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Ecological and spatial variations of legacy and emerging contaminants in white-tailed sea eagles from Germany: Implications for prioritisation and future risk management

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ABSTRACT

The increasing use of chemicals in the European Union (EU) has resulted in environmental emissions and wildlife exposures. For approving a chemical within the EU, producers need to conduct an environmental risk assessment, which typically relies on data generated under laboratory conditions without considering the ecological and landscape context. To address this gap and add information on emerging contaminants and chemical mixtures, we analysed 30 livers of white-tailed sea eagles (*Haliaeetus albicilla*) from northern Germany with high resolution-mass spectrometry coupled to liquid and gas chromatography for the identification of >2400 contaminants. We then modelled the influence of trophic position ($\delta^{15}N$), habitat ($\delta^{13}C$) and landscape on chemical residues and screened for persistent, bioaccumulative and toxic (PBT) properties using an *in silico* model to unravel mismatches between predicted PBT properties and observed exposures. Despite having generally low PBT scores, most detected contaminants were medicinal products with oxfendazole and salicylamide being most frequent. Chemicals of the Stockholm Convention such as *4,4′*-DDE and PCBs were present in all samples below toxicity thresholds. Among PFAS, especially PFOS showed elevated concentrations compared to other studies. In contrast, PFCA levels were low and increased with $\delta^{15}N$, which indicated an increase with preying on piscivorous species. Among plant protection products, spiroxamine and simazine were frequently detected with increasing concentrations in agricultural landscapes. The *in silico* model has proven to be reliable for predicting PBT properties for most chemicals. However, chemical exposures in apex predators are complex and do not solely rely on intrinsic chemical properties but also on other factors such as ecology and landscape. We therefore recommend that ecological contexts, mixture toxicities, and chemical monitoring data should be more frequently considered in regulatory risk assessments, e.g. in a weight of evidence approach, to trigger risk management measures before adverse effects in individuals or populations start to manifest.

1. Introduction

The use of an increasing number of chemicals over the last century has resulted in environmental emissions and wildlife exposures (Chiaia-Hernández et al., 2020; González-Rubio et al., 2020a). The global chemicals production (excluding pharmaceuticals) are expected to double by 2030, with the European Union (EU) being the second biggest producer accounting for ~17% of the global sales (EC, 2020). Among the currently produced chemicals, >70% are classified as hazardous to human health and ~30% as hazardous to the environment (EUROSTAT, 2020). In the EU, producers and importers are responsible for conducting health and environmental risk assessments and compile the information in dossiers with guidance from different regulatory frameworks, depending on their intended use. These comprise e.g. industrial

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chemicals (REACH - Registration, Evaluation, Authorisation and Restriction of Chemicals, EC 1907/2006), plant protection products (EC 1107/2009), and medicinal products (veterinary medicinal products (VMP): Regulation (EU) 2019/6; human medicinal products (HMP): Directive 2001/83/EC). A first step for registering or approving a chemical requires a hazard identification, including the assessment of persistence, bioaccumulation, and toxicity (PBT properties). A second step requires a risk assessment, where the predicted environmental concentration (PEC) is supposed to remain below the so-called predicted no-effect concentration (PNEC). However, (eco)toxicological data and information on exposure scenarios of the registered substances are often missing (EEA, 2019). This is emphasized in a study that demonstrated that 58% of the registration dossiers for REACH chemicals with tonnages above 1000 tons (t) per year were non-compliant (Springer et al., 2015). Both hazard and risk assessments are usually based on data generated under laboratory conditions, typically lacking information on the ecological, landscape and management context (Schäfer et al., 2019). This has resulted in inaccurate predictions of environmental exposures (Knäbel et al., 2014; Knäbel et al., 2012). As a consequence, the term chemicals of emerging concern (CECs) has been established for a wide range of contaminants and transformation products (TPs) that entered the environment recently (Dulio and Slobodnik, 2009).

Besides regulations on a nationwide or continental scale such as REACH, treaties like the Stockholm Convention have been established on a worldwide scale to restrict the use of persistent organic pollutants (POPs). POPs are classified as PBT or very persistent and very bioaccumulative (vPvB) substances that result in long-lasting environmental exposures, have the potential for long-range transport as well as for toxic effects in biota (de Wit et al., 2020; Sonne et al., 2020). Whereas many POPs are biomagnified in food webs even after mitigation measures were established (e.g. de Wit et al., 2020), much less information is available on the behaviour of CECs and complex chemical mixtures, as established analytical procedures mainly focussed on the target analysis of a limited number of contaminants (i.e. < 100) without considering metabolites and TPs. This represents a critical knowledge gap as identifying chemical mixtures is essential for conducting hazard and risk assessments. Recent developments in analytical techniques resulted in wide-scope target screening techniques based on high resolution-mass spectrometry (HR-MS) coupled to both liquid (LC) and gas chromatography (GC) that allow for the simultaneous quantification of a large set of chemicals (i.e. >2400) within each sample (Alygizakis et al., 2020; Gago-Ferrero et al., 2020).

Most studies on CECs focussed on abiotic matrices such as water or sediment (Chiaia-Hernández et al., 2020; Diamanti et al., 2020), whereas information on the occurrence of CECs in biota, in particular apex predators is scarce (González-Rubio et al., 2020a). An important means for wildlife biomonitoring is the selection of sentinel species for which suitability is expected to depend on ecological traits such as migratory behaviour, diet and habitat preference (Badry et al., 2020). This becomes especially important when a species forages on both the aquatic and the terrestrial food web or feeds on different trophic levels. An efficient way to control for dietary plasticity is the use of stable isotopes of nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) (Elliott et al., 2009; Eulaers et al., 2013). Nitrogen isotopes are commonly used as proxies for estimating the trophic position of animals since consumers get enriched with ^{15}N in relation to ^{14}N by ~2 to 3.4 ‰ compared to their prey (Vanderklift and Ponsard, 2003), whereas the stable isotope of carbon can be used to distinguish different carbon sources of terrestrial and aquatic environments (Kelly, 2000).

In the present study, we focussed on the white-tailed sea eagle (*Haliaeetus albicilla*; hereafter HAAL), a mixed food web feeder that forages mostly on fish but also on water birds, carrion and game species depending on season and availability (Nadjafzadeh et al., 2016). In northern Germany, HAALs inhabit mainly inland and coastal habitats of the Baltic Sea region. The Baltic Sea region is influenced by industrial and agricultural pollution, where apex predators including HAALs have suffered from population declines during the 20th century (de Wit et al., 2020; Helander et al., 2002; Sonne et al., 2020). As a consequence, HAALs have been included as an indicator species for biodiversity and anthropogenic pressures in the EU Marine Strategy Framework Directive for the Baltic Sea environment (Zampoukas et al., 2014).

With this study, we specifically aim to (i) characterise the food web of German HAALs by using stable isotope values of $\delta^{15}N$ and $\delta^{13}C$, (ii) to determine >2400 POPs and CECs via wide-scope target screening (iii) to compare contaminant concentrations with those detected in other HAAL subpopulations (iv) to determine the influence of trophic position, habitat and land cover on POPs and CECs and (v) to predict PBT properties based on a (quantitative) structure–activity relationship ((Q)SAR) model to unravel potential mismatches between predicted PBT properties and observed exposures.

2. Methods

2.1. Study area and sampling

The study included 30 liver samples from HAALs that were found dead between 2015 and 2018 in the north of Germany (Fig. 1). One exception represents MV542, which died within 24 h of being kept in captivity. Carcasses were frozen at −20 °C and thawed at room temperature for necropsy. During necropsy, a liver aliquot of 10–20 g (wet weight, ww) was taken and stored at −20 °C. Prior to shipment to the analytical laboratory, the liver samples were stored at −80 °C and subsequently lyophilised. Most birds were found as adults (≥5 years, n = 23) including one sub-adult (4 years) with a minor proportion of immature birds (2–3 years; n = 6) (Table SI-1). Most birds were of good nutritional status (n = 19) based on the measurement/presence of subcutaneous fat tissue, fat in the body cavity and in the coronary sulcus (moderate: n = 8; bad: n = 3) (Table SI-1). GPS coordinates were manually assigned to samples that had only a written description of the location where a carcass was found (Fig. 1).

2.2. Stable isotope analysis

As a first step, 0.5 mg sub-samples of dried, lyophilised and homogenised HAAL livers were weighed into tin cups and combusted in a Flash Elemental Analyser (Thermo Finnigan, Bremen, Germany). Further analyses of resultant N_2 and CO_2 gases were performed using an elemental analyser (Flash EA, Thermo Fisher, Bremen, Germany) connected in sequence via a Conflo (Thermo Fisher, Bremen, Germany) to a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher, Bremen, Germany). The isotope ratios were reported as δ-values and expressed as relative difference per mil [‰] according to the following equation, shortened according to Coplen (2011): $\delta X = R_{sample} = R_{standard} - 1$, where X is ^{13}C or ^{15}N, R_{sample} is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and $R_{standard}$ refers to the ratio of the international references Pee Dee Belemnite (V-PDB) for carbon and atmospheric N_2 (AIR) for nitrogen. The $\delta^{13}C$ and $\delta^{15}N$ values of the protein laboratory standards were −24.0‰ and 4.4‰, respectively, for tyrosine and −30.3‰ and 11.0‰, respectively, for leucine. The precision of repeated measurements of laboratory standards was better than 0.04‰ (1 standard deviation, sd) for carbon and 0.04‰ (1 sd) for nitrogen.

