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Wide-scope screening for contaminants of emerging concern in archived biota

Method development, suspect prioritisation, and non-target screening in a novel identification tool

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Cover: Time-trend analysis of non-target screening features in white-tailed sea eagle, lynx, and perch

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Abstract

Environmental monitoring of hazardous chemicals in wildlife conventionally uses target screening for selected contaminants, but relatively few contaminants are monitored and knowledge of potentially hazardous contaminants of emerging concern (CECs) in wildlife is lacking. In this thesis, a non-target screening (NTS) method combined with temporal trend analysis was developed and applied as a prioritisation tool for identification of CECs in top predators, using high-resolution mass spectrometry (HRMS). A multi-residue sample extraction and HRMS screening method was developed and validated for various biota tissue types and species, to capture chemicals with a broad range of physiochemical properties (Paper I). Minimised sample pre-treatment and clean-up resulted in a non-specific extraction method for NTS in biota. A tool for creating suspect lists for screening of CECs in biota was developed based on an extensive database of chemicals (Paper II). Systematic ranking of chemicals based on relevant physicochemical properties was used to prioritize CECs relevant for biota and water. Finally, a NTS workflow was developed for prioritizing CECs in time series of archived biological tissue of top predators. The samples included time series of muscle tissue from white-tailed sea eagle (*Haliaeetus albicilla*) (1965-2017) and Eurasian lynx (*Lynx lynx*) (1969-2017) obtained from the environmental specimen bank (ESB) at the Swedish Museum of Natural History (SMNH). The prioritisation method was validated with an artificial time series using spiked matrix samples of increasing concentrations (Paper III). A total of 14 compounds (six of anthropogenic origin) with increasing time trends were tentatively identified in white-tailed sea eagle samples, while two compounds with increasing time trends and one compound with a decreasing time trend were tentatively identified in lynx samples (Paper IV). The tentatively identified compounds originated from different chemical categories (pharmaceuticals, personal care products, industrial chemicals, herbicides). These results showed that, despite the high matrix effect and low expected concentrations in terrestrial species (lynx), it was possible to tentatively identify new CECs in wildlife. The novel prioritisation strategy and NTS workflow developed in this thesis can provide a useful tool for future identification of CECs in biota. The overall findings can help government agencies expand their monitoring programmes for identification of CECs in biota.

Keywords: contaminants of emerging concern, top predators, environmental specimen bank, time series analysis; prioritisation; mass spectrometry; non-target screening

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Breda analyser av oönskade organiska ämnen i arkiverade biologiska prover

Metodutveckling, prioritering och förutsättningslös screening i en ny identifieringsmetodik

Sammanfattning

Miljöövervakning av hälsofarliga kemikalier i vilda djur använder konventionell analys för utvalda miljöföroreningar. Dock övervakas endast en liten del av tänkbara föroreningar och kunskap om nya, potentiellt hälsofarliga ämnen (CEC) i vilda djur saknas. I denna avhandling förutsättningslös screening (non-target screening, NTS) genom högupplöst masspektrometri (HRMS) i tidstrender utvecklades och används för att kunna identifiera nya föroreningar av CEC i arkiverade prover från vilda djur. En bred extraktionsmetod och en HRMS metod utvecklades för olika typer av biota och validerades för att upptäcka kemikalier med ett brett spektrum av fysikalisk-kemiska egenskaper (Artikel I). Minimerad förbehandling och upparbetning av proverna resulterade i en icke-specifik extraktionsmetod lämpad för NTS i biota. Ett verktyg utvecklades för utformning av smarta screeningslistor baserat på en omfattande databas med oönskade ämnen (Artikel II). Systematisk rangordning av kemikalier baserad på fysikalisk-kemiska egenskaper samt data om kemikaliernas användning utfördes och listor över relevanta CECs kunde därmed skapas för vatten och biota. Slutligen utvecklades ett NTS arbetsflöde för att prioritera CEC i tidsserier av arkiverad biologisk vävnad från rovdjur. Proverna inkluderade muskelvävnad från havsörn (*Haliaeetus albicilla*) och lodjur (*Lynx lynx*) insamlade från 1965 till 2017 och erhölls från miljöprovbanken via Naturhistoriska riksmuseet. Prioriteringsmetoden validerades med hjälp av artificiella tidsserier som tillverkades genom att tillsätta referenssubstanser till extrakt från vävnadsprover i en gradient med ökande koncentrationer (Artikel III). Totalt identifierades 14 kandidater (sex av antropogen ursprung) med ökande tidstrender i havsörnsproverna (Artikel III). Dessutom identifierades preliminärt två ämnen (en med antropogent ursprung) med ökande tidstrender och ett ämne med minskade tidstrend i lodjur (Artikel IV). De preliminärt identifierda ämnen tillhör olika kemiska kategorier (läkemedel, kosmetika, industriella kemikalier , herbicid). Trots kraftig matriseffekt och låga förväntade halter av CEC i lodjur var det möjligt att preliminärt identifiera CEC i vilda djur. Prioriteringsstrategierna och NTS arbetsflöde som utvecklats i denna avhandling utgör nya verktyg för identifikation av oönskade ämnen i biologiska prover. På sikt kan dessa nya verktyg hjälpa samhället att förbättra miljöövervakningen och leda till ytterligare upptäckter av nya miljöföroreningar i biota.

Nyckelord: nya miljöföroreningar, rovdjur, havsörn, lodjur, miljöprovbank, tidsserieanalys, prioritering, masspektrometri, förutsättningslös screening

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Screening von neu detektierten organischen Schadstoffen in archivierten Biotaproben

Methodenentwicklung, Priorisierung von Substanzen und Screening nach unbekannten organischen Schadstoffen

Zusammenfassung

Im Rahmen der Umweltüberwachung zum Schutz vor gefährlichen Chemikalien werden Tiere hinsichtlich des Vorkommens von Schadstoffen in ihrem Körper analysiert. Allerdings wird bisher nur ein kleiner Teil von existierenden organischen Schadstoffen überwacht, da es an Erkenntnissen mangelt, welche Schadstoffe in Tieren vorkommen. In dieser Arbeit wurden Zeitreihenanalysen und "non-target screening" (NTS) zur Prioritisierung von Schadstoffen verwendet um unbekannte Schadstoffe in archivierten Biotaproben mittels hochauflösender Massenspektrometrie nachzuweisen. Die Probenaufarbeitung wurde optimiert und validiert um Schadstoffe mit einem breiten Spektrum physikochemischer Eigenschaften nachzuweisen (Artikel I). Die minimierte Probenaufarbeitung führte zu einer unspezifischen Extraktionsmethode für NTS in verschiedenen Biotaproben. Ein Werkzeug zur Erstellung von Schadstofflisten wurde entwickelt, welches auf einer umfangreiche Datenbank von bekannten Schadstoffen basiert (Artikel II). Systematisches Sortieren der Chemikalien nach deren Eigenschaften resultierte in relevante Schadstofflisten für Biota und Wasser. Abschließend wurde ein NTS-Workflow zur Priorisierung von Schadstoffen in Zeitreihen von archiviertem biologischem Gewebe von Raubtieren entwickelt. Die Proben umfassten Zeitreihen von Muskelgewebe des Seeadlers (*Haliaeetus albicilla*) und Luchses (*Lynx lynx*), die von 1965 bis 2017 von der Umweltprobenbank des Schwedischen Naturkundemuseums gesammelt wurden. Zur Kontrolle wurde die Priorisierungsmethode durch eine künstliche Zeitreihe, welche mit steigenden Konzentrationen bekannter Schadstoffe angereichert war, validiert (Artikel III). Insgesamt wurden vorläufig 14 Schadstoffe (sechs mit anthropogenem Ursprung) mit zunehmendem Zeittrend im Adler identifiziert. Zusätzlich wurden im Luchs vorläufig zwei Schadstoffe mit zunehmendem und ein Schadstoff mit abnehmendem Zeittrend identifiziert (Artikel IV). Trotz des hohen Matrixeffekts während der Analyse und der geringen erwarteten Konzentrationen bei terrestrischen Arten war es möglich, Schadstoffe vorläufig in Biota zu identifizieren. Die in dieser Arbeit entwickelten Methoden und Priorisierungsstrategien bieten ein neues Identifikationswerkzeug für potenziell schädliche Stoffen in Tieren. Die Ergebnisse dieser Arbeit können Regierungsbehörden oder anderen Stakeholders helfen, ihre Umweltüberwachungsprogramme zur Identifizierung von neuen Schadstoffen zu erweitern.

Schlüsselwörter: organische Schadstoffe, Raubtiere, Umweltbank, Zeitreihenanalyse, Priorisierung, Massenspektrometrie, non-target screening

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Brede analyse voor nieuwe verontreinigende stoffen in gearchiveerde biologische monsters

Methode ontwikkeling, prioritering van verdachte stoffen en onbevooroordeeld screening voor een nieuwe identificatiemethode

Samenvatting

Milieumonitoring van gevaarlijke stoffen in weefsel van dieren maakt vaak gebruik van doelgerichte analyses voor geselecteerde verontreinigende stoffen. Er wordt echter slechts een klein deel van deze stoffen gecontroleerd en er is een gebrek aan kennis van nieuwe stoffen waarvan vermoed wordt dat ze een risico vormen voor het milieu (CECs). In dit proefschrift werd non-target screening (NTS) en temporele trendanalyse gebruikt als een prioteringsstrategie om met behulp van hoge resolutie massenspectrometrie nieuwe CECs in gearchiveerde monsters te identificieren. Er werd een multi-residu extractie voor verschillende monster ontwikkeld en gevalideerd om stoffen met een breed scala aan fysiochemische eigenschappen te analyseren (Artikel I). Minimale monstervoorbehandling resulteerde in een niet-specifieke extractiemethode voor NTS in verschillende biologische monsters. Er werd een tool ontwikkeld op basis van een uitgebreide chemische database voor het creëren van relevante screeningslijsten van verdachte stoffen (Artikel II). Systematische rangschikking van chemicaliën op basis van relevante fysiochemische eigenschappen leide tot gerichte lijsten van verdachten voor water en biota. Ten slotte werd een NTS-workflow ontwikkeld om CECs in een tijdsreeks van gearchiveerd biologisch weefsel van roofdieren te prioriteren en identificeren. De monsters omvatten tijdsreeksen van zeearend- en lynxspierweefsel dat tussen 1965 en 2017 door de mileubank van het Zweedse natuurhistorische museum werd verzameld. De prioriteitsmethode werde gevalideerd met behulp van een kustmatige tijdreeks van matrixmonsters gespiked met oplopende concentraties van bekende CECs (Artikel III). In totaal werden 14 stoffen (waarvan zes met antropogene oorsprong) met toenemende tijdstrends in de adelaar voorlopig geïdentificeerd. Bovendien werden twee stoffen met stijgende tijdstrends en één stof met een dalende tijdstrend in lynx voorlopig geïdentificeerd (Artikel IV). Ondanks een groot matrixeffect en lage verwachte concentraties bij terrestrische dieren, was het mogelijk om CECs met de ontwikkelde prioriteringsstrategieën en NTS workflow voorlopig te identificeren. De ontwikkelde methoden en prioriteringsstrategieën in dit proefschrift vormen een nieuw identificatiemethode voor CECs in dieren. De bevindingen van dit proefschrift kunnen overheidsinstanties helpen om hun monitoringprogramma's uit te breiden naar nieuwe verdachte verontreinigende stoffen in dieren.

