Screening of organic micropollutants in wastewater and treated wastewater by Liquid Chromatography coupled with High Resolution Mass Spectrometry (LC-HRMS)

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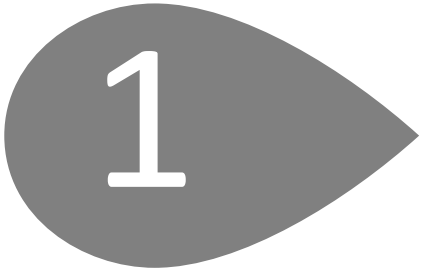
29/11/12

Workshop Norman

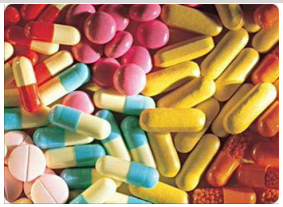
Contents



Context

Context

- Human uses and consumption of organic compounds :
  - Pharmaceuticals
  - Pesticides
  - Personal care products



↳ Contamination of wastewaters → risk of rivers contamination

- Water quality is based on regulations
  - Increasing but limited lists of compounds
  - Ex : pharmaceuticals non yet regulated
- Quantification methods → limited
- GC-MS screenings not enough (volatile and apolar compounds)

↳ ***Bad knowledge on micropollutants identification and occurrence***

Necessity to develop screening methods to identify micropollutants in wastewaters ⇨ LC-HRMS

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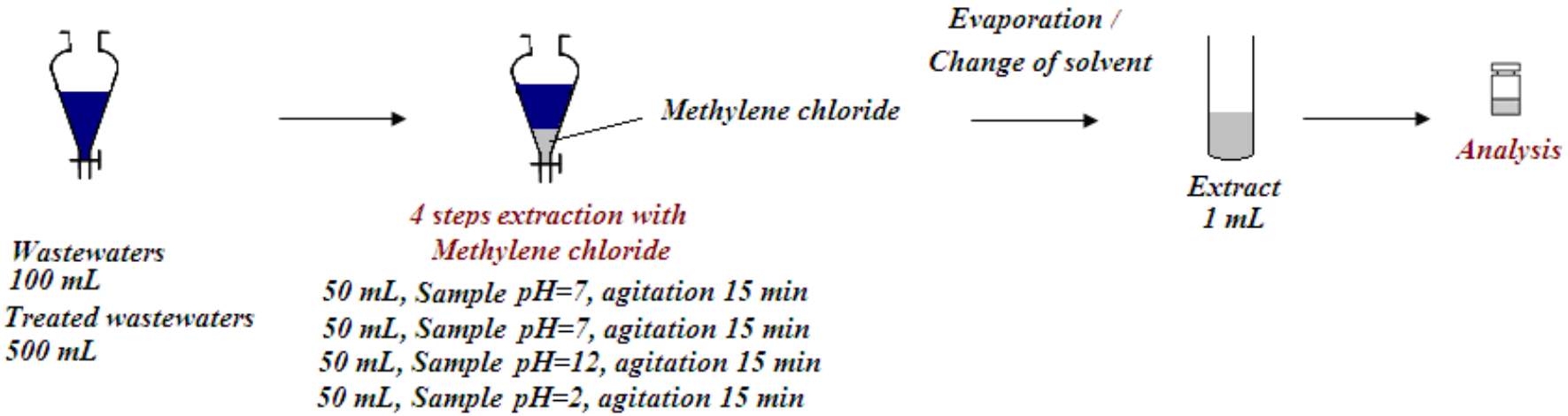
Identification strategy

- Samples preparation

Wastewater preparation

- Extraction step before analysis
  - Not specific to extract a maximum of pollutants
  - Without filtration step to treat whole sample

  ↳ Liquid/liquid extraction at different pH



2

Identification strategy

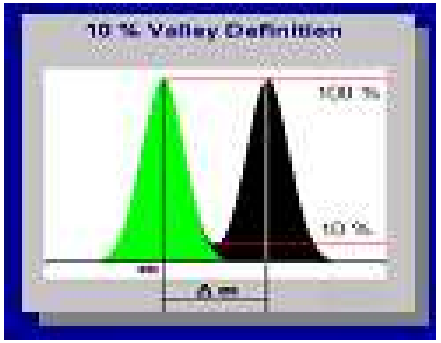
- LC-HRMS apparatus and conditions

Why high resolution system ?

