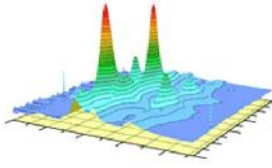




National and Kapodistrian University of Athens
School of Science
Department of Chemistry
Laboratory of Analytical Chemistry



HRMS wide-scope target and suspect screening methodologies for the monitoring of emerging contaminants in raptors

Dr. Nikolaos S. Thomaidis, Professor of Analytical Chemistry, National and Kapodistrian University of Athens (NKUA)



European Raptor
Biomonitoring Facility

ERBFacility WG3 Collections - Virtual Meeting

The role of collections for contaminant monitoring in raptors across Europe: State of play and next steps

11 February 2021

Our Research Team

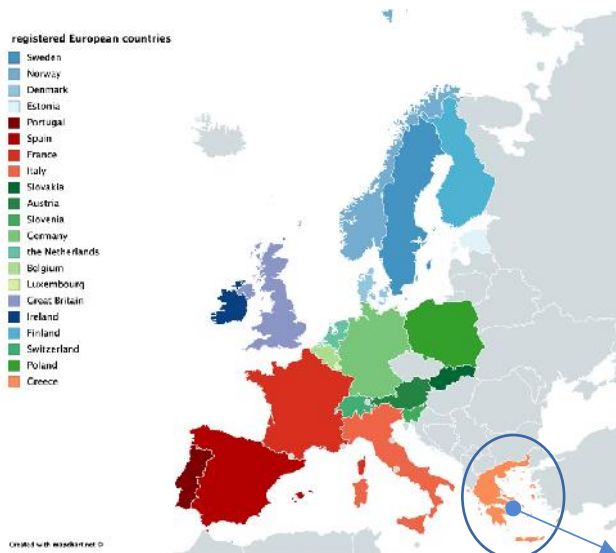


<http://trams.chem.uoa.gr>

National and Kapodistrian University of Athens Laboratory of Analytical Chemistry Trace Analysis and Mass Spectrometry (Tr.A.M.S.) group



7 post-doctoral researchers
15 PhD Candidates
10 Master Students



Athens

Advanced analytical methodologies
Cutting-edge instrumental techniques

Environmental Chemistry
Food Chemistry
Pharmaceutical Chemistry
Industrial Chemistry

TrAMS group
208 Tweet

MS lovers ❤️

TrAMS
Trace Analysis and Mass Spectrometry

TrAMS group
@ThomaidisLab

Passionate about Analytical Chemistry & Mass Spectrometry.
#EmergingContaminants #metabolomics #foodomics PI: @nikos_thomaidis
(tweets by @DimitrisDamalas)

Our mission



Answers in **environmental problems** on emerging contaminants and their (bio)transformation products (**holistic research approach**; monitoring, fate studies and ecotoxicology)



Development of **novel analytical methodologies** on sample preparation



State-of-the-art **mass spectrometric** instrumentation



Development of **advanced chemometric tools and data processing methods** (supporting non-target tools)

Instrumentation

GC-APCI-QTOF



GC-EI-MS/MS



volatiles
non-polars



RPLC/HILIC
ESI-QTOF



Semi-polars/Polars
Non-volatiles

NanoLC / UHPLC
ESI-TIMS-QTOF



MALDI-QTOF



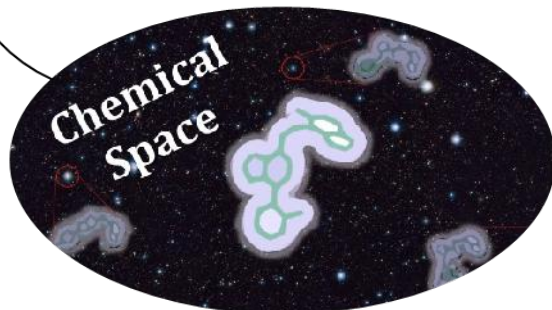
High-throughput
proteomics

Metabolomics
Lipidomics
Proteomics

Multidimensional Chromatography-HRMS data



- polarity
- volatility
- thermostability
- ionization type
- polymers
- separation of isomers



Chemical
Space

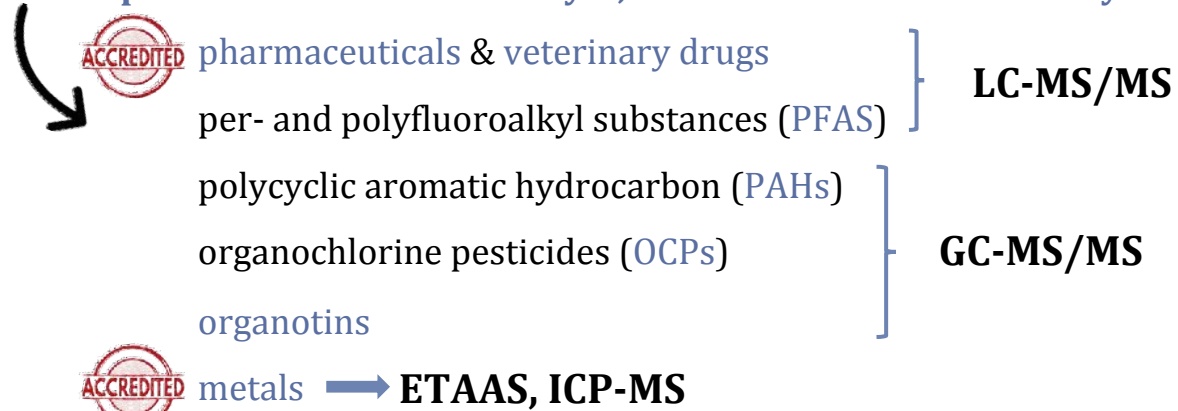


timsTOF flex

Experience & Expertise

Laboratory of Analytical Chemistry (LAC) → great experience in the field of emerging contaminants' identification in biota

developed & validated multianalyte, multiresidue sensitive analytical methodologies