Trefwoorden: opkomende verontreinigende stoffen; roofdieren; milieubank; tijdreeksanalyse; prioritering; massaspectrometrie; non-target screening

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Dedication

To Remco, Maxim and Yuki, who give me joy and inspiration on filling the time given to me.

“All we have to decide is what to do with the time that is given us.”

J.R.R. Tolkin, *The Fellowship of the Ring*, 1954

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I **Dürig, W.**, Kintzi, A., Golovko, O., Wiberg, K. & Ahrens, L. (2020). New extraction method prior to screening of organic micropollutants in various biota matrices using liquid chromatography coupled to high-resolution time-of-flight mass spectrometry. *Talanta* 219, 121294.

II **Dürig, W.***, Tröger, R.*, Andersson, P. L., Rybacka, A., Fischer, S., Wiberg, K. & Ahrens, L. (2019). Development of a suspect screening prioritisation tool for organic compounds in water and biota. *Chemosphere* 222, 904-912.

III **Dürig, W.**, Alygizakis, N., Menger, F., Golovko, O., Wiberg, K. & Ahrens, L. Novel prioritisation strategies for evaluation of temporal trends in archived white-tailed sea eagle muscle tissue in non-target screening. Under review for publication in *Environmental Science and Technology.*

IV **Dürig, W.**, Alygizakis, N., Wiberg, K. & Ahrens, L. Application of a novel prioritisation strategy using non-target screening for evaluation of temporal trends (1969-2017) in contaminants of emerging concern (CECs) in archived lynx muscle tissue samples (manuscript).

*Shared first authorship

Papers I and II are reproduced with the permission of the publishers.

The contribution of Wiebke Dürig to Papers I-IV was as follows:

I Planned the study together with the co-authors and had the main responsibility for conducting the experiment, analysing the data, and writing the manuscript, with the support of all co-authors.

II Together with Rikard Tröger had the shared equal main responsibility for the study, analysis of the data and writing the manuscript, with the support of all co-authors.

III Had the main responsibility for designing and conducting the experiment, analysing the data, and writing the manuscript, with the support of all co-authors.

IV Had the main responsibility for designing and conducting the experiment, analysing the data, and writing the manuscript, with the support of all co-authors.

Abbreviations

ACN	Acetonitrile
ATS	Artificial time series
BCF	Bioconcentration factor
CECs	Chemicals of emerging concern
ECHA	European Chemical Agency
ESB	Environmental specimen bank
ESI	Electron spray ionisation
FA	Formic acid
FR	Flame retardants
GC	Gas chromatography
HRMS	High-resolution mass spectrometry
IS	Internal standard
LC	Liquid chromatography
MDL/MQL	Method detection/quantification limit
NTS	Non-target screening
PFASs	Per- and polyfluoroalkyl substances
PMT	Persistent, mobile, and toxic

POP	Persistent organic pollutant
qToF	Quadrupole time of flight
QuEChERS	Quick, easy, cheap, effective, robust, and safe
RSD	Relative standard deviation
RT	Retention time
SL	Suspect list
SMILES	Simplified molecular-input line-entry system
SPE	Solid-phase extraction
SusTool	Customizable *in silico* prioritisation tool
UPLC	Ultra-performance liquid chromatography
WTSE	White-tailed sea eagle

1 Introduction

Did you ever wonder what time does to our environment? We improve our living standards constantly by the development of novel technologies and products involving the use of a wide range of chemicals. These chemicals are beneficial for human lifestyles, but over time can cause harm to all living organisms in the wider environment. Can we identify compounds that are becoming more abundant in certain animals over time? What is needed to identify these compounds?

1.1 Chemicals of emerging concern

There is increasing awareness and anxiety about chemicals of emerging concern (CECs) in the environment. Government agencies and conventions aim to restrict emissions of these chemicals via regulations (*e.g.*, the Stockholm Convention, lists of CECs on the website of the European chemical agency (ECHA) *etc*.). These restrictions aim to protect humans and the environment from adverse health effects. Monitoring of known chemicals of concern, *e.g.*, persistent organic pollutants (POPs), in different programmes has been in place since the early 1960s, to follow the trends in certain compounds over time. In this regard, environmental specimen banks of archived samples can be a valuable source for retrospective analysis. Organic compounds in different matrices can be detected by *e.g.*, chromatography and mass spectrometry. Subsequent identification can be made by one of three different analytical workflows for screening.

1.2 Target, suspect and non-target screening

There are three analytical approaches available to identify chemicals in various matrices: target screening, suspect screening, and non-target screening (NTS) (Krauss *et al*., 2010). Wide-scope target screening is well-established, while suspect screening approaches are becoming more sophisticated. The past decade has seen rapid development of NTS workflows, which to date have been applied in analysis of water (Schymanski *et al*., 2015), sediment (Albergamo *et al*., 2019), soil (Veenaas *et al*., 2017) and biota (Heffernan *et al*., 2017).

From an analytical point of view, matrix-rich samples (*e.g*. wastewater, biota) are challenging, as these types of samples contain many biotic compounds that can interfere with the analytical method. Therefore, most extraction methods for biota are developed for specific compounds in certain species or tissues (Huerta *et al*., 2015; Ziarrusta *et al*., 2018). Gas chromatography (GC) approaches are commonly applied when investigating biological samples, since hydrophobic compounds tend to bioaccumulate and thus can be expected to be detected by GC analysis (Fernando *et al*., 2018; Neugebauer *et al*., 2018). However, more hydrophilic compounds such as pharmaceuticals, pesticides and per- and polyfluoroalkyl substances (PFASs), which are typically separated using liquid chromatography (LC) approaches, are often more mobile and can also be bioaccumulative and harmful to biota (Ahrens *et al*., 2014). Thus, GC and LC are complementary approaches to separate organic compounds for subsequent detection in biota.

Target screening, suspect screening and NTS are described below, focusing on screening of biological matrices using LC coupled to high-resolution mass spectrometry (HRMS).

Target screening

Within target screening, a selected number of compounds is analysed in a desired matrix. These so-called target compounds are of interest in the specific matrix analysed and reference standards are available for identification purposes (Schymanski *et al*., 2014). Multi-residue methods have been developed to capture many target compounds with a wide range of physicochemical properties (Neugebauer *et al*., 2018; Paper I). For example, environmental monitoring campaigns apply target screening to follow trends over time in known chemicals of concern in various biological matrices (Odsjö, 2006). However, this approach only captures a small fraction of the many substances occurring in the environment and overlooks *e.g.*, trans-

formation products and compounds potentially harmful to wildlife species (Millow *et al*., 2015). Use of analytical HRMS data processing workflows for suspect screening could shed light on overlooked compounds.

Suspect screening

In suspect screening workflows, there is no need for reference standards until the confirmation stage, thus saving time and money and allowing inclusion of an extensive list of substances (Gago Ferrero *et al*., 2018). These suspect lists can be developed based on *e.g*., expected transformation products (Zonja *et al*., 2015), regulatory databases (Gago-Ferrero *et al*., 2018) or physicochemical properties (Paper II). Lists containing structural information in terms of *e.g*., chemical structure file format (mol files) for the suspected compounds can be accompanied by data on predicted retention times (RT) and/or predicted fragments (Aalizadeh *et al*., 2016). Applying the list developed to the acquired dataset assigns suspects to features (mass-to-charge ratio (*m/z*), RT, intensity). These hits must be elucidated against mass spectral libraries. *In-silico* fragmentation can assist in elucidating suspects by comparing measured and predicted MS/MS fragmentations with each other, resulting in a list of likely suspects present.

The presence of endogenous compounds in biological samples makes comparison of the chromatogram and spectra acquired with mass spectral libraries challenging. Extensive component co-elution and associated matrix effects limit successful identification for suspect screening (Hollender *et al*., 2017; Diamanti *et al*., 2020). Predicted RTs can aid in differentiating interfering and isobaric compounds from the suspects (Du *et al*., 2017). However, suspects without available standards remain a primary challenge for HRMS analysis.

Non-target screening

In contrast to suspect screening, NTS starts without any *a priori* information on the compounds to be detected. NTS workflows are therefore based on prioritisation approaches that can be experiment-driven (*e.g*., elimination/formation, transformation product formation, effect-directed analysis) or data-driven (*e.g*., signal intensity, frequency, characteristic isotopic pattern, spatial/temporal trends) (Hollender *et al*., 2017). The dataset acquired, of all detected masses, needs to be prioritised to give a realistic number of relevant features for further elucidation. The prioritised features can then be handled as a suspect list for further elucidation.

Previous studies have shown that prioritizing features in NTS by means of temporal trend analysis is possible and beneficial for reducing the high

amount of data produced and focusing on the most relevant compounds (Plassmann *et al*., 2016; Albergamo *et al.,* 2019; Alygizakis *et al*., 2019b; Anliker *et al*., 2020). Historical archived samples from *e.g*., environmental specimen banks (ESB) provide opportunities to identify new CECs by means of temporal trend analysis and NTS in wildlife species. However which species are representative for this purpose?

1.3 Environmental specimen banks and selection of species for biota monitoring

Archived environmental samples, collected in accordance with standardised protocols by *e.g*., ESBs, provide opportunities for detection of CECs in many different species over a long -time span (Bignert, 2002). The Swedish Museum of Natural History has systematically collected a wide variety of environmental samples since the 1960s (Odsjö, 2006), providing opportunities for development of new prioritisation strategies through temporal trend analysis of non-target features obtained in HRMS analysis.

The literature highlights several criteria that should be met to qualify a species as suitable for use as a sentinel species in contaminant monitoring, including migration and distribution knowledge, known variation within and between samples (Miller *et al.*, 2014) and knowledge on species biology and ecology (Furness *et al*., 1997). In addition, the following criteria should be met for species selection regarding NTS:

(i) Availability of archived samples (according to Animal Welfare Acts and Animal Welfare Ordinance).

(ii) Presence of sufficient sample tissue and a complete time series (at least every five years) to avoid large gaps in temporal data.

(iii) High trophic feeding level and ability to accumulate pollutants.

The distribution of compounds may be species-specific (Mateo *et al*., 2012) and/or tissue-specific (Jasper *et al*., 2013), depending on diet, habitat, and specific biotransformation of the species. Considering the above-mentioned criteria, certain species are more suitable, and others less suitable, for NTS regarding time trend analysis (Table 1).