- Definition of resolution
  - → Resolution is the capacity to differentiate 2 masses
  - → R=m/Δm

    m : mass of the first peak

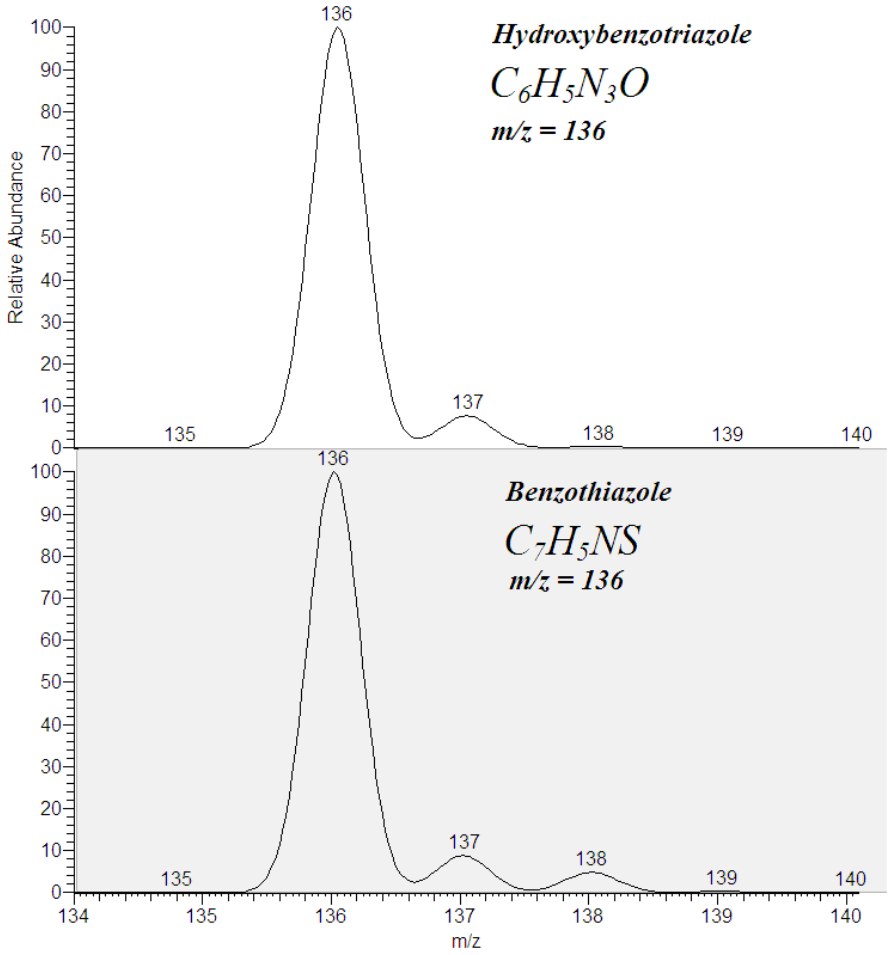
    Δm : difference of mass between two consecutive peaks



- LC-MS low resolution is not a good tool for screening :
  - → No or few spectral databases in LC-MS
  - → Mass spectrum too simple to be specific
  - → Not able to dissociate 2 molecules with the same unit mass

Why high resolution system ?