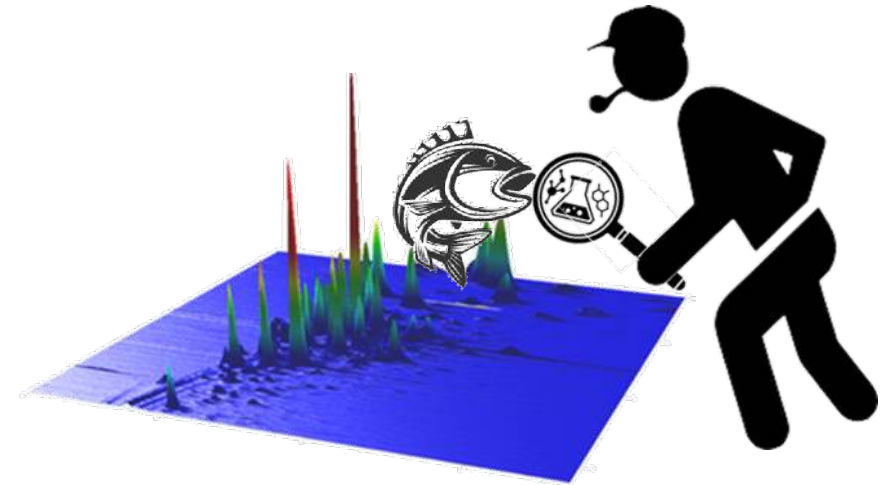
analytical methodologies for the determination of **WFD** compounds

Application in numerous national and European funded projects

2013 - today → **HRMS**

developed and validated **HRMS analytical methodologies** for the simultaneous determination of thousands of chemicals

↪ *smart validation → representative mix of compounds*

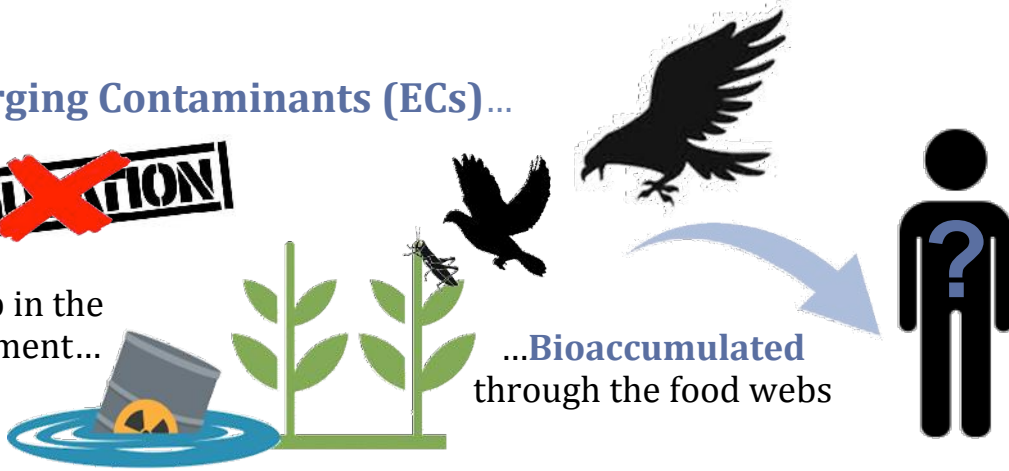


The analytical problem

Emerging Contaminants (ECs)...

~~REGULATION~~

...end-up in the environment...



Thousands of ECs

- Diverse classes of compounds
- Different physicochemical properties
- Multilevel concentrations

Known ECs

Unknown ECs

Long-term **monitoring studies** of ECs using **wildlife**:

- ✓ **effectiveness** of chemicals risk mitigation measures in reducing exposure
- ✓ **better chemicals management** (prioritization of substances for PBT assessment)
- ✓ **possible candidates** for future EU legislation

Why raptor specimens?

- ✓ **upper trophic level** predator
- ✓ **relatively long lifespan** → accumulation of ECs
- ✓ **measurable responses** to ECs (e.g. population decline due to DDE)
- ✓ **catholic diet** (avian prey from the aquatic & terrestrial environments)
- ✓ **global distribution**
- ✓ **diverse habitat types**

Why HRMS?

HRMS gives access to a larger number of **Diagnostic Tools**

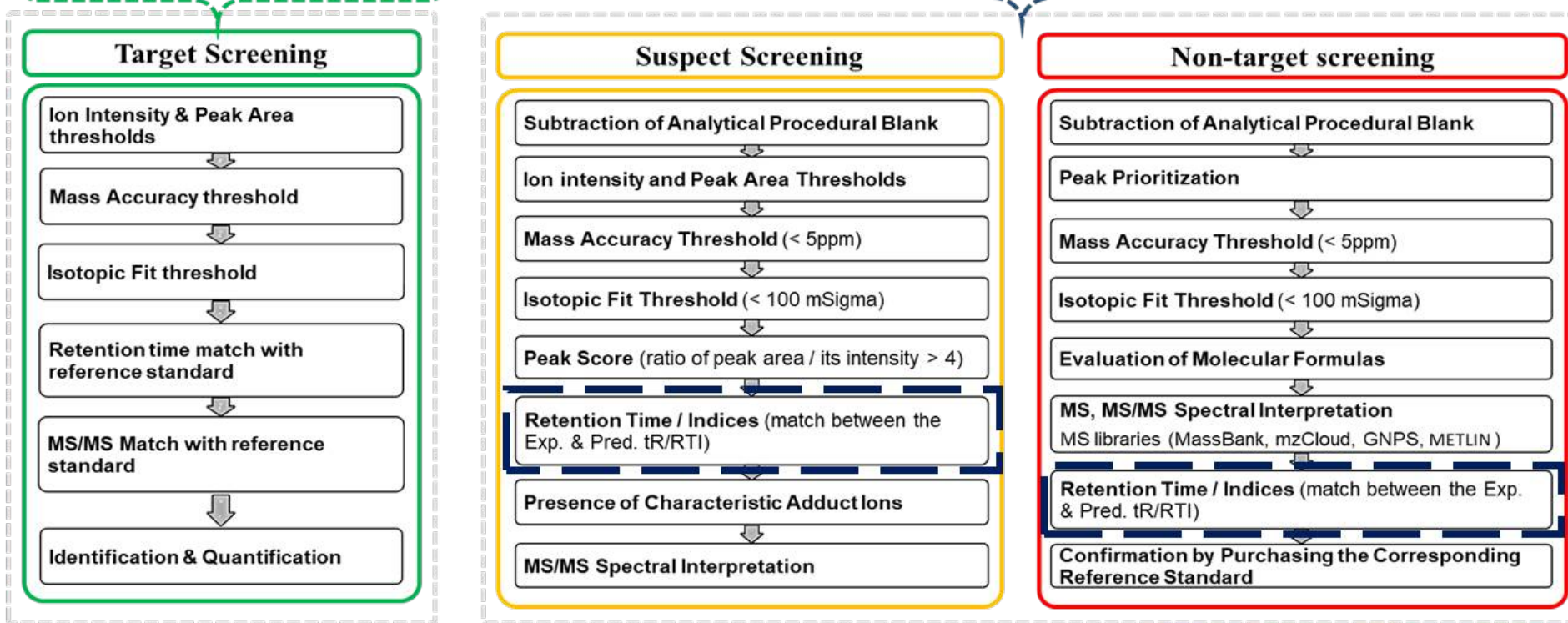
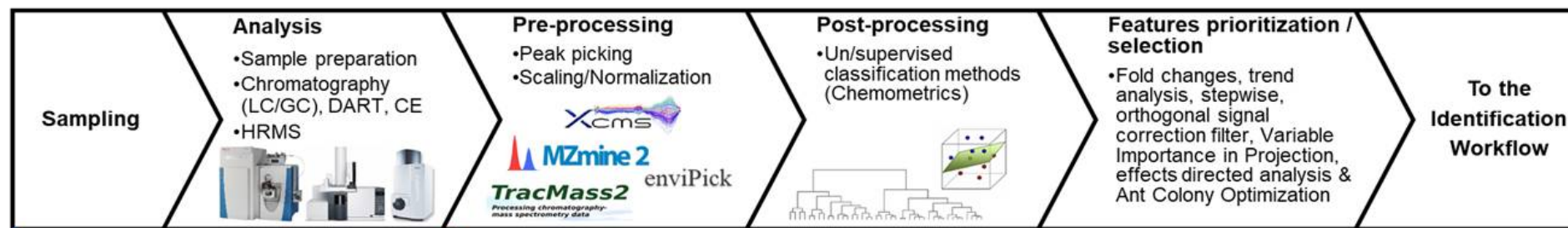
- *Accurate **mass** measurements from **full scan** measurements*
- ***Isotopic** information*
 - *elemental composition of both precursor and fragment ions*
- *Data Dependent MSⁿ or Data Independent MS/MS features*
 - *excellent tools for the identification of compounds*
 - *allow differentiating isobaric compounds*
- ***Retrospective Analysis***
- *Quantitative applications*
 - *advances regarding matrix effect, sensitivity and dynamic range*
- **Target, Suspect & Non-target Screening**

