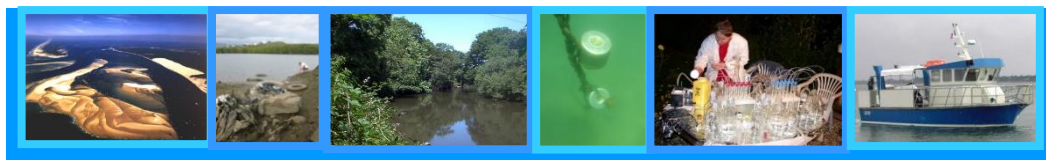




# High resolution mass spectrometry as a tool to elucidate the fate and behaviour of emerging contaminants, specially drugs and metabolites, in waste waters

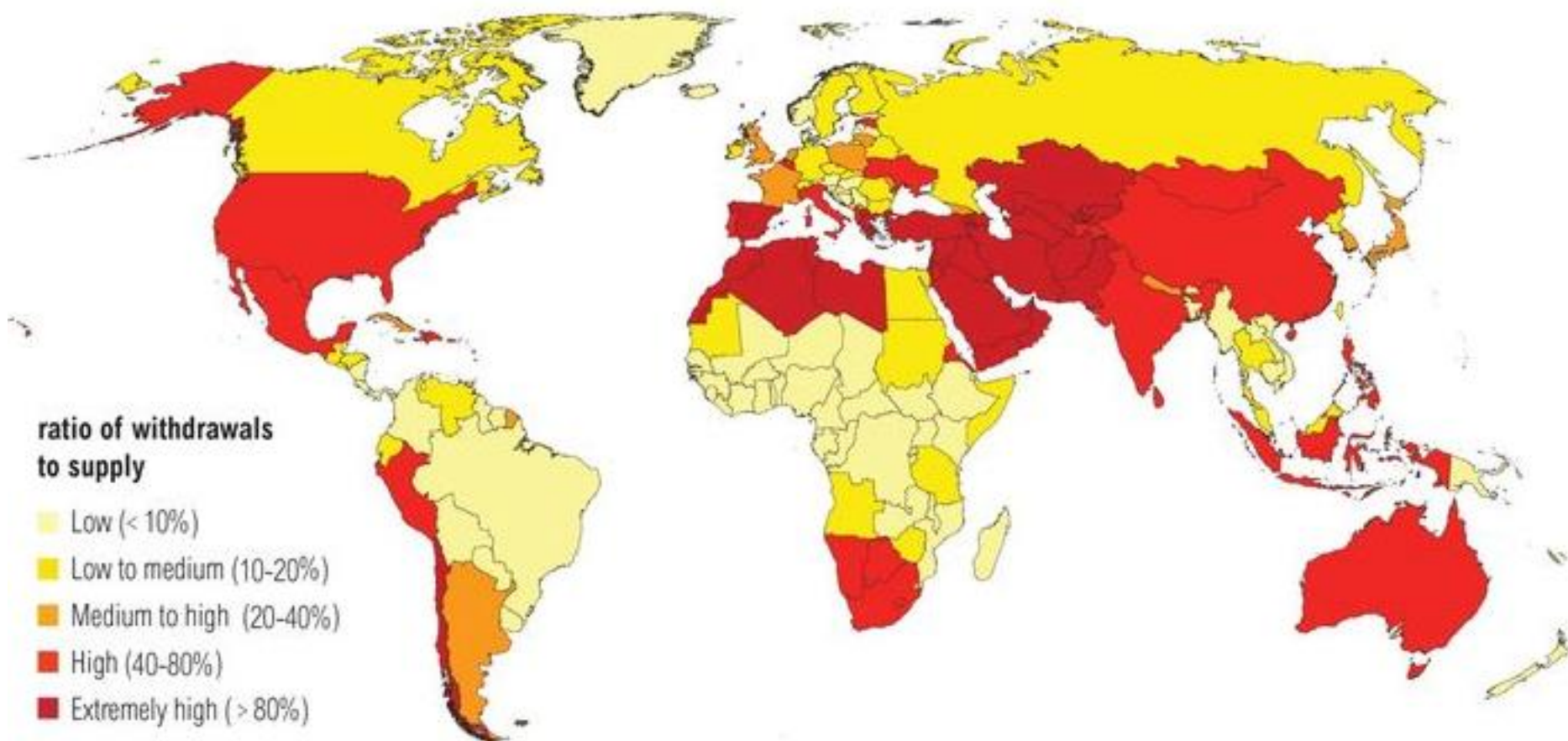
*H. Budzinski, MH. Dévier, C. Gardia-Parège*

*LPTC – EPOC- UMR 5805 CNRS – University of Bordeaux*



université  
de **BORDEAUX**

## Water Stress by Country: 2040



**NOTE:** Projections are based on a business-as-usual scenario using SSP2 and RCP8.5.

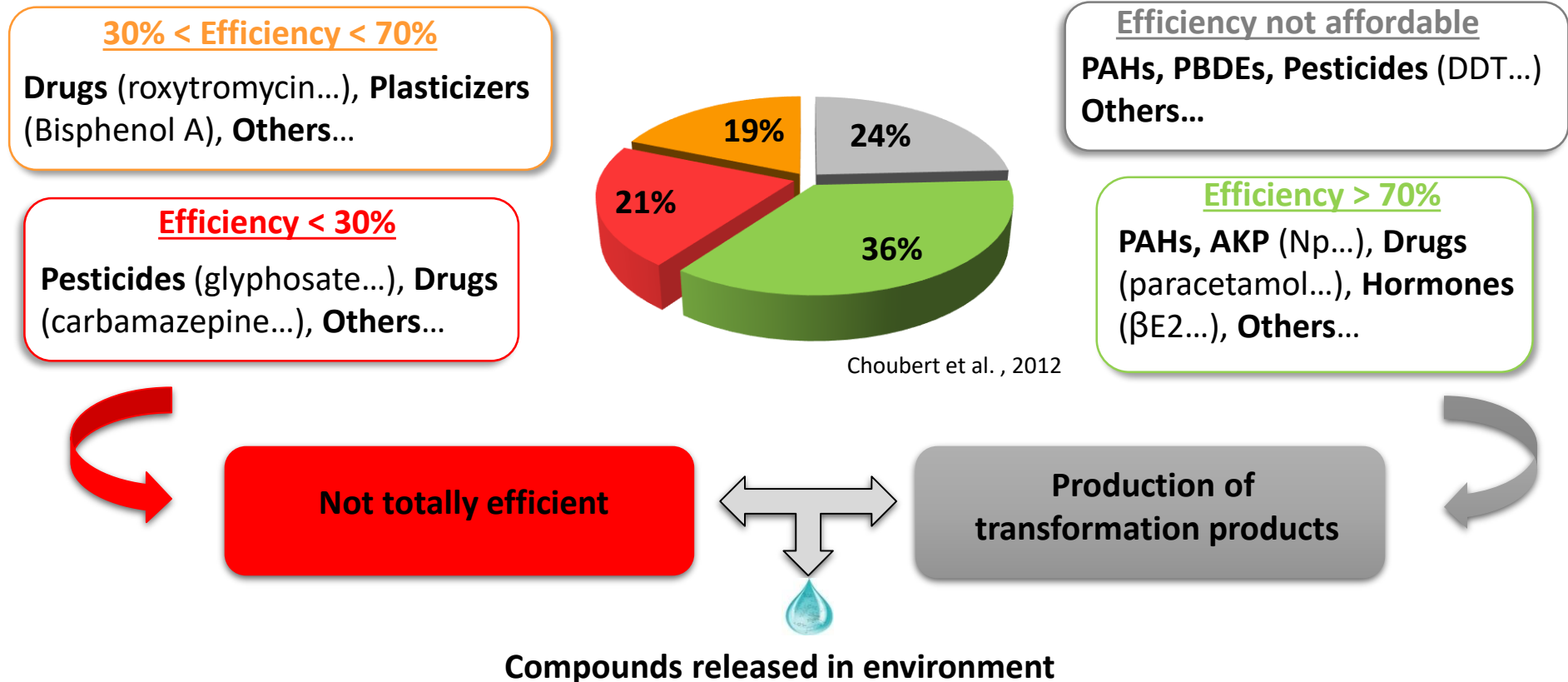
For more: [ow.ly/RiWop](https://ow.ly/RiWop)





# Sources : Wastewater treatment plant (WWTP)

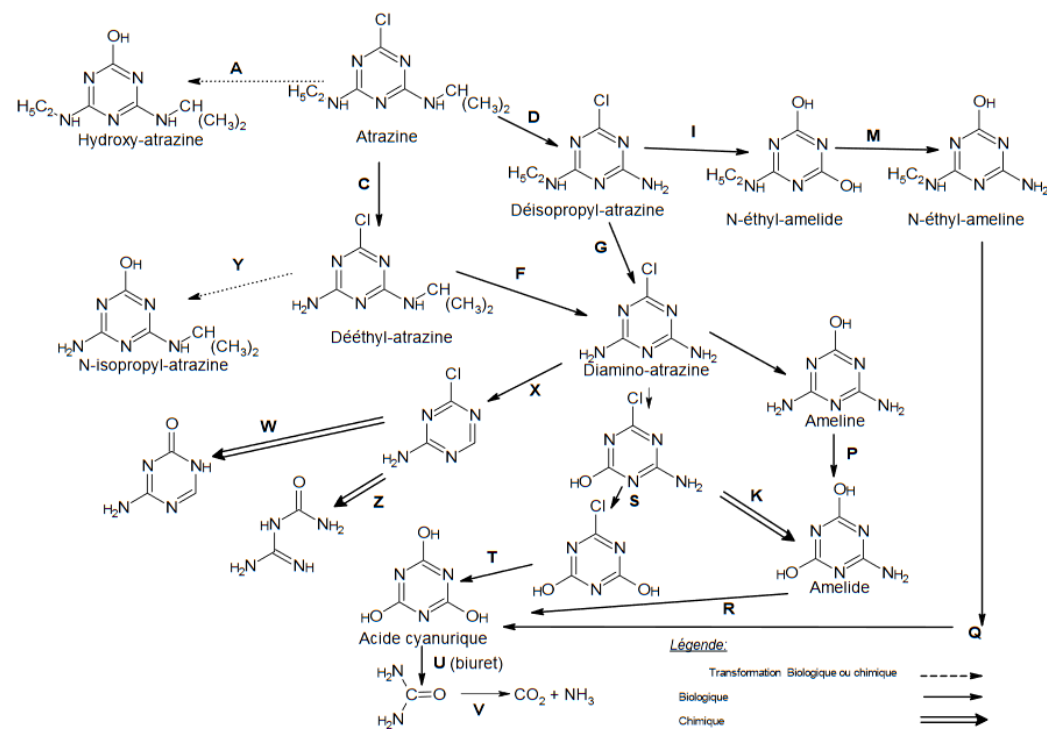
- Waste Water Treatment Plants : AMPERES and Armistiq projects



- Half of compounds are not or partially removed
  - Half of compounds are removed but they could produce transformation products
- **Environmental impact?**

# Context

In environment (during collection, transport, treatment, discharge of waste waters and after in the aquatic ecosystems), compounds could be transformed by biotic and abiotics processes

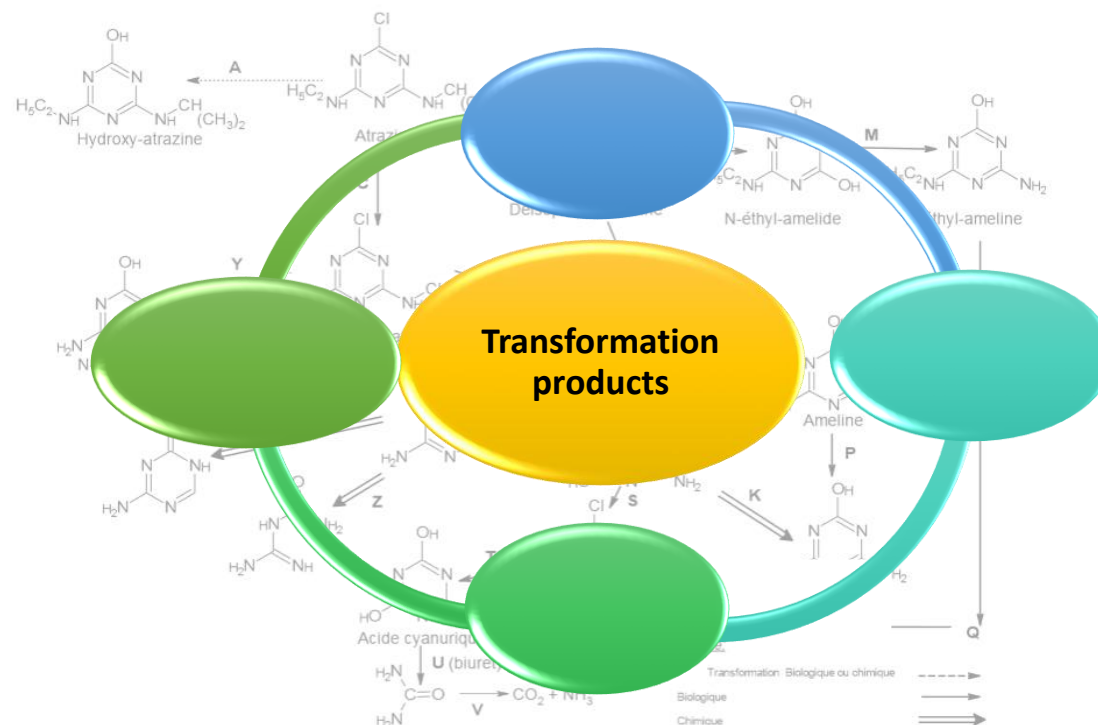


(Canard, 2003)



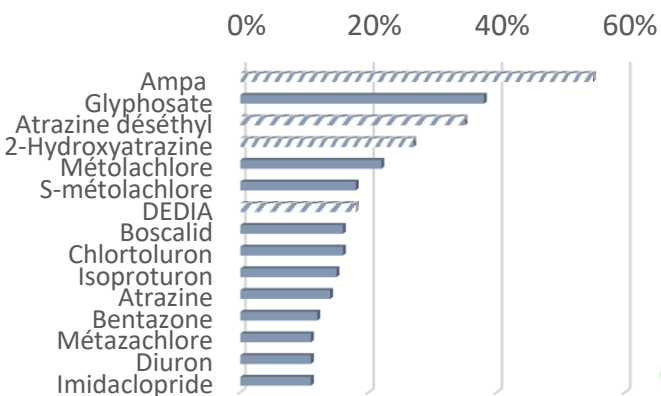
# Context

To date, studies do not (or rarely) consider transformation products, though...

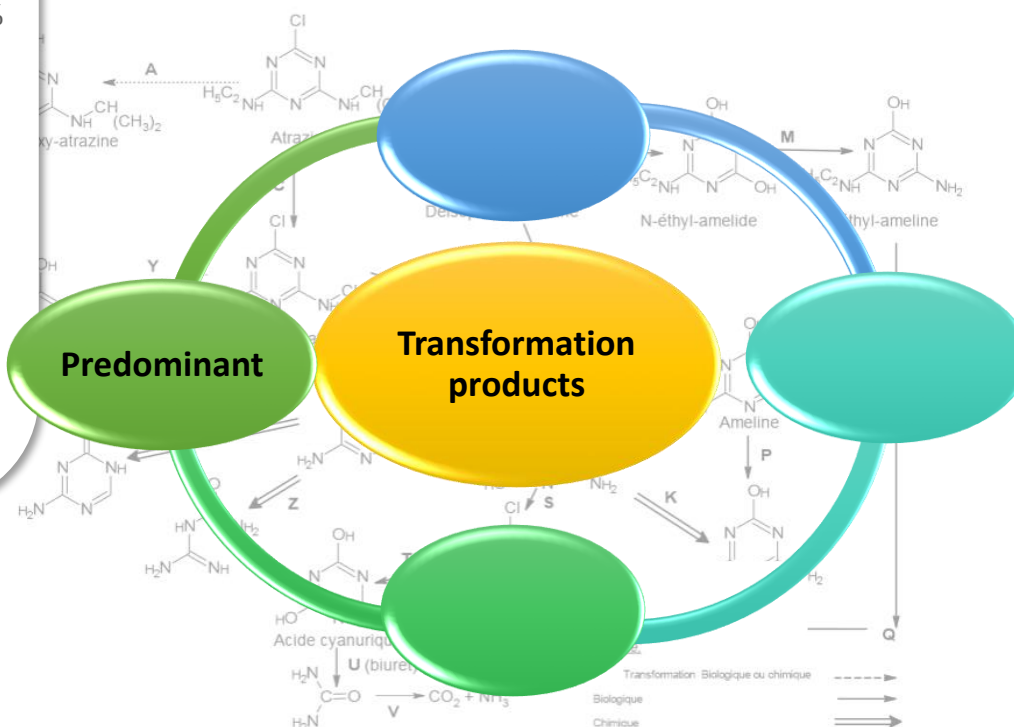


# Context

Pesticide detection rate in rivers in France in 2013



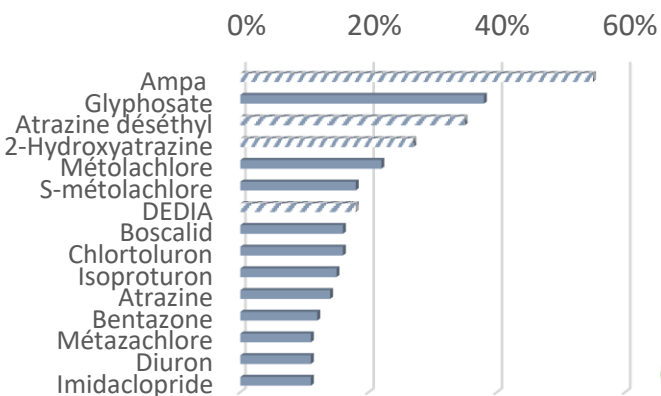
(Commissariat général au développement Durable, n°697, novembre 2015)



