline** of **environmental contamination** caused by several decades of production and uses of EDCs
- Characterise relevant **exposure routes** from **diffuse** and **point sources**
- Facilitate the assessment of the **effectiveness of EDC management actions**

Methodology

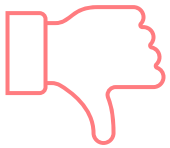
- Conducting an **effect-based assessment** with broad spectrum of species-specific endocrine activities, representative of different modes of action

in parallel

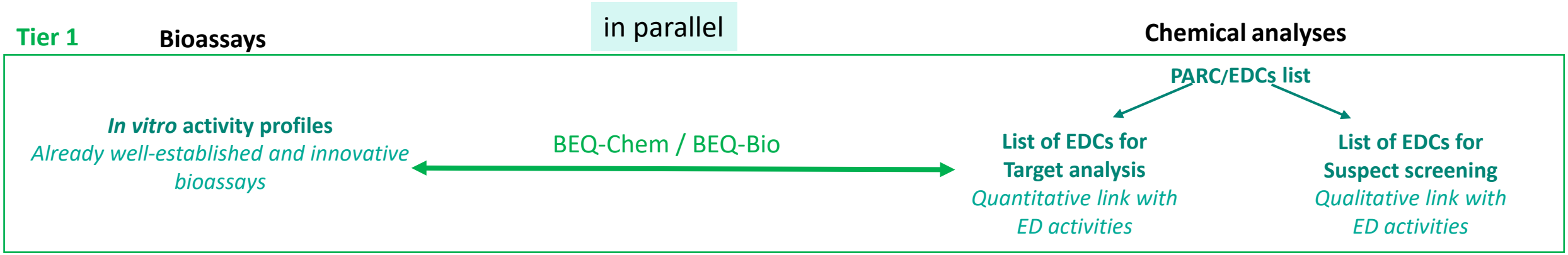
- Conducting **chemical analysis**:
  - target analysis for selected compound groups
  - suspect screening of all identified candidate EDCs

Integration of effect-based assessment and chemical analysis

Effects of chemicals depend on the concentration, toxicity, solubility, bioavailability, duration of exposure and the sensitivity of the exposed organisms.

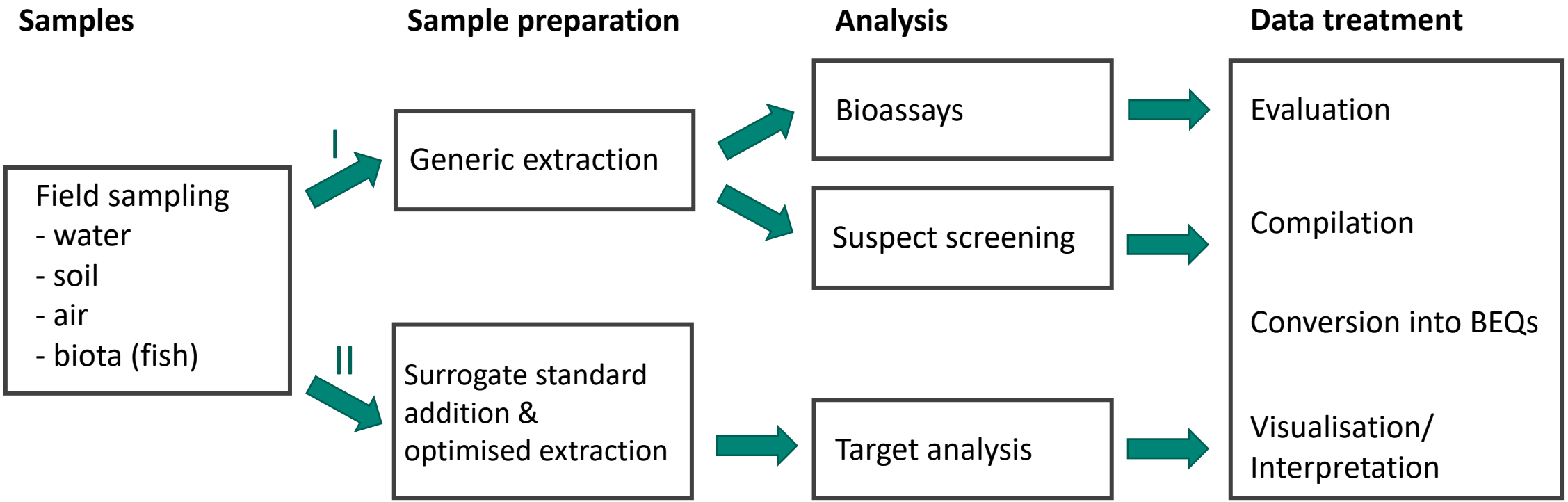
Target analysis of EDCs	Suspect screening of EDCs	*In vitro* bioassays for EDC properties
Concentration x toxicity ↓	↓	↓
Predicted biological effect	Measured presence of EDCs	Measured biological effect
 • Not accounting for mixture toxicity • Not accounting for less studied substances	• Qualitative determination: presence/absence	• No identification of the cause, i.e. ED substances causing the effect
 • Monitoring easy to implement for known substances • Quantitative	• View on less monitored (potential) EDCs	• Integration of mixture effect for specific endpoints

