IDENTIFICATION AND QUANTIFICATION OF TRANSFORMATION PRODUCTS IN THE AQUATIC ENVIRONMENT BY HIGH RESOLUTION MASS SPECTROMETRY

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Transformation Products (TPs)/Metabolites in European Directives and Guidelines

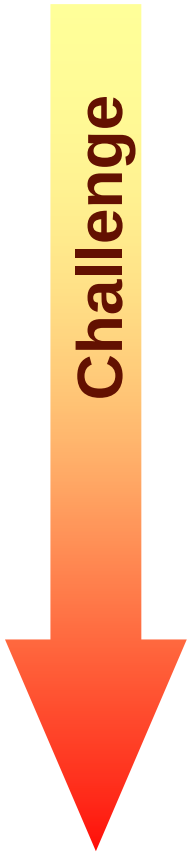
Drinking Water Directive:
…pesticides and their **relevant metabolites** in drinking water must not exceed 0.1 μg/L.

Council Directive 91/414/EEC concerning the placing of plant protection products on the market

…64 times „**relevant metabolites**“

EMEA-Guideline on the environmental risk assessment of medical products for human use (June 2006)
… **relevant metabolites** ….

Analytical strategy to identify and quantify transformation products (TPs)



Target screening
with reference standards

Kolpin et al. Groundwater 2004
Boxall et al. ES&T, 2004, ...

Suspects screening
without reference standards

Non-target screening
without reference standards

Analytical procedure

Challenges:

Low concentrations

Broad set of compounds

Ionization in positive and negative mode

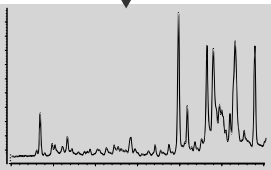
Identification without reference standards

Sample - ground, surface, waste water
- 1/0.5/0.2 liter, filtration,
- pH 6.8, 90 isotope labeled internal standards

Enrichment (SPE)
- mixed bed cartridge (RP, Ionic exchange mode)

Chromatography
- Reversed Phase Xbridge 50 x 2mm
- H_2O/MeOH gradient with 0.1% formic acid

LTQ-Orbitrap-MS
- Electrospray ionisation (positive/negative run)
- HR scan with data dependent MSMS-scan



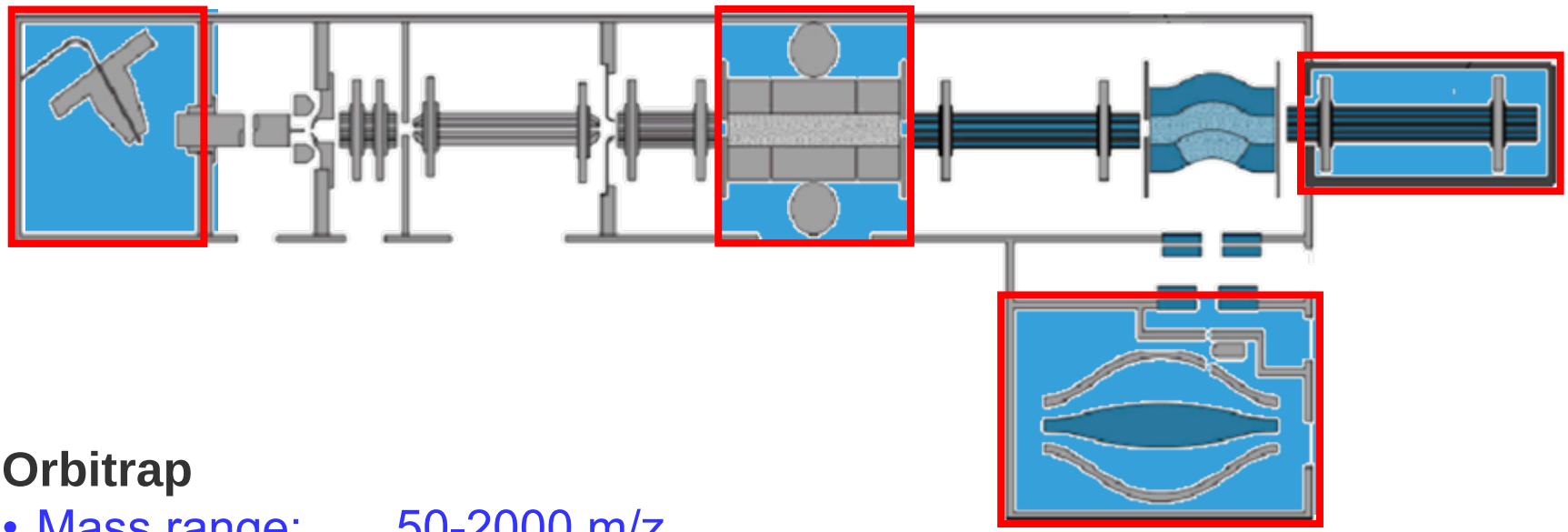
HR Orbitrap-Chromatogram
- Quantification of ~240 target analytes
- Target, suspects and non-target screening