screening of thousands of chemicals

Molecular Fingerprint



HRMS Screening workflows



Time, Effort & Number of False Positives....

Generic Sample Preparation

Several developed and validated generic sample preparation protocols (**polar** & **non-polar** chemicals)

Pre-treatment

Lyophilization



Extraction

Bead-beating



1st

MeOH:H₂O (1:1)

2nd

DCM:MeOH (2:1)

Accelerated Solvent Extraction



1st

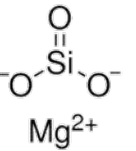
MeOH:ACN (2:1)

2nd

Hexane:DCM (2:1)

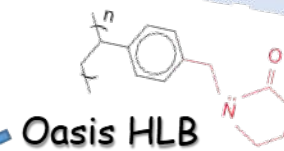
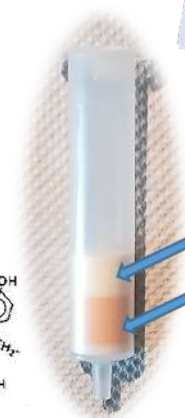
Clean up

Florisil cartridges

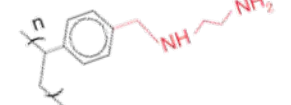
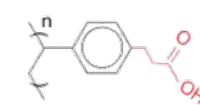
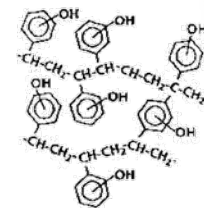


SPE

mixed-mode cartridges



Mixture of Strata X-CW, Strata X-AW & Isolute ENV+



Wide-scope target analysis

NKUA Databases

>**2,400** compounds in 4 datasets; **LC-ESI(+/-)-QToF MS** & **GC-APCI(+/-)-QToF MS**

- ✓ Pharmaceuticals (>450)
Antibiotics (>50)
- ✓ Personal care products
- ✓ Illicit drugs & New Psychoactive Substances (>500)
- ✓ Industrial Chemicals (>100)
- ✓ Plant Protection Products (>900)
- ✓ Sweeteners
- ✓ Surfactants
- ✓ Steroids & hormones
- ✓ **Transformation Products/ Metabolites**