Table 1. *Examples of marine, limnic and terrestrial wildlife species and their suitability for non-target screening of contaminants of emerging concern (CECs).*

	Marine species	Limnic species	Terrestrial species
+ (suitable)	White-tailed sea eagles, herring gulls, glaucous gulls, fulmars, murres, guillemots, common eider	Fish (burbot, trout, eels, mullet, perch, cod, rusk, halibut, char, salmon *etc*.), mink, otters	Lynx, birds of prey (peregrine falcons, red-tailed hawks, great homed owls, tawny owls, golden eagles *etc.*)
O (neutral)	Artic foxes, polar bear, ringed seals, whales, oysters, mussels		Semi-domesticated reindeers, red foxes
– (less suitable)	Shrimp	Reptiles	Domestic animals (rodents, chickens *etc*.)

Species high in the food web (*e.g*., birds of prey, bears, seals) are more susceptible to bioaccumulation of CECs to high concentrations as result of biomagnification (Badry *et al.*, 2020; de Wit *et al*., 2020). In particular, marine species with fat-rich tissue allow for accumulation of hydrophobic compounds and magnification in the aquatic food chain, making detection of CECs in these species feasible and relevant. However, ESBs collect samples from top predators mainly on an occasional basis (*i.e*., stranded specimens or traffic kills), resulting in limited availability of material. In addition, concentrations of CECs in these individuals may not reflect that in the general species population, which could compromise conclusions on identified CECs.

Sea birds are diurnal, large, wide-ranging (widespread habitat), conspicuous, abundant, long-lived, easily observed and monitored, and of interest to the public, making them suitable species for NTS (Moore, 1966). The ESB at the Swedish Museum of Natural History collects a broad variety of sea birds for contaminant analysis. Migrating species are less suitable for monitoring, as they suffer exposure from different sites. The diversity resulting from this can be minimised by pooling samples from individuals (Bignert *et al*., 2014). The habitat and numbers of white-tailed sea eagles living in the northern hemisphere decreased between the mid-1950s to early 1980s (Helander *et al*., 2002), raising concern and prompting extensive collection of samples of this species. White-tailed sea eagle is a top predator in the aquatic food chain, where it mainly feeds on fish and marine bird species, which are prone to accumulate CECs.

Regarding the limnic environment, fish have been studied extensively. Their proximity to pollutant sources, high abundance and human consumption makes them relevant and suitable for monitoring (Moore, 1966). As

availability and presence of tissue is less of an issue for these species, it is important to select fish at the top of the food chain. In addition, it could be relevant to select fish from a location impacted by environmental stressors, increasing the chances of detection of anthropogenic CECs.

Most studies of CECs concentrate on aquatic species, while terrestrial species are rarely studied. Slow movement of contaminants in the terrestrial environment and fewer trophic levels compared with the aquatic environment make detection of accumulative compounds in terrestrial species challenging (Moore, 1966). The concentrations of most chemicals in terrestrial animals are considered to be very low to nearly undetectable (Swackhamer *et al*., 2009). A review by Movalli *et al.* (2019) highlighted the importance of raptor collection and monitoring in relation to chemical regulation and showed that 75% of natural history museums/ESBs in Europe receive and collect raptors, providing the opportunity to screen in time series for CECs. In previous studies, conventional POPs like pesticides and polychlorinated biphenyls have been investigated in Iberian lynx (*Lynx pardinus*) in Spain (Mateo *et al*., 2012) and conventional flame retardants have been found in Norwegian Eurasian lynx (*Lynx lynx*) livers (Mariussen *et al*., 2008). CECs have not previously been investigated in the latter species, but the accessibility of lynx tissue in ESBs and their opportunistic foraging behaviour make lynx attractive for NTS.

Many ESBs freeze incoming carcasses from top predators, raptors and collected fish on arrival and then process and freeze wet tissues (*e.g*., muscle, heart, liver *etc*.). The tissue most suitable for contaminant monitoring depends highly on the question in place. Yu and Cohen (2004) advise avoiding “hair, bone or cartilage as sample matrix as these require extreme measures for extraction”. Blood reflects recent exposure for many substances, but when stored over a long period compounds are likely to transform or break down. Liver has become a standard tissue for contaminant testing, particularly for organic compounds, as many compounds accumulate in this tissue. Brain and kidney levels indicate impacts on the investigated species (Yu & Cohen, 2004). Muscle concentrations reflect risks for predators, particularly species that avoid eating organs. Sample tissues should be selected based on availability and the possibility to extract as many different compound groups as possible with time- and cost-efficient extraction and clean-up techniques. In this thesis, muscle tissues from white-tailed sea eagle, perch, and Eurasian lynx were selected for development and application of an extraction method and NTS workflow.

2 Objective and research questions

Environmental monitoring and assessment are essential to follow trends in pollutants in the environment. Knowledge gained by identifying these trends can be used to regulate emissions of chemicals to the environment and thereby protect the environment. Currently, legacy, or regulated pollutants which are known to cause harm to the environment are monitored regularly. However, only a small number of CECs circulating in the environment are monitored. Thus, a tool for identification of potential persistent and bioaccumulative chemicals in environmental samples is urgently needed. In this thesis, the newest developments in suspect screening and NTS using HRMS were combined with trend analysis in biota obtained from ESBs. The following research questions guided the thesis work and addressed the overall aim of building a novel prioritisation tool for NTS in archived biological tissues:

(i) How can a broad range of CECs in different biota tissues be extracted and subsequently analysed using LC-HRMS with respect to suspect and NTS? (**Paper I**).

(ii) Can CECs from an extensive database be systematically ranked, based on their physicochemical properties, in terms of their likelihood to be detected in biota? (**Paper II**).

(iii) Can archived biological samples be used as a NTS prioritisation strategy for CECs in biota? (**Papers III and IV**).

(iv) Which CECs can be detected and tentatively identified in top predators (white-tailed sea eagle and lynx) using time trend analysis as a prioritisation tool? (**Papers III and IV**).

3 Materials and methods

Sample selection and sample preparation determine the accuracy of the results obtained in the subsequent steps of chemical analysis, data handling and workflow creation. Therefore, it is of critical importance to choose samples carefully and to develop a robust, reliable, and in the present case wide-scope, sample preparation method.

3.1 Sample selection

Based on the criteria and suitability aspects described in section 1.3 of this thesis, muscle tissue from white-tailed sea eagle (*Haliaeetus albicilla*) (**Papers I** and **III**) and Eurasian lynx (*Lynx lynx*) (**Paper IV**) were analysed. The tissues were provided by the ESB at the Swedish Museum of Natural History and stored at -20 ºC until extraction.

In **Paper I**, muscle tissue from white-tailed sea eagle (WTSE) collected in 2014 and 2015 was used for development of a generic sample extraction method. For application of the final extraction method developed, heart, liver, and muscle tissue from 10 European perch (*Perca fluviatilis*) were analysed individually (**Paper I**).

In **Paper III**, fresh WTSE muscle tissue (wet weight) sampled and stored according to standardised protocols at the ESB were obtained (Odsjö, 2006). Pooled WTSE muscle tissue collected in 1965 to 2017, mainly from birds killed by traffic, was analysed year-wise. Selection criteria for the individual samples were: (i) availability of individual samples per year, (ii) close proximity of sample locations, (iii) equal sex ratio per year (1:1, male: female), (iv) preferably adult birds, and (v) feed intake mainly by marine feed sources. Feed intake was determined by analysis of

carbon and nitrogen isotopes in all individual muscle tissue samples (**Paper III**).

In **Paper IV**, fresh muscle tissue (wet weight) from lynx, collected during 1969 to 2017 according to standardised protocols set by the ESB, was studied. For this, the extraction method developed in **Paper I** and the prioritisation method and NTS workflow developed in **Paper III** were applied. Selection criteria for the individual samples were: (i) availability of individual samples per year, (ii) close proximity of sample locations, (iii) equal sex ratio per year (1:1, male: female), and (iv) preferably adult.

3.2 Chemicals

Across the studies (**Papers I, III** and **IV**), a total of 272 CECs and 72 isotopically labelled internal standards (IS) were included in the target analysis component of the work. Target compounds were selected based on environmental relevance and availability. Together, they represented a broad range of physicochemical properties, including pharmaceuticals (n = 107), pesticides (n = 92), flame retardants (FR) (n = 15), PFASs (n = 14), industrial chemicals (n =12), personal care products (n = 8), phthalates (n = 6), food additives (n = 3), isoflavones (n = 3), fatty acids (n = 3), benzotriazoles (n = 2), siloxanes (n = 2), surfactants (n = 2), stimulants (n = 2), and contrast media (n = 1).

3.3 Sample preparation methods

Preparing biological tissue for analysis remains a tedious and time-consuming laboratory task, prone to loss of analytes and to contamination. Several extraction and clean-up techniques are assessed in the literature with regard to single and multiclass target analysis for a great diversity of biota samples (Huerta *et al*., 2015; Neugebauer *et al*., 2018). In most studies, acetonitrile is used as extraction solvent, which reduces the amount of co-extracted lipids. Further lipid removal and removal of waxes, sugars, and other components with low solubility in acetonitrile can be achieved by freezing out the extracts (Payá *et al*., 2007). These components may negatively affect the results of GC and LC analysis.

All biological tissues included in the studies in this thesis (**Papers I, III** and **IV**) were extracted using the extraction method developed in **Paper I** (Figure 1). In brief, 1 g wet-weight tissue in total (pooled samples in **Pa-**

pers III and **IV**) was weighed into a homogenisation tube (15 mL), together with ceramic beads (Precellys, Bertin Technologies, France) without solvent. The material was spiked with 50 ng of each IS and the solvent was left to evaporate at room temperature for 30 minutes. Then 1 mL extraction solvent (acetonitrile + 0.1% formic acid) was added to the tubes and the samples were extracted (2 x 40 s at 5000 rpm) in a Precellys evolution tissue homogeniser. After centrifugation and filtration, aliquots were frozen at -20 °C for at least 16 h to denature the proteins. After another centrifugation for 3 min at -10 °C, aliquots of 250 µL were transferred to autoinjector vials for analysis.

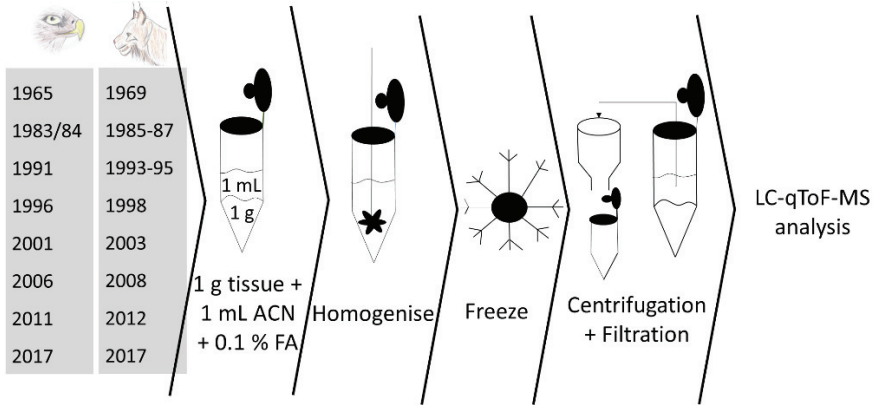

Figure 1. Schematic overview of sample preparation for biological tissues used in **Papers I, III** and **IV** in this thesis.

