Determination of more than 2,400 emerging contaminants in apex predators and their prey from European Specimen Banks and Natural History Museums by novel and complementary High Resolution Mass Spectrometry Techniques

Gkotsis G.[1], Alygizakis N.[1,2], Cincinelli A.[3], Dekker R.[4], Duke G.[5], Glowacka N.[2], Knopf B.[6], Koschorreck J.[7], Martellini T.[3], Movalli P.[4], Nika M.C.[1], Nikolopoulou V.[1], Ruedel H.[6], Shore R.[8], Thomaidis N.S.[1*], Treu G.[7], Slobodnik J.[2]

[1]National and Kapodistrian University of Athens, Panepistimiopolis Zographou, 15771, Athens, Greece.
[2]Environmental Institute, Okruzna 784/42, 97241, Kos, Slovak Republic
[3]University of Florence, 50121 Firenze, Italy
[4]Naturalis Biodiversity Center, 2333 CR Leiden, Netherlands
[5]Environmental Change Institute, University of Oxford, OX1 3QY Oxford, United Kingdom
[6]Fraunhofer Institute for Molecular Biology and Applied Ecology, 57392 Schmallenberg, Germany
[7]German Environment Agency, 06844 Dessau-Roßlau, Germany
[8]Center for Ecology and Hydrology, LA1 4AP Lancaster, United Kingdom

*corresponding author: Thomaidis N.S.: e-mail: ntho@chem.uoa.gr

Abstract

Overwhelming evidence over many decades has shown that many organic compounds have been released in the environment due to anthropogenic activities. The term "chemicals of emerging concern (CEC)" has been established for chemicals, which are not subjected to marketing restrictions and regulatory monitoring programmes but are candidates for future regulation, due to their frequent detection in environmental samples and their potential hazardous properties (Thomaidis et al, 2012; Gavrilescu et al, 2015; Dulio et al, 2018). Despite the fact that their chemical and physical properties allow them to enter marine, freshwater and/or terrestrial ecosystems, data for the potential bioaccumulation and biomagnification of CECs though the food are missing, which increases the concern about their effects on the ecosystems, biota and human health. CECs, in many studies, are determined by targeted methods, based on the analysis of reference standards (e.g., plant protection products, OCPs, PAHs, PFAS, pharmaceuticals and veterinary medicines). However, despite the high selectivity and sensitivity of targeted methods, using mainly selected reaction monitoring (SRM) mode of detection, they are limited to the determination of a restricted number of compounds. On the other hand, High Resolution Mass Spectrometry (HRMS) techniques, given the high resolution capability (R=35,000) and mass accuracy in full scan mode, have enabled the simultaneous determination of hundreds of emerging contaminants and their transformation products, even if reference standards are not commercially available (Picó et al, 2012; Du et al, 2017).

Although thousands of chemicals are marketed in Europe, so far only 600 chemicals have been screened and identified as PBT (persistent, bioaccumulative, toxic), ED (endocrine disrupting) and/or CMR (carcinogenic, mutagenic, toxic for reproduction), because human and environmental hazard assessment is laborious and often obstructed due to the lack of data (ECHA's 2016 General Report, ECHA 2017). The determination of contaminants in apex predators and their prey (AP&P) allows to identify the emergence of chemicals including substitutes of regulated compounds which may present similar levels of hazard. Apex predators play a key role in the monitoring of environmental contaminants and in risk assessment studies for a number of reasons including: their position at the top of food webs, a relatively long lifespan over which to accumulate contaminants, integration of exposure both over time and relatively large spatial areas, relative ease with which samples can be obtained, and relative ease with which populations can be quantified and monitored (Movalli et al, 2017).

The EU funded LIFE APEX project (LIFE17 ENV/SK/000355, 2018-2022), demonstrates the use of AP&P in monitoring contaminants in the environment and assessing the effectiveness of chemicals risk management measures under EU regulations. The aim of LIFE APEX (https://www.lifeapex.eu) is to improve systematic use, by regulators, of chemical monitoring data from apex predators (Harbour Seal, Common Buzzard, Eurasian Otter) and their prey (freshwater fish: Bream/Roach, marine fish: Eelpout/Herring) for better chemicals management, thereby reducing exposure to harmful substances and protecting human health and the environment. LIFE APEX links biota samples from

different sample collections with novel analytical methods, for a more thorough understanding of chemicals' occurrence and bioaccumulation through the food chain. These data can be used for a better prioritization of hazardous substances, their regulation and the effectiveness evaluation of chemicals management.