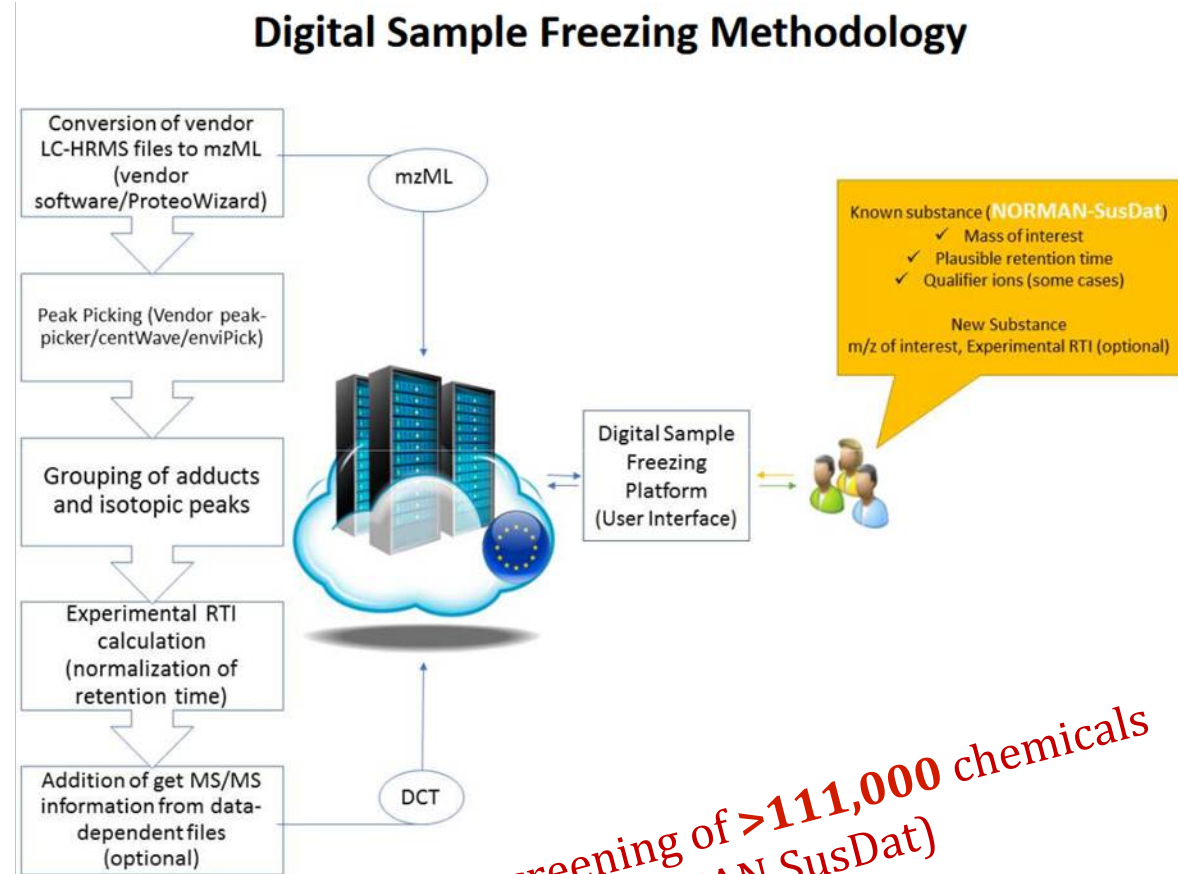
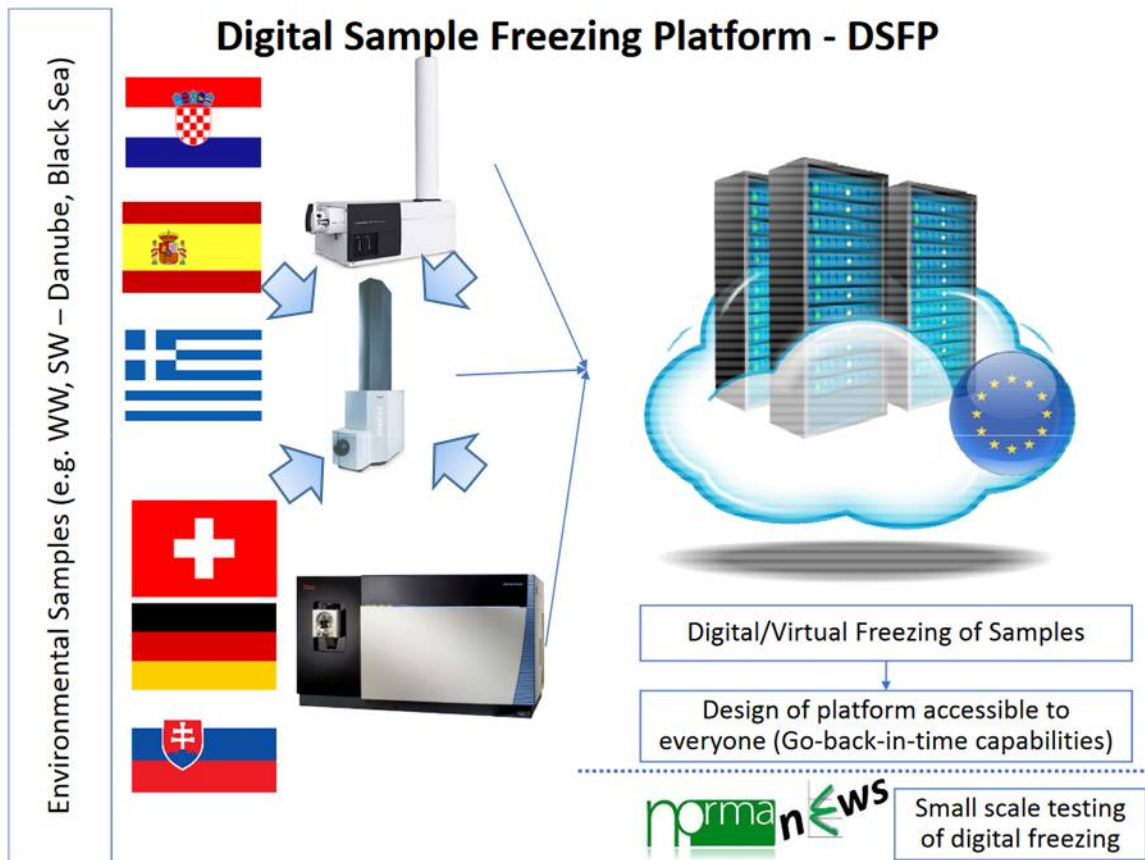
- ✓ PAHs
- ✓ OCPs
- ✓ PCBs
- ✓ Plant Protection Products
- ✓ PCNs
- ✓ PBDEs
- ✓ FAMES

 <https://zenodo.org/record/3753372>

The databases are **continuously** being **updated**!

Suspect screening workflows

Digital Sample Freezing Platform (DSFP)



- ✓ Automated tool
- ✓ Digital archiving
- ✓ Retrospective suspect screening

Suspect screening of >111,000 chemicals (NORMAN SusDat)

Environmental Analysis – Projects

Biomonitoring European Projects
(aquatic organisms from different trophic levels)



EMBLAS project (2016-2018, 2019-2020)

- I. EQS 2013/39/EC target screening (**LC-QqQ, GC-QqQ**)
- II. Wide-scope target screening of (>2,200 chemicals, **LC-QToF MS**)
- III. Non-target screening (**LC-QToF MS, GC-APCI-QToF MS, GC-EI-MS**)

Samples

- Wastewater (influent & effluent)
- Sewage sludge
- Surface water
- Sediment & Soil
- **Biota** (mussels, fish, apex predators)



Joint Danube Survey 4 (JDS 4) (2019-2020)

- I. Target analysis of drugs of abuse (**LC-QqQ**)
- II. Wide-scope target screening of (>2,200 chemicals, **LC-QToF MS**)
- III. Non-target screening (**LC-QToF MS**)



+ **raptors** (common Buzzard)
→ terrestrial ecosystem



LIFE17 ENV/SK/000355 (LIFE APEX)

"Systematic use of contaminant data from apex predators and their prey in chemicals management"
(2018-2022)

- I. Samples' pre-treatment (lyophilization, homogenization) and distribution
- II. Wide-scope target screening of (>2,200 chemicals, **LC-QToF MS**)
- III. Non-target screening (**LC-QToF MS, GC-APCI-QToF MS**)

Environmental Analysis – Projects



CONNECT project (2020-2021)

