Future challenges for using in silico molecular typology for risk assessment of pesticides metabolites - the example of Typol

Pierre Benoit, Laure Mamy, Yoce Aprianto, Dominique Patureau, Eric Latrille, Virginie Rossard, Rémi Servien, Fabienne Bessac, Sophie Hoyau, Christelle Margoum, et al.

Future challenges for using in silico molecular typology for risk assessment of pesticide metabolites - the example of Typol

Benoit P.[1*], Mamy L.[1], Aprianto Y.[1], Patureau D.[2], Latrille E.[2], Rossard V.[2], Servien R.[2], Bessac F.[3], Hoyau S.[3], Margoum C.[4], Rocco R.[4], Martin-Laurent F.[5]

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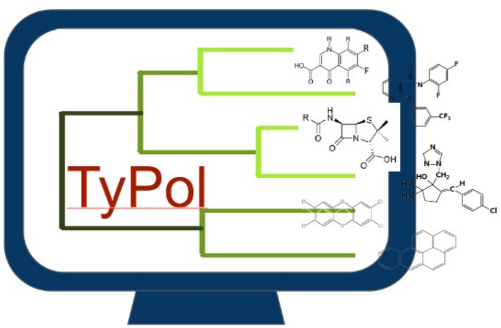
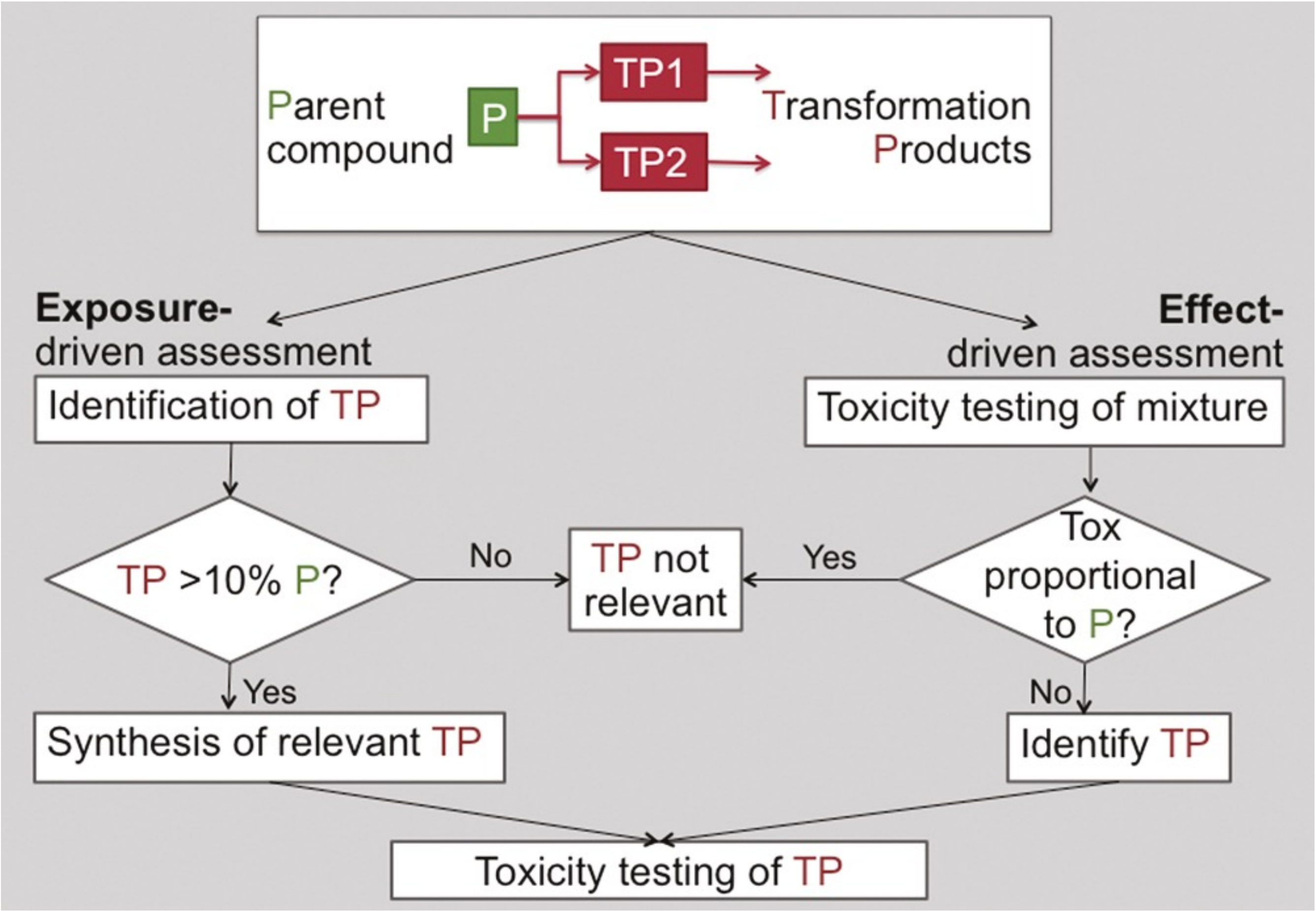
[4] UR RiverLy, INRAE, 69625 Villeurbanne, France
[5] UMR Agroécologie, INRAE, 21065 Dijon, France



Environmental Risk Assessment of Pesticide Transformation Products (TPs)