Hybrid mass spectrometer - Orbitrap XL

Electrospray Interface

Linear Ion trap LTQ

- Mass range: 50-2000 m/z
- Resolution: Unit resolution
- Fragmentation: CID



Orbitrap

- Mass range: 50-2000 m/z
- Resolution: 100,000 (@ 400m/z)
- Accuracy: < 5 ppm

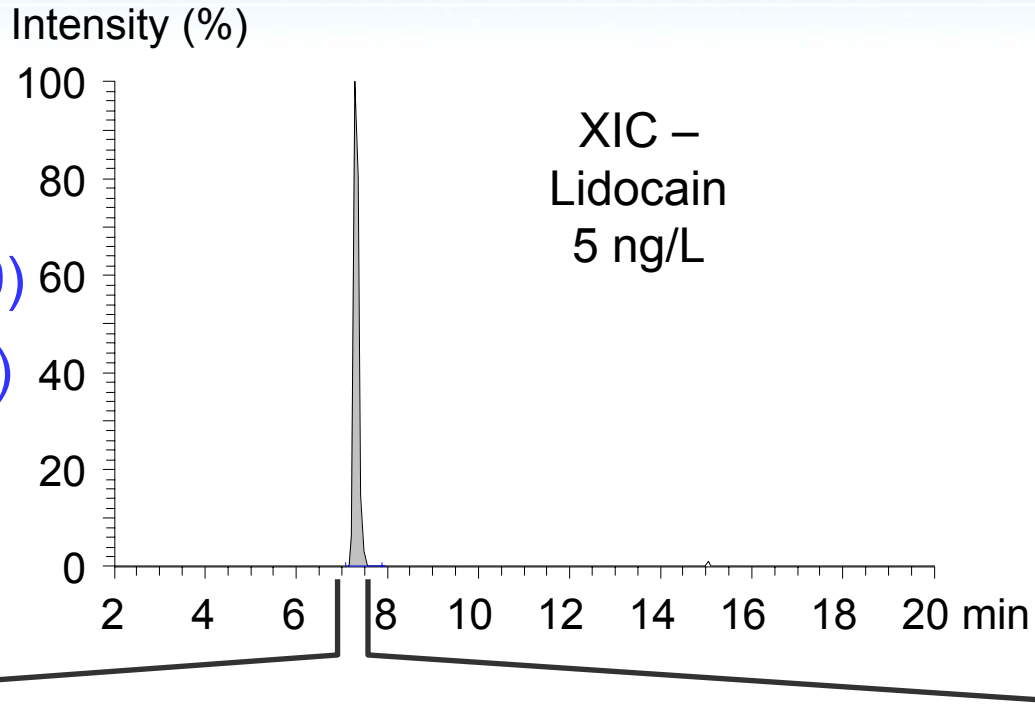
Collision Cell

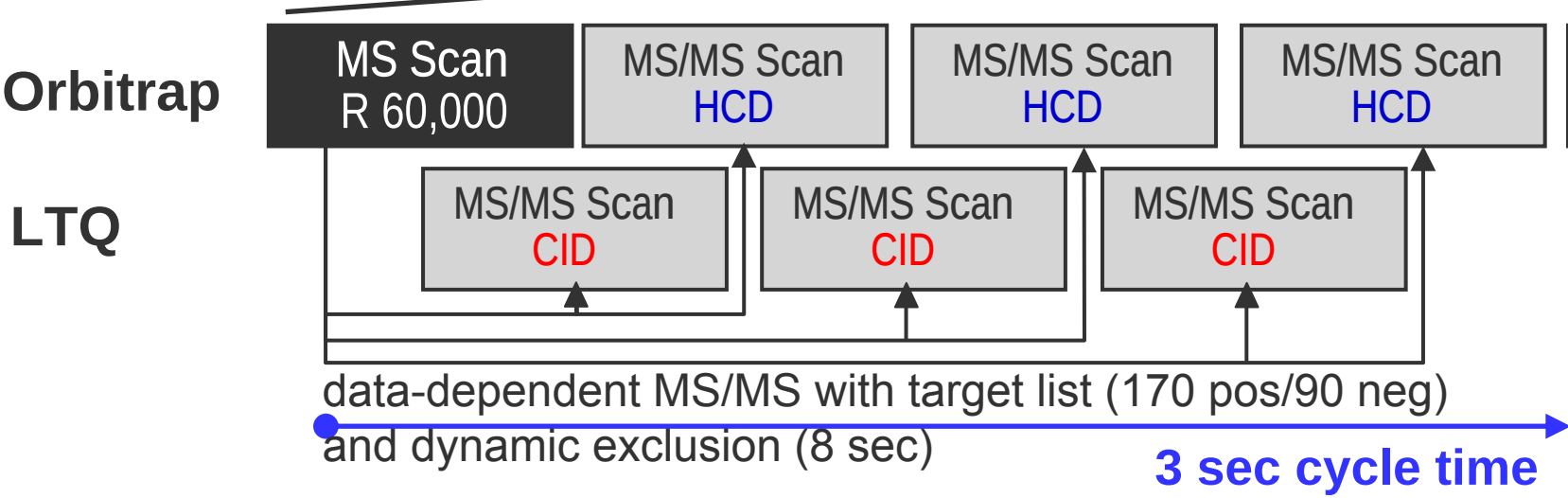
- Fragmentation: higher energy collision dissociation (HCD)

MS settings for screening

Per 30sec LC-peak:

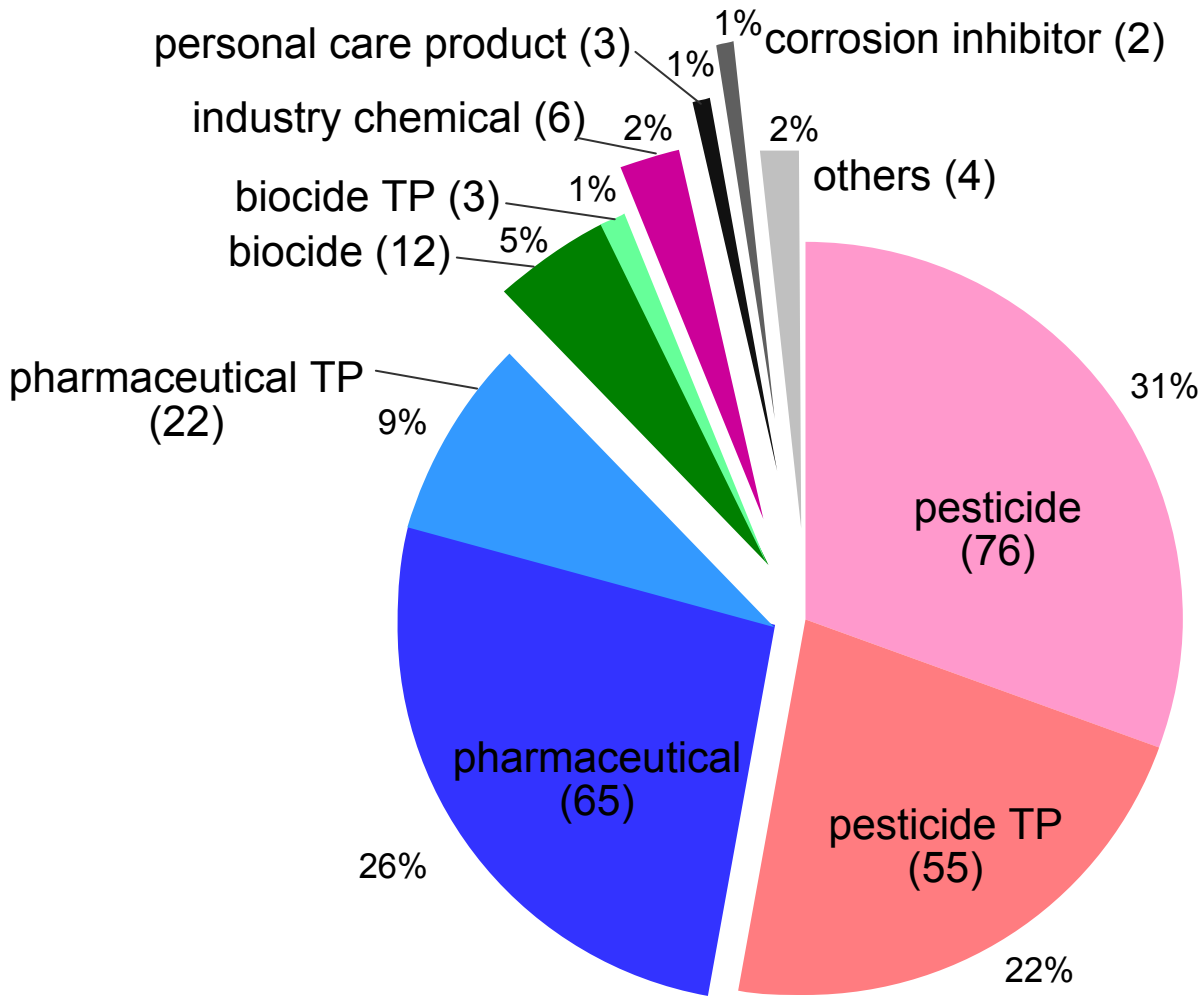
- 10 MS scans (R 60,000)
- 30 HCD MSMS scans (R 7,500)
- 30 CID MSMS scans (Unit res.)