2.3. Chemical analysis

Simultaneous extraction of contaminants with different physicochemical properties from lyophilised and homogenised HAAL livers was carried out using generic sample preparation protocols (Androulakakis et al., 2021; Gkotsis et al., 2019). An Accelerated Solvent Extraction (ASE) and Solid Phase Extraction (SPE) were employed prior to the analysis by LC-/GC-HR-MS. Two generic sample preparation methods per sample were performed. More polar, less volatile, and thermally unstable compounds were extracted by the method-specific for LC-

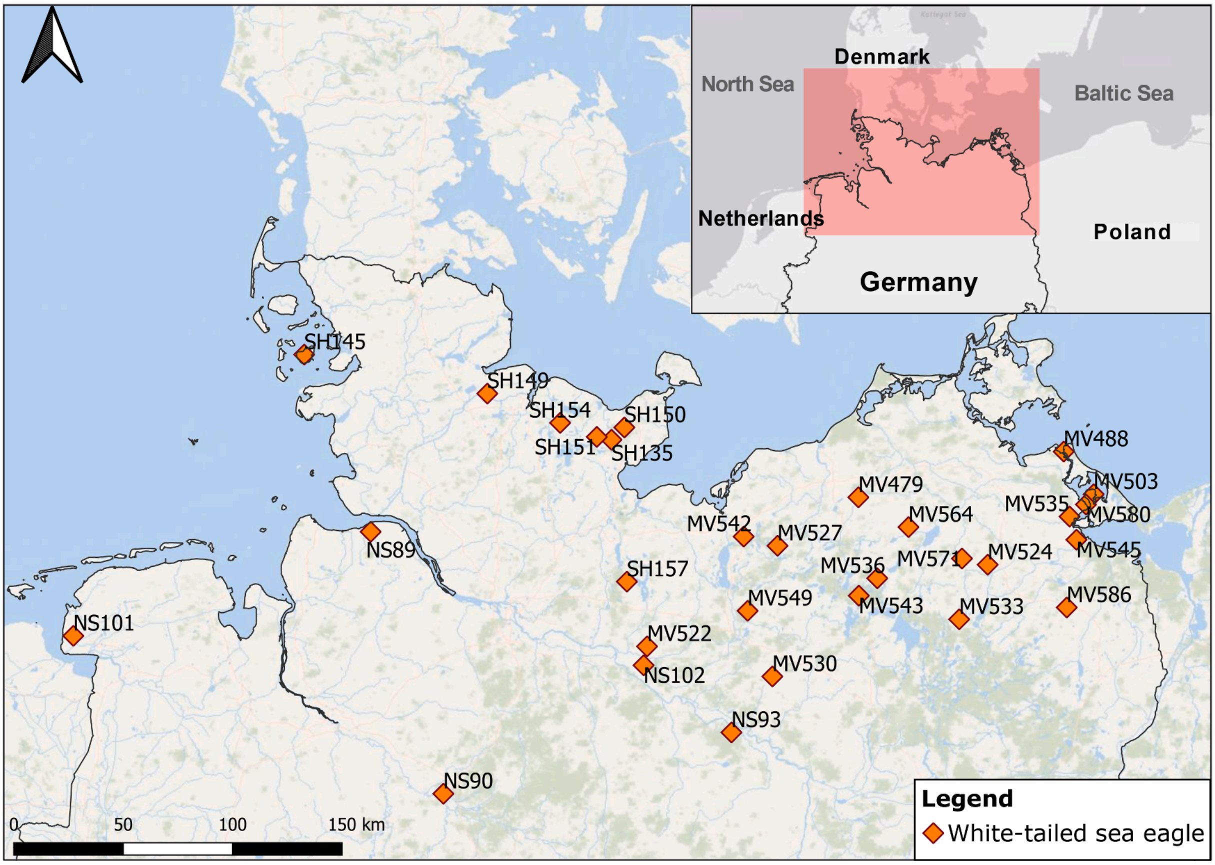

Fig. 1. Sampling locations of white-tailed sea eagles (HAAL) in the north of Germany. Letters indicate the federal state followed by continuous enumeration: MV = Mecklenburg-Western Pomerania, NS: Lower Saxony, SH: Schleswig-Holstein.

amenable compounds, whereas a different sample preparation method was followed for the extraction of more volatile and thermostable GC-amenable compounds. Detailed information on the extraction of LC- and GC-amenable compounds can be found in the supplementary information (SI-1.1/1.2).

2.4. *Instrumental analysis*

The analysis for LC-amendable compounds was conducted using Ultra High Performance Liquid Chromatography (UHPLC) apparatus with an HPG-3400 pump (Dionex UltiMate 3000 RSLC, Thermo Fisher Scientific) and Acclaim TM RSLC 120 C18 column (100 × 2.1 mm, 2.2 μm; Thermo Fisher Scientific). The gradient elution program for the LC system is given in Table SI-3. The system is coupled to a Hybrid Quadrupole Time of Flight Mass Analyzer (QTOF-MS) (Maxis Impact, Bruker Daltonics) with an electrospray ionization interface (ESI) operated in both positive and negative mode. Two scan modes were used: 1st run in Data Independent mode comprised a broadband Collision Induced Dissociation (bbCID) acquisition mode (acquisition of full scan MS spectra (4 eV) and MS/MS (25 eV) spectra in a single run). The second mode in data dependent mode consisted of a full scan MS spectra and MS/MS spectra of the 5 most abundant ions per MS scan in a single run.

The GC-APCI-QToF system consisted of a Bruker 450 GC coupled to the same MS as the LC system. GC was operated in splitless injection mode and the splitless purge valve was activated 1 min after injection. The injection volume was 1 μL. A Restek Rxi-5Sil MS column of 30 m (0.25 mm i.d. × 0.25 μm film thickness) was used with Helium as carrier gas in a constant flow of 1.5 mL min^{-1}. The GC oven was programmed as follow: 55 °C initial hold for 3 min, increase at a rate of 15 °C min^{-1} to 180 °C, then increase with a step of 6.5 °C min^{-1} to 280 °C and hold for 5 min followed by an increase of 10 °C min^{-1} to 300 °C and hold for 5.28 min. The temperature of splitless injector port, GC–MS transfer line and MS source were maintained at 280, 290 and 250 °C, respectively. The QToF mass spectrometer was equipped with an atmospheric pressure chemical ionization (APCI) source operated in positive ionization mode. The operating parameters of APCI interface were: capillary voltage, 5000 V; corona voltage, 2000 V; endplate offset, 500 V; nebulizer, 3.5 bar; drying gas, 1.5 L min^{-1}. The QTOF MS system operated in the same two different acquisition modes as described above for LC.

2.5. *Data treatment*

Target screening was performed using in-house developed databases of 2441 contaminants (the LC target list (https://zenodo.org/record/3723478) is available as S21 UATHTARGETS in Suspect List Exchange (https://www.norman-network.com/nds/SLE/) and the GC target list (https://doi.org/10.5281/zenodo.3753372) is available in the following link: https://zenodo.org/record/3753372. The target lists included: 1099 MPs (&TPs), 762 legacy and modern PPPs (&TPs), 313 drugs of abuse (&TPs), 232 legacy and modern industrial chemicals, and 35 contaminants from various other categories. The data treatment was performed using TASQ Client 2.1 and DataAnalysis 5.1 (Bruker Daltonics, Bremen, Germany) software. The detection was based on specific screening parameters [mass accuracy < 2 mDa, retention time shift ±0.2 min, isotopic fitting <100 mSigma (only for confirmation of positive findings), whereas the presence of adduct and fragment ions confirmed the analytes]. The Screening Detection Limit (SDL) was calculated from spiked samples; specifically, it refers to the lowest concentration level for which the identification of 95% of the target analytes was reliable (Gago-Ferrero et al., 2020). In the in-house developed method, the SDL was established as the concentration at which the thresholds of (i) retention time and (ii) mass accuracy of the precursor ion were satisfied (SI-1.3). The SDL was not compound-specific, but a generic reporting value derived after method validation (Gago-Ferrero et al., 2020). Further, thorough compound-specific validation was performed for quantification purposes of the compounds detected with the screening method. Compound-specific limit of detection (LOD) and limit of quantification (LOQ) values were calculated after the treatment and analysis of samples spiked with the detected compounds and structure-related isotope-labelled compounds. Both LOD and LOQ as well as the analytical techniques (LC vs. GC) used for the determination of the detected contaminants are given in Table SI-4.

For quantification, a representative mix of isotopically labelled reference standards (IS) covering a wide range of classes, polarities, and other physicochemical properties was added to every sample before extraction for quality control/assurance reasons (tracing and correcting potential sample preparations and instrumental analysis variations) as commonly suggested in HRMS screening studies (Kruve et al., 2021; Ng et al., 2020). The quantification of the detected analytes was based on the standard addition method (spiked matrix curve of 5 concentration levels), which achieves reliable and accurate quantification in cases of high matrix effects. In selected cases when the internal standard was available, a combination of the standard addition method and the isotopic dilution was used. The equation for quantification is provided in the supplementary information (SI-1.3).

2.6. *Statistical analysis*

We conducted all statistical analyses in R version 3.6.3 (R Core Team, 2020) and set the level of significance to $p < 0.05$. Heat maps were generated using the R-package ggplot2 (Wickham et al., 2016) and Inkscape 0.92.4. The sampling map was created using QuantumGIS software version 3.10.2 (QGIS Development Team, 2020). Concentrations ranging between LOD and LOQ were substituted with LOQ/2 in accordance with Directive 2009/90/EC. Concentrations below LOD were given a value of zero for modelling. Isotopic values of the HAALs were normally distributed (Shapiro-Wilk test, $p > 0.05$) and given as mean ± 2*Standard Error (SE). Concentrations of contaminants are given as median with interquartile range (IQR) as most contaminant concentrations were not normally distributed (Shapiro-Wilk test, $p < 0.05$). Summary statistics (median, IQR) refer to samples with detectable residues.

2.6.1. *Extraction and transformation of land cover variables*

We selected a buffer radius of 5 km (~78.5 km^2) for the quantification of land cover variables from the Corine land cover data set (EEA, 2018) as the recommended 3 km buffer zones for wind parks has shown to not be sufficient for HAALs in the Mecklenburg Lake District (Krone and Treu, 2018). The contribution of land cover class for each individual is given in Table SI-6. We applied a detrended correspondence analysis (DCA) using the R-package "vegan" (Oksanen et al., 2020) and extracted the scores of the first axis to create a single synthetic variable encompassing information from the different land cover classes while removing collinearity between them (Dormann et al., 2013). The DCA separated agricultural inland areas (negative scores) from mainly water bodies and wetlands (positive scores) on the first axis (Figure SI-5).