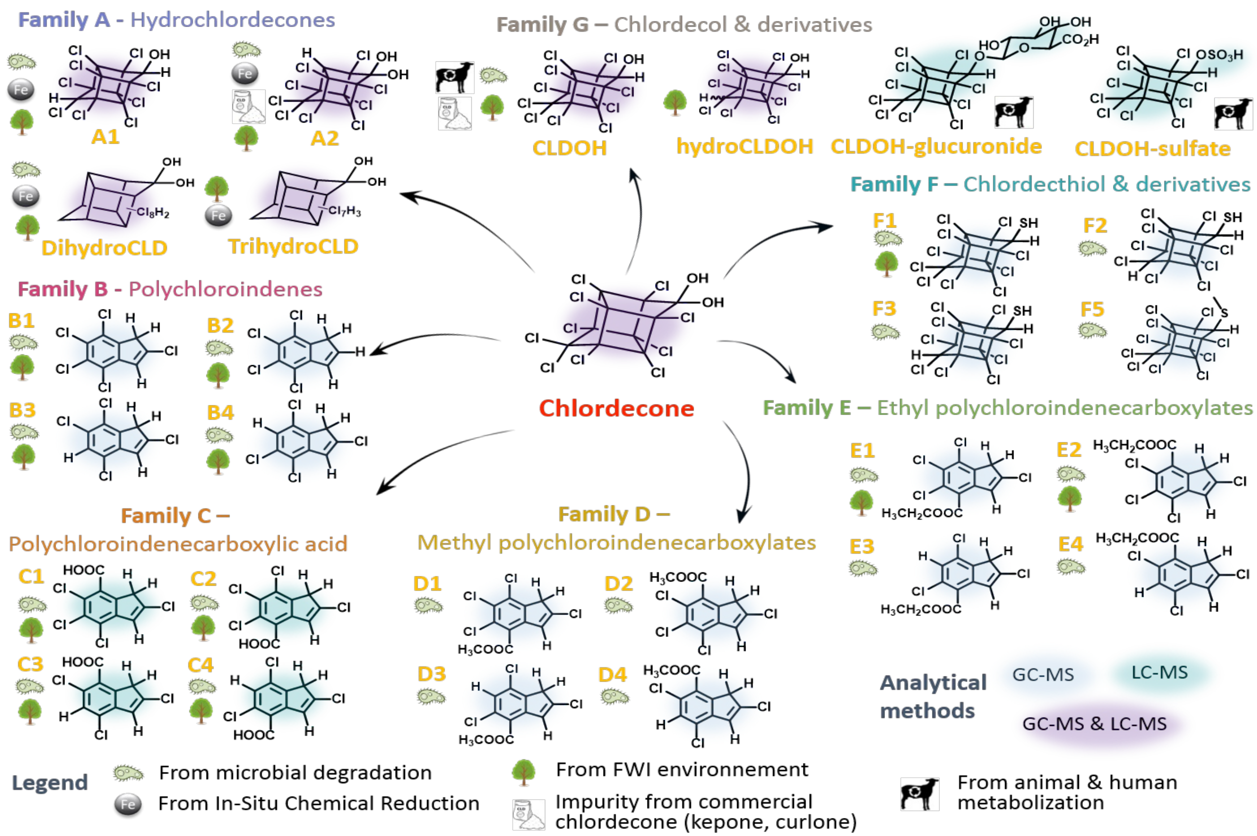
Escher & Fenner, 2011

Pesticide TPs : What do we know ?

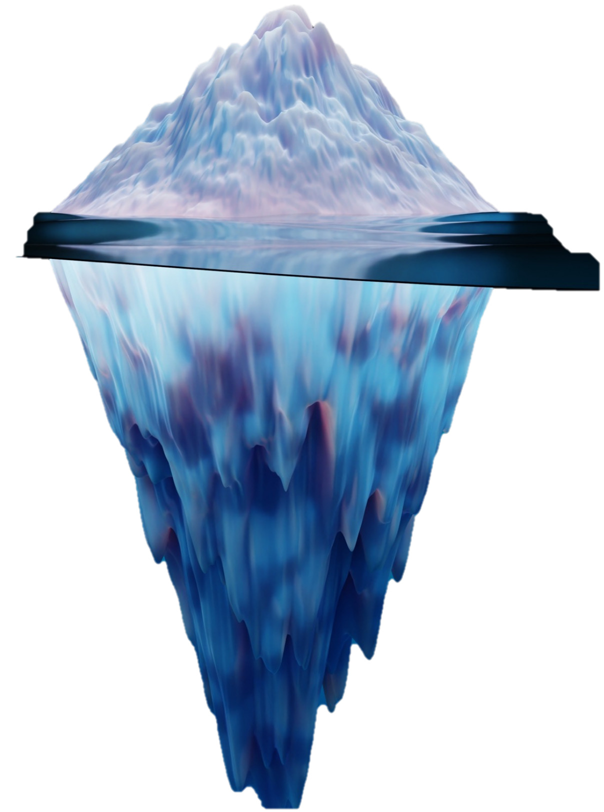
The immerged face of the iceberg

Large number of potential TPs are still unknown but

- Increasing knowledge related to analytical methods improvement (non target methods)



Saaidi et al., 2022



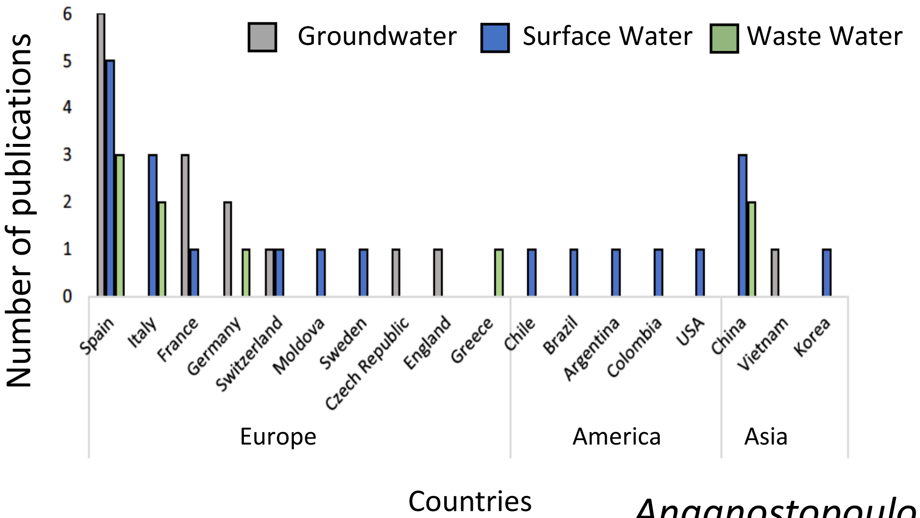
Changes in molecular structures induce changes in :

- Environmental fate
- Toxicity and ecotoxicity

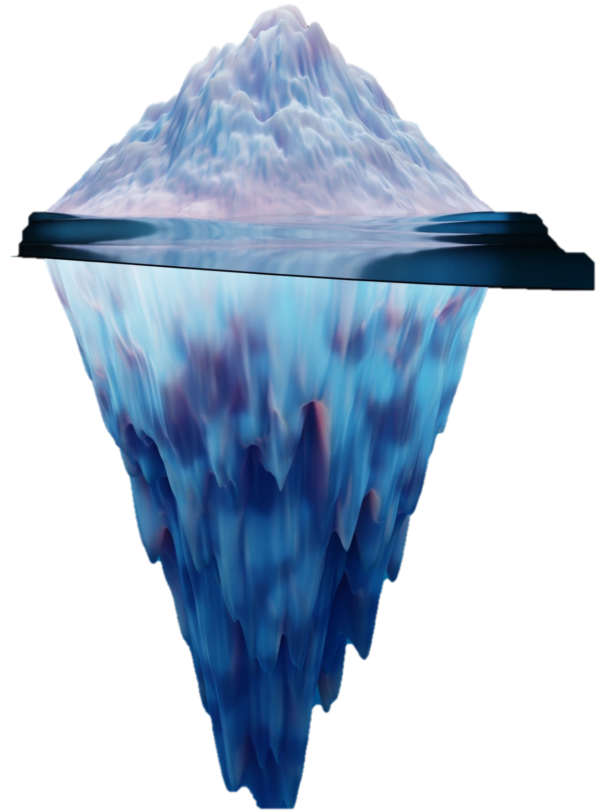
Pesticide TPs : What do we know ?

Knowledge Gaps

About identification, quantification



Anagnostopoulou et al., 2022



Increasing number of scientific publications

- Occurrence in environmental compartments
  - Water resources
  - Less information on soil, air, biota

About Fate and Effects (toxicity, ecotoxicity)

- Mostly via in silico approaches

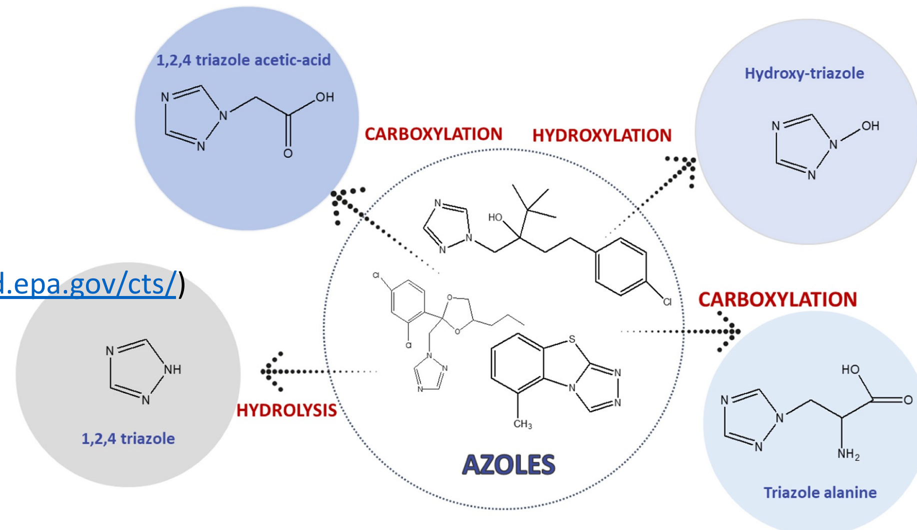
In Silico Approach applied on pesticide TPs

