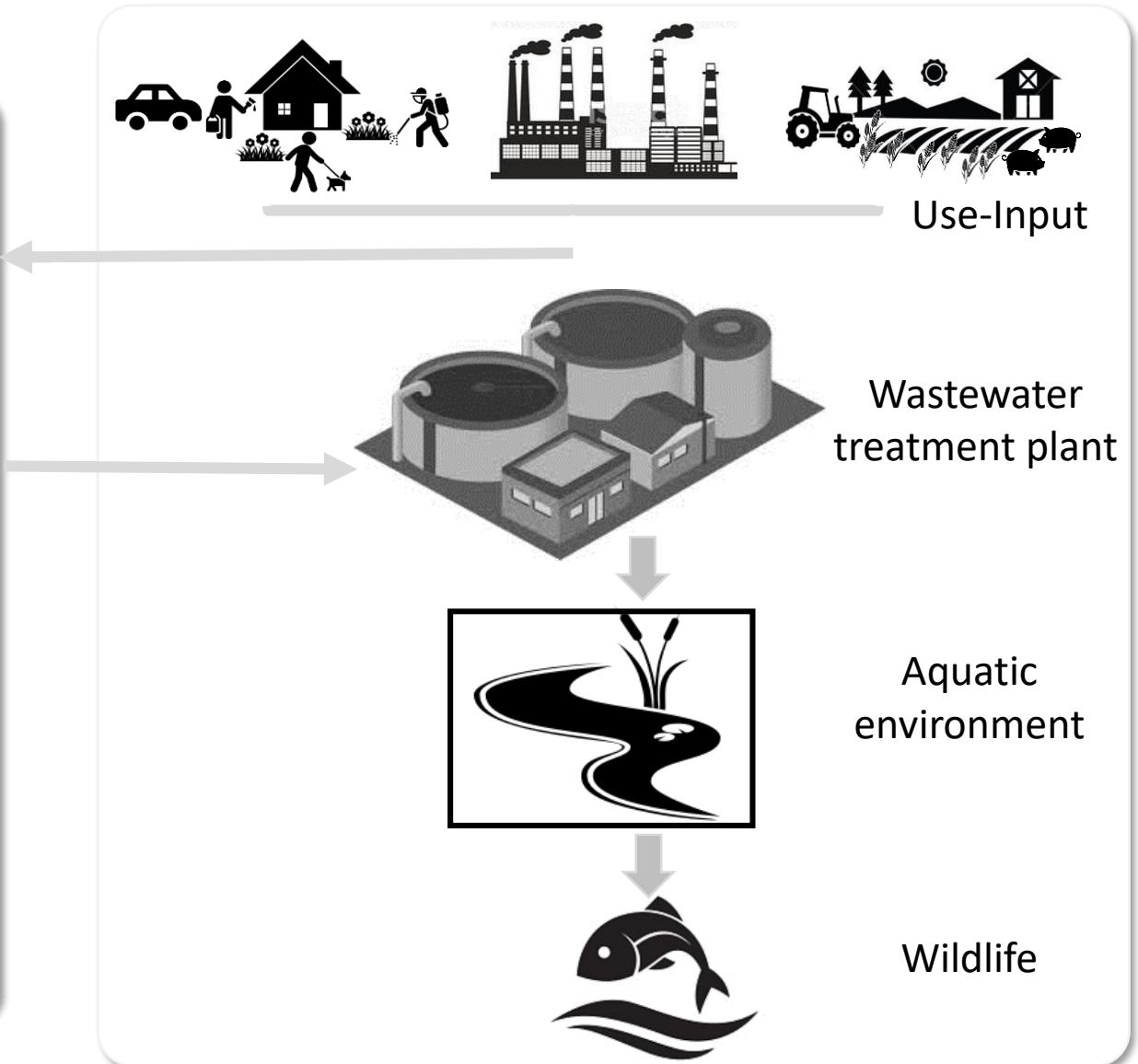
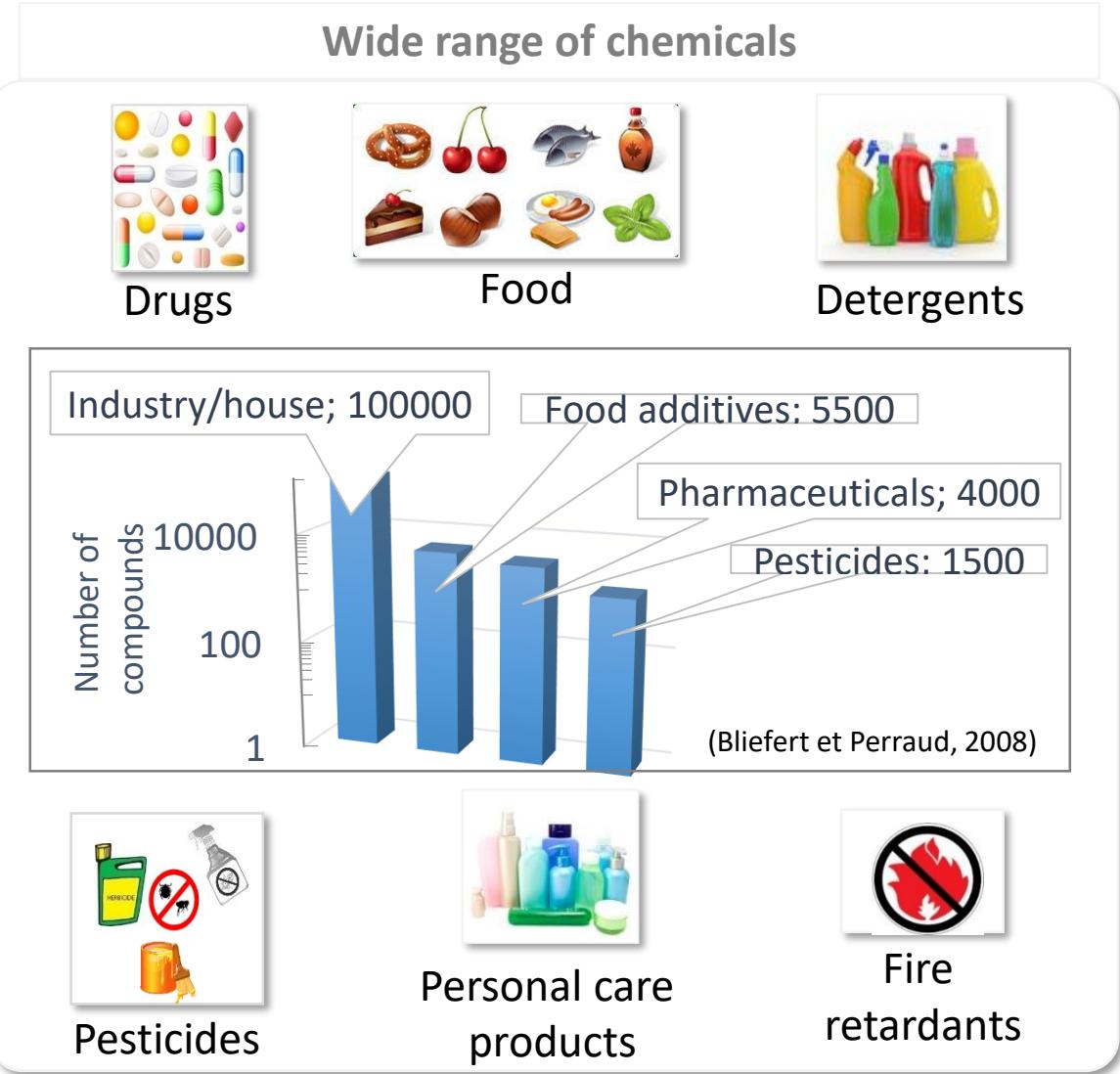


CECs and their TPs in urban wastewaters: results from REGARD project in France”

Caroline Gardia-Parège¹, Marie-Hélène Dévier¹, Emmanuel Geneste¹, Selim Aït-Aïssa²,
Hélène Budzinski^{1,3}

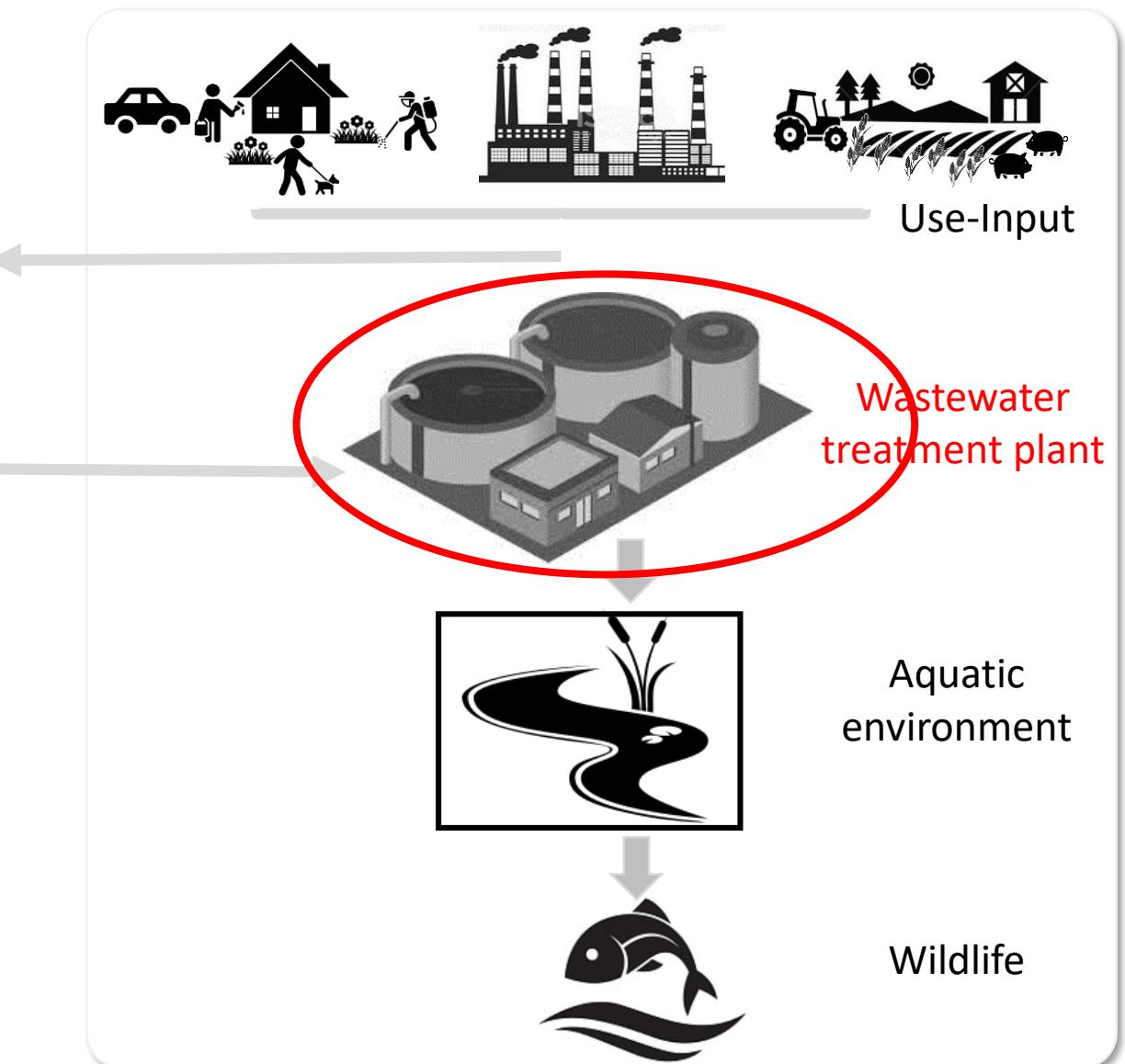
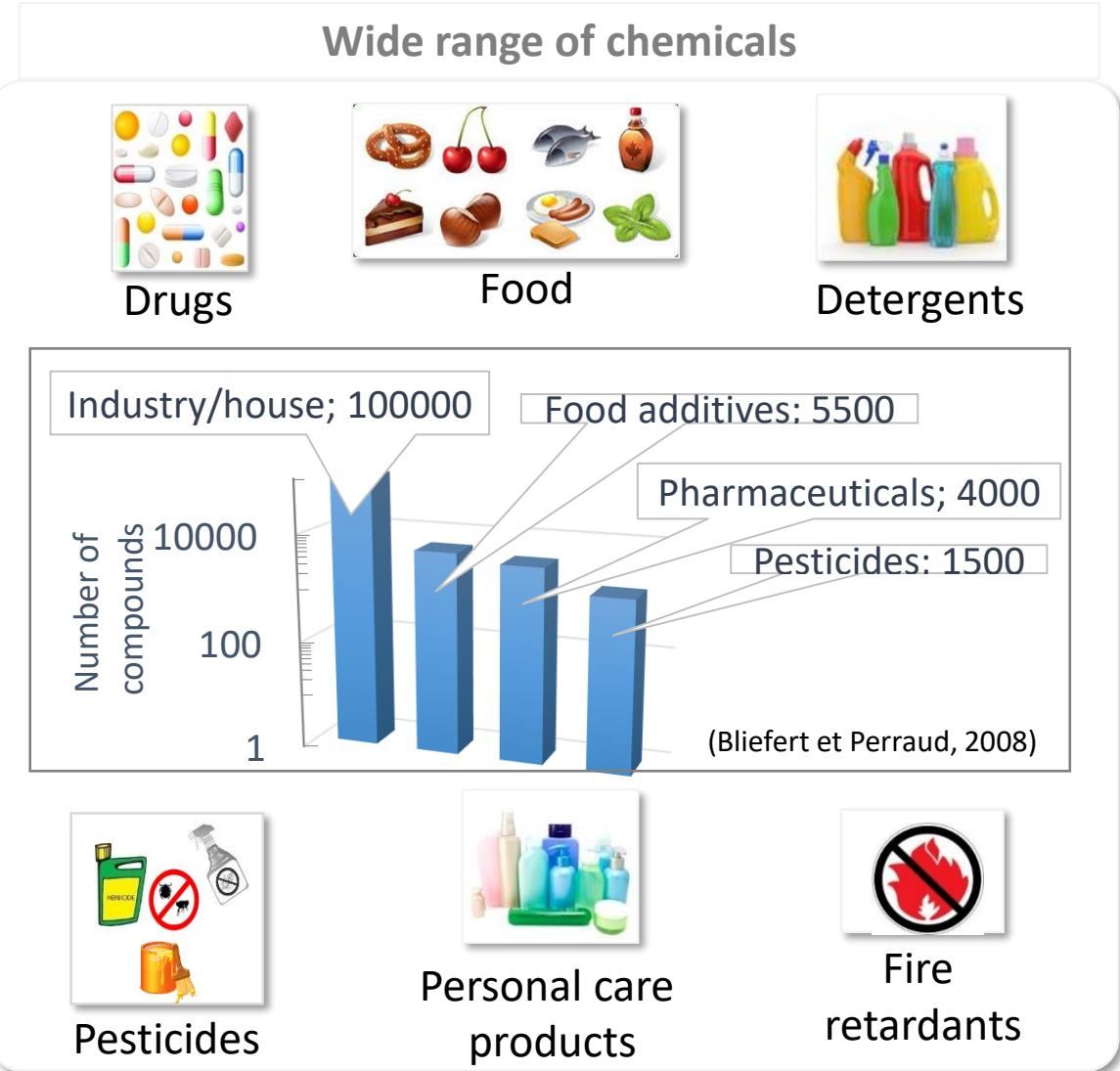


Context



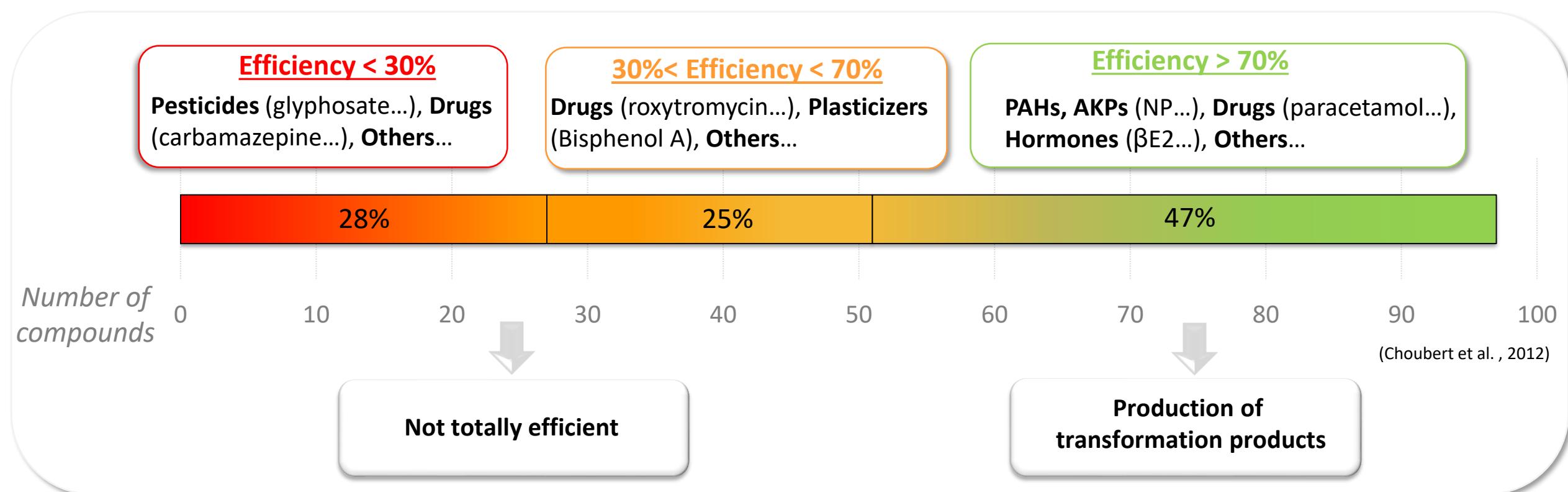
- Environmental contamination is induced by multiple pollutants coming from various sources and multiple uses

Context



- Environmental contamination is induced by multiple pollutants coming from various sources and multiple uses

Indeed...