Suitability of screening procedure

167 parent compounds, 81 transformation products (TP):
Mr: 115 – 1000; Kow: -2.2 up to 5.7, 52 % neutral, 48 % ionic



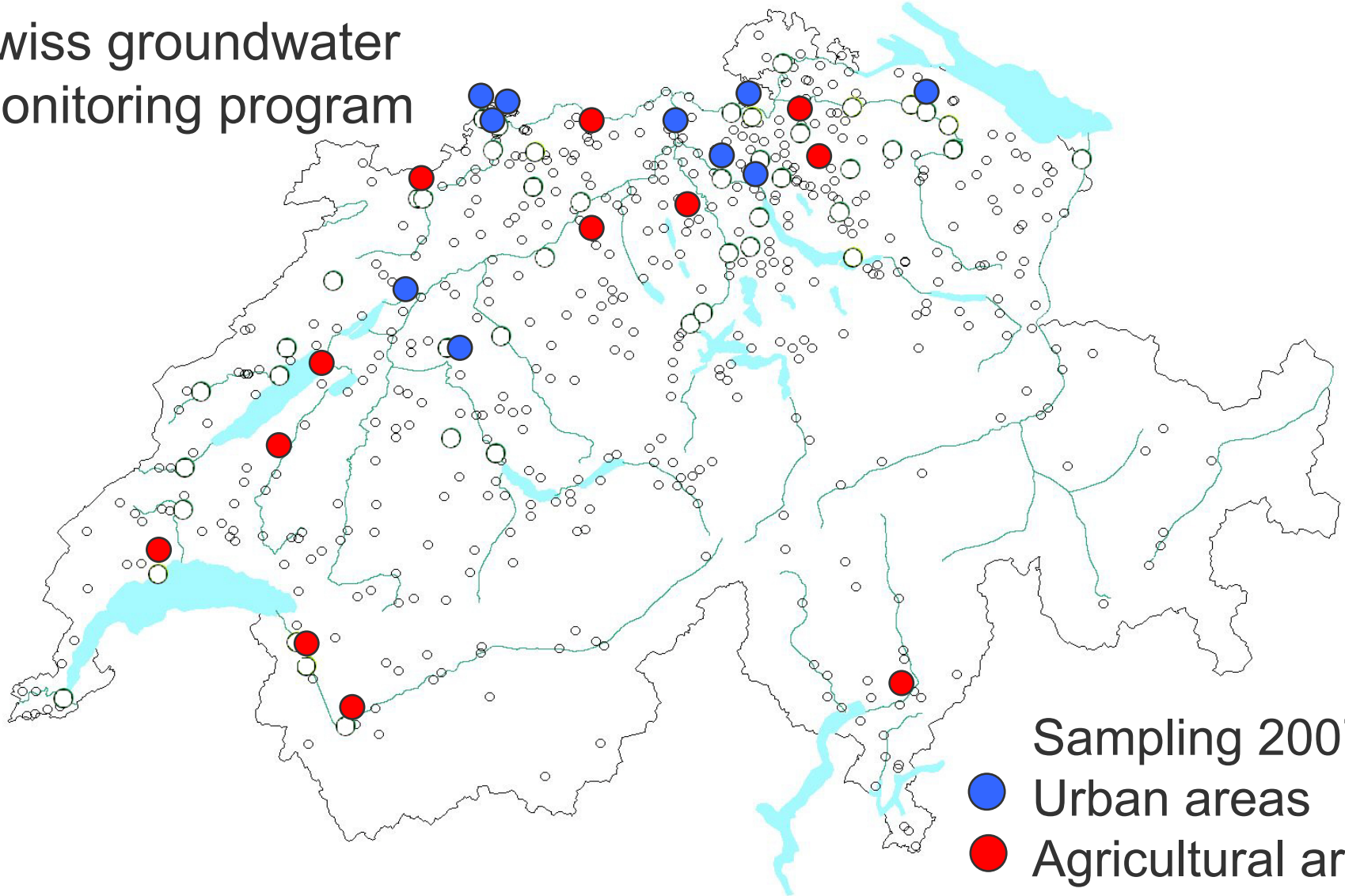
Method detection limits:
(surface water)
75 % ≤ 10 ng/L
20 % 10 - 100 ng/L
5 % < 1000 ng/L

Relative recoveries:
(surface water)
80 % > 80 %
15 % 20-80 %
5 % < 20 %

Singer et al., in prep.

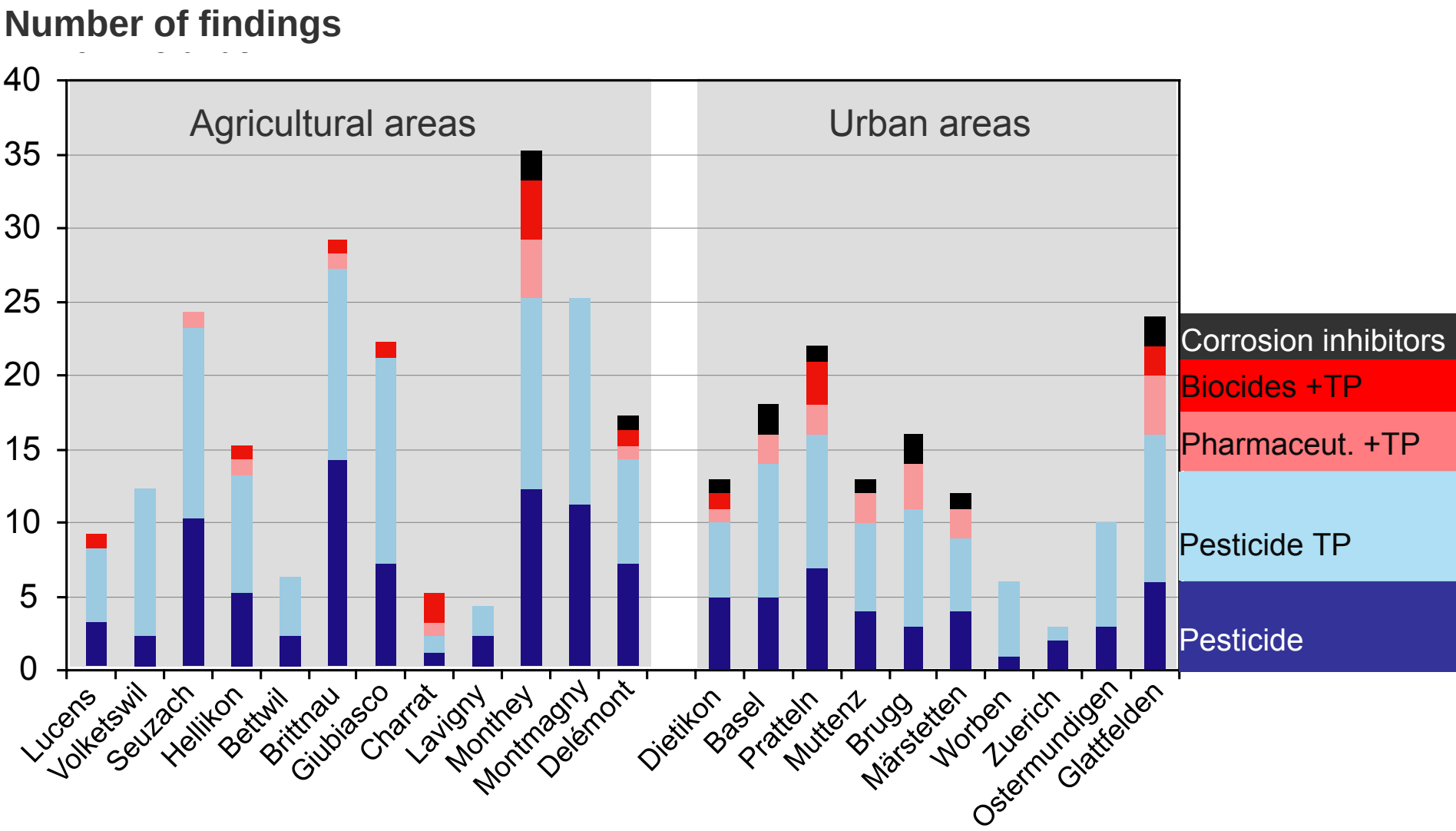
Target screening: Case study groundwater