- **Computational tools and softwares to predict degradation pathways**

The pathway prediction system provides different relative reasoning models to predict likely biotransformation pathways and products

Examples :

- **EnviPath*** (https://envipath.org)
- **Eawag_BBD*** (http://eawag-bbd.ethz.ch/predict/)
- **PathPred*** (https://www.genome.jp/tools/pathpred/)
- **Chemical Transformation Simulator** (https://qed.epa.gov/cts/)



- **Computational tools and softwares to calculate fate and ecotoxicity endpoints**

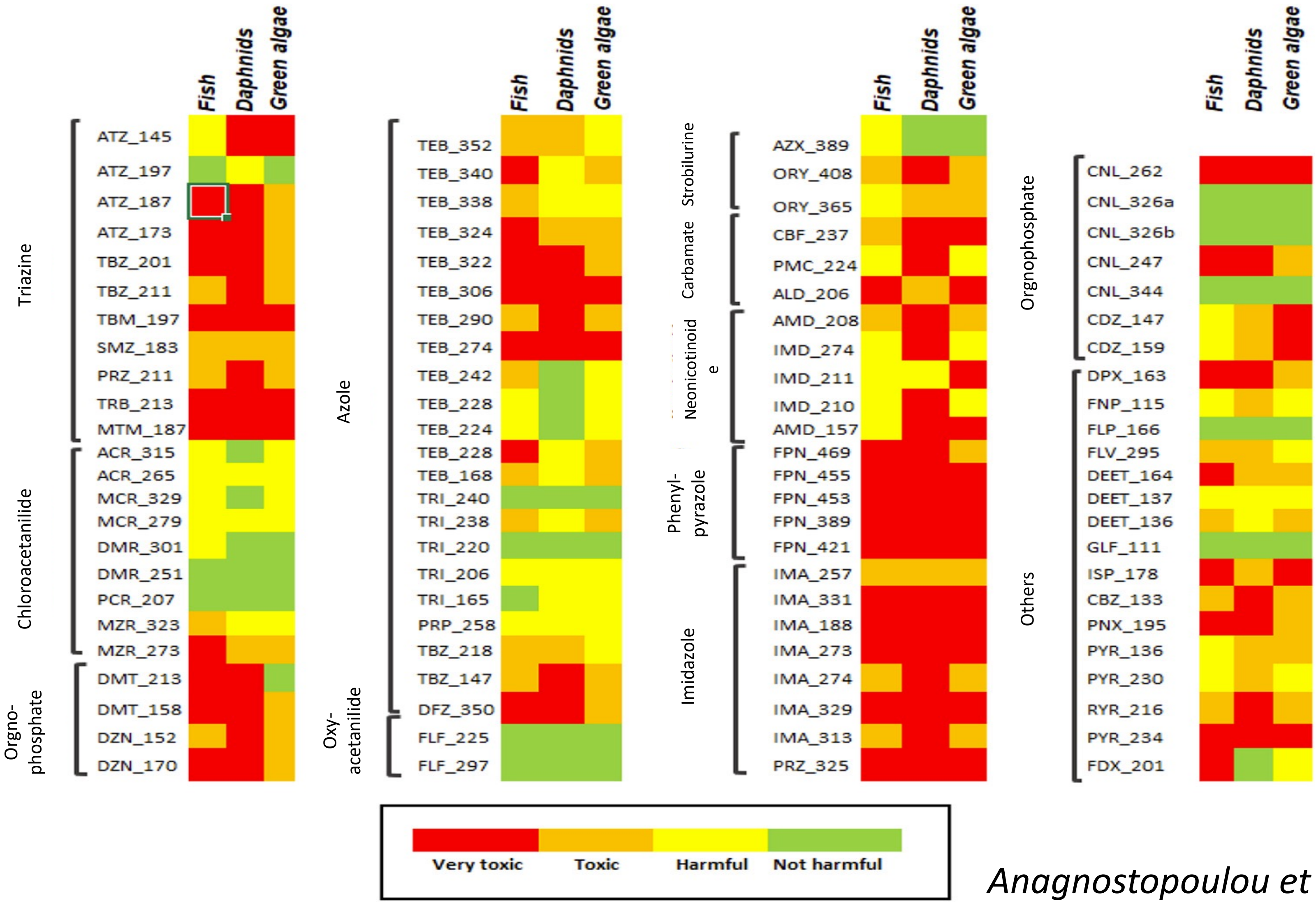
From the molecular structure knowledge – QSARs

Examples

- **ECOSAR™** (Ecological Structure Activity Relationships) : based on a linear relationships between the predicted log Kow values (**EPI Suite** KOWWIN prediction) and the associated log of the evaluated toxicity values (mmol/L) for fish, daphnids, and green algae

Predicting ecotoxicological endpoints

ECOSAR : chronic toxicty for aquatic organisms



Anagnostopoulou et al., 2022

Clustering approaches

A large amount of TPs

Not feasible for experimentation

***In silico* approaches (QSAR/QSPR)**

To predict environmental properties, fate, and toxicity of organic compounds

New approach

Rapid & comprehensive tool to handle numerous TPs for future risk assessment

Hensen *et al.* (2020)

Servien *et al.* (2014)

TyPol

- PPCPs[5]
- Several pesticides[6]
- Chlordecone[7]
- Tebuconazole[8]

[5]Bonnot (2021), [6]Traore et al. (2018), [7]Benoit et al. (2017), [8]Storck et al. (2016)

Hypothesis

The structure of molecules is **quantitatively correlated** with molecular descriptors and reflect their physicochemical properties, which enable to predict the behavior of pesticide TPs in the environment respecting their parent compounds (PCs)

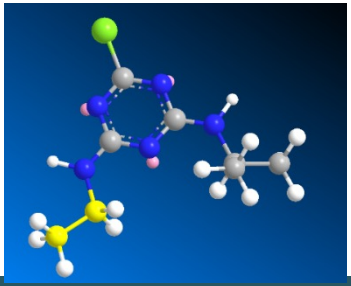
TyPol : Typology of Organic Contaminants

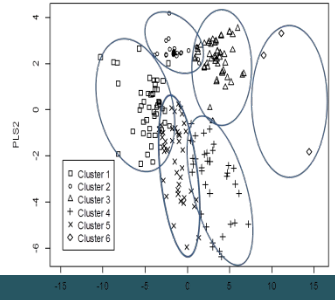
An operational tool for mapping and choosing "model" molecules to study their fate and impact in environment

- To select “model” molecules according to phenomenological (different behaviors) and operational (intensity of current or future risks) criteria

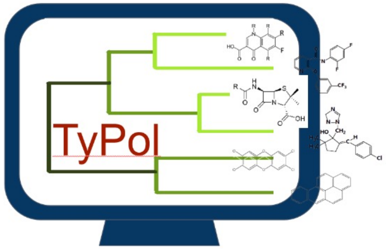
- Achieve a mutualizable tool (database) which makes possible to argue the choice of model contaminants to carry out studies concerning their fate and their ecotoxicological effects