- Half of compounds are not or partially removed
- Half of compounds are treated but they could produce transformation products

Indeed....

Efficiency < 30%

Pesticides (glyphosate...), Drugs

30% < Efficiency < 70%

Drugs (roxytromycin...), Plasticizers

Efficiency > 70%

PAHs, AKPs (NP...), Drugs (paracetamol...),

The study aims to use :

- **Non Target Screening**, to assess the overall contamination of wastewater treatment plant
- Alternative approach, **Effect Directed Analysis**, to identify unknown biological active compounds that could be released in aquatic environment

- Half of compounds are not or partially removed
- Half of compounds are treated but they could produce transformation products

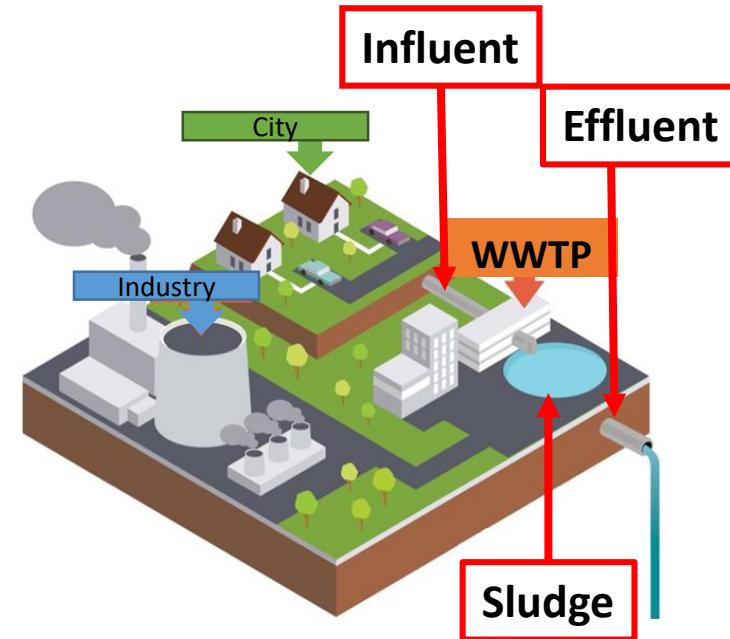
Study site

Urban wastewater treatment plants :

- Influent and effluent :
 - 85 000 population equivalent
 - Secondary treatment : Biofilters
- Sludges :
 - 250 to 250 000 population equivalent
 - Various treatments : activated sludge, aerated pond, biological contractor...

24h composite samples

- ✓ WWTPs classically encountered in France
- ✓ Influent and effluent were sampled



