

Highlights

- Rodenticide exposure increased with the contribution of urban habitat.
- Red kites and urban goshawks were at greatest risk for rodenticide poisoning. •
- Rodenticide exposure may not be limited to terrestrial food webs.
- Ibuprofen and fluoroquinolones were the most frequently detected medicinal products.

FEEDBACK 🖵

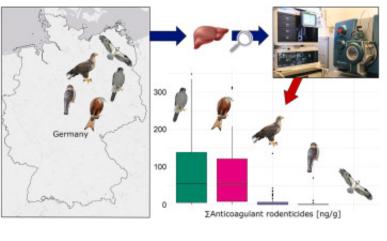
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• Most analysed and currently used plant protection products were not detected.

Abstract

Intensification of agricultural practices has resulted in a substantial decline of Europe's farmland bird populations. Together with increasing urbanisation, chemical pollution arising from these land uses is a recognised threat to wildlife. Raptors are known to be particularly sensitive to pollutants that biomagnify and are thus frequently used sentinels for pollution in food webs. The current study focussed on anticoagulant rodenticides (ARs) but also considered selected medicinal products (MPs) and frequently used plant protection products (PPPs). We analysed livers of raptor species from agricultural and urban habitats in Germany, namely red kites (MIML; Milvus milvus), northern goshawks (ACGE; Accipiter gentilis) and Eurasian sparrowhawks (ACNI; Accipiter nisus) as well as white-tailed sea eagles (HAAL; Haliaeetus albicilla) and ospreys (PAHA; Pandion haliaetus) to account for potential aquatic exposures. Landscape composition was quantified using geographic information systems. The highest detection of ARs occurred in ACGE (81.3%; n = 48), closely followed by MIML (80.5%; n = 41), HAAL (38.3%; n = 60) and ACNI (13%; n = 23), whereas no ARs were found in PAHA (n = 13). Generalized linear models demonstrated (1) an increased probability for adults to be exposed to ARs with increasing urbanisation, and (2) that species-specific traits were responsible for the extent of exposure. For MPs, we found ibuprofen in 14.9% and fluoroquinolones in 2.3% in individuals that were found dead. Among 30 investigated PPPs, dimethoate (and its metabolite omethoate) and thiacloprid were detected in two MIML each. We assumed that the levels of dimethoate were a consequence of deliberate poisoning. AR and insecticide poisoning were considered to represent a threat to red kites and may ultimately contribute to reported decreased survival rates. Overall, our study suggests that urban raptors are at greatest risk for AR exposure and that exposures may not be limited to terrestrial food webs.

Graphical abstract



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Keywords

Raptors; Agriculture; Rodenticides; Medicinal products; Pesticides

1. Introduction

Intensification of agricultural practices resulted in a substantial decline of Europe's farmland bird populations during the past 50 years (Busch et al., 2020; Donald et al., 2001; Emmerson et al., 2016). Besides factors such as declined landscape heterogeneity and habitat fragmentation, the increased use of agriculturally related chemicals was identified as a driver of population declines (Emmerson et al., 2016; Tscharntke et al., 2005). Exposure to agriculturally related chemicals has been shown to negatively impact populations of many wildlife species including raptors (Köhler and Triebskorn, 2013; Shore and Taggart, 2019). However, the application of pesticides is not restricted to agricultural habitats as anticoagulant rodenticides (ARs) are frequently used in urban areas to control rodent populations (López-Perea and Mateo, 2018). Certain pesticides have been classified as being persistent, bioaccumulative, or toxic (PBT) and raptors have shown to be particularly sensitive to compounds that bioaccumulate or biomagnify (Gómez-Ramírez et al., 2019; Shore and Taggart, 2019). Raptors are typically apex predators of high conservation value that are frequently used sentinels for contamination in food webs (Gómez-Ramírez et al., 2014). The current study focusses on chemical pollution arising from agricultural intensification and urbanisation such as ARs, medicinal products (MPs) and plant protection products (PPPs), which were identified as current threats for raptors in Europe (Badry et al., 2020).

Many ARs are classified as PBT substances and are divided into first-generation ARs (FGARs) and second-generation ARs (SGARs) (Elliott et al., 2016; Regnery et al., 2019a). SGARs are more persistent in animal tissues than FGARs and have been developed as more acute toxic substances due to increasing resistance of rodents against FGARs (Eason et al., 2002; Rattner et al., 2014b). Both FGARs and SGARs inhibit the synthesis of clotting factors in the liver, which represents a risk for many wildlife species as the clotting system is highly conserved among vertebrates (Eason et al., 2002; Rattner et al., 2014b). Non-target wildlife species such as raptors are indirectly exposed to ARs due to the delayed death of poisoned rodents taken alive as well as through scavenging on carcasses (López-Perea and Mateo, 2018). Besides agricultural and urban applications, ARs are frequently used in sewage systems, which has resulted in fish exposures in Germany (Kotthoff et al., 2018; Regnery et al., 2019b). Due to their global use, high toxicity to vertebrate wildlife and potential to bioaccumulate in food webs, the current study had a large focus on AR exposure in avian top predators.