Integration of effect-based assessment and chemical analysis



BEQ = bioanalytical equivalents
BEQ-Bio = activity measured in bioassays
BEQ-Chem = measured bioactive chemicals' concentration

Monitoring workflow from sample collection to data treatment

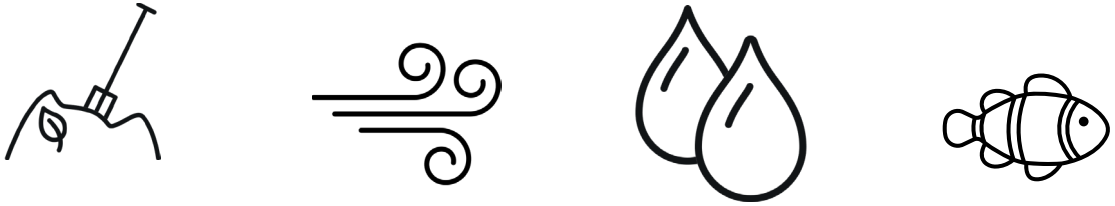


Matrix	Chemical Analyses	Bioassays
Water	✓	✓
Soil	✓	✓
Air	✓	✓
Biota (Fish)	✓	X

Samples

>200 samples throughout Europe, covering all regions

4 matrices:



Different pressures: industry, agriculture, urban, WWTP, ...

Sampling: Until end of April 2024

Lesson learned

- For alignment of sampling with (national) monitoring programmes:
  need notice long time in advance (1 year ideally)

Sample matrices & geographical coverage – example Soil

Region	Country	Organisation	urban	Industrial	Agricultural (cropland)	grass land	Reference	Total per country	Total per region
E	Czech Republic	RECETOX (MU)	2	2	2	1	0	7	13
E	Poland	UG-PL	1	1	1	2	1	6	
N	Latvia	UL	1	1	1	1	0	4	10
N	Norway	NILU	1					1	
N	Sweden	IVL	1	1	1	1	1	5	
S	Greece	NKUA	1	1	1	1	0	4	10
S	Spain	IDAEA-CSIC	1	1	2	1	1	6	
W	France	INRAE	1	0	2	2	1	6	10
W	Belgium	OVAM	0	0	1	1	0	2	
W	Belgium	ISSeP	1	1	0	0	0	2	
Per region intended			2 to 3	2 to 3	2 to 3	2 to 3	1		10 to 11
Total intended			9	9	10	10	4		42
Total provided			10	8	11	10	4		43

Other matrices:
- fish: # 21
- water: # 117
- air: # 29

Sampling campaign = logistically challenging!