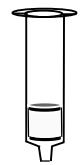
Methodology

Sample preparation

3.5 L Influent



6 L Effluent



Filtration : 0.7µm

Solid phase extraction
HLB Oasis® cartridge



2.5g Sludge

Lyophilisation

Accelerated solvent
extraction

Alumina Purification

Chemical characterisation

Non target
screening



Methodology

Sample preparation



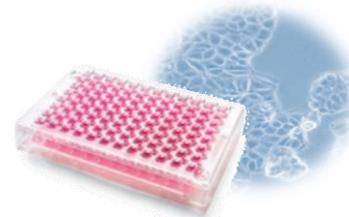
3.5 L Influent

6 L Effluent

Filtration : 0.7µm

Solide phase extraction
HLB Oasis® cartridge

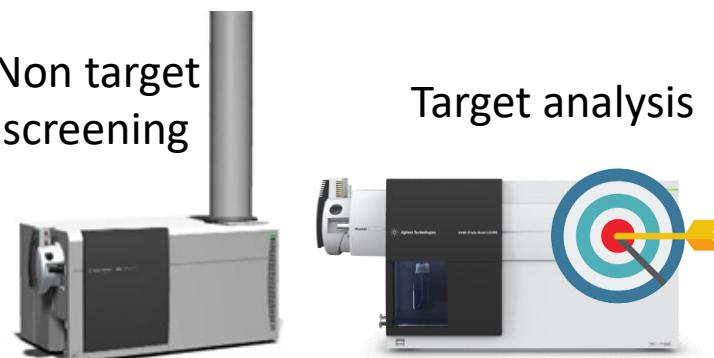
In vitro bioassays



Characterisation of biological activities

Chemical characterisation

Non target screening



Target analysis

Methodology

Sample preparation

3.5 L Influent
6 L Effluent
Filtration : 0.7µm
Solid phase extraction
HLB Oasis® cartridge

In vitro bioassays



Characterisation of biological activities

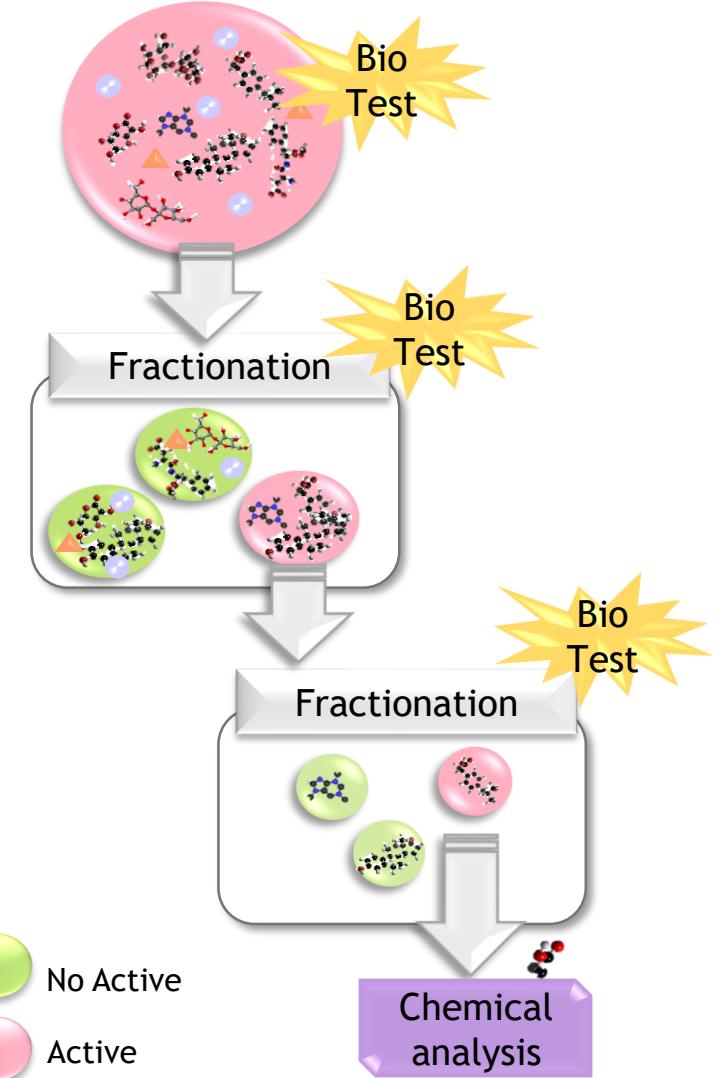
Chemical characterisation

Non target screening



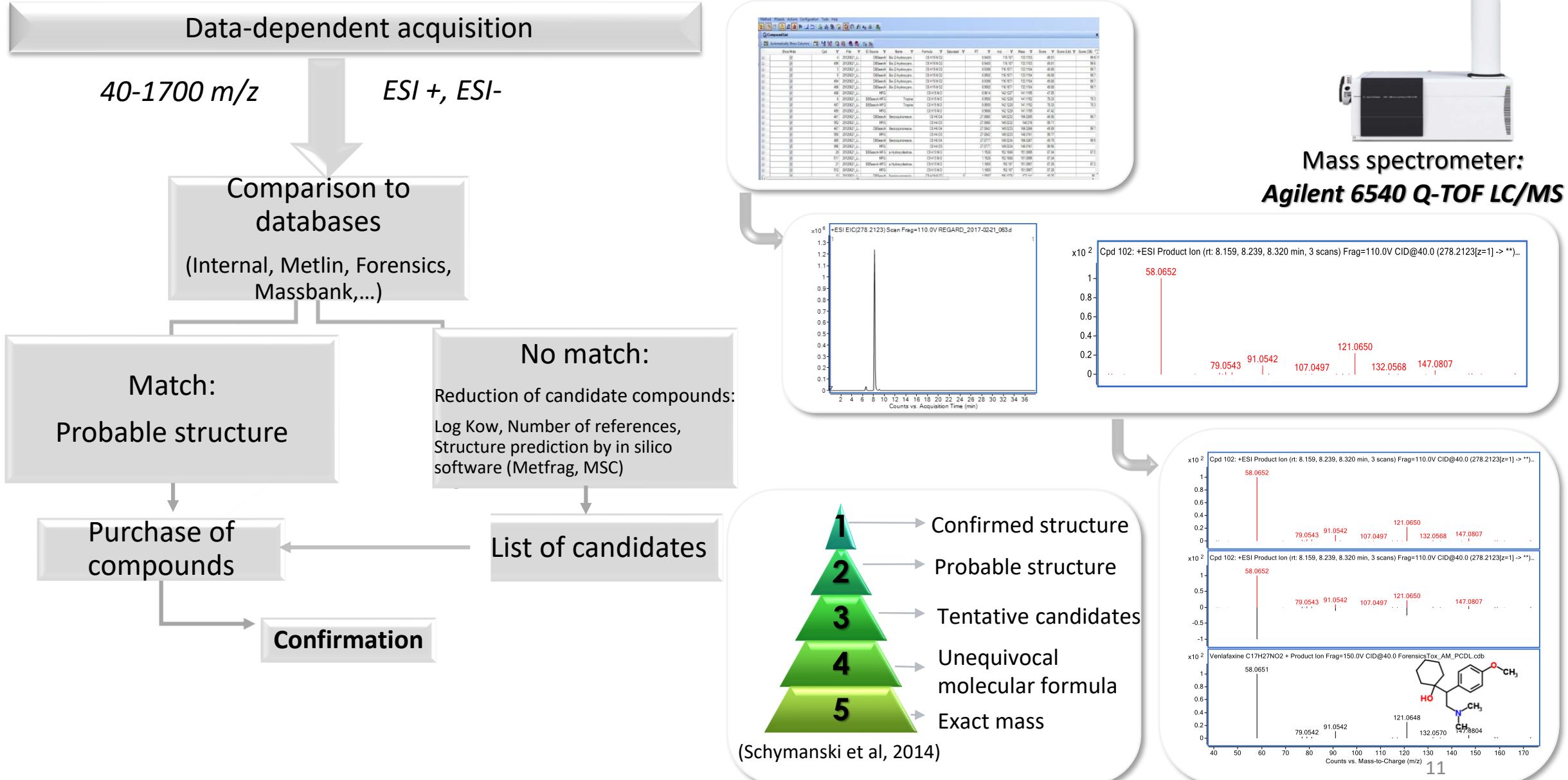
Target analysis

Effect directed analysis

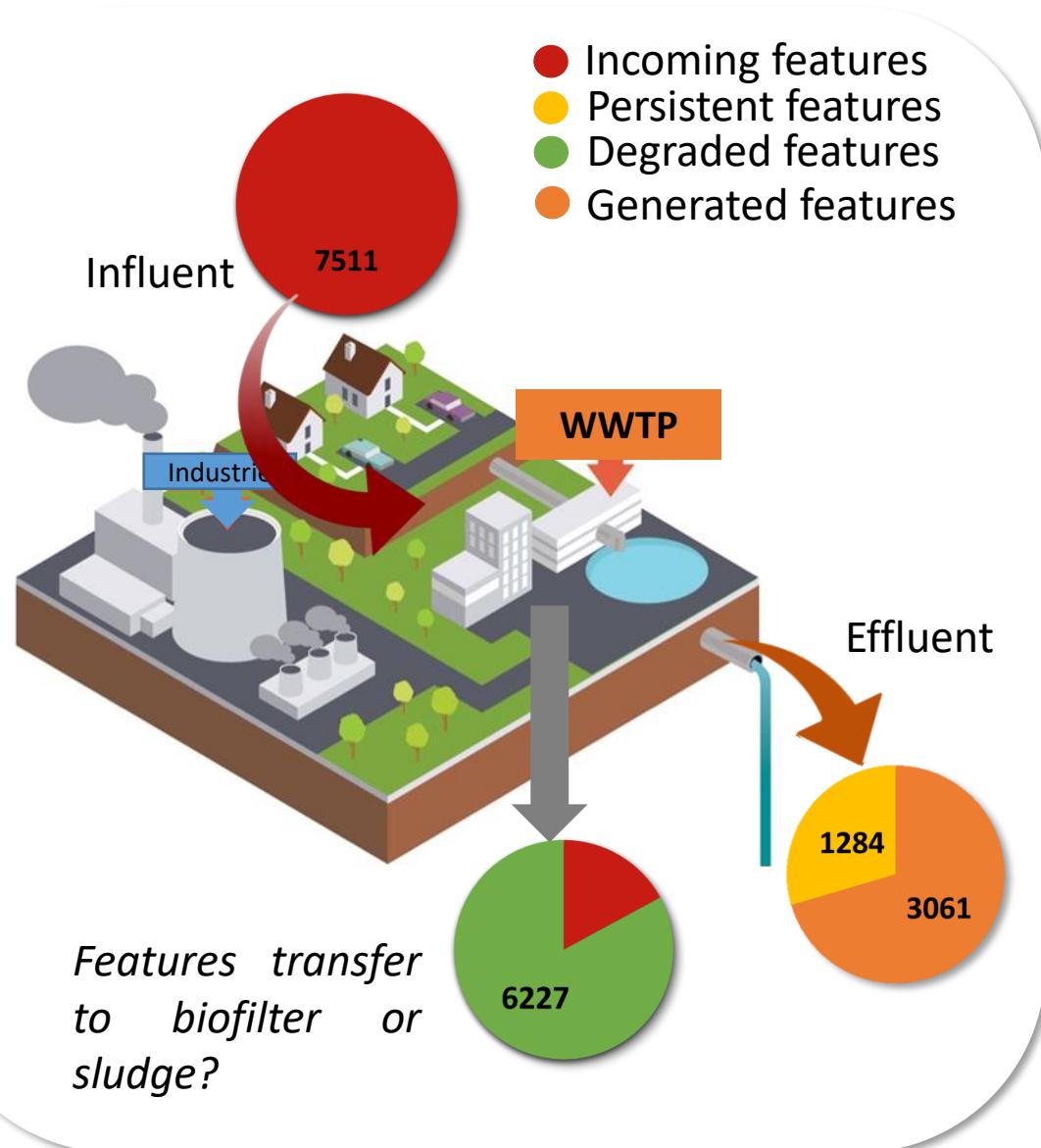


Identification workflow

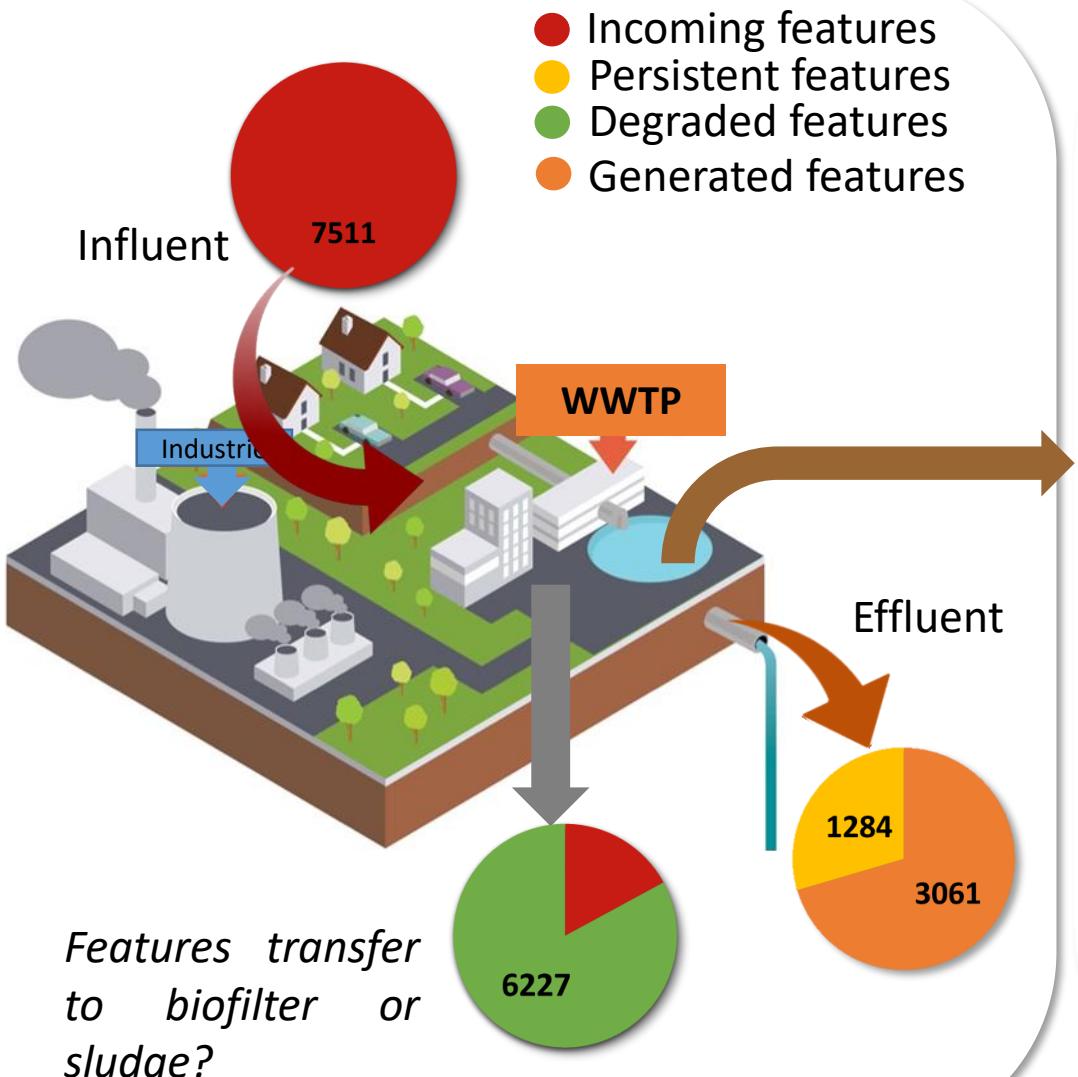
MS/MS mode



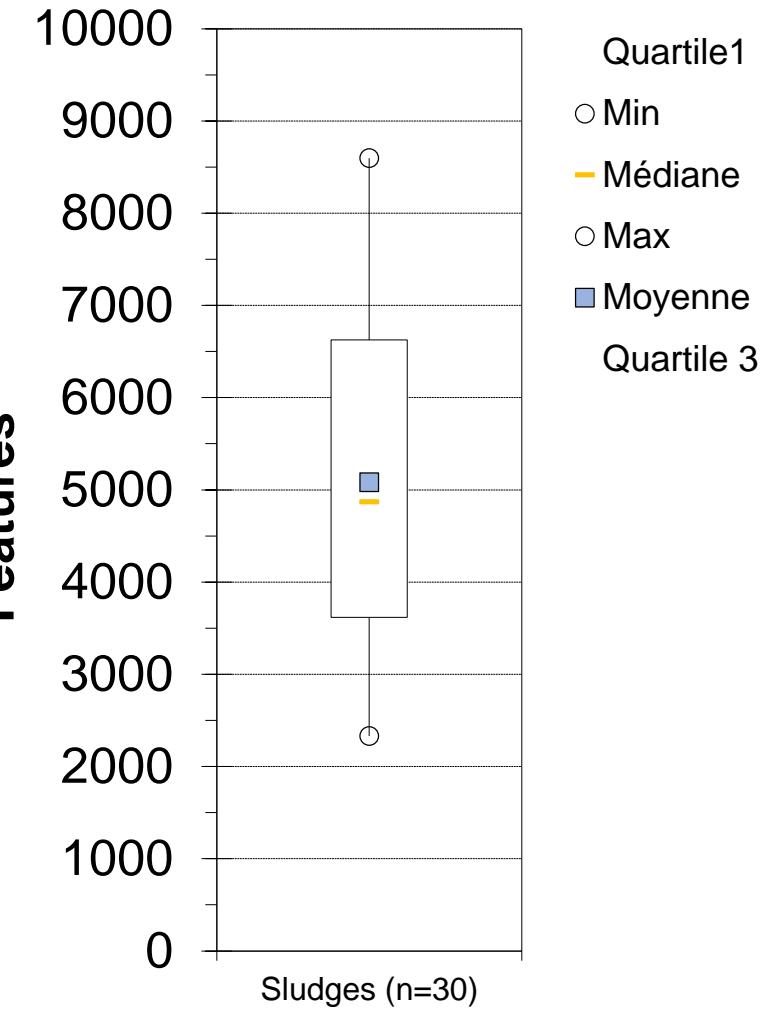
Overall contamination



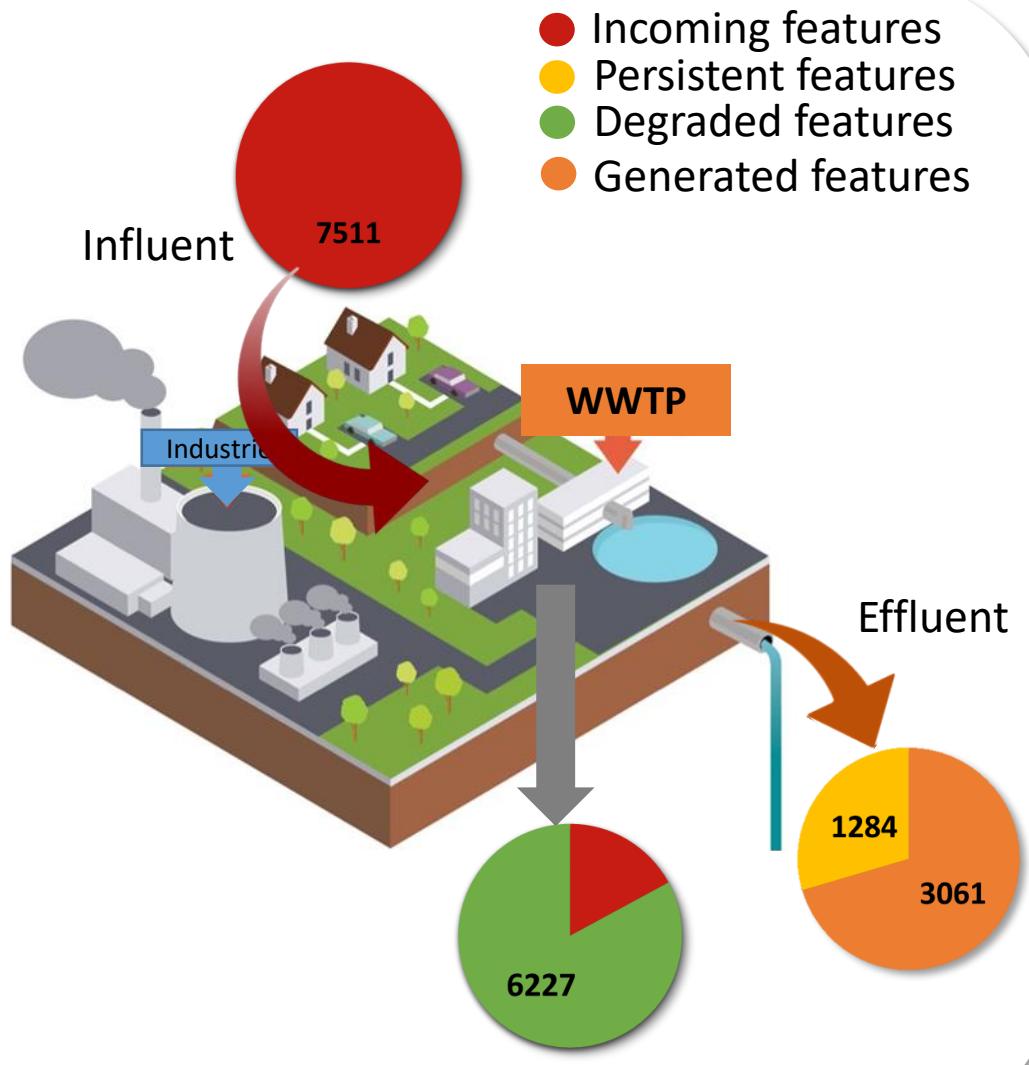
Overall contamination



Overall contamination of sludges :



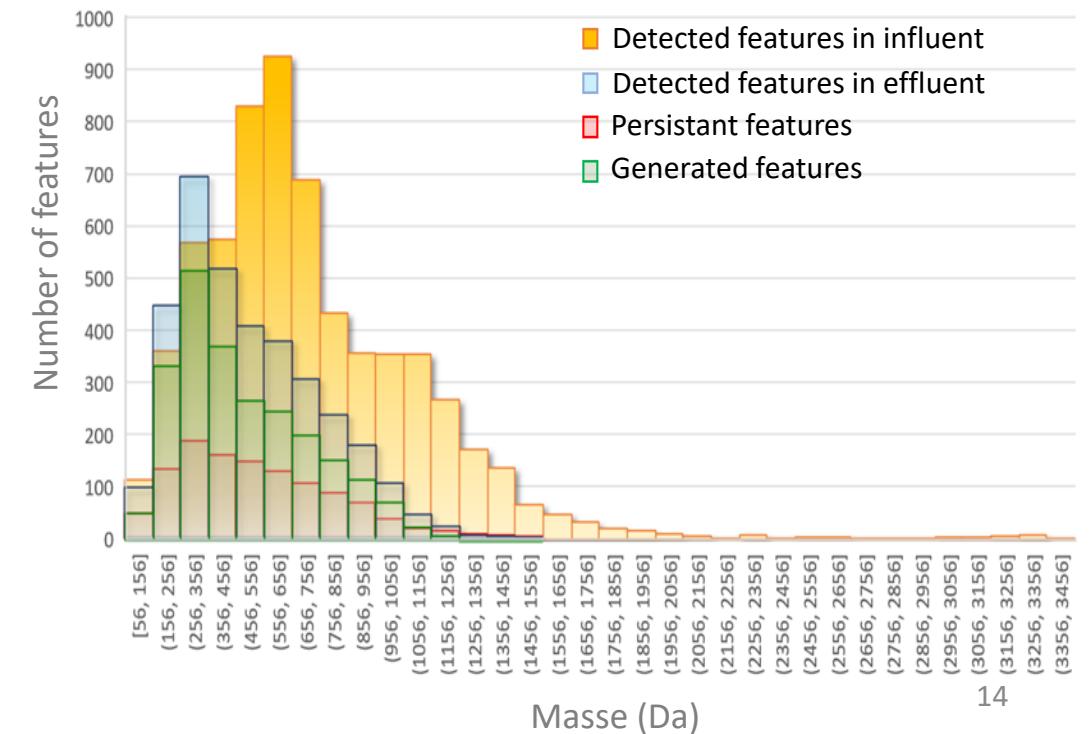
Overall contamination



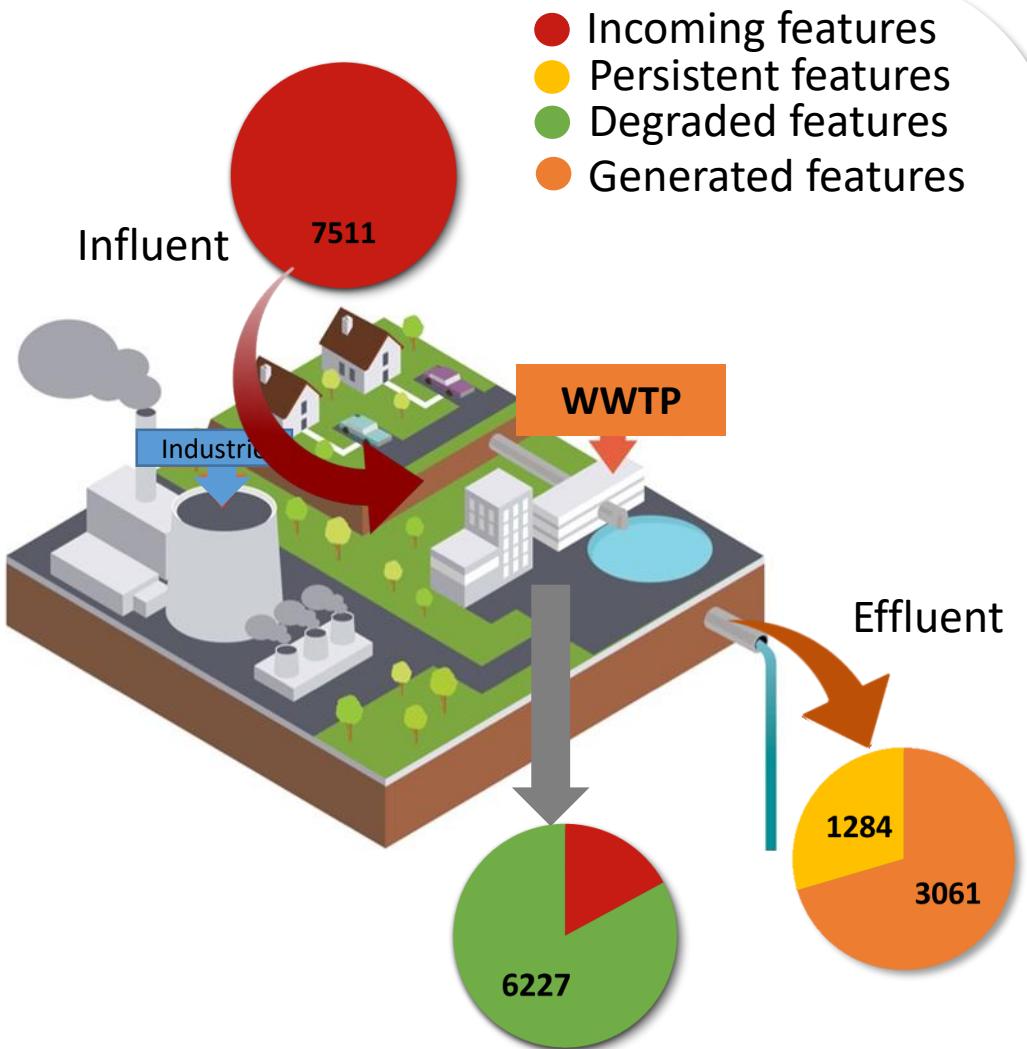
Overall efficiency of WWTP:

Resistant features	17%
Degraded features	83%
Generated features	70% of features in effluent

Transformation products?