2.6.2. *Generalised linear modelling (GLM)*

For the statistical modelling of individual substances, we only considered those with ≥ 80% detection frequency (excluding nornicotine as potential sources remain difficult to assess in the current study). For polychlorinated biphenyls (PCBs), the sum of the six indicator PCB congeners ($\sum_6$PCB 28, 52, 101, 138, 153 and 180) was used as the response variable of the model as it comprises about half of the amount of total non-dioxin-like PCBs (NDL-PCBs) present in feed and food according to Regulation (EU) No 277/2012. For approximating DDT exposure, *4,4′*-DDD and *4,4′*-DDE were summed to $\sum_2$DDT. For per- and polyfluoroalkyl substances (PFAS), we used the sum of perfluorosulfonic acids ($\sum_4$PFSAs: C_6-C_8: PFHxS, PFHpS. PFOS linear&branched) and the sum of perfluorinated carboxylic acids ($\sum_6$PFCAs: C_8–C_{13}: PFOA, PFNA, PFDeA, PFUnA, PFDoA, PFTrD) as the responses (similar to Sun et al. (2020b)). For PPPs, we used spiroxamine and simazine and for MPs, we used oxfendazole as the response. Full compound names and CAS numbers of all detected chemicals are given in Table SI-5.

For modelling, we tested the effect of δ^{15}N as proxy for the trophic position (Fig. 2), δ^{13}C as proxy for the food web (Fig. 2), and DCA1 land cover scores (Figure SI-5) separately on the respective responses. We only considered univariate models due to the small sample size (n = 30), which limits the statistical power of our analysis and can lead to an increase of false positives due to multiple testing. Therefore, we adjusted (adj) *p*-values by controlling the expected proportion of false discoveries amongst the rejected hypotheses (false discovery rate) using the approach from Benjamini and Hochberg (1995), which is more powerful than that of the traditional Bonferroni correction (Benjamini and Hochberg, 1995). Additionally, we simulated data for our model system (n = 30; 7 responses referring to chemical (groups) with detection frequency ≥80%, and 3 univariate fixed effects) assuming either a gamma or a gaussian distribution of the response to assess the risk of false positives in our particular setting. Model assumptions (linearity of the predictor, independence of errors and expected dispersion) were checked by simulating data from the fitted model and comparing the residuals of the model fitted on such simulated values to the residuals of the model fitted on the observed data using the R-package "DHARMa" (Hartig, 2020). A $\log_e(x + 1)$ transformation was applied to simazine to normalise the distribution. Predictor effect plots and confidence intervals were depicted in the scale of the linear predictor using the R-package "effects" (Fox and Weisberg, 2019; Fox and Weisberg, 2018).

2.7. *Screening for PBT properties*

All chemicals detected in the study were assessed by the JANUS tool (https://www.vegahub.eu/portfolio-item/janus/) for PBT properties (Table SI-5). The JANUS software is based on a battery of (Q)SAR models integrated with a specific workflow for each endpoint (UBA, 2016). The final predictions are combined in a PB and PBT score, which allows to rank and prioritize the list of detected target compounds. A score of 0–0.3 means that the compound is predicted to not meet the PB/PBT criteria, a score of 0.3–0.6 means that no conclusion can be drawn, while a score above 0.6 indicates that PB/PBT properties are likely to be met (Pizzo et al., 2016; UBA, 2016).

3. Results

3.1. *Stable isotope analysis*

The stable isotope values of livers from 30 HAALs are plotted together with those of muscle tissue from common prey species from the study area (taken from Nadjafzadeh et al. (2016)) in Fig. 2. Summary statistics as well as Latin species names are given in Table SI-7. The stable isotope values of HAALs (δ^{15}N: 12.4 ± 0.7‰; δ^{13}C: −24.7 ± 0.5‰) are positioned within the isotopic range of selected prey species, yet closer to freshwater fish and piscivorous birds than to ungulates. In particular, omnivorous fish species such as common rudd showed values close to those of the investigated HAALs, whereas predatory species such as European perch and northern pike showed enriched δ^{15}N values. Similar observations were made for aquatic birds, where piscivorous species (great cormorant and great crested grebe) had enriched δ^{15}N values compared to omnivorous (black-headed gull, mallard, Eurasian coot) and herbivorous species (geese: *Anser* sp.). Among the terrestrial game mammals, only carnivorous/omnivorous species such as racoon dog and red fox showed overlapping stable isotope values with some HAAL individuals, whereas ungulates had considerably lower δ^{15}N values.

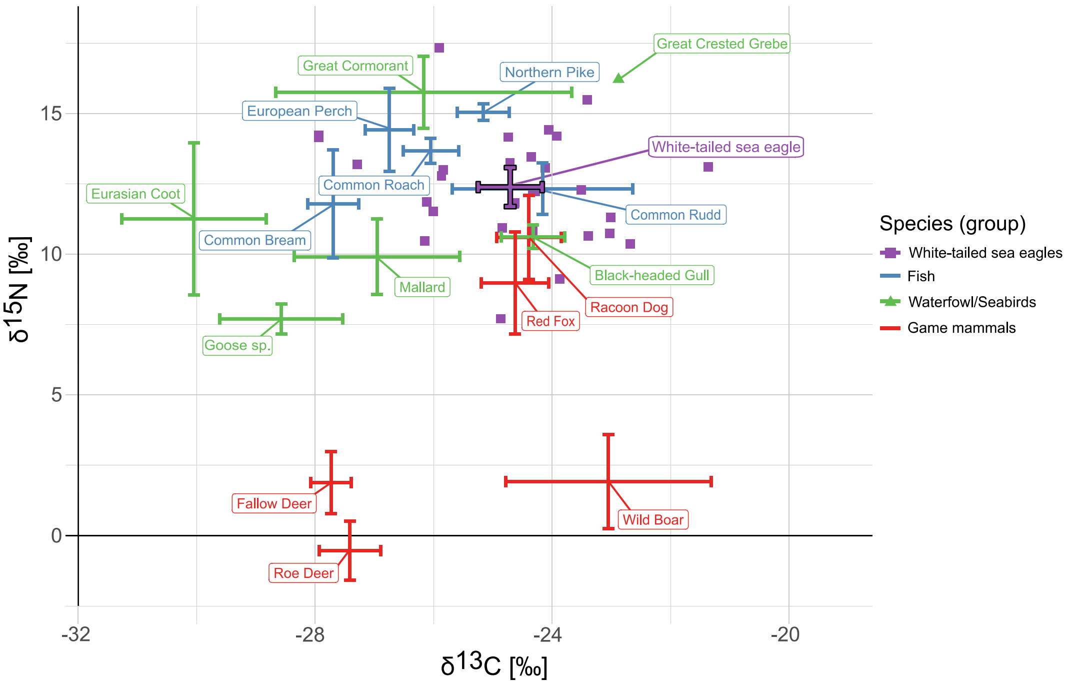

Fig. 2. Stable isotope values of δ^{15}N and δ^{13}C (cross: mean ± 2.0 * SE) for livers of white-tailed sea eagles (HAAL, n = 30, this study) and muscles of common prey species (taken from Nadjafzadeh et al., 2016). European perch (n = 5), northern pike (n = 6), common bream (n = 6), common roach (n = 6), common rudd (n = 6), great cormorant (n = 6), great crested grebe (n = 1), black-headed gull (n = 6), mallard (n = 5), Eurasian coot (n = 6), goose sp. (n = 7), red fox (n = 6), racoon dog (n = 6), wild boar (n = 6), fallow deer (n = 6), roe deer (n = 6). Summary statistics and Latin species names are given in Table SI-7. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Wide-scope target screening

In total, we detected 85 chemicals of which 27.1% were medicinal products (MPs) including TPs followed by POPs regulated under the Stockholm Convention (23.5%) and plant protection products (PPPs) (20%). Industrial chemicals regulated under REACH accounted for 17.6% of the identified compounds. Furthermore, 7.1% were stimulants and 4.7% belonged to chemicals regulated under various other legislations. Non-detected compounds of the target list were below the method SDL of 1.83 ng g^{-1} ww. Seven individuals were previously analysed by a multi-target method (Badry et al., 2021) for a subset of the 2441 target compounds analysed in this study (Table SI-15/16). Reported concentrations (median, IQR) in both studies refer to individuals with detected residues. A comparison of both analytical methods indicates that rodenticides such as brodifacoum may need compound-specific extraction and analysis protocols as concentrations were detected only in the individual with the previously highest residue. A comparison between the results of both methods can be found in the supplementary information (SI 2.1 and Table SI-15/16).

3.2.1. Chemicals of the Stockholm Convention

Among the chemicals regulated under the Stockholm Convention, PCB138 (median ww concentration (IQR): 238 (384) ng g^{-1}) and PCB180 (113 (321) ng g^{-1}) as well as *4,4′*-DDE (169 (159) ng g^{-1}) and *4,4′*-DDD (2.18 (5.41) ng g^{-1}) were detected in all individuals (Fig. 3; Table SI-8). For the investigated PCBs the order of detection is as follows: PCB138/180: 100% > PCB101: 93% (6.91 (13.0) ng g^{-1}) > PCB153: 80% (90.1 (217) ng g^{-1}) > PCB28: 53% (0.6 (1.53) ng g^{-1}) > PCB52: 43% (0.52 (5.17) ng g^{-1}) > PCB209: 17% (1.76 (12.0) ng g^{-1}). PFOS as well as PFOA are included in the Stockholm Convention as well but are presented together with other PFAS to allow for comparisons among chemicals of the same group. For the other POPs, dicofol was detected in 70% (0.77 (1.1) ng g^{-1}) and its metabolite 4,4′-dichlorobenzophenone in 60% (0.51 (1.25) ng g^{-1}) of the individuals followed by β-hexachlorocyclohexane (β-HCH) in 33% (4.35 (13.2) ng g^{-1}), heptachlor epoxide in 17% (0.87 (1.44) ng g^{-1}) and pentachlorobenzene in 10% (0.24 (0.21) ng g^{-1}). Hexachlorobenzene (44.9 (25.7) ng g^{-1}), *cis*-chlordane (4.4 (1.95) ng g^{-1}) and *trans*-chlordane (11.9 (1.3) ng g^{-1}) were detected in two individuals each. None of the tested univariate models (δ^{13}C, δ^{15}N and DCA1 land cover scores) significantly (adj $p < 0.05$) explained the variation of $\sum_6$PCB and $\sum_2$DDT (Table 1).