Other agriculturally related substance classes that have negatively impacted avian top predators in the past include MPs and PPPs. MPs are increasingly used as a result of an ageing human population and intensification of food production (Arnold et al., 2014; Shore et al., 2014). Environmental emissions of human medicinal products (HMPs) are associated with liquid waste effluents from domestic or hospital sewage whereas veterinary medicinal products (VMPs) enter the environment through livestock production and manure fertilisation (Arnold et al., 2014; Kümmerer, 2009; Shore et al., 2014). Even though risks for mammalian consumption are well characterised for MPs, much less data is available on birds (Shore et al., 2014). This can lead to fatal consequences as exemplified for the metabolism of the non-steroidal anti-inflammatory drug (NSAID) diclofenac in Gyps vultures (Oaks et al., 2004).

PPPs refer to pesticides i.e. insecticides, herbicides or fungicides that are used to protect agricultural crops. Persistent and bioaccumulative PPPs have been shown to negatively affect raptors (Helander et al., 1982; Ratcliffe, 1967) and residues of persistent PPPs in agricultural habitats are still detectable in raptors after their ban (Gómez-Ramírez et al., 2019). Besides chronic exposures, threats to raptors include deliberate pesticide abuses and wildlife

poisoning (Berny et al., 2015; Coeurdassier et al., 2014). Even though currently applied PPPs are tested for PBT properties, usually under laboratory conditions, current legislation is lacking information on the ecological and landscape context (Schäfer et al., 2019). As a result, data on the actual exposure levels of currently registered PPPs to wildlife including apex predators is scarce.

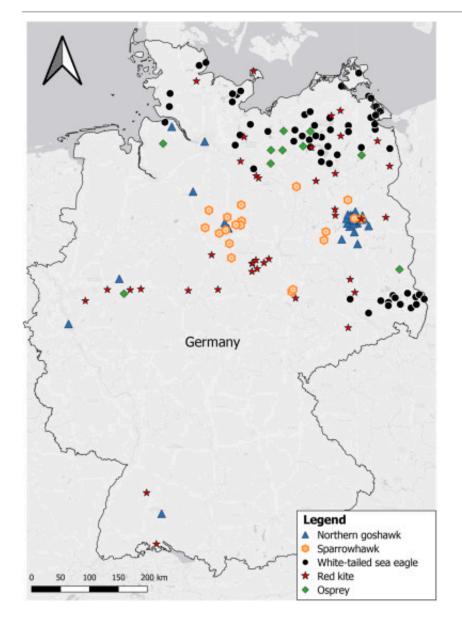
The present study focuses on the analysis of livers from raptors that died in Germany, where agricultural intensification has shown to negatively impact farmland bird populations (Busch et al., 2020). We specifically focused on the red kite (MIML; Milvus milvus), a species of high conservation value for Germany as more than 50% of all worldwide breeding pairs live there (Heuck et al., 2013). Red kites are facultative scavengers in agricultural habitats and show a decline in survival, which was suggested to be related to agricultural intensification and pesticide poisonings (Katzenberger et al., 2019). Moreover, we included the northern goshawk (ACGE; Accipiter gentilis), a species that has recently established stable populations in urban areas such as Berlin as well as the Eurasian sparrowhawk (ACNI; Accipiter nisus) sampled in predominantly agricultural habitats to investigate AR exposure risks associated with avivorous trophic pathways (Vyas, 2017; Walker et al., 2015). Two aquatic species, namely the white-tailed sea eagle (HAAL; Haliaeetus albicilla), a facultative scavenger feeding on fish, waterbirds and game carcasses, as well as the osprey (PAHA; Pandion haliaetus), a migratory species feeding exclusively on fish were included as previous studies suggested potential AR exposure risks for fish-eating predators in Germany (Regnery et al., 2020a). Including species feeding on aquatic food webs further allows to account for agricultural runoffs and incomplete wastewater removals.

Monitoring and determining factors that influence exposures is crucial for understanding population declines and to inform chemicals management. Therefore, the current study specifically aims (i) to assess exposures of the investigated environmental pollutants among the different species and associated feeding guilds and, where applicable, to evaluate concentrations regarding toxicity thresholds, (ii) to model the influence of landscape composition and (iii) to model the influence of biometric and individual-level factors on the probability and extent of exposure.

2. Methods

2.1. Sampling and study areas

The study included a total of 186 birds of prey that died in Germany between 1996 and 2018. Most birds were found as carcasses and a few alive, which died within 24 h in veterinary clinics. Carcasses were frozen at –20 °C and thawed at room temperature for necropsy. A complete veterinary and parasitological investigation was conducted for all individuals (Krone, 2000). Most individuals in the study were apparently healthy and died from collisions or intraspecific fights but we also included individuals that died from poisonings or infections (Table SI-1). During necropsy, a liver aliquot of 1–1.5 g was derived from 42 red kites (1996– 2019), 48 northern goshawks (1998–2018), 23 sparrowhawks (1996–2012), 60 white-tailed sea eagles (2004–2015) and 13 ospreys (2003–2016). We defined two age classes, five categories for the cause of death (similar to López-Perea et al., 2019) and three groups of nutrition condition based on the measurement/presence of subcutaneous fat tissue, fat in the body cavity and in the coronary sulcus (Table SI-1). Most samples (n = 149) originated from (sub-)adults with a minor proportion of juveniles (n = 36). GPS coordinates were manually assigned to samples that had only a written description of the location where a carcass was found. The majority of birds originated from the north of Germany (Fig. 1).