Overall contamination

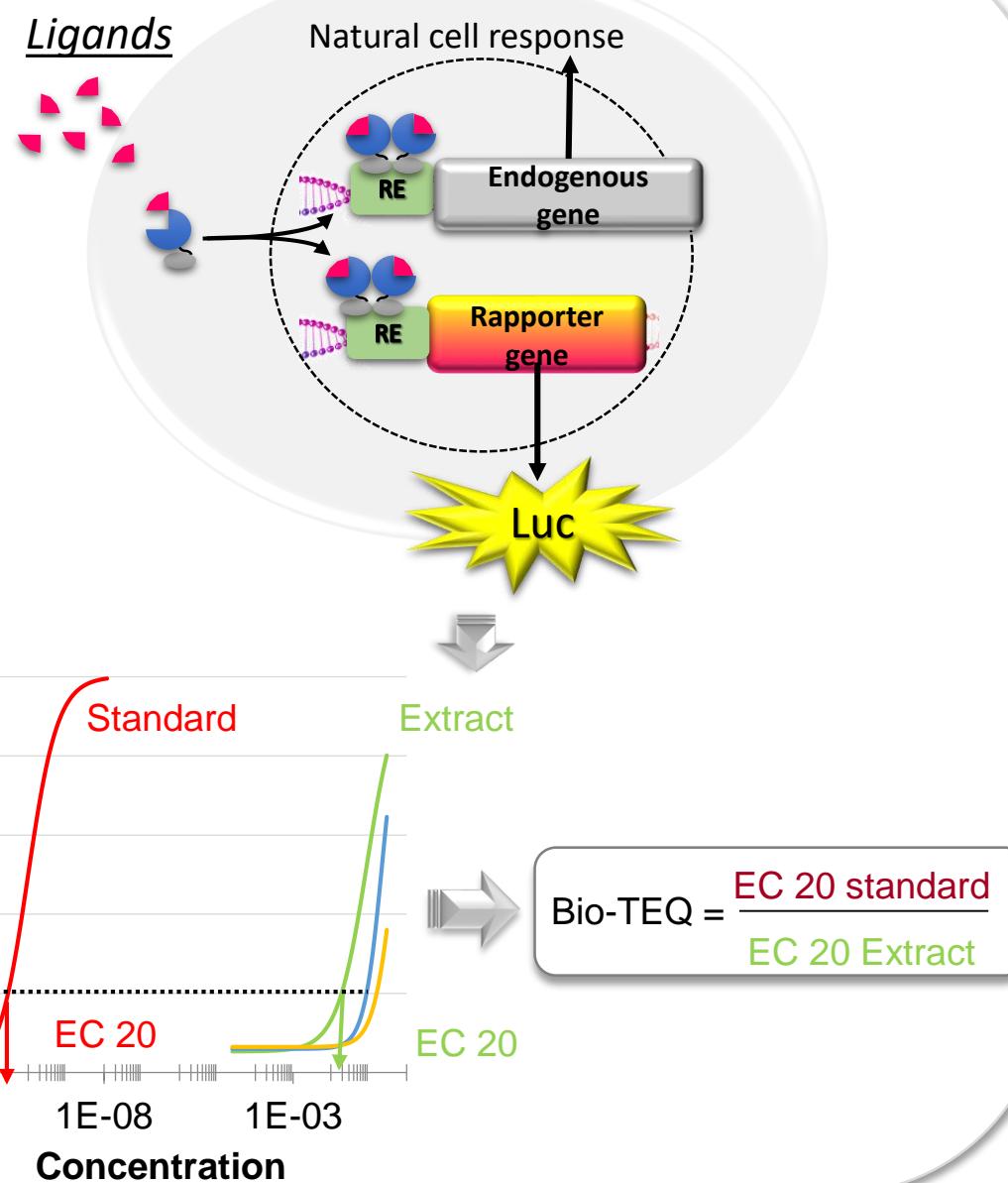


Overall efficiency of WWTP:

Resistant features	17%	
Degraded features	83%	
Generated features	70% of features in effluent	Transformation products?

- ✓ Extracts are very complex
- We need an alternative analytical methodology to focus on identification

In vitro bioassays



- ✓ Based on reporter gene expression under control by receptor
- ✓ Cell signal proportional to the concentration of ligand

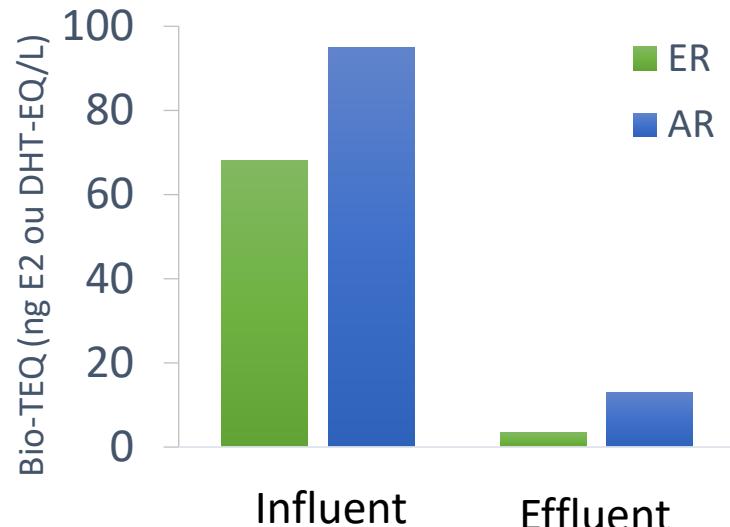
Receptors	Cell lines	Environmental ligands
Steroid receptors		
Estrogen (ER)	MELN	<i>E1, E2, EE2, alkylphenols, BPA, pharmaceuticals</i>
Androgen (AR) Glucocorticoid (GR)	MDA-kb2	<i>DHT, BPA, vinclozolin Pharmaceuticals</i>



Detection of Endocrine Disrupting Compounds

Mass balance analysis

Biological activities



Chemical analysis

161 Targeted compounds
(pharmaceuticals, pesticides, PAHs, PCBs,
OCPs, AKPs, BTEXs, COVs, phthalates,
hormones, bisphenols)

75 detected molecules

5 known agonist ER ligands
(no ligand for AR)

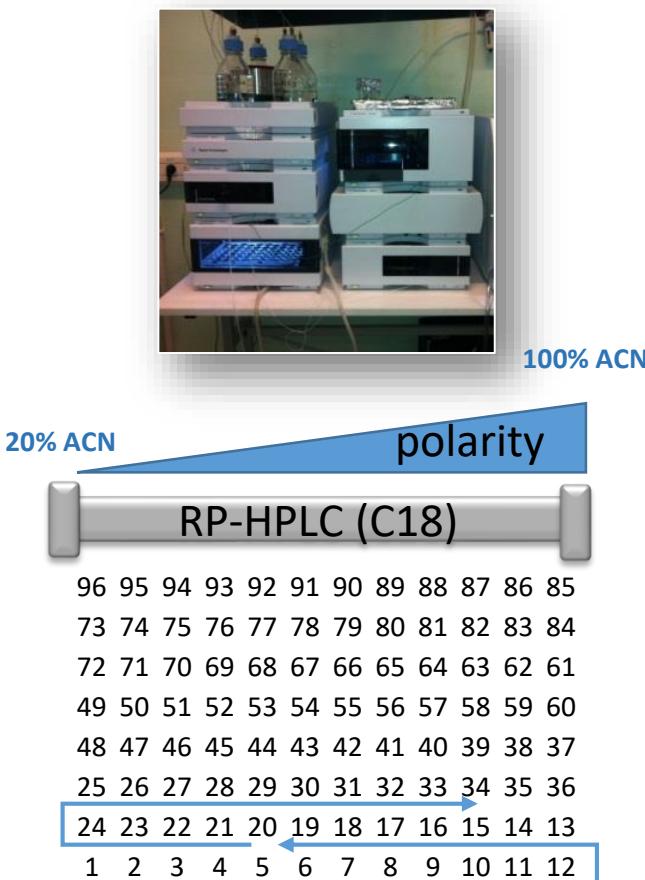
WARNING

Mass balance analysis

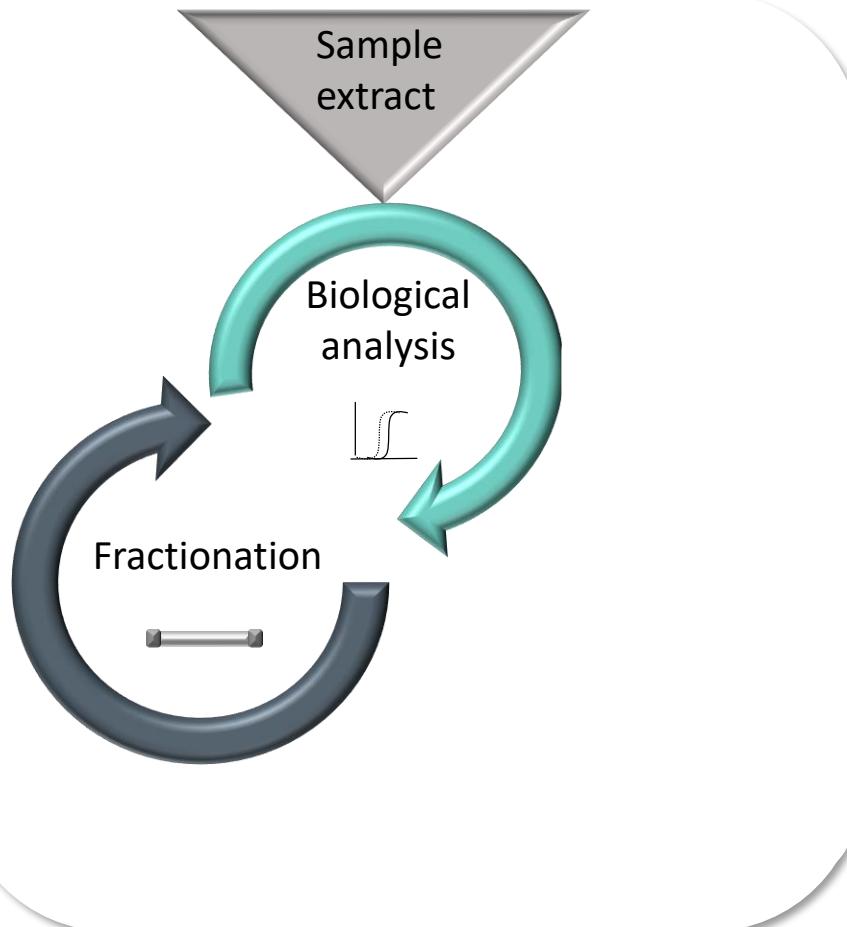
Activities remained unexplained
Explained activity <1%
(except for effluent <4%)

Deconvolution of information : Effect directed analysis

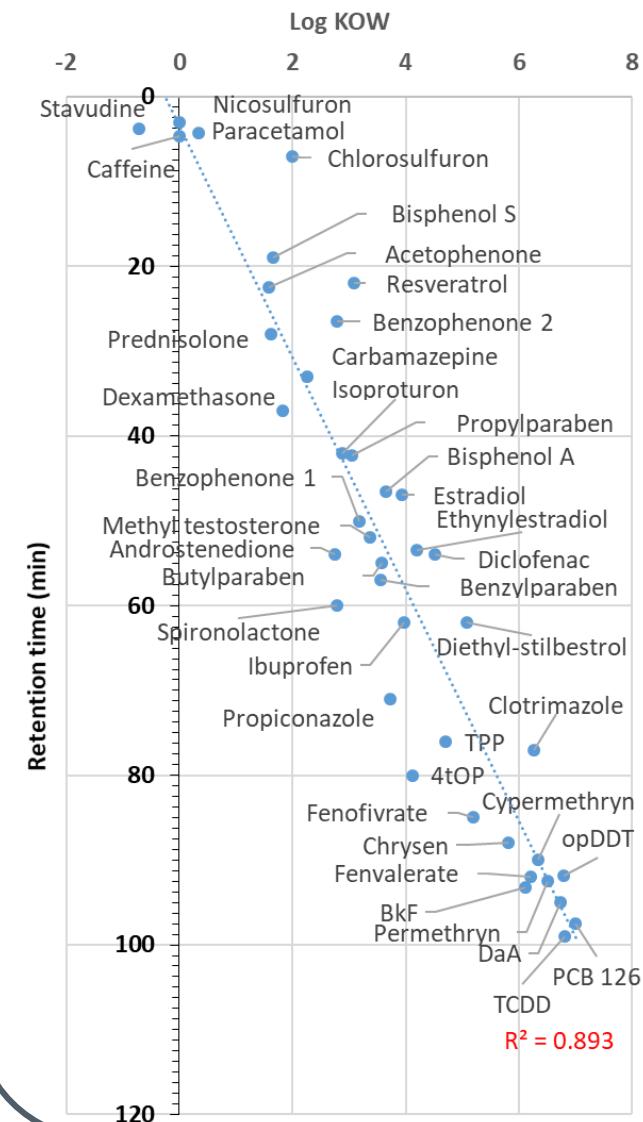
Simplification



96 collected fractions

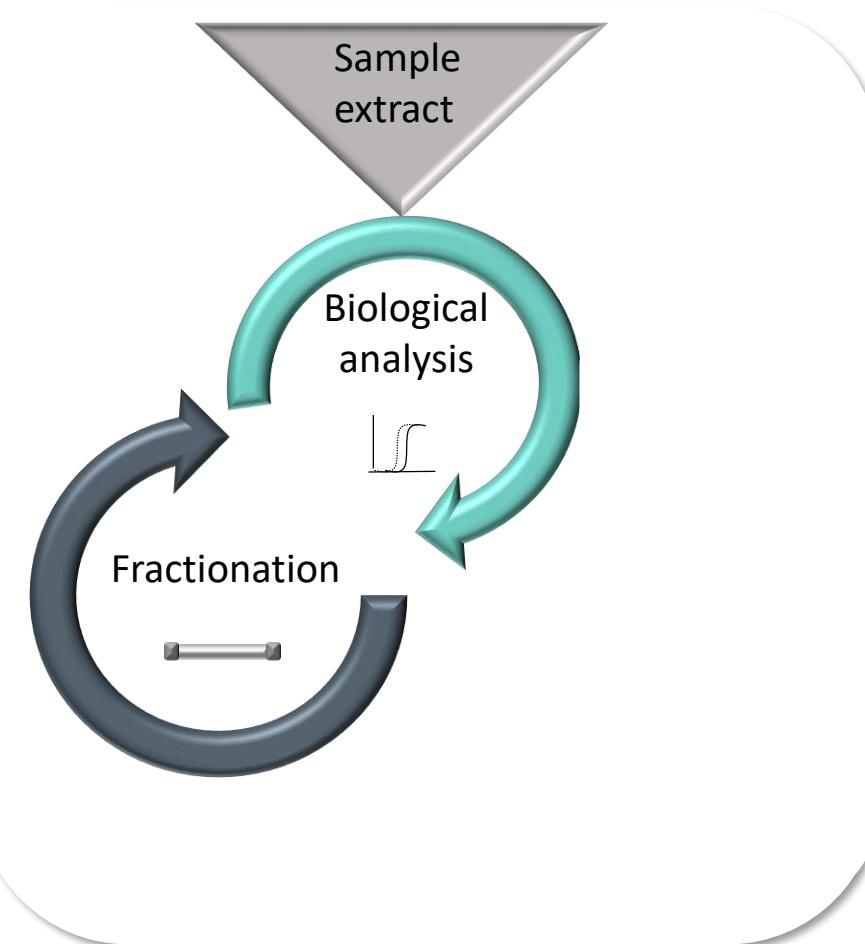
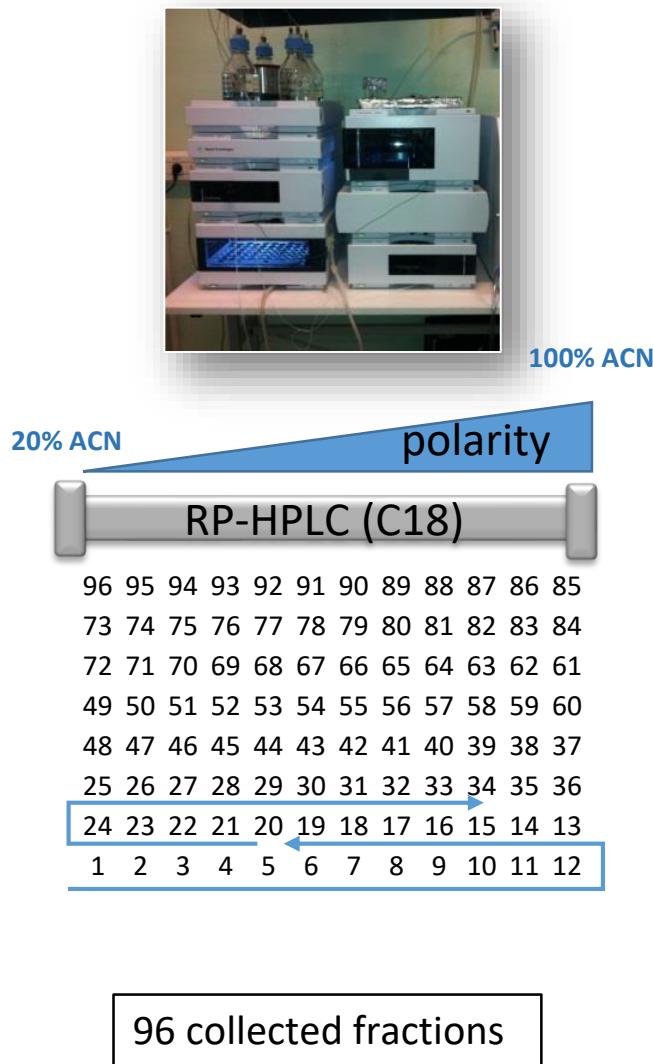