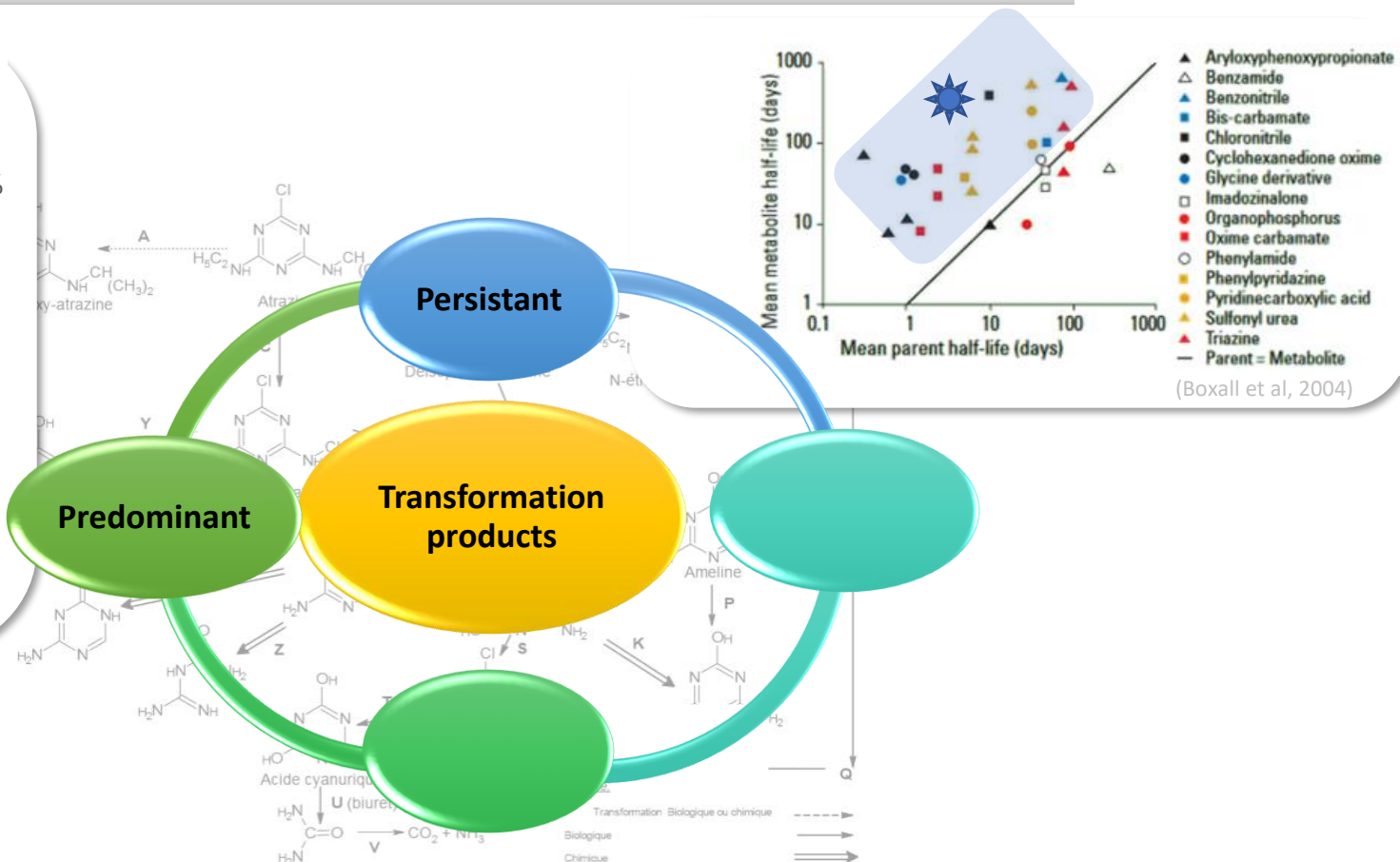


# Context

Pesticide detection rate in rivers in France in 2013

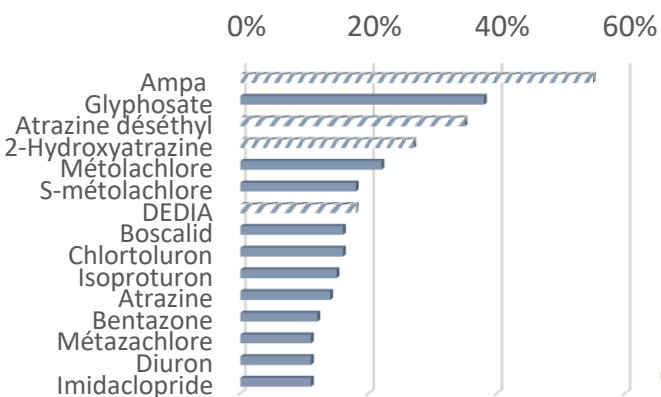


(Commissariat général au développement Durable, n°697, novembre 2015)

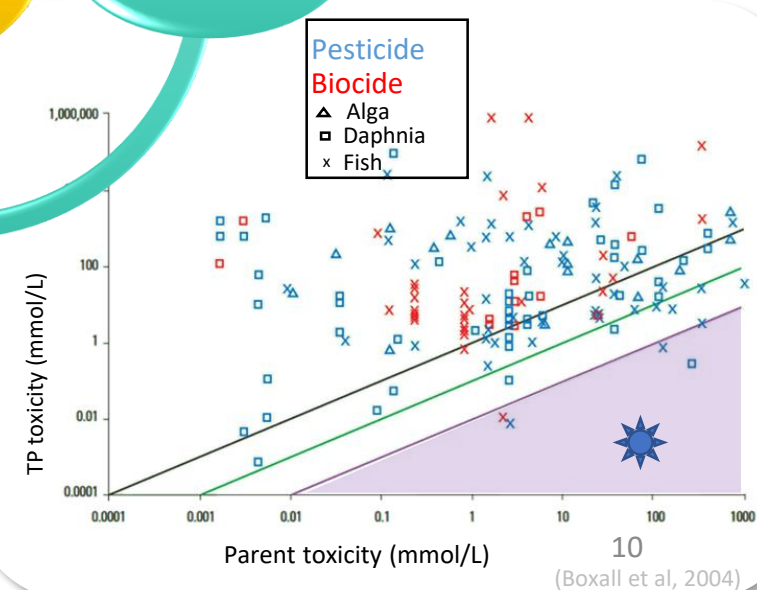
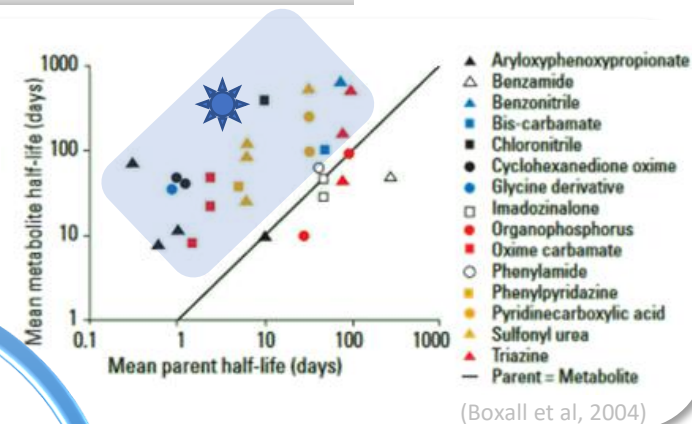
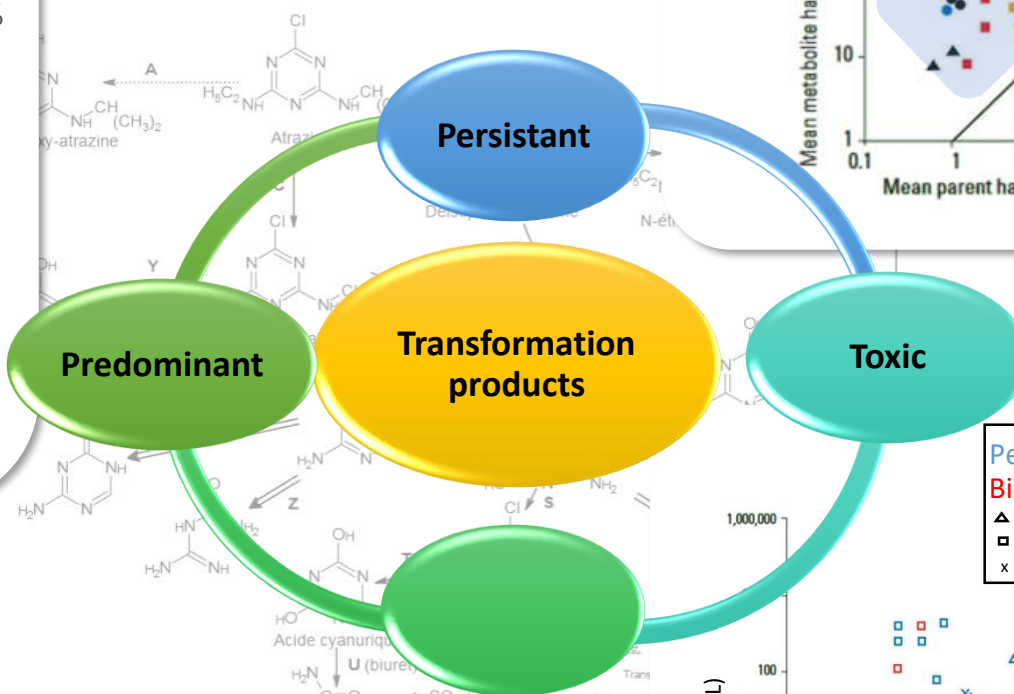


# Context

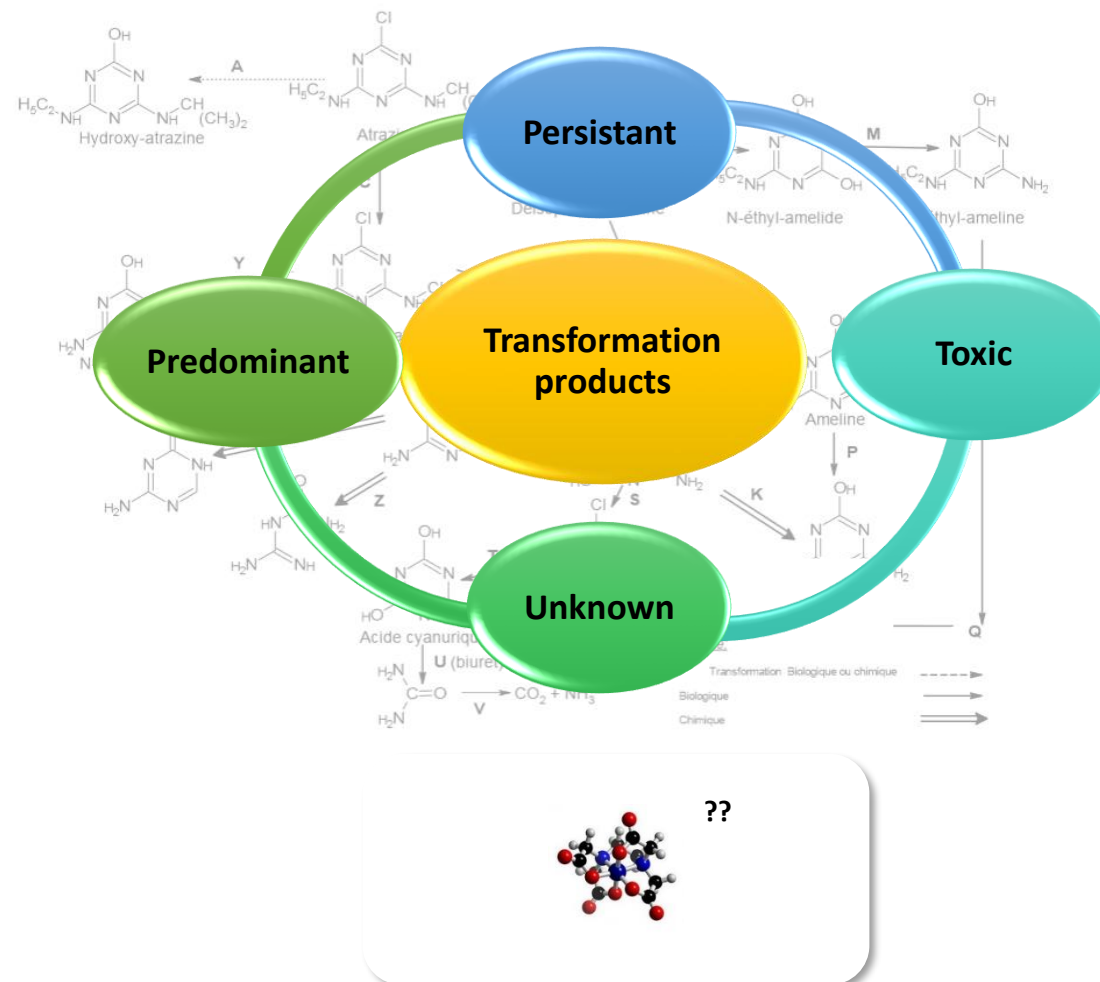
Pesticide detection rate in rivers in France in 2013



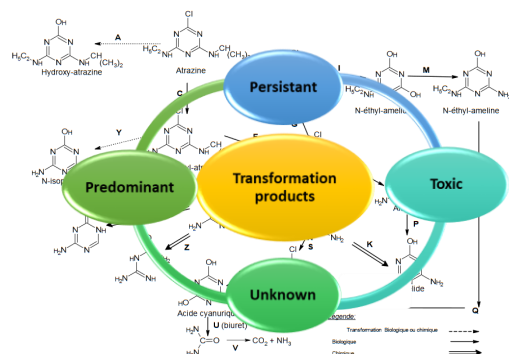
(Commissariat général au développement Durable, n°697, novembre 2015)



# Context



# Aims

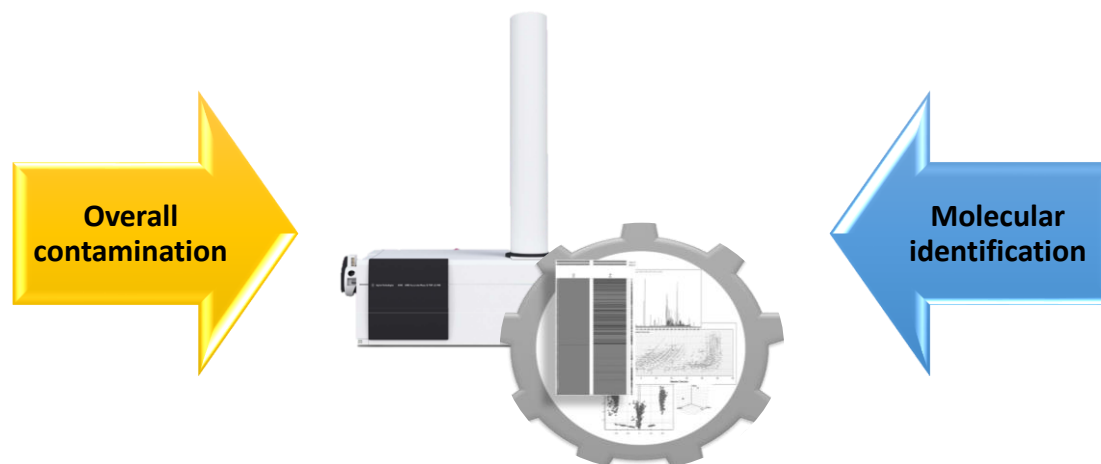


To date, main studies and assessments of chemical quality of aquatic environments are based on **target chemical analysis and pre-selected set of compounds**