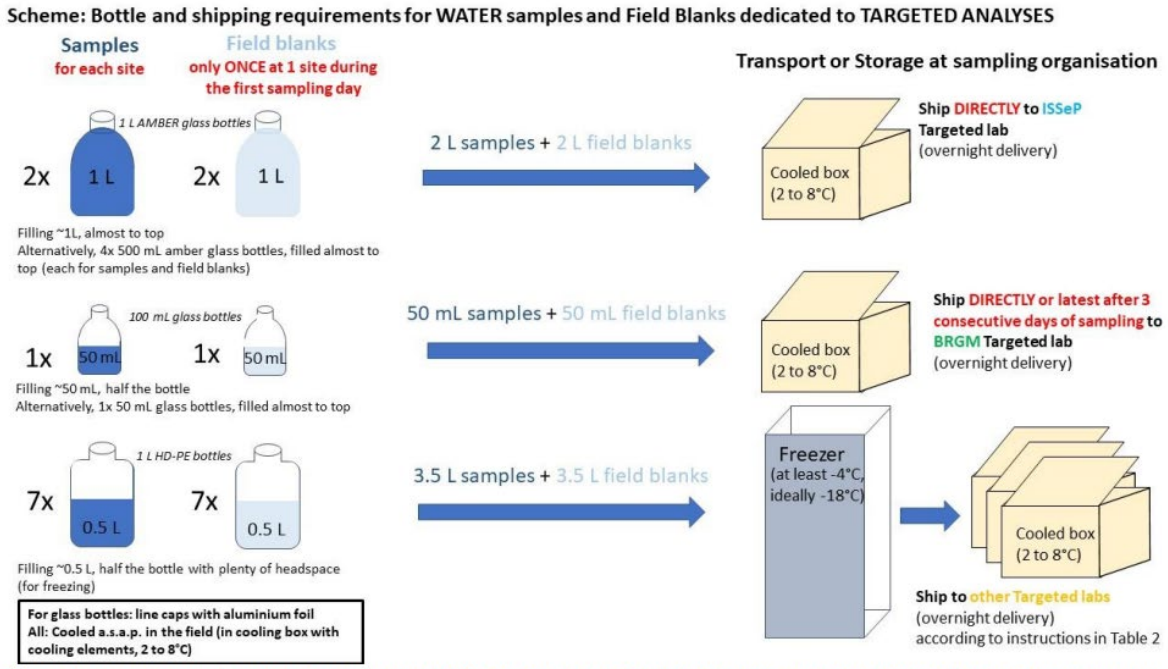