- Test novel molecules (including transformation products) *in silico* , extrapolate (behaviours and impacts)





Biodegradation: DT50



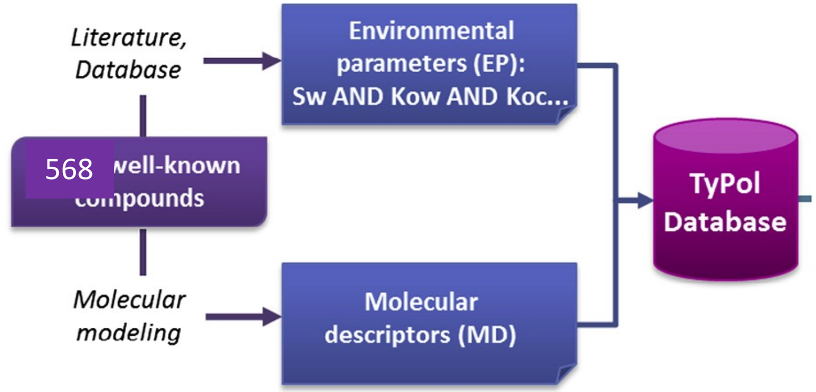
TyPol - Basic principles

Chemosphere 111 (2014) 613–622
Contents lists available at ScienceDirect
Chemosphere
journal homepage: www.elsevier.com/locate/chemosphere

TyPol – A new methodology for organic compounds clustering based on their molecular characteristics and environmental behavior

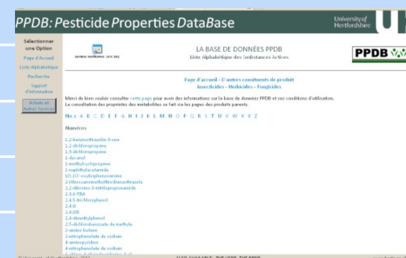
Rémi Servien[a,b,*], Laure Mamy[c], Ziang Li[d], Virginie Rossard[b], Eric Latrille[b], Fabienne Bessac[e,f,g], Dominique Patureau[b], Pierre Benoit[d]

Servien et al., 2014



Issues	Process
Atmospheric release (Post application)	Volatilization
Atmospheric dissemination (polluted site)	Adsorption
Soil purification capacity	Absorption (plants)
Transfer to organisms & plants (Biavailability)	Biodegradation
Biological effects	Abiotic degradation
Persistence in wastewater treatment plant	Dissolution
Groundwater transfer	Ecotoxicity
Surface water transfer	

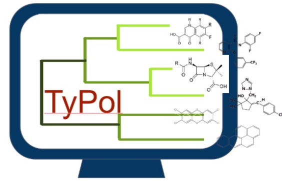
Parameters
Pvap, K_H
Koc
$DT50_a$
$DT50_b$
Kow
Sw, Kow
BCF, EC50, LC50, DL50 (various organisms) *ADI, AOEL*



K_H: Henry's constant
DT50: Half-life time
BCF: Bioconcentration factor
EC50: Concentration producing 50 % effect
LC50: Lethal concentration
LD50: Lethal dose 50
ADI : Acceptable daily intake
AOEL : Acceptable operator exposure level

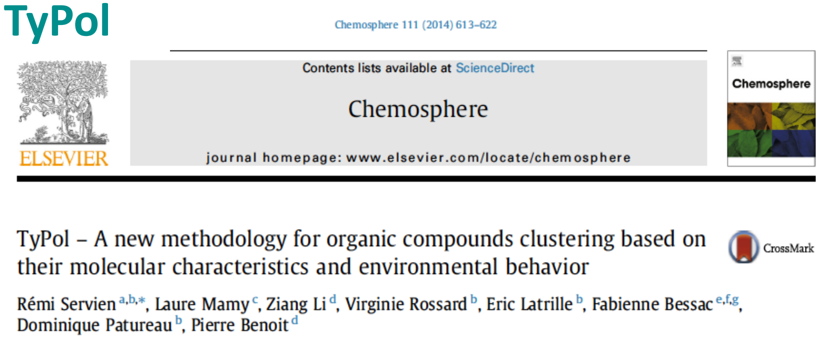
Environmental and ecotoxicological parameters retrieved from existing databases and litterature

TyPol - Basic principles



in silico and statistical approaches

TyPol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TyPol – A new methodology for organic compounds clustering based on their molecular characteristics and environmental behavior

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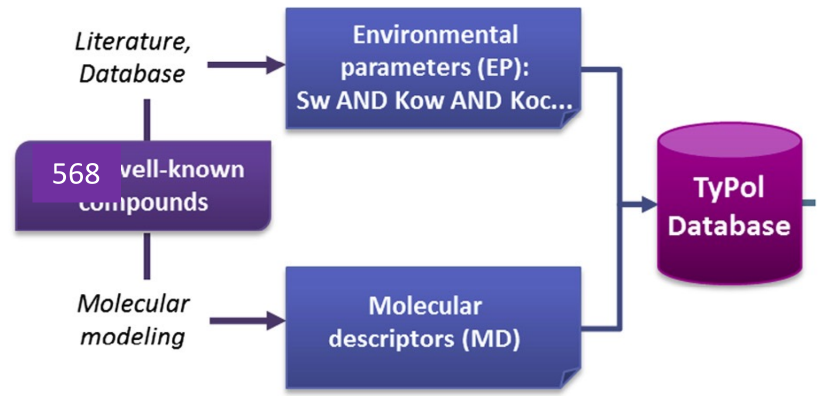
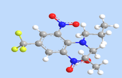
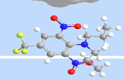
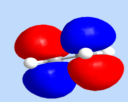
Servien et al., 2014



Descriptor type	Molecular descriptors
Constitution	Number and types atoms and bonds Molecular weight 
Geometry	Surface 
Topology	Connectivity Index 
Quantum	Polarizability Dipolar moment Orbital energies (homo, lumo) Ionization potential 

➡ 40 most generic descriptors *Mamy et al., 2015*

	August 2023
Molecules	568 parents/214 TPs
Descriptors	32939
Parameters	10569

Questions adressed

- To what extent can the TyPol tool effectively classify pesticide parent compounds and their corresponding TPs and be used to highlight the relationships between chemical structures and the environmental fate or effects of putative pesticide TPs?

- To what extent can the clusters obtained with TyPol tool be used for a first approach in the risk assessment of pesticide TPs?

Hypothesis

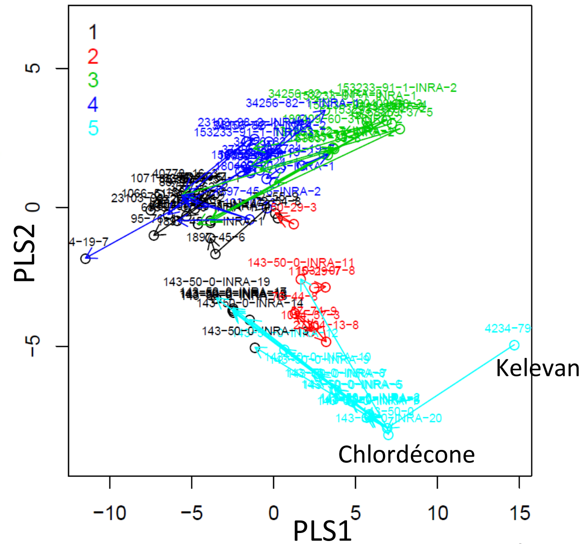
TyPol tool allows clustering pesticide parent compounds and their TPs **based on similarities** in molecular attributes, physicochemical parameters, environmental fate, and ecotoxicological effects