Artificial time series in **Papers III** and **IV** were prepared from a homogenised pool of each individual WTSE and lynx muscle tissue, respectively. The homogenised tissue was split between triplicate homogenisation tubes (15 mL each) and spiked with IS mixture (50 ng) and target compounds at five (WSTE) and six (lynx) levels of gradient concentrations of the selected CECs. Extraction was performed in the same way as described above. This artificial time series was used for development (**Paper III**) and validation (**Papers III** and **IV**) of the NTS workflow.

In **Paper I**, the performance of three extraction and clean-up methods was evaluated. These were: Quick, easy, cheap, effective, robust, and safe (QuEChERS) with solid-phase extraction (SPE), ultrasonication with SPE, and extraction without SPE clean-up. For QuEChERS analysis, 3 mL aliquots were extracted with 900 mg $MgSO_4$ and 300 mg Z-sep$^+$ QuEChERS salts. For ultrasonication extraction, the samples were ultrasonicated for 30

min, aliquots were transferred to new vials and ultrasonication was repeated two times more. The extracts were then cleaned by SPE with Oasis PRiME HLB cartridges. For optimization, the extraction procedure without SPE clean-up was tested, using acetonitrile with 0.1% formic acid, H_2O/acetonitrile 50/50 with 0.1% formic acid and isopropanol/acetonitrile 50/50 with 0.1% formic acid **(Paper I**).

3.4 Instrumental analysis

All prepared extracts in **Papers I, III** and **IV** were analysed on the same instrument with the same analytical method, as reported in the individual papers. In brief, analytes were separated using a Waters Acquity I-Class ultra-performance liquid chromatograph (UPLC) system equipped with a quaternary pump. For chromatographic separation in positive ionisation mode, a reversed-phase HSS T3 C_{18}-column was used, while for negative ionisation mode a BEH C_{18}-column was used. Mobile phases used in positive ionisation mode were Milli-Q + 0.01% formic acid + 5 mM ammonium formate for phase A and acetonitrile + 0.01% formic acid for phase B. In negative ionisation mode, Milli-Q + 0.01% ammonium hydroxide + 5 mM ammonium acetate was used for phase A and acetonitrile + 0.01% ammonium hydroxide for phase B. A linear gradient was used with a flow rate of 0.5 mL min^{-1} and a total run time of 21 min in both ionisation modes. The injection volume was 5 µL.

The UPLC system was coupled to a Xevo G2-S qToF-MS (Waters Corporation, Manchester, UK) with an electrospray ionisation (ESI) interface working in positive and negative ionisation modes. All data were collected in separate injections for positive and negative ionisation mode using data-independent resolution mode (MS^E-resolution) with low collision energy at 4 eV and a high collision energy ramp from 10-45 eV at a mass range of 100-1200 *m/z*. Leucine enkephalin was continuously infused for lock mass correction. The software UNIFI Waters Scientific Information System (v 1.9.4) was used for instrument control and for identification of compounds during the target analysis step, by searching for $[M+H]^+$ and $[M-H]^-$ adducts with one absolute charge for adduct combinations and 3 mDa mass tolerance.

3.5 Databases

For **Paper II**, three databases (recent U.S. EPA database with chemicals posing a potential risk in human exposure, the Swedish medical products list, the Norman Network list of emerging substances) containing a broad variety of organic compounds were merged into a finale database (~32 000 compounds). The compounds in the final database were characterized using a total of 15 parameters, including physicochemical properties (n = 4), environmental fate characteristics (n = 2), endocrine disruption potential (n = 3), exposure indices (n = 5) and quantity index (n = 1), obtained via a variety of software (*i.e.,* EPI suite, Chemaxon, OCHem and SPIN Toolbox).

3.6 Data handling and statistics

The prioritisation tool (SusTool) developed in **Paper II** was completely developed in Microsoft Excel. Parameter values from the physiochemical properties used were converted into scores ranging from 0 to 1, with a high score representing a high rank in the suspect list and *vice versa*. Before summing up the scores, an adjustable weighting factor in accordance with specific aims of the future application was developed.

Appropriate pre-processing workflows must be applied to obtain high-quality data in NTS (Pochodylo *et al*., 2017; Hohrenk *et al*., 2019). The workflow applied to the data collected after sample extraction and analysis for **Papers III** and **IV** is summarised in Figure 2. Raw data recorded using the vendor software UNIFI (v 1.9.4) were converted to a standardised format (*mzML*) using ProteoWizard (version 4.7.2), and then the data were processed using an automated workflow described elsewhere (Alygizakis *et al*., 2019b). Due to a gradual decrease in sensitivity over the entire instrument run, intensities of all detected features were corrected using the average sensitivity loss of all IS. Subsequently, features were only considered if: i) their intensity was at least 10-fold higher than that of the solvent blank injections (when present in the blank), ii) they were present in at least two of the three replicates, and iii) they had relative standard deviation (RSD) of less than 50% across the triplicates. Finally, time trend analysis was performed for prioritisation of increasing features, based on Spearman rank and Mann-Kendall tests on the average response of each year, using R (v 4.0) software. Features with an increasing intensity trend at significance level $\alpha = 0.05$ and Spearman correlation coefficient (ρ) >0.8 were prioritized. Unequivocal molecular formula for prioritised features was predicted using Waters Corporation software UNIFI (version 1.9.4). Further elucida-

tion was performed in MetFrag using the unequivocal predicted molecular formula for candidate collection. PubChem was used as a search database, candidates were retrieved with a mass error of 3 mDa, and $[M+H]^+$, and $[M+H]^-$ adducts were searched for features prioritised in positive and negative ionisation mode, respectively. The candidates were then scored in MetFrag using the patent and reference counts in PubChem and the *in-silico* fragment score, which was based on the experimental high-collision energy spectra. The final identification status was assigned based on all available information. For confirmation, a reference standard will be analysed in the matrix to achieve the highest identification status.

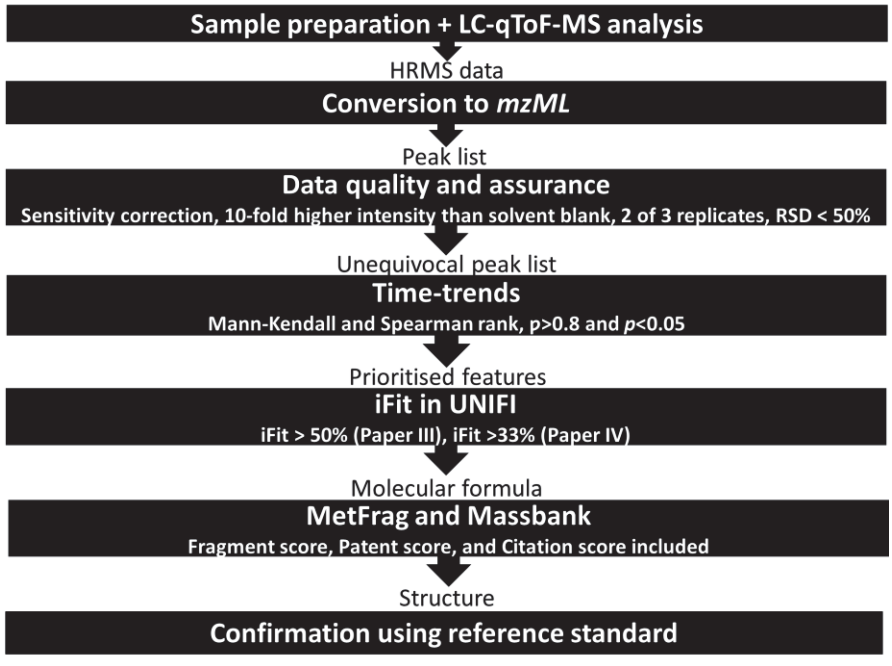

Figure 2. Data handling and non-target screening workflow for tentative identification of contaminants of emerging concern (CECs) in time-trends of archived samples.

3.7 Quality assurance and quality control (QA/QC)

For monitoring contamination during laboratory work and the possibility of cross-over contamination, regent blanks consisting of acetonitrile were analysed. All samples were extracted in triplicate, to check the reproducibility of the extraction method and the data obtained. Data from the triplicates were also used to select unequivocal features in **Papers III** and **IV**.

A quality control sample was prepared by pooling 50 µL extract from each (pooled) year in **Papers III** and **IV**. This quality control sample was injected multiple times throughout the chromatographic sequence to monitor the performance of the UPLC-qToF-HRMS system.

Method detection limit (MDL) and quantification limit (MQL) (**Paper I**) were calculated based on the standard deviation (SD) from the lowest detectable point in a matrix-matched calibration curve as follows:

$$MDL = 3 * SD\ (C_{\text{Lowest in matrix-matched calibration}} - C_{\text{Biota blank}}) \quad (1)$$

$$MQL = 10 * SD\ (C_{\text{Lowest in matrix-matched calibration}} - C_{\text{Biota blank}}) \quad (2)$$

For calculation of matrix effect (**Papers I, III** and **IV**), matrix-matched standards were prepared by adding the target compounds after extraction (equation 3).

$$\text{Matrix effect (\%)} = \left(\left(\frac{Area_{Post-spiked} - Biota\ blank}{Area_{Solvent-spiked}}\right) - 1\right) * 100 \quad (3)$$

For absolute recovery calculations (**Papers I, III** and **IV**), the matrix-matched standard was compared with a sample fortified with the target compounds before extraction according to equation 4.

$$\text{Absolute recovery (\%)} = \frac{Area_{Post-spiked}}{Area_{Pre-spiked}} * 100 \quad (4)$$

For analysis of target compounds, five- to six-point solvent calibration curves at concentrations of 0.5, 5, 10, 25, 50 and 75 ng mL^{-1} were run in the beginning of the sequence and after every 6-9 injections of matrix-rich sample.

4 Results and Discussion

The work in this thesis encompassed extraction method development, suspect list creation, non-target workflow development and application of the tools developed to time series muscle tissue samples of two top predators (Figure 3). The main findings in **Papers I-IV** are summarized and discussed in the following sections.

Figure 3. Conceptual diagram showing combined use of the methods developed in **Papers I-IV** in this thesis.

4.1 Extraction method (Papers I, III and IV)

Extraction of chemicals from environmental samples will always discriminate between chemicals present in the samples. Depending on the extraction solvent, clean-up method and analytical method chosen, certain compounds will be extracted or retained with the chosen chromatography, while

others will not. Extracting biological tissue for a broad range of chemicals remains a challenge due to co-extracted lipids, sugars, proteins, and other compounds that might interfere with the analytical method.