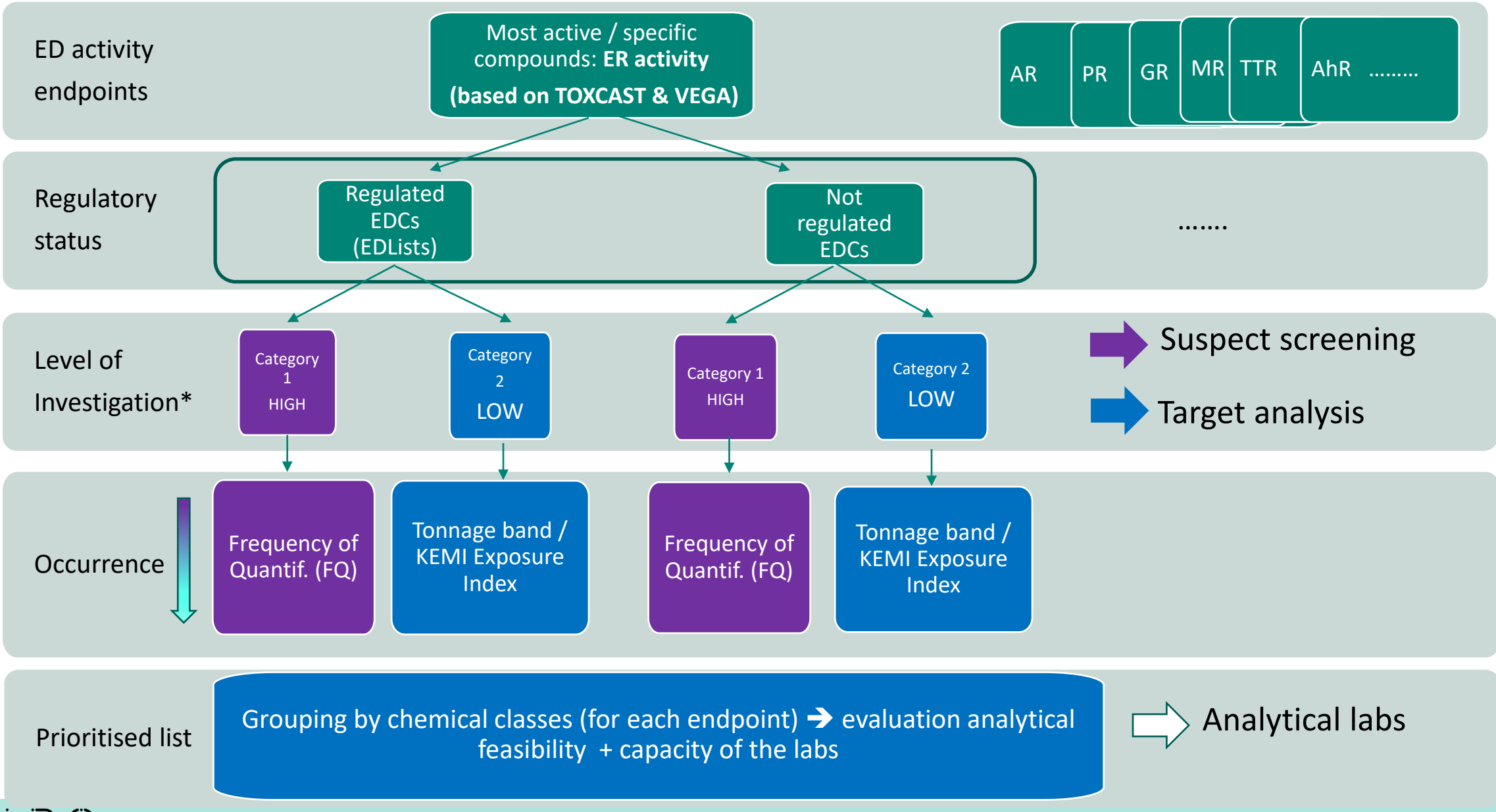