Filiation parent compound –> transformation products

➢ Screening chlordecone putative metabolites

Chlordecone dehalogenation products and potential congeners * (Dolfing et al., ES&T 2012)

- 6-hydrochlordécone
- 8-hydrochlordécone*
- 9-hydrochlordécone
- 10-hydrochlordécone
- 3,7-dihydrochlordécone*
- cis 8,10-dihydrochlordécone*
- 3,7,10-trihydrochlordécone*
- 8,10,10-trihydrochlordécone*
- 3,7,10,10-tetrahydrochlordécone*
- 2,3,7,8,10,10-hexahydrochlordécone
- 2,3,7,8,9,10,10-heptahydrochlordécone
- 1,2,3,7,8,9,10,10-octahydrochlordécone
- 1,2,3,4,6,7,8,9,10-nonahydrochlordécone
- 1,2,3,6,7,8,9,10,10-nonahydrochlordécone
- 1,3,4,6,7,8,9,10,10-nonahydrochlordécone*
- 2,3,4,6,7,8,9,10,10-nonahydrochlordécone
- décahydrochlordécone



Benoit et al. STOTEN 2017

➢ Visualization of class change for certain metabolites
➢ Potential consequences in terms of behavior and therefore risk

Projet Biodechlord « Search for the biological signature of chlordecone degradation in the soils of the French West Indies »
Coord : F. Martin Laurent (INRAE, Agroécologie, Dijon)

Combining TyPol to suspect screening approach

Environmental Pollution 208 (2016) 537–545

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Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

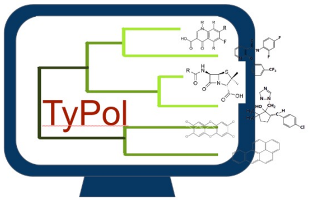
Short communication

Identification and characterization of tebuconazole transformation products in soil by combining suspect screening and molecular typology

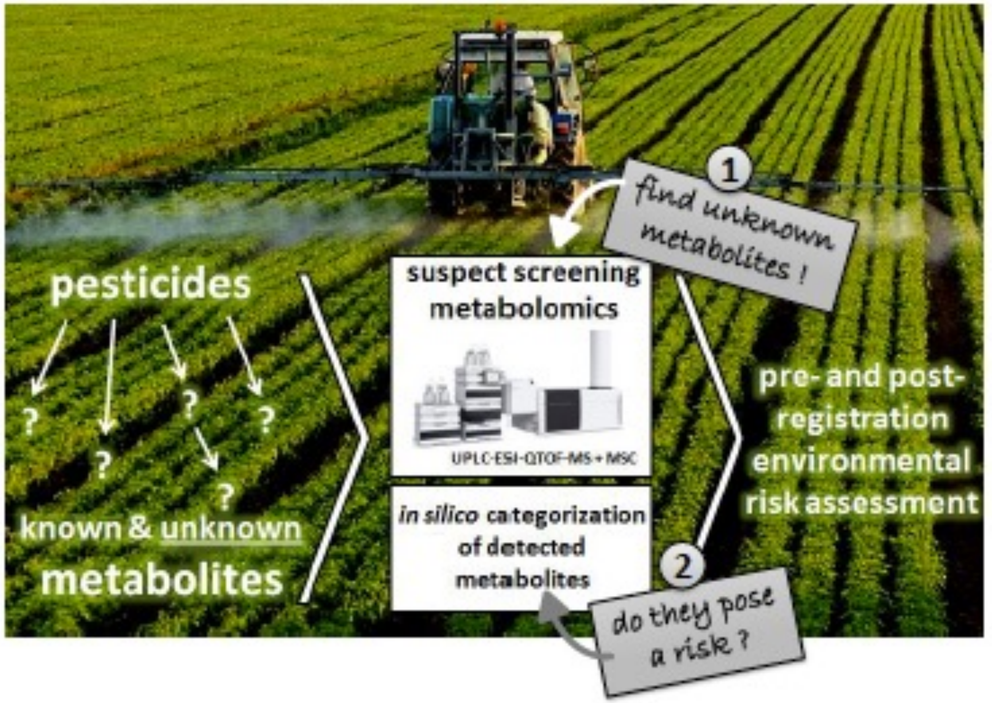
CrossMark

Veronika Storck [a, b, c], Luigi Lucini [c, **], Laure Mamy [d], Federico Ferrari [b], Evangelia S. Papadopoulou [e], Sofia Nikolaki [f], Panagiotis A. Karas [e], Remi Servien [g], Dimitrios G. Karpouzas [e], Marco Trevisan [c], Pierre Benoit [d], Fabrice Martin-Laurent [a, *]

[a] INRA, Mixed Research Unit 1347 Agroecology, Dijon, France
[b] Aeiforia srl, Spinoff Catholic University of the Sacred Heart, Fidenza, Italy
[c] Catholic University of the Sacred Heart, Department of Agronomy and Environmental and Chemistry, Piacenza, Italy
[d] INRA, Mixed Research Unit 1402 ECOSYS, Thiverval-Grignon, France
[e] University of Thessaly, Department of Biochemistry and Biotechnology, Larissa, Greece
[f] University of Patras, Department of Environmental and Natural Resources Management, Agrinio, Greece
[g] INRA, Mixed Research Unit 1331 Toxalim, Toulouse, France