- 40 organisms from the lower trophic levels (**mussels**, oysters, flounders) and sediments, gathered from the **North-East Atlantic** ecosystem
- **Wide-scope target** analysis (>2,400 substances) and **suspect** screening (>111,000 NORMAN SusDat substances) by **LC-HRMS** and **GC-APCI-HRMS**



HELCOM project (2020-2021)

- Marine mammals, gathered from the **Baltic Sea**
- **Wide-scope target** analysis (>2,400 substances) and **suspect** screening (>111,000 NORMAN SusDat substances) by **LC-HRMS** and **GC-APCI-HRMS**



Priority pollutants and Chemicals of emerging concern in polar regions

Phase I (2019-2020): Organisms from the lower trophic levels
(*macrophytes, sea star, sea urchin, fish specimens*)

Phase II (2020-2021): Apex Predators → different matrices (e.g. **placenta, eggs**)



Surveys in the framework of NORMAN network activities

Biomonitoring studies (raptor specimens)

- **Leibniz Institute for Zoo and Wildlife Research (IZW)**, Germany
white-tailed sea eagle (liver)
- **Naturalis Biodiversity Center & Dutch Peregrine Working Group**, the Netherlands
peregrine falcon (liver)
- **Landesanstalt für Umwelt, Messungen und Naturschutz Baden-Württemberg (LUBW)**, Germany
little owl, great curlew, peregrine falcon, eagle owl (egg)



LIFE APEX



The *AIM* of **LIFE APEX** is to improve the *systematic use of chemical monitoring data from apex predators and prey* for protecting human health and the environment.

Tier 1: Wide-scope screening

12/67; common Buzzard specimens → 3 countries of Northern Europe (Germany, United Kingdom, Netherlands)

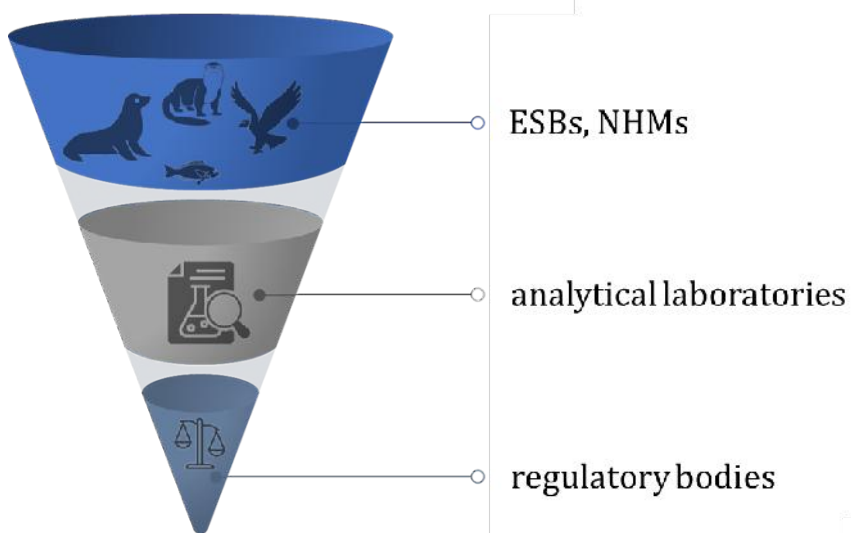
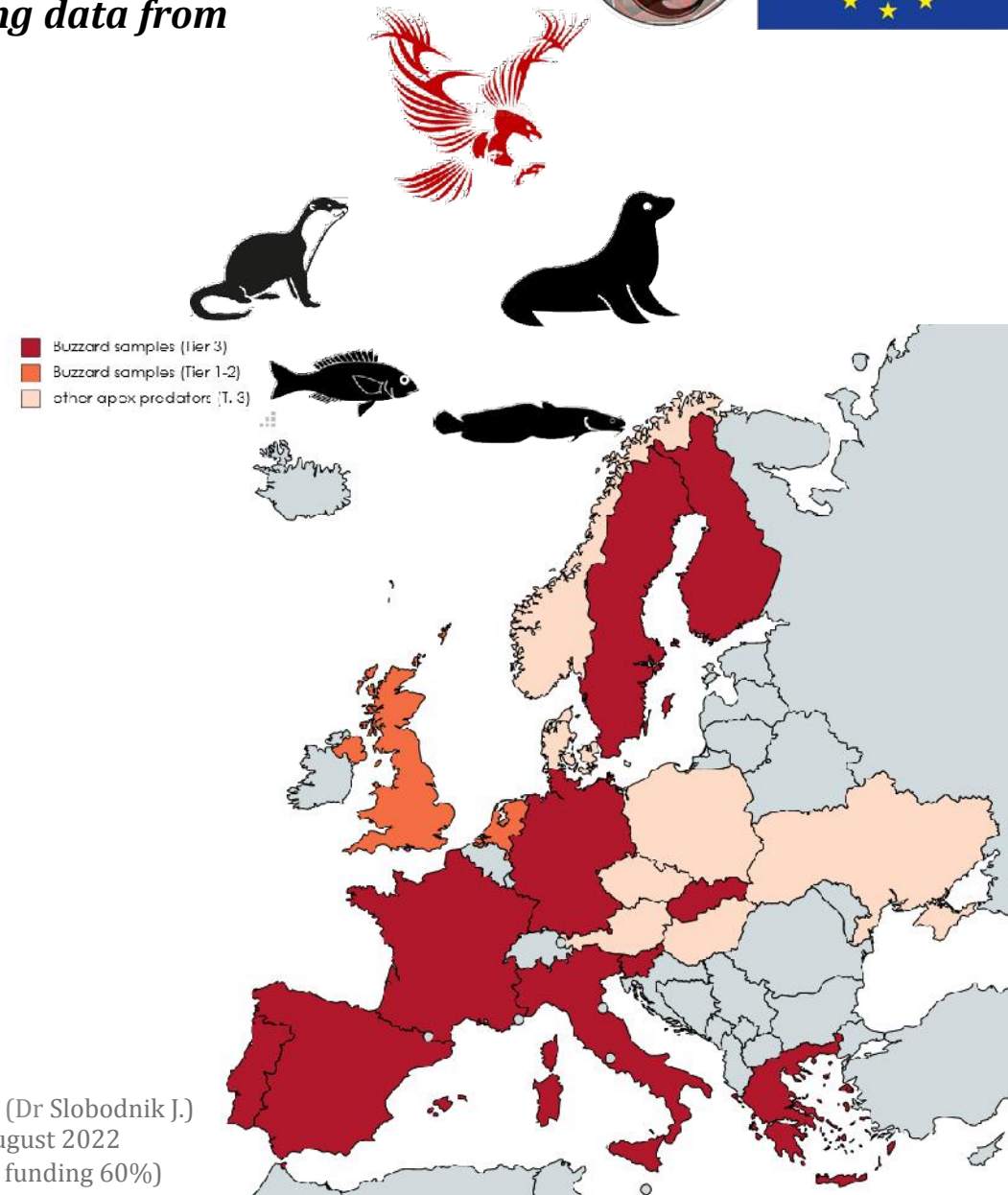
Tier 2: Time trend analyses

