

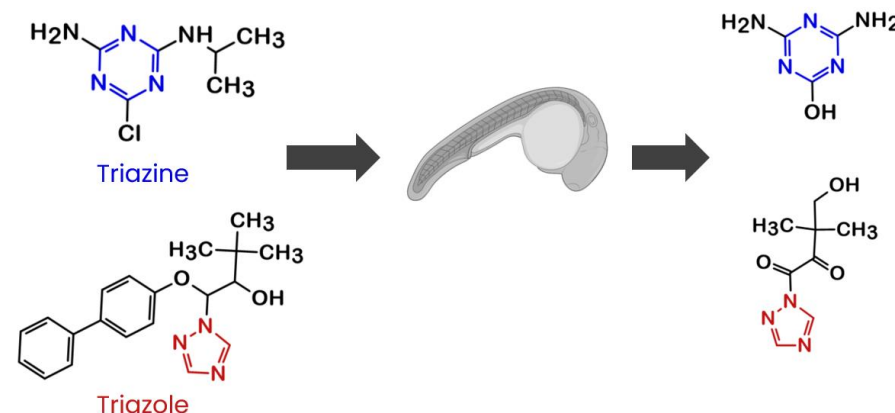
# Identifying persistent substructures in TPs using Zebrafish embryos

**Parviel Chirsir<sup>1</sup>**, Maria Lorena Cordero-Maldonado<sup>1</sup>, Maxim P. Carlier<sup>2</sup>, Timo Hamers<sup>2</sup>, Emma L. Schymanski<sup>1</sup>

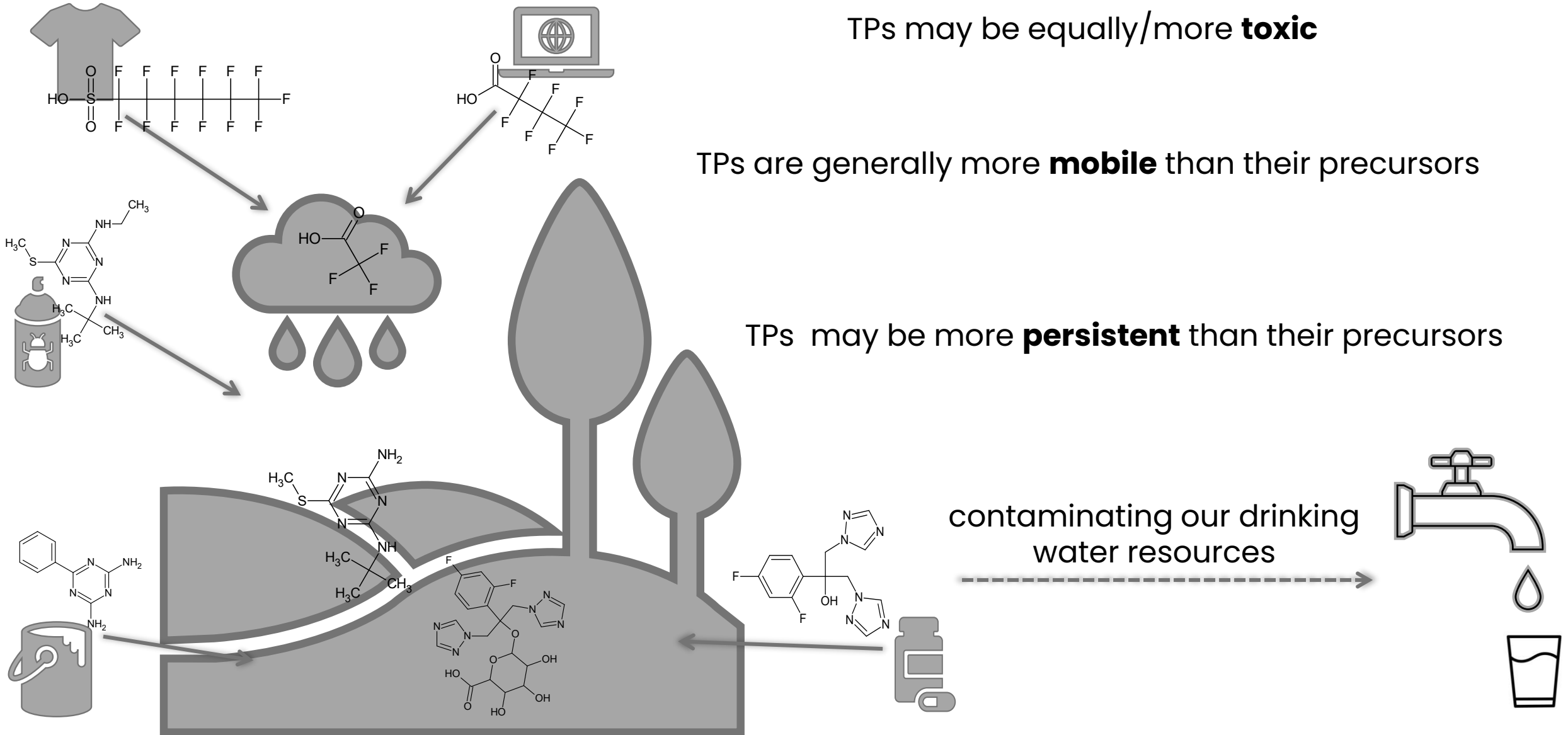
<sup>1</sup> LCSB, University of Luxembourg

<sup>2</sup> Vrije Universiteit Amsterdam

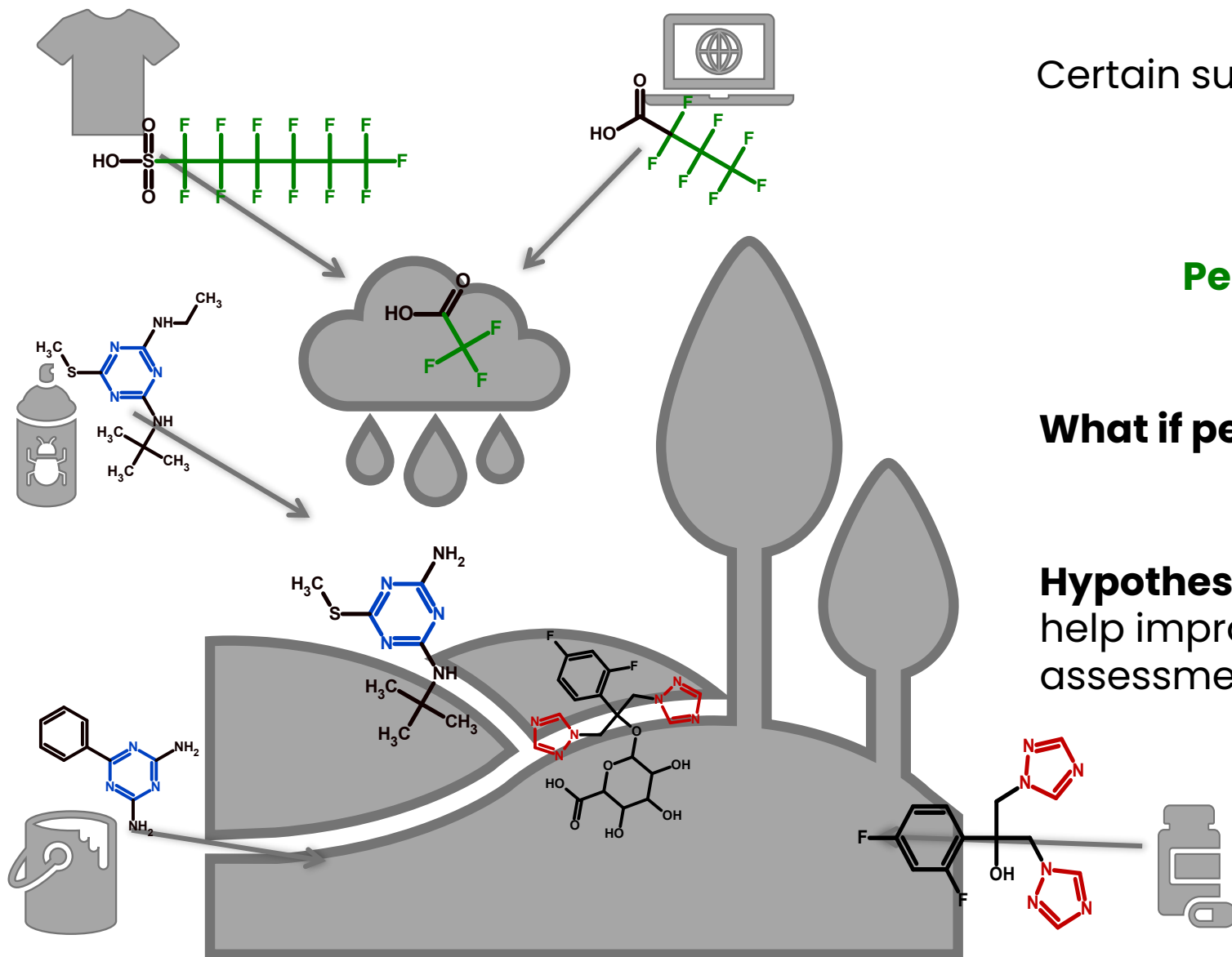
Contact: [parviel.chirsir@uni.lu](mailto:parviel.chirsir@uni.lu)