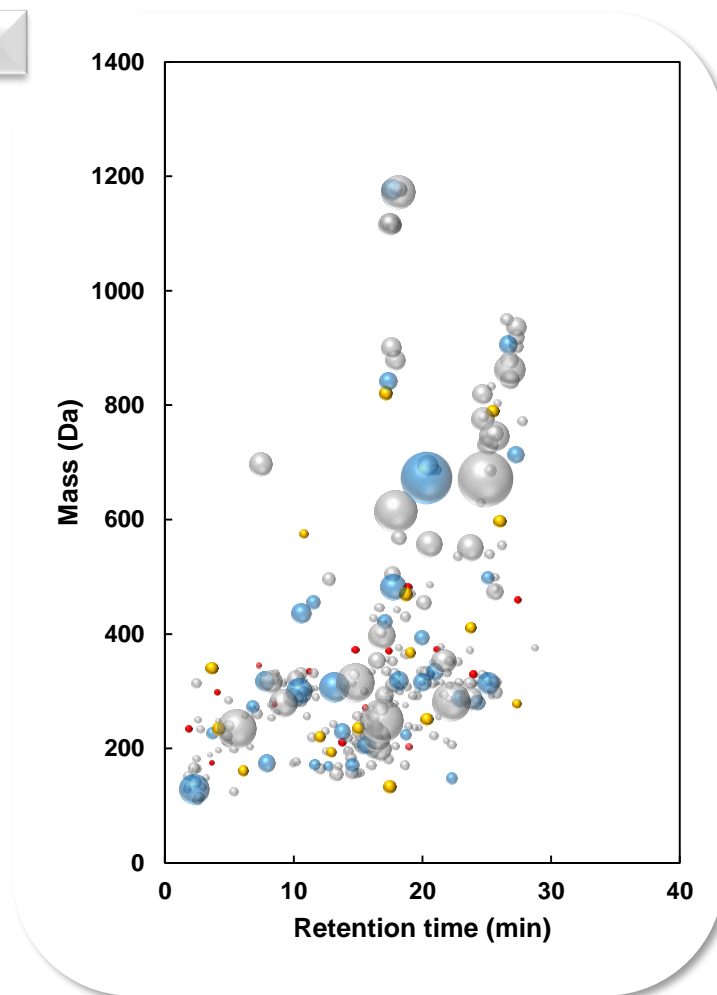
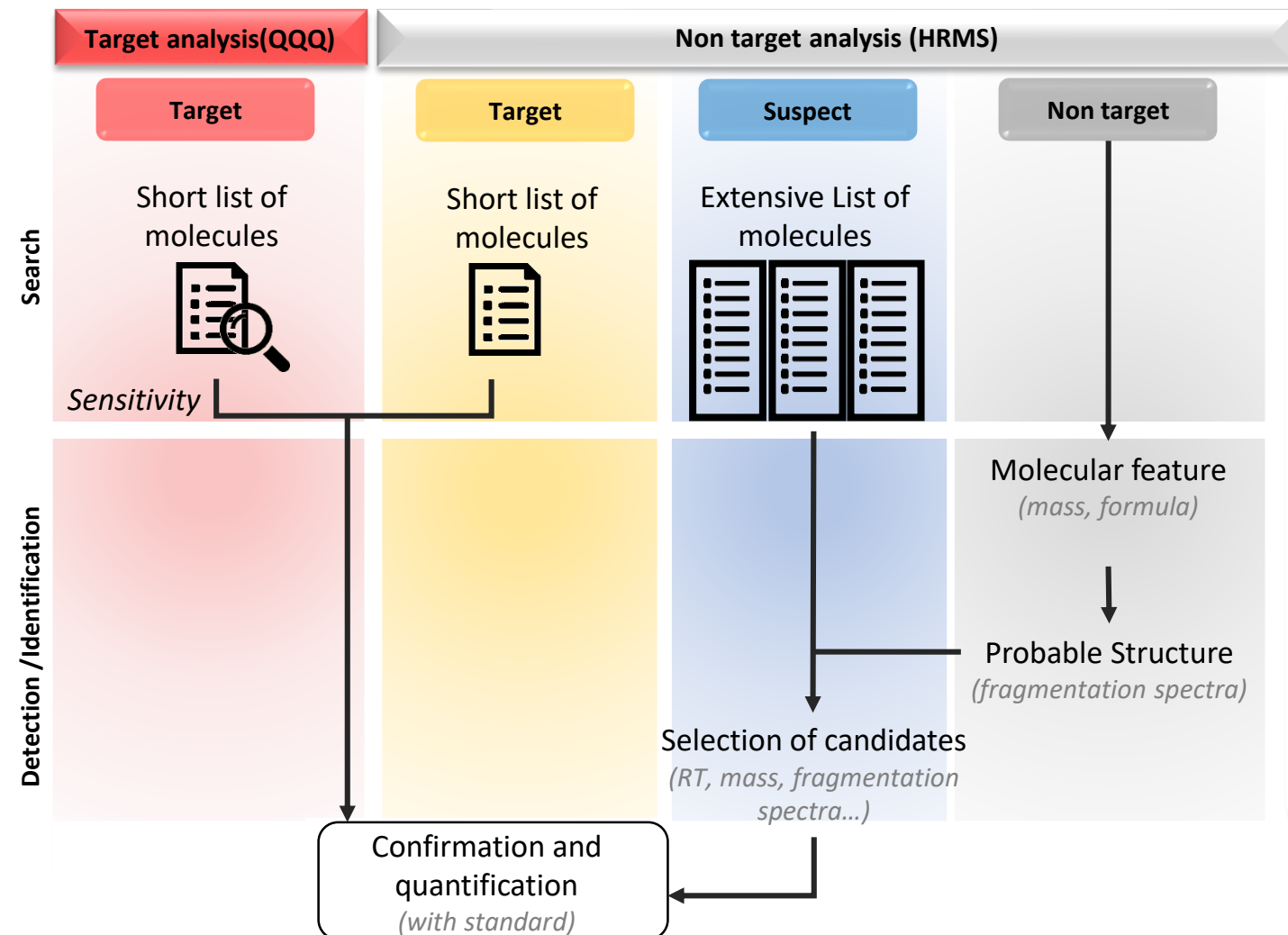


We are only looking for what we know

- Need to use new technologies and alternative approaches to characterise more deeply chemical contamination: Non target analysis



# Analytical strategies



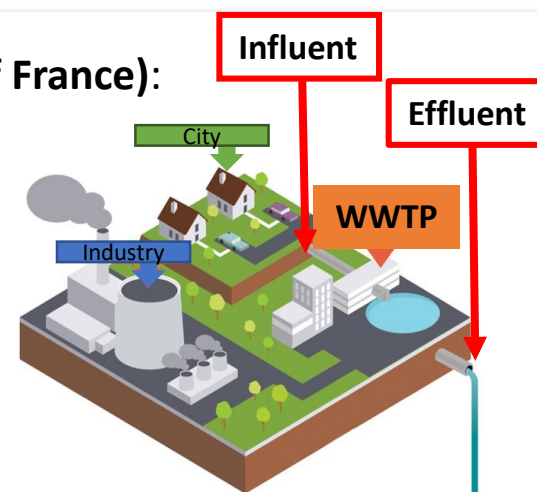
# Study site and methodology

## Urban wastewater treatment plant (Arcachon Area, South west coast of France):

- Influent and effluent :
  - 150 000 population equivalent
  - Secondary treatment : Biofilters

24h composite samples

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



### Sample preparation



3.5 L Influent  
6 L Effluent

Filtration : 0.7 $\mu$ m

Solide phase extraction  
HLB Oasis® cartridge

### Chemical characterisation

Non target  
screening



# Non target analysis workflow

extract influent, effluent and sludges of WWTPs

100-1700  $m/z$

ESI +, ESI-

Molecular screening  
(*Molecular Feature extraction algorithm software*)

10 fold threshold

List of ions

List of features and molecular fingerprint of extract  
(*Mass Profiler*)

Substraction of procedural blank

C, H, O, N, S, Cl, Br, F, P

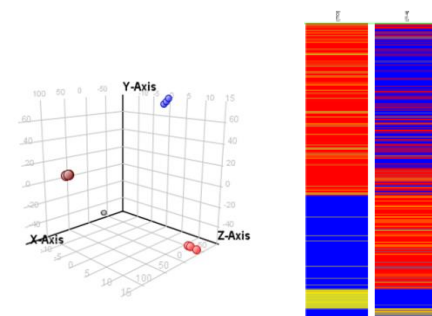
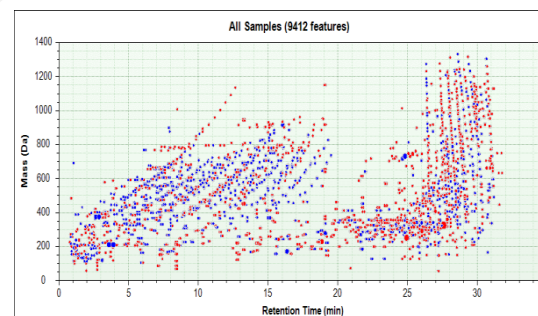
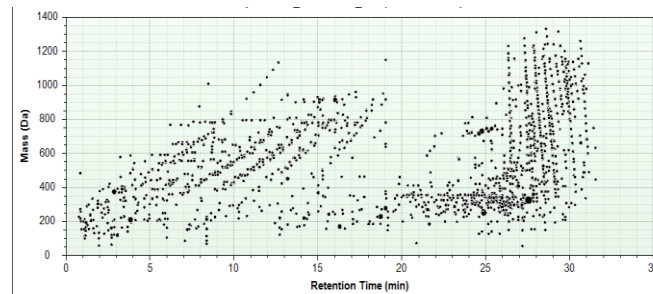
Attribution of formula to sample features

Statistical data analysis  
(*Mass Profiler pro, R*)

Sample	Time	Mass	Intensity	Retention Time	Mass	Intensity	Retention Time	Mass	Intensity	Retention Time
1	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
2	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
3	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
4	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
5	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
6	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
7	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
8	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
9	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
10	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
11	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
12	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
13	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
14	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
15	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
16	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
17	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
18	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
19	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
20	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
21	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
22	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
23	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
24	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
25	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
26	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
27	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
28	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
29	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
30	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
31	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
32	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
33	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
34	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000
35	10.000	100.000	1000000	10.000	100.000	1000000	10.000	100.000	1000000	10.000



Mass spectrometer:  
**Agilent 6540 Q-TOF LC/MS**

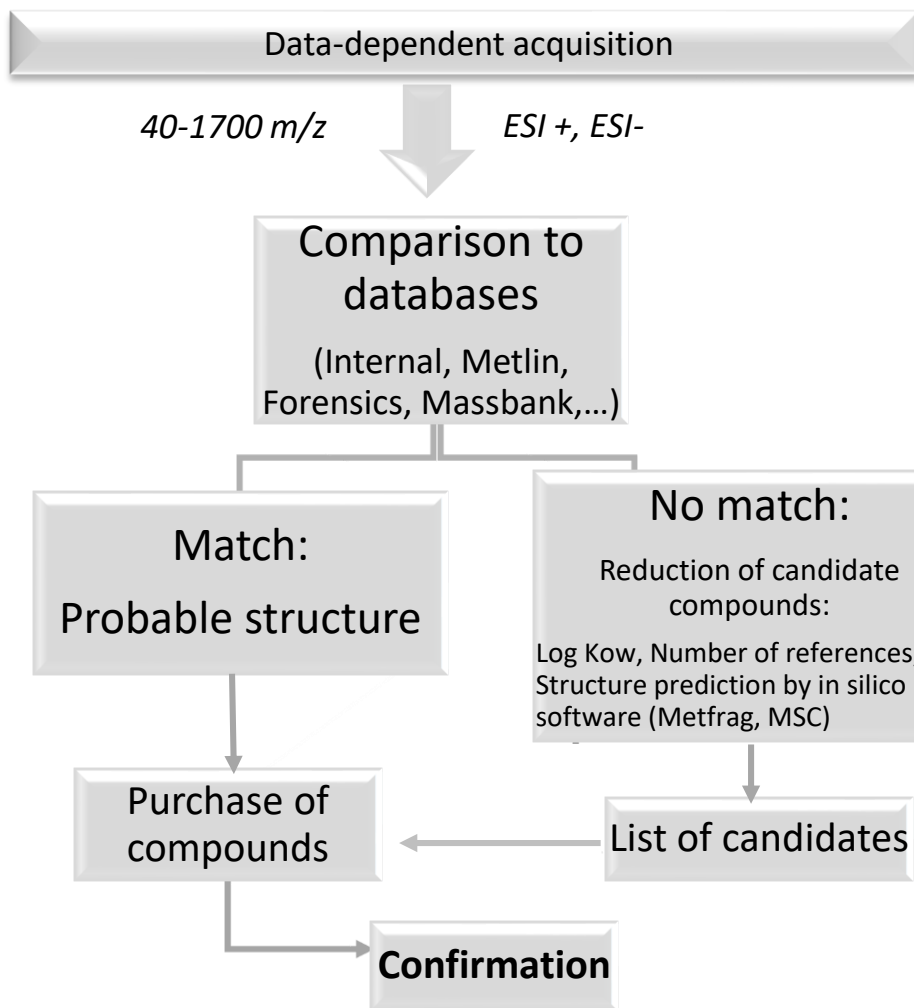


MS mode



# Identification workflow

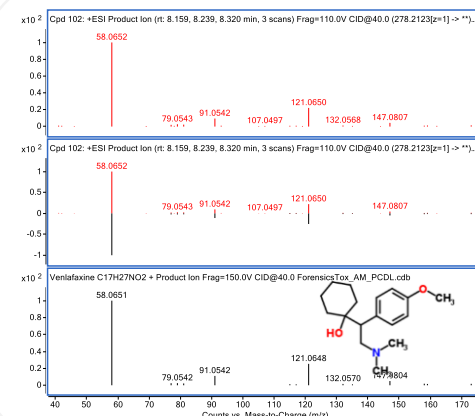
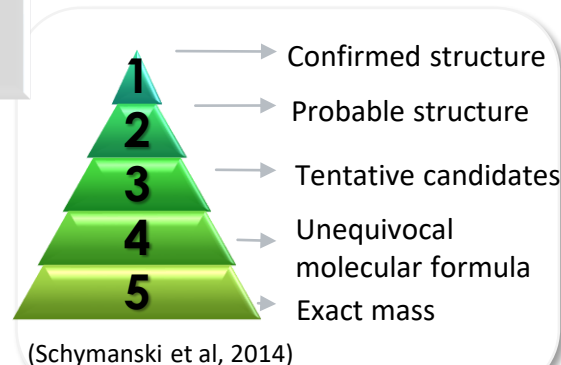
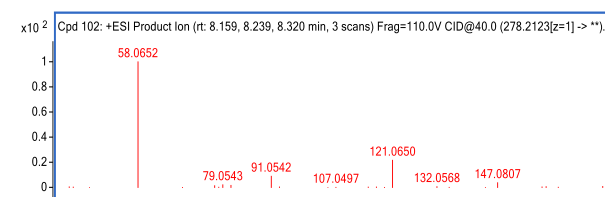
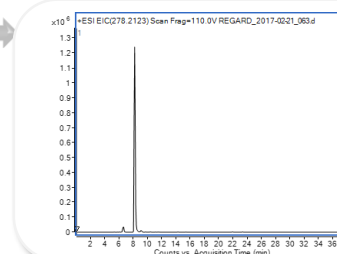
MS/MS mode



Sample	Time	Scan	Mass	Intensity	Retention	Scan	Mass	Intensity	Retention
1	8.159	110	58.0652	1000000	8.159	110	58.0652	1000000	8.159
2	8.239	110	58.0652	1000000	8.239	110	58.0652	1000000	8.239
3	8.320	110	58.0652	1000000	8.320	110	58.0652	1000000	8.320