19/65; common Buzzards (United Kingdom) → Sampling Years: **2002-2018**

Tier 3: Replication with R&T partners

Apex predator specimens from different European countries
Replication of the Tier 1 approach in a pan-European level (e.g., **Buzzard** samples)

↪ wider European picture of occurrence of ECs



Project Coordinator: Environmental Institute (Dr Slobodnik J.)
Project Duration: September 2018 – August 2022
Project budget: € 3.35M (of which EU LIFE funding 60%)



Tier 1 (common Buzzards)

Detected ECs in Tier 1 → 164

Detected ECs → **86** (Buzzards)

Pharmaceuticals & TPs → 28%

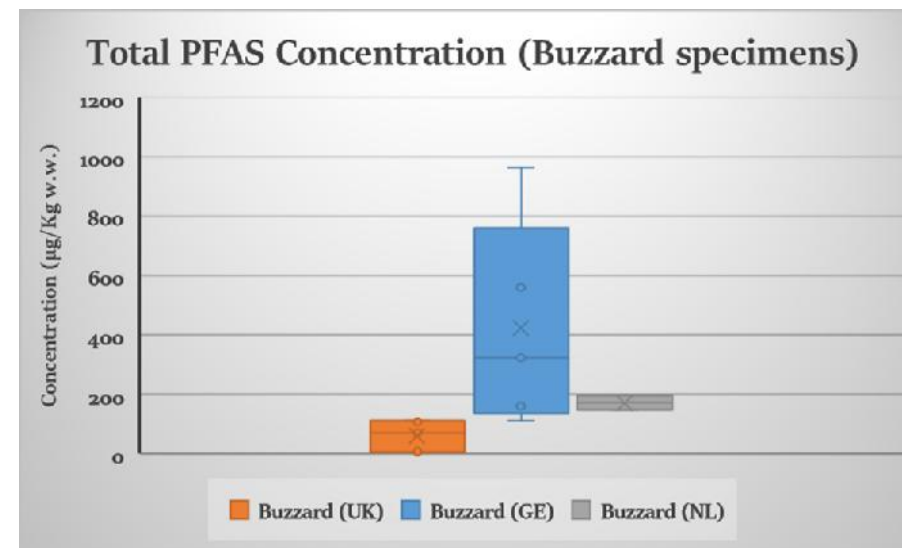
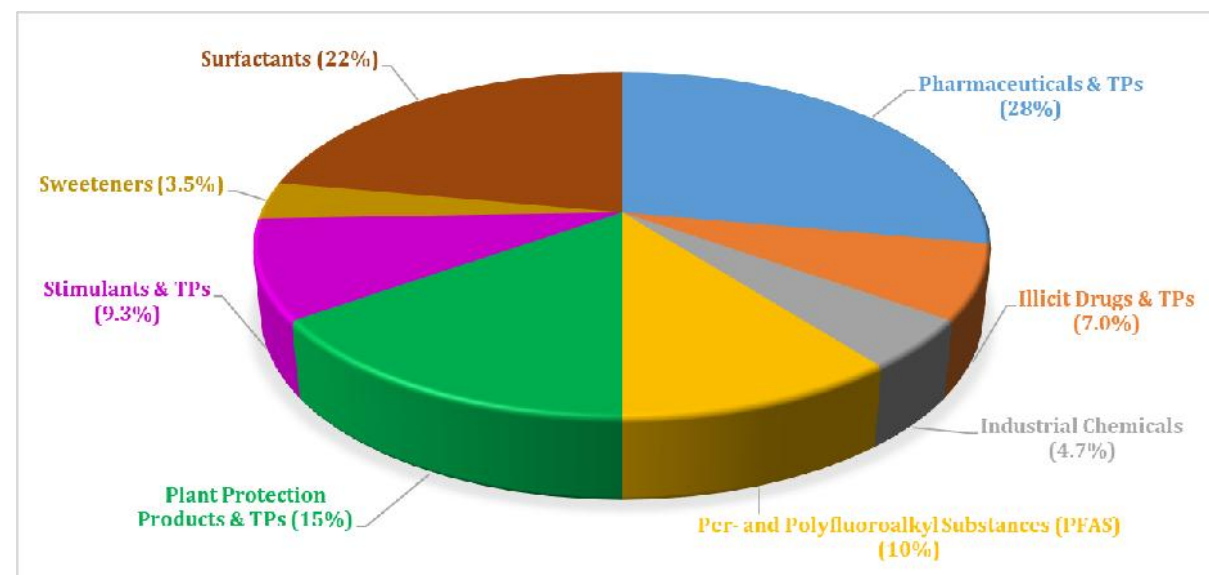
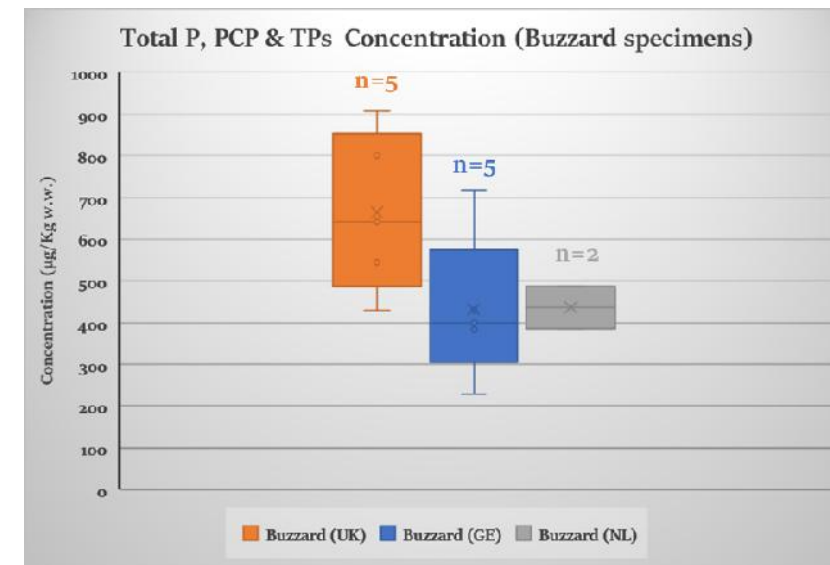
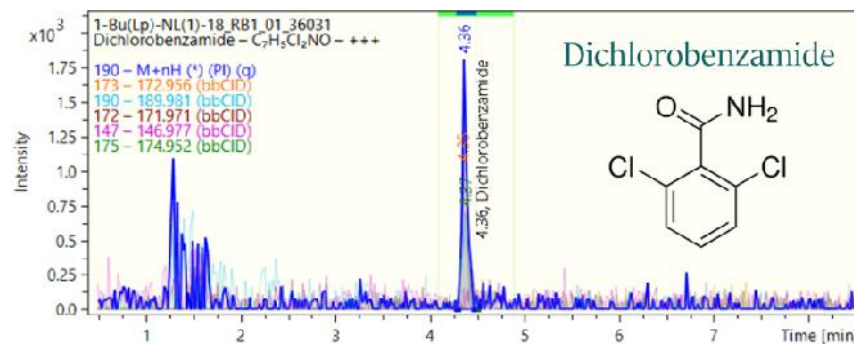
Caffeine, Nicotine and their TPs (Theobromine, Theophylline, Cotinine, Hydroxy-Cotinine, Nor-Nicotine)