In **Paper I**, a generic sample extraction method for a broad range of CECs and subsequent detection via UPLC-qToF-HRMS in biota was developed. The performance of three extraction methods (QuEChERS + SPE, ultrasonication + SPE, solvent extraction without SPE) and different extraction solvents (acetonitrile + 0.1% formic acid, acetonitrile + isopropanol + 0.1% formic acid, acetonitrile + H_2O + 0.1% formic acid) was compared and evaluated based on absolute recovery and matrix effect on a broad range of target compounds. White-tailed sea eagle (WTSE) tissue was used as the sample matrix in **Paper I**.

A reduced matrix effect was observed for the extraction methods that included a clean-up step (*i.e.*, QuEChERS + SPE, ultrasonication + SPE), which agrees with findings in previous studies (*e.g.*, Baduel *et al.*, 2015). On the other hand, the average absolute recovery for those methods (*i.e.*, QuEChERS + SPE, ultrasonication + SPE) was lower than for the method without clean-up (*i.e.*, solvent extraction without SPE). For NTS, it is generally preferable to have higher recovery over a broad range of compounds rather than low matrix effects, since false negative results are more likely to be avoided by not losing possible important compounds. False positive results can be excluded during data treatment by *e.g.,* blank subtraction, reference/contaminated comparison, or any other prioritisation strategy (Bader *et al.*, 2016). Matrix effect suppression was observed for WTSE and lynx samples with solvent extraction without SPE (Figure 4). Higher matrix effect enhancement was seen in **Paper I** compared with **Paper III** (WTSE muscle tissue in both studies). This might have resulted from analysing one individual bird which had a high matrix load (**Paper I)** compared with a pool of multiple birds (**Paper III**) or from different instrument conditions (*e.g.*, dirty curtain plate). Absolute recovery for the method was satisfactory across the different species (Figure 4).

As extraction solvent, acetonitrile + 0.1 % formic acid was chosen over acetonitrile + H_2O + 0.1% formic acid and acetonitrile + isopropanol + 0.1% formic acid (**Paper I**). Choosing acidified acetonitrile as the extraction solvent has two advantages: i) co-extraction of lipids is reduced and ii) compounds containing basic groups form a soluble salt and become more water soluble. Formic acid is a good choice for adjusting the pH because it is volatile and compatible with LC-MS applications. Freezing out the ex-

tract further removes lipids, as well as waxes, sugars, and other compounds with low solubility in acetonitrile (Payá *et al*., 2007).

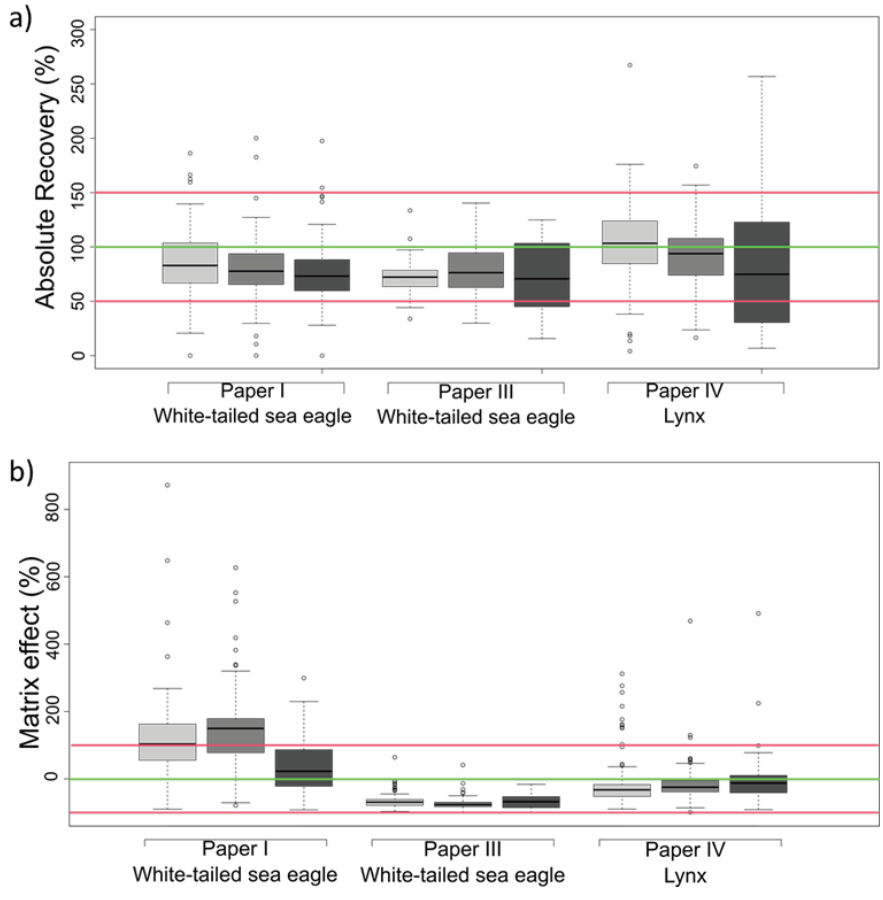

Figure 4. Boxplots comparing findings in **Paper I** (white-tailed sea eagle), **Paper III** (white-tailed sea eagle) and **Paper IV** (lynx) in terms of **a)** absolute recovery and **b)** matrix effect for pesticides (light grey; n = 89, 89, 94, resp.), pharmaceuticals (dark grey; n = 74, 74, 101) and other compounds (black; n = 54, 54, 60).

4.2 SusTool (Paper II)

Prioritizing relevant chemicals in a certain matrix is a key requirement for suspect and NTS approaches. Without prioritisation, the acquired mass spectral dataset is too large for elucidation and chemicals of less interest (*e.g*., proteins and lipids) will be included.

The prioritisation tool developed in **Paper II** for creating relevant suspect lists, called SusTool, employs a systematic approach by scoring chemicals based on their physicochemical properties relevant for the selected matrix. A database of relevant chemicals was created in **Paper II** by merging three databases of CECs. These were i) a recent U.S. EPA database consisting of chemicals posing a potential risk in human exposure (32 464 compounds) (Mansouri *et al*., 2016), ii) the Swedish medical products list FASS (Farmaceutiska specialiteter Sverige) covering 900 pharmaceuticals used in Sweden (FASS, 2017), and iii) the Norman Network list of emerging substances (920 compounds), which contains CECs previously detected in the environment (Norman Network, 2017). Removing duplicates (based on CAS number), compounds without CAS numbers and compounds with metal counter-ions resulted in a database with 31 832 entries, spanning a wide range of compound classes and properties. All compounds were characterized using 15 different parameters calculated based on their canonical simplified molecular-input line-entry system (SMILES) notations, which provides information on molecular structure.

Two suspect lists (SL) were created for biota, to capture compounds with a wide range of hydrophobicity (analysable with LC-HRMS and GC-HRMS). The two lists were differentiated based on log K_{ow}, one comprising hydrophobic compounds ($SL_{Biota\ Kow>3}$) tailored for GC-HRMS analysis and the other with less hydrophobic compounds ($SL_{Biota\ Kow<5}$) generally more suitable for LC-HRMS analysis. Physicochemical properties considered to be of relevance for creating suspect lists in biota included the octanol-water distribution coefficient (D) calculated assuming pH 7, the partitioning coefficient for organic carbon and water (K_{oc}), and the aqueous solubility (S_w). The octanol-air partitioning coefficient (K_{oa}) was included only in the creation of $SL_{Biota\ Kow>3}$ to include chemicals undergoing long-range atmospheric transport as aerosol-sorbed chemicals (Muir *et al*., 2006). Ultimate biodegradation of organic compounds in the presence of mixed populations of environmental organisms and bioconcentration factor (BCF) were also deemed to be relevant environmental fate characteristics for biota. Estimates of quantity and potential exposure to specific environmental compartments/recipients were included using indices provided by the SPIN database compiled by the Nordic Council of Ministers Chemical Group (SPIN Database, 2017). Compound-specific index values ranging from 0 to 5 for chemical quantity (QI) and emissions (EI) for three different compartments (EI_{Air}, EI_{Water}, EI_{Soil}), for air, surface water and soil, respectively, were selected for SL_{biota}. However, due to lack of data in the SPIN data-

base, only 17% and 15% of the compounds in the database were assigned an EI and QI value, respectively. Missing index values were replaced with average values for the same category, to avoid over- or underestimation in the scoring of compounds with missing data.

For systematic scoring of the chemicals in the database, SusTool first introduces a cut-off value in order to mitigate outliers with unrealistic values generated during properties predictions in EPI Suite (EIP SUITE 4.1, 2000-2012). All parameters are then converted into relative values ranging from 0 to 1, with 1 representing a high rank for that parameter (Table 2). Minimum and maximum parameter score limits (PLLS and PLMS, respectively) and vertex points (VP) were set for this purpose. Linear scoring is applied for parameters using PLLS and PLMS, whereas a bell curved-shaped model is applied for parameters using vertex point scoring. These scoring parameters can easily be modified depending on the sample matrix for which the final created suspect list is intended. The values which gave the highest score in testing were based on the persistent, mobile, and toxic (PMT) principle of chemicals proposed by Kalberlah *et al*. (2014) and Schulze *et al.* (2018). Adjustable weighting factors can be applied to each individual parameter with regard to the importance for the created suspect list, before summing up the scores to a final score. The final score for $SL_{Biota\ Kow>3}$ and $SL_{Biota\ Kow<5}$ was calculated as:

$$Final\ Score = P_1 * W_1 + P_2 * W_2 + [\dots] + \left(\frac{E_1 * WEI_1 + E_1 * WEI_2 +}{Sum\ WEI_x}\right) * QI * WQI \quad (5)$$

where P_x is the score (0-1) of each individual parameter included and W_x is the weight assigned to that parameter, EI_x is the score of each emission index included and WEI_x is the weight assigned to that index, QI is the score of the quantity index and WQI is the weight assigned to the quantity index.

Table 2. *Equations used for linear and vertex point scoring and weighting factors for parameters included in the suspect lists $SL_{Biota\ Kow<5}$ and $SL_{Biota\ Kow>3}$ developed using SusTool* (modified from **Paper II**)

Parameter	Equations	Weighting factor $SL_{Biota\ Kow<5}$	Weighting factor $SL_{Biota\ Kow>3}$
Linear scoring			
BCF	$\frac{P}{PLMS}$	5	5
EI_{Water}		3	2
EI_{Air}		0	3
EI_{Soil}		2	1
QI		5	5
Biodegradation		4	4
Vertex scoring			
Log *D*	$\frac{1}{1+\lvert(P-VP)\rvert}$	2	3
Log K_{oc}		2	2
Log S_w		4	1
Log K_{oa}		0	4

SusTool is the first tool to include weighting of physicochemical properties for suspect list creation. With the weighting factors applied, the 500 top-ranked compounds assigned to a suspect list developed for water were not included in either $SL_{Biota\ Kow>3}$ or $SL_{Biota\ Kow<5}$ (**Paper II**). Only a small overlap of 15% was observed for $SL_{Biota\ Kow>3}$ and $SL_{Biota\ Kow<5}$, implying a well-defined suspect list for screening in biota with GC and LC, respectively. Some overlap was expected due to similar scoring parameters and weighting factors applied for the two suspect lists.