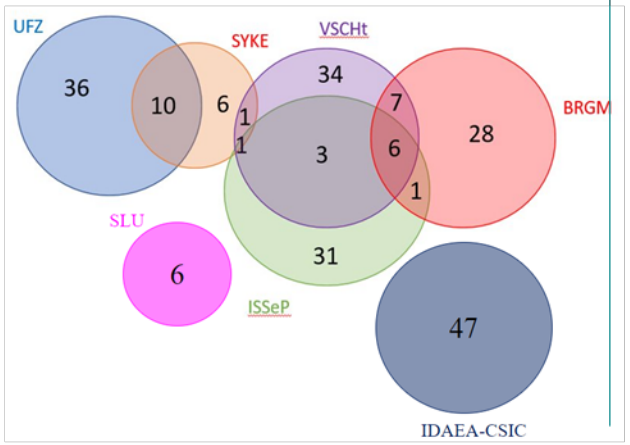
*Figure 1: Water sampling and field blank scheme within the PARC T4.2 EDC campaign for targeted analyses, volumes and number of bottles given per sampling site for the water samples and for the first sampling day for the field blanks. Note: Glass bottles should have **pyrolyzed/rinsed aluminium foil lining** of the screw caps.*

Lab	Required bottle type	Number of bottles and sample volumes	Condition to be achieved as soon as arriving with cooled samples at sampling organisation	Max. days of storage before shipping	Shipping plan
Coordinating Lab	HDPE	6 x 1 L HDPE filled with 1 L filled to the top	Cooled	0-1	Ship immediately after sampling, **latest** the day after, i.e. on Tuesday, send cooled
ISSeP	Amber glass	2 x 1 L amber glass bottles (OR 4x 500 mL amber glass bottles) filled to the top	**Cooled SHIPPED directly**	**0**	Ship **directly** after sampling (same day), ideally early in the week, send cooled
BRGM	Glass	1 x 100 mL OR 1x 50 mL glass bottle filled with 50 mL	Cooled	0-3	Ship immediately after sampling, **latest** after 3 consecutive sampling days, i.e. on Wednesday, send cooled
SYKE	HDPE	2 x 1 L HDPE filled with 0.5 L water	Frozen	7	Keep frozen and collect samples for max. a week and send samples lates after 7 days, i.e. on the Monday after, send frozen
UFZ	HDPE	1 x 1 L HDPE filled with 0.5 L water	Frozen	7	Keep frozen and collect samples for max. a week and send samples latest after 7 days, i.e. on the Monday after, send frozen
IDAEA-CSIC	HDPE	1 x 1 L HDPE filled with 0.5 L water	Frozen	7	Keep frozen and collect samples for max. a week and send samples latest after 7 days, i.e. on the Monday after, send frozen
VSCHT	HDPE (stored in dark)	1 x 1 L HDPE filled with 0.5 L water (stored dark)	Frozen (protected from light)	14-21	Keep frozen and collect samples for max. 2-3 weeks, send frozen, ideally on a Monday
SLU	HDPE	2 x 1 L HDPE filled with 0.5 L water	Frozen	21-28	Keep frozen and collect samples during up to 3-4 weeks, send frozen, ideally on a Monday

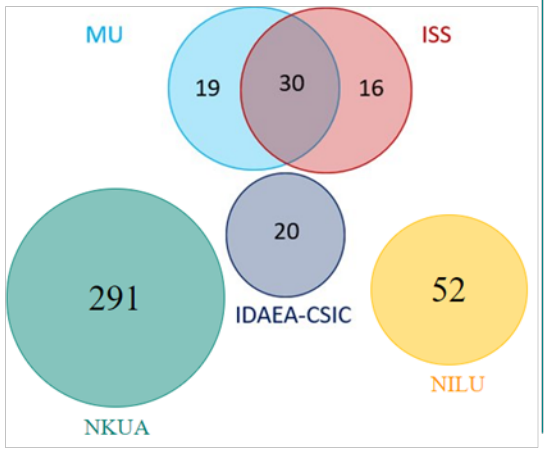
Prioritisation of compounds for target vs suspect screening