Surfactants → highest concentrations among the detected compounds

PFOS and its' branched isomers → 100% FoA, high concentrations (up to 953 µg/Kg w.w.)

Bioaccumulation? → supporting information from prey (terrestrial ecosystem)

Plant Protection Products → Aldicarb-sulfoxide, Dichlorobenzamide, Ethiofencarb-sulfone





wide-scope target analysis

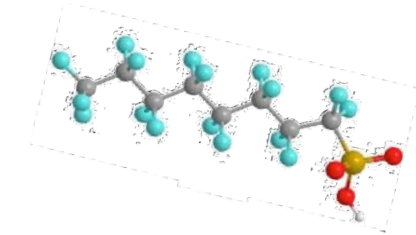
Tier 2: time-trend analysis



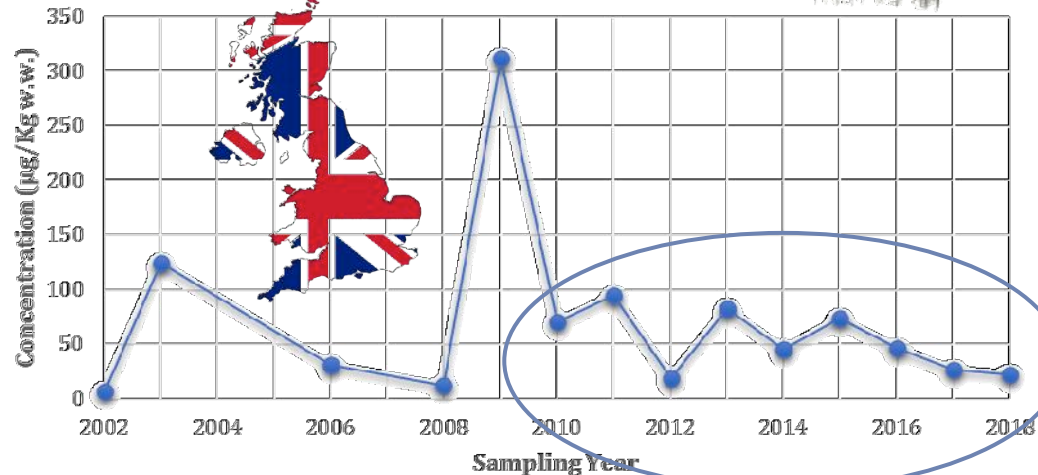
The case of Perfluorooctanesulfonic acid (PFOS)

2010-2018 → trend to lower concentrations of PFOS

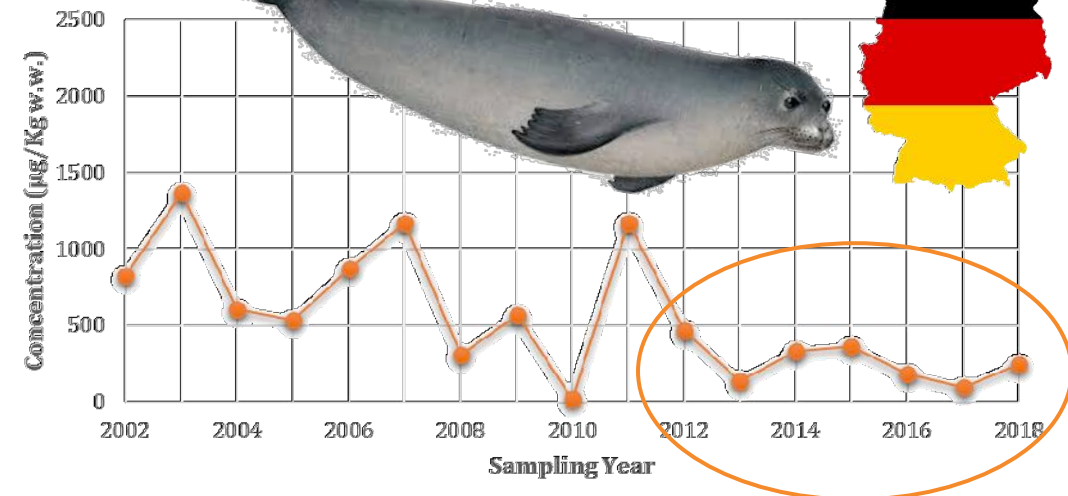
Attributed to PFOS replacement compounds (GenX, ADONA)?