# Chemicals do not disappear – they transform



# Persistent substructures in TPs

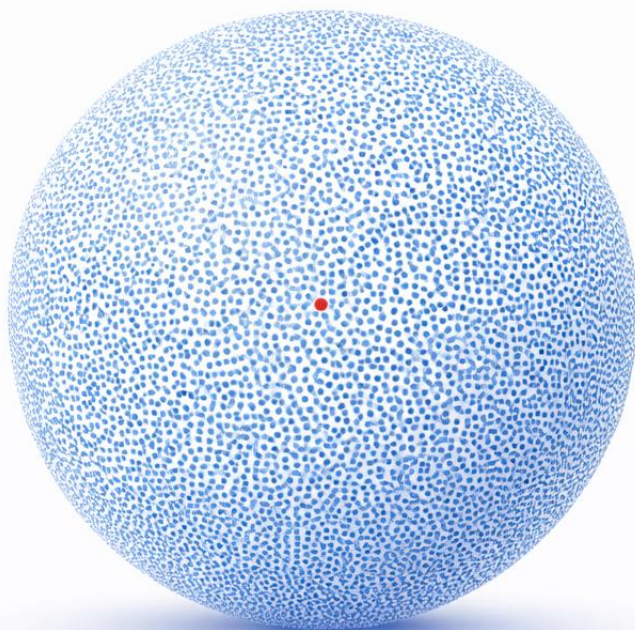


Certain substructures remain unchanged during the transformation process

**Persistent substructures in TPs**

**What if persistence is driven by substructure?**

**Hypothesis:** Persistent substructures in TPs can help improve substance prioritisation and risk assessment of PMT compounds and their TPs



123,000,000 Compounds in PubChem  
9,483 with TPs

## FAIR-TPs Statistics

An overview and key stats for FAIR-TPs, all in one page



Compounds  
**9,483**

Nodes



Reactions  
**11,260**

Edges



Transformations  
**9,371**

Unique






Sources  
**11**

Datasets

### Top 10 Compounds by Total Reactions

Here, all REACTION edges are counted, including multiple reactions between the same two compounds

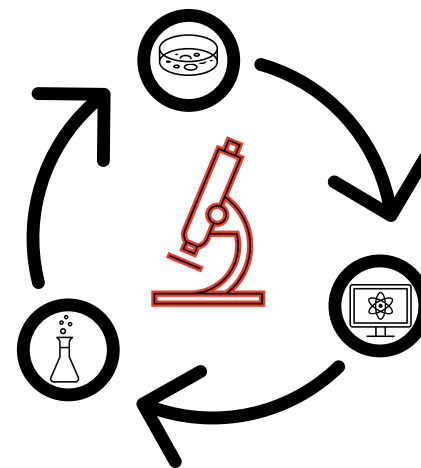
↓ PNG

Compound	Reactions
 Atrazine	126
 Diclofenac	71
 Diuron	53

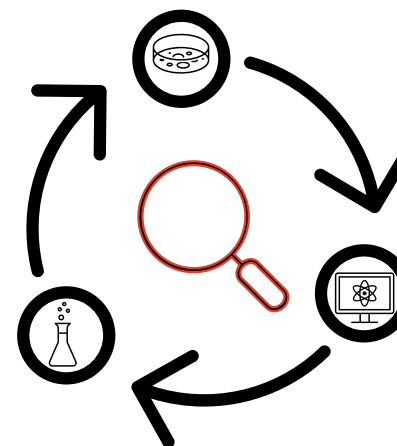
### Top 10 Compounds by Total Reactions in FAIR-TPs

Atrazine	126
Diclofenac	70
Diuron	53
Bupropion	46
Nicotine	40
Ranolazine	40

How do we investigate these persistent substructures?



Develop a **suspect screening method** to **generate TPs** for **data-poor compounds**



Use the method to **characterize persistent substructures in TPs** of 36 potential PMT compounds




ChemRxiv®

This is a preprint and has not been peer reviewed. Data may be preliminary.

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f x b in e

## Identification of persistent substructures in transformation products with zebrafish embryos using cheminformatics and a suspect screening approach

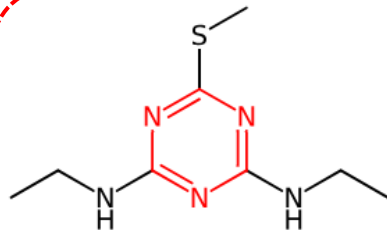
**Authors:** [Mr. Parviel Chirsir](#) , [Dr. Maria Lorena Cordero-Maldonado](#), [Mr. Maxim P. Carlier](#) , [Dr. Timo Hamers](#), and [Prof. Emma L. Schymanski](#)  | [Authors Info & Affiliations](#)