Swiss groundwater monitoring program



Quelle Geobasisdaten: Eurostat-GISCO (1992) via UNEP-GRID-Genf

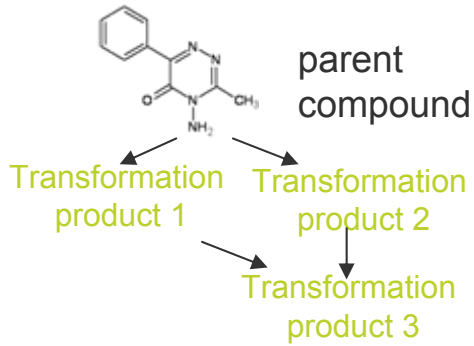
Target screening: Case study groundwater



Suspects screening

1. Step

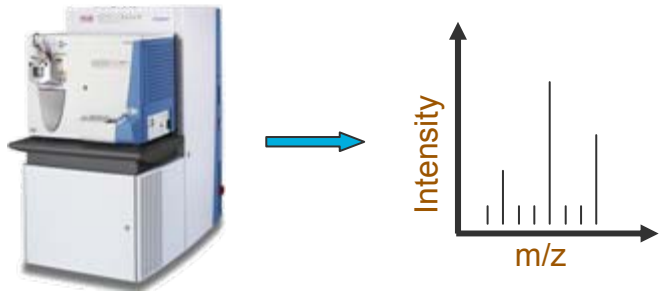
Prediction of possible transformation products using structure-biodegradation relationships & data mining



List of target transformation products

2. Step

Identification of transformation products in environmental samples by high resolution mass spectrometry



Identified transformation products

Identification procedure without reference standards

1. Exact mass:

→ extracted chromatogram

2. Retention time:

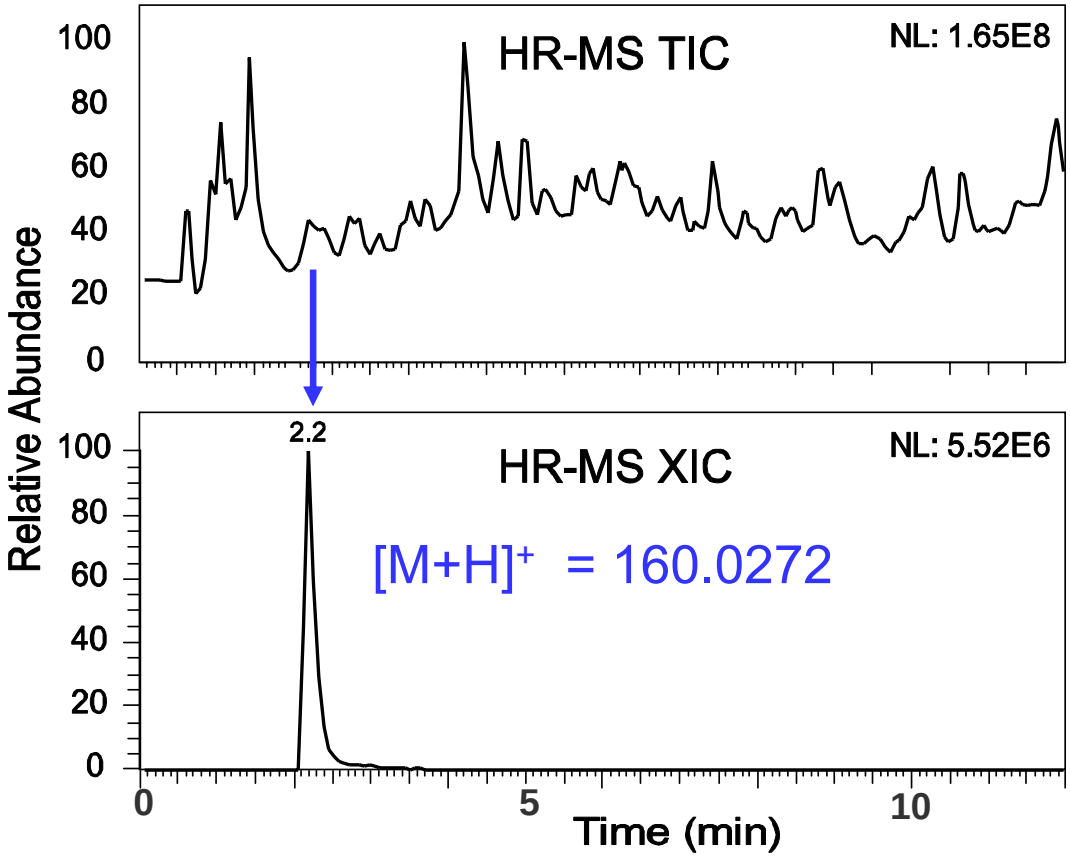
→ comparison to parent compound
or prediction based on log Kow

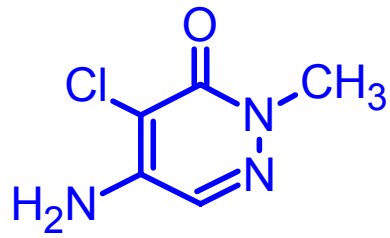
3. Molecular structure:

→ interpretation of MS/MS fragments

Suspects screening: 1. Exact mass & isotope pattern

Compound detection: filtering with 5 ppm extraction window

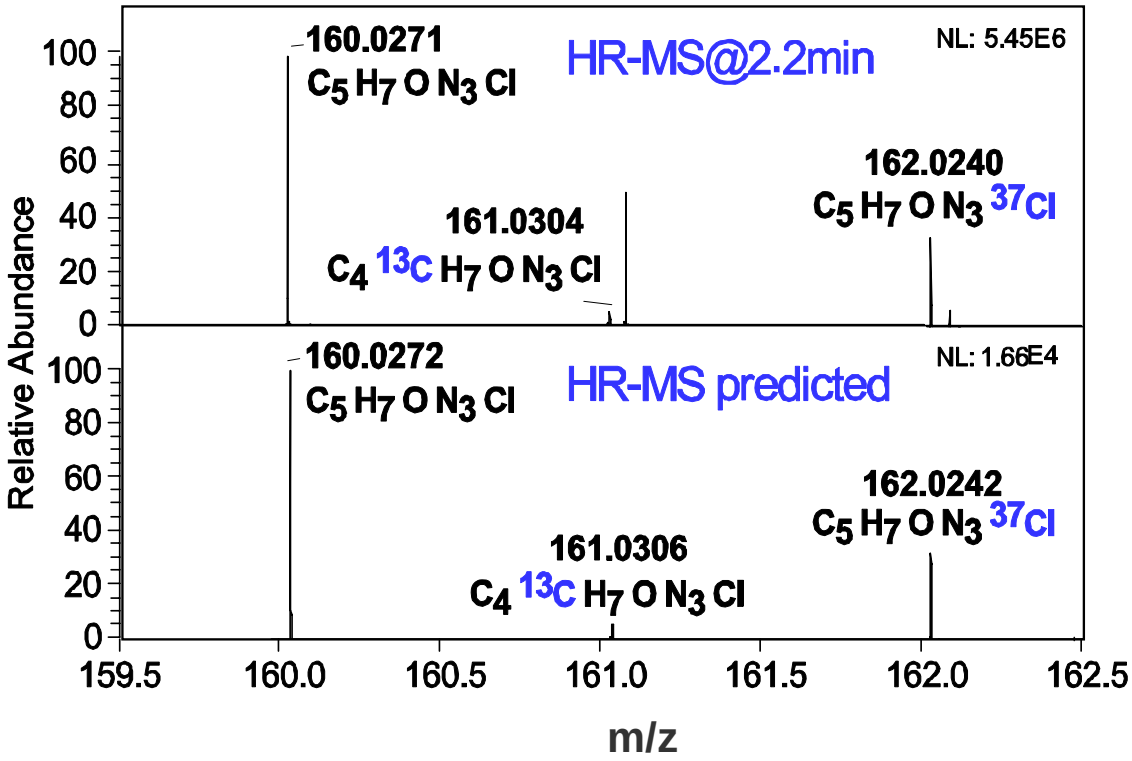


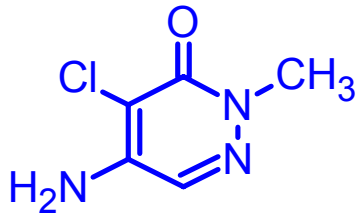


Chloridazon-methyl-desphenyl

Suspects screening: 1. Exact mass & isotope pattern

Isotope pattern: $C_5H_6ClN_3O$

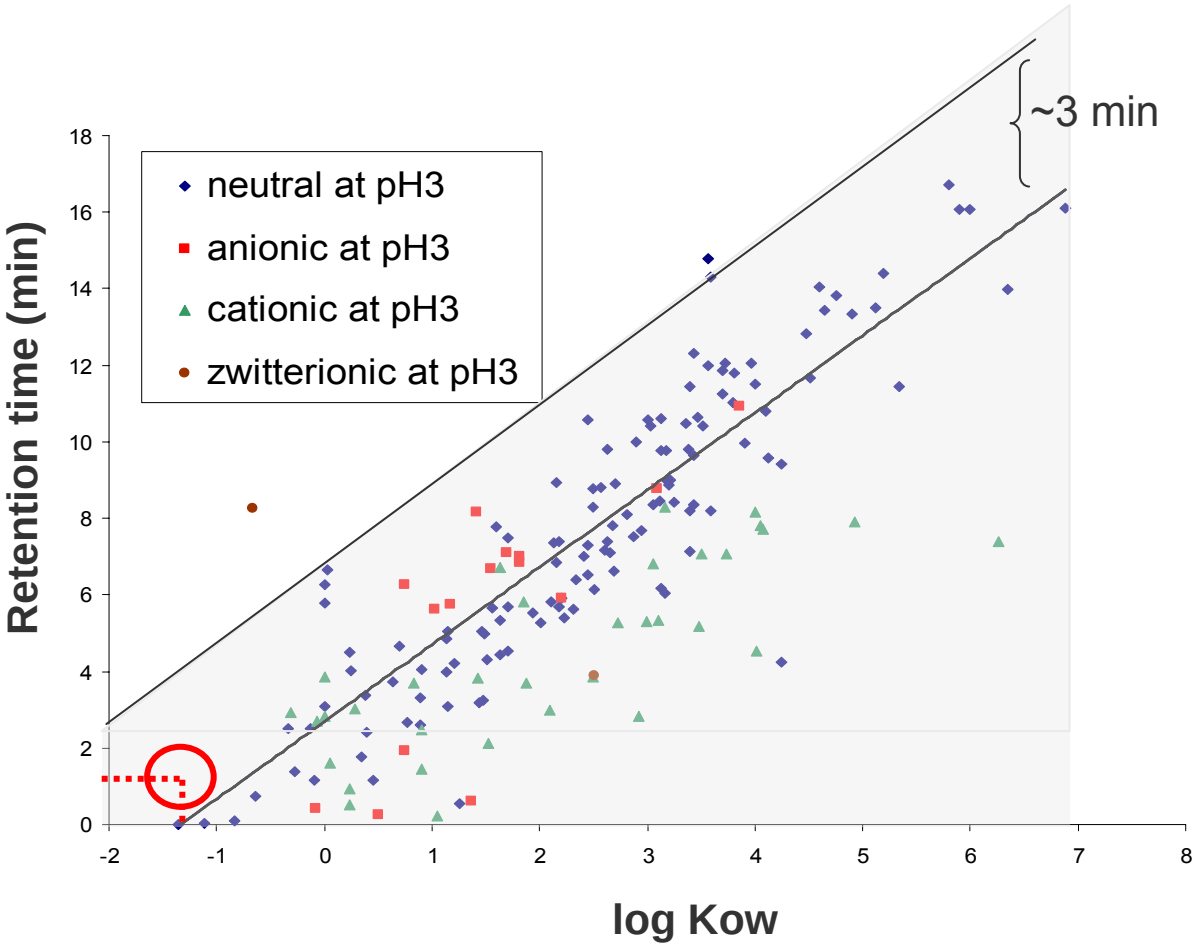


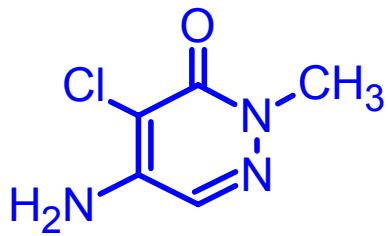


Chloridazon-methyl-desphenyl

➔ **~100 possible structures in Pubchem/Scifinder data bases**

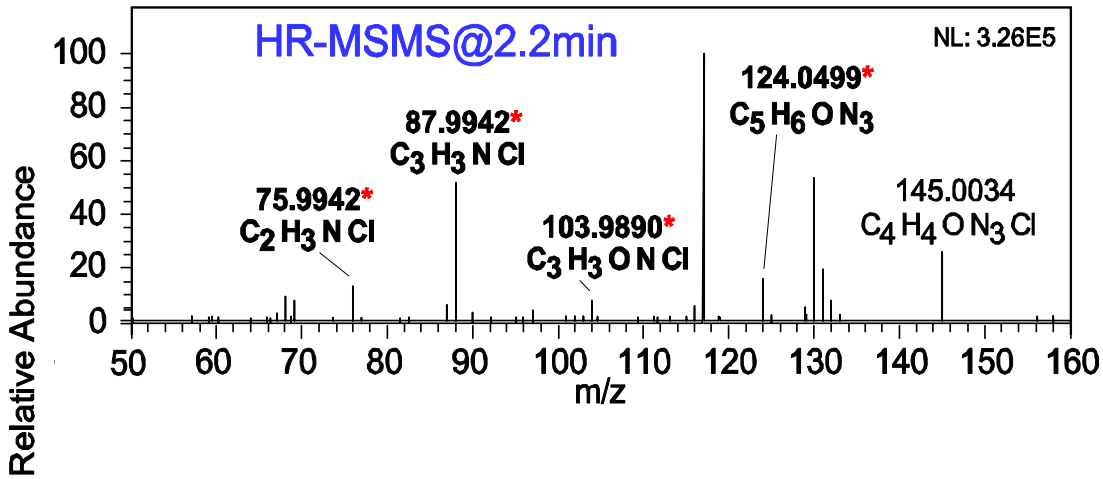
Suspects screening: 2. Retention time