HPLC calibration (42 compounds)



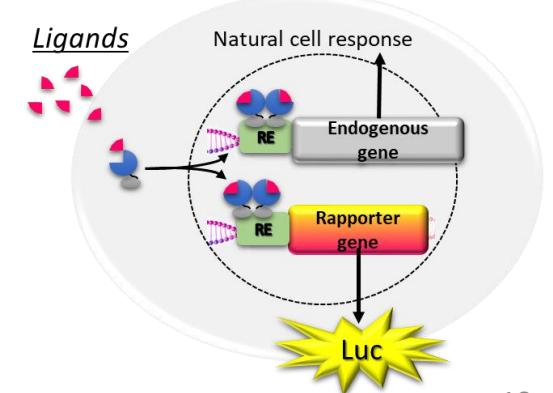
Deconvolution of information : Effect directed analysis

Simplification



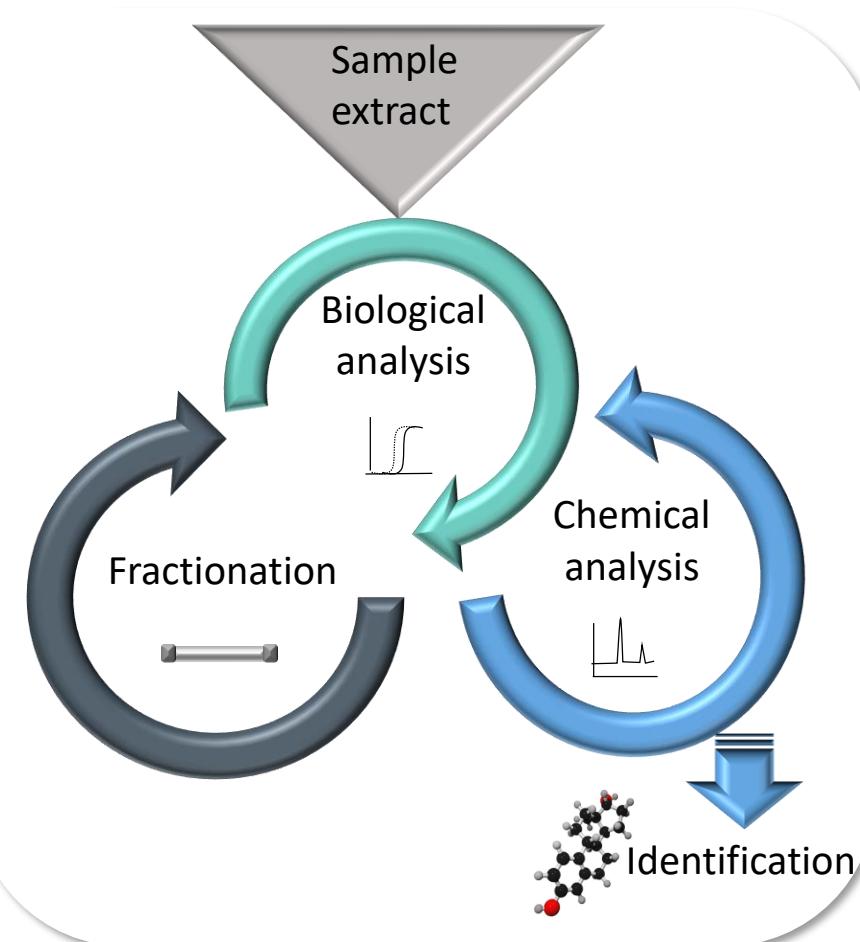
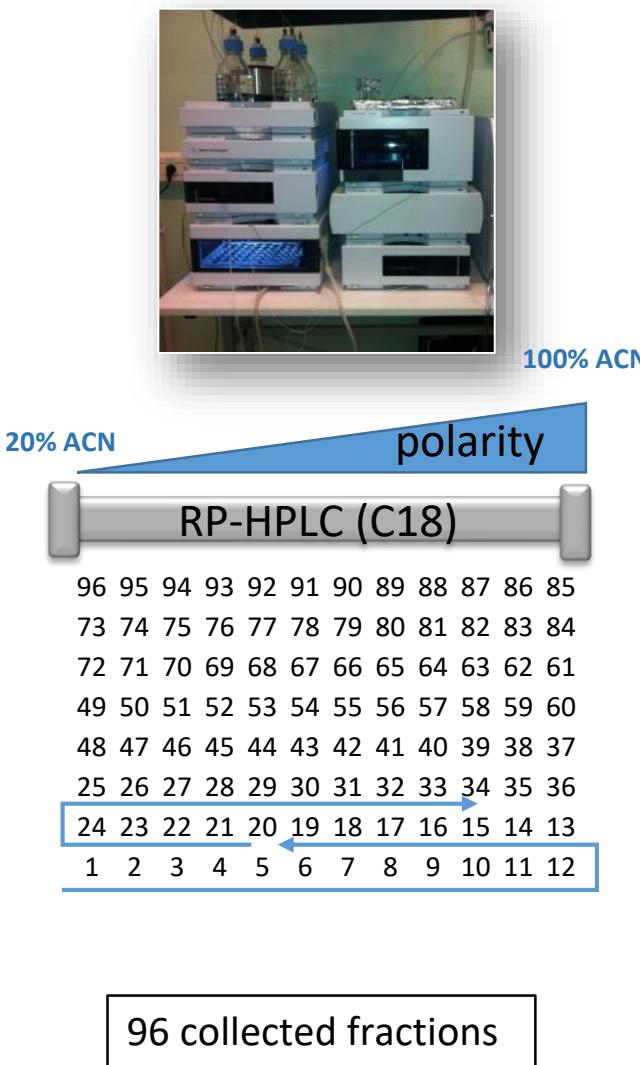
Directed

- ✓ high throughput screening on ER, AR/GR bioassays



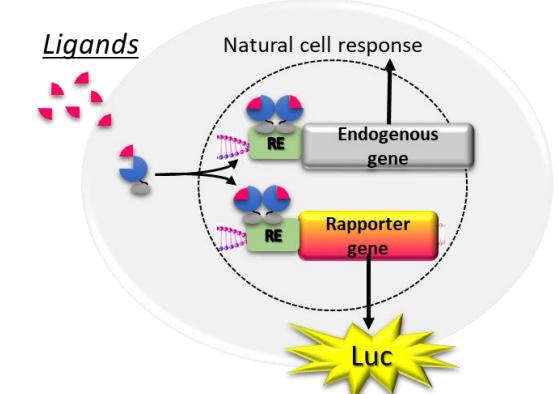
Deconvolution of information : Effect directed analysis

Simplification



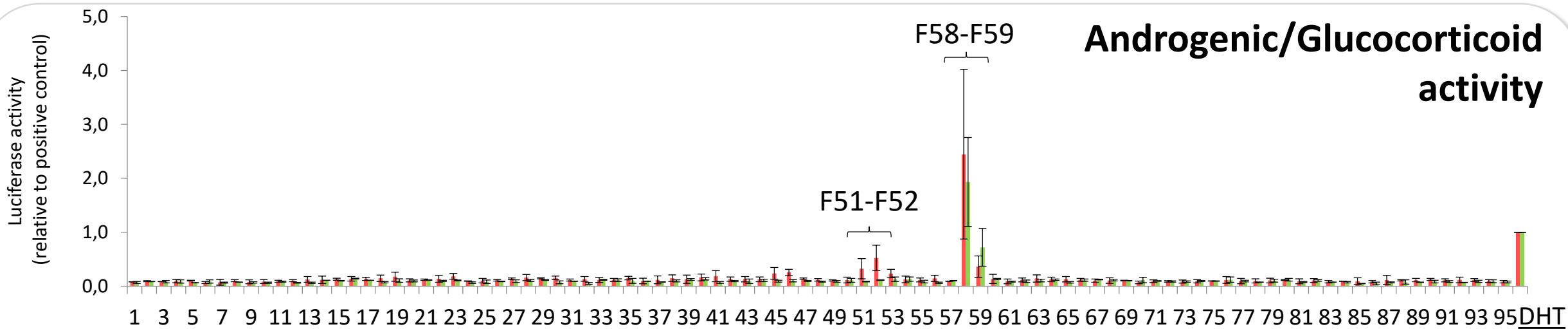
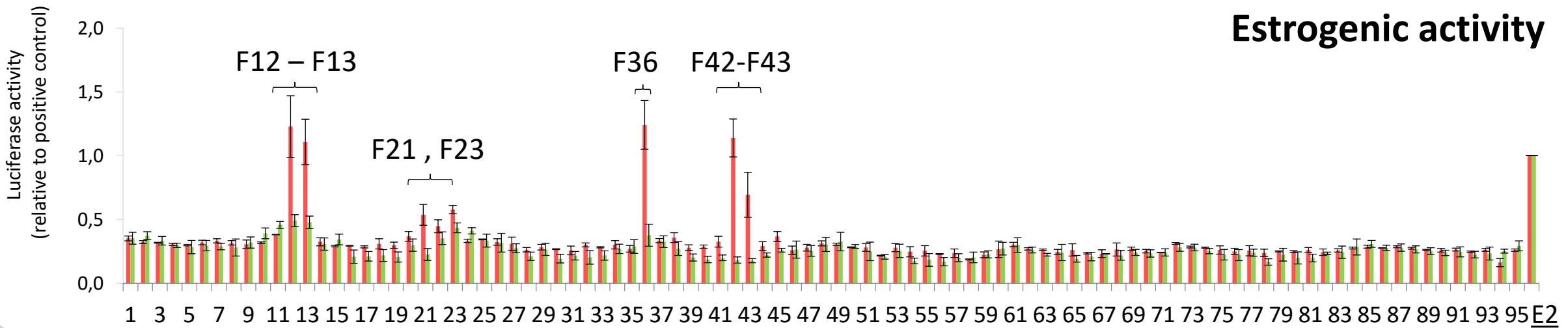
Directed

- ✓ high throughput screening on ER, AR/GR bioassays



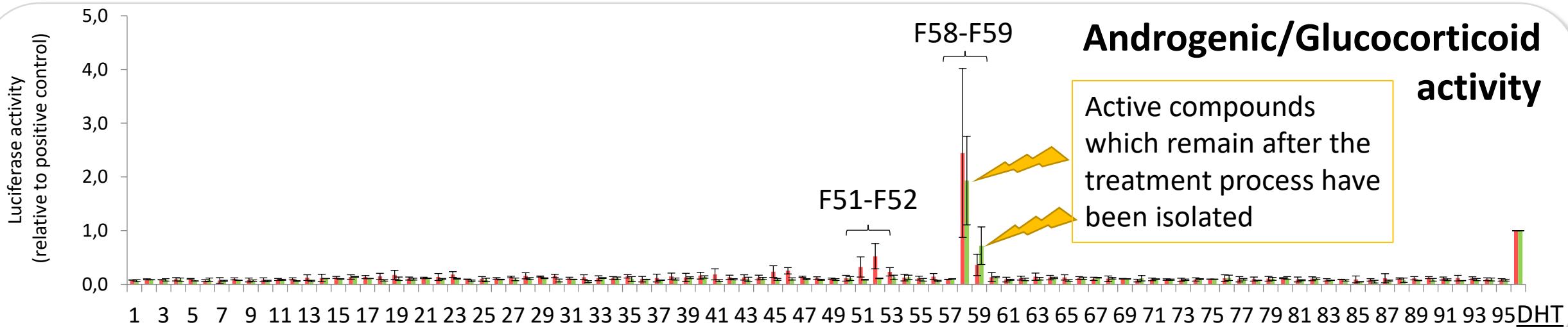
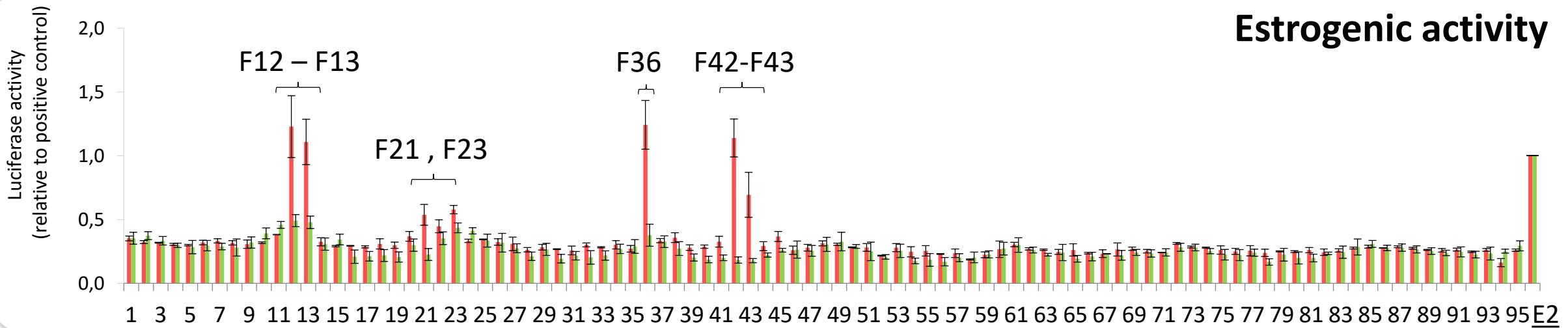
Biological activities