29 15

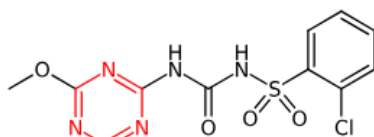


# Methods: Data-poor compounds for TP elucidation

**5 compounds with limited Literature  
TP data selected for experiments...**

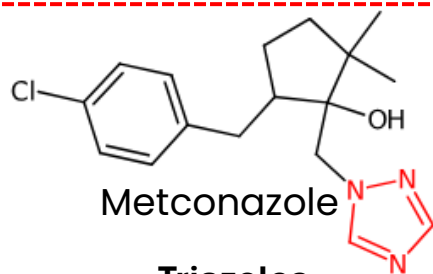


Simetryn



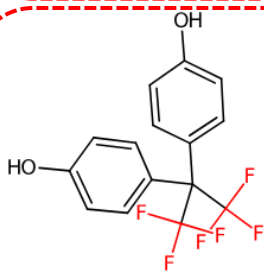
Chlorsulfuron

**Triazines**

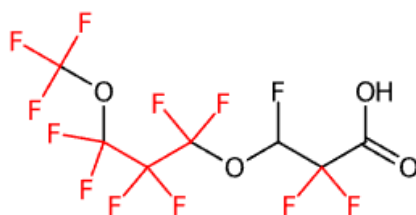


Metconazole

**Triazoles**



Bisphenol AF



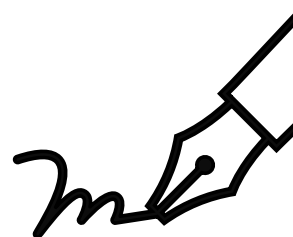
ADONA

**PFAS**

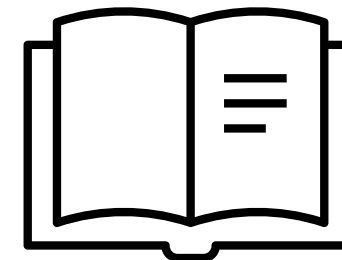


NORMAN S90 ZeroPMBBox1  
structural classes and  
**38 compounds**

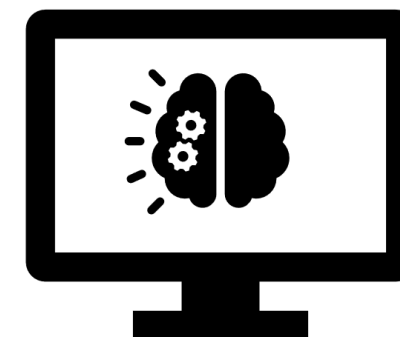
**Analogous TPs**



**Literature TPs**



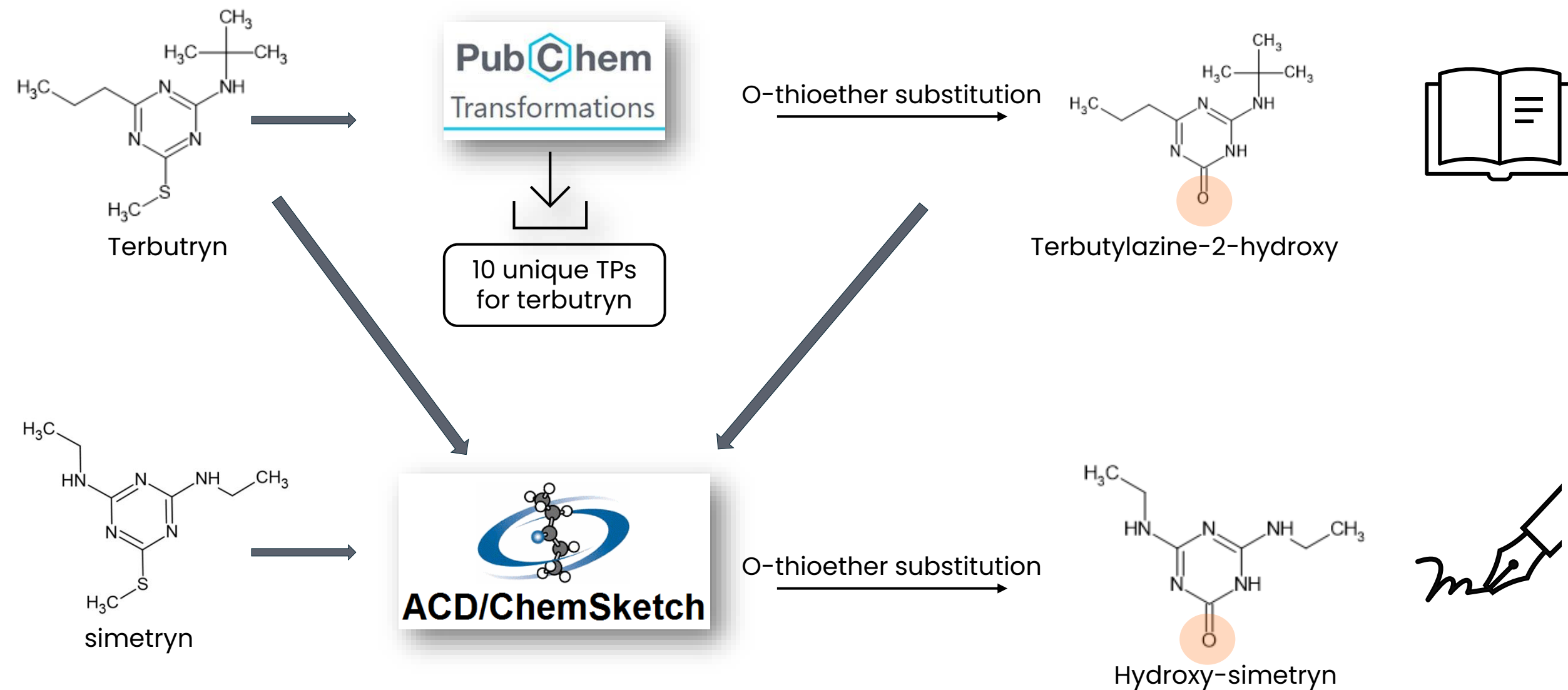
**Predicted TPs**



**BioTransformer v4.0 beta  
(env + allhuman models)**



# Methods: Analogous TP generation workflow

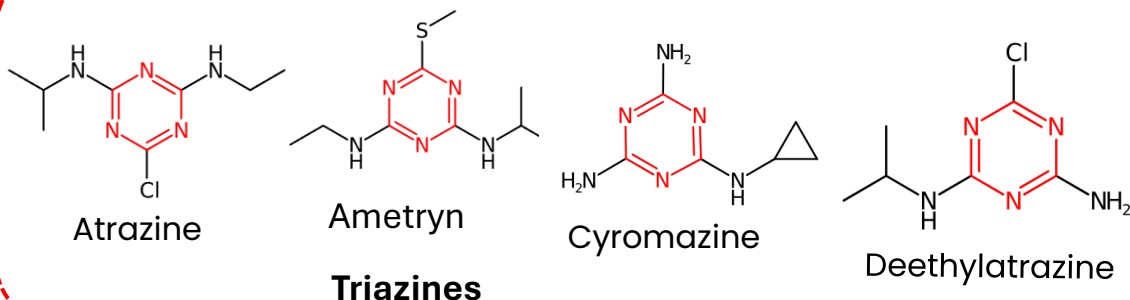


# Methods: Persistent substructures characterisation

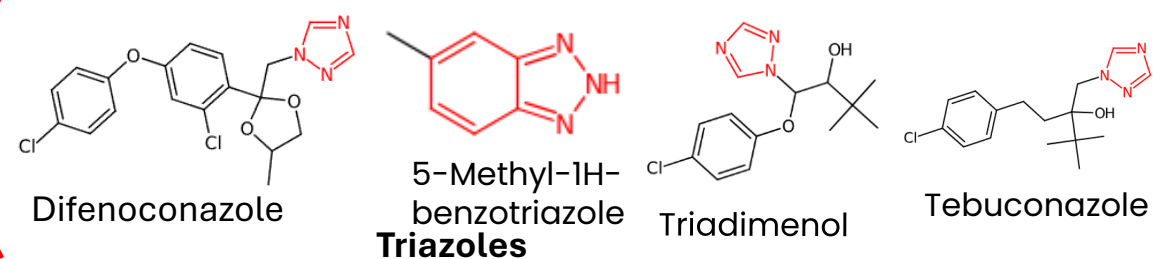
**36 PM compounds from Carlier et al. (2024)  
based on ZeroPMBOX1 substructural classes**



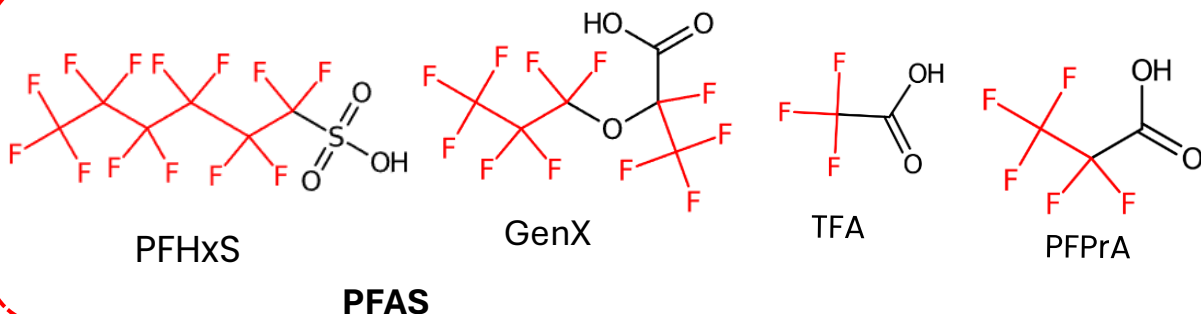
Maxim



9 Triazine compounds

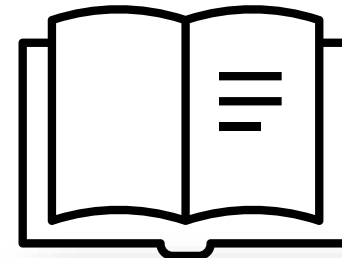


13 triazoles compounds  
&  
3 benzotriazoles



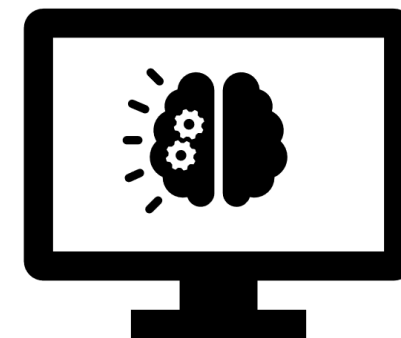
11 PFAS compounds

**Literature TPs**



**PubChem**  
Transformations

**Predicted TPs**



**BioTransformer v4.0 beta  
(env + allhuman models)**

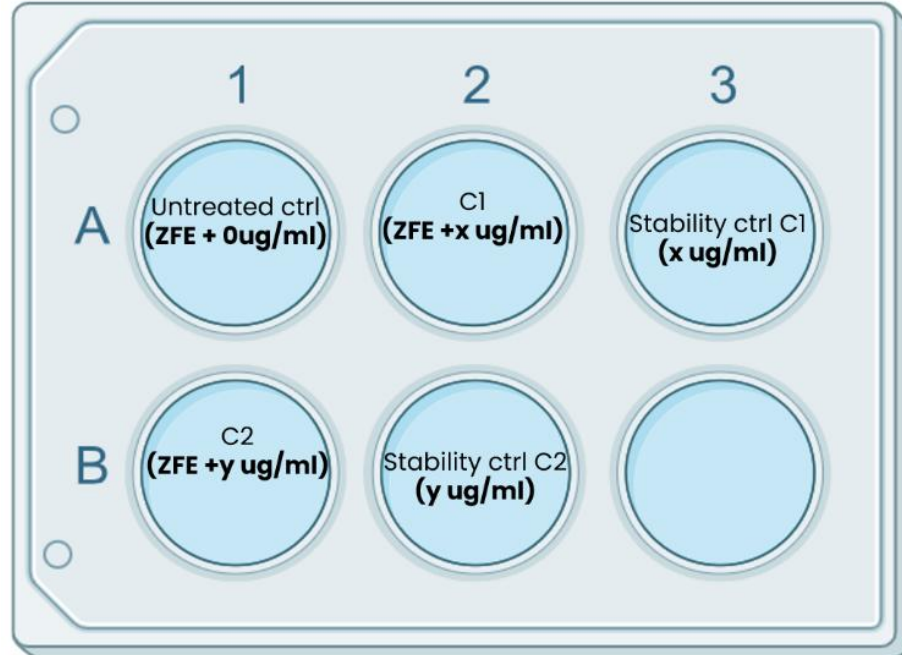


## Five Compounds with limited TPs in Literature for TP elucidation



Individual exposure in 6 well plates with 35 embryos per well

Exposure duration: 72hrs (**24–96hpf**)