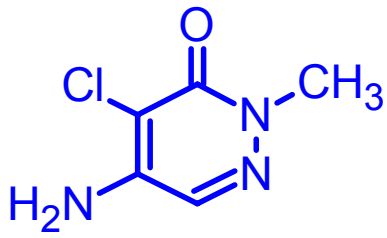




Chloridazon-
methyl-desphenyl

Suspects screening:
3. Molecular structure - Interpretation of MS/MS fragments

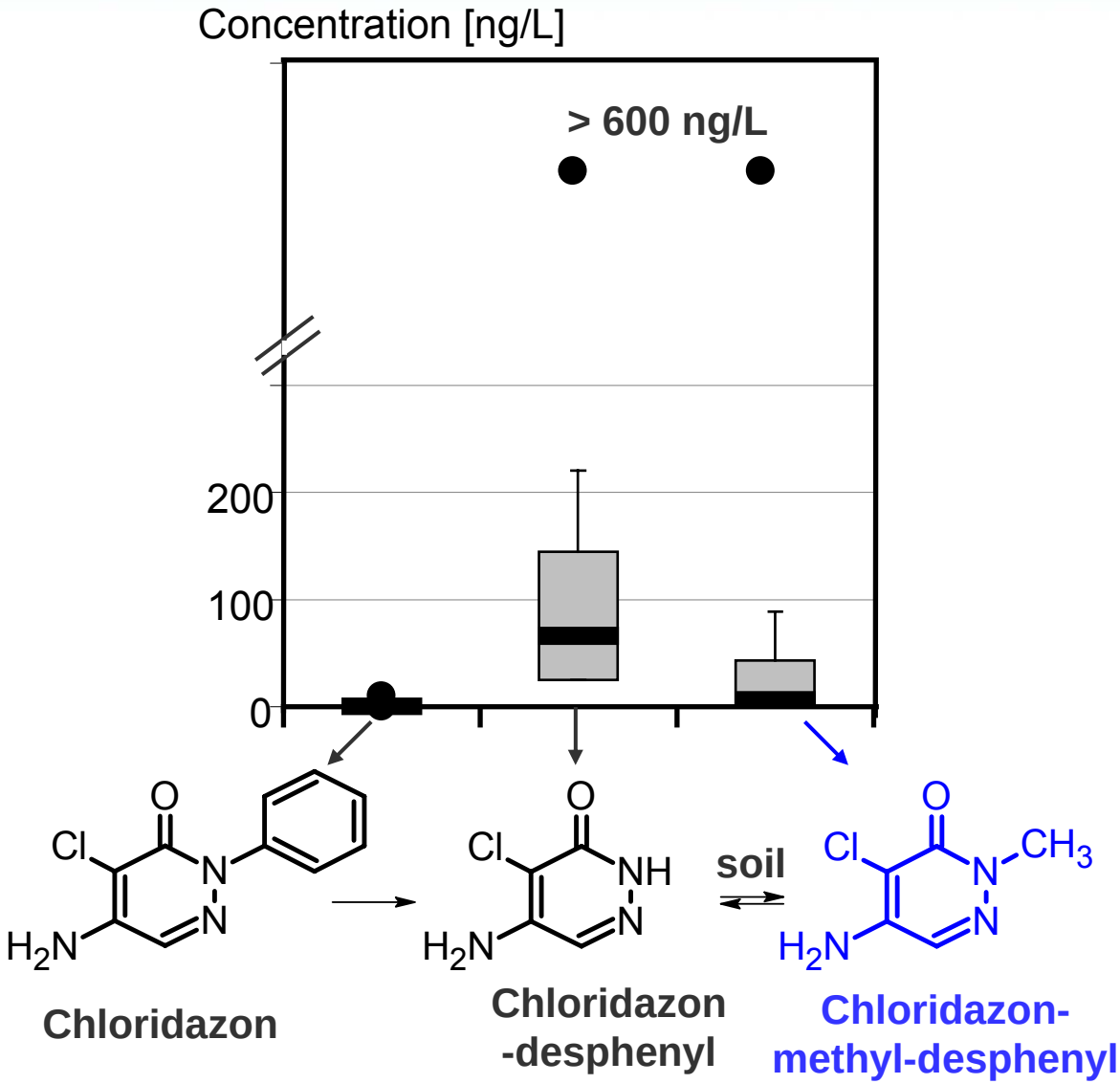




Chloridazon-methyl-desphenyl

*predicted by fragmentation software (Mass frontiers)

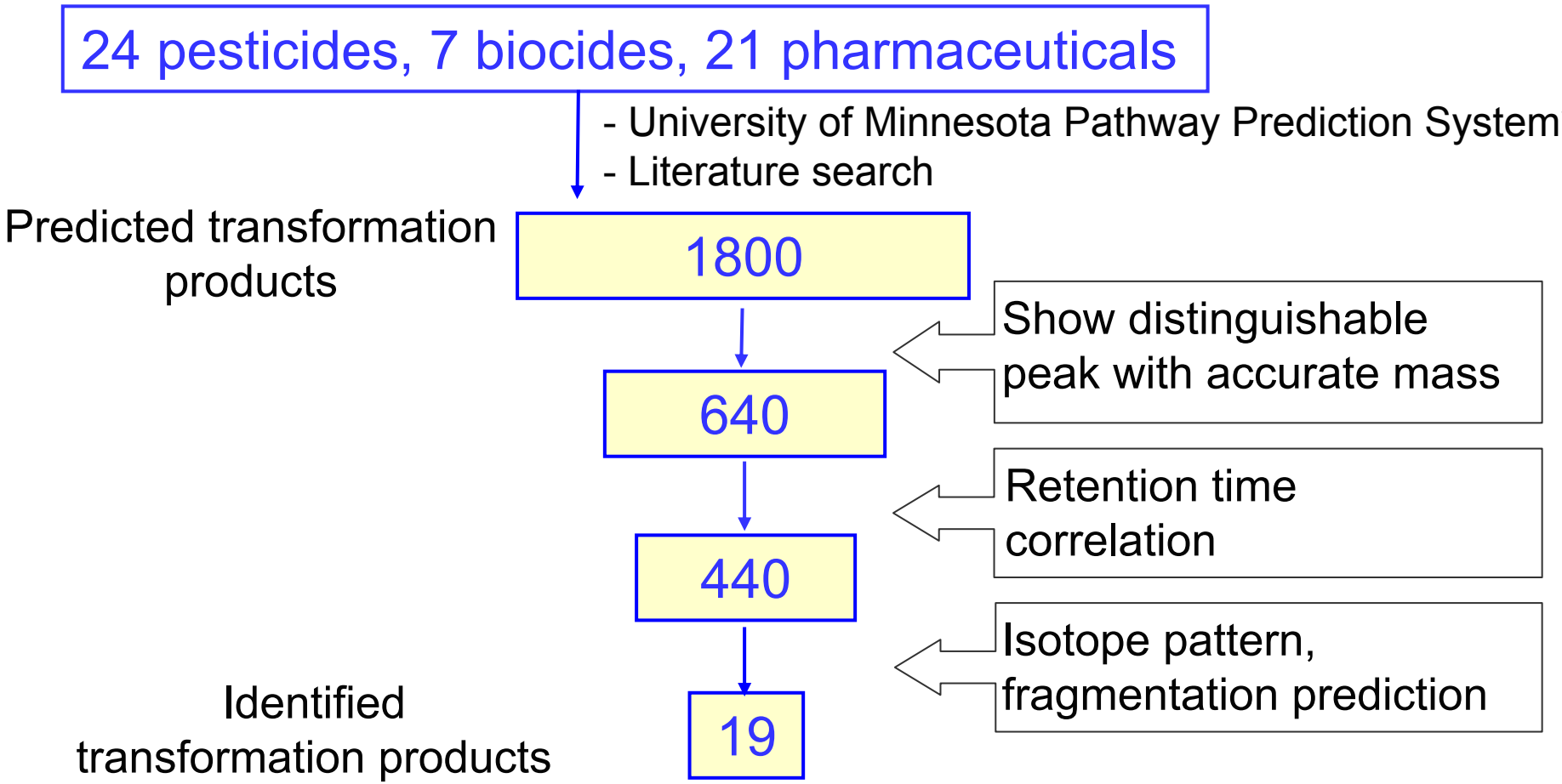
Chloridazon TPs in Swiss groundwater samples



Roberts & Hutson et al., Metabolic Pathways of Agrochemicals, 2002
Weber et al. Vom Wasser 2007

Suspects screening: Case study surface waters

7 water samples (agricultural areas, downstream wastewater treatment plants)