Influent
Effluent



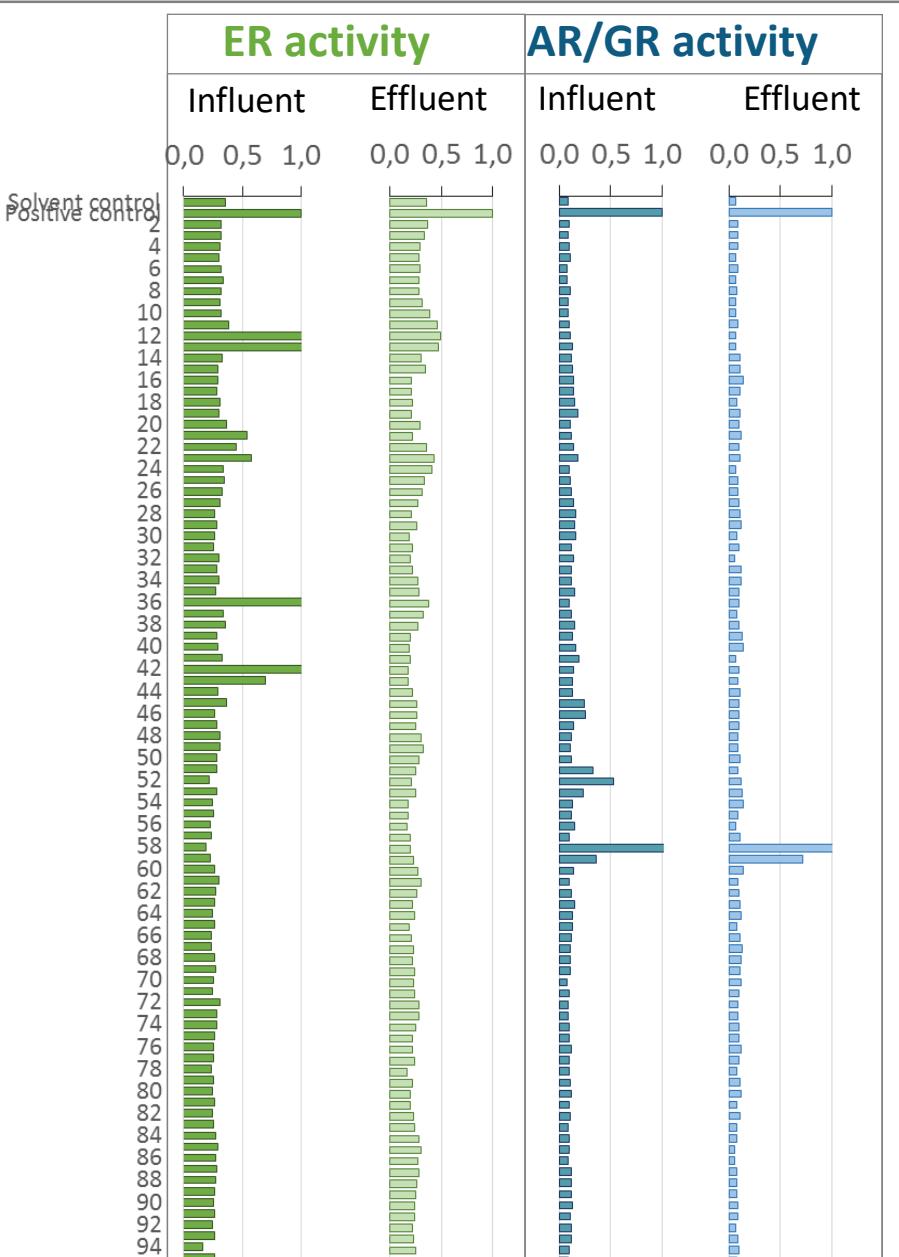
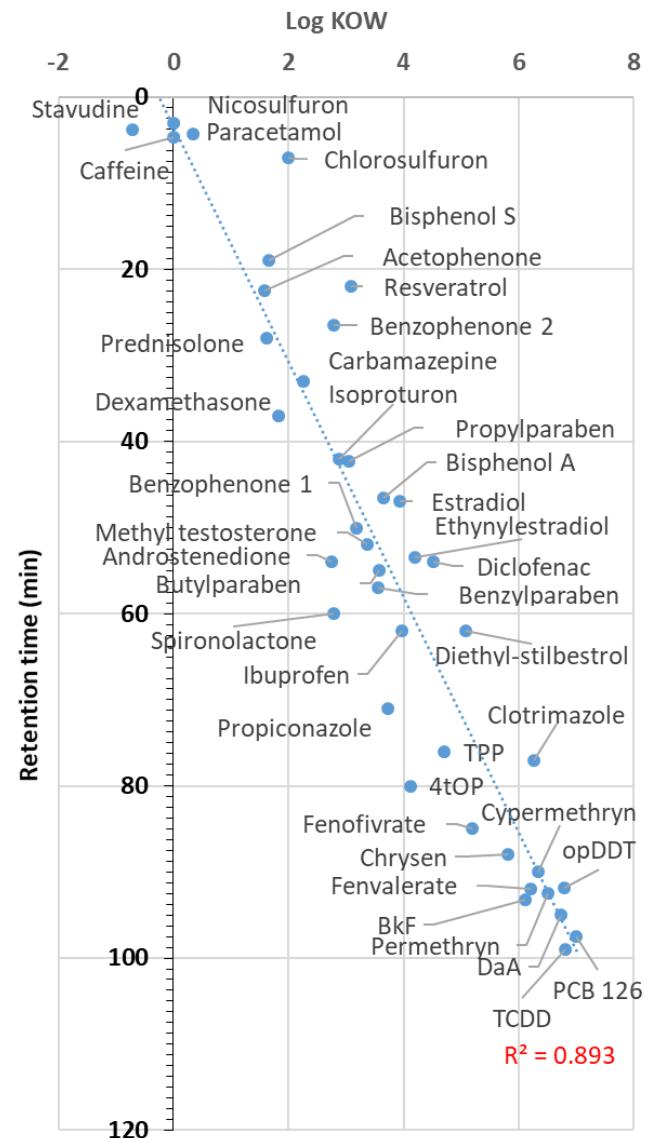
Biological activities

Influent
Effluent

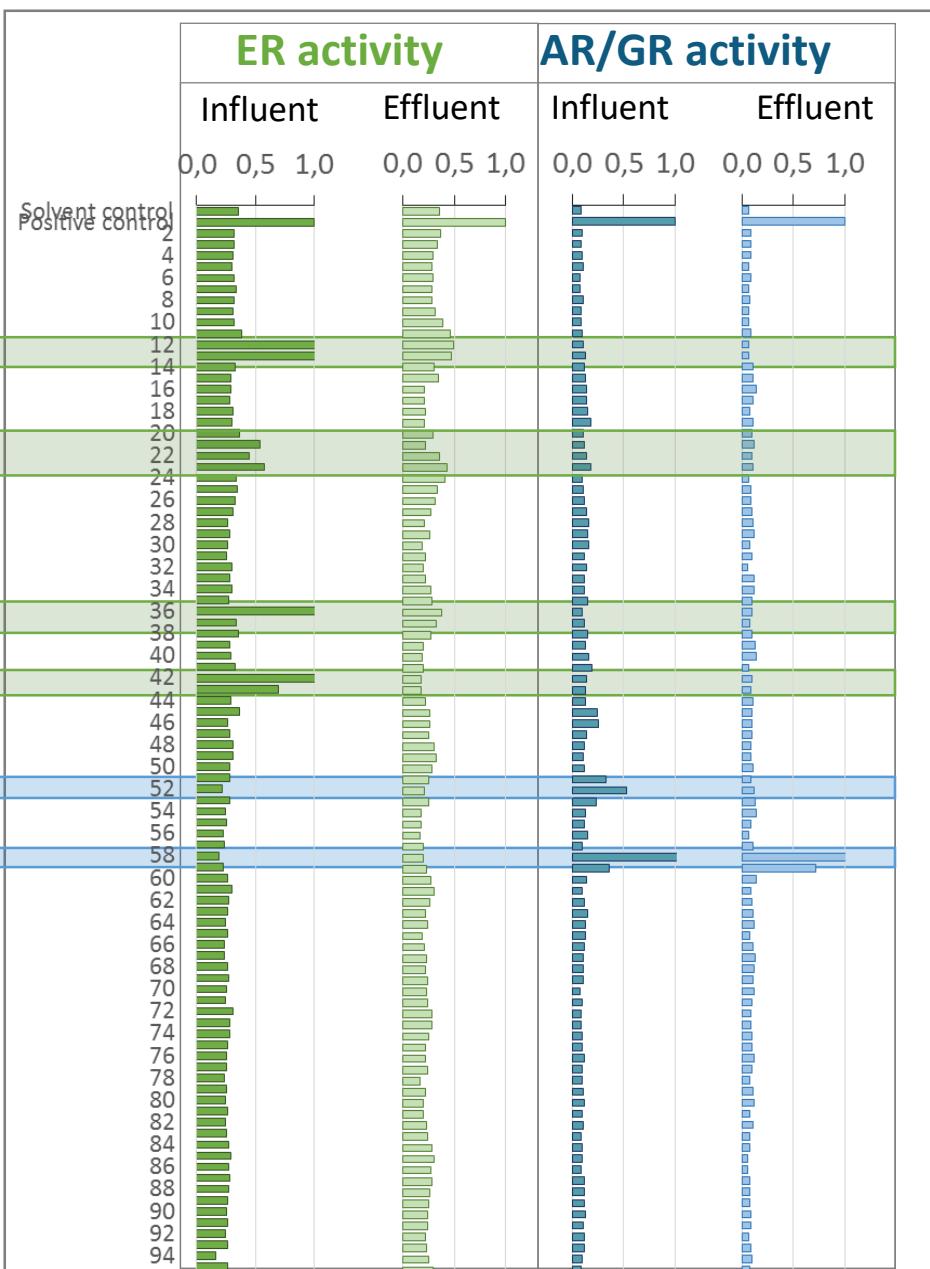
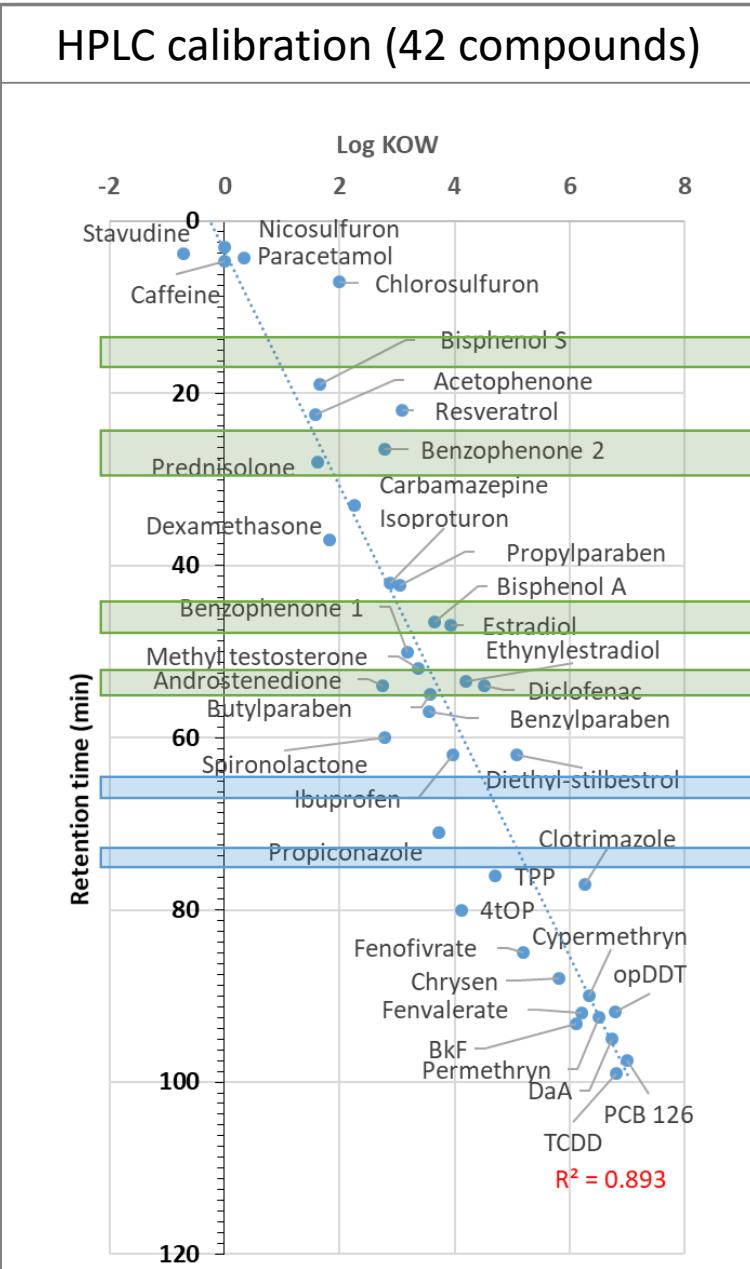


Comparison of biological profile and calibration

HPLC calibration (42 compounds)



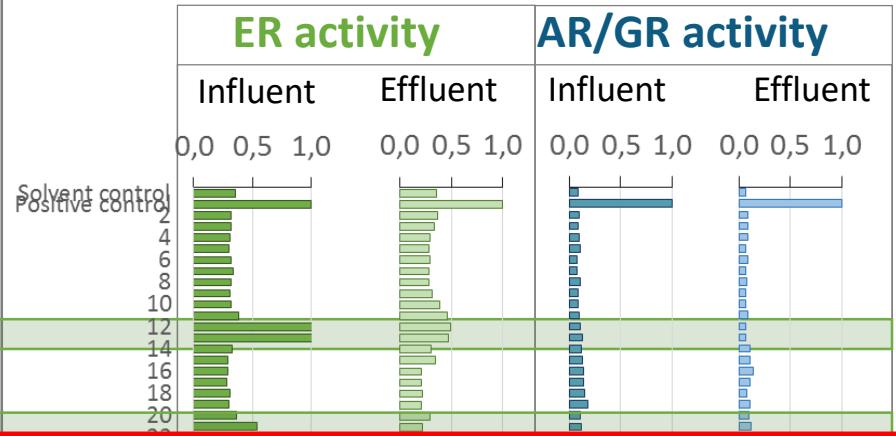
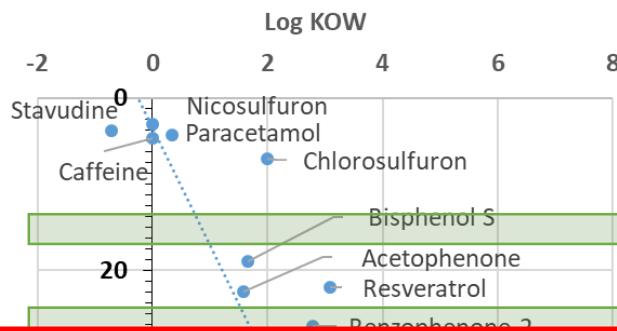
Comparison of biological profile and calibration



F12-F13 → Unexplained
 F21-F23
 F36
 F42-43 → Target compounds (BPA, estrogens)
 F52
 F58-59 → Unexplained

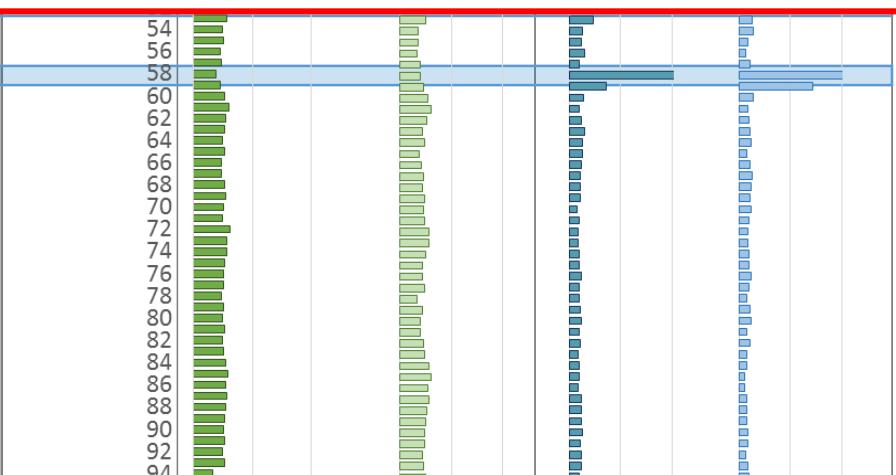
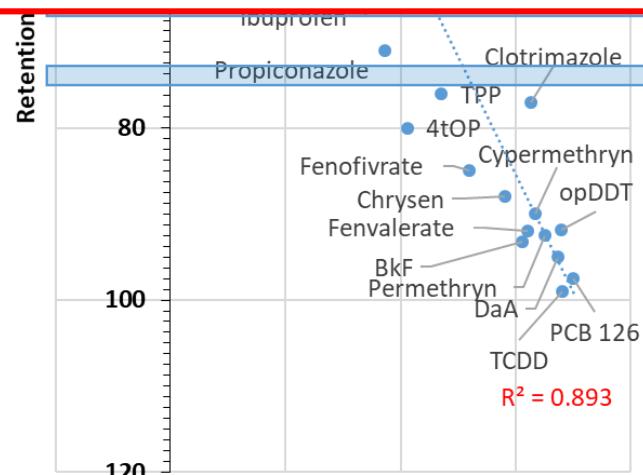
Comparison of biological profile and calibration