- Example
  - No dissociation of the 2 compounds



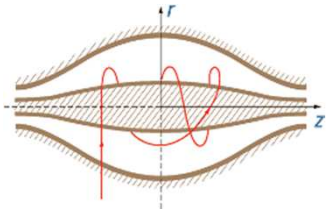
Need to work with high resolution systems

LC-HRMS apparatus (1)

- LQT-ORBITRAP Discovery *(Thermo fisher scientific)*

LC-HRMS apparatus (2)

- LQT-ORBITRAP properties :
  - hybrid system
    - ➜ Linear trap
    - ➜ Orbitrap analyser : oscillation of ions under an electric field. The oscillation frequency of ion is dependant of the m/z. The measurement of this frequency with high precision gives the m/z with high precision.

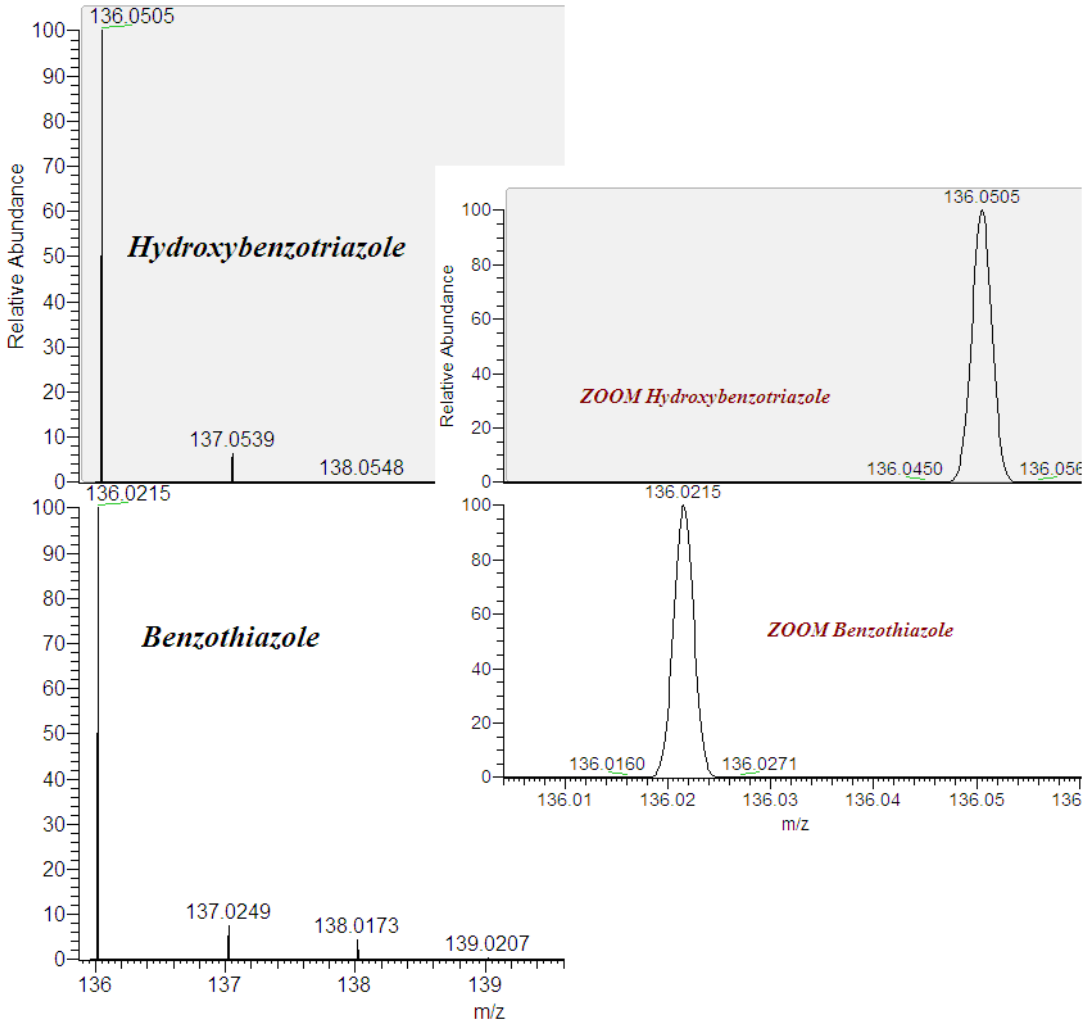


  - Resolution of 30 000 at m/z= 400

High resolution leads to the raw formulae of the compounds

LC-HRMS apparatus (3)