+ 10 low concentrated transformation products identified by reference compounds

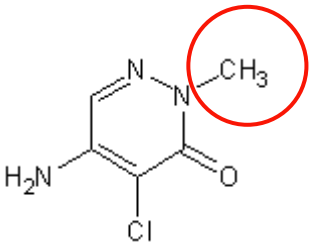
*http://umbbd.msi.umn.edu/servlets/predict.jsp

Kern et al., ES&T 2009

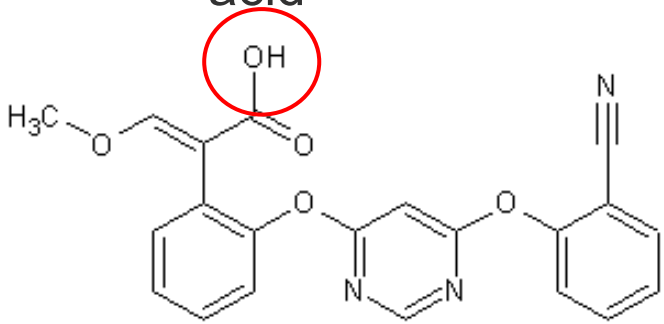
Examples for identified transformation products

Pesticide transformations products:

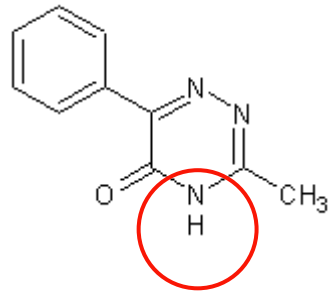
Chloridazon-methyl -desphenyl



Azoxystrobin acid



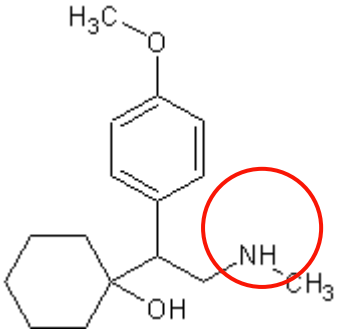
Metamitron-desamino

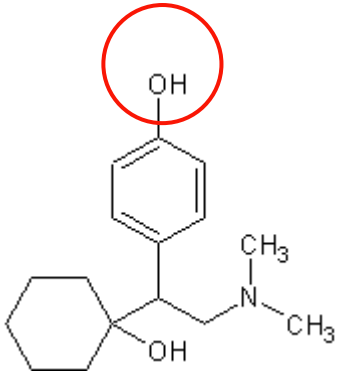


Pharmaceutical transformation products:

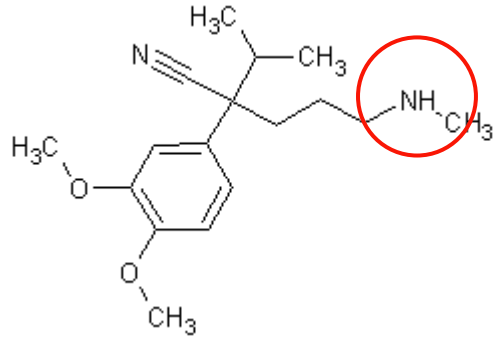
N-Desmethylvenlafaxin

O-Desmethylvenlafaxin





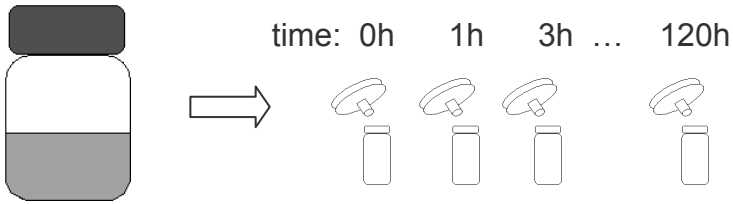
D617 (Verapamil-TP)



Kern et al., ES&T 2009

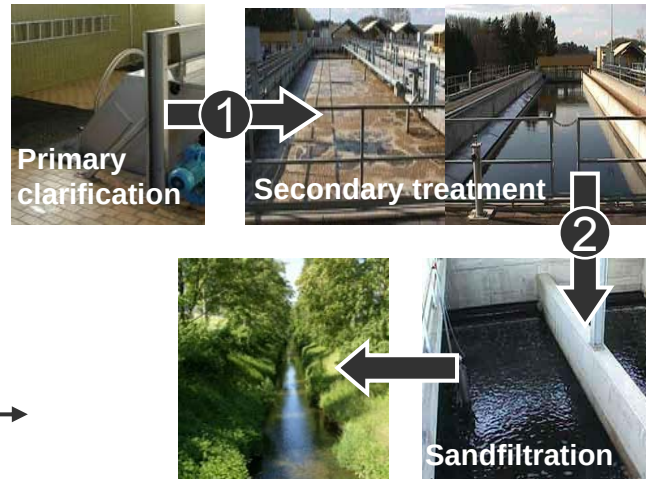
Suspects screening

Strategy for identification of relevant pharmaceutical TPs



50 mL, X_{SS}: 3 $g_{SS}L^{-1}$, dark, O_2 : >0.5 mg/L, T : 19°, autoclaved controls

Qualitative agreement?



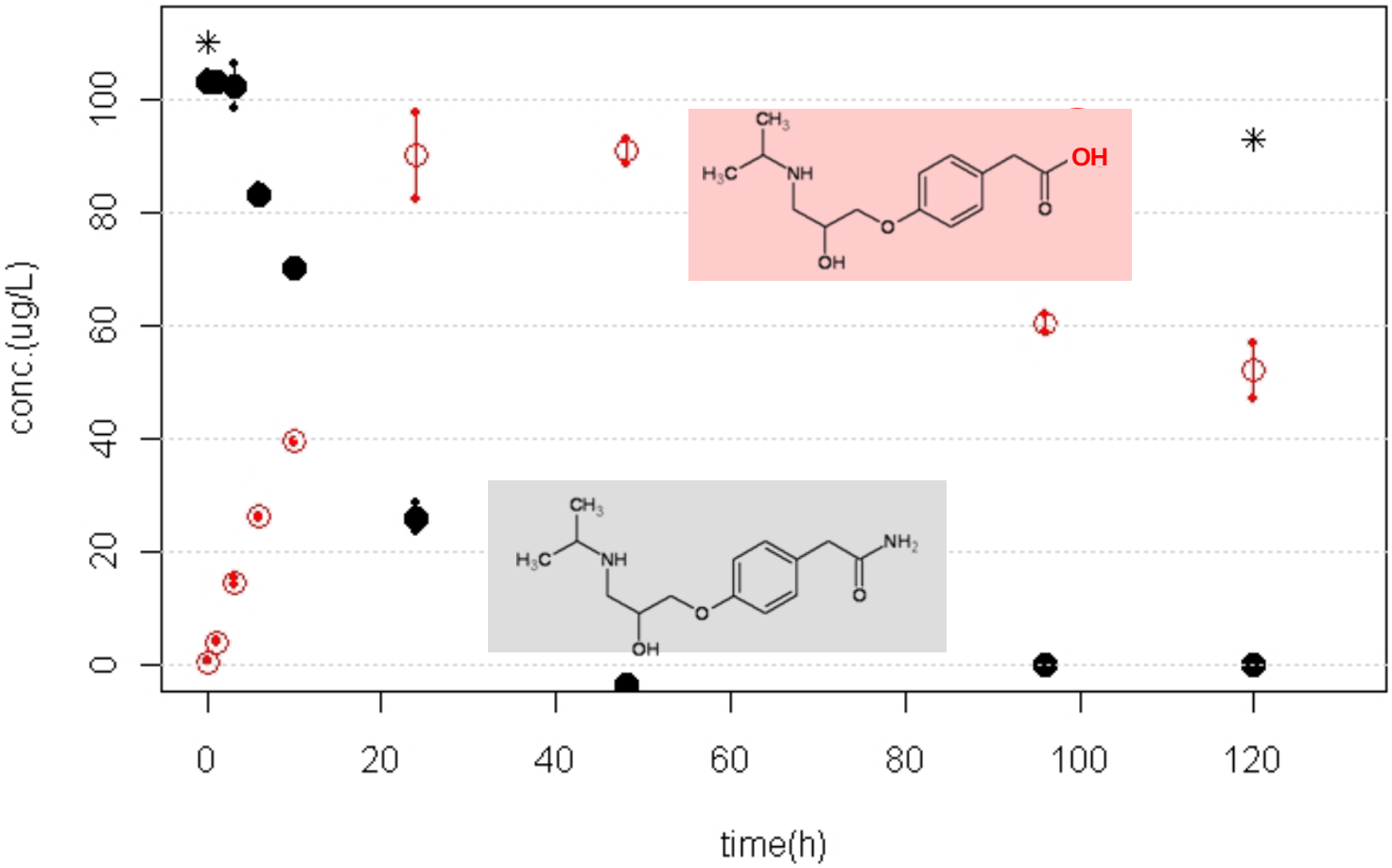
TP formed in batch experiments with sewage sludge from operating sewage treatment plant (STP)