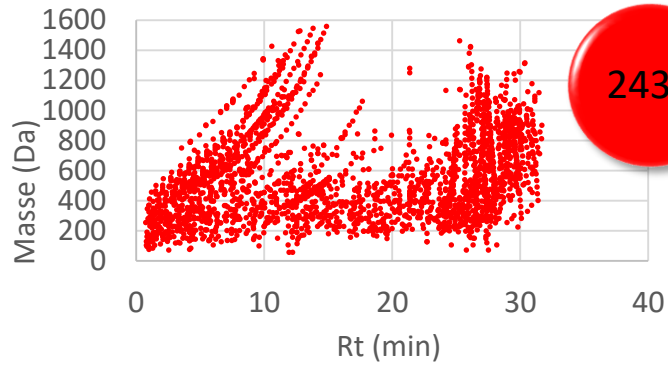


Mass spectrometer:  
**Agilent 6540 Q-TOF LC/MS**

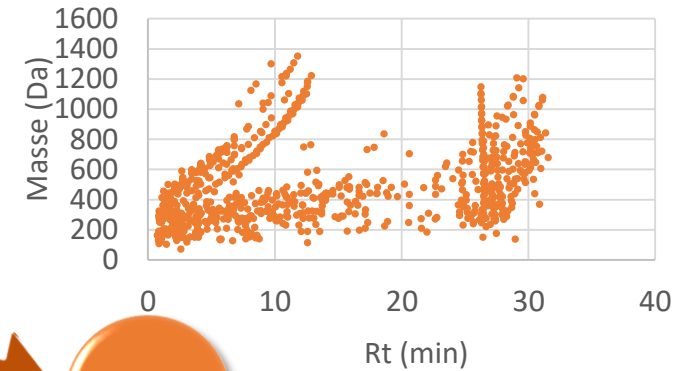


# Overall analysis – wastewater treatment plant

Influent

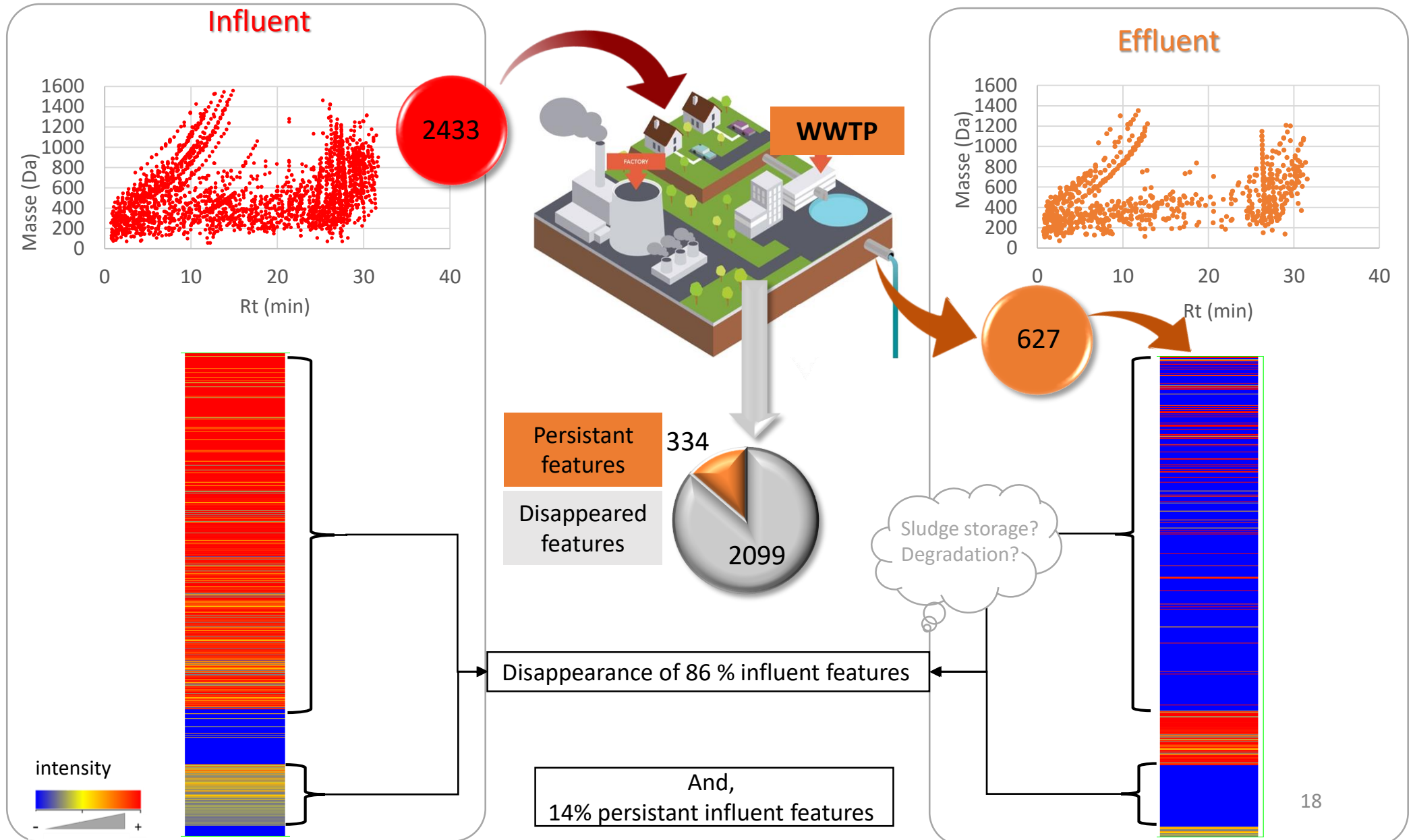


Effluent

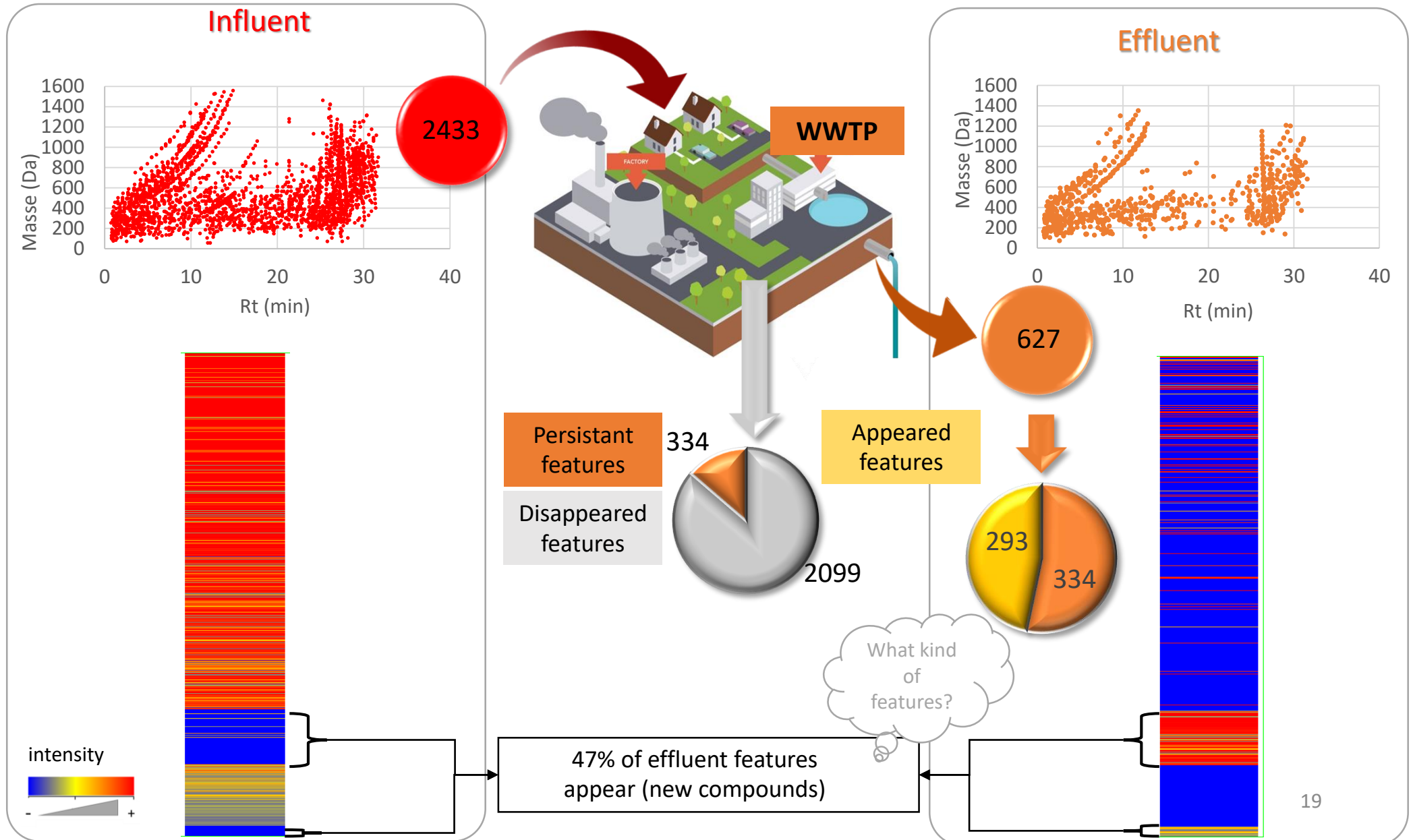


In terms of total number of features:  
Decrease of 74% between influent and effluent

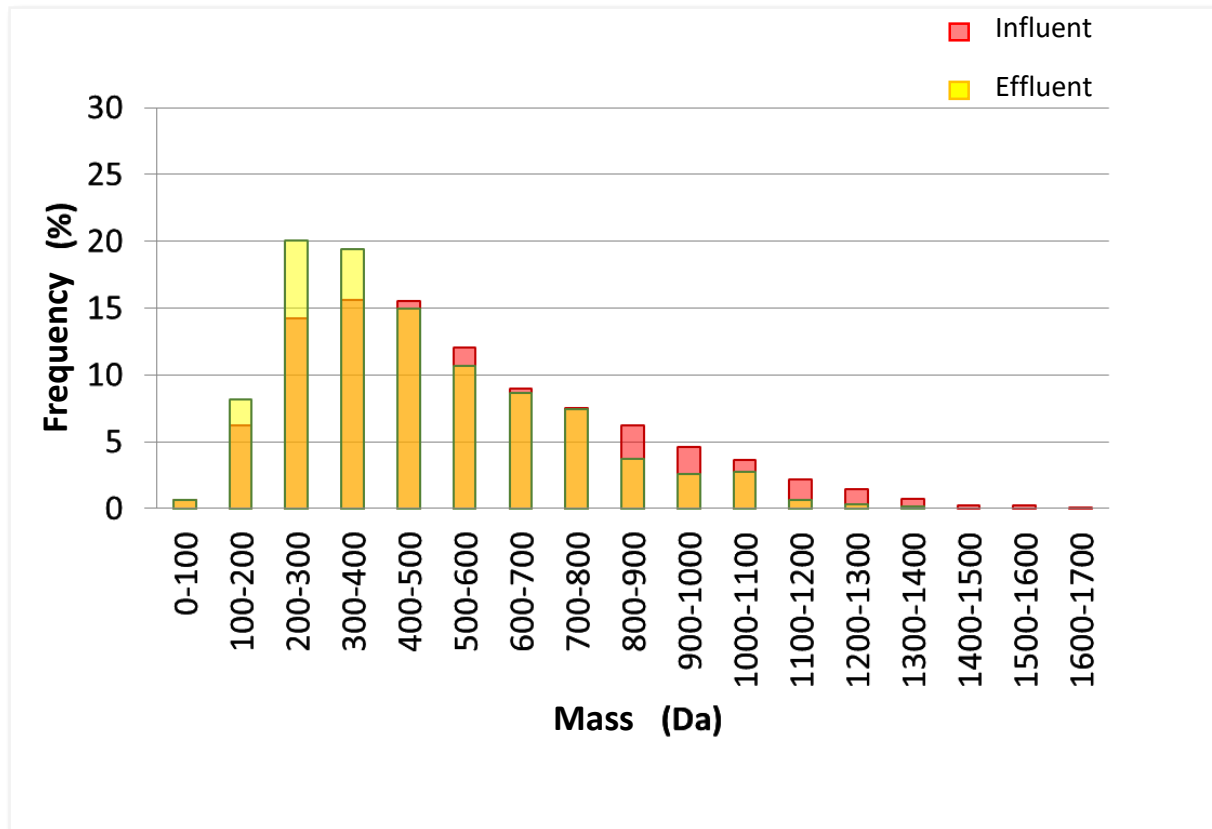
# Overall analysis – wastewater treatment plant



# Overall analysis – wastewater treatment plant



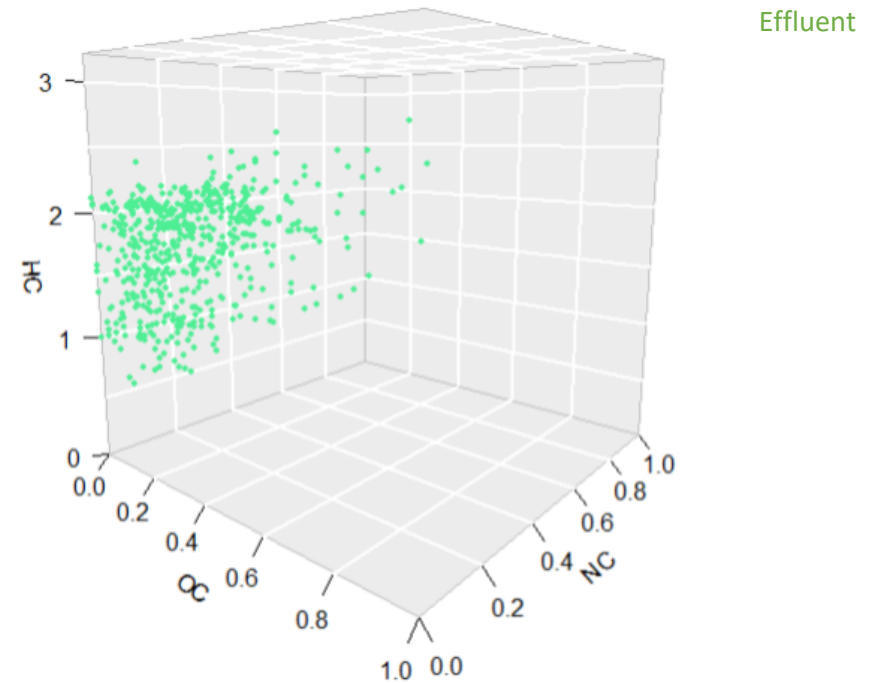
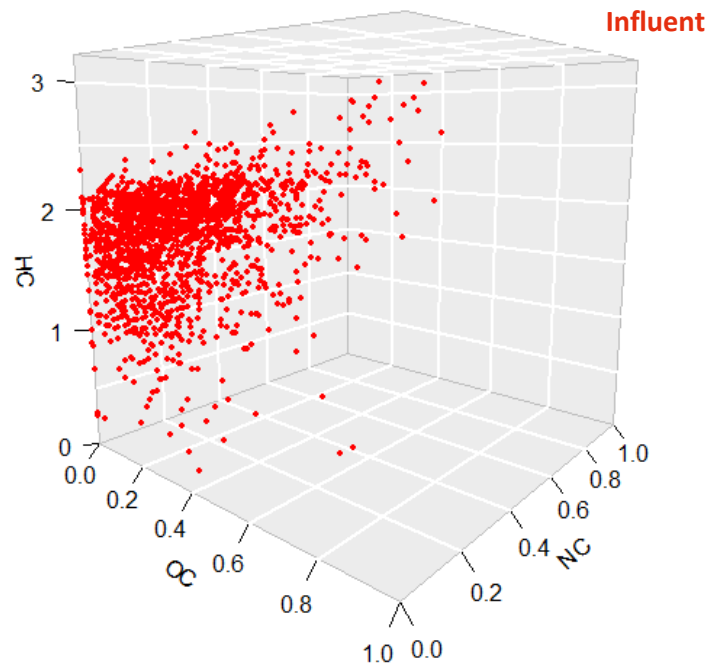
# Overall characterisation



## Mass distribution

- Weak difference between influent and effluent
  - Higher masses observed in influent
  - Lower masses observed in effluent

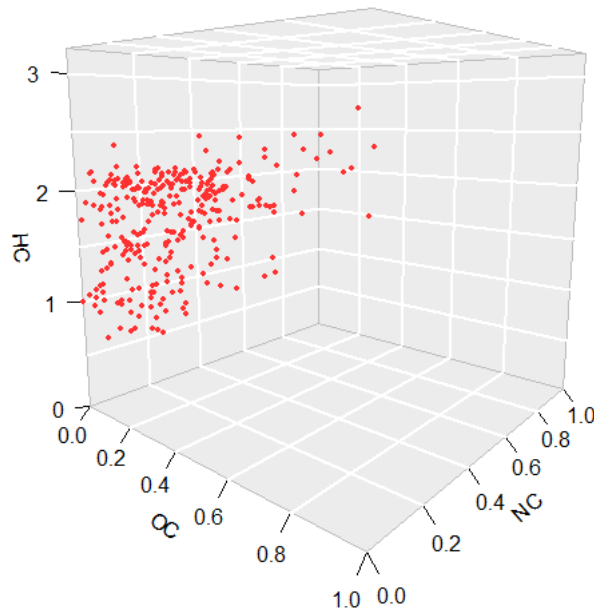
# Overall characterisation



- Loss of several features
- But, distribution is globally conserved : low structural evolution (low oxidation, low hydrogenation or deshydrogenation)

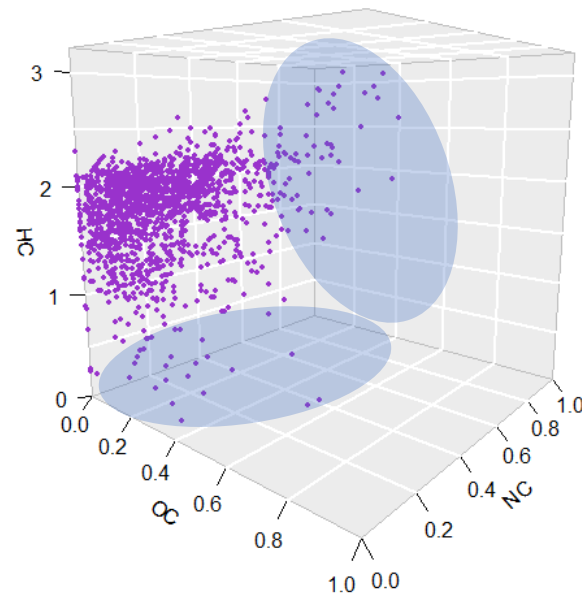
# Overall characterisation

Influent



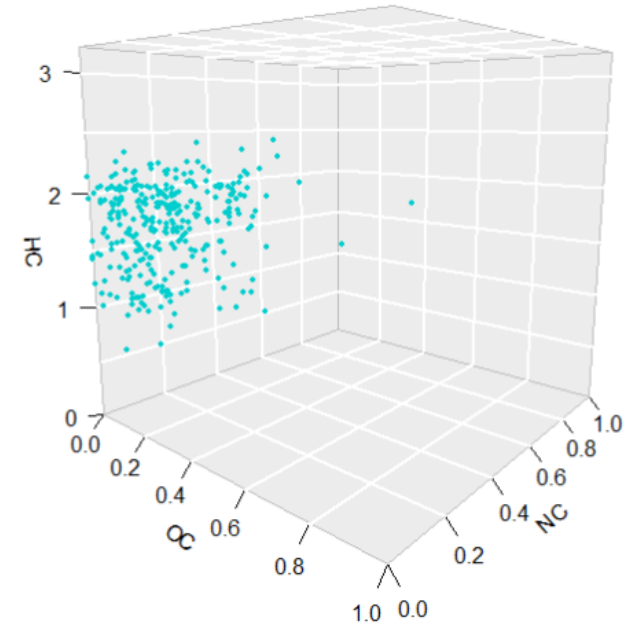
*Persistent features  
Appear slightly less hydrogenated  
and oxygenated*