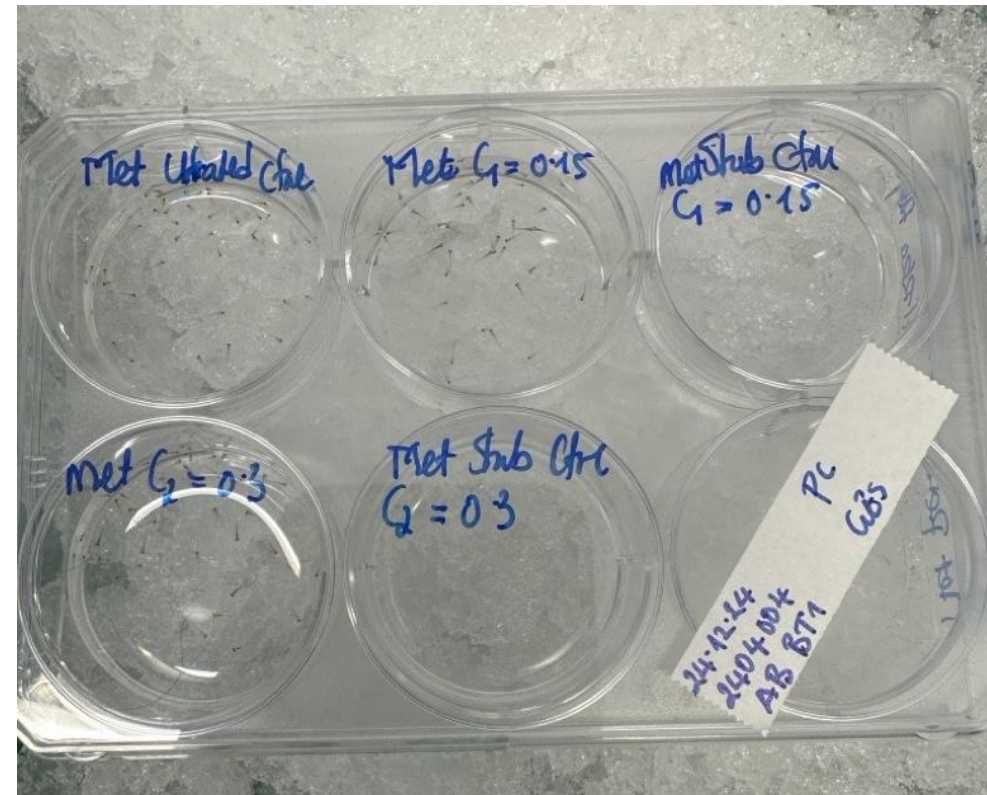
## 36 potential PM compounds for persistent substructure characterization



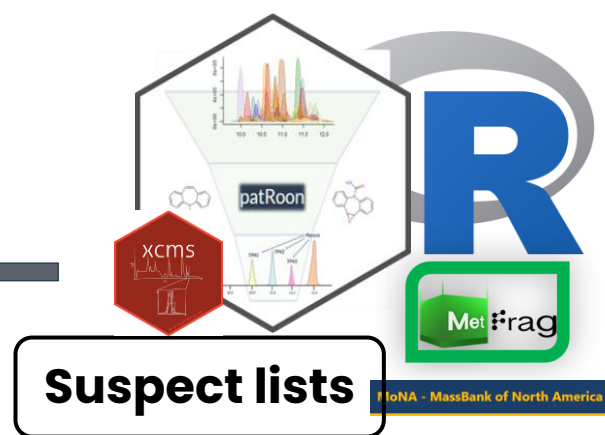
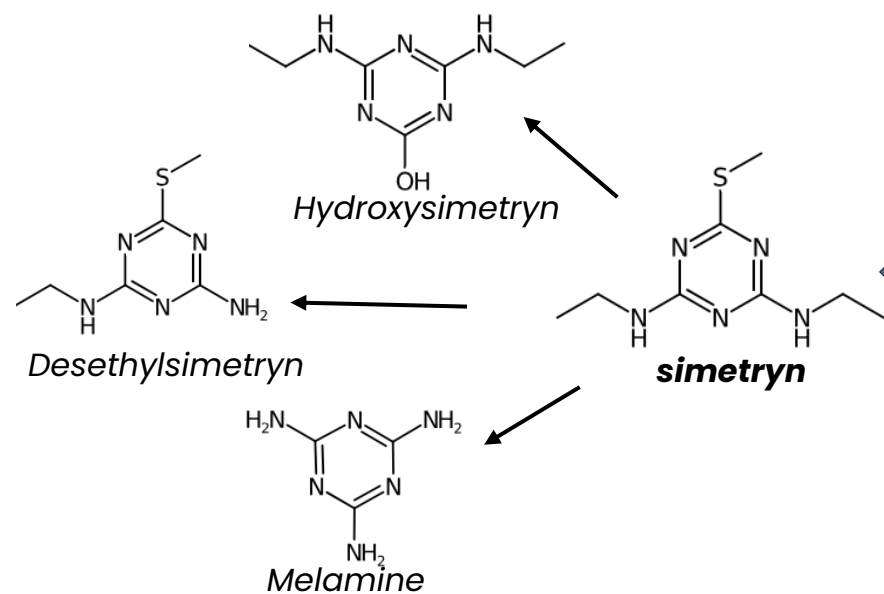
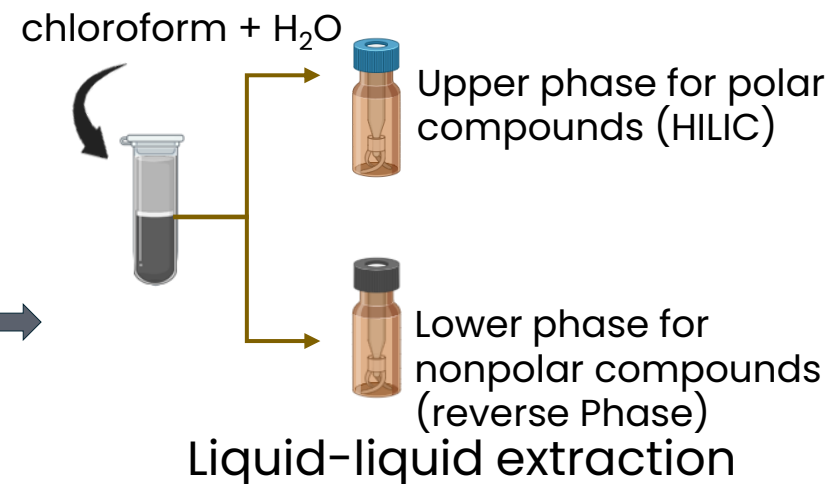
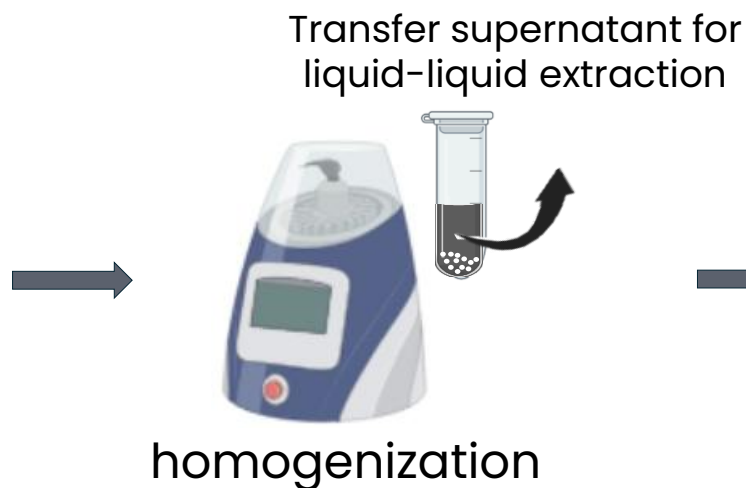
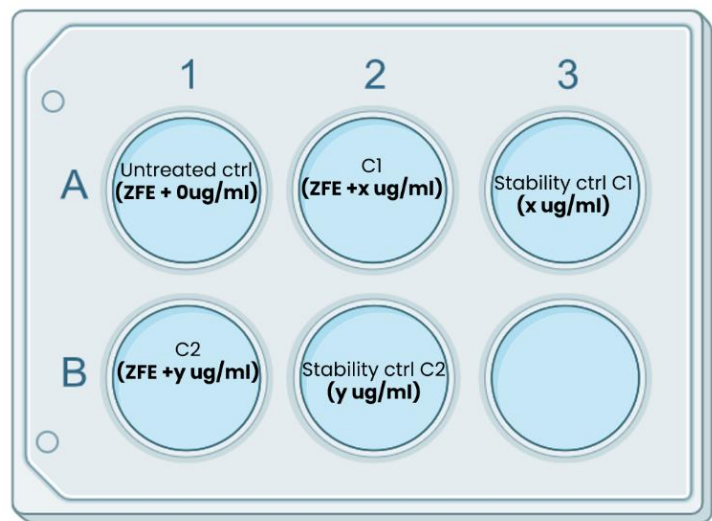
Maxim