- Previous example



↳ Dissociation of the 2 compounds

LC-HRMS conditions

- LC usual conditions:
  - Column : Hypersil Gold 100x2.1 mm, 3 µm
  - Solvents and gradient

→ Solvent ESI+ :

A : Water + 0,05% Formic acid

B : Methanol + 0,05% Formic acid

→ Solvent ESI- :

A : Water

B : Methanol

→ Gradient :

Flow (µl/min)	Time (min)	A en %	B en %
0	0	95	5
25	25	5	95
30	30	5	95
30,5	30,5	95	5
40	40	95	5

- HRMS :
  - Acquisition in fullscan mode

→ Electrospray positif

→ Electrospray negatif

→ Mass range : m/z : 80- 1500

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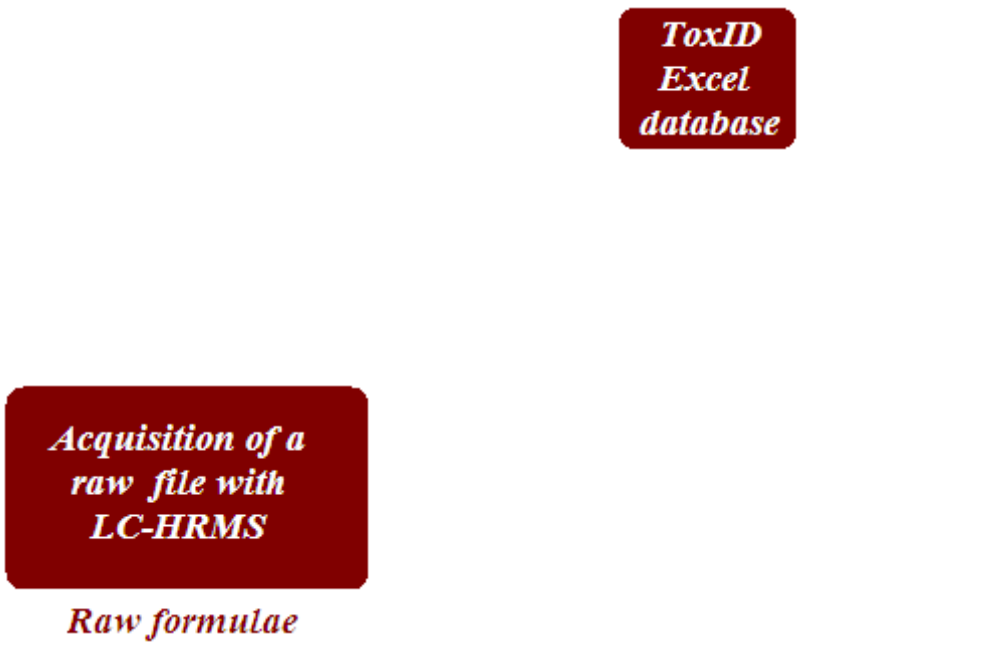
Identification strategy

- ToxID database

Tox ID database (1)