Influent / effluent concentrations in corresponding STP

Strategy for identification of relevant pharmaceutical TPs

Illustrative results from batch experiments

Duplicate batches for atenolol (β-blocker):

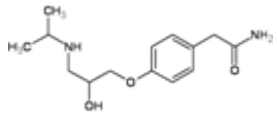
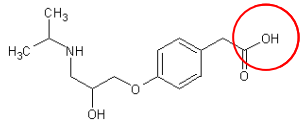
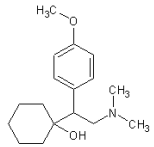
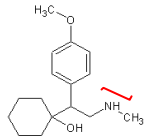
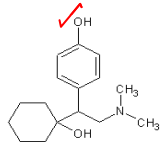


● : atenolol, ○ : atenolol acid (TP), * : control

Strategy for identification of relevant pharmaceutical TPs

TPs in batch experiments and STP influent/effluent

TPs with reference standards

parent compound	transformation product	structure	influent	effluent
Atenolol			✓	✓↓
	Atenolol acid		✓	✓↓
Venlafaxine			✓	✓↓
	N-desvenlafaxine		-	✓
	O-desvenlafaxine		✓	✓↑

Conclusions

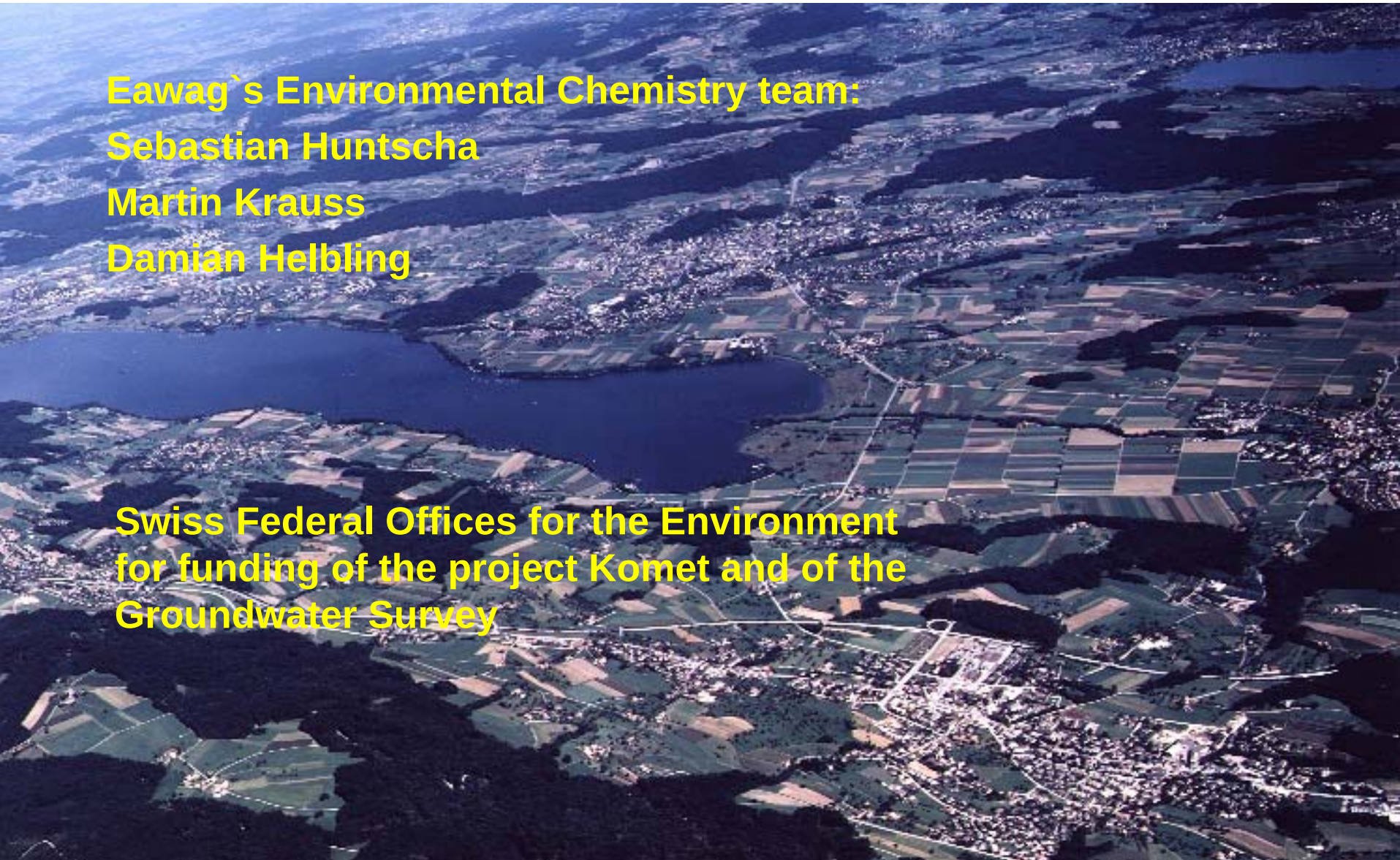
Chemical analysis

- Combination of target and suspects screening enables evaluating the exposure to transformation products in the aquatic environment
- SPE-HPLC-ESI-MS method is necessary to enrich, separate, ionize and detect mostly polar transformation products
- High resolution mass spectrometry is indispensable to identify polar transformation products without reference standards
- Combination of laboratory batch experiments and screening of STP samples is suitable to identify new transformation products

Exposure to transformation products

- confirmed that especially pesticide transformation products are important in groundwater
- For half of the parent compounds 1-2 transformation products were detected in surface and groundwater

Thanks to



Eawag`s Environmental Chemistry team:
Sebastian Huntscha
Martin Krauss
Damian Helbling

Swiss Federal Offices for the Environment for funding of the project Komet and of the Groundwater Survey

Environmental transformation of organic compounds:

Towards a joint perspective on the importance of transformation products as environmental contaminants

12-17 September, 2010
Monte Verità, Ascona, Switzerland

Organizers: Kathrin Fenner, John Sumpter, Juliane Hollender

Centro Stefano Franscini

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