3.2.2. Industrial chemicals regulated under REACH

Except for PFAS, eight industrial compounds regulated under REACH were detected in the HAAL livers. The synthetic musk galaxolide was detected in 30% (11.3 (15.4) ng g^{-1}) of the individuals followed by tributylamine in 20% (2.71 (all LOQ/2) ng g^{-1}) and phenanthrene in 20% (1.74 (2.65) ng g^{-1}). Furthermore, benzenesulfonamide (10.7 (all LOQ/2) ng g^{-1}) and didecyldimethylammonium (8.4 (all LOQ/2) ng g^{-1}) were detected in two individuals and 2-OH-benzothiazole (9.59 ng g^{-1} (LOQ/2)), methylparaben (7.95 ng g^{-1}), and lauric isopropanolamide (1.6 ng g^{-1} (LOQ/2)) were detected in one individual (Table SI-9).

3.2.2.1. Per- and polyfluoroalkyl substances (PFAS). Linear and branched PFOS isomers were the most frequently detected PFSAs and PFASs (100%) with higher concentration of the linear isomer 480 (518) ng g^{-1} vs. the branched isomer: 8.41 (12.7) ng g^{-1} (Fig. 4A, Table SI-10). The other PFSAs were detected in fewer individuals with PFHpS in 30% (0.22 (0.29) ng g^{-1}) and PFHxS in 23% (0.05 (0.12) ng g^{-1}) of the individuals at lower concentrations. The order of detected PFCAs is as follows: PFDeA: 97% (1.78 (2.5) ng g^{-1}) > PFNA: 90% (3.97 (2.23) ng g^{-1}) > PFUnA: 60% (1.98 (1.57) ng g^{-1}) > PFOA: 23% (0.67 ng g^{-1} (all LOQ/2)) > PFDoA: 20% (0.6 ng g^{-1} (LOQ/2)) and PFTrDA: 20% (0.68 (1.38) ng g^{-1}). Linear PFOS concentrations made up 98.2% of $\sum_4$PFSA concentrations (Fig. 4B) and 96.8% of $\sum_{10}$PFAS concentrations. Among $\sum_6$PFCA concentrations, PFNA contributed to 47.2% followed by PFDeA (32.5%) and PFUnA (13.7%) (Fig. 4C). For $\sum_6$PFCA, concentrations significantly increased with δ^{15}N (1.38 times higher per ‰; adj $p < 0.01$; Fig. 4D), whereas no significant relationships were observed

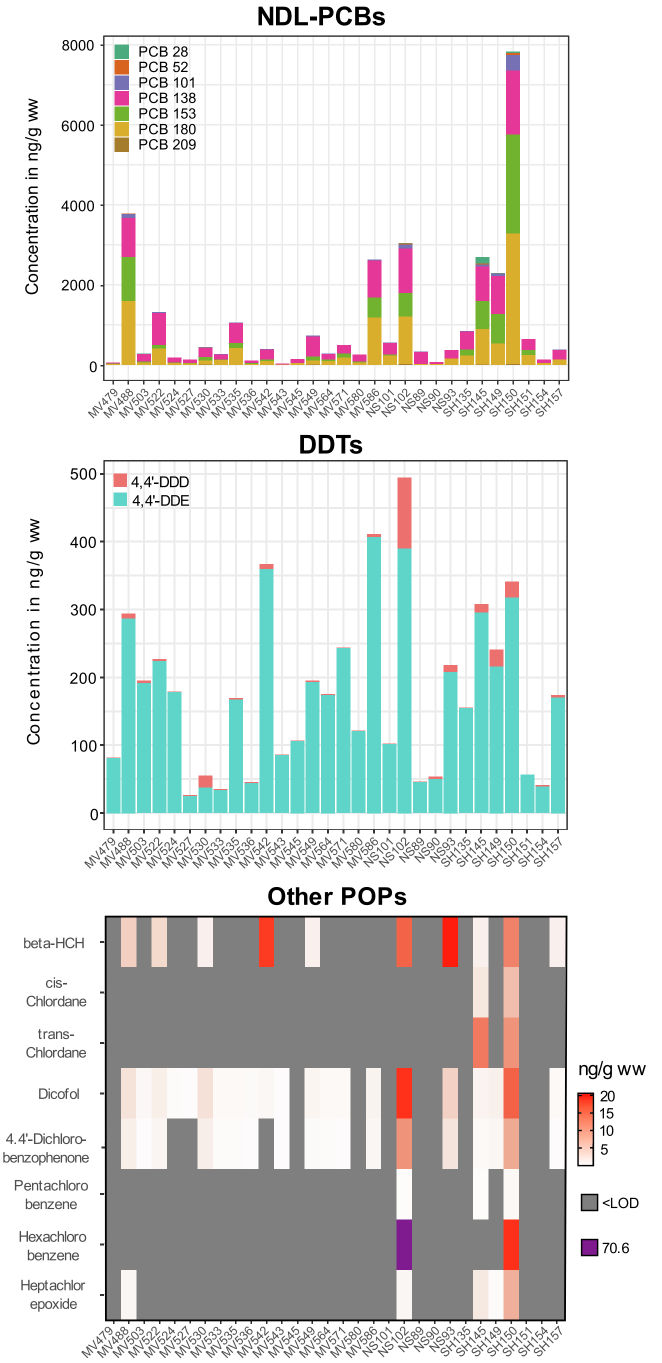

Fig. 3. Detection of non-dioxin-like PCBs and *4,4′*-DDD/*4,4′*-DDE in white-tailed sea eagles (HAAL, n = 30) given as bar charts. Other persistent organic pollutants (POPs) of the Stockholm Convention are visualised as heat map. Grey tiles in the heat map refer to samples below the limit of detection (LOD). Summary statistics are given in Table SI-8. Model estimates of all univariate models are given in Table 1. None of the univariate models significantly (adj $p < 0.05$) explained the variation of $\sum_6$PCB and $\sum_2$DDT.

for $\sum_4$PFSA (Table 1).

3.2.3. *Plant protection products (PPPs)*

The majority of detected PPPs was approved in Germany during the sampling period (2015–2018). Spiroxamine was detected in all individuals (3.03 (1.79) ng g^{-1}) followed by napropamide in 17% (1.75 ng g^{-1} (all LOQ/2)) and pymetrozine in 13% (7.71 (5.33) ng g^{-1}) of the individuals (Figure-5A). Bromoxynil, dimethachlor metabolites ESA-&OXA, dichlorobenzamide (parent compounds: fluopicolide/dichlobenil), metalaxyl, myclobutanil, propamocarb and pyrethrin I were detected in three or fewer individuals (Table SI-11). Besides the approved PPPs, five non-authorised PPPs were detected with simazine in 93% (4.67 (4.21) ng g^{-1}) of the individuals showing the highest detection rate followed by the metabolites ethiofencarb-sulfone in 33% (2.85 (2.52) ng g^{-1}) and alachlor-OXA in 30% (15.54 ng g^{-1} (LOQ/2)). Carbofuran, dikegulac and propachlor were detected in two individuals each with carbofuran showing the highest concentrations (1334 (1143) ng g^{-1}). For spiroxamine and simazine, concentrations significantly decreased with DCA1 land cover scores (adj $p < 0.05$; Fig. 5 B&C), which can be interpreted as a proxy for agricultural land cover within the 5 km buffer zone (Figure SI-5).

3.2.4. *Medicinal products (MPs)*

MV542 was excluded from the analysis of MPs due to potential deliberate treatments. Oxfendazole (VMP) was detected in all individuals (40.6 (9.2) ng g^{-1}) followed by salicylamide (HMP) in 72% (36.5 (33.8) ng g^{-1}) and meptazinol (HMP) in 55% (25.3 (27.0) ng g^{-1}) of the individuals (Fig. 6, Table SI-12). Residues of O-desmethylnor-tramadol were detected in 59% (19.0 (25.2) ng g^{-1}) along with other tramadol metabolites (O-desmethyldinor-tramadol, N-bisdesmethyl-tramadol, nor tramadol) and its parent compound (tramadol: HMP/VMP), which were were found at lower concentrations and detection rates. The venlafaxine (HMP) metabolite D L-N N didesmethyl-venlafaxine was detected in 38% of the individuals (5.38 (4.45) ng g^{-1}), whereas D L-N O didesmethyl-venlafaxine was detected in 10% of the individuals (7.77 (3.73) ng g^{-1}). The metabolite N-desmethyl-tapentadol (HMP) was detected in 17% (17.2 (9.08) ng g^{-1}) of the individuals. Other MPs were detected in three or fewer individuals. None of the fixed effects significantly explained the variation observed for oxfendazole (Table 1).

3.2.5. *Stimulants and others*

Residues of tobacco-related substances such as nor-nicotine were detected in 83% (194 (129) ng g^{-1}) of the individuals followed by nicotine in 20% (92.6 (26.2) ng g^{-1}), hydroxy cotinine in 7% (388 (34.6) ng g^{-1}) and cotinine (58.9 ng g^{-1}) in one individual. In general, metabolites showed higher concentrations and detection rates compared to their parent compounds (Table SI-13). We furthermore detected the drug of abuse methamphetamine in three individuals (6.49 ng g^{-1} (all LOQ/2)). Detection rates and concentrations of chemicals from various origins such as the artificial sweetener aspartame, which was detected in 40% (14.8 (22.3) ng g^{-1}) of the individuals or the biocide 1,2-benzisothiazolinone (13.3 ng g^{-1}; one individual) are given in Table SI-14.