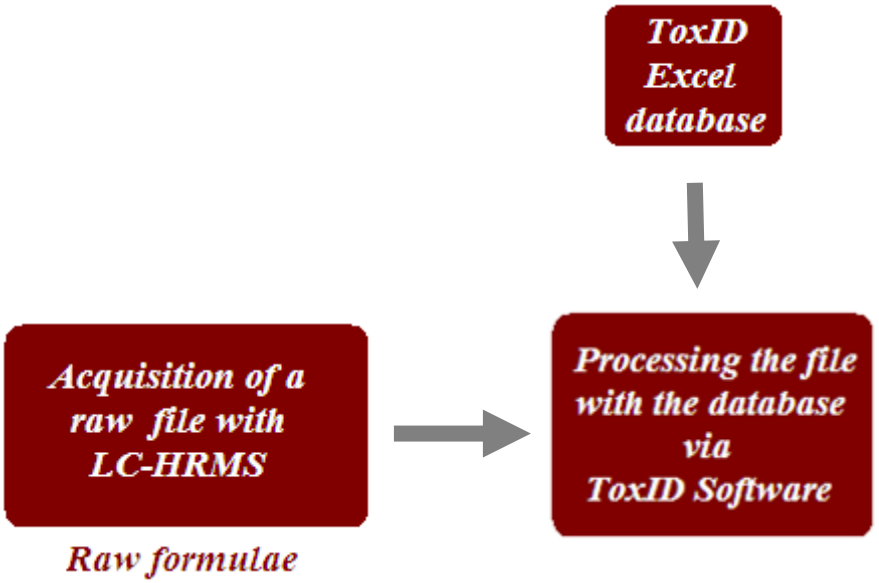
Tox ID database (2)

- Homemade Excel database containing more than 1000 compounds
  - Type of compounds : pharmaceuticals, pesticides, personal care products, anti-UV filters, hormones, phytoestrogens, artificial sweeteners.
  - Base is built with :
    - → Identification of compound : Name, cas number, type of compound and elemental composition
    - → Conditions of detection : analysis polarity (ESI+/ESI-), intensity threshold
    - → Experimental information if known : retention time, fragmentation data

ToxID 2.1.2 Configuration File

Index	Compound Name	Elemental Composition	Polarity	Analyte Type	Expected RT	Intensity Threshold	Adduct1	Adduct2	Adduct3	Fragment1	Fragment2	Fragment3
326	Amitraz	C19H23N3	+	Pesticides	1	100000	1	1	1			
327	Amitriptyline	C20H23N	+	Produit pharma	21.54	100000	1	1	1	233.15		
328	Amlodipine	C20H25ClN2O5	+	Produit pharma	1	100000	1	1	1			
329	Amoxapine	C17H16ClN3O	+	Produit pharma	1	100000	1	1	1			
330	Amoxicillin	C16H19N3O5S	+	Produit pharma	1	100000	1	1	1			
331	Amphetamine	C9H13N	+	Produit pharma	1.69	100000	1	1	1	90.9		
332	AmphotericineB	C47H73NO17	+	Produit pharma	1	100000	1	1	1			
333	Ampiciline	C16H19N3O4S	+	Produit pharma	1	100000	1	1	1			
335	Ancymidol	C15H16N2O2	+	Pesticides	1	100000	1	1	1			

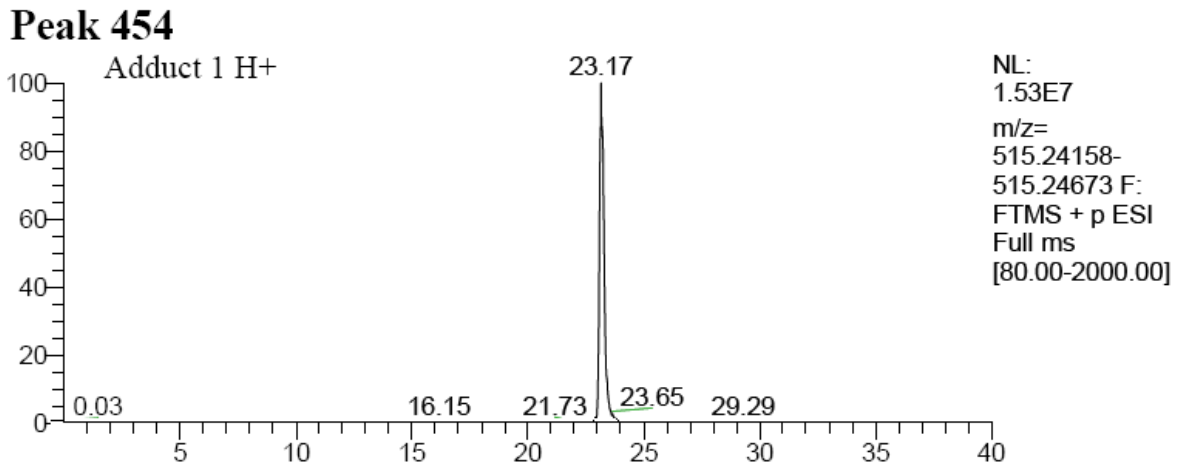
Tox ID database (3)