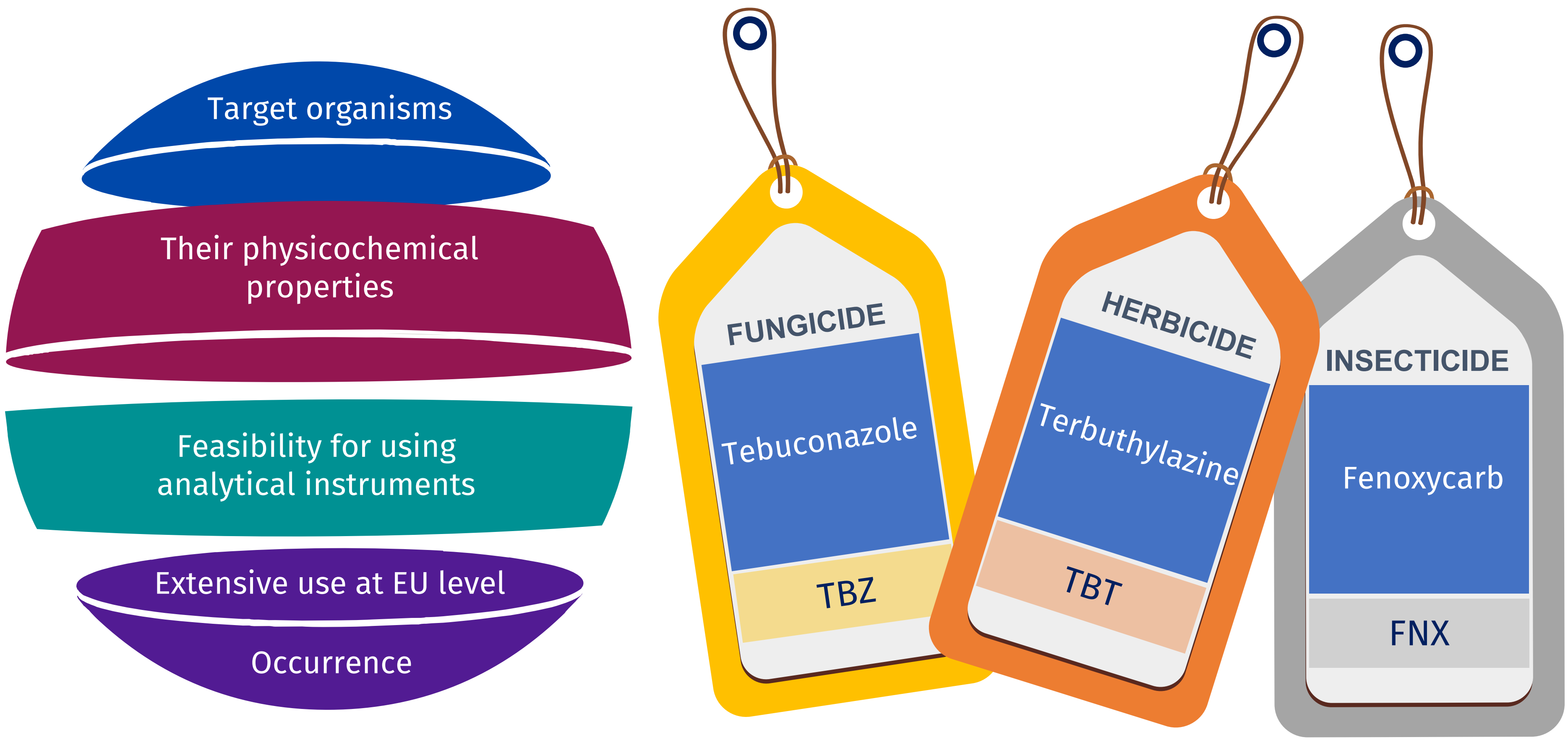


- *76 tebuconazole metabolites*
- *Complementary diagnostic from in silico clustering by TyPol*



Storck V., Lucini L., Mamy L., Ferrari F., Papadopoulou E.S., Nikolaki S., Karas P.A., Servien R., Karpouzas D.G., Trevisan M., Benoit P., Martin-Laurent F. 2016. Env. Pollution, 208, 537-545.

Characterizing the Environmental Fate and Ecotoxicological Effects of Pesticide Transformation Products



Aprianto 2023

Data preparation for Typol Database

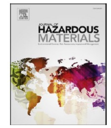
Journal of Hazardous Materials 440 (2022) 129706

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

ELSEVIER



Enhanced database creation with *in silico* workflows for suspect screening of unknown tebuconazole transformation products in environmental samples by UHPLC-HRMS

Kevin Rocco*, Christelle Margoum, Loïc Richard, Marina Coquery

INRAE, UR RiverLy, 69625 Villeurbanne, France

Transformation Predictors

Envipath | CTS | Eawag_BDD

PathPred | Meteor | Zeneth

Input data:
chemical identifier of TBZ, TBT, FNX (**SMILES**)

Output data:
Various format (depends on predictors)

Output converter: OpenBabel (V2.4.1)
*https://sourceforge.net/projects/**openbabel**/*

Output harmonization:
InChiKey format, one-way readable identifier

OUTPUT

Tebuconazole	Terbuthylazine	Fenoxycarb
257 TPs	**21 TPs**	**11 TPs**

In total 289 TPs

List of TPs was retrieved from Rocco *et al*. (2022; 2023), INRAE-UR RiverLy, Villeurbanne

Data preparation for Typol Database

Descriptors Calculation

40 molecular descriptors

Dragon 7.0

Constitutional

- No of atoms
- Molecular weight
- No of rings
- No of bonds
- Etc..

Topological

- Connectivity index (CI)
- Valence CI

ChemOffice Ultra 12.0+Excel

Geometric

- Connolly molecular surface area

Computational Chemistry (DFT)

3D Quantum-chemical

- Dipole moment
- HOMO energy
- LUMO energy
- Polarizability
- Ionization energy

- Using Gaussian 16
- Executed in terminal macOS Ventura 13
- Access to CALMIP supercomputing

Environmental & Ecotoxicological Parameters

EPI Suite (v4.11) + **ECOSAR (v.2.0.2)**

Environmental parameter

Ecotoxicological parameter (acute & chronic endpoints)

Environmental Process	Parameter
Dissolution	Sw (mg/L); log Kow
Volatilization	Pvap (mPa); K_H (Pa m^3/mol)
Adsorption	Koc (L/kg)
Degradation	DT_{50} (days)
Bioaccumulation	BCF
Ecotoxicity	LC_{50} / EC_{50}, NOEC (mg/L)

fish, daphnia, & green algae

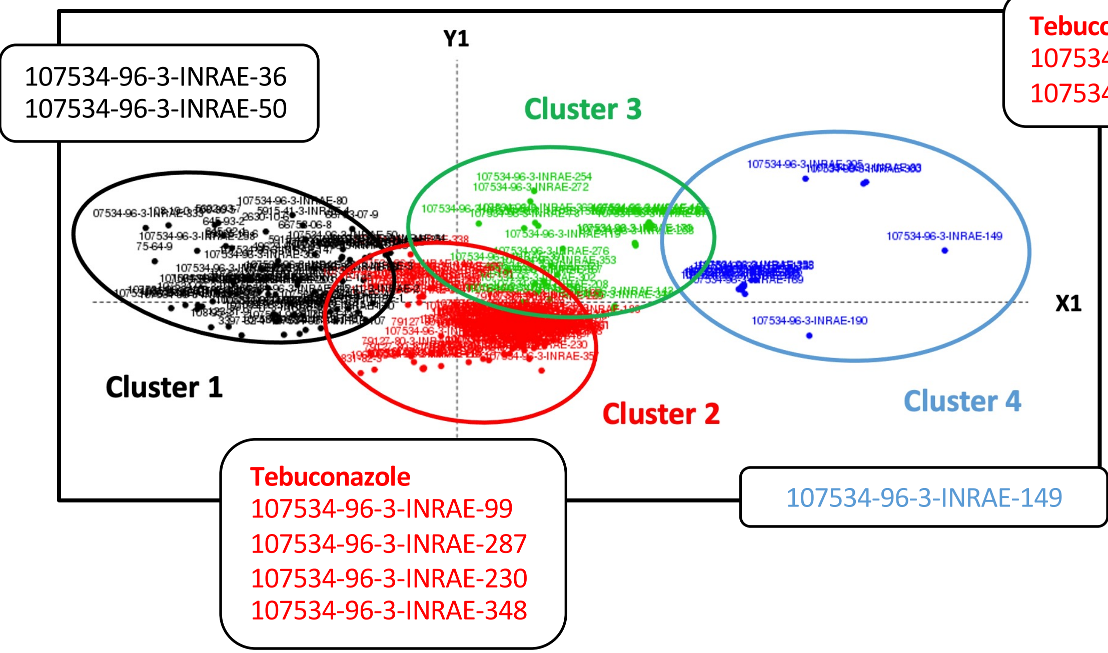
Most values were retrieved from Rocco *et al.* (2022; 2023), except DT_{50} & K_H

