

Unveiling the chemical fingerprints in organisms from different trophic levels using advanced HRMS workflows: The case study of the Baltic Sea

K. Diamanti¹, G. Gkotsis¹, E. Panagopoulou¹, M.-C. Nika¹, N. Alygizakis^{1,2}, K. Vasilatos¹, A. Konomi¹, N. Boinis¹, N. Maragou¹, P. Oswald², S. Rohner³, U. Siebert³, F. Reif⁴, M. Dähne⁴, S. Persson⁵, A. Galatius⁶, I. Pawliczka⁷, A. Künitzer⁸, E. Vähä⁹, A. Lastumäki⁹, A. Grajewska¹⁰, T. Zalewska¹⁰, E. Usin¹¹, M. Laht¹¹, J. Mitrulevičiūtė¹², N. Suhareva¹³, L. Brokmar⁵, C. Engelke¹⁴, H. Johansson¹⁵, H. Ruedel¹⁶, U. Pirntke⁸, D. Frank-Kamenetsky¹⁷, M. M. Larsen¹⁸, J. Slobodnik², N. S. Thomaidis¹

¹ National and Kapodistrian University of Athens, Panepistimiopolis Zografou, 15771 Athens, Greece; ² Environmental Institute, Okružná 784/42, 97241 Koš, Slovak Republic; ³ Institute for Terrestrial and Aquatic Wildlife Research, University of Veterinary Medicine Hannover, Werftstr. 6, 25761 Bismum, Germany; ⁴ German Oceanographic Museum, Katharinenberg 16-20, 18439 Stralsund, Germany; ⁵ Naturhistoriska riksmuseet / Swedish Museum of Natural History, Frescativägen 40, SE-104 05 Stockholm, Sweden; ⁶ Aarhus University, Høegh-Guldbergs Gade 4A 8000 Aarhus, Denmark; ⁷ University of Gdańsk, street Bazynskiego 8, 80-309 Gdańsk, Poland; ⁸ Umweltbundesamt, Wörlitzer Platz 1 06844, Dessau-Roßlau, Germany; ⁹ Finnish Environment Institute, Latokartanonkaari 11, 00790 Helsinki, Finland; ¹⁰ Institute of Meteorology and Water Management (IMGW-PIB), Podleśna 61, 01-673 Warszawa, Poland; ¹¹ Estonian Environmental Research Centre, Marja 4d, 10617 Tallinn, Estonia; ¹² Lithuanian Environmental Protection Agency, A. Juozapavičiaus g. 9, 09311 Vilnius, Lithuania; ¹³ Latvian Institute of Aquatic Ecology, Voleļu iela 2, Kurzemes rajons, Rīga, LV-1007, Latvia; ¹⁴ State Office for the Environment, Nature Conservation and Geology Mecklenburg-Vorpommern (LUNG MV), Goldberger Str. 12b, 18273 Güstrow, Germany; ¹⁵ Swedish Agency for Marine and Water Management, Gullbergs Strandgata 15, 411 04 Göteborg, Sweden; ¹⁶ German Environmental Specimen Bank, Wörlitzer Platz 1, 06844 Dessau-Roßlau, Deutschland; ¹⁷ HELCOM Secretariat, Katajanokanlaituri 6 B, 00160 Helsinki, Finland; ¹⁸ Aarhus University, Nordre Ringgade 1, 8000 Aarhus C, Denmark

Introduction

Organic micropollutants in the environment (priority pollutants & emerging contaminants)

marine biodiversity **threatened** by anthropogenic impairments

Bioaccumulation in marine food webs long-term biomonitoring studies of organic micropollutants

- effectiveness of chemicals risk mitigation measures
- prioritization of substances for PBT assessment (REACH)
- possible candidates for future legislation
- insights into wider ecological health

*PBT: Persistent, Bioaccumulative, Toxic

Aim of the study

Assessment of the occurrence of organic micropollutants in marine biota from the Baltic Sea