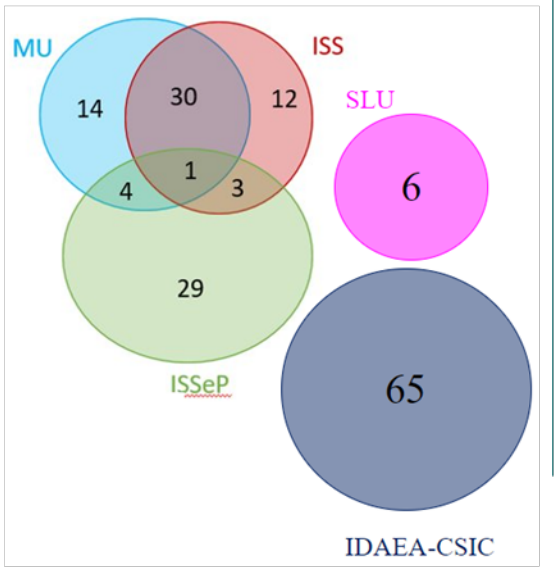
Substances selected for target analysis



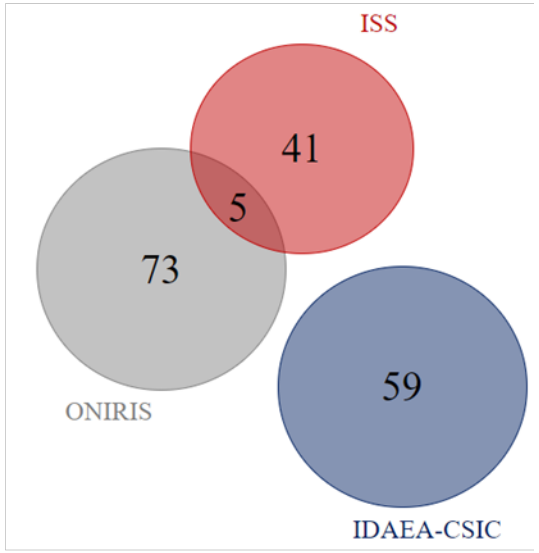
~260 compounds



~350 compounds

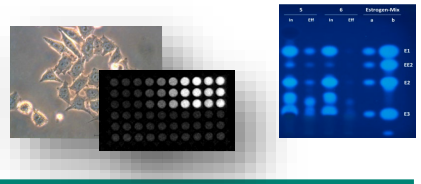


~200 compounds



~200 compounds

Selected *in vitro* bioassays



Endocrine pathway/function	Endpoints	Bioassays / Human receptors	Bioassays / Fish receptors*
Steroid hormones	ER	MELN pYES	-
	AR	UALH-hAR MDA-kb2 pYAS	-
	GR	HMLN-hGR	-
	MR	UG5LN-hMR	UG5LN-zfMR
	PR	HELN-PRB	UEL-zfPR
Thyroid	*TTR*	*TTR-FITC-T4***	-
Retinoid	RAR/RXR	HRLN	-
Xenobiotic and endogenous metabolism	AhR	PAH-CALUX EROD/HepG2	- EROD/PLHC1
	PXR	HG5LN-hPXR	HG5LN-zfPXR
	PPARg	HG5LN-hPPARg PPARg-GenBLAzer	HG5LN-zfPPARg -

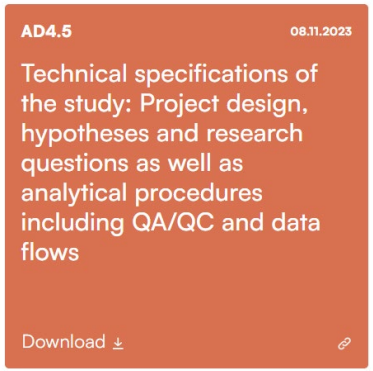
- Well-established bioassays : ER, AR, AhR
- Innovative bioassays : PXR, PR, GR, MR, PR, RAR, RXR, PPARg, TTR

ER: estrogen receptor
GR: glucocorticoid receptor
MR: mineralocorticoid receptor
PR: progesterone receptor
TTR: thyroid receptor
RAR: retinoic acid receptor
RXR: retinoid X receptor
AhR: aryl hydrocarbon receptor
PXR: pregnane X receptor
PPARg: peroxisome proliferator-activated receptor

** To be applied on water samples only ; ** knowledge transfer*

Conclusions and take home messages

A detailed technical specifications document is available online:
www.eu-parc.eu/deliverables



Practical – large scale monitoring

- Good organisation required – centralised management
- Alignment with other monitoring campaigns hard (to impossible) due to difference in timing, protocols,...
- Sample acquisition from environmental specimen banks is hard as often small amounts available only

Expected outcomes

- Overlapping patterns among countries, i.e. sources/pressures connected to certain EDCs;
- Identifying EDCs that both overlap among and are specific for certain matrices;
- Close the gap between BioTEQs and ChemTEQs

Collaboration is key

T4.2 Task leaders

Valeria Dulio (INERIS), Katrin Vorkamp (Aarhus University)

EDC Project leaders

Valeria Dulio, Elise Chatillon, Abd-El-Rahman El-Mais, Nina Huynh and Azziz Assoumani (INERIS), Gunnar Thorsén and Kerstin Pütz (IVL)

PARC T4.2 EDC group

UBA, AU, SYKE, INRAE, UFZ, IDAEA-CSIC, ANSES, BfG, BPI, BRGM, CNRS, CSTB, EAWAG, EFET, Fraunhofer-IME, FISABIO, INERIS, ISS, ISSeP, IVL, JSI, LNS, MU, NILU, NKUA, NMBU, ONIRIS, OVAM, SCIENSANO, UAntwerpen, UL, VSCHT, VU-E&H, VITO

Co-funded by the European Union

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