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Fig. 1. Sampling locations of investigated birds of prey within Germany. Blue triangles to northern goshawks (ACGE), orange hexagons to sparrowhawks (ACNI), black dots refer to white-tailed sea eagles (HAAL), red stars to red kites (MIML) and green squares to ospreys (PAHA). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.2. Selection of analytes

All currently registered ARs were analysed (brodifacoum, bromadiolone, chlorophacinone, coumatetralyl, difenacoum, difethialone, flocoumafen and warfarin). For MPs and PPPs, individual substances within the prioritised pollutant classes were selected based on the sales figures (BVL, 2017; Wallmann et al., 2018), the fate and behaviour, the general toxicity of the substances (Lewis et al., 2016) as well as the financial framework available for the realisation of the study. These criteria resulted in the selection of three fluoroquinolone antibiotics (ciprofloxacin, enrofloxacin and marbofloxacin), one sulfonamide antibiotic (sulfamethazine) as well as one pyrethroid (permethrin) and two NSAIDs (diclofenac and ibuprofen). For PPPs, eight herbicides, nine insecticides (+one metabolite) and 12 fungicides were selected for analysis (Table SI-2).

2.3. Sample extraction and analysis

The frozen liver aliquots were stored at -80 °C after arrival at the analytical laboratory and were thawed before analysis. The sample treatment is presented step by step in Table SI-3 (Geduhn et al., 2014). The liver tissues (0.3–1 g) were weighed in polypropylene tubes, spiked with a surrogate mixture for ongoing validation of analytical performance and subsequently homogenized in methanol/water (2:1) using an Ultra Turrax. After centrifugation, a saturated sodium chloride solution was added to the aliquots of the supernatant. The mixture was subsequently transferred to a diatomaceous earth column (ChemElut, Agilent) and completely absorbed. After 15 min, the analytes were eluted with dichloromethane. Aliquots were reduced to dryness and resuspended in internal standards for LC-MS/MS (methanol/water (1:1); Tables SI-4, SI-5and GC-MS/MS (acetonitrile; Tables SI-6, SI-7). The separation of analytes by LC was performed using four different methods: two reversed-phase columns, each with two different gradient programmes (Table SI-4). The measurement of analytes was performed with a QTRAP-Triple Quad Linear Ion Trap 6500+ (SCIEX) in electrospray ionisation mode. The identification and quantification of analytes were done with precursor - product ion transition (Table SI-5). We used the linear ion trap mode with dynamic fill time to confirm the identity of a substance. A substance was accepted when its enhanced product ion spectra in the sample (with intensity > 500 cps) matched more than 80% of those in the matrix standards in the same sequence. The GC separation of the semi-volatile substances was done on a column with low polarity (Table SI-6). The qualification and quantification of the substances (pyrethroids) were carried out in two runs by electron impact ionisation mode and negative chemical ionisation mode of a TSQ Quantum GC XLS (Thermo Scientific). Two ion transitions were extracted in the selected reaction monitoring mode for each substance. A substance was confirmed when the ion ratio of the two ion transitions of the sample was within ±30% of the

average of the reference standards in the same sequence. All analytes in all samples (LC and GC) were quantified against a matrix-matched standard and the criterion for the acceptance of the calibration curve was the correlation coefficient ($r^2 > 0.99$). The validation of the analytical procedure was checked by recovery tests using blank chicken liver. Sample preparation and extraction did not cause interferences in the blank liver samples. The reporting limit refers to the lowest calibration level with a signal to noise ratio >6:1 and relative standard deviation < 20% in the sequence (Table SI-2). The measured concentrations of the analytes were neither surrogate nor recovery corrected.

2.4. Statistical analysis

We conducted all statistical analysis in R version 3.6.3 (R Core Team, 2020) and set the threshold for the level of significance to p = 0.05. Graphical visualisations were generated using the Rpackage "ggplot2" (Wickham, 2016) and Inkscape 0.92.4.

2.4.1. Extraction of environmental variables

All land cover types defined by the Corine Land Cover 2018 (artificial structures, agricultural areas, forest and semi-natural areas, water bodies and wetlands; EEA, 2018) were extracted within circular buffer zones of 5 (i.e. 78 km²) and 10 km (i.e. 314 km²) around the location where a bird of prey was found to approximate potentially used foraging habitats (Badry et al., 2019). The contribution of the five land cover classes in the 10 km buffer zone is given in Tables SI–8. Artificial structures were used as a proxy for urbanisation since they mainly refer to urban-like features such as industrial units and urban fabrics (Kosztra et al., 2017). All land cover variables were extracted using QuantumGIS software version 3.10.2 (QGIS Development Team, 2020). To fit interdependent land cover data into statistical models, we summarise the information in a single variable by extracting the axis from either a principal component analysis (PCA) using the R-package "FactoMineR" (Lê et al., 2008), or from a detrended correspondence analysis (DCA) using the R-package "vegan" (Oksanen et al., 2013). Both methods used to create a synthetic variable from the land cover data resulted in similar results and effectively separated anthropogenic land cover types (artificial (urban) and agricultural areas) from non-anthropogenic land cover types (Figure SI-1) irrespective of the radius of the buffer zone selected. We selected the 10 km radius to minimise the risk of bias and relied on the PCA to approximate anthropogenic areas.