Previously developed prioritisation approaches commonly use hard cut-off lines base on guideline values for *e.g.,* persistence (*e.g.* Arp *et al*., 2017), whereas in the approach developed in **Paper II** compounds are scored gradually. Compounds are not directly discarded if a parameter scores low and high scores for other parameters can compensate, so the compound may still end up as relevant for the suspect list. Other prioritisation strategies for suspect lists involve extensive literature searches (*e.g*., Richardson *et al*., 2017) or focus on emerging compounds that have been detected or are expected to be detected in the near future based on expert judgment (Sobek *et al*., 2016; Singer *et al*., 2016; Avaguyan *et al*., 2017).

While these approaches are very useful, the approach developed in **paper II** is complementary, as it instead uses systematic selection based on a large variety of parameters. Recently, SusTool was applied successfully in a world-wide study screening of raw and drinking water from Europe and Asia and identified problematic CECs for drinking water treatment plants (Tröger *et al*., 2020). In future work, this tool should be applied to biota and other matrices in order to identify relevant CECs in those matrices.

4.3 Non-target screening workflow and time trend analysis (Papers III & IV)

SusTool applies a gradual scoring system for systematic selection based on a large variety of parameters, to capture as many as suspects as possible. However, hard cut-off values are needed for development of NTS workflows for prioritisation of the mass spectral data acquired, to reduce the data drastically and yield a manageable number for elucidation.

As previously demonstrated in other studies (Pochodylo *et al*., 2017; Hohrenk *et al*., 2019), an appropriate pre-processing workflow must be applied to obtain high-quality data. A NTS workflow based on an artificial time series (ATS) spiked with gradient concentrations of CECs with a broad range of physicochemical properties was developed in **Paper III** and further validated in **Paper IV**. In the ATS, a total of 36 783 and 24 013 features were detected for the WTSE and lynx samples, respectively (Figure 5). After blank subtraction, 19 272 and 11 587 features remained in WTSE and lynx ATS, respectively. The smaller number of features obtained in the lynx series agreed with expectations of lower contamination levels for terrestrial species compared with marine species. A noteworthy data reduction was achieved by considering features detected in at least two of three replicates and with RSD <50% (7 205 and 5 994 features remained in WTSE and lynx ATS, respectively). This reduction is similar to that obtained in previous studies applying strict selection criteria on data acquired from matrix-rich extracts (Hohrenk *et al*., 2019; Purschke *et al*., 2020). After prioritisation using Spearman rank and Mann-Kendall test, similar numbers of features were prioritised for the WTSE and lynx ATS (126 and 128 features, respectively) for both statistical tests, indicating that the spiked compounds were dominant (higher signal intensity) and therefore picked up by both statistical approaches and in both matrixes.

All spiked target compounds were treated as non-target features and retrospective checking was performed to determine which features belonged to the spiked target compounds. In contrast to the WTSE ATS (65 targets), only 28 targets were prioritised in the lynx ATS. Greater matrix interferences in the lynx ATS compared with WTSE ATS could be a possible explanation for this. An unequivocal molecular formula with iFit value >50% using Waters Corporation software UNIFI (version 1.9.4) was predicted for 62 features (40 targets) for the WTSE ATS and 38 features (15 targets) for the lynx ATS. The great loss of target features in the lynx series during this step was possibly due to low sensitivity of the target compounds or high influence of the matrix on the mass spectra, which resulted in molecular formula prediction with low credibility. The loss was improved by considering a softer cut-off with iFit value >33%, resulting in 52 features (19 targets) for the lynx ATS. Further elucidation with MetFrag and EU Mass bank allowed tentative identification of 37 and 40 structures (26 and 14 targets) for WTSE and lynx, respectively. The loss of only a few target and non-target features in the lynx ATS during this step compared with previous steps indicates that, after molecular formula assignment, relatively clean mass spectra remained, which could in turn be used for positive fragment matching in MetFrag. Combining the prioritisation and elucidation workflows demonstrated that 26 of 233 (11%) theoretically possible detectable target compounds in the WTSE ATS, and 14 of 182 (8%) theoretically possible detectable target compounds in lynx ATS, could be tentatively identified when treated as non-targets. The low detection rate of target compounds can be explained by matrix effects and low sensitivity of the target compounds. However, the number of identified targets was shown to be acceptable for a NTS identification workflow in biota (**Paper III**).

Development of a NTS workflow by means of an ATS spiked with known CECs is beneficial as the workflow can be developed with matrix samples, which is especially advantageous for matrix-rich samples. The developed NTS workflow reduced the number of features for both matrices (WTSE, lynx) drastically and provided a manageable number of curated features to focus upon during structural elucidation. However, the conservative approach applied led to losses of target compounds using the workflow, indicating underestimation during application of the workflow on real time-series samples.

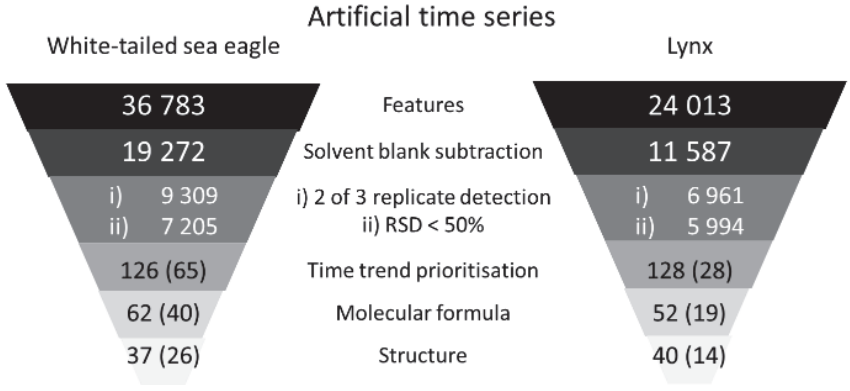

Figure 5. Number of features remaining in the white-tailed sea eagle (**Paper III**) and lynx (**Paper IV**) artificial time series during each step of the non-target data treatment workflow. Values in brackets are number of spiked target compounds identified if treated as non-target compounds in the highest concentration level (50 and 75 ng mL^{-1} in white-tailed sea eagle and lynx, respectively). Time trend prioritisation by Mann-Kendall $p<0.05$ and Spearman rank correlation coefficient $\rho>0.8$.

The validated data treatment workflow was applied to a real WTSE muscle tissue time series (1965-2017) and lynx muscle tissue time series (1969-2017) (**Papers III** and **Paper IV)**. After blank subtraction, 26 597 and 12 941 features were obtained for WTSE and lynx, respectively (Figure 6). This was a higher number of features than in the ATS and was probably due to the separate analysis of pooled samples (*i.e.*, not combining them to one pool as for the artificial time series). A likely explanation for the lower number of features for lynx compared with WTSE is that terrestrial animals have lower contamination load than marine species. A total of 14 409 and 7 241 features were considered in statistical analysis of WTSE and lynx time series, respectively. Finally, a total of 207 and 264 features were prioritised for WTSE and lynx, respectively, using univariate statistical approaches. Interestingly, in the real time series of WTSE the two statistical approaches prioritised different features, whereas in the lynx time series they prioritised the same features. One likely cause for this could be that the compounds in the lynx time series were dominant (high signal intensity) in the matrix and therefore picked up by both statistical approaches.

Visual inspection allowed for removal of more than half of the prioritised features in the WTSE time series. The prioritised features in the lynx time series consisted to a great portion of split or double peaks, which can lead to difficulties in assigning molecular formula, resulting in less tenta-

tively identified features. However, for 51 of 207 prioritised features (25%) in the WTSE time series, it was possible to predict an unequivocal molecular formula with iFit value >50%, while for six of the 104 prioritised features (6%) in the lynx time series an unequivocal molecular formula was predicted with iFit >33%. Comparison of the experimental high-collision energy spectra for those features with MetFrag (Ruttkies *et al*., 2016) and EU MassBank resulted in 14 and two tentatively identified structures for WTSE and lynx, respectively.

A strong matrix effect was observed in **Papers III** and **IV**, partly explaining the low rate of target compounds identified in ATS. **Paper I** highlighted the importance of minimising sample pre-treatment and clean-up for NTS methods to be non-specific and extract a broad range of compounds. However, the drawback, in particular for biota samples, is that this can lead to strong matrix effects, which reduces the sensitivity and increasing the number of background masses in the chromatogram. This in turn complicates identification of compounds using NTS (Baduel *et al*., 2015; Hefferman *et al*., 2017; Plassmann *et al*., 2018). Thus, it is challenging to find a compromise between extensive sample preparation to reduce matrix effects and minimal sample preparation to avoid losing NTS compounds. The results from the ATS and application of the NTS workflow showed that only small fractions of target compounds (11% and 8% for WTSE and lynx, respectively) were prioritised and tentatively identified. There is thus a need for improved sample preparation methods without losing non-target compounds.

The above-mentioned workflow could also be applied to prioritise decreasing intensity time trends as proof of concept to identify compounds with decreasing trends, such as legacy pollutants (Falk *et al*., 2019; Sun *et al*., 2020). Features with a decreasing intensity trend in lynx at significance level $\alpha = 0.05$ and Spearman coefficient $\rho > -0.8$ were prioritised in **Paper IV**. A total of 61 features were prioritised with both statistical approaches, and subsequently elucidated. For 18% of the prioritised features, it was possible to predict an unequivocal molecular formula with iFit value >33%. For structural elucidation, comparison of the experimental high-collision energy spectra of the 11 features with MetFrag (Ruttkies *et al*., 2016) resulted in one tentatively identified structure (octadecanenitrile). Decreasing time trends of identified CECs could indicate positive effects of risk management measures taken by chemical agencies.

Prioritisation of CECs in biological tissue by means of temporal trend analysis based on features intensity is beneficial with regard to relevance, but also brings some disadvantages. This approach gives preference to compounds that are more sensitive with the analytical method applied and might miss features of high relevance (*i.e*., toxic features) with lower sensitivity. Effect-directive analysis in combination with a data-driven prioritisation approach could be a solution for identifying relevant CECs with lower sensitivity (Weiss *et al*., 2011; Simon *et al*., 2013; Brack *et al*., 2019). In addition, data gaps (*i.e*., under limit of detection or intensity cut-off) and contaminants with very low sensitivity and/or intensity can prevent statistical approaches from identifying increasing/decreasing time trends. High concentration and co-elution of endogenous compounds might hamper prioritisation of exogenous compounds present in lower concentrations in the sample. Time trends of novel PFASs recently identified in individual marine species show a steady shallow increase (Wang *et al*., 2021; Barrett *et al*., 2021) and might not be prioritised in pooled matrix-rich samples with the statistical tools applied in this thesis. Ultimately, the prioritisation method and NTS workflow developed in this thesis provide an alternative novel approach to reduce the acquired mass spectral data and enable identification of new CECs in biota.