Individual exposure in 24 well plates

Exposure duration: 92hrs (**4–96hpf**)



# Methods: Sample extraction and measurement



# Methods: Identification confidence levels

## Identification confidence levels assignment

**Level 1**

**MS, MS<sup>2</sup>, RT and reference Std.**

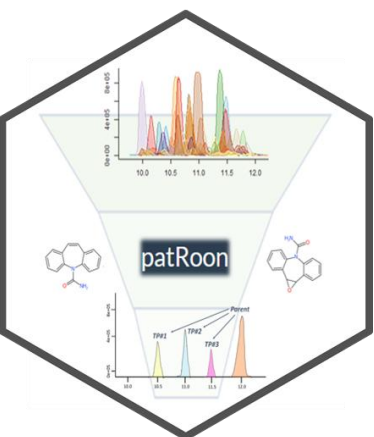
**Level 2a**

**MoNA score > 0.9**

**Level 3**

**MS, MS<sup>2</sup> and experimental data**

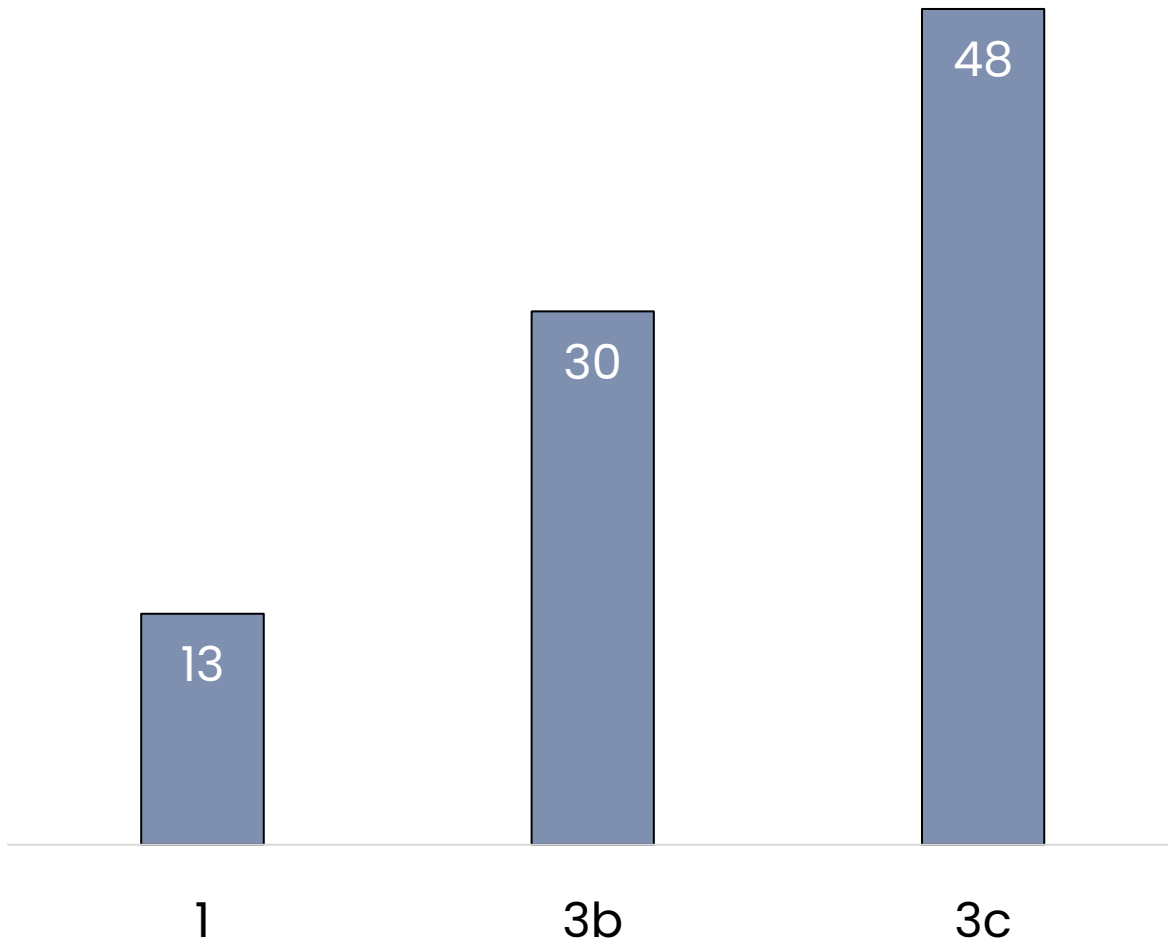
- **3a** = 0.9 < MoNA Score < 0.7;
- **3b** = at least **three fragments** match between experimental data **MetFrag *in silico*** fragmentation
- **3c** = **less than 3 fragments** match and the candidate **compound listed** in suspect list



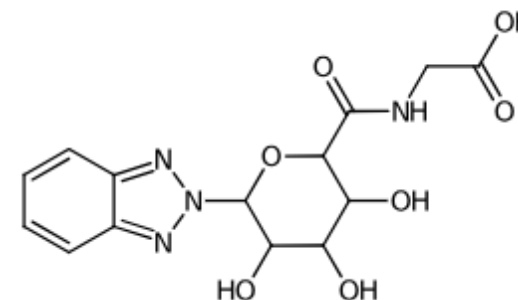
- **MoNA score** = spectral matching score from MassBank of North America (between 0-1)

# Results: Overview of TPs & ID confidence levels

Number of TPs per ID level



- Some standard are **not available** e.g.,

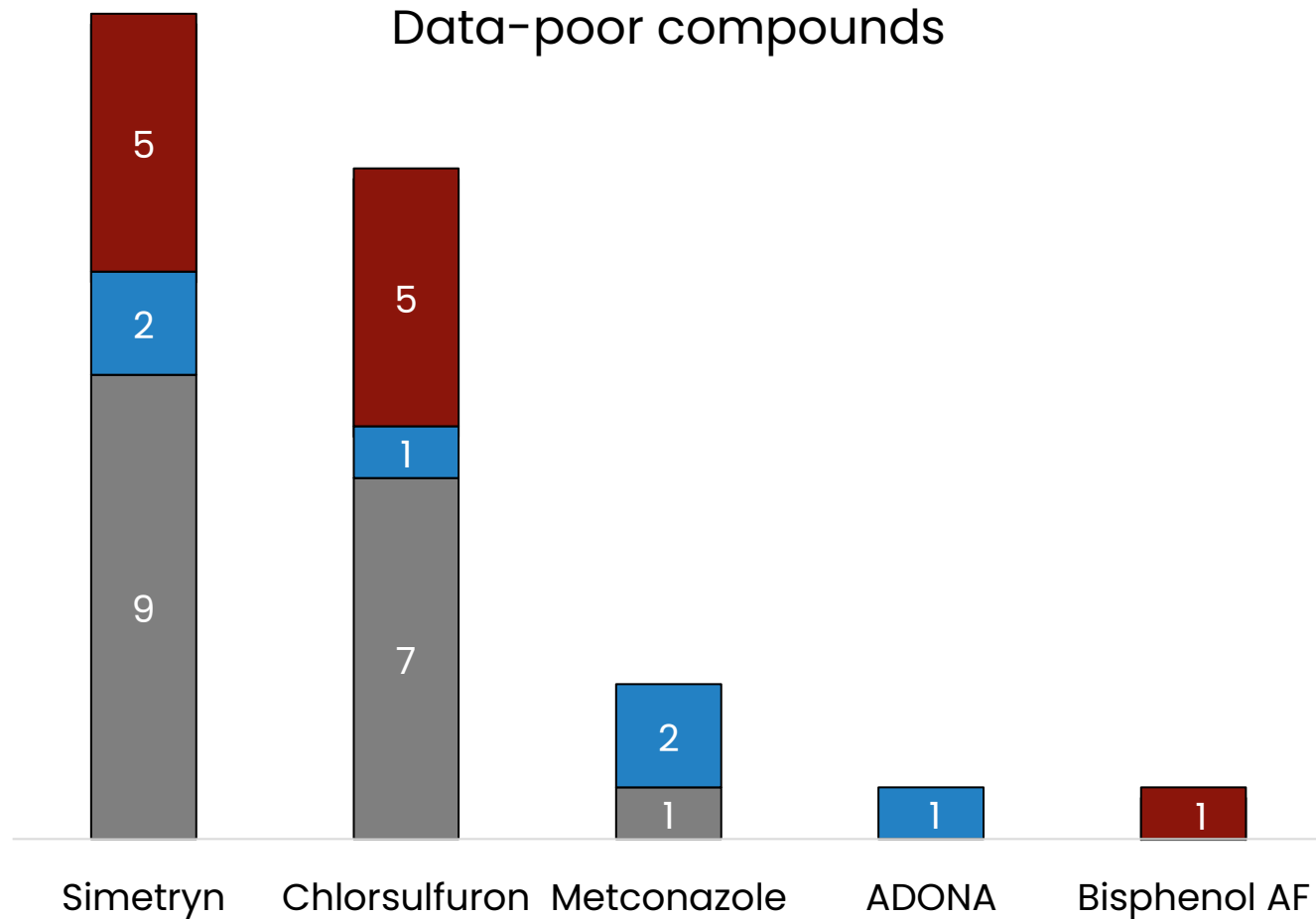


Benzotriazole glycine-glucuronide conjugate[3b]