Aprianto 2023

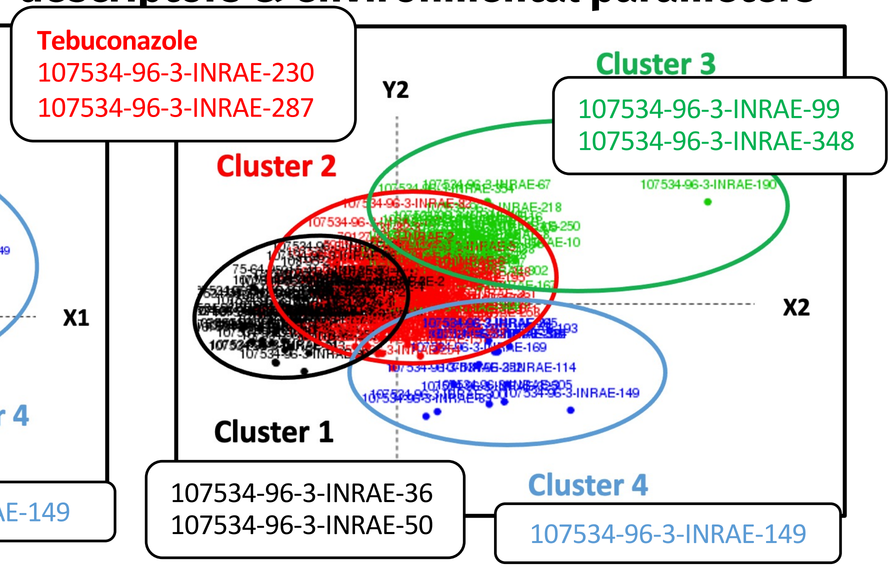
Example of results

217 compounds (3 Parent Compounds Tebuconazole, Terbuthylazine et Fenoxycarb and 214 TPs)

Clustering 1 : Based on molecular descriptors



Clustering 2 : Based on molecular descriptors & environmental parameters



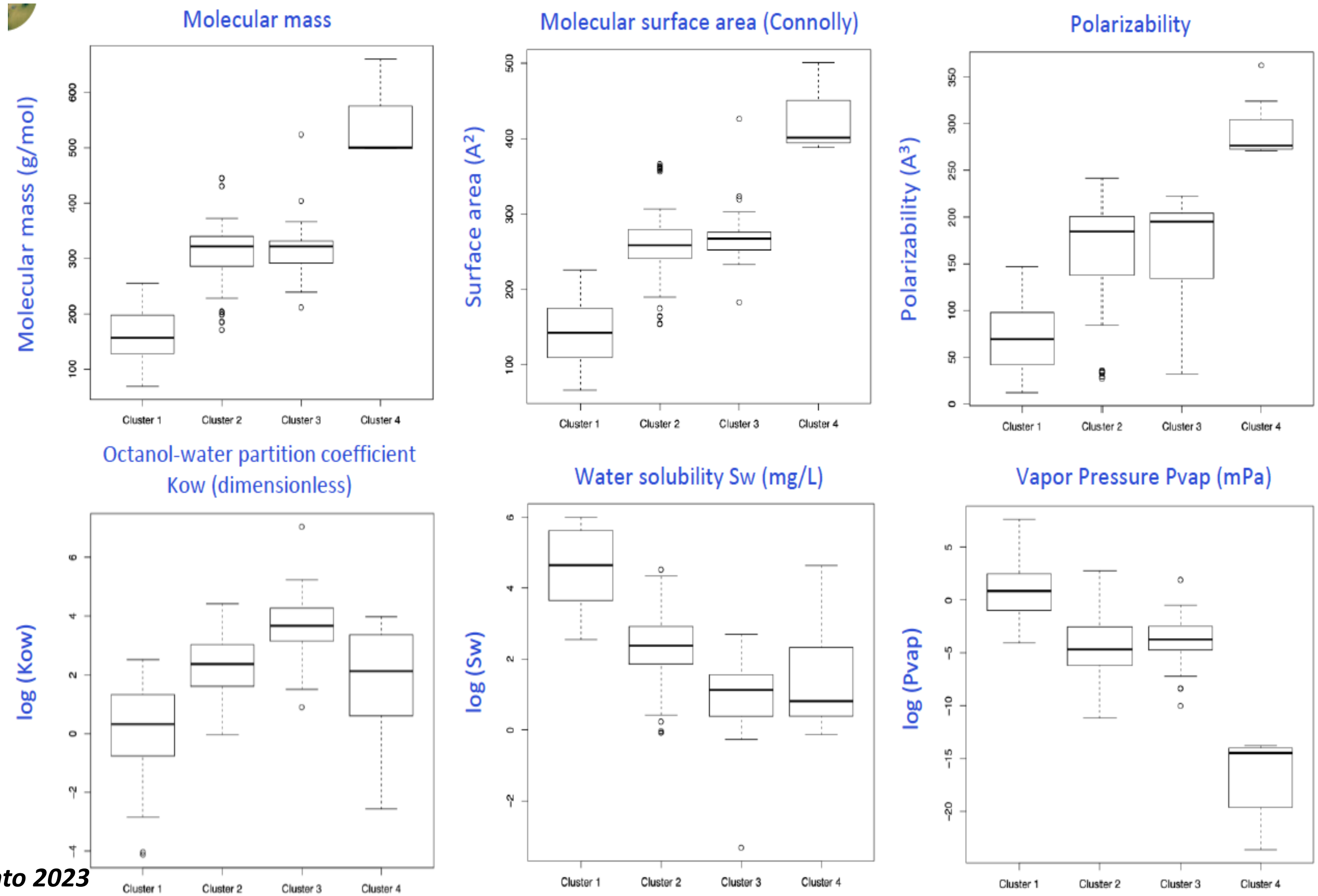
≡ TPs that **are clustered away** from parent compounds **may have different behavior** due to marked changes in molecular structure

Aprianto 2023

Example of results

217 compounds (3 Parent Compounds Tebuconazole, Terbuthylazine et Fenoxycarb and 214 TPs)