For revealing the presence of CECs in AP & P samples, during the first year of LIFE APEX implementation, 67 recent samples (2015-2019) of apex predators (livers) & their prey (muscles) from four Northern Europe's countries (United Kingdom, Germany, Netherlands and Sweden), from Environmental Specimen Banks (ESB's), Natural History Museums (NHM's) and other scientific collections, were analyzed following state-of-the-art wide-scope target screening methodologies.

All samples were lyophilized before analysis, in order to enhance extraction efficiency, improve the precision and achieve lower detection limits. The analytes were extracted from the dry matrices through generic methods of extraction, using Accelerated Solvent Extraction (ASE), followed by a clean-up step using Solid Phase Extraction. The final extracts were analyzed by different chromatographic and ionization techniques (both liquid and gas chromatography) coupled to High Resolution Mass Spectrometry (using electrospray and atmospheric pressure ionization, respectively), in order to broaden the chemical domain accessible to wide-scope target analysis. The samples were screened for the presence of more than 2,400 organic pollutants, included in the target list of the University of Athens (UoA). UoA database includes compounds of different classes (such as pharmaceuticals, personal care products, biocides, plant protection products, illicit drugs, stimulants, sweeteners, and industrial chemicals, e.g. per- and polyfluorinated compounds (PFASs), flame retardants, corrosion inhibitors, plasticizers, surfactants), as well as their transformation products and metabolites. The target list is being updated on a regular basis, since new compounds of concern are continuously being identified.

Strict criteria of mass accuracy (<2mDa), isotopic fitting (mSigma<50), retention time (<0.40 min) and fragmentation pattern match were applied during the screening process, while the standard addition method was used for quantification purposes. Preliminary results indicate the presence of several plant protection products (including DEET, myclobutanil and terbuthylazine), stimulants (such as nicotine and its metabolites), sweeteners, industrial chemicals (including benzotriazole and tolytriazole), pharmaceuticals and psychoactive drugs (including sertraline and quetiapine) in the tested samples. Furthermore, numerous transformation products (including propachlor-OXA, Nor-tramadol, 4-acetamido-antipyrine) were detected in livers from apex predators, underlying the power of wide-scope target screening. Moreover, more than 15 PFASs were detected in samples from all tested countries with high detection frequency and at high concentration levels. Based on the results, significantly higher concentrations of chemicals (including perfluorodecanoic acid -PFDA- and propachlor-OXA) were detected in livers from apex predators than in the muscle samples from their prey, implying a potential bioaccumulation through the food chain.

Keywords:
Apex Predators; Emerging Contaminants; High Resolution Mass Spectrometry; Biomonitoring; Bioaccumulation.

Acknowledgments
This research has received funding from the European Union through the program LIFE17 ENV/SK/000355 "Systematic use of contaminant data from apex predators and their prey in chemicals management".

References

Du B., Lofton J., Peter K., Gipe A., James A., McIntyre J., Scholz N., Baker J., Kolodziej E. (2017), Development of Suspect and Non-Target Screening Methods for Detection of Organic Contaminants in Highway Runoff and Fish Tissue with High-Resolution Time-of-Flight Mass Spectrometry, *Environmental Science: Processes & Impacts*, **19(9)**, 1185-1196.

Dulio V., Van Bavel B., Brorström-Lundén E., Harmsen J., Hollende J., Schlabach M., Slobodnik J., Thomas K., Koschorreck J. (2018), Emerging pollutants in the EU: 10 years of NORMAN in support of environmental policies and regulations, *Environmental Sciences Europe*, **30(5)**.

Movalli P., Dekker R., Koschorreck J., Treu G. (2017), Bringing together raptor collections in Europe for contaminant research and monitoring in relation to chemicals regulations, *Environmental Science and Pollution Research*, **24(31)**, 24057-24060.

Gavrilescu M., Demnerová K., Aamand J., Agathos S., Fava F. (2015), Emerging pollutants in the environment: Present and future challenges in biomonitoring, ecological risks and bioremediation, *New Biotechnology*, **32(1)**, 147-56.

Picó Y., Barceló D. (2012), Emerging contaminants in biota, *Analytical and Bioanalytical Chemistry*, **404 (9)**, 2525–2526.

Thomaids N.S., Asimakopoulos A.G., Bletsou A.A. (2012), Emerging contaminants: A tutorial mini- review, *Global NEST Journal*, **14(1)**, 72–79.