HPLC calibration (42 compounds)



F12-F13 → Unexplained
F21-F22

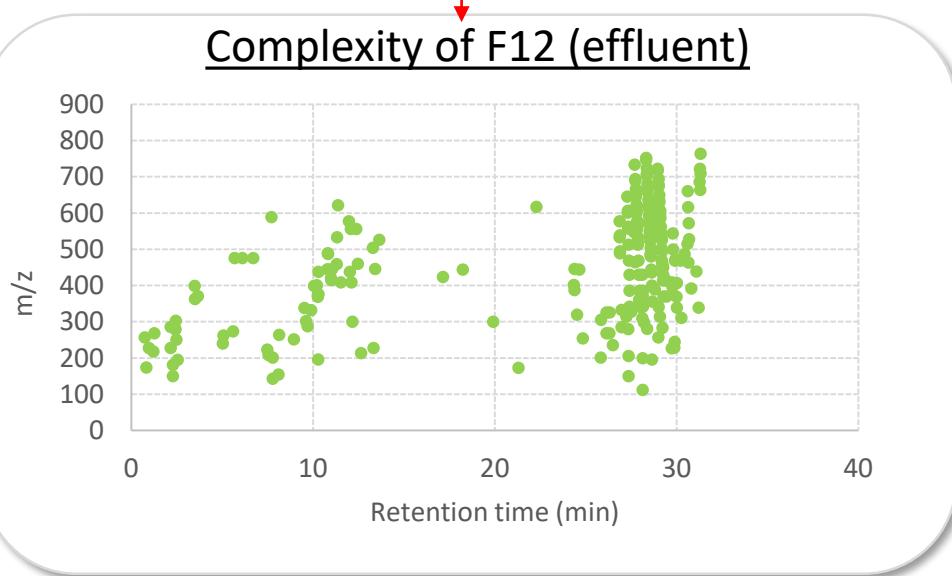
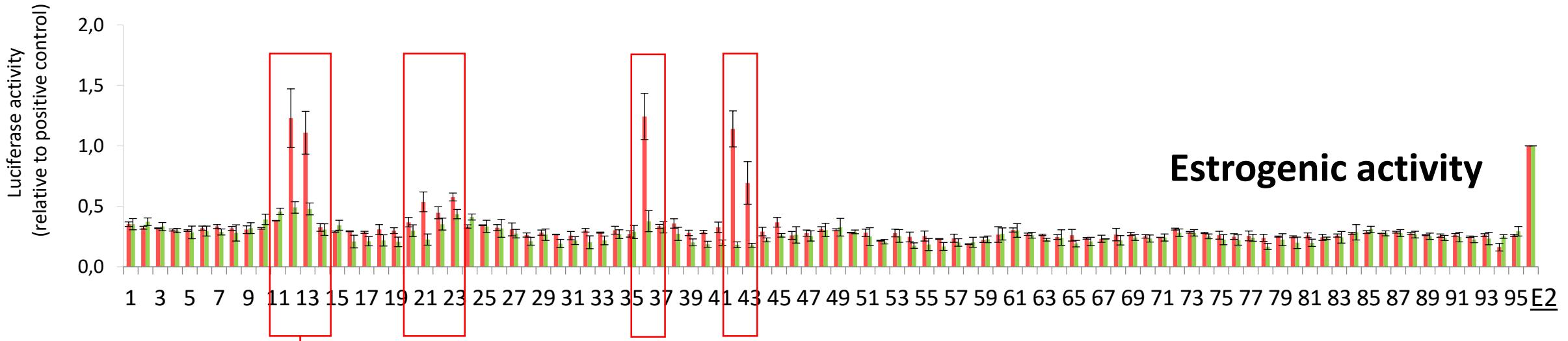
➤ Many active fractions remained unexplained



F58-59 → Unexplained

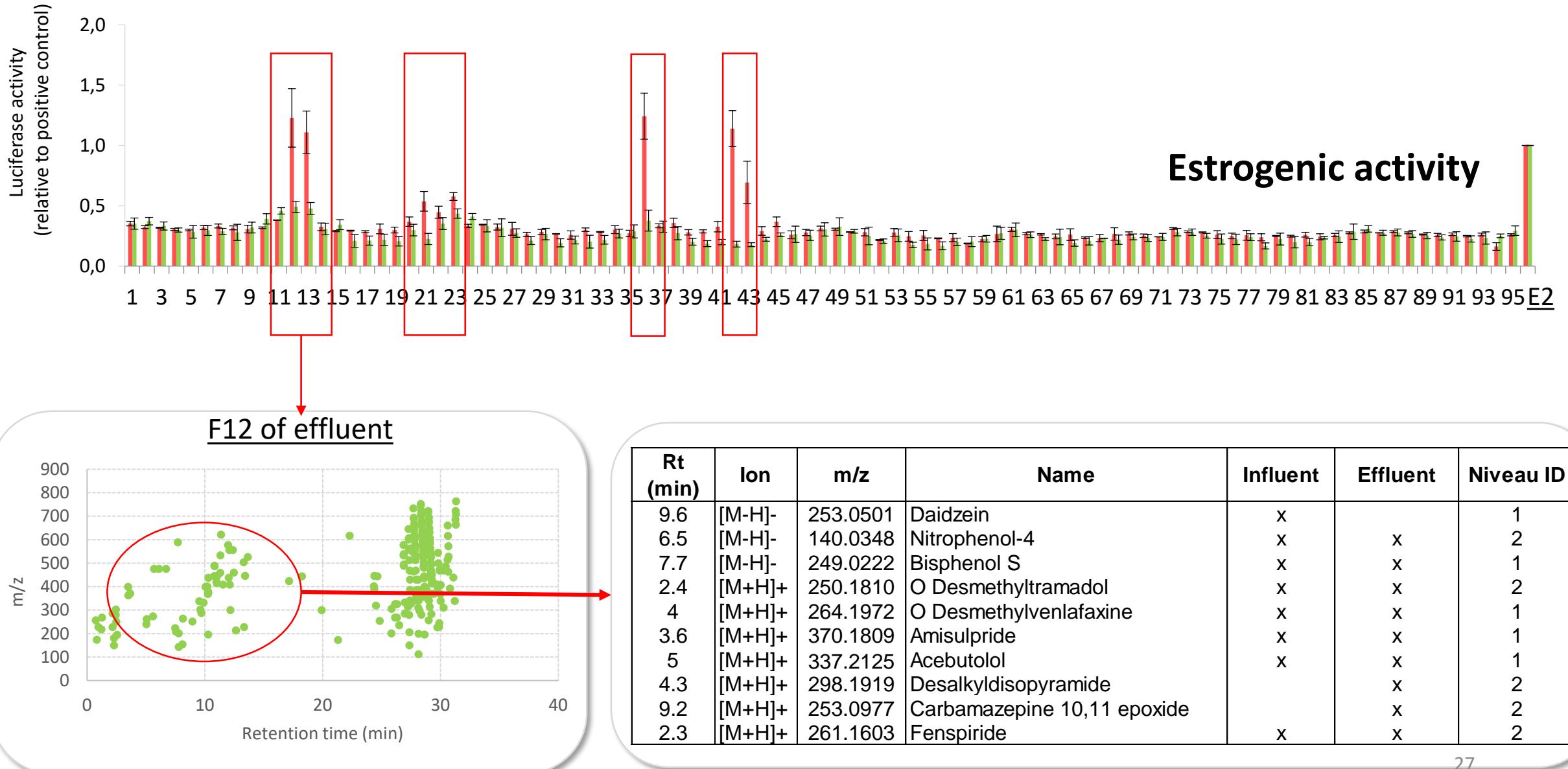
bounds
gens)

Identification of compounds

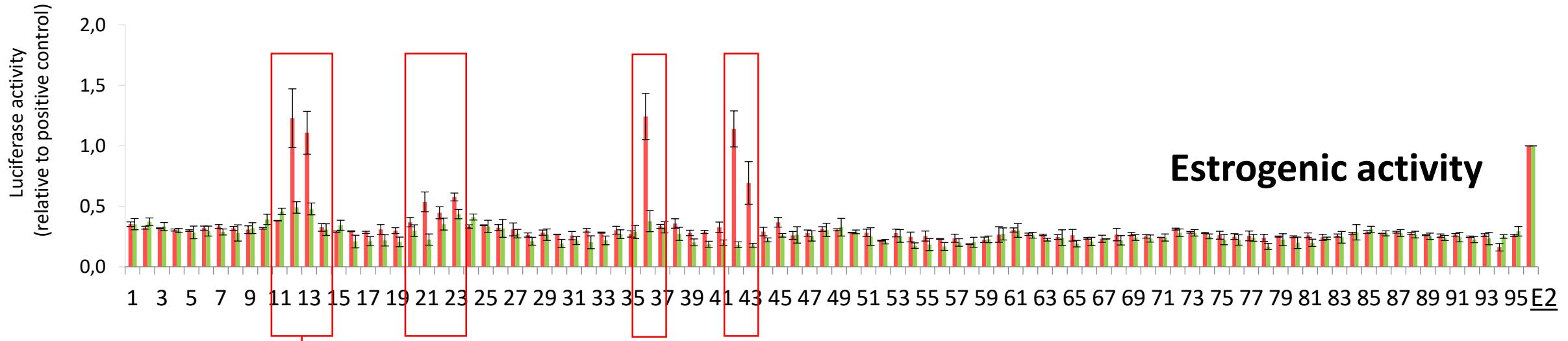


➤ More than 100 features in each fraction

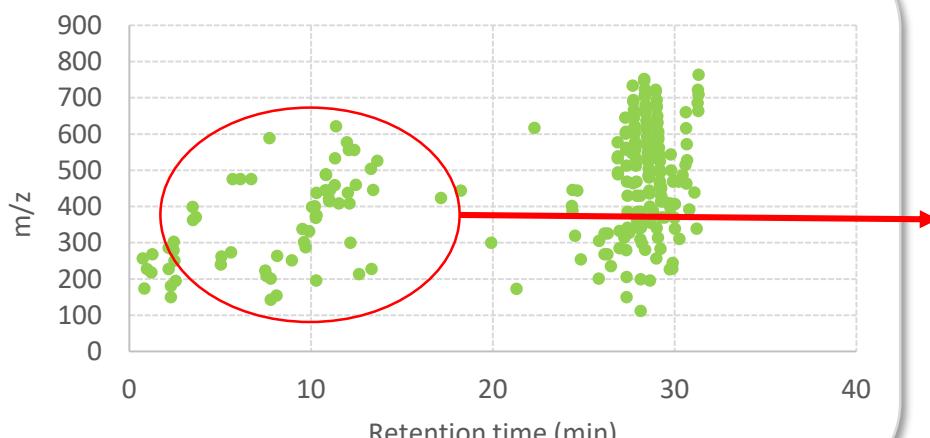
Identification of compounds



Identification of compounds

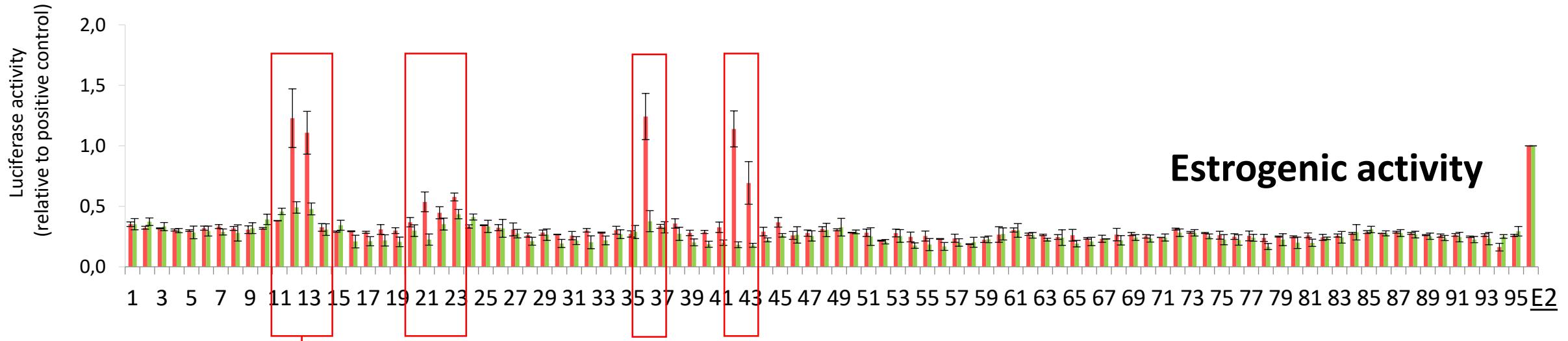


F12 of effluent

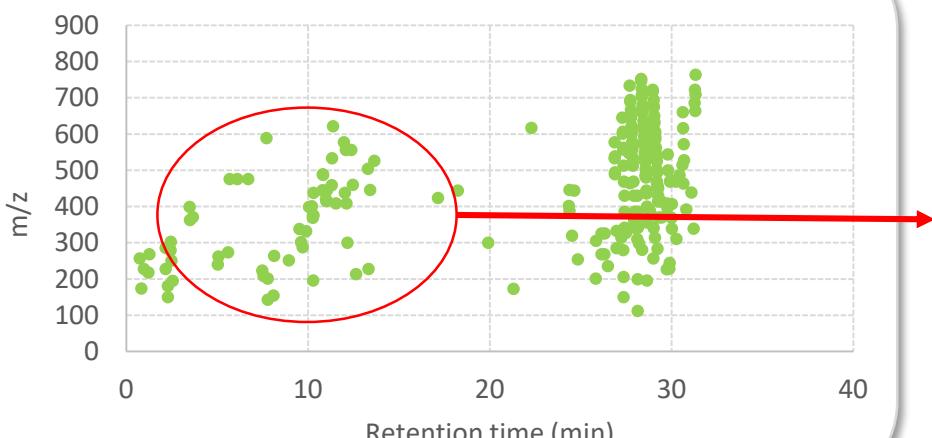


Rt (min)	Ion	m/z	Name	Influent	Effluent	Niveau ID
9.6	[M-H] ⁻	253.0501	Daidzein	x		1
6.5	[M-H] ⁻	140.0348	Nitrophenol-4	x	x	2
7.7	[M-H] ⁻	249.0222	Bisphenol S	x	x	1
2.4	[M+H] ⁺	250.1810	O Desmethyltramadol	x	x	2
4	[M+H] ⁺	264.1972	O Desmethylvenlafaxine	x	x	1
3.6	[M+H] ⁺	370.1809	Amisulpride	x	x	1
5	[M+H] ⁺	337.2125	Acibutolol	x	x	1
4.3	[M+H] ⁺	298.1919	Desalkyldisopyramide		x	2
9.2	[M+H] ⁺	253.0977	Carbamazepine 10,11 epoxide		x	2
2.3	[M+H] ⁺	261.1603	Fenspiride	x	x	2