- Generally low MS1 intensities (<10E5) so **little/no** MSMS fragments
  - 33 with MS2 (>3 fragments)**
  - 17 with <3 MS2 fragments** and
  - 30 with no MS2 fragments**

# Results: Data-poor compounds for TP elucidation

Number of TPs & information source for Data-poor compounds



 50% were analogous TPs

 32 % were both analogous &  BioTransformer TPs

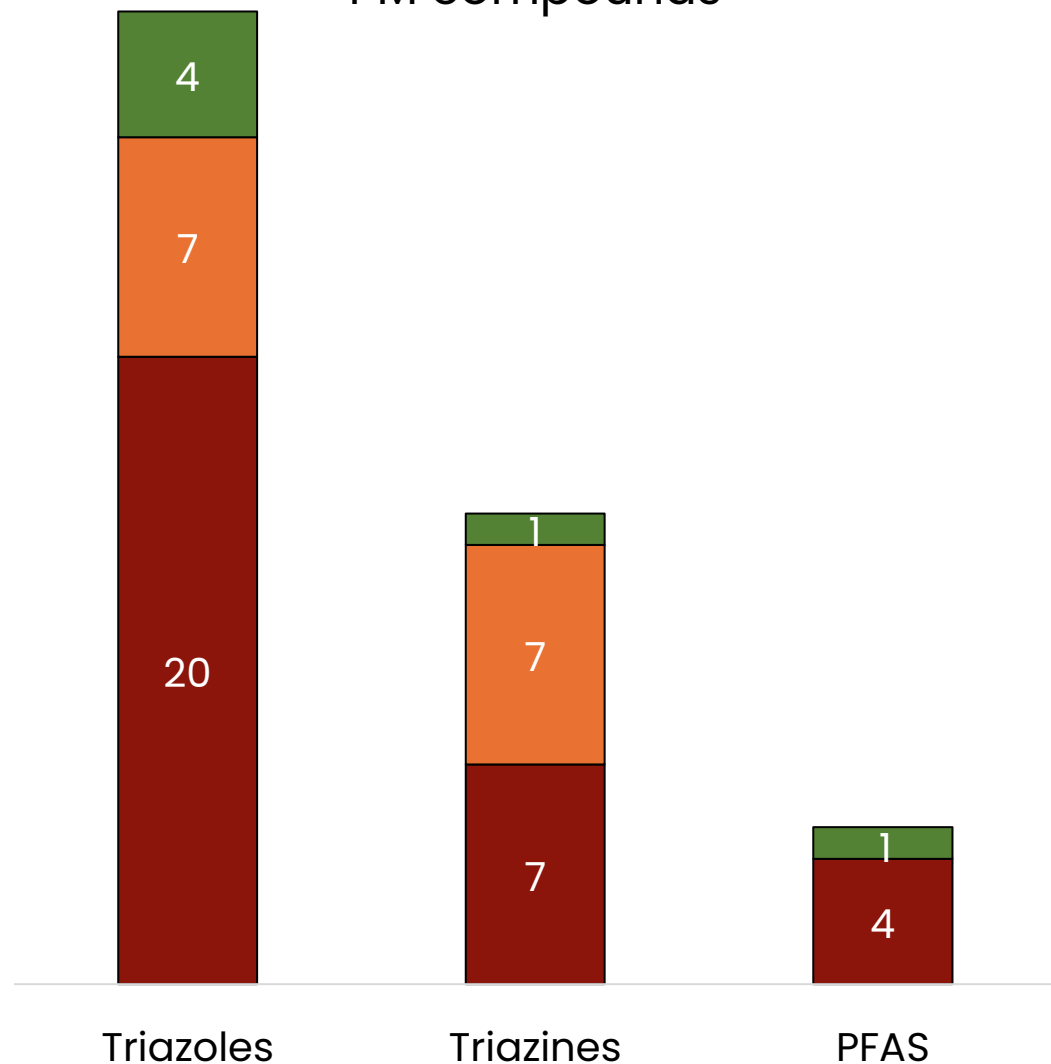
 18% were BioTransformer TPs

**Analogous TPs are a good source of TP data when structurally similar compounds have TP data**


**Show the need for more data to improve predictive models**

# Results: Persistent substructures characterisation

Number of TPs & TP source for 36 PM compounds

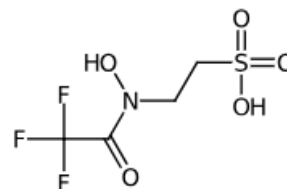


 64% were **BioTransformer TPs**

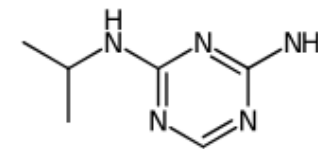
 25 % were **both BioTransformer & Literature TPs**

 11 % were **Literature TPs**

- **Predicted TPs are a complementary source to literature TP data**
- **Source of novel or poorly described TPs** e.g., TFA-  
taurine conjugate and Dechloro-deethylatrazine