3.3. *Estimated PBT scores*

Estimated PBT scores are given for better accessibility as separate Excel file in the supplementary information (Table SI-5). High estimated P, B, and/or T scores (>0.6) were most frequently observed for chemicals regulated under the Stockholm Convention. Among the chemicals regulated under REACH, especially PFAS had high P and/or B scores. Furthermore, 2-OH-benzothiazol had high P and galaxolide high B and T scores. Among the approved PPPs, bromoxynil, myclobutanil, napropamide, pymetrozine and spiroxamine were predicted P candidates similar to the expired PPPs alachlor-OXA, propachlor, and simazine. None of the PPPs had B or T scores > 0.6, which is in line with results for the MPs. In contrast to PPPs most MPs also had a P score < 0.6. Among the MPs with P score > 0.6 were the HMPs pindolol, desethylhydroxychloroquine and the VMPs oxfendazole, sulfadoxine as well as lidocaine and lidocaine-N-oxide (HMP/VMP). Except for hydroxy-cotinine (P > 0.6), all detected stimulants (and other compounds) had P, B and T scores < 0.6 (Table SI-5).

Table 1
Estimates of the univariate models on contaminant concentrations in livers of white-tailed sea eagles (HAAL, n = 30; medicinal products: n = 29). *p*-values were adjusted due to multiple testing by controlling the false discovery rate. Associations with negative DCA1 scores can be interpreted as a proxy for agricultural land cover and associations with positive DCA1 scores as a proxy for aquatic land cover (Figure SI-5).

	Estimates (multiplicators)	Estimates (log scale)	Std. Error	t value	Adjusted p-values
$\sum_6$PCB concentration [ng g^{-1}] gamma (log link)					
$\delta^{13}C$	1.10	0.10	0.19	0.52	0.98
$\delta^{15}N$	1.24	0.21	0.14	1.52	0.33
Land cover (DCA1 scores)	1.13	0.12	0.77	0.15	0.88
$\sum_2$DDT concentration [ng g^{-1}] gamma (log link)					
$\delta^{13}C$	1.07	0.07	0.09	0.78	0.98
$\delta^{15}N$	1.05	0.05	0.07	0.78	0.78
Land cover (DCA1 scores)	1.07	0.07	0.35	0.20	0.88
$\sum_4$PFSAs concentration [ng g^{-1}] gamma (log link)					
$\delta^{13}C$	1.01	0.01	0.12	0.09	0.99
$\delta^{15}N$	1.15	0.14	0.09	1.62	0.33
Land cover (DCA1 scores)	0.83	−0.18	0.46	−0.39	0.88
$\sum_6$PFCAs concentration [ng g^{-1}] gamma (log link)					
$\delta^{13}C$	1.05	0.05	0.10	0.50	0.98
$\delta^{15}N$	1.38	0.32	0.07	4.36	$p < 0.01$
Land cover (DCA1 scores)	1.32	0.28	0.42	0.66	0.88
Spiroxamine [ng g^{-1}] gamma (log link)					
$\delta^{13}C$	0.93	−0.07	0.04	−1.59	0.86
$\delta^{15}N$	1.02	0.02	0.04	0.46	0.90
Land cover (DCA1 scores)	0.65	−0.44	0.16	−2.75	$p < 0.05$
		Estimate (ng g^{-1})	**Std. Error**	**t value**	**Adjusted p-values**
Log_e(Simazine + 1) gaussian (identity link)					
$\delta^{13}C$		0.00	0.09	−0.01	0.99
$\delta^{15}N$		−0.01	0.07	−0.08	0.93
Land cover (DCA1 scores)		−0.80	0.31	−2.62	$p < 0.05$
Oxfendazole [ng g^{-1}] gaussian (identity link)					
$\delta^{13}C$		−0.48	1.22	−0.39	0.98
$\delta^{15}N$		0.10	0.94	0.11	0.93
Land cover (DCA1 scores)		−2.25	4.74	−0.48	0.88

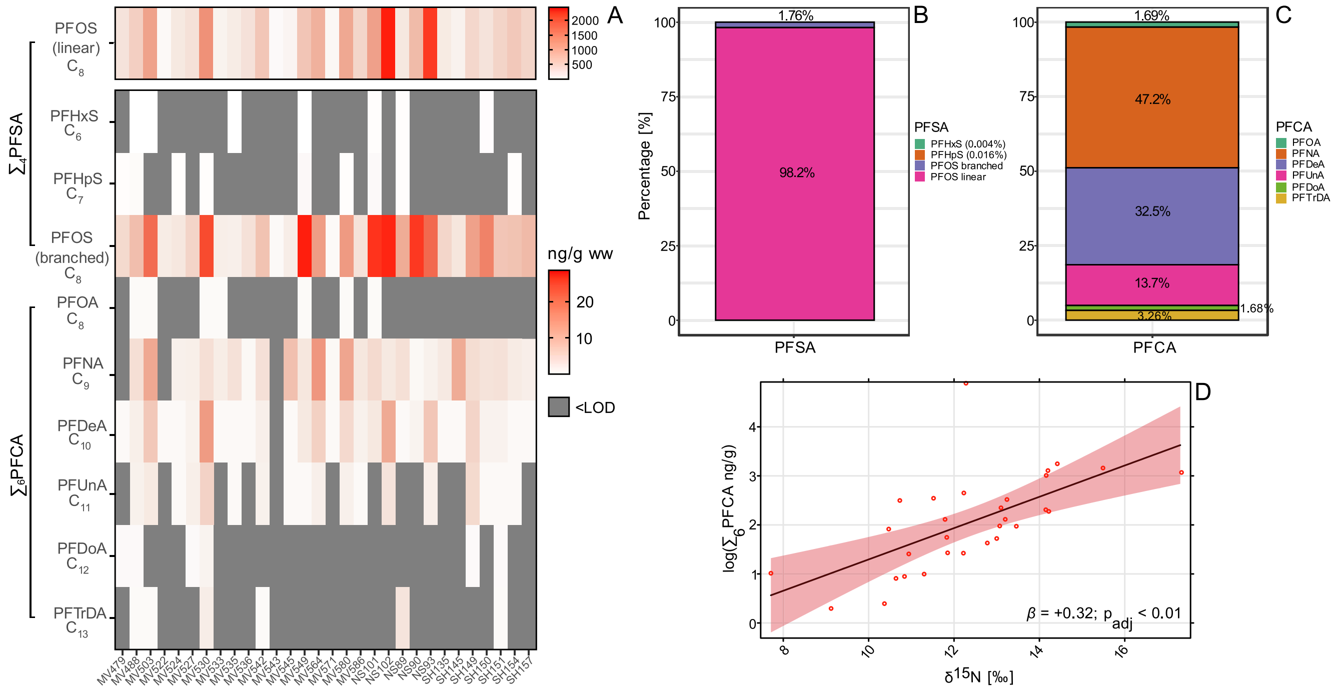

Fig. 4. Detection of PFSAs and PFCAs in white-tailed sea eagles (HAAL, n = 30) given as heat map (A). Grey tiles refer to samples below the limit of detection (LOD). Summary statistics are given in Table SI-10. Percentage of individual PFSA (B) and PFCA (C) concentrations are given as stacked bar plots. The significant effect (adj $p < 0.01$) of $\delta^{15}N$ on $\sum_6$PFCA is given as predictor effect plot with regression line, residuals (dots), and 95% confidence interval (D). Model estimates of all univariate models are given in Table 1.

4. Discussion

Whereas raptors played a critical role in developing awareness of chemical pollution and policy, they have so far played a much smaller role in research on CECs. Recent research efforts have led to the detection of novel CECs in European raptors (recently reviewed by González-

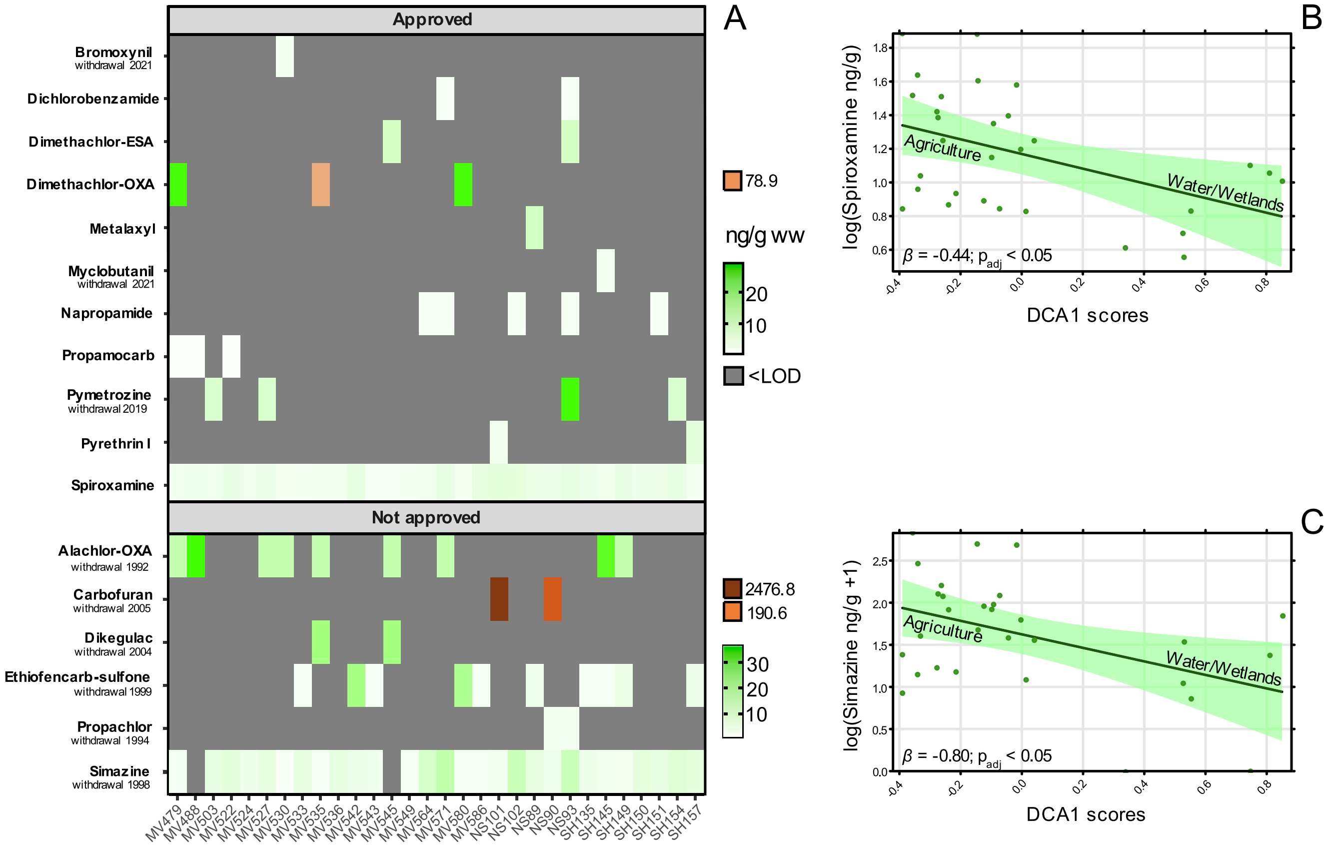

Fig. 5. Detection of approved and expired plant protection products at the time of sampling (until 31/12/2018) in white-tailed sea eagles (HAAL, n = 30) given as heat map (A). Grey tiles refer to samples below the limit of detection (LOD). Summary statistics are given in Table SI-11. Date of withdrawal (in Germany) refers to the parent compound in case of metabolites/transformation products. Significant effects (adj $p < 0.05$) of DCA land cover scores on spiroxamine (B) and simazine (C) are given as predictor effects plots with regression line, residuals (dots), and 95% confidence intervals. Model estimates of all univariate models are given in Table 1.