Identification of compounds

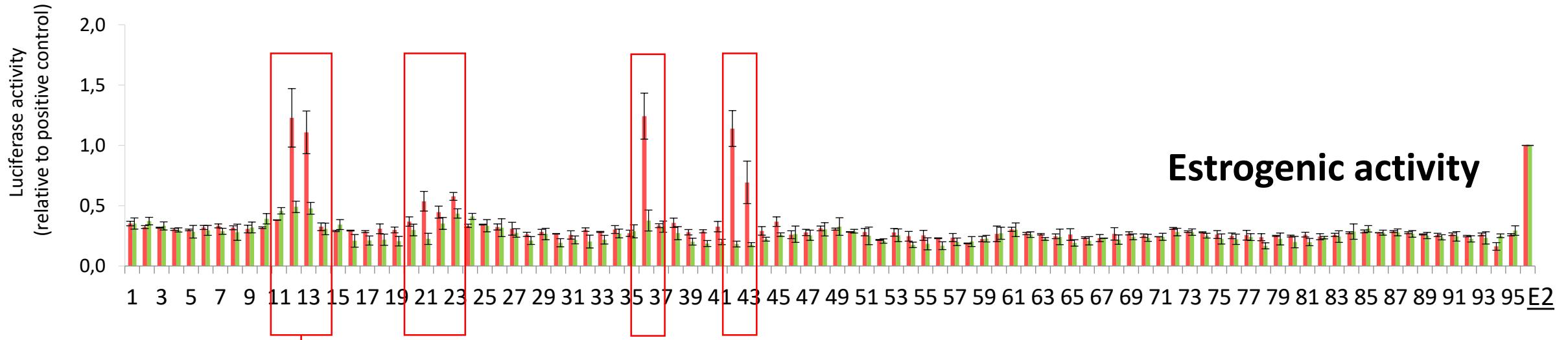


F12 of effluent



Rt (min)	Ion	m/z	Name	Influent	Effluent	Niveau ID
9.6	[M-H]-	253.0501	Daidzein	x		1
6.5	[M-H]-	140.0348	Nitrophenol-4	x	x	2
7.7	[M-H]-	249.0222	Bisphenol S	x	x	1
2.4	[M+H]+	250.1810	O Desmethyltramadol	x	x	2
4	[M+H]+	264.1972	O Desmethylvenlafaxine	x	x	1
3.6	[M+H]+	370.1809	Amisulpride	x	x	1
5	[M+H]+	337.2125	Acebutolol	x	x	1
4.3	[M+H]+	298.1919	Desalkyldisopyramide		x	2
9.2	[M+H]+	253.0977	Carbamazepine 10,11 epoxide		x	2
2.3	[M+H]+	261.1603	Fenspiride	x	x	2

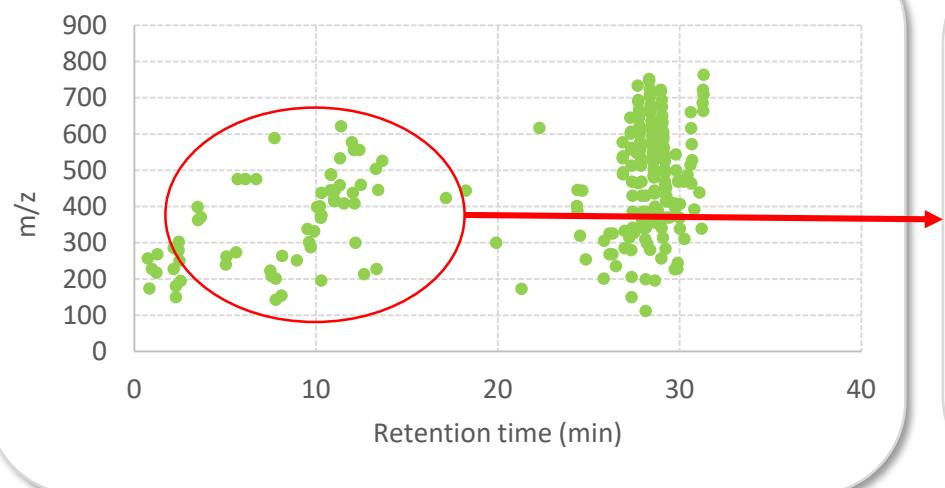
Identification of compounds



Estrogenic activity

- Persistant TPs
- TPs detected only in fraction of effluent

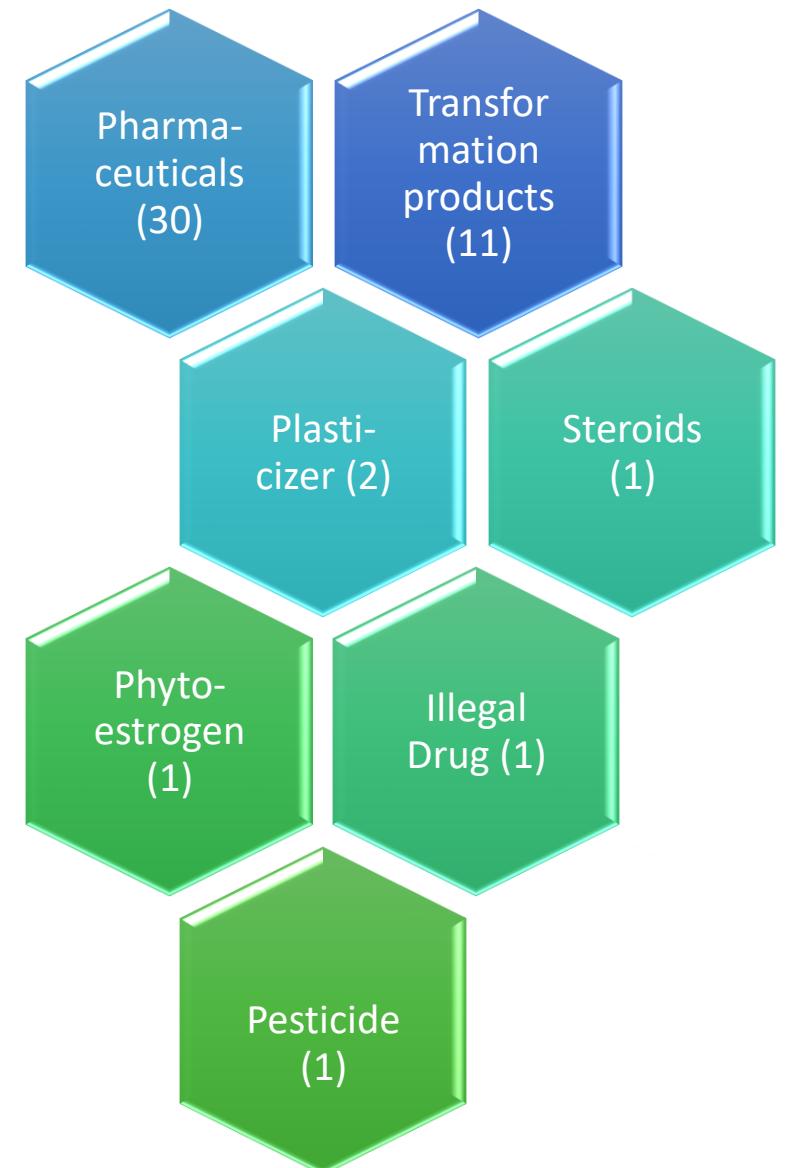
F12 of effluent



Rt (min)	Ion	m/z	Name	Influent	Effluent	Niveau ID
9.6	[M-H]-	253.0501	Daidzein	x		1
6.5	[M-H]-	140.0348	Nitrophenol-4	x	x	2
7.7	[M-H]-	249.0222	Bisphenol S	x	x	1
2.4	[M+H]+	250.1810	O Desmethyltramadol	x	x	2
4	[M+H]+	264.1972	O Desmethylvenlafaxine	x	x	1
3.6	[M+H]+	370.1809	Amisulpride	x	x	1
5	[M+H]+	337.2125	Acebutolol	x	x	1
4.3	[M+H]+	298.1919	Desalkyldisopyramide		x	2
9.2	[M+H]+	253.0977	Carbamazepine 10,11 epoxide		x	2
2.3	[M+H]+	261.1603	Fenspiride	x	x	2

Identification of compounds

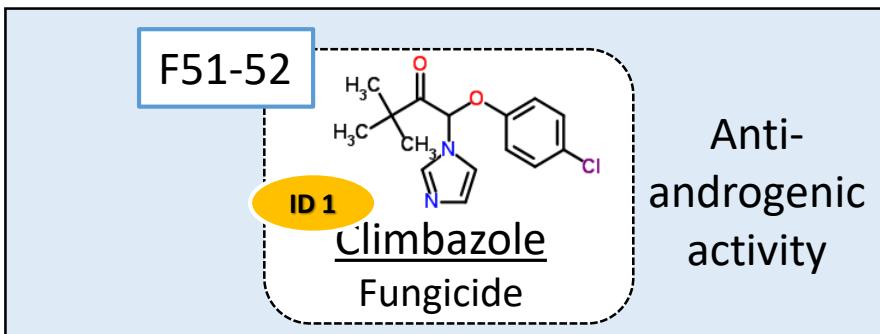
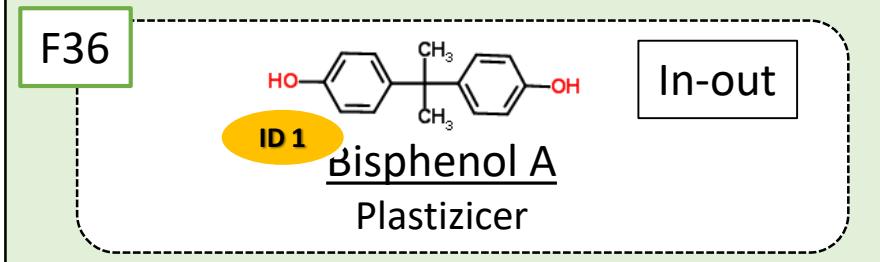
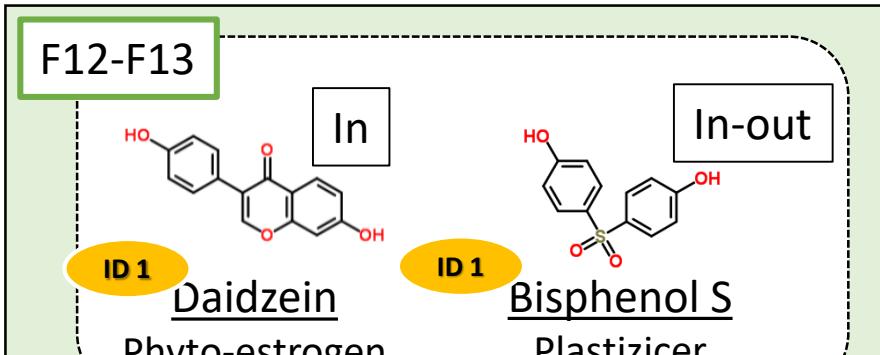
Fraction activity	Fraction	Name	Influent	Effluent	Niveau ID	Family
ER	F12-F13	Diadzein	x		Identified	Phytoestrogen
		Nitrophenol-4	x	x	Suspected	Transformation product (paracetamol, fongicides...)
		Bisphenol S	x	x	Identified	Plasticizer
		O Desmethyltramadol	x	x	Suspected	Transformation product (Tramadol)
		O Desmethylvenlafaxine	x	x	Identified	Transformation product (Venlafaxine)
		Amisulpride	x	x	Identified	Psychotropic
		Acibenzolo	x	x	Identified	Beta blockers
		Desalkylisopyramide		x	Suspected	Transformation product (Disopyramide)
		Carbamazepine 10,11 epoxide		x	Suspected	Transformation product (carbamazepine)
	F21-F23	Fenspiride	x	x	Suspected	Anti-inflammatory
AR/GR	F36	Metoclopramide	x	x	Suspected	Pharmaceutical
		Tramadol	x		Suspected	Analgesic
		Celiprolol	x	x	Suspected	Beta blockers
		Articaine	x		Suspected	Analgesic
		11beta-hydroxyandrost-4-ene-	x		Suspected	Steroid
	F42-F43	Bisphenol A	x	x	Identified	Plasticizer
		Disopyramide	x	x	Identified	Pharmaceutical
		Nordiazepam	x	x	Suspected	Transformation product (Diazepam)
		N Desalkylverapamil (D617)		x	Suspected	Transformation product (verapamil)
		Tiemonium		x	Suspected	Antispasmodic
AR/GR	F51-52	Bisoprolol	x	x	Identified	Beta blockers
		Telmisartan	x	x	Suspected	Pharmaceutical
		Pheniramine	x		Suspected	Antihistamine
		(3 α ,5 β ,6 α)-3-Hydroxy-6-methyl-20-oxopregn-17-yl acetate	x		Suspected	Metabolite (Steroid)
		Propranolol	x	x	Identified	Pharmaceutical
	F58-59	5-hydroxypropafenone		x	Suspected	Transformation product (Propafenone)
		Dipyridamole	x		Identified	Platelet antiaggregant
		Sitagliptin	x		Suspected	Antidiabetic
		Cocaïne	x		Identified	Illegal Drug
		Trospium		x	Suspected	Pharmaceutical
AR/GR	F51-52	Erythromycin	x		Identified	Antibiotic
		Diltiazem	x	x	Suspected	Pharmaceutical
		Climbazole	x	x	Identified	Antifungal
		Citalopram	x	x	Identified	Psychotropic
		Norverapamil		x	Suspected	Transformation product (Verapamil)
	F58-59	8 Hydroxyefavirenz	x	x	Suspected	Transformation product (Efavirenz)
		Atazanavir	x	x	Suspected	Antiretroviral
		Raloxifene	x		Suspected	Hormonal substitution treatments
		Diphenhydramine	x	x	Suspected	Antihistamine
		Betaxolol	x		Suspected	Beta blockers



Ubiquitous compounds

ER
AR/GR

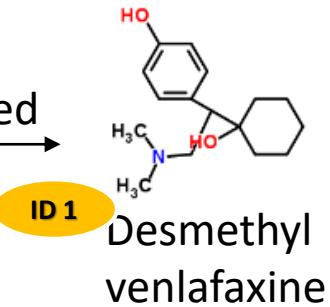
Endocrine disrupting compounds



Transformation products

Fraction	Name	In	Out	Niveau ID
F12-F13	O Desmethyltramadol	x	x	2
	O Desmethylvenlafaxine	x	x	1
F36	Nordiazepam	x	x	2
F42-F43	(3α,5β,6α)-3-Hydroxy-6-methyl-20-oxopregnan-17-yl acetate	x		2
F51-52	8 Hydroxyefavirenz	x	x	2

Confirmed



Need to purchase other suspected TPs

Parent compounds

Fraction	Rt (min)	Ion	m/z	Name	In	Out	Niveau ID
F12-F13	3.6	[M+H] ⁺	370.1809	Amisulpride	x	x	1
	5	[M+H] ⁺	337.2125	Acebutolol	x	x	1
F21-F23	4.5	[M+H] ⁺	300.1479	Metoclopramide	x	x	2
	5.2	[M+H] ⁺	264.1950	Tramadol	x	x	2
	7.7	[M+H] ⁺	380.2546	Celiprolol	x	x	2
	4.3	[M+H] ⁺	285.1257	Articaine	x	x	2
	13.603	[M+H] ⁺	303.1960	11beta-hydroxyandrost-4-ene-	x	x	2
F36	8.4	[M+H] ⁺	326.2337	Bisoprolol	x	x	1
	17.6	[M+H] ⁺	515.2447	Telmisartan	x	x	2
F42-F43	10	[M+H] ⁺	260.1651	Propranolol	x	x	1
F51-52	11.2	[M+H] ⁺	392.2249	Trospium	x	x	2
	13.5	[M+H] ⁺	415.1700	Diltiazem	x	x	2
	15.2	[M+H] ⁺	293.1060	Climbazole	x	x	1
	11	[M+H] ⁺	308.2204	Betaxolol	x	x	2
F58-59	13.3	[M+H] ⁺	328.1217	Loxapine	x	x	2

Conclusion

- ✓ Non target screening allows detecting wide range of compounds and thus allows assessing the overall contamination and generating useful fingerprints (profiling)
- ✓ Usefulness of the EDA based strategy
 - Simplification of information by the selection of only active fractions containing EDCs
 - Identification of several metabolites, drugs, plasticizers, phyto-estrogens with some well known EDCs (Daidzein, BPA, BPS)
 - Several transformation products were detected only in effluent

What remains to do?

- To compare compounds which disappeared in effluent with its which appeared in sludge
- Confirmation of suspected compounds
- Concentrations and activities of confirmed compounds
- Occurrence in aquatic compartment
- Focus on AR activity and AR ligands in relation with no negligible remaining AR activity in effluent (\neq ER activity)

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Research project : **REGARD**

Thank you for your attention