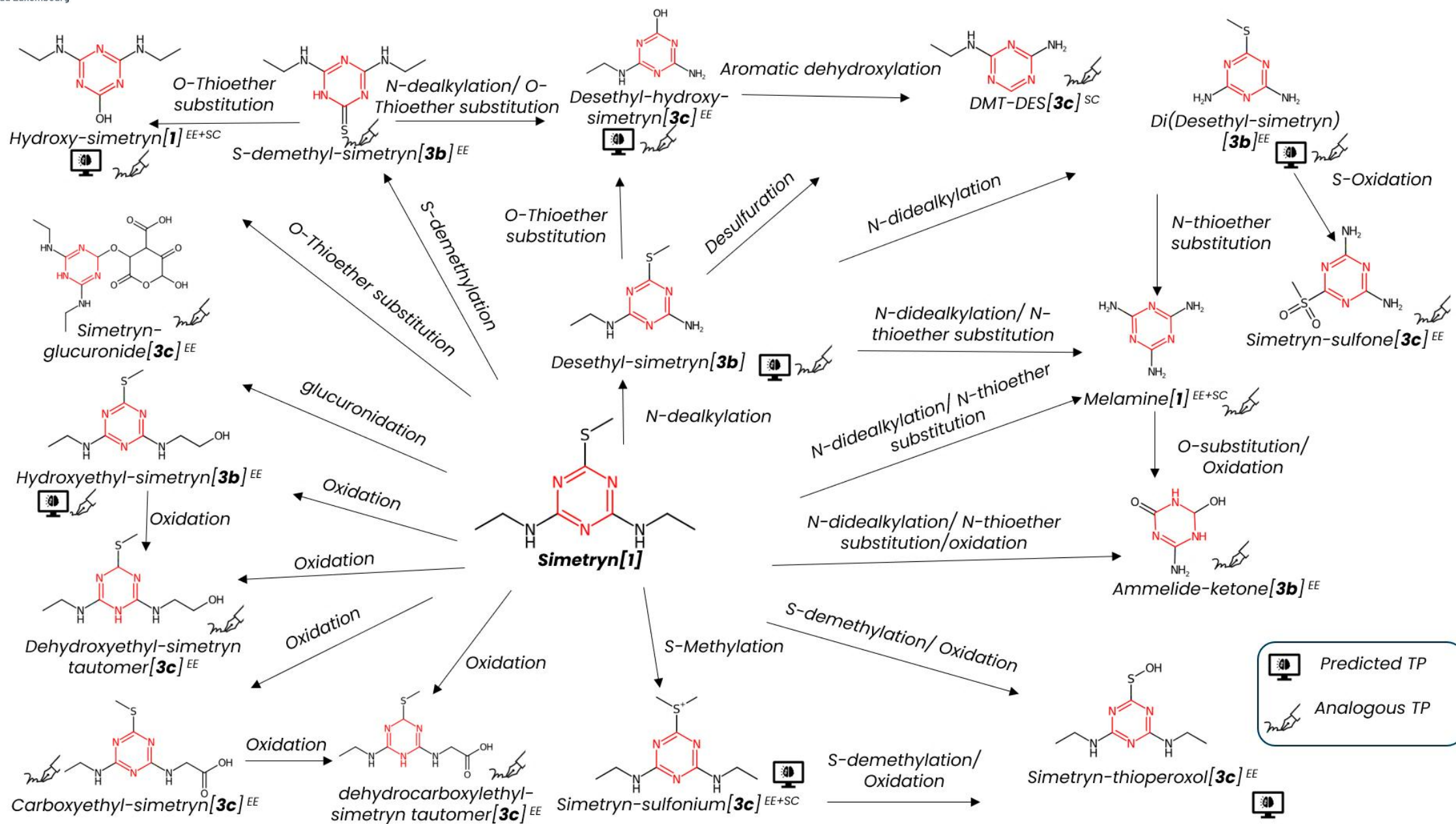
TFA-aurine

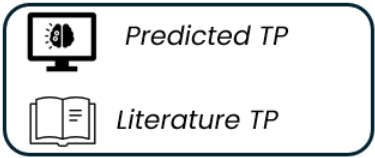


Dechloro-deethylatrazine

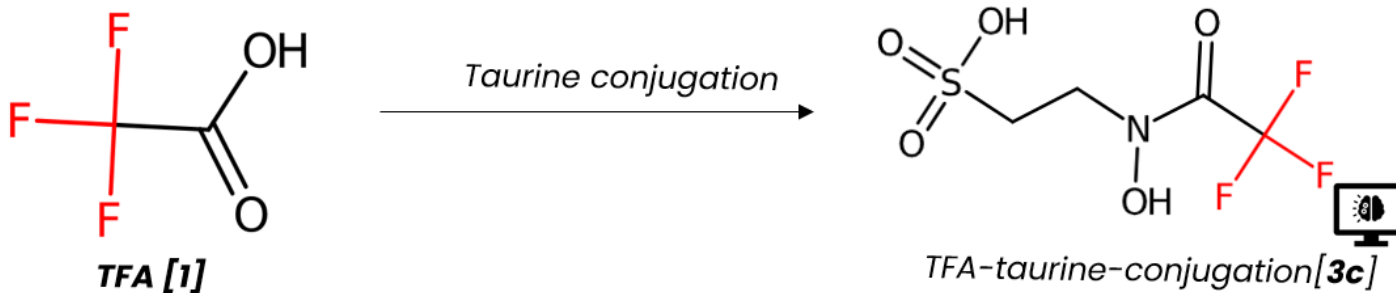
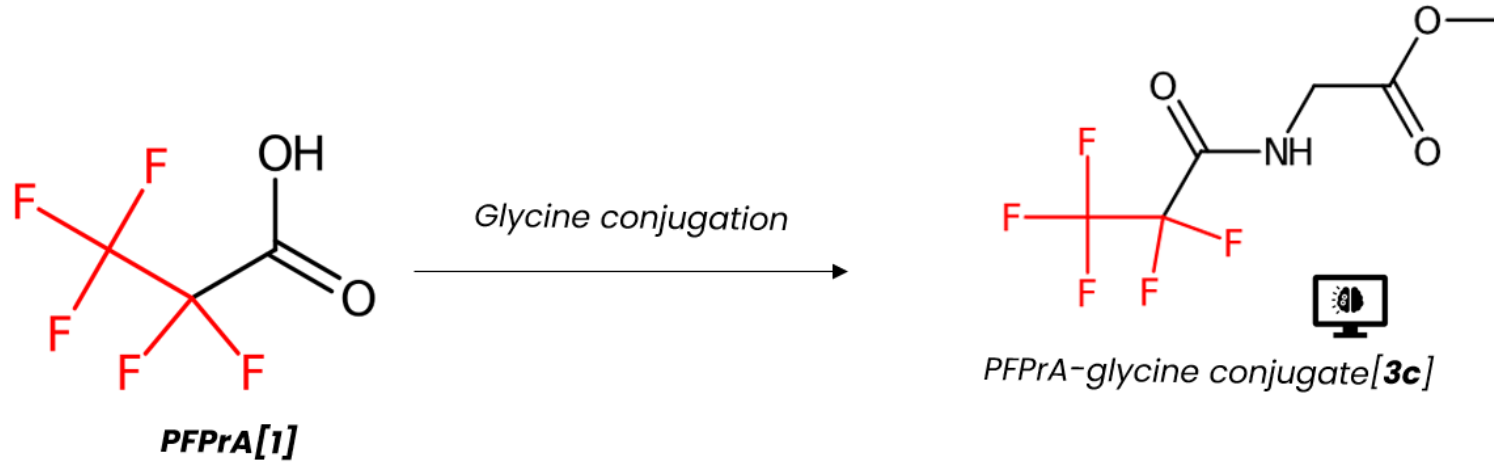



# Results: Persistent substructures – 1,3,5-triazine ring





# Results: Persistent substructures– CF<sub>3</sub>/CF<sub>2</sub> groups

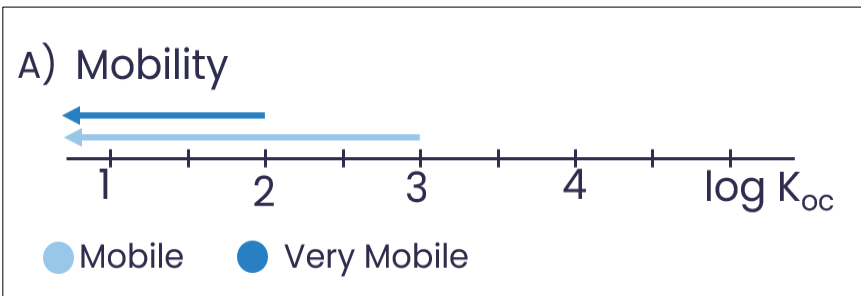



Predicted TP

- TFA-Taurine conjugation previously reported by Han et al., (2021)

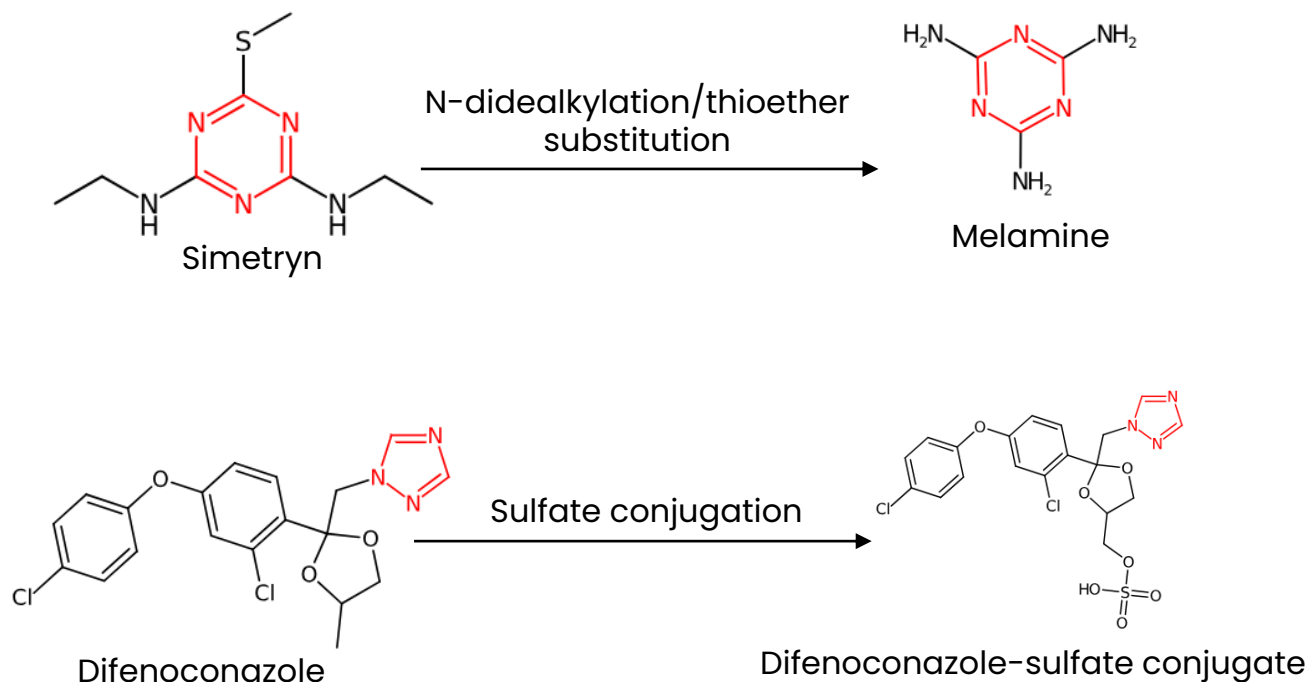
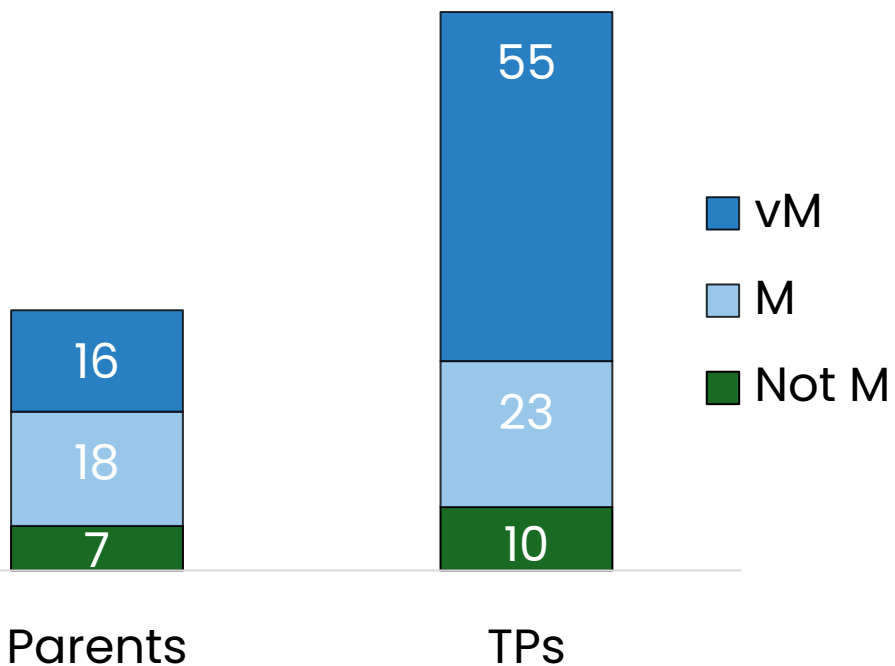
# PMT relevance of annotated TPs