Influent



*Features which disappear*

Effluent

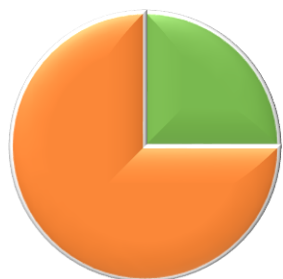


*Features which appear  
Appear less oxygenated  
and less nitrogenous ones*



# Identification – spectral database

Effluent



■ Suspected Compounds

■ Unidentified features

Name	id	Family	Influent	Effluent
Cetirizine	1	Drug	x	x
Dipyridamole	1	Drug	x	x
Propanolol	1	Drug		x
Venlafaxine	1	Psychotropic	x	x
Clarithromycin	2	Antibiotic	x	x
Diltiazem	2	Drug	x	x
5-Hydroxypropafenone	2	TP		x
Climbazole	2	Antifungal	x	x
Denatonium	2	Repulsive	x	x
Deoxyadenosine	2	Metabolite		x
Fexofenadine	2	Drug	x	x
Flecanaide	2	Drug	x	x
Irbesartan	2	Drug	x	x
N-Bis(desalkyl)verapamil (D620)	2	TP	x	x
N-Desalkylverapamil	2	TP		x
Norcitalopram	2	TP	x	x
Perindopril	2	Drug		x
Roxithromycin	2	Antibiotic	x	x
Tiemonium	2	Quaternary ammonium	x	x
Trospium	2	Quaternary ammonium	x	x
Verapamil	2	Drug	x	x
Memantine	2	Drug	x	x
Clarithromycin-N-oxide	3	TP	x	x
N-monodesmethyldiltiazem	3	TP	x	x
O- Desmethylvenlafaxine	3	TP	x	x

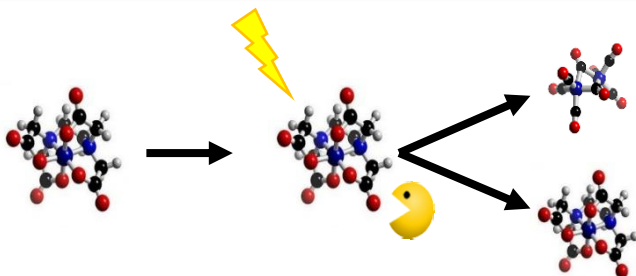
Spectral libraries are limited to some classes : drugs are predominant families in databases

➤ drugs were pointed out in majority

# Identification – case of transformation products

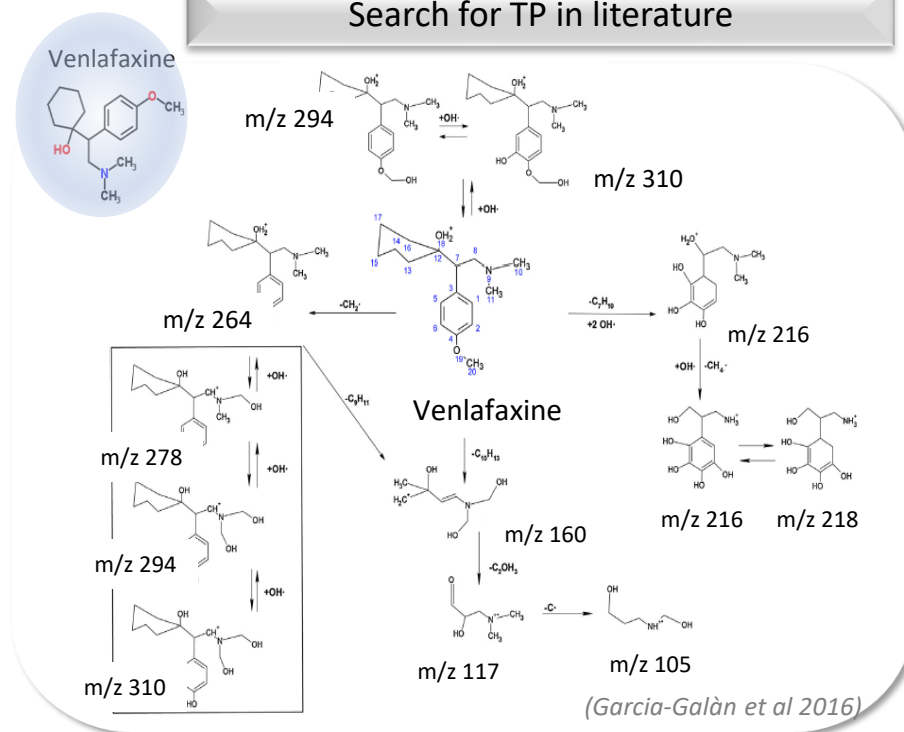
- Establishment of a list of suspects based on known and referenced transformation products or on theoretical transformation products (oxidation, hydrolysis, etc.)

Search for TPs based on experimentation and on in silico calculation



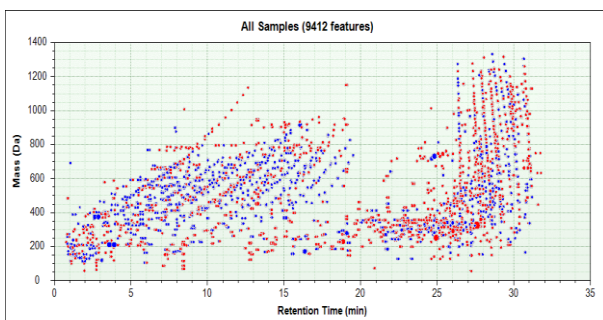
- To create transformation products using lab experimentation
  - Identification of transformation products
- And/or
- To establish lists of suspects based on transformation processes (*in silico*; home-made macro, Eawag PSS)

Search for TP in literature



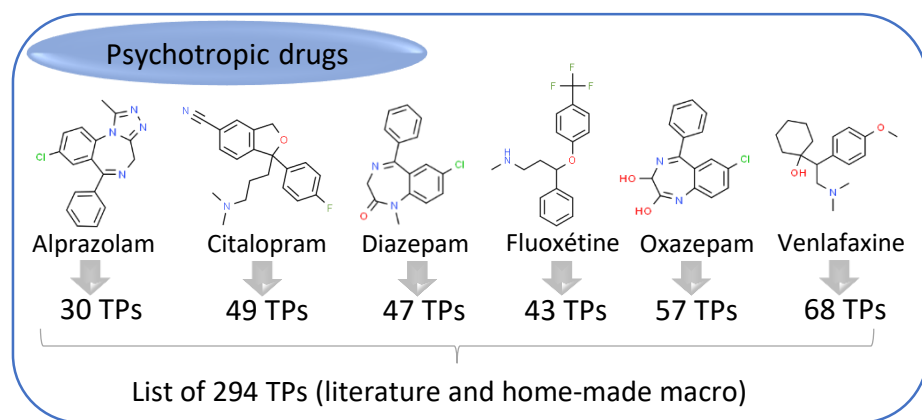
## Identification – case of transformation products

- Establishment of a list of features based on the comparison of chemical fingerprint (features which appear)



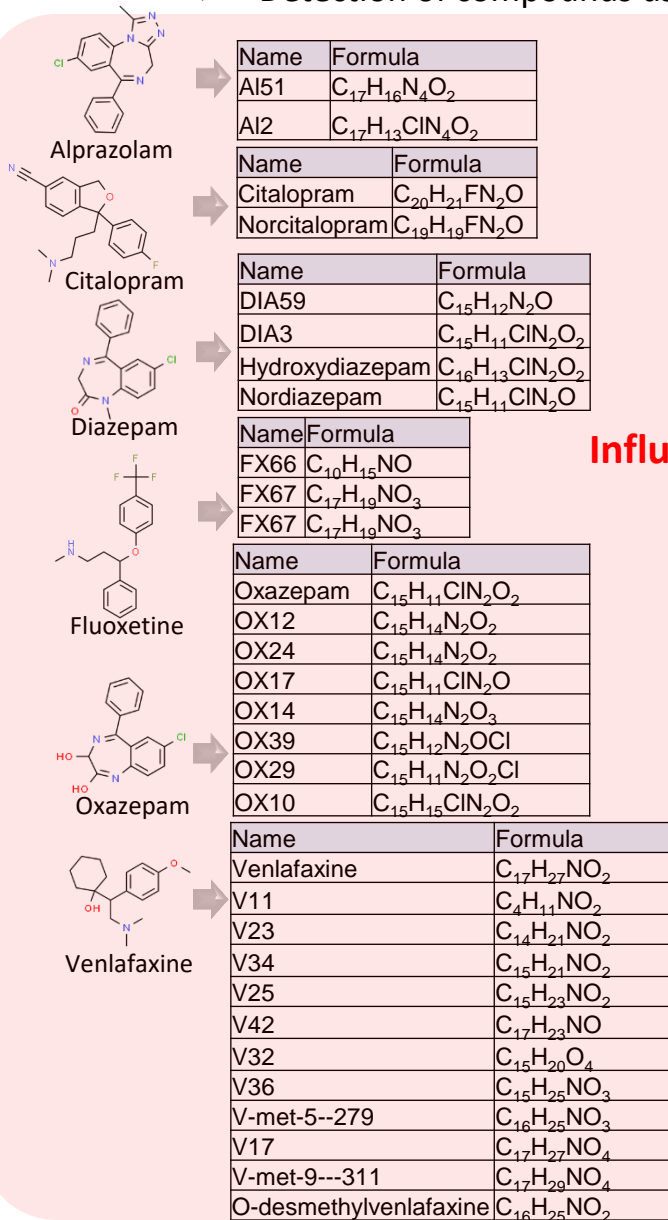
	Mans				Mans				Mans				RT						
0.705	161.027	3.05	204.0838	6.775	627.3125	10.158	440.2617	13.684	322.2253	24.871	356.2333	26.505	52.3477	28.351	521.7496				
0.712	152.2978	3.295	154.1106	6.781	272.1377	10.168	259.1577	13.73	306.204	24.902	356.3449	26.545	220.1297	29.049	360.2875				
0.719	143.0625	3.54	106.0838	6.787	162.1864	10.174	186.2177	13.77	256.204	24.933	356.4569	26.589	112.1268	30.163	311.228				
0.828	282.0273	3.84	652.2969	6.805	656.4207	10.572	882.4684	13.923	462.2382	26.009	356.3353	27.925	728.2343	28.521	520.3675				
0.875	267.0971	3.78	183.1371	6.809	471.1113	10.598	366.2262	13.973	442.2110	25.009	434.1324	26.815	685.0599	28.625	363.3835				
0.922	261.0971	3.78	183.1371	6.809	471.1113	10.598	366.2262	13.973	442.2110	25.009	434.1324	26.815	685.0599	28.625	363.3835				
0.944	182.0971	3.878	133.1842	6.787	281.0556	10.627	1215.6860	14.012	247.2674	25.304	654.4173	26.847	674.3137	28.847	674.3137	762.5455			
0.948	155.1536	3.934	444.2117	7.129	1036.1422	10.631	483.2838	14.559	436.2828	25.426	200.2091	26.908	480.1743	29.039	1150.7498				
0.955	155.1536	3.934	444.2117	7.129	1036.1422	10.631	483.2838	14.559	436.2828	25.426	200.2091	26.908	480.1743	29.039	1150.7498				
1.03	192.0977	3.845	245.1775	7.238	1082.2001	10.852	323.1673	15.005	492.309	25.544	556.4629	26.855	266.0267	28.789	701.1799				
1.052	268.1523	3.845	524.2442	7.255	345.1149	10.883	484.2875	15.361	212.0979	25.677	778.5044	27.023	560.4003	28.831	508.4189				
1.055	268.1523	3.845	524.2442	7.255	345.1149	10.883	484.2875	15.361	212.0979	25.677	778.5044	27.023	560.4003	28.831	508.4189				
1.1	306.0988	4.05	604.3308	7.395	866.3335	10.905	418.2541	15.738	364.2479	25.749	422.324	27.023	560.3475	28.928	441.3163				
1.122	408.1182	4.12	181.1212	7.395	744.4129	10.903	369.213	15.778	456.3562	26.018	466.159	27.097	340.243	29.123	536.3033				
1.125	408.1182	4.12	181.1212	7.395	744.4129	10.903	369.213	15.778	456.3562	26.018	466.159	27.097	340.243	29.123	536.3033				
1.211	207.1314	4.19	604.331	7.395	328.1799	11.109	554.511	15.826	357.307	26.123	1143.7752	27.137	646.3635	28.987	626.8305				
1.232	186.1869	4.21	444.2172	7.5	277.1137	11.399	391.2145	15.865	468.3927	26.24	334.2252	27.142	428.275	29.098	1208.8017				
1.235	186.1869	4.21	444.2172	7.5	277.1137	11.399	391.2145	15.865	468.3927	26.24	334.2252	27.142	428.275	29.098	1208.8017				
1.307	219.1691	4.474	250.0709	7.676	665.3337	11.498	357.1938	16.387											
1.308	219.1691	4.474	250.0709	7.676	665.3337	11.498	357.1938	16.387											
1.388	150.1514	4.604	307.2288	7.745	885.4851	11.542	386.1825	16.605	482.3088	26.248	638.9645	27.127	722.4526	29.184	638.4595				
1.391	150.1514	4.604	307.2288	7.745	885.4851	11.542	386.1825	16.605	482.3088	26.248	638.9645	27.127	722.4526	29.184	638.4595				
1.525	365.1551	4.661	315.1043	7.886	912.2693	11.912	1074.6048	16.605	454.2631	26.248	1027.7663	27.255	816.4622	29.566	718.4598				
1.526	365.1551	4.661	315.1043	7.886	912.2693	11.912	1074.6048	16.605	454.2631	26.248	1027.7663	27.255	816.4622	29.566	718.4598				
1.635	262.1638	4.68	260.1884	7.956	153.1152	11.956	307.1572	17.107	514.2372	26.275	101.1829	27.284	216.2564	29.575	1066.789				
1.636	262.1638	4.68	260.1884	7.956	153.1152	11.956	307.1572	17.107	514.2372	26.275	101.1829	27.284	216.2564	29.575	1066.789				
1.733	265.1677	4.68	546.2686	8.097	173.1671	12.139	1058.5732	17.109	304.1688	26.276	304.1688	27.284	216.2564	29.575	1066.789				
1.734	265.1677	4.68	546.2686	8.097	173.1671	12.139	1058.5732	17.109	304.1688	26.276	304.1688	27.284	216.2564	29.575	1066.789				
1.829	221.1771	4.703	686.2019	8.115	436.2464	12.197	1088.653	17.144	333.2668	26.28	607.6672	27.413	238.4525	29.669	518.751				
1.830	221.1771	4.703	686.2019	8.115	436.2464	12.197	1088.653	17.144	333.2668	26.28	607.6672	27.413	238.4525	29.669	518.751				
1.957	222.1276	5.038	280.1885	8.386	956.3864	12.924	131.1482	17.243	373.1616	26.305	508.5958	27.491	450.6283	29.733	652.4775				
1.958	222.1276	5.038	280.1885	8.386	956.3864	12.924	131.1482	17.243	373.1616	26.305	508.5958	27.491	450.6283	29.733	652.4775				
2.097	471.2625	5.146	650.3728	8.411	277.0289	12.22	666.6667	17.465	358.2658	26.306	524.5958	27.507	624.547	29.733	556.895				
2.098	471.2625	5.146	650.3728	8.411	277.0289	12.22	666.6667	17.465	358.2658	26.306	524.5958	27.507	624.547	29.733	556.895				
2.111	300.1308	5.345	305.1737	8.417	117.0798	12.245	749.4562	18.02	402.3454	26.322	878.614	27.652	265.6268	29.822	638.9575				
2.112	300.1308	5.345	305.1737	8.417	117.0798	12.245	749.4562	18.02	402.3454	26.322	878.614	27.652	265.6268	29.822	638.9575				
2.191	474.1371	5.512	692.3805	8.592	164.0307	12.48	1102.5895	18.081	839.5248	26.338	856.5742	27.684	476.4249	30.074	710.518				
2.194	474.1371	5.512	692.3805	8.592	164.0307	12.48	1102.5895	18.081	839.5248	26.338	856.5742	27.684	476.4249	30.074	710.518				
2.28	168.0509	6.099	324.2145	8.888	528.1841	12.628	324.1637	21.506	273.2677	26.34	659.482	28.01	434.3505	30.386	768.9585				
2.281	168.0509	6.099	324.2145	8.888	528.1841	12.628	324.1637	21.506	273.2677	26.34	659.482	28.01	434.3505	30.386	768.9585				
2.339	222.1445	6.377	277.1598	8.918	748.5089	12.853	1184.6759	21.506	273.2677	26.34	756.1568	27.789	522.379	30.475	534.505				
2.34	222.1445	6.377	277.1598	8.918	748.5089	12.853	1184.6759	21.506	273.2677	26.34	756.1568	27.789	522.379	30.475	534.505				
2.36	168.0509	6.099	324.2145	8.888	528.1841	12.628	324.1637	21.506	273.2677	26.34	659.482	28.01	434.3505	30.386	768.9585				
2.361	168.0509	6.099	324.2145	8.888	528.1841	12.628	324.1637	21.506	273.2677	26.34	659.482	28.01	434.3505	30.386	768.9585				
2.489	168.0509	6.139	736.4204	9.033	366.1299	12.868	278.2091	22.641	271.2512	26.411	109.1306	27.9	484.332	30.362	667.627				
2.492	237.0522	6.166	366.1182	9.01	1039.5754	12.874	763.4719	23.10	427.3016	26.414	615.4562	28.004	763.9073	30.413	1078.5887				
2.505	237.0522	6.166	366.1182	9.01	1039.5754	12.874	763.4719	23.10	427.3016	26.414	615.4562	28.004	763.9073	30.413	1078.5887				
2.605	148.11	6.344	314.1158	9.309	323.1634	13.064	444.2281	24.012	538.378	26.431	576.3851	27.912	595.455	30.448	984.6151				
2.648	243.0518	6.389	336.1351	9.323	421.8719	12.96	324.2021	24.45	364.2821	26.431	571.4295	27.925	595.455	30.448	984.6151				
2.649	243.0518	6.389	336.1351	9.323	421.8719	12.96	324.2021	24.45	364.2821	26.431	571.4295	27.925	595.455	30.448	984.6151				
2.655	168.0509	6.502	345.1837	9.518	276.1337	13.035	410.2878	24.509	430.2543	26.454	326.2071	28.041	289.7212	30.797	104.7584				
2.715	438.2292	6.519	303.1472	9.674	1030.9293	13.2	516.1541	24.700	396.3468	26.465	696.3722	28.041	720.371	30.804	1019.771				
2.716	438.2292	6.519	303.1472	9.674	1030.9293	13.2	516.1541	24.700	396.3468	26.465	696.3722	28.041	720.371	30.804	1019.771				
2.804	154.1637	6.602	696.2654	9.949	200.1997	13.472	322.2362	24.718	518.307	26.513	497.3503	28.251	288.2664	31.547	678.564				
2.806	154.1637	6.602	696.2654	9.949	200.1997	13.472	322.2362	24.718	518.307	26.513	497.3503	28.251	288.2664	31.547	678.564				
2.836	168.0509	6.716	317.1455	10.081	262.147	13.439	504.501	24.871	562.3331	26.514	502.3122	28.274	341.3616	31.647	678.564				