HOW? 11 mammal pooled liver samples & 64 mussel and fish pooled samples gathered from 9 different regions of the Baltic Sea (Germany, Sweden, Denmark, Poland, Finland, Estonia, Lithuania, Latvia, Russian Federation) during 2015-2021

- comprehensive environmental biomonitoring by exploiting the HRMS capabilities
 - wide-scope target analysis (>2,400 chemicals)
 - suspect screening (>65,000 chemicals)
- Archiving of the HRMS data in the NORMAN Digital Sample Freezing Platform (DSFP) for future retrospective screening

Advanced analytical workflows

Lyophilized & homogenized samples

Accelerated Solvent Extraction (ASE)

MeOH:ACN 50°C

HEX:DCM 100°C

defatting (HEX) SPE (in-house mixed-mode cartridges)

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

SPE (florisil cartridges)

RP LC-ESI(+/-)-QTOF MS

GC-APCI(+)-QTOF MS

HRMS analyses performed by:

- complementary chromatographic techniques (RP LC, GC)
- different ionization sources (ESI, APCI)
- data independent and dependent acquisition modes (DIA, DDA)

Data treatment by targeted and untargeted approaches

Strict identification criteria:

- Mass accuracy ($\Delta m/z \pm 2mDa$)
- Retention time shift ($\Delta RT \pm 0.2min$)
- Isotopic pattern fitting
- Presence of qualifier ions (characteristic fragments and/or adduct ions)

Results

Target analysis

- Wide-scope target analysis revealed the presence of **99** chemicals in the organisms collected from the Baltic Sea.
- 28% industrial chemicals, 22% pharmaceuticals, 20% plant protection products, 13% PFAS, 17% other.
- 15 (bio)TPs were detected in the analyzed organisms, underlining the importance of HRMS-based monitoring.
- One order of magnitude higher concentration levels observed in the apex predators compared with their prey.
 - Indications of chemicals with possible bioaccumulative properties
 - Apex predators → ideal human simulators
- 23 compounds were determined in organisms from both high (marine mammals) and low (fish, mollusks) trophic levels.
 - Most of them were conventional micropollutants [6 PFAS -PFOA, PFNA, PFDA, PFUnA, PFHxS, PFOS-, 4 PCBs -PCB 101, PCB 138, PCB 153, PCB 52-, 2,4-DDT and its TP 4,4-DDE, as well as Hexachlorobenzene].

Suspect screening

- 151 additional organic micropollutants were tentatively identified in the tested marine biota samples
- Identification levels 2A & 3 based on Schymanski et al., 2014
- Industrial Chemicals (mainly), Pharmaceuticals
- Semiquantification of the identified substances

High trophic levels

Low trophic levels

Conclusions

- First extensive HRMS-based biomonitoring survey using wildlife in the Baltic Sea ecosystem
- Unique depiction of the chemicals' spatial distribution in the Baltic Sea
- Insights in the status of the Baltic Sea ecosystem's quality
- Unravelling the presence of organic micropollutants and their (bio)transformation products
- Indications for possible biomagnification of chemicals through the food web to higher trophic levels
- Risk assessment and inclusion of prioritized chemicals in future monitoring programmes
- Upload of identified chemicals in NORMAN EMPODAT and digital storage of acquired HRMS data (specimens' library)

Acknowledgements

The studies presented herein were financed by:

- the German Environment Agency (Umweltbundesamt)
- the NEFCO Baltic Sea Action Plan Fund and varying co-contributions from the HELCOM Contracting Parties

Contact details

ntho@chem.uoa.gr
kdiamanti@chem.uoa.gr
geogkotsis@chem.uoa.gr

@ThomaidisLab
@nikos.thomaidis
@Diamanti_Kon
@GeorgiosGkotsis

Nikos Thomaidis
Konstantina Diamanti
Georgios Gkotsis

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