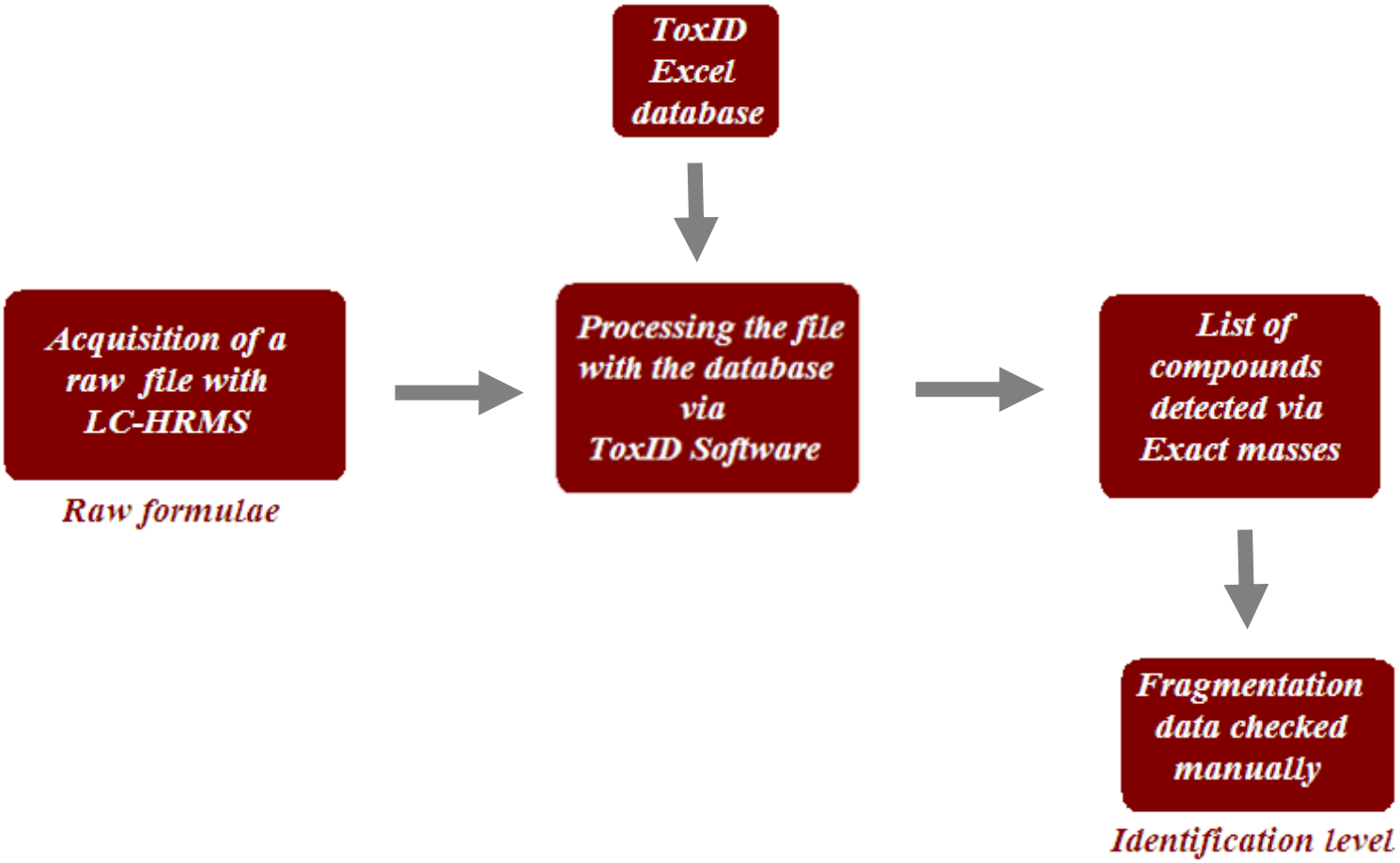
Tox ID database (4)