- Establishment of a list of theoretical suspects based on known and referenced TPs or simulated ones

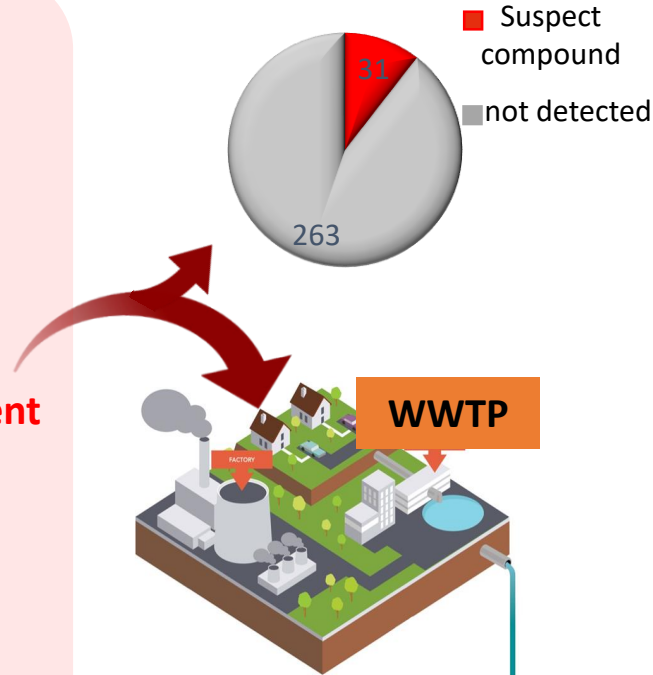


# Identification – case of transformation products

- Detection of compounds using a list of 294 theoretical TPs (294 TPs) and parent compounds (suspect MS mode)



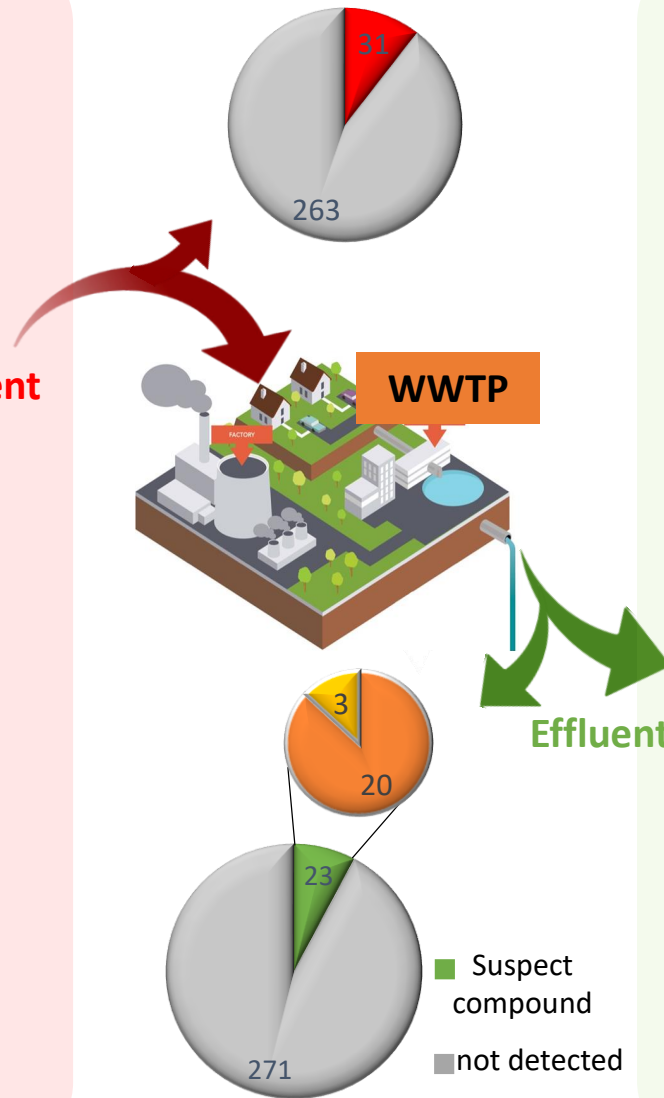
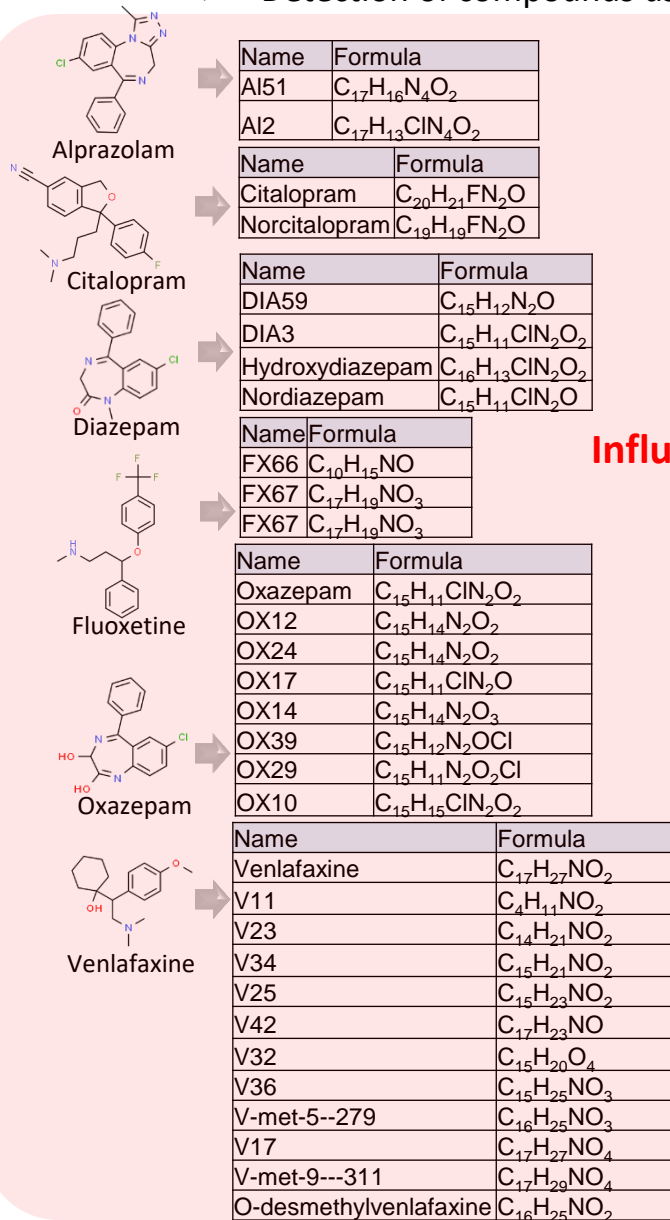
Influent



(Levels 4 and 5 of Schymanski classification)

# Identification – case of transformation products

- Detection of compounds using a list of 294 theoretical TPs (294 TPs) and parent compounds (suspect MS mode)



(Levels 4 and 5 of Schymanski classification)

Name	Formula		Name	Formula
Al51	C <sub>17</sub> H <sub>16</sub> N <sub>4</sub> O <sub>2</sub>		CTR-45	C <sub>20</sub> H <sub>24</sub> N <sub>2</sub> O <sub>3</sub>
Al2	C <sub>17</sub> H <sub>13</sub> ClN <sub>4</sub> O <sub>2</sub>		CTR-44	C <sub>20</sub> H <sub>24</sub> N <sub>2</sub> O <sub>2</sub>
Citalopram	C <sub>20</sub> H <sub>21</sub> FN <sub>2</sub> O	+		
Norcitalopram	C <sub>19</sub> H <sub>19</sub> FN <sub>2</sub> O			
Name	Formula			
DIA59	C <sub>15</sub> H <sub>12</sub> N <sub>2</sub> O			
DIA3	C <sub>15</sub> H <sub>11</sub> ClN <sub>2</sub> O <sub>2</sub>			
Hydroxydiazepam	C <sub>16</sub> H <sub>13</sub> ClN <sub>2</sub> O <sub>2</sub>			
Nordiazepam	C <sub>15</sub> H <sub>11</sub> ClN <sub>2</sub> O			
Name	Formula			
FX66	C <sub>10</sub> H <sub>15</sub> NO			
FX67	C <sub>17</sub> H <sub>19</sub> NO <sub>3</sub>			
FX67	C <sub>17</sub> H <sub>19</sub> NO <sub>3</sub>			
Name	Formula			
Oxazepam	C <sub>15</sub> H <sub>11</sub> ClN <sub>2</sub> O <sub>2</sub>			
OX12	C <sub>15</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub>			
OX24	C <sub>15</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub>			
OX17	C <sub>15</sub> H <sub>11</sub> ClN <sub>2</sub> O			
OX14	C <sub>15</sub> H <sub>14</sub> N <sub>2</sub> O <sub>3</sub>			
OX39	C <sub>15</sub> H <sub>12</sub> N <sub>2</sub> OCl			
OX29	C <sub>15</sub> H <sub>11</sub> N <sub>2</sub> O <sub>2</sub> Cl			
OX10	C <sub>15</sub> H <sub>15</sub> ClN <sub>2</sub> O <sub>2</sub>			
Name	Formula			
Venlafaxine	C <sub>17</sub> H <sub>27</sub> NO <sub>2</sub>			
V41	C <sub>14</sub> H <sub>11</sub> NO <sub>2</sub>	+		
V23	C <sub>14</sub> H <sub>21</sub> NO <sub>2</sub>		V23b	C <sub>14</sub> H <sub>21</sub> NO <sub>2</sub>
V34	C <sub>15</sub> H <sub>21</sub> NO <sub>2</sub>			
V25	C <sub>15</sub> H <sub>23</sub> NO <sub>2</sub>			
V42	C <sub>17</sub> H <sub>23</sub> NO			
V32	C <sub>15</sub> H <sub>20</sub> O <sub>4</sub>			
V36	C <sub>15</sub> H <sub>25</sub> NO <sub>3</sub>			
V-met-5--279	C <sub>16</sub> H <sub>25</sub> NO <sub>3</sub>			
V17	C <sub>17</sub> H <sub>27</sub> NO <sub>4</sub>			
V-met-9---311	C <sub>17</sub> H <sub>29</sub> NO <sub>4</sub>			
O-desmethylvenlafaxine	C <sub>16</sub> H <sub>25</sub> NO <sub>2</sub>			

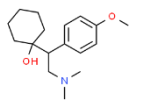
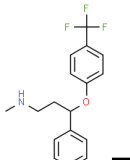
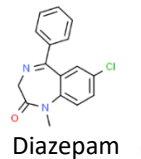
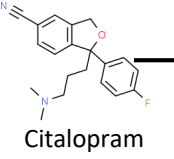
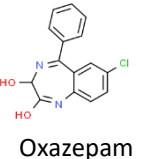
Persistent

Appear

Disappear

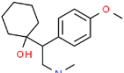
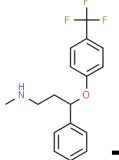
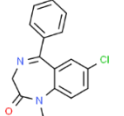
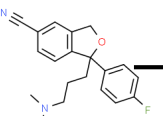
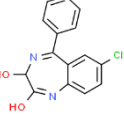
# Identification – case of transformation products

Use of fragmentation spectra to identify/confirm the compounds which are persistent and appear : based on MS/MS databases (suspect mode)

Parents	Name	Formula
 Venlafaxine	Venlafaxine	C17 H27 N O2
	V-met-5--279	C16H25NO3
	V23	C14 H21 N O2
	V23b	C14 H21 N O2
	V34	C15 H21 N O2
	V25	C15 H23 N O2
	V36	C15 H25 N O3
	V-met-5--279	C16 H25 N O3
	V17	C17 H27 N O4
	O-desmethylvenlafaxine	C16 H25 N O2
 Fluoxetine	Fluoxetine	FX66 C10 H15 N O
	FX67	C17 H19 N O3
 Diazepam	Nordiazepam	C15 H11 Cl N2 O
	Hydroxydiazepam	C16 H13 Cl N2 O2
	DIA59	C15 H12 N2 O
	DIA3	C15 H11 Cl N2 O2
 Citalopram	Citalopram	C20 H21 F N2 O
	Norcitalopram	C19 H19 F N2 O
	CTR-45	C20 H24 N2 O3
	CTR-44	C20 H24 N2 O2
	Oxazepam	C15 H11 Cl N2 O2
 Oxazepam	OX24	C15 H14 N2 O2
	OX14	C15 H14 N2 O3
	OX29	C15 H11 N2 O2 Cl

# Identification – case of transformation products

Use of fragmentation spectra to identify/confirm the compounds which are persistent and appear : based on MS/MS databases (suspect mode)

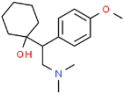
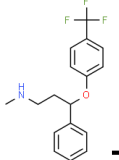
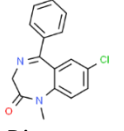
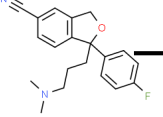
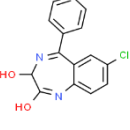
Parents	Name	Formula
 Venlafaxine	Venlafaxine	C <sub>17</sub> H <sub>27</sub> N O <sub>2</sub>
	V-met-5--279	C <sub>16</sub> H <sub>25</sub> NO <sub>3</sub>
	V23	C <sub>14</sub> H <sub>21</sub> N O <sub>2</sub>
	V23b	C <sub>14</sub> H <sub>21</sub> N O <sub>2</sub>
	V34	C <sub>15</sub> H <sub>21</sub> N O <sub>2</sub>
	V25	C <sub>15</sub> H <sub>23</sub> N O <sub>2</sub>
	V36	C <sub>15</sub> H <sub>25</sub> N O <sub>3</sub>
	V-met-5--279	C <sub>16</sub> H <sub>25</sub> N O <sub>3</sub>
	V17	C <sub>17</sub> H <sub>27</sub> N O <sub>4</sub>
	O-desmethylvenlafaxine	C <sub>16</sub> H <sub>25</sub> N O <sub>2</sub>
 Fluoxetine	Fluoxetine	C <sub>10</sub> H <sub>15</sub> N O
	FX66	C <sub>10</sub> H <sub>15</sub> N O
	FX67	C <sub>17</sub> H <sub>19</sub> N O <sub>3</sub>
 Diazepam	Nordiazepam	C <sub>15</sub> H <sub>11</sub> Cl N <sub>2</sub> O
	Hydroxydiazepam	C <sub>16</sub> H <sub>13</sub> Cl N <sub>2</sub> O <sub>2</sub>
	DIA59	C <sub>15</sub> H <sub>12</sub> N <sub>2</sub> O
	DIA3	C <sub>15</sub> H <sub>11</sub> Cl N <sub>2</sub> O <sub>2</sub>
 Citalopram	Citalopram	C <sub>20</sub> H <sub>21</sub> F N <sub>2</sub> O
	Norcitalopram	C <sub>19</sub> H <sub>19</sub> F N <sub>2</sub> O
	CTR-45	C <sub>20</sub> H <sub>24</sub> N <sub>2</sub> O <sub>3</sub>
	CTR-44	C <sub>20</sub> H <sub>24</sub> N <sub>2</sub> O <sub>2</sub>
	Oxazepam	C <sub>15</sub> H <sub>11</sub> Cl N <sub>2</sub> O <sub>2</sub>
 Oxazepam	Ox24	C <sub>15</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub>
	OX14	C <sub>15</sub> H <sub>14</sub> N <sub>2</sub> O <sub>3</sub>
	OX29	C <sub>15</sub> H <sub>11</sub> N <sub>2</sub> O <sub>2</sub> Cl