2.4.2. Anticoagulant rodenticides (ARs)

First, we built a generalized linear model (GLM) with logit link including all individuals for fitting exposure probability using the presence/absence of ARs as binary response (0/1) of a binomial distribution. Fixed factors included anthropogenic areas identified by PCA (Figure SI-1); sex (to observe potential differences in exposure for males and females); age class ("adult" and "juvenile"); year of death as well as the cause of death ("trauma": unspecific trauma and traffic collisions; "Pb-poisoning"; "other poisonings": such as insecticide poisonings; "infection"; "unclear", and "other": such as intraspecific fight, starvation or predation; Table SI-1). Nine individuals had missing information for one of the fixed factors and were thus excluded by the GLM resulting in n = 176. Values below the reporting limit (Table SI-2) were given a value of zero.

We then built a second GLM with gamma distribution and log link including only AR-positive individuals using untransformed Σ AR-residue concentration [ng g⁻¹] as the response. Fixed factors in this GLM included anthropogenic areas; species ("ACGE", "MIML", "HAAL", "ACNI"); year of death and nutrition condition ("bad", "moderate", "good"; Table SI-1) to observe potential mobilisation of lipid-soluble pollutants that may decrease residue concentrations in starved birds. For the analysis, all contaminant concentrations were tested visually for influential outliers. One red kite (Bra305) had an unusual high brodifacoum concentration (4853.47 ng g⁻¹), which was considered to be a consequence of deliberate poisoning, and thus excluded from statistical analysis. Four individuals with missing information for one of the fixed factors were excluded from the GLM resulting in n = 94.

All model assumptions (linearity of the predictor, independence of errors and expected dispersion) were checked (Figures SI-2, 3) 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). We assessed collinearity among the investigated fixed factors by computing Generalized Variance Inflation Factors (GVIFs^{1/(2*Df)}), where Df refers to the number of coefficients in a subset (Fox and Monette, 1992). Only variables showing GVIFs^{1/(2*Df)} < 2 were included in the model (Zuur et al., 2010). The overall significance of all fixed effect structures was checked using a likelihood ratio test by comparing the fitted model to that of a model fitted without the fixed factors of interest. Model predictions, predictor effect plots and confidence intervals were visualised using the R-package "effects" (Fox and Weisberg, 2018, 2019).

2.4.3. Medicinal products (MPs)

For the analysis of MPs, we only included individuals that were found dead to ensure that

birds had been environmentally exposed to MPs and to exclude deliberate treatments prior to death. This approach reduced the samples size to 87 birds (ACGE = 15, MIML = 19, HAAL = 42, ACNI = 3, PAHA = 8; Table SI-1). Statistical modelling was not possible due to low detection rates.

3. Results

3.1. Anticoagulant rodenticides (ARs)

3.1.1. Exposure among species

Overall, one or more ARs were found in 98 (53%) of the investigated samples (Fig. 2A). Exposure to a single AR was found in 41 samples (22.2%), whereas 57 (30.8%) had combinations of more than one AR (two: 33; three: 18; four: 3; five: 2; six: 1; Fig. 2B). The detection rate of ARs was highest in northern goshawks (81.3%), closely followed by red kites (80.5%), whitetailed sea eagles (38.3%) and sparrowhawks (13%). Exposure of ARs among individuals of the respective species is given in Figure SI-4. The most frequently detected AR was difenacoum with an overall detection rate of 34.6% followed by brodifacoum (31.9%), bromadiolone (19.5%), difethialone (9.7%), coumatetralyl (4.3%) and flocoumafen (2.2%) (Table SI-9). Except for coumatetralyl in northern goshawks, no FGARs (chlorophacinone and warfarin) were detected in any of the samples.

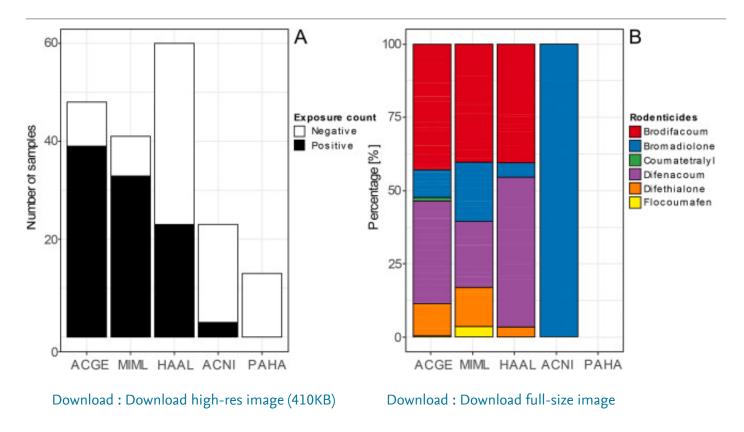


Fig. 2. A: Exposure count of Σ ARs per species (0/1) and **B**: Percentage of AR concentrations per species. ACGE: Accipiter gentilis (n = 48); MIML: Milvus milvus (n = 41); HAAL: Haliaeetus albicilla (n = 60); ACNI: Accipiter nisus (n = 23); PAHA: Pandion haliaetus (n = 13). One MIML (Bra305) was excluded due to deliberate poisoning.