Rubio et al. (2020a)) including studies on HAALs (Badry et al., 2021; González-Rubio et al., 2020b; Oró-Nolla et al., 2021). Through the application of comprehensive analytical tools using both GC and LC coupled to HR-MS, the current study adds information on novel CECs in combination with underlying food web characterisation as well as on risk assessment with regards to their chemical regulations in Europe.

Our study demonstrated the presence of a large variety of contaminants in the livers of an apex predator from Germany, some of which have not been reported in the literature before. However, 2300+ chemicals remained undetected and their absence (SDL: <1.83 ng g^{-1}) remains difficult to assess as not all investigated target analytes were liver-specific and might be present in other environmental matrices such as wastewater, surface water or soils. Nevertheless, the liver represents the metabolic most competent organ and was therefore chosen as matrix for analysing contaminants with different physicochemical properties. The fact that a compound was not detected in a field biomonitoring study does not relieve a compound from the suspicion of being PBT since the environmental occurrence also always depends on e.g. its use and (local) emission sources. Furthermore, the generic sample preparation protocols and the full scan acquisition mode applied in HRMS instrumentation may have accounted for increased detection limits and lower %recoveries compared to conventional LC- or GC- MS/MS targeted methodologies for a pre-selected and restricted number of compounds (usually from the same chemical class) using Selected Reaction Monitoring (SRM) mode. However, one major advantage of our HRMS analysis is that apart from wide-scope target screening, the acquired chromatograms are accessible for retrospective data treatment, without the need for additional analysis, using suspect and non-target screening strategies that will expand the number of detected compounds in future (Menger et al., 2020).

4.1. Stable isotope values and food web characterization

An important means to control for dietary variations of apex predators feeding on mixed food webs represents the analysis of stable isotopes. During the summer season between 1996 and 2008, linear mixing models using stable isotope values in muscles (n = 75) of HAALs from the same study area revealed that the aquatic environment represents the main food source (91%) with fish accounting for 60% of the diet followed by waterfowl (27%) and game mammals (13%) (Nadjafzadeh et al., 2016). During winter, the contribution of wild ungulates can increase up to 29.5% (Nadjafzadeh et al., 2016). In general, stable isotope values of the present study were lower compared to those in livers of 30 HAALs sampled in the same study region between 1996 and 2003 (δ^{13}C: –23.5 ± 1.1 ‰; δ^{15}N: +13.6 ± 2.4 ‰; Nadjafzadeh et al. (2016)). The lower δ^{15}N values may be caused by a higher proportion of game mammals throughout the seasons, whereas the lower δ^{13}C values might reflect an increased uptake of freshwater (vs marine) prey. However, both δ^{13}C and δ^{15}N values have shown to decline in species of the coastal food web from northern Germany between 1988 and 2016, which was suggested to be related to an increase in terrestrial carbon sources and changes in nutrient inputs (Corman et al., 2018). In general, German HAALs use, compared to other HAAL subpopulations from Norway and Greenland, a higher proportion of freshwater food sources, which results in considerably lower δ^{13}C values (Løseth et al., 2019; Nadjafzadeh et al., 2016). Similar to HAALs from southern Sweden, marine HAALs from Germany (e.g. from Usedom) mainly forage on the brackish water of the Baltic Sea, which is characterised by lower salinity and slow water exchange with the North Sea, which might have contributed to the generally lower δ^{13}C values observed by the current study.

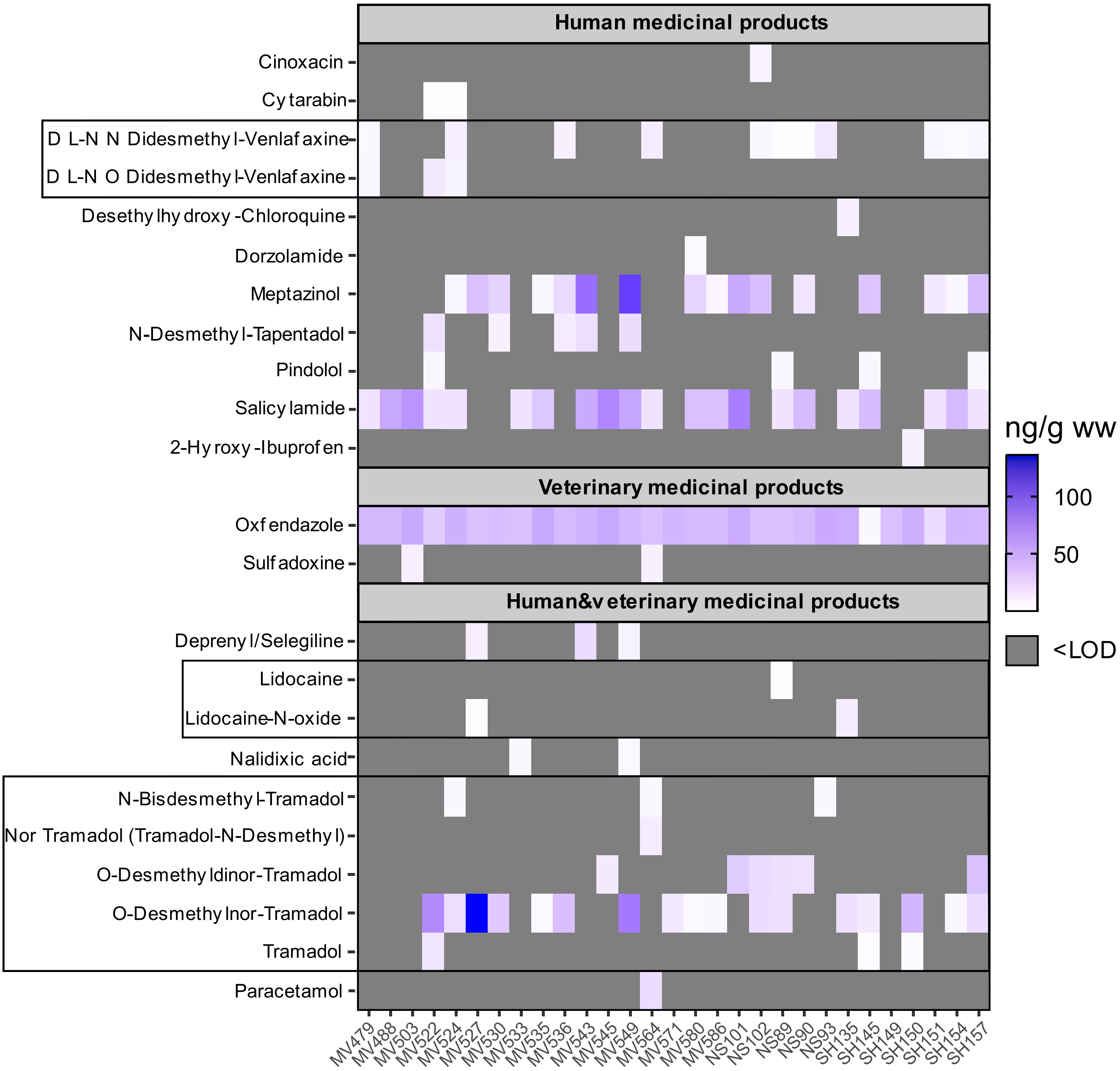

Fig. 6. Detection of medicinal products classed by their registration (human, veterinary, both) in white-tailed sea eagles found dead (HAAL, n = 29) given as heat map. Grey tiles refer to samples below the limit of detection (LOD). Summary statistics are given in Table SI-12. MV542 has been excluded due to potential deliberate treatments. Model estimates of all univariate models are given in Table 1. None of the univariate models significantly (adj $p < 0.05$) explained the variation of oxfendazole.