- Samples are processed with the database via the ToxID software *(Thermo fisher scientific)*
  - ToxID software calculates the exact mass of expected ions of compounds listed in the base
  - ToxID checks if these exact masses are present in the sample raw file
  - Results are presented on Excel and PDF format

Peak Number:	454
Index in Config:	1757
Compound Name:	Telmisartan
Formula:	C33H30N4O2
Polarity:	+
Compound Info:	pharmaceuticals
Expected m/z with Adduct:	515.24415
Detected m/z:	515.24426
Delta (ppm):	0.2
Expected RT (min):	22.88
Actual RT (min):	23.17
Adduct H+:	15300828*
Adduct H+:	15300828
Adduct H+:	15300828
Fragment 1:	Expected m/z: 497.30000, Detected: ---
Fragment 2:	--
Fragment 3:	--



Tox ID database (3)



Fragmentation data-Identification levels

- Fragmentation data are checked manually
- Fragmentation data are obtained thanks to:
  - Injection of commercial standard
  - Spectral databases like Massbank
  - Bibliographic data
- Identification levels (Veolia levels):
  - Level 1 : identification only based on exact mass
  - Level 2 : identification based on exact mass + retention time Ok
  - Level 3 : Confident level : identification based on exact mass + fragmentation data OK (experimental and bibliographic fragmentation data

Why being interested in level 1 compounds ?

- *Detection of recurrent or very intense compounds ⇨ compounds of interest*
- *Work specifically on their identification and forget the others*

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Some experimental results

- Samples studied

Samples studied

- 13 French sewage treatment plants
- Raw wastewater and treated wastewater
- Sampling winter/summer and week/weekend
- Different locations : north-west, north-east, south-west and south-east
- Different sizes

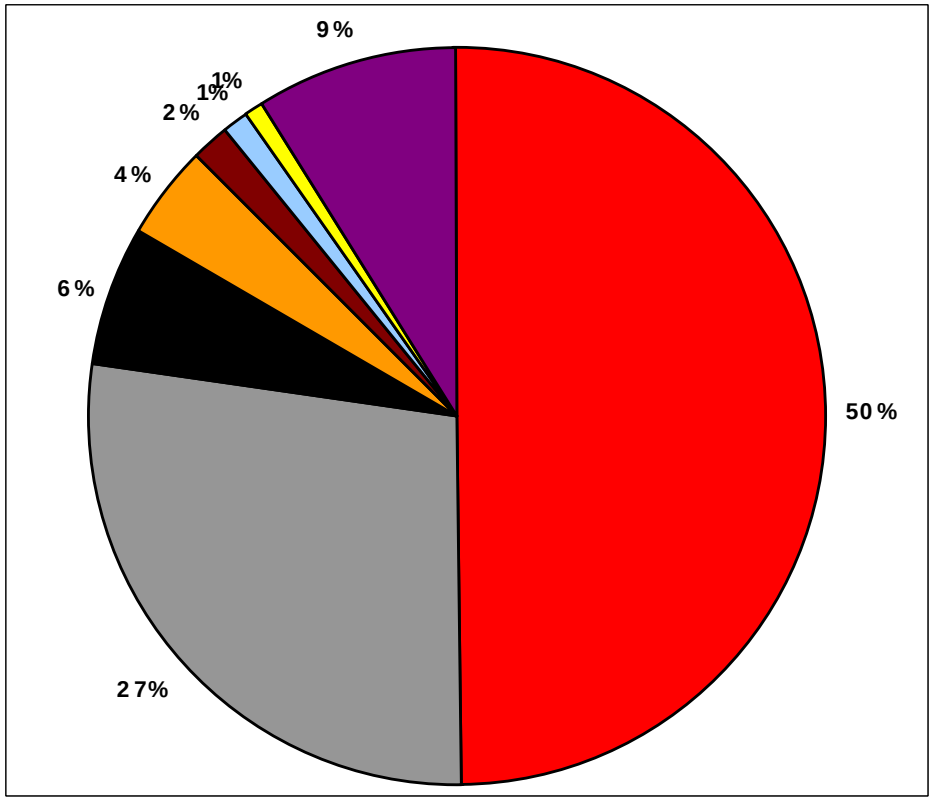
Small (< 10000 P.E.)	Medium (~ 30000 P.E.)	Large (> 100000 PE)
4 plants	4 plants	5 plants