The highest detection rate of difenacoum was found in northern goshawks with 66.7% (median wet weight concentrations (interquartile range; IQR): 37.03 (66.79) ng g⁻¹; Fig. 3) followed by red kites with 46.3% (18.35 (46.46) ng g⁻¹) and white-tailed sea eagles with 21.7% (18.04 (23.08) ng g⁻¹). The detection rate of brodifacoum was in the same order as difenacoum with northern goshawks being most frequently exposed (34.9 (89.85) ng g⁻¹; 60.4%), followed by red kites (37.38 (49.15) ng g⁻¹; 46.3%) and white-tailed sea eagles (5.85 (10.23) ng g⁻¹; 18.3%). Bromadiolone was detected in 37.5% of northern goshawks (15.01 (28.87) ng g⁻¹), again followed by red kites (42.88 (25.26) ng g⁻¹; 29.3%) and was the only AR detected in sparrowhawks (10.71 (33.29) ng g⁻¹; 13%). Furthermore, 5% of the white-tailed sea eagles were exposed to bromadiolone (9.28 (3.17) ng g⁻¹). Difethialone showed the highest detection rate in red kites (20.99 (26.78) ng g⁻¹; 19.5%) followed by northern goshawks (25.73 (108.52) ng g⁻¹; 16.7%) and white-tailed sea eagles (8.03 (0.89) ng g⁻¹; 3.3%). Coumatetralyl was detected only in

northern goshawks (5.37 (8.65) ng g⁻¹; 16.7%), whereas flocoumafen was detected in both, red kites (59.44 (56.49) ng g⁻¹; 4.9%) and northern goshawks (10.87 (5.58) ng g⁻¹; 4.2%). No ARs residues were detected in ospreys (Fig. 2, Fig. 3).

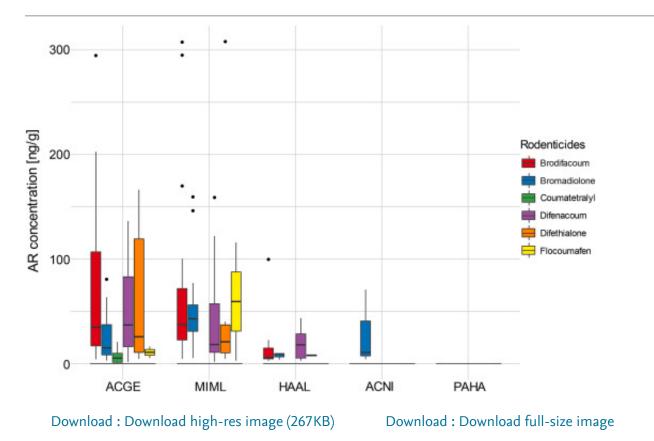


Fig. 3. Box plots of detected ARs among the different species. The lower and upper hinges of the box correspond to the 25th and 75th percentile. The upper whisker extends from the hinge to the largest value no further than 1.5*IQR from the hinge. The lower whisker extends from the hinge to the smallest value at most 1.5*IQR of the hinge. Data points beyond are plotted individually by black dots. ACGE: *Accipiter gentilis*, MIML: *Milvus milvus*; HAAL: *Haliaeetus albicilla*; ACNI: *Accipiter nisus*; PAHA: *Pandion haliaetus*. One MIML (Bra305; brodifacoum: 4853.47 ng g⁻¹; difenacoum: 69.41 ng g⁻¹) was excluded due to deliberate poisoning.

3.1.2. Variation in the presence and absence of AR residues

The estimates of Table 1 refer to changes in log(odds) of AR exposure when a continuous fixed factor increases by one unit (and all other fixed effects are at a fixed value). When returning to the original scale for interpretation purposes, exponentiation of fixed factors results in a

multiplicative effect on the response (odd ratios; Table 1). For each factor, one level needs to be chosen as reference so that parameter estimates are shown in reference to the intercept. Selecting a reference category is the default contrast used by R to express parameter values. It does not influence the model fit. Parameter estimates relative to each other are presented by predictor effect plots in Figure SI-6. The presence of AR residues increased with the contribution of artificial areas as shown by the negative relationship between the log odds of AR exposure and the first PCA axis (anthropogenic areas) which can be interpreted as a measure of urbanisation (p < 0.01; Table 1; Figure SI-1). Age class revealed that adults are 3.42 times more likely to have AR residuals compared to juveniles (p = 0.01; Table 1). Year of death tended to have a weak positive effect (p = 0.07) on the presence of ARs, whereas no effect was observed for sex (p = 0.86). Birds of prey that died from unclear reasons tended to have an increased odd for being exposed to ARs compared to birds of prey that died from trauma (p = 0.06). A similar trend was observed for birds of prey that died from infection (p = 0.18; Table 1). Poisonings as well as other causes of death did not show an effect on the presence of AR residues.

Table 1. Estimates (changes in log(odds) and odd ratios) of the fixed effects on the presence and absence of ARs in livers of northern goshawks (n = 45), red kites (n = 39), white-tailed sea eagles (n = 60), sparrowhawks (n = 22) and ospreys (n = 10). The reference category for cause of death was set to trauma, for age class to juvenile and for sex to female.