Clusters main characteristics



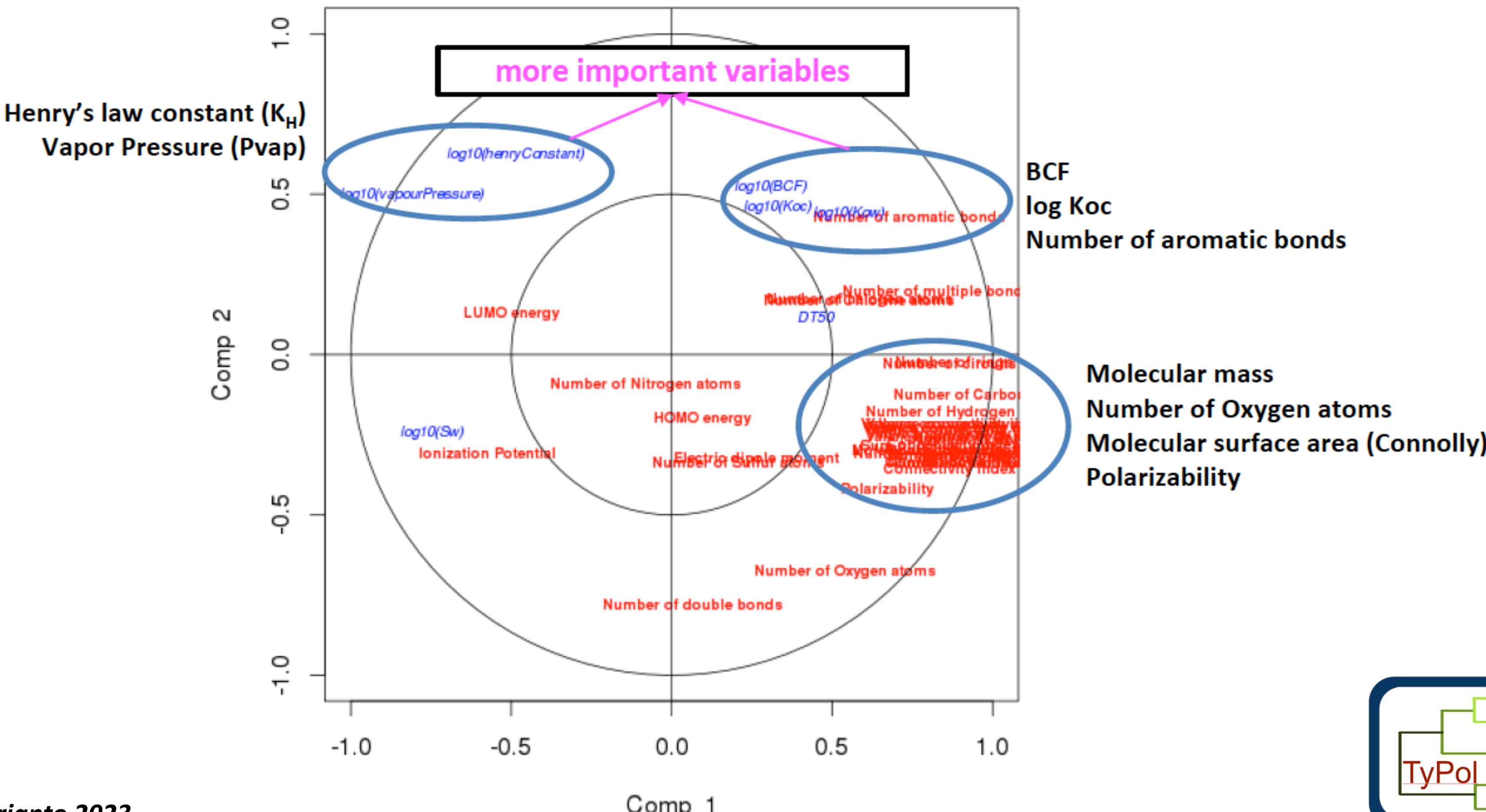
Molecular descriptors

Environmental parameters

Aprianto 2023

Identifying molecular descriptors driving the fate of TPs

217 compounds (3 Parent Compounds Tebuconazole, Terbuthylazine et Fenoxycarb and 214 TPs)

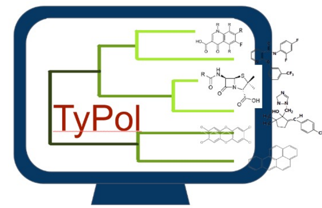


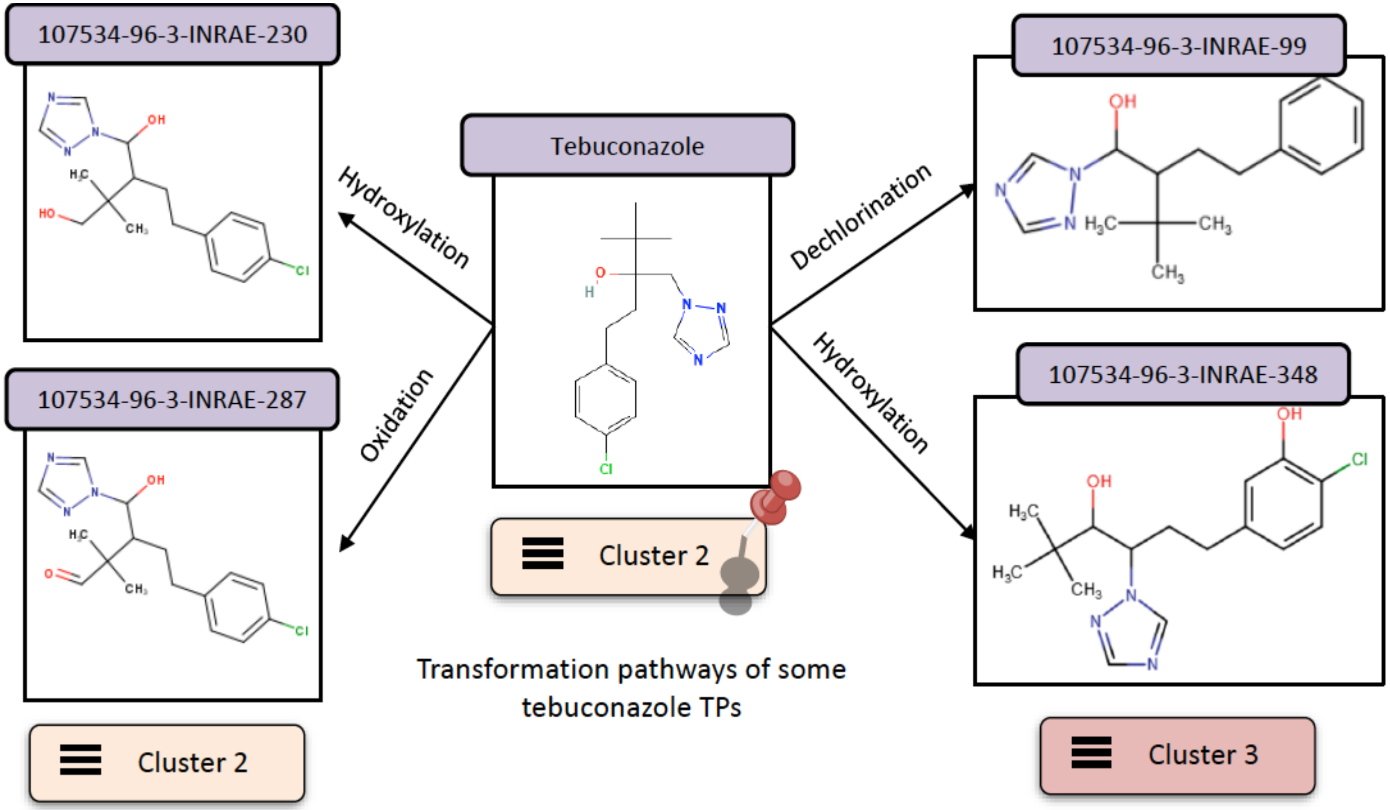
Aprianto 2023

Relating cluster changes to pathways, fate and impacts

217 compounds (3 Parent Compounds Tebuconazole, Terbuthylazine et Fenoxycarb and 214 TPs)

Understanding why some TPs shifted from one cluster to another





Transformation pathways of some tebuconazole TPs

Cluster 3 was characterized by higher log Kow (lower Sw), higher Pvap, higher K_{H}, high persistence

➡ Higher Risk to bioaccumulate in food webs compared to cluster 2

Pose risk to **aquatic organisms**

Some TPs have been detected in water samples in France (Rocco et al., 2022)

Some TPs have been identified in laboratory experiment (Richard, 2021)

Aprianto 2023

Conclusions

- TyPol allows to classify the pesticides and their TPs in different clusters characterized by distinct properties: molecular descriptors, environmental parameters (log Kow, Sw, Koc, BCF, Pvap, and KH) & some ecotoxicity endpoints (LC50 and NOEC)

- Some TPs can be assumed to have the same behavior as their parent compounds while others would be more persistent and/or toxic or, on the contrary, less persistent and/or toxic

- TyPol relies on the robustness of Input Data
  -> Increased knowledge from **suspect screening approach**
  -> Increased knowledge from predictive tools for exposure
  -> Increased knowledge from predictive tools for effects
  -> **Also integrating** more experimental values

- Potential use of TyPol for Pesticide TPs Environmental Risk Assessment
  -> Screening and prioritization framework
  -> Selecting compounds for experimental testing
  -> Selecting compounds for exposure and effects modeling scenarios

Thanks for your attention

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Servien R., Mamy L., Li Z., Rossard V., Latrille E., Bessac F., Patureau D., Benoit P. 2014. TyPol – A new methodology for organic compounds clustering based on their molecular characteristics and environmental behavior . *Chemosphere , 111, 613-622.*

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