EU CLP classification criteria for PMT/vPvM substances (OPERA KOC model)

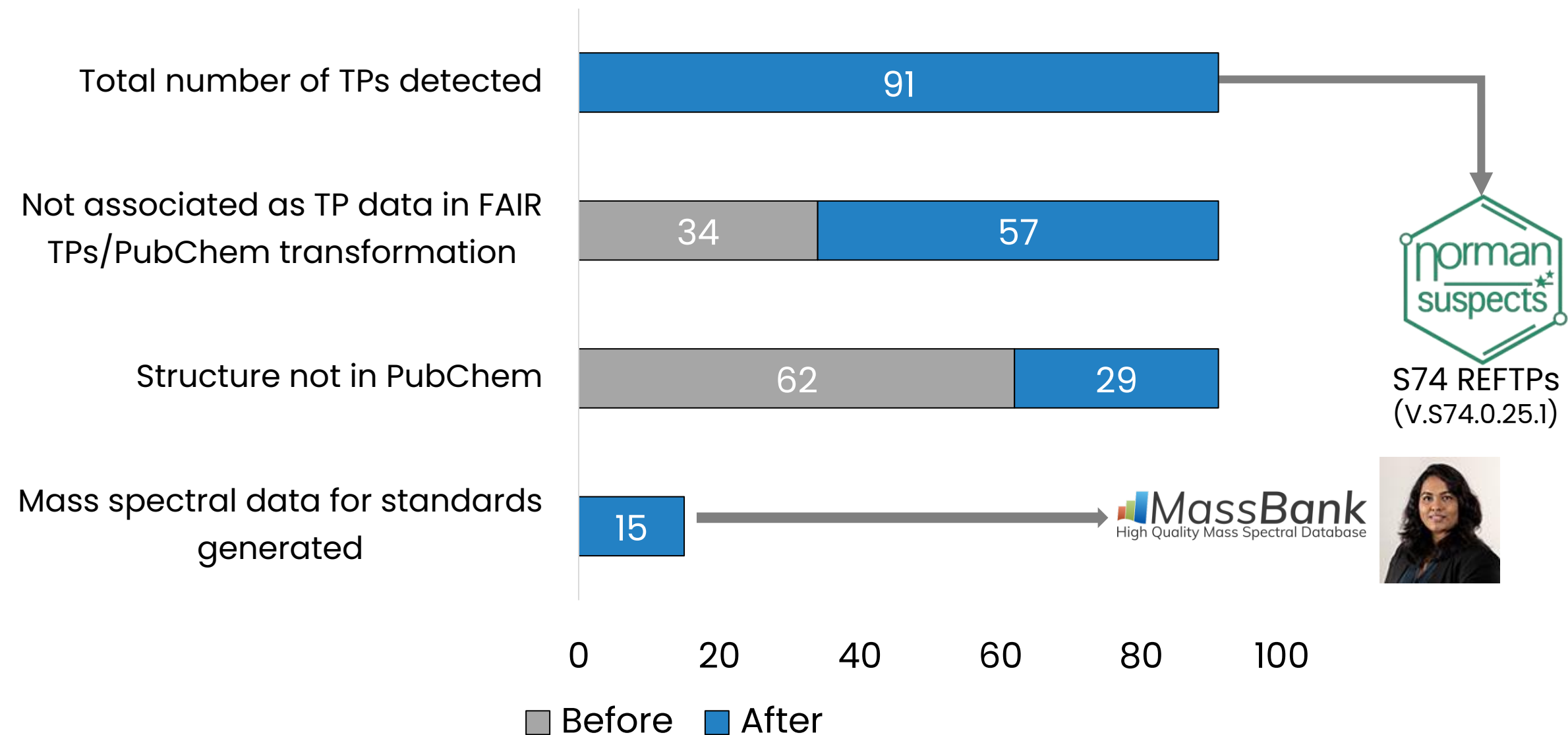


- **TPs** are **more mobile** than their **parent compounds**
  - **65% of TPs** fall under the **very Mobile** (vM) range compared to **35% of the parents**
- Degradation/conjugation -> polarity -> mobility

## Mobile compounds



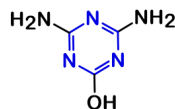
# Filling the data gaps for data-poor TPs?



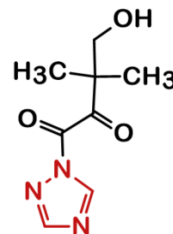
# Summary of Persistent substructures

- Persistent substructures in TPs in this zebrafish embryo study are:

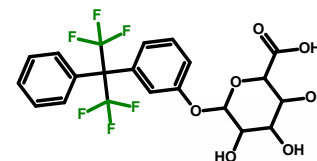
- 1,3,5 triazine ring **for Triazines**



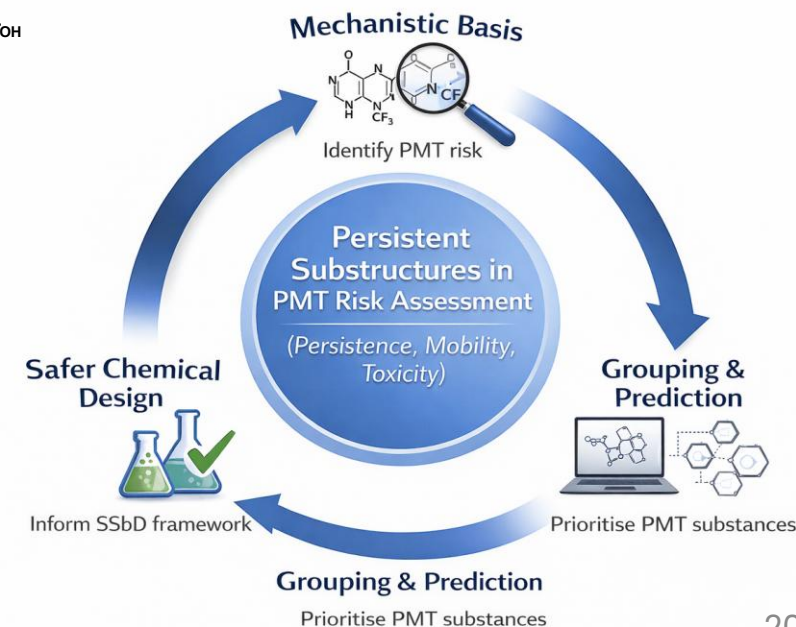
- 1,2,4 triazole ring **for Triazoles**



- Contains saturated **CF2 or CF3** part **for PFAS**



- Could be used as structural markers of persistence**
- Supporting risk assessment through grouping strategies**
- Guide and develop strategies for safer chemical design**







Lorena

**FAIR-TPs**



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European Union**

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