- Suspected presence of several compounds : Parent compounds and TPs (good confidence)



# Identification – case of transformation products

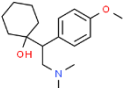
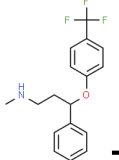
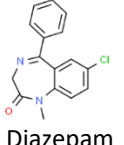
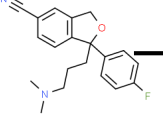
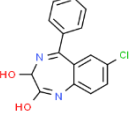
Use of fragmentation spectra to identify/confirm the compounds which are persistent and appear : based on MS/MS databases (suspect mode)

Parents	Name	Formula	
 Venlafaxine	Venlafaxine	C <sub>17</sub> H <sub>27</sub> N O <sub>2</sub>	
	V-met-5--279	C <sub>16</sub> H <sub>25</sub> NO <sub>3</sub>	Tramadol-N-oxide
	V23	C <sub>14</sub> H <sub>21</sub> N O <sub>2</sub>	Meperidine (Pethidine)
	V23b	C <sub>14</sub> H <sub>21</sub> N O <sub>2</sub>	
	V34	C <sub>15</sub> H <sub>21</sub> N O <sub>2</sub>	
	V25	C <sub>15</sub> H <sub>23</sub> N O <sub>2</sub>	O-Desméthyltramadol
	V36	C <sub>15</sub> H <sub>25</sub> N O <sub>3</sub>	
	V-met-5--279	C <sub>16</sub> H <sub>25</sub> N O <sub>3</sub>	
	V17	C <sub>17</sub> H <sub>27</sub> N O <sub>4</sub>	
	O-desmethylvenlafaxine	C <sub>16</sub> H <sub>25</sub> N O <sub>2</sub>	
 Fluoxetine	Fluoxetine	FX66	
		FX67	Piperine
 Diazepam	Diazepam	Nordiazepam	
		Hydroxydiazepam	
		DIA59	Carbamazépine
		DIA3	
 Citalopram	Citalopram	Citalopram	
		Norcitalopram	
		CTR-45	
		CTR-44	Quinidine
 Oxazepam	Oxazepam	Oxazepam	
		OX24	
		OX14	
		OX29	

- Suspected presence of several compounds : Parent compounds and TPs
- Invalidated presence for some TPs

# Identification – case of transformation products

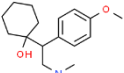
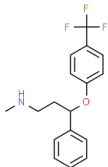
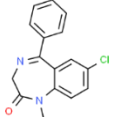
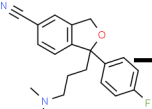
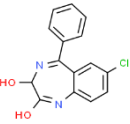
Use of fragmentation spectra to identify/confirm the compounds which are persistent and appear : based on MS/MS databases (suspect mode)

Parents	Name	Formula	
 Venlafaxine	Venlafaxine	C <sub>17</sub> H <sub>27</sub> N O <sub>2</sub>	
	V-met-5--279	C <sub>16</sub> H <sub>25</sub> NO <sub>3</sub>	Tramadol-N-oxide
	V23	C <sub>14</sub> H <sub>21</sub> N O <sub>2</sub>	Meperidine (Pethidine)
	V23b	C <sub>14</sub> H <sub>21</sub> N O <sub>2</sub>	
	V34	C <sub>15</sub> H <sub>21</sub> N O <sub>2</sub>	
	V25	C <sub>15</sub> H <sub>23</sub> N O <sub>2</sub>	O-Desméthyltramadol
	V36	C <sub>15</sub> H <sub>25</sub> N O <sub>3</sub>	
	V-met-5--279	C <sub>16</sub> H <sub>25</sub> N O <sub>3</sub>	
	V17	C <sub>17</sub> H <sub>27</sub> N O <sub>4</sub>	
	O-desmethylvenlafaxine	C <sub>16</sub> H <sub>25</sub> N O <sub>2</sub>	
 Fluoxetine	Fluoxetine	C <sub>10</sub> H <sub>15</sub> N O	
	FX67	C <sub>17</sub> H <sub>19</sub> N O <sub>3</sub>	Piperine
 Diazepam	Nordiazepam	C <sub>15</sub> H <sub>11</sub> Cl N <sub>2</sub> O	
	Hydroxydiazepam	C <sub>16</sub> H <sub>13</sub> Cl N <sub>2</sub> O <sub>2</sub>	
	DIA59	C <sub>15</sub> H <sub>12</sub> N <sub>2</sub> O	Carbamazépine
	DIA3	C <sub>15</sub> H <sub>11</sub> Cl N <sub>2</sub> O <sub>2</sub>	
 Citalopram	Citalopram	C <sub>20</sub> H <sub>21</sub> F N <sub>2</sub> O	
	Norcitalopram	C <sub>19</sub> H <sub>19</sub> F N <sub>2</sub> O	
	CTR-45	C <sub>20</sub> H <sub>24</sub> N <sub>2</sub> O <sub>3</sub>	
	CTR-44	C <sub>20</sub> H <sub>24</sub> N <sub>2</sub> O <sub>2</sub>	Quinidine
 Oxazepam	Oxazepam	C <sub>15</sub> H <sub>11</sub> Cl N <sub>2</sub> O <sub>2</sub>	
	OX24	C <sub>15</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub>	
	OX14	C <sub>15</sub> H <sub>14</sub> N <sub>2</sub> O <sub>3</sub>	
	OX29	C <sub>15</sub> H <sub>11</sub> N <sub>2</sub> O <sub>2</sub> Cl	

- Suspected presence of several compounds : Parent compounds and TPs
- Invalidated presence for some TPs
- Identification in progress : in silico software

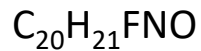
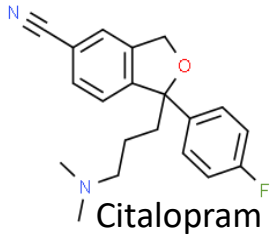
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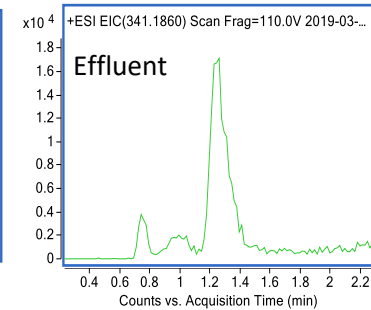
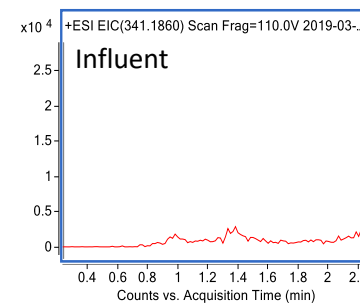
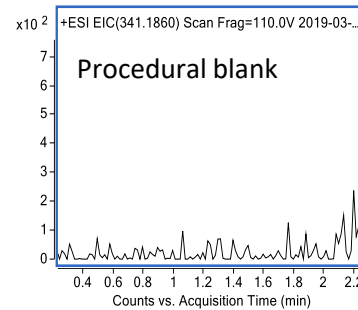
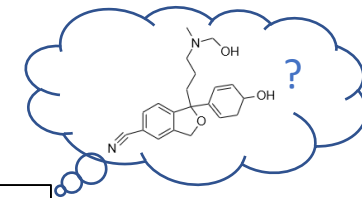
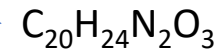
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# Identification – case of transformation products

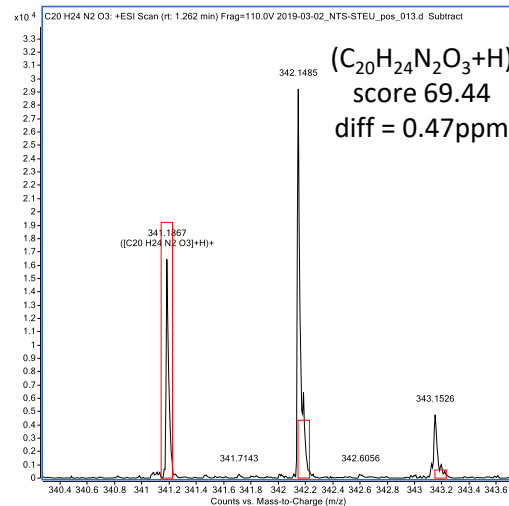
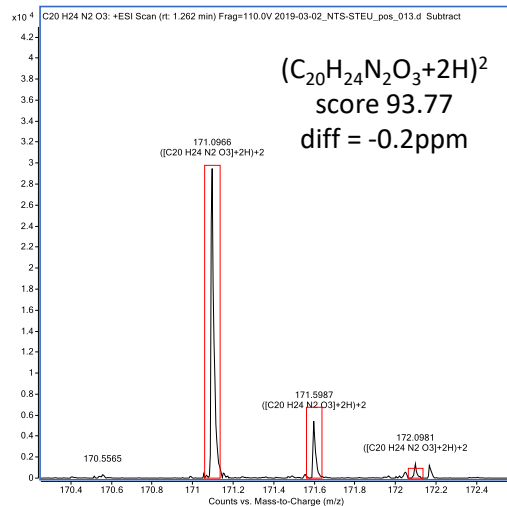


→ Oxidative displacement of Fluorine → +OH-F

→ + Hydration → +H<sub>2</sub>O

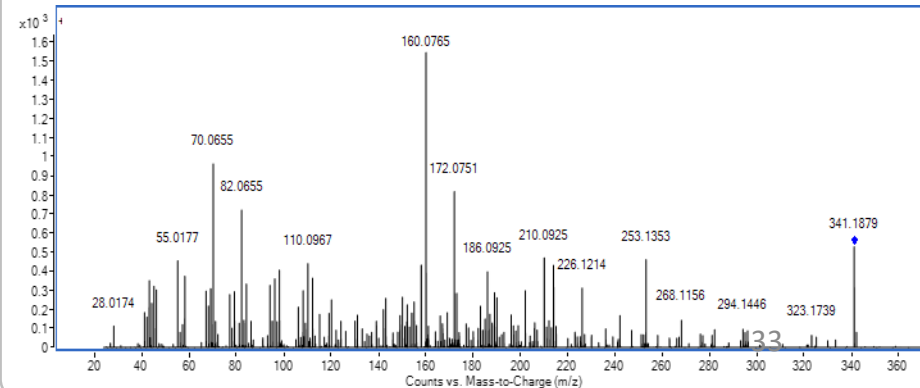


MS Spectrum Rt 1.3min



21566 Candidates

MSMS Spectrum Identification in progress : in silico software





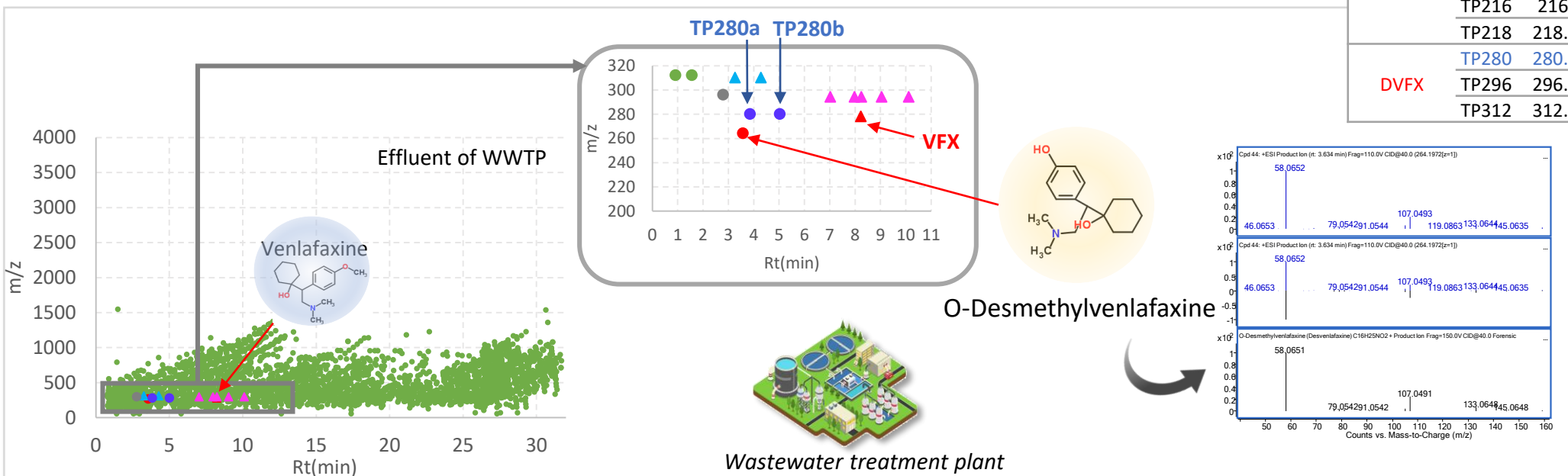
## **Study of TPs in natural environment (surface water)**

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# Transformation products in natural environment

## List of suspected TPs

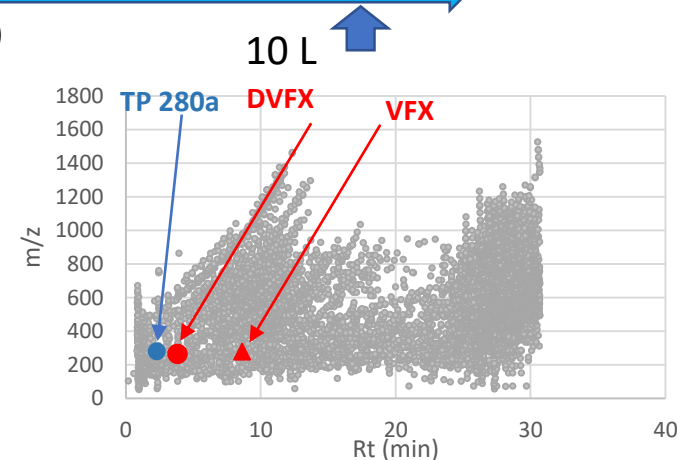
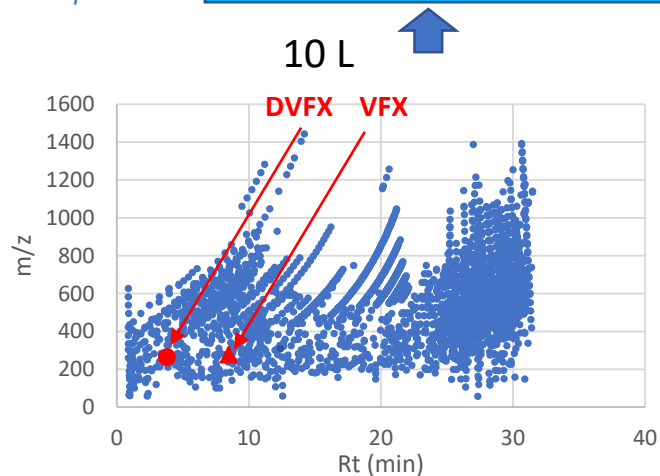
	TPs	[M+H] <sup>+</sup>
Venlafaxine	DVFX	264.1958
	TP294	294.2053
	TP310	310.2008
	TP216	216.086
DVFX	TP218	218.1012
	TP280	280.1903
	TP296	296.1852
	TP312	312.1799