4.2. Assessment of POP and CEC concentrations among HAAL subpopulations and other predators

POPs listed in the Stockholm Convention were banned on a global scale due to their PBT properties but many POPs are still threatening apex predators in the Baltic Sea region due to biomagnification (de Wit et al., 2020). Among POPs, *p,p*'-DDE was together with $\sum$PCBs the main driver for population declines of HAALs during the 20th century (Helander et al., 2002; Roos et al., 2012). DDE as well as PCBs represent the dominant legacy POPs in the present study, which is in agreement with other marine apex predators in the Baltic Sea region (de Wit et al., 2020). In western Germany (Federal Republic of Germany), DDT was banned in the early 1970ties (id: SH&NS, Fig. 1), whereas it was still in use until 1988 by the German Democratic Republic (GDR, id: MV, Fig. 1). Concentrations of $\sum_3$DDT (95% *p,p*'-DDE) showed declining trends in eggs of HAALs from northern Germany between 1969 and 2001 (Scharenberg and Struwe-Juhl, 2006), which is in agreement with Swedish HAALs (Roos et al., 2012; Sun et al., 2020a). Concentrations of *4,4'*-DDE in the present study were considerably lower compared to liver concentrations of HAALs from Eastern Germany sampled between 1979 and 1998 (n = 24, median: 6400 (738) ng g^{-1}; Kannan et al. (2003)), which is considered to be related to the local use patterns of DDT in the GDR. In contrast to DDT, PCBs are still suspected to threaten marine apex predators (e.g. Desforges et al., 2018) but associations with reproduction failure in HAALs was considered to be lower compared to *p,p*'-DDE (Helander et al., 2002). Western Germany had one of the highest PCB productions between 1930 and 1983, accounting for ~12% of the global production, whereas production in the GDR was considered to be lower (Breivik et al., 2002). Among northern HAAL subpopulations, $\sum$PCB levels were, similar to *p,p*'-DDE, highest in Sweden, where concentrations show a declining trend between 1968 and 2011 (Sun et al., 2020a). Concentrations of $\sum_6$PCBs in the current study were lower compared to those in livers of previously sampled (1979–1998) HAALs from Eastern Germany (dioxin-like $\sum$PCBs median: 6500 (1443) ng g^{-1}; Kannan et al. (2003)) and HAALs from Greenland sampled between 1997 and 2009 ($\sum_{28}$PCBs, median: 540 ng g^{-1}; Jaspers et al. (2013a)). The detected DDE and PCB levels in the current study were below thresholds for reproductive impairment in HAALs (DDE: 120 μg g^{-1} lipid weight (lw); $\sum$PCBs: 500 μg g^{-1} lw; Helander et al. (2002)) based on the conversion from wet weight to lipid weight for DDTs and PCBs in HAAL livers (~1:20) (calculated based on Jaspers et al. (2013a)). However, both POPs showed considerable variations among individuals and may still cause subclinical effects on immunity and oxidative stress as observed for HAAL nestlings from Norway (Hansen et al., 2020; Sletten et al., 2016). Another frequently detected POP was dicofol, which was used in Germany as miticide until 1992. Dicofol was not detected in muscle tissue of bream (*Abramis brama*) from German freshwater sites (LOQ: 10 ng g^{-1}) and therefore not considered to be a relevant aquatic contaminant (Fliedner et al., 2016). Concentrations of the current study were mainly below the LOQ reported by Fliedner et al. (2016), indicating that the frequent detection may be related to biomagnification of comparably low residues in the food web. Other legacy POPs such as β-HCH, hexachlorobenzene and chlordanes were previously detected in HAAL subpopulations (Hansen et al., 2020; Løseth et al., 2019; Sun et al., 2020a). The detection of those POPs in the current study is expected to reflect exposures through remobilisation from soils and sediments as some POPs were ceased out comparably late in the GDR.

PFAS are used in a large variety of products including fire-fighting foams, paints and varnishes or outdoor clothing, which resulted in a ubiquitous distribution in the environment (Land et al., 2018). PFOS represented the main PFSA (98.2%) and PFAS (96.8%) in the current study, which is in line with results observed for eggs of Swedish HAALs and livers of Swedish otters (*Lutra lutra*) (Faxneld et al., 2016; Roos et al., 2013). In Norway, the contribution of PFOS is also dominant but appears to have a lower relative contribution to $\sum$PFAS (Jouanneau et al., 2020; Roos et al., 2013). PFOS levels in livers of 36 HAALs from Eastern Germany (1979–1998) ranged between < 3.9–127 ng g^{-1} with a median of ~29 ng g^{-1} (Kannan et al., 2002). These results indicate that PFOS levels in HAALs considerably increased over the past decades in the study area (47.2–2440 ng g^{-1}; this study). This is in line with increasing PFOS trends in feathers of HAALs from Sweden (1968–2011) (Sun et al., 2019), whereas other northern HAAL subpopulations showed declining PFOS trends after the phase-out in the early 2000s (Jouanneau et al., 2020; Sun et al., 2019). Furthermore, liver concentrations in the current study were higher compared to PFOS concentrations in livers from terrestrial raptors sampled in the vicinity of a PFAS point source in Antwerp, Belgium (barn owl (*Tyto alba*), median: 304.5 ng g^{-1}; Jaspers et al. (2013b): and Eurasian sparrowhawk (*Accipiter nisus*), mean: 236 ng g^{-1}; Meyer et al. (2009)). The high PFOS levels of HAALs from northern Germany (this study) in combination with the high levels from southern Sweden (Faxneld et al., 2016) indicate that emission sources from Germany might contribute to a delayed onset of declining PFOS trends in biota from the Baltic Sea region.

The dominant PFCAs in the present study were PFNA (C_9) > PFDeA (C_{10}) > PFUnA (C_{11}), which is in line with patterns found in livers of Swedish otters (Roos et al., 2013), whereas PFNA (C_9), PFUnDA (C_{11}), PFTrDA (C_{13}) were most dominant in eggs of Swedish HAALs (Faxneld et al., 2016). A dominant contribution of odd chain PFCAs appears to be more pronounced in marine (vs freshwater) environments (Roos et al., 2013) and might, besides matrix specific differences, explain the observed differences in PFDeA detection. In Kannan et al. (2002), the only targeted PFCA, PFOA, was not detected in HAALs from Eastern Germany, which might have been related to the comparably high LOQ of 40 ng g^{-1} ww. In the study by Jaspers et al. (2013b), PFCAs were except for PFOA only sporadically detected in livers of barn owls but at higher concentrations compared to PFCAs in the present study. Similar to PFOS, PFCAs showed an increasing trend in feathers and eggs of HAALs populations from Sweden (Faxneld et al., 2016; Sun et al., 2019). For long-chain PFCAs, atmospheric transport and transformation of precursors were suggested to result in a uniform distribution among northern HAAL subpopulations, whereas for PFOS, spatial water-bound contamination was suggested to be most influential (Faxneld et al., 2016; Roos et al., 2013; Sun et al., 2019). In central Europe, where most industrial activity is located, direct PFCA emissions might have a considerable impact as well. However, since PFCA concentrations in the present study were comparably low, direct emission sources of the targeted PFCAs in the study area seem unlikely.

Among approved PPPs (until 31/12/2018), the fungicide spiroxamine was detected in all individuals. Quantities of sold spiroxamine in Germany ranged between 100 and 1000 tonnes (t) per year during the sampling period (BVL, 2020), making it a frequently sold PPP. Spiroxamine was not detected in a recent multi-target analysis of blood (LOQ: 0.1 ng ml^{-1}) and muscle tissues (LOQ: 10 ng g^{-1}) of terrestrial raptors from Spain (Rial-Berriel et al., 2020; Sabater et al., 2020), whereas 3% of herbivorous game mammals from Poland had residues in their muscles (n = 136, mean: 1.1 ng g^{-1}) (Kaczyński et al., 2021). For spiroxamine, the highest levels are expected to occur in livers (mammals), where it is relatively fast metabolized and excreted (EFSA, 2010). Despite the matrix differences compared to our study, these results indicate that terrestrial spiroxamine exposure via scavenging might only be responsible for a minor part of the observed exposures. Other approved PPPs were only occasionally present in HAALs with dimethachlor metabolites being present in 17% of the individuals, which might be related to aquatic exposure due to their frequent detection in wastewater treatment plant (WWTP) effluents (Gago-Ferrero et al., 2020). Among the expired PPPs, the herbicide simazine was detected in almost all individuals and was also detected in WWTP effluents as well as in soil and sediments from Europe (Chiaia-Hernández et al., 2020; Gago-Ferrero et al., 2020). Furthermore, simazine was only occasionally detected in blood (5%; n = 148) of terrestrial raptors from Spain (Rial-Berriel et al., 2020), which indicates, similar to spiroxamine, that terrestrial exposure might only be responsible for a minor part of the observed exposures. In contrast, carbofuran is despite its ban frequently used to deliberately poison HAALs and other raptors in Europe (Kitowski et al., 2020). Carbofuran poisoning was confirmed as cause of death for the HAAL NS101 via gut and gizzard content and was suspected during necropsy for NS90.

Among the MPs, the anthelmintic agent oxfendazole (VMP; livestock) was detected in all individuals. In a previous study, oxfendazole was together with another anthelmintic agent detected in a liver of a pine marten (*Martes martes*) from the UK and suspected to be related to scavenging on treated livestock (Taylor et al., 2019). In contrast, no oxfendazole residues (LOQ: 0.1 ng mL^{-1}) were found in blood of non-scavenging raptors from Spain (Rial-Berriel et al., 2020), which indicates that scavenging on livestock might have influenced the observed exposures. The second most common MP, salicylamide (HMP, analgesic) is not used in Germany (Table SI-5), which raises the question if salicylamide is a potential metabolization/transformation product from more common and structurally similar MPs such as acetylsalicylic acid. However, salicylamide is also pre-registered under REACH as it used as an intermediate during the manufacture of other substances (Table SI-5). Previous studies detected salicylamide in freshwater along with paracetamol, (hydroxy-)cotinine, DADMAC (C10:C10) and 2-OH-benzothiazole (Diamanti et al., 2020) as well as together with venlafaxine, lidocaine and meptazinol in WWTP effluents (Gago-Ferrero et al., 2020). In the current study especially, coastal HAALs from the island Usedom (Baltic Sea) but also the HAALs close to the North Sea (e.g. NS101&SH145) had considerable residues, which indicates the presence of potential marine exposure sources. Other detected MPs comprise opioid analgesics such as tramadol and its metabolites, which were together with (nor-)lidocaine and (O-desmethyl-) venlafaxine previously detected in aquatic biota from Germany (bream) and the UK (amphipods) and suspected to be related to WWTP effluents (Boulard et al., 2020; Miller et al., 2021). Both tramadol and lidocaine metabolites were found at similar or higher concentrations in bream compared to their parent compounds (Boulard et al., 2020), which indicates at least some of the metabolites are transferred in the food web of HAALs. Among the stimulants, nicotine metabolites were most frequently detected even though nicotine derivates are in general efficiently eliminated in WWTPs (Buerge et al., 2008). Therefore, the assessment of potential routes of exposure for nicotine in the present study remains difficult to assess and requires further investigation.