↳ 50 samples studied

Some results (1) – general points

- About 600 compounds found

- Distribution by use



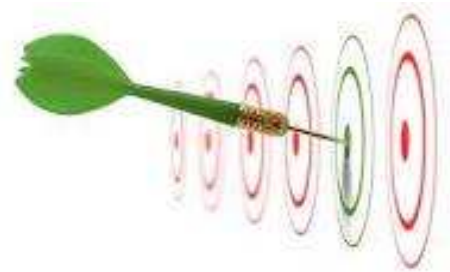
- **Lot of pharmaceuticals, compounds not under regulations**
- **Many pesticides**

~ Distribution influenced by the database building

Some results (2) – recurrent compounds

- 39 micropollutants found in all samples. 25 with a level 3
- 41 micropollutants found in more than 80% of the samples. 11 with a level 3

  ↳ **Mainly pharmaceuticals**



- Examples of molecules found
  - Pharmaceuticals :

  Level 3 : Acebutolol, Bisoprolol, Carbamazepine, Cetirizine, Codeine, Diacetolol, Diclofenac, EDDP (methadone metabolite), Flecainide, Diltiazem, Ketoprofen, Irbesartan, Telmisartan, Tramadol, Valsartan …

  Level 1 : Alprenolol, Lamotrigine, Varenicline …

  - Pesticides : Level 1 : Cyromazine, Isoprocarb ….
  - Industrial agent: Level 1 : Benzotriazole ….
  - Consumption product: Level 3 : Caffein ….

Identification of micropollutants not listed in our quantification methods nor prioritization lists

Advantages and limits of the method

- **Identification of new compounds, not targeted before.**
- **Possibility in the future to search new molecules in the raw data file previously acquired without reinjection of samples.**
- **Possibility to increase the database with new compounds easily.**

- **Nowadays, qualitative method.**
- **Some compounds may not be extracted or detected.**
- **Need to increase fragmentation data (pooling of fragmentation information)**

Conclusions and perspectives

- **Innovative tool which allows the detection of recurrent pollutants**
- **Identification of emerging micropollutants not studied in quantification methods before.**
- **Possibility to extract a lot of information**

  Information on treatment efficiency (already done)

  Statistical analysis (ongoing)
- **Need to be done :**

  Increase information on fragmentation data.

  Automated the data processing.

Thanks to

- ✓ **Delphine BRILLANT** – ***Laboratory technician***
- ✓ **Thomas NEFAU** – ***PhD Student***
- ✓ **Luis CASTILLO** – ***Expert and project manager***
- ✓ **Valérie INGRAND** – ***Head of Chemical analysis and innovation team***

Thank you for your attention