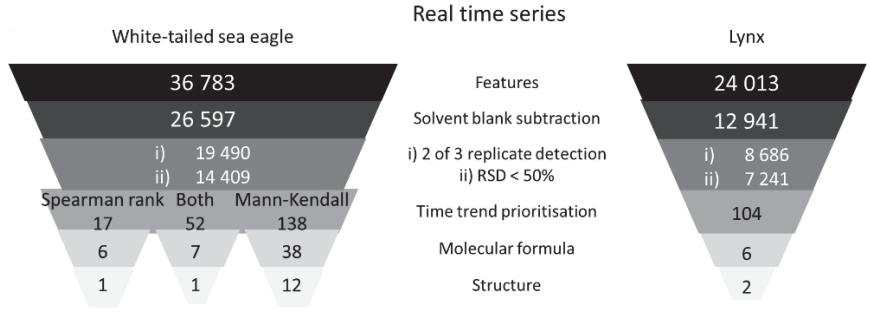

Figure 6. Number of features remaining in the white-tailed sea eagle times series (1965-2017) (**Paper III**) and lynx time series (1969-2017) (**Paper IV**) during each step of the non-target data treatment workflow. Time trend prioritisation via Mann-Kendall $p<0.05$ and Spearman rank correlation coefficient $\rho > 0.8$.

4.4 Tentatively identified compounds in WTSE and lynx (Papers III & IV)

With the NTS workflow developed in this thesis, it was possible to prioritise and tentatively identify 14 structures (six anthropogenic compounds) with statistically significant increasing temporal trends in the WTSE muscle tissue samples and two structures (one anthropogenic compound) with statistically significant increasing temporal trends in the lynx muscle tissue samples. In the WTSE samples, the prioritised anthropogenic compounds belonged to the compound categories pharmaceuticals (*i.e*. R-(+)-tolterodine, (+)-aphidicolin, cholamid), cosmetics (*i.e*. octoxynol-2) and industrial chemicals (*i.e*. 4'-hydroxy-stearanilide, 1-chloro-N1,N1",N2-trimethyl-butane-1,1,2,4-tetramine).These pharmaceuticals, personal care products and industrial chemicals enter the aquatic environment via *e.g*., wastewater treatment plants (Ferrer *et al*., 2012; Grabicova *et al*., 2014). Therefore, aquatic organisms like fish and their predators *(e.g*., WTSE) are exposed to these compounds (Huerta *et al*., 2012). For lynx, the anthropogenic compound S-ethyl dipropylthiocarbamate (EPTC), which is used in plant protection products (US EPA, 2021; MSB, 2021), was tentatively identified. Plant protection products are directly applied to agricultural fields, making predators feeding in the terrestrial environment, such as lynx, potentially more exposed and prone to accumulation (Mateo *et al*., 2012).

Some tentatively identified structures (*viz*. dodecanoyl-L-carnitine, 6,9,12,15-octadecatetraenoic acid, L-phenylalaninamide, retinol, PKI166, L-(-)-tyrosine and PD-128042 for WTSE; pyrrolidine-2-carbaldehyde for lynx) were endogenous compounds such as fatty acids, vitamins, amino acids, or inhibitors previously mentioned in metabolomics papers (Pekala *et al.,* 2011; Johnson, 2017). Identification of anthropogenic compounds by NTS is desired. Filtering out anthropogenic compounds in NTS is challenging, as naturally occurring compounds co-exist with anthropogenic compounds (Hollender *et al*., 2017). In addition, naturally occurring compounds could be released from human products. Plassmann *et al*. (2016) suggest comparing the original feature list against known metabolite databases to exclude endogenous compounds, which could be beneficial when dealing with many prioritised features. On the other hand, selecting such a database for biological tissue from different species is difficult.

Significant time trends in tentatively identified compounds are shown in Figure 7. The WTSE samples showed a greater increase in tentatively iden-

tified compounds than the lynx samples. The difference could possibly be attributable to the low contamination load in terrestrial species (lynx) compared with WTSE feeding in the marine environment, as has been shown in previous studies (de Wit *et al*., 2020). Steadily increasing trends for all tentatively identified features in the WTSE muscle tissue samples, by 160% from 1965 to 1996 and by 500% from 1996 onwards, were observed, except for cholamid, PD-12804 and 6,9,12,15-octadecatetraenoic acid. The signal intensity of the herbicide EPTC, tentatively identified in the lynx samples, increased steadily by 200% from 1969 to 2008. The tentatively identified endogenous compound pyrroline-2-carbaldehyde in the lynx samples increased steadily by 1000% from 1969 to 2017. The standard deviation for pyrroline-2-carbaldehyde intensity in the samples from 2003, 2008 and 2017 indicated that the time trend stagnated around the year 2000. From 1991 to 2011, a rapid increase was observed for 6,9,12,15-octadecatentraenoic acid (an increase of 2100%), PD12804 (+2600%) and cholamid (+1500%). A decreasing trend for octadecanenitrile, a fatty nitrile used in cosmetics and chemical manufacturing, was observed in lynx muscle tissue. Octadecanenitrile was tentatively identified with a 3-fold decreasing trend from 1985 to 1998.

Data gaps (*i.e*., below intensity cut-off) for the tentatively identified features in the WTSE samples occurred mainly before 1990, indicating that fewer environmental stressors were released to the environment some decades ago. No data were observed for EPTC in lynx after 2008. This product was sold between 1972 and 1997 in Sweden but has been banned for application in Sweden since 1999 (KEMI 2021). This example shows that imposing restrictions on compounds can result in a fast response in terms of decreasing concentrations in the environment.

Ultimately, NTS of biological matrices is of high relevance to pinpoint unknown, potentially harmful compounds showing an increasing trend, which can indicate persistence and bioaccumulation potential of these compounds. Despite the disadvantages of high matrix effect and low expected concentrations in terrestrial species like lynx, the prioritisation approach developed in this thesis provided some useful results and proved capable of capturing an anthropogenic compound. Univariate statistics proved to be useful for prioritisation of increasing intensity trends in LC-HRMS data. The strategies developed here can be used as a complement to conventional target screening monitoring (Menger *et al*., 2021). The use of archived biological tissue provides more possibilities for successful prioritisation and identification of CECs in biota using NTS.

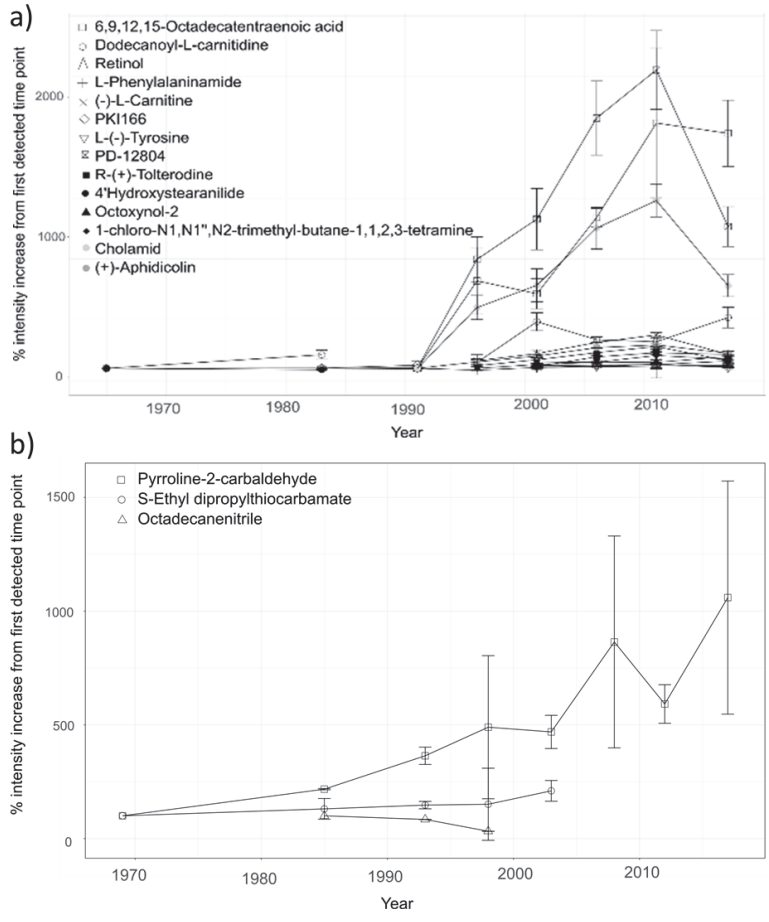

Figure 7. Time trends in tentatively identified compounds displaying a statistically significant increasing trend (Mann-Kendall $p<0.05$, Spearman rank correlation coefficient (ρ) >0.8) from **a)** 1965 to 2017 in white-tailed sea eagle and **b)** 1969 to 2017 in lynx given as average % peak intensity increase/decrease from the first detected time point. Error bars represent standard deviation of triplicate samples (modified from **Papers III** and **IV**).

5 Conclusions and future perspectives

The main conclusions of this thesis with respect to the research questions were as follows:

(i) How can a broad range of CECs in different biota tissues be extracted and subsequently analysed using LC-HRMS with respect to suspect and NTS? (Paper I)

Method comparison showed that the selected polar compounds were best extracted using acetonitrile + 0.1% formic acid and limited clean-up, resulting in reduced co-extracted lipids. Further removal of lipids, waxes, sugars and other components with low solubility in acetonitrile was achieved by freezing out the extracts. However, strong matrix effects remain a challenge for NTS in biota.

(ii) Can CECs from an extensive database be systematically ranked, based on their physicochemical properties, in terms of their likelihood to be detected in biota? (Paper II)

Prioritising relevant and novel CECs is a key requirement for suspect screening and can easily be performed in various matrices using the SusTool approach. Systematic ranking of physicochemical properties made it possible to prioritise chemicals for various matrices and different purposes.

(iii) Can archived biological samples be used as a NTS prioritisation strategy for CECs in biota? (Papers III and IV)

Temporal trend analysis with univariate statistics proved to be suitable in reducing the amount of data produced in HRMS analysis, allowing a focus on increasing time trends. Workflow development on an artificial time series in the desired matrix is highly recommended before application on a valuable time series.

(iv) Which CECs can be detected and tentatively identified in top predators (white-tailed sea eagle and lynx) using time trend analysis as prioritisation tool? (Papers III and IV)

Anthropogenic compounds and naturally occurring compounds can be prioritised and tentatively identified with the tools developed in this thesis. The tentatively identified compounds originated from different chemical categories (pharmaceuticals, personal care products, industrial chemicals, herbicides). Detecting an increasing trend of these compounds could indicate increased usage, an increasing possible threat for the environment and changes occurring in the environment, so changes in intensity trends of these chemicals are relevant to consider.