4.3. Ecological and spatial variation of POPs and CECs

Many legacy POPs have shown to be biomagnified in food webs of HAALs (de Wit et al., 2020; Helander et al., 2002). However, no significant association between POPs of the Stockholm Convention ($\sum_6$PCB, $\sum_2$DDT) and δ^{15}N was observed, which is in line with observations for $\sum$OCPs/$\sum$PCBs (Løseth et al., 2019), but contrasts observations for *p,p*'-DDE and PCB 153 in Norwegian HAAL nestlings (Eulaers et al., 2013). For freshwater sites, which represent together with brackish water the main food source for German HAALs, lower admixture and higher spatial variation are suggested to result in increased heterogeneity of contaminants levels, which might obscure trophic signals (Elliott et al., 2009). Another confounding factor for the assessment of trophic influences on contaminant levels might have been the use of δ^{15}N bulk values, as baseline δ^{15}N values have shown to vary not only with trophic position but also with agricultural inputs, which might obscure or create spurious relationships with δ^{15}N (Elliott et al., 2021). Due to the unaccounted variation of baseline δ^{15}N, δ^{13}C (as well as δ^{34}S) has especially in marine ecosystems proven to be a better predictor for contaminants in top-predators (Elliott et al., 2021; Løseth et al., 2019). In contrast, the lack of a significant relationship between agricultural land cover scores and legacy POPs in the present study is assumed to be related to the lack of temporal overlap between the time of the latest POP application/emission (1970s/1990s), sample collection (2015–2018) and extraction of the data from the 2018 Corine data set.

For PFOS, the major PFSA in the current study, significant associations with δ^{15}N were reported for trend models in feathers of Norwegian and Swedish HAALs, whereas δ^{15}N was no significant predictor for $\sum$PFCA (Sun et al., 2019). In the blood of peregrine falcon nestlings (*Falco peregrinus*) from Canada, both $\sum$PFSA and $\sum$PFCA significantly increased with δ^{15}N (Sun et al., 2020b). In the current study, only $\sum_6$PFCA levels significantly increased with δ^{15}N, which indicates higher $\sum_6$PFCA levels with preying on piscivorous prey species. However, the non-significant relationship of $\sum_4$PFSA with δ^{15}N (adj $p = 0.33$) was unexpected as PFSAs generally have higher biota to soil accumulation factors compared to PFCAs of equal chain length (Zhao et al., 2013). A possible reason might be related to specific point sources that have potentially obscured trophic signals within the home range of the eagles. For example, both NS93 and NS102 were found in the same catchment area of the river Elbe and showed the highest PFOS concentrations (>2000 ng g^{-1}) among individuals of the present studies as well as compared to raptors from a PFAS contaminated area in Belgium (see above). Potential PFAS sources in our study area (including MV530: 1291 ng g^{-1} PFOS) include a military training ground in Lübtheen (used until 2013) as well as private airports, e.g. from the use of firefighting foams. The lack of association of both $\sum_6$PFCA and $\sum_4$PFSA/PFOS with δ^{13}C is however consistent with results for feathers of adult HAALs from Sweden, whereas δ^{13}C significantly explained $\sum$PFCAs variations in Norwegian HAALs (Sun et al., 2019) and peregrine falcon nestlings from Canada (Sun et al., 2020b).

Among the PPPs, the concentrations of the approved fungicide spiroxamine and expired herbicide simazine increased with the proportion of inland agricultural land cover, which indicates that exposures might be related to local sources. For example, surface runoff and spray drift of spiroxamine might have caused aquatic emissions in the direct vicinity of agricultural fields as spiroxamine rapidly binds to sediments in water systems (EFSA, 2010). We therefore recommend further studies on spiroxamine in bioturbating organisms such as bream from agricultural influenced areas to further investigate potential exposure risks. For simazine, regular monitoring for the EU Water Framework Directive is only recommended for water but not sediments or biota due to its low log K_{ow} (2.2) and low bioconcentration factor (1) (EC, 2010). However, residues of simazine were previously detected in sediments as well as in apex predators (see 4.2), which calls for further studies on potential exposures from simazine impurities (up to 3%) in terbuthylazine formulations (herbicide, sold up to 1000 tons per year BVL (2020)) as well as for studies on potential legacy applications of simazine as algicide in fishing ponds, where HAALs are known to forage.

4.4. Risk assessment and implications for chemicals management

The JANUS software is a helpful tool to unravel a mismatch between predicted laboratory data on PBT properties and observed exposures (Pizzo et al., 2016). As expected, many of those compounds that were predicted to have PBTs or PBs properties (JANUS scores > 0.6; Table SI-5), e.g. most legacy POPs and PFAS, also had high detection rates (>70%). However, some estimated and already regulated PBTs, e.g. PFHxS and PFTrDA had low detection rates, which may reflect the complexity of exposure events for apex predators that are not solely related to intrinsic chemical properties. Furthermore, some of the identified PBTs that were not or hardly detected were used in low quantities in Germany (e.g. fluorene), regulated for a long time (e.g. hexachlorobenzene), or may have been removed by WWTPs.

Among the non-restricted industrial chemicals regulated under REACH, PFASs (particularly PFDeA; PFHpS; PFNA, PFuNA) and galaxolide were the chemicals of highest concern based on their detection rate (>30%) and/or their predicted PBT properties (JANUS: P and/or B score > 0.6). In 2017, the REACH restriction of PFOA, its salts and precursors came into force and was implemented in 2020 (EC, 2017). Our data further support the urge for regulatory action on polyfluorinated compounds, which are currently evaluated as part of the ongoing restriction of PFAS (ECHA, 2020). We have submitted our results to the authorities responsible for the EU-wide restriction of PFAS to support their weight of evidence assessment. Another industrial compound regulated under REACH, galaxolide, is used as synthetic musk in washing and personal care products up to 10,000 tons per year (EU) and is assumed to be bioaccumulative and toxic but not persistent according to JANUS (Table SI-5). On request of the French authority, our data have also been used for the ongoing REACH substance evaluation concerning potential PBT properties. These examples demonstrate that strengthening the collaboration between biomonitoring networks and authorities might help to further promote the regulatory use of centralized, open assess databases like NORMAN (https://www.norman-network.com/nds/) and IPCHEM (https://ipchem.jrc.ec.europa.eu).

In contrast to the other chemical classes, data on use and tonnage are available for most PPPs. Based on a P score of > 0.6 and sold amounts of > 100 tons per year in Germany, exposures to bromoxynil, spiroxamine, and propamocarb were expected. However, only spiroxamine appeared to have a wide-spread exposure in HAALs. The fact that all other approved PPPs were found in few individuals indicates that the majority of PPPs are either not bioaccumulative, rapidly metabolized and excreted by HAALs or are just not applied in the study area as indicated by their low tonnages (Table SI-5). Among the non-approved PPPs, the widely detected PPPs simazine and alachlor-OXA are predicted to be vP/P by JANUS and have high absorption affinity, which might indicate that both substances bind to soils and sediments and are potentially remobilised as described in section 4.3. In contrast, the frequent detection of ethiofencarb-sulfone indicates potential illegal applications in the study area based on the low P and B scores.

Among the MPs, most of the detected substances had a P, B or T score

< 0.6, indicating that the substances are degradable in the environment (low P) and can be metabolised/excreted by organisms (low B). Observed exposures to detected MPs might therefore be related to emissions that have exceeded metabolization/transformation rates. In contrast, oxfendazole (VMP) may persist in the environment as predicted by JANUS (Table SI-5). However, exposure routes via scavenging are not accounted for during registration and should be considered for VMPs used in livestock as exemplified for diclofenac poisoning of scavengers in Spain (Herrero-Villar et al., 2021). Therefore, a regulatory follow-up check of the environmental fate and behaviour of oxfendazole including future monitoring studies is necessary to investigate if oxfendazole contamination is of concern for scavengers. Taken together, the JANUS software has proven to be a reliable tool for the rapid identification of P, B or T properties for the majority of the detected compounds. However, for some known PBT compounds such as brodifacoum, the JANUS tool fails to predict PBT properties, which indicates that hazard assessments cannot yet be based on *in silico* tools alone.

5. Conclusion

Our study shows that HAALs from Germany are exposed to a large cocktail of chemicals across different regulations including more than fifteen POPs and their metabolites, which demonstrates that contamination in German HAALs is still widespread. Even though wildlife species are exposed to multiple chemicals, chemical mixtures are so far not adequately assessed in the European risk assessment (Drakvik et al., 2020). There is an urgent need to promote strategies on how exposure to multiple hazardous chemicals can be more effectively assessed to cover field conditions. Since data on bird toxicity, sales and use of MPs, industrial chemicals regulated under REACH as well as on biocides are non-obligatory within the EU approval or registration, no link can be drawn between predicted environmental emission rates, measured concentrations in HAALs and their effects. However, especially the combination of legacy POPs and PFAS (e.g. for NS93&102, SH150), is suspected to result in cumulative or synergistic effects that may exceed toxic thresholds (Sonne et al., 2021; Sun et al., 2020a) and requires further investigation. Our study supports the general trend, that whilst in recent years great efforts have been undertaken in terms of analytical development to quantify the presence of CECs in biota, our understanding of the risks and possible chronic impacts posed to wildlife species, particularly to apex predators, still lags behind. We therefore recommend that data on the occurrence of CECs in apex predators should be more commonly considered in risk assessments under the different regulatory frameworks, e.g., in a weight of evidence approach, to trigger timely risk management measures before adverse effects in organisms or populations start to manifest.

CRediT authorship contribution statement

Alexander Badry: Conceptualization, Writing – original draft, Formal analysis (statistics), Data curation, Visualization. **Gabriele Treu:** Conceptualization, Writing – original draft, Formal analysis (risk assessment), Data curation, Funding acquisition. **Georgios Gkotsis:** Validation (wide-scope target target methods), Investigation (extraction of CECs and HRMS analysis), Writing - review & editing. **Maria-Christina Nika:** Formal analysis (wide-scope target screening), Writing – review & editing. **Nikiforos Alygizakis:** Formal analysis (analytical data), Writing – review & editing. **Nikolaos S. Thomaidis:** Writing – review & editing, Supervision, Resources. **Christian C. Voigt:** Investigation, Resources, Writing – review & editing. **Oliver Krone:** Conceptualization, Investigation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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