Presence/absence of ARs	Estimates (odd ratios)	Estimates (log odds)	Std. Error	z value	Pr (> z)
Anthropogenic areas	0.58	-0.55	0.19	-2.87	<0.01
Age class - adult	3.42	1.23	0.48	2.55	0.01
Sex -male	1.06	0.06	0.32	0.17	0.86
Year of death	1.05	0.05	0.03	1.8	0.07
Cause of death - infection	2.44	0.89	0.67	1.34	0.18
Cause of death - other	1.17	0.15	0.48	0.32	0.75
Cause of death – Pb-poisoning	1.34	0.29	0.57	0.52	0.6

Cause of death – other- poisonings	2.25	0.81	0.9	0.9	0.37
Cause of death - unclear	2.51	0.92	0.48	1.91	0.06

3.1.3. Variation of AR concentrations in AR-positive birds of prey

Similar to the GLM described above, the estimates of the gamma GLM are on the log scale and assumed to be additive in their effect on the response (Σ AR concentration [ng g⁻¹]; Figure SI-5). All model predictions are visualised relative to each other in Figure SI-7. In contrast to the binomial model including all individuals, the current model revealed no effect of anthropogenic areas on the concentration of Σ ARs in AR-positive individuals (Table 2). However, concentrations of northern goshawks were 4.77 times higher and those of red kites 5.09 times higher than those of white-tailed sea eagles (p < 0.01; Table 2). Σ AR concentrations in sparrowhawks were similar to those of white-tailed sea eagles (p = 0.36) but interpretation warrants caution due to a low number of AR-positive sparrowhawks (n = 3). Year of death tended to increase with increasing Σ AR concentrations (p = 0.1; Table 2). Good and bad nutrition condition did not significantly affect Σ AR concentrations compared to moderate nutrition condition.

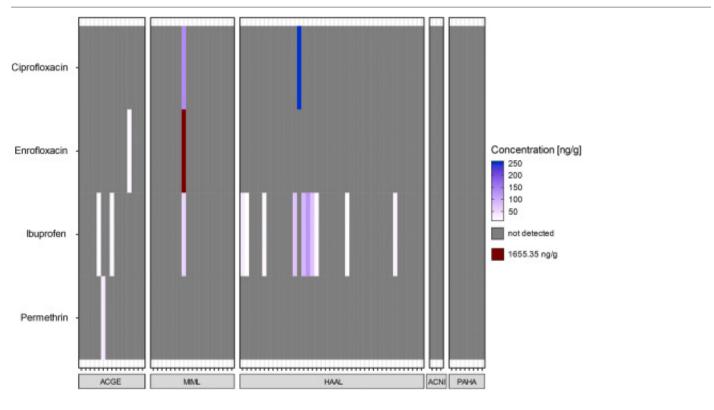
Table 2. Estimates of the fixed effects on AR concentration in livers of northern goshawks (n = 38), red kites (n = 31), white-tailed sea eagles (n = 22) and sparrowhawks (n = 3) with detected residues. The reference category for species was set to HAAL and for nutrition condition to moderate.

ΣAR concentration [ng g^{-1}]	Estimates (multiplicators)	Estimates (log scale)	Std. Error	t value	Pr (> t)
Anthropogenic areas	0.9	-0.11	0.15	-0.73	0.47
Species – ACGE	4.77	1.56	0.4	3.9	<0.01
Species - ACNI	1.88	0.63	0.69	0.92	0.36
Species – MIML	5.09	1.63	0.29	5.62	<0.01
Year of death	1.03	0.03	0.02	1.67	0.1

Nutrition condition - bad	1.05	0.05	0.32	0.14	0.89
Nutrition condition - good	1.11	0.1	0.28	0.37	0.71

3.2. Medicinal products (MPs)

Among all investigated MPs, we detected the NSAID ibuprofen, two fluoroquinolone antibiotics (enrofloxacin and its metabolite ciprofloxacin) and permethrin in individuals that were found dead (Fig. 4; Table SI-10). Ibuprofen was detected in 14.9% of all samples with white-tailed sea eagles (33.6 (53.05) ng g⁻¹; 23.8%) showing the highest detection rate followed by northern goshawks (18.55 (3.73) ng g⁻¹; 13.3%). Furthermore, one red kite (NRW42; 55.45 ng g⁻¹) had residues of ibuprofen whereas no residues were detected in sparrowhawks and ospreys (Fig. 4). Both fluoroquinolones were detected in two individuals (2.3%) with one red kite being exposed to both antibiotics (NRW42; enrofloxacin: 1655.35 ng g⁻¹; ciprofloxacin: 135.34 ng g⁻¹), one northern goshawk only to enrofloxacin (B97; 21.12 ng g⁻¹) and one white-tailed sea eagle only to ciprofloxacin (MV327; 257.3 ng g⁻¹). Additionally, permethrin was found in one northern goshawk from Berlin (B194; 35 ng g⁻¹; Fig. 4). Marbofloxacin, diclofenac, and sulfamethazin were not detected.



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Fig. 4. Heat map of detected MPs among individuals that were found dead. ACGE: Accipiter gentilis (n = 15); ACNI: Accipiter nisus (n = 3); HAAL: Haliaeetus albicilla (n = 42); MIML: Milvus milvus (n = 19); PAHA: Pandion haliaetus (n = 8). Not detected = concentration below reporting limit (Table SI-2). Summary statistics are given in Table SI-10.