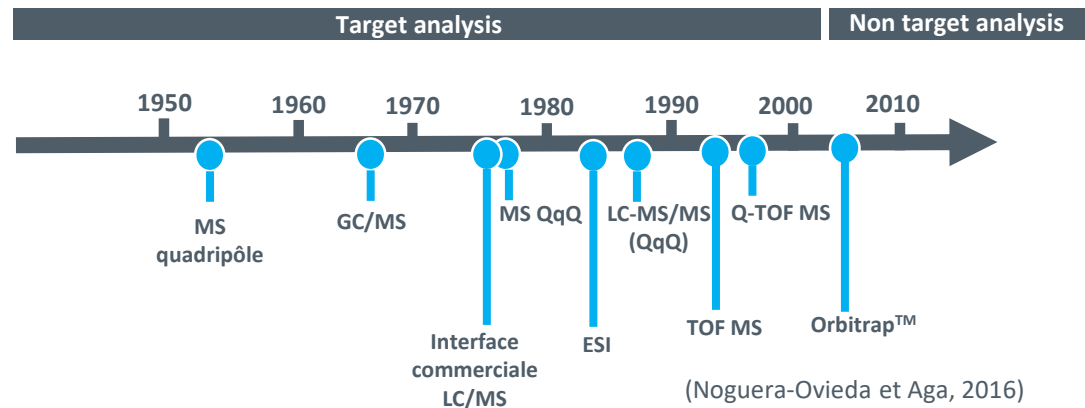
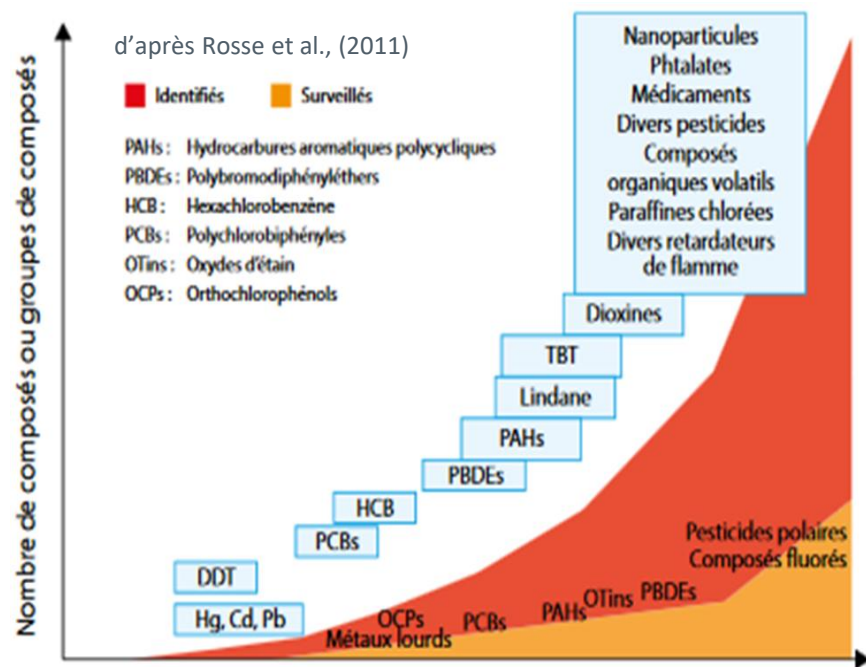
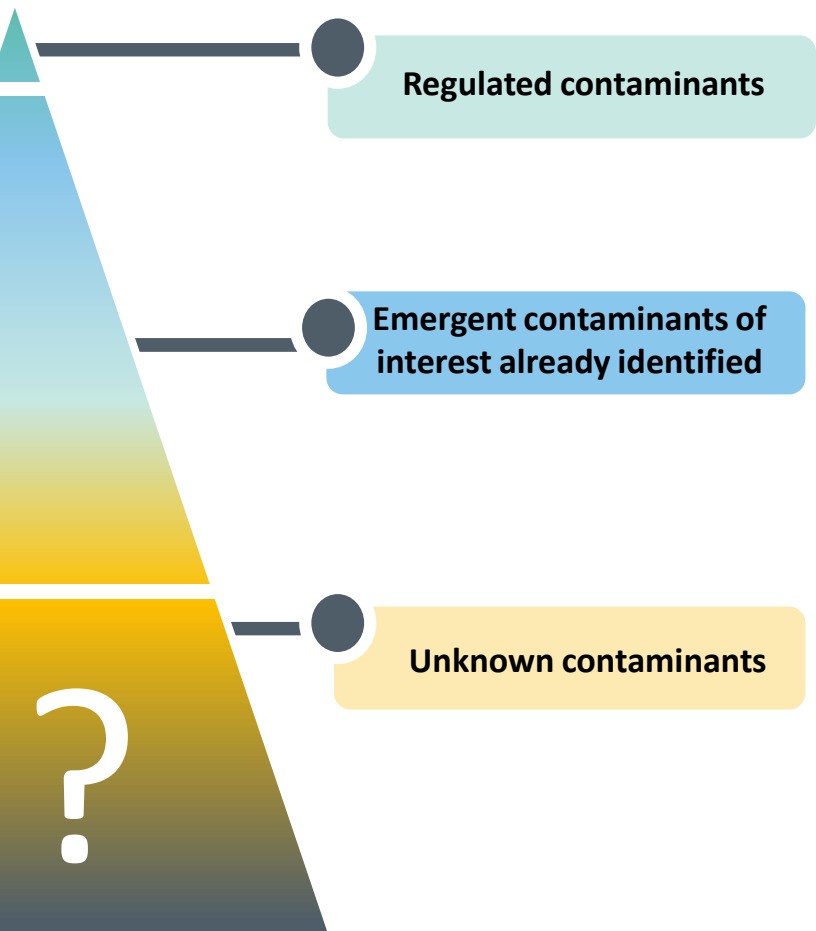
Wastewater treatment plant

Upstream → Downstream

Jalle de Blanquefort (Bordeaux)



# CONCLUSION





# CONCLUSION AND NEEDS

- Demonstration of presence of more and more compounds
- Characterization of more and more complex mixtures
- Able to address low concentrations (lower and lower)
- Able to start to address TPs

## But needs

- Lower LQ (HRMS towards LQ QQQ)
- To continue to characterize TPs (treatment of waste water – oxidation – « re-use »)
- **To improve treatment and storage of HRMS data** (databases, softwares, statistics, interoperability, ...)
- Impossible to characterize all – Mixtures effects : strategies?
- Link presence/effects – Effect Directed Analysis



## **Researching TPs using bioassays**

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# Methodology

## Sample preparation

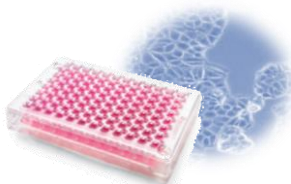


3.5 L Influent  
6 L Effluent

Filtration : 0.7µm

Solid phase extraction  
Cartridge HLB Oasis®

In vitro bioassays



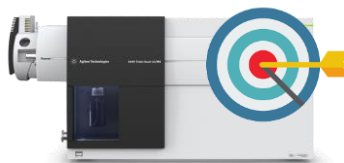
## Characterisation of biological activity

## Chemical characterisation

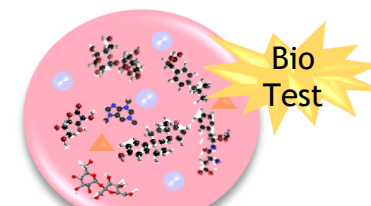
Non target analysis



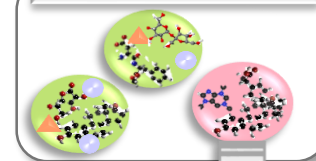
Target analysis



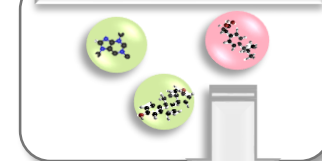
## Effect directed analysis



Fractionation



Fractionation

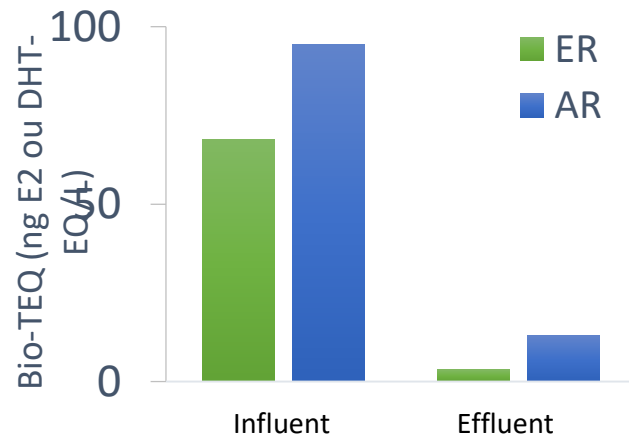


Chemical analysis



# Mass balance

## Biological activities



## Target chemical analyses

161 targeted compounds

(pharmaceuticals, pesticides, PAHs, PCBs, OCP, AKP, BTEX, COV, phtalates, hormones, bisphenols)

75 molecules detected

5 known ER-ligands (agonists)  
( no Ar-ligand)



## « Mass balance » analysis

Activities remain unexplained

*Explained activities <1%  
(except effluent <4%)*

# Information deconvolution : Effect directed Analysis

## Simplification



100% ACN

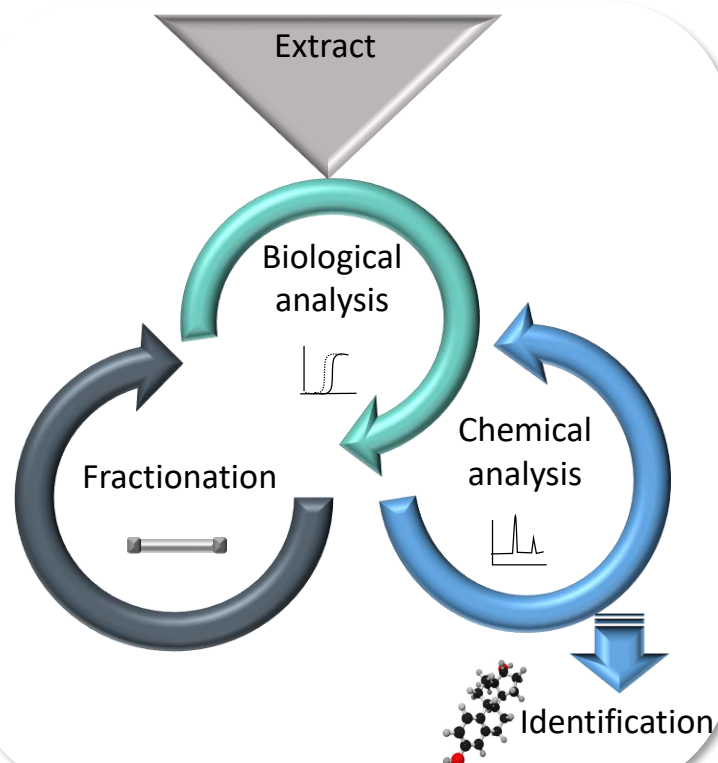
20% ACN

polarité

RP-HPLC (C18)

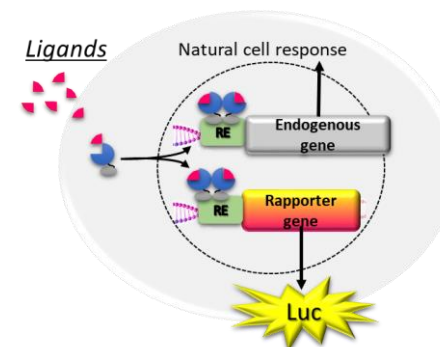
96	95	94	93	92	91	90	89	88	87	86	85
73	74	75	76	77	78	79	80	81	82	83	84
72	71	70	69	68	67	66	65	64	63	62	61
49	50	51	52	53	54	55	56	57	58	59	60
48	47	46	45	44	43	42	41	40	39	38	37
25	26	27	28	29	30	31	32	33	34	35	36
24	23	22	21	20	19	18	17	16	15	14	13
1	2	3	4	5	6	7	8	9	10	11	12

96 collected fractions



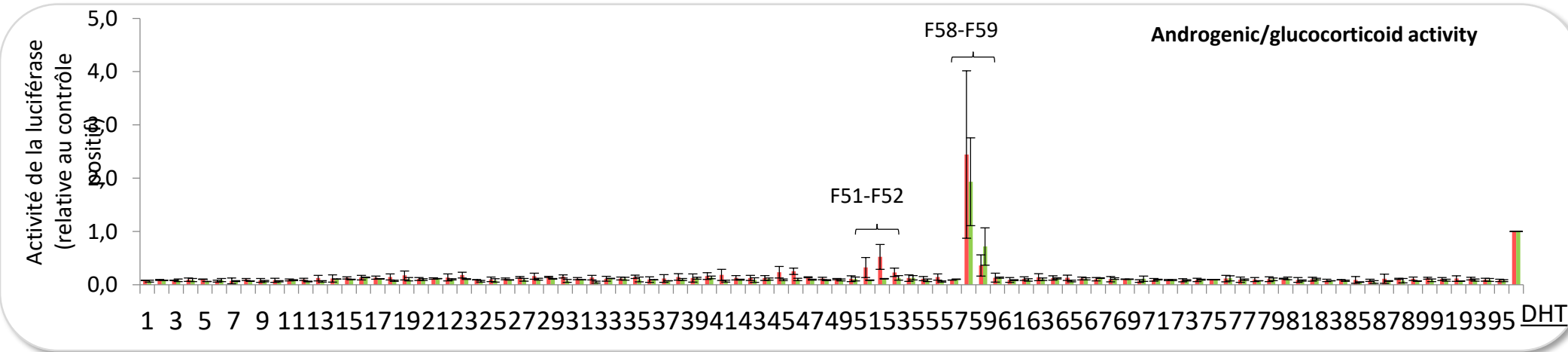
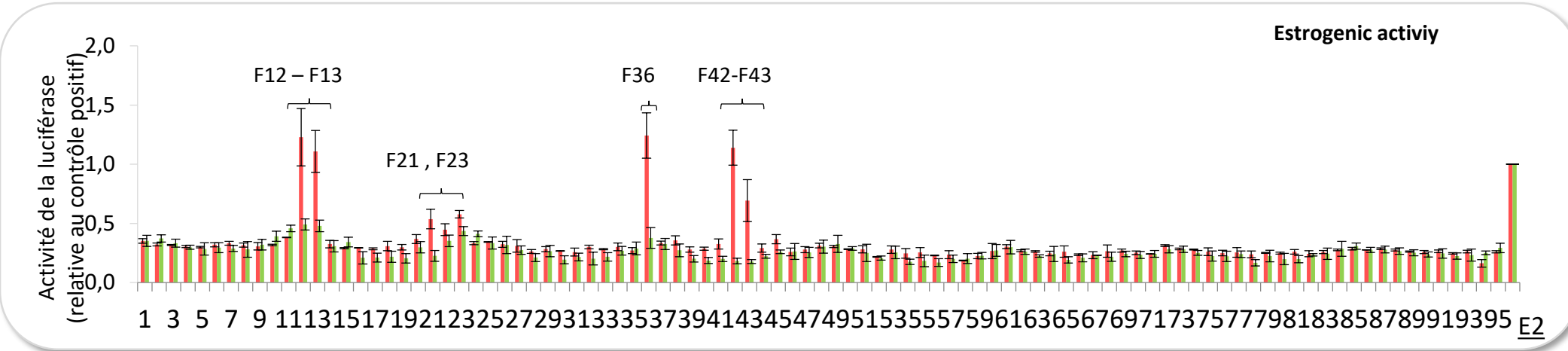
## Directed

- ✓ High-throughput screening using ER, AR/GR-ligands



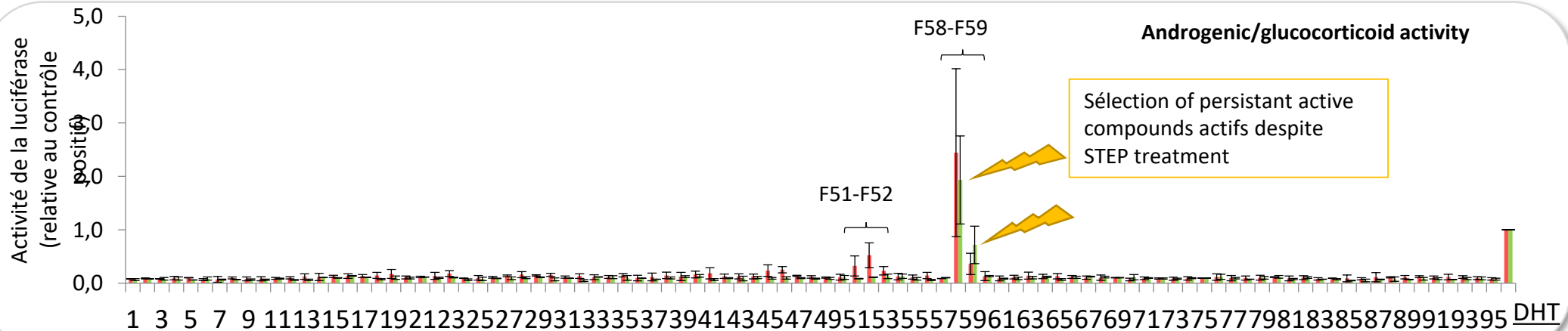
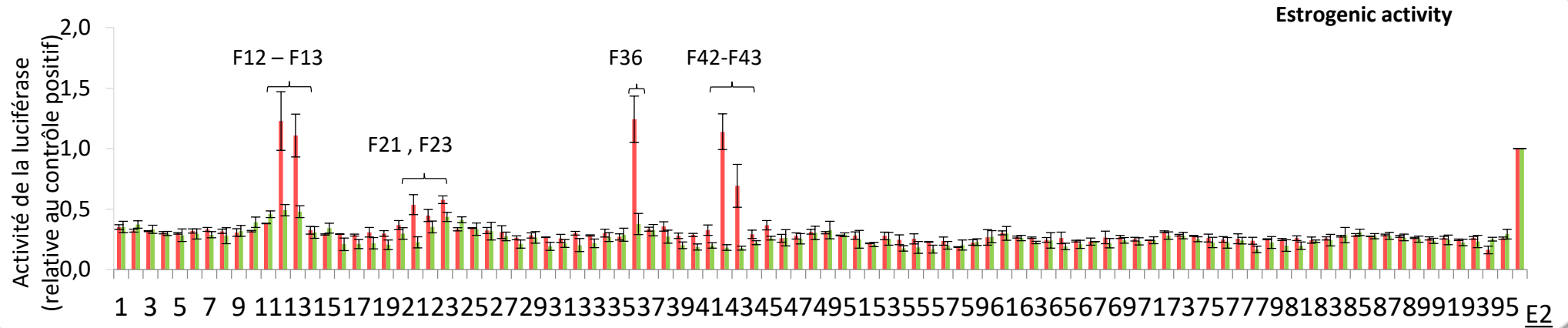
# Biological activities

Influent  
Effluent



# Biological activities

■ Influent  
■ Effluent



Clobetasol propionate  
Fluticasone propionate



# TEAM AND FUNDING



AGENCE DE L'EAU  
ADOUR-GARONNE



AGENCE FRANÇAISE  
POUR LA BIODIVERSITÉ

Établissement public du ministère de l'Environnement







Thank you for your attention 😊