Monitoring of CECs in archived biota is challenging, but possible. Before the tool developed in this thesis can be implemented in current monitoring programmes, more research is needed with regard to degradation of chemicals during storage, automated screening workflows and more simplified software for elucidation and identification. The following observations can be addressed to specific stakeholders:

Environmental specimen banks: In light of the findings in this thesis, it might be worth considering collection of top predators. Storage of the samples is important for analysis of known and unknown pollutants. Monitoring campaigns for target compounds should not be replaced by the methods described in this thesis but should be accomplished with wide-scope screening techniques. Analysing samples with HRMS makes it possible to digitally archive the acquired data for retrospective analysis (*e.g.*, the digital sample freezing platform of the Norman Network provides a great tool for this). The knowledge acquired over the years with regard to biology and ecology, and on decreasing the variation between and within samples at ESBs, will be beneficial when new CECs have been identified.

Government and regulatory bodies: The results presented in this thesis highlight the possibility to tentatively identify CECs with an increasing trend in biota. This information can be used to guide regulatory environmental monitoring campaigns to pinpoint CECs early for establishing risk management measures, and to assess whether risk management measures are actually reducing the impacts on the environment and wildlife health.

The general public: Think about the products you use, as parts of these products will end up in the environment and can cause harm to humans and wildlife.

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Popular science summary

Did you notice that our environment changes a lot over time? Technologies and chemicals improve our lives but could also cause harm to wildlife. The question is how to identify chemicals that become more abundant in top predators over time.

This thesis presents a method for identifying man-made compounds of increasing concern in white-tailed sea eagle and lynx tissue samples. Different extraction methods were compared with each other for many different chemicals and different animals (*e.g.*, eagle, fish, lynx). A tool (called SusTool) was created to make it easier to rank and select chemicals of concern in different environments (*e.g.*, water, biota). SusTool generates lists based on systematic ranking of chemicals according to their environmental characteristics. These ranking lists can be used to identify relevant contaminants of concern in specific environments.

Increasing time trends in new contamiants in two predators in Sweden, white-tailed sea eagle and lynx were identified. These animals were selected because they may potentially contain higher concentrations of chemicals as they are located high in the food chain. Muscle tissue samples from white-tailed sea eagle and lynx, collected from 1965 to 2017, were obtained from the Swedish Natural History Museum. The instruments and methods used did not always distinguish man-made compounds from naturally occurring compounds in the biological tissues tested. However, it was possible to tentatively identify six man-made compounds (pharmaceutical, cosmetics, industrial chemical) in white-tailed sea eagle samples and one man-made compound (pesticide) in lynx muscle tissue samples. Identification of new contaminants in animals living and feeding on land, such as lynx, is more challenging than for animals feeding in the marine environment, such as white-tailed sea eagle.

Vast amounts of different chemicals are released and will continue to be released into the environment every day. Not all of these chemicals are regulated, so it is critical that researchers continue to develop screening methods for pinpointing new chemicals that should be targeted in actions by government agencies. The methods developed in this thesis can form part of a fruitful approach to prioritise chemicals in top predators. Above all, everyone should be aware of the chemicals they release into the environment, consider alternative products, and dispose of used products and their packaging in a correct manner to avoid contamination. If fewer harmful chemicals are released into nature, we might be able to spot more healthy bears, wolves, lynx, and white-tailed sea eagles in the world around us in the future.

Populärvetenskaplig sammanfattning

Visste du att vår miljö ändrar sig med tiden? Teknik och kemikalier hjälper oss i vardagen men kan också skada oss och vår miljö genom så kallade faroämnen som läcker ut i miljön. Frågan är om det är möjligt att identifiera kemikalier med ökande trender i vilda djur?

I denna avhandling identifierades faroämnen i havsörn och lodjur. Olika metoder för provberedning för upptäckt av oönskade ämnen i biologiska prover (havsörn, aborre, lodjur) utvecklades. Ett verktyg, SusTool, som gör det enklare att ranka och välja relevanta kemikalier för analys av ämnen i miljöprover (t.ex. vatten, biologisk vävnad). SusTool skapar listor av relevanta kemikalier baserade på ämnenas egenskaper och användning. Den rankade listan kan användas för att upptäcka nya faroämnen i olika miljöer.

Ökande tidstrender för nya miljöföroreningar i havsörn och lodjur från Sverige identifierades. Rovdjur innehåller höga halter av faroämnen eftersom många miljöföroreningar ansamlas succesivt i födokedjor. Muskelprover från havsörn och lodjur som samlats in från 1965 till 2017 av naturhistoriska riksmuseet analyserades. De verktyg och metoder som används kan inte alltid åtskilja konstgjorda ämnen från naturligt förekommande ämnen i biologisk vävnad. Dock var det möjligt att preliminärt identifiera sex syntetiska ämnen (läkemedel, kosmetika, industriella kemikalier) i muskelproverna från havsörn och ett växtskyddsmedel med ökande trend i lodjur. Att upptäcka nya miljöföroreningar i rovdjur som lever på land är mer utmanande jämfört med att finna nya ämnen i rovdjur som lever på vattenlevande organismer. Detta beror bland annat på att akvatiska födovävar är långa och ansamlar högre halter av miljöföreningar.

Kemikalieanvändningen ökar i samhället och oönskade syntetiska ämnen läcker kontinuerligt ut till miljön. De flesta av kemikalierna är inte reglerade, så det är viktigt att forskningen fortsätter att utveckla metoder för att hitta nya, potentiellt hälsofarliga kemikalier. Studierna och metoderna som utvecklats i denna avhandling är bevis på fungerade prioriteringsverktyg för upptäckt av nya, oönskade miljöföreningar i rovdjur. Sammanfattningsvis bör alla vara medvetna om kemikalier som släpps ut i miljön, överväga alternativa produkter och hantera förbrukade produkter på rätt sätt för att undvika onödig miljöbelastning. På så sätt släpps mindre skadliga kemikalier ut i naturen och vi kan kanske behålla och upptäcka fler och friska björnar, vargar, lodjur och havsörnar i framtiden.

Zusammenfassung für Kinder

Dieses lange Buch (meine Doktorarbeit) beschreibt, was Mama all die Jahre gemacht hat, während ihr im Kindergarten ward. Jetzt will ich euch gerne eine kleine Geschichte dazu erzählen, um Euch zu erklären, was in dem Buch für Erwachsene steht. Alles um uns herum besteht aus Elementen, diese Elemente kann man sich so vorstellen wie ganz kleine Bällchen, die man aber nicht sehen kann. Mehrere dieser kleinen Teile (Elemente) zusammen ergeben eine Sache, wie zum Beispiel euer Spielzeug, Medizin oder Shampoo. Wenn wir diese Dinge benutzen, werden manche Elemente (also ein Teil dieser kleinen Bällchen) frei und können in die Natur gelangen. Leider sind nicht alle dieser Elemente gut für die Natur. Manche können sogar gefährlich sein und sowohl Menschen als auch Tiere krank machen. Darum ist es sehr wichtig, dass wir so viel wie möglich von den Sachen, die wir benutzen, wiederaufarbeiten oder in den richtigen Müll geben, wie z.B. zum Verpackungsmüll, zum Papier- oder Restmüll.

In meiner Arbeit möchte ich herausfinden, welche von diesen Elementen wir in der Natur und in Tieren wiederfinden. Natürlich habe ich dafür keine Tiere getötet. Deswegen habe ich mich an das Naturhistorische Museum in Stockholm gewandt. Die sammeln nämlich für das Museum Tiere, die tot in Schweden gefunden werden und untersuchen diese Tiere auf bereits bekannte Elemente. Das Museum hat schon angefangen Tiere zu sammeln als Oma und Opa noch Kinder waren. Kleine Mengen dieser Tiere haben sie in einem großen Gefrierschrank aufgehoben. Mein Ziel war es, Elemente zu finden, die in den Tieren die gerade erst gestorben waren, viel öfter vorkommen als in den Tieren, die schon vor längerer Zeit gestorben sind. Zudem sollten die Tiere, die ich untersuche, Raubtiere sein. Denn Raubtiere haben relativ viele Elemente in sich, da sie andere Tiere fressen, die auch schon diese Elemente in sich haben. Das Museum hatte

viele Seeadler und Luchse die lange in die Vergangenheit reichen in ihrem Gefrierschrank, welche ich zum Glück für meine Arbeit verwenden durfte.

Dieses Buch besteht aus 4 Kapiteln, welche erklären was ich heraus gefunden habe. Das erste Kapitel beschreibt eine Vorgehensweise, die ich entwickelt habe, um so viel Elemente wie möglich aus dem Fleisch des Seeadlers heraus zu bekommen, die eigentlich gar nicht in den Seeadler gehören. Das zweite Kapitel zeigt, wie man eine Liste von Elementen zusammenstellen kann, die wir sehr wahrscheinlich in Raubtieren finden können. Nach den Elementen auf der Liste kann man dann in den Raubtieren suchen. Im dritten Kapitel habe ich dann in den Seeadlern von früher und auch in denen von heute, nach neuen Elementen gesucht, die über so viele Jahre immer häufiger vorkommen und die Tiere eventuell krank machen können. Dort habe ich 6 Elemente gefunden, die nicht in den Seeadler gehören und die wegen uns Menschen in dem Tier vorkommen. Drei davon waren Medikamente, eines kam aus der Kosmetik und zwei weitere werden in der Industrie verwendet. Ob der Seeadler von diesen Elementen krank wird, weiß ich leider nicht, aber dies kann man mit anderen Untersuchungen herausfinden. Im letzten Kapitel dieses Buches, habe ich dann noch nach neuen Elementen in Luchsen gesucht. Hier war es schwieriger etwas zu finden, da es im Vergleich zum Seeadler weniger von den Elementen im Luchs gibt. Für das Instrument (Werkzeug), welches ich benutze um die Elemente zu finden, ist es schwierig die Elemente die zum Luchs gehören und die, die nicht zum Luchs gehören auseinander zuhalten. Manche Elemente sehen sehr ähnlich aus. Trotzdem habe ich ein Element gefunden das wir dafür benutzen dass unsere Pflanzen besser wachsen, aber das gehört doch nicht in den Luchs.

Die Moral von dieser kleinen Geschichte ist, dass jeder gut überlegen muss welche Produkte er verwendet und wie er diese entsorgt. So kommen hoffentlich keine schädlichen Elemente in die Natur und wir können vielleicht mehr gesunde Raubtiere wie Bären, Wölfe, Luchse und Seeadler sehen.

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Acta Universitatis Agriculturae Sueciae

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Little is known about the occurrence and fate of contaminants of emerging concern (CECs) in wildlife. In this thesis, a multi-residue sample extraction method and high-resolution mass spectrometry method for various biota tissue types was developed. It was applied, in combination with temporal trend analysis, as a prioritisation tool in non-target screening (NTS) for identification of CECs in time series (1965-2017) muscle tissue samples from top predators (white-tailed sea eagle and lynx). The findings in this thesis can help government agencies expand their monitoring programs for identification of CECs in biota.

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