3.3. Plant protection products (PPPs)

Among all 30 investigated PPPs, dimethoate, omethoate and thiacloprid were detected in red kites (Figure SI-8; Table SI-11). Dimethoate (21042.21 (10682.33) ng g^{-1}) and its metabolite omethoate (4077.85 (3649.86) ng g^{-1}) were detected in the same two individuals whereas thiacloprid (99.95 (46.43) ng g^{-1}) was found in two different individuals.

4. Discussion

4.1. Anticoagulant rodenticides (ARs)

4.1.1. Concentrations among feeding guilds

AR poisoning represents an important cause of death for raptors even when no misuses were reported to authorities (Coeurdassier et al., 2014). Species that facultatively scavenge or that are feeding specifically on small mammals have shown to be at highest risk (López-Perea and Mateo, 2018). Surprisingly, the northern goshawk was the most highly exposed species in the current study. The northern goshawk is characterised as a forest inhabiting species that mainly preys on other birds and to a lesser degree on mammals, depending on latitude and availability (Kenward, 2006). However, individuals of the present study originated from Berlin, where northern goshawks have established stable populations in recent years. Despite not being specialised on mammalian prey, the current study demonstrates high ARs exposures for avivorous predators in urban habitats. Thresholds related to acute toxic effects from ARs such as coagulopathy and haemorrhage have been associated with liver concentrations exceeding 100 ng g⁻¹ and 200 ng g⁻¹ wet weight (Berny et al., 1997; Rattner et al., 2014a; Thomas et al., 2011). When applying the acute toxicity threshold of >200 ng $g^{-1} \Sigma$ SGAR, nine individuals (18.8%) exceeded this level, while these were not identified as being poisoned during necropsy. During necropsy, a special emphasis was put on pathological indications of AR poisoning such as generalized hemorrhages, subcutaneous bleedings and un-coagulated blood in large vessels

or the heart. However, the decomposition of the carcasses often makes it impossible to find clear indications of AR poisoning. Few studies with small sample sizes exist on AR residues in northern goshawks in Europe (López-Perea and Mateo, 2018), demonstrating that northern goshawks have generally not been considered for AR poisoning. Interestingly, a study investigating potential prey species detected high AR exposures of passerine birds from Germany, which was linked to AR applications in local bait boxes (Walther et al., 2021). The high exposure of northern goshawks and accessibility of bait boxes by prey species calls for further investigations of northern goshawks in urban habitats. The observed lower concentration in sparrowhawks, a species that is specialised on avian prey, indicates that the quality of habitat might be most influential for AR exposure as sparrowhawks from the current study originated predominantly from agricultural and forest habitats. Previous studies demonstrated that sparrowhawks have comparable exposure rates to raptors that forage on mammals but concentrations were generally lower (Hughes et al., 2013; Ruiz-Suárez et al., 2014; Walker et al., 2015). Nevertheless, the potential that ARs are transferred in avian trophic pathways in combination with the use of habitats where AR are frequently applied is considered to pose a threat to avivorous raptors.

In contrast to species foraging on the avian trophic pathways, facultative scavengers such as the red kite are known to be at high risk for ARs poisoning as they frequently forage on (dead) mammals in agricultural habitats (Coeurdassier et al., 2012; Heuck et al., 2013; López-Perea and Mateo, 2018). The results of the current study confirm the high risk for AR poisoning of red kites in Germany, where more than 50% of the breeding population lives. The detection frequency of ARs in the current study is comparable to red kites from the UK (1994–2018, 80.4%, n = 214; Hughes et al., 2013; Walker et al., 2019; Walker et al., 2008) and Spain (2005– 2016, 80.1%, n = 21; López-Perea et al., 2019; Sánchez-Barbudo et al., 2012), whereas in France they seem to be lower (1992–2011, 61%, n = 95; Berny and Gaillet, 2008; Coeurdassier et al., 2014). Six individuals (14.3%) exceeded the applied threshold of >200 ng g⁻¹ Σ SGAR, which was, except for one individual, not associated with AR-poisoning during necropsy. In particular, the high levels of brodifacoum in red kites are considered to be a threat for scavengers as prolonged effects that increase in toxicity with subsequent exposure were reported (Rattner et al., 2020). The results of the current study add evidence that AR exposure contributes to reported declined survival rates of red kites in Germany (Katzenberger et al., 2019). However, further measures such as the observation of blood clotting in rehabilitation centres for raptors that are known to be at high risk for AR poisoning are recommended to further investigate the impact of chronic AR exposures on survival rates (Hindmarch et al., 2019).

Besides AR exposure in species foraging on terrestrial prey, the white-tailed sea eagle showed a considerable exposure to ARs as well. White-tailed sea eagles are mixed food web feeders that predominantly forage on fish but also on water birds, game species and carrion (Nadjafzadeh et al., 2016). Besides exposure routes from scavenging on poisoned prey, aquatic AR residues might also be taken up from the aquatic food web since ARs were detected in fish from Germany (Kotthoff et al., 2018; Regnery et al., 2019b, 2020a). Almost 80% of the municipalities in Germany indicated in a nation-wide survey that they were using ARs in sewage systems in 2017 (Regnery et al., 2020b), which was suggested to result in aquatic trophic transfers (Regnery et al., 2020a). AR residues were previously detected in a white-tailed sea eagle from Scotland (Hughes et al., 2013) whereas no AR residues were detected in sea eagles from Finland (Koivisto et al., 2018). AR concentrations in the current study were lower compared to species feeding dominantly on terrestrial prey, which could be a result of the feeding ecology and the use of rather pristine habitats in the north of Germany. However, further studies including stable isotope analyses are needed to determine the sources of contaminants since no ARs were detected in ospreys, a species that is known to exclusively prey on fish (Häkkinen, 1978). Even though samples sizes for ospreys were comparably small, the results indicate that the primary source of ARs for white-tailed sea eagles might be carcasses of smaller mammals.