Perfluorooctanesulfonic acid (PFOS)
common Buzzards (UK)



Perfluorooctanesulfonic acid (PFOS)
Harbour Seals (GE)



Surveys with raptor specimens

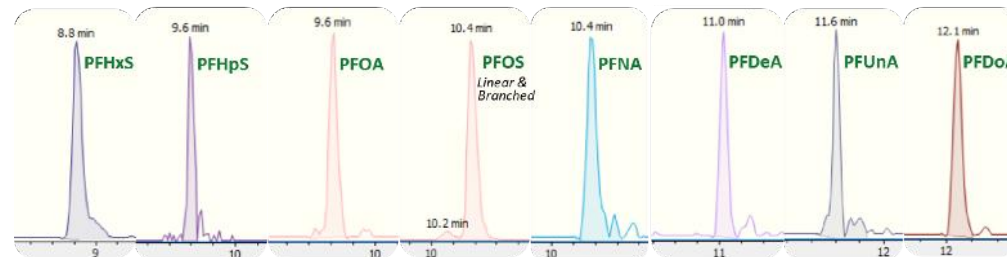
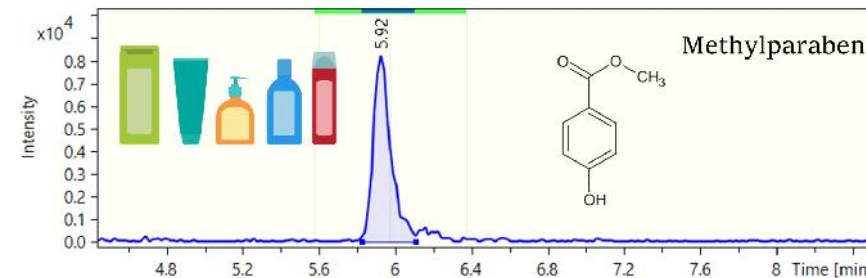


Wide-scope target and suspect screening of emerging contaminants and their transformation products in Peregrine Falcon samples gathered from the Netherlands



8 liver samples (2015 & 2017)
Adults (Males & Females)
7 different sampling regions

- 43 ECs were detected through **wide-scope target analysis**
 - Pharmaceuticals & TPs; 35%, PFAS; 26%
 - PFOS & its' branched isomers → high %FoA
 - Antibiotics** (Enrofloxacin, Ciprofloxacin, Ofloxacin) → high concentrations in one specimen, (found alive in poor condition, died by Trichomonas → **treated** in a recovery center)
 - Plant Protection Products [*Myclobutanil*, *Aldicarb-sulfone (Aldoxycarb)* and *Aldicarb-sulfoxide*]



- 54 emerging contaminants were identified through **suspect screening**
 - mainly **industrial chemicals**, pharmaceutical, personal care products
 - acquired HRMS chromatograms → **future retrospective screening** (NORMAN DSFP)



SETAC SciCon
SETAC Europe 30th Annual Meeting

- ✓ poster presentation in SETAC SciCon2020 (virtually meeting)
- ✓ article is under preparation

Surveys with raptor specimens



LU:W

Determination of emerging contaminants in livers of white-tailed sea eagle from Germany using novel and complementary High Resolution Mass Spectrometry techniques



white-tailed sea eagle (*Haliaeetus albicilla*) specimens
30 liver samples (2015 – 2018)
3 different federal states in Northern Germany

mainly Antibiotics (like Nalidixic acid & Cinoxacin),
Analgesics (such as Paracetamol & Tramadol)

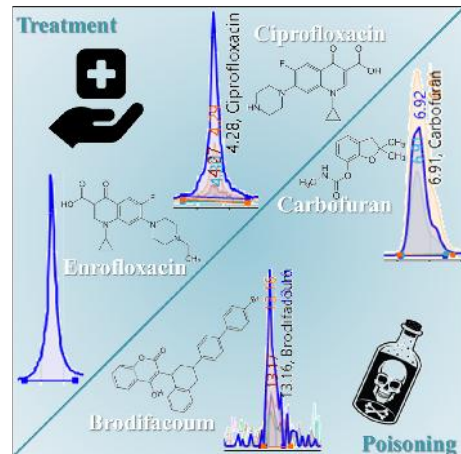
27 raptor egg samples
Sampling years: 2005 - 2020

- 87 emerging contaminants were detected through wide-scope target analysis
 - mainly Plant Protection Products & TPs (25%), Pharmaceuticals & TPs (25%)
 - PFOS and its' branched isomers (sum) → 100% FoA
 - detection of many (bio)TPs → should be included in the future monitoring programmes
- 30 emerging contaminants were identified through suspect screening
 - Industrial Chemicals (53%), Pharmaceuticals, Plant Protection Products
 - most abundant: N-Benzylformamide
- acquired HRMS chromatograms → **future retrospective screening** (NORMAN DSFP)

IN PROGRESS



wide-scope target analysis (>2,400 substances)
suspect screening (>111,000 NORMAN SusDat substances)
by LC-HRMS and GC-APCI-HRMS





Leibniz Institute for Zoo and Wildlife Research
IN THE FORSCHUNGSVERBUND BERLIN E.V.



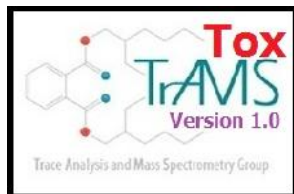
Umwelt Bundesamt

Determination of emerging contaminants in eggs of raptors from Germany using novel and complementary HRMS techniques

Future Objectives

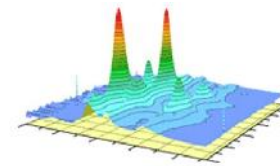
- 🎯 Collaboration with NHMs and ESBs → European Raptor Biomonitoring Facility
 - Application of the developed analytical protocols in raptor specimens (liver, muscles, eggs)
 - Investigation of the presence and distribution of ECs in **different matrices** (blood, feathers,...) of raptor specimens
 - Collection of prey samples (terrestrial ecosystem) → supporting information on the **bioaccumulation** of ECs
- 🎯 Investigation of the presence of ECs in **feathers** (*lack of knowledge*) collected from raptors (the Netherlands) collaboration with Naturalis Biodiversity Center and Peregrine and Dutch Peregrine Working Group  
- 🎯 Collaboration within NORMAN network through the Joint Activity “*Contaminants of Emerging Concern (CECs) in terrestrial organisms from different trophic levels*”
- 🎯 Enrichment of NORMAN Databases with monitoring data from raptors
- 🎯 Collaboration for the development of **chemometric tools** for the toxicity prediction of ECs adapted to raptors (**ToxTrAMS**).

Enrichment of NORMAN Ecotoxicological database with PNECs for terrestrial biota





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Acknowledgements

Dr. Nika Maria-Christina

Dr. Alygizakis Nikiforos

Dr. Aalizadeh Reza

M.Sc. Nikolopoulou Varvara

M.Sc. Gkotsis Georgios

Orfanoti Anastasia

*Thank
you*



European Raptor
Biomonitoring Facility