4.1.2. Modelling exposure probability and influence on residue concentrations

Exposure to ARs is expected to occur at a very local scale where baiting stations are placed. Previous studies reported exposures to small mammals less than 100 m around baiting stations (Geduhn et al., 2014; Tosh et al., 2012). This can lead to a high risk for secondary poisoning in the direct surrounding of baiting stations. In the current study, secondary exposures increased with increasing urban habitat, which might be related to an enhanced use of ARs on public and private property rather than on the countryside. Similar observations were made for predators (including raptors) in Spain where the presence of SGARs was related to urban area and human population density rather than agricultural activity (López-Perea et al., 2019). Furthermore, associations between AR occurrence and urban areas were reported for red foxes (*Vulpes vulpes*) in Germany (Geduhn et al., 2015) as well as for urban wild boars (*Sus scrofa*) in Spain, where AR occurrence was positively related to human population and anthropization (Alabau et al., 2020). Other factors that have shown to be important determinants for AR residue occurrence were related to cattle and pig farm density (Geduhn et al., 2015; López-Perea et al., 2019), which might represent a risk for raptors in the northwestern parts of Germany where animal farming is most frequent. Although the degree of urban land cover was linked to the AR exposure probability in the current study, it was not related to the extent of exposure. This indicates that habitat composition, i.e. patterns of anthropogenic land use, may determine the probability of AR exposure, whereas species-specific ecological factors such as feeding ecology seem to be the main drivers for the extent of exposure. Due to the high risk of AR exposure in urban habitats, we recommend further studies on trophic magnification (e.g. songbirds, rodents, wild boars, foxes, raptors) in urban terrestrial food webs as done for legacy pollutants in Canada (Fremlin et al., 2020).

The higher risk of adults to be exposed to ARs compared to juveniles is expected to be attributed to the larger number of exposure events over time, which may ultimately lead to accumulation of compounds that reach detectable levels at a certain point. Similar observations were made for sparrowhawks in the UK (Walker et al., 2015) as well as for brodifacoum in raptors from Denmark (Christensen et al., 2012). However, red kites showed similar exposures of adults and juvenile birds in the UK (Walker et al., 2019), which is in line with a previous study reporting high exposures of juvenile red kites (Hughes et al., 2013). This emphasizes the susceptibility of red kites to be exposed to toxic AR concentrations.

Similar to our study, Christensen et al. (2012) and López-Perea et al. (2019) found no significant influence of cause of death on AR residues in various raptors from Denmark and Spain. ΣAR residues in the present study tended to be higher in raptors that died from unclear reasons compared to raptors that died from trauma, which indicates that AR poisoning often remains unnoticed during necropsy. The same trend was observed for raptors that died from infections. Interactions between infectious diseases and AR exposure were previously reported for voles (Vidal et al., 2009) but associations between chronic AR exposure and effects in wildlife species remain poorly characterised (Rattner et al., 2014b), which complicates the evaluation to which degree AR exposure contributes to the respective cause of death.

Sex did not influence the probability of AR exposure, which is in line with previous research (Christensen et al., 2012; Walker et al., 2015). Both the risk for AR exposure as well as the exposure extent tended to increase during the sampling period, which might be related to the intrinsic properties of AR as being persistent and bioaccumulative (Rattner et al., 2014b). Furthermore, ARs such as brodifacoum and difenacoum were expected to have an increased potential to partition and accumulate in fatty tissues as indicated by their octanol-water partition coefficient (log Kow > 4). We therefore expected higher AR concentrations in individuals with good nutritional status and associated lipid-rich livers, which was not

observed in the current study. This indicates that other factors such as the binding affinity of ARs to the active side of the vitamin K epoxide reductase (Rattner et al., 2014b) might be more influential for the accumulation in livers.

4.2. Medicinal products (MPs)

The NSAID ibuprofen (HMP) was the most frequently detected MP in the current study followed by the fluoroquinolone antibiotics, enrofloxacin (VMP) and its metabolite ciprofloxacin (HMP). Between 2002 and 2012, consumption of ibuprofen increased from 250 to 975 tonnes (t) per year making it one of the most sold HMPs in Germany (Küster and Adler, 2014). Ibuprofen has shown to be frequently detected (57%) in wastewater treatment plant effluents across Europe (Loos et al., 2013) as well as in surface waters in Germany (Bergmann et al., 2011). The highest detection rate of ibuprofen was found in white-tailed sea eagles, indicating that feeding on the aquatic food web may be responsible for exposures to ibuprofen. This is supported by the detection of ibuprofen in otters (Lutra lutra) from the UK as otters feed predominantly on fish (Richards et al., 2011). However, no ibuprofen residues were detected in ospreys, which might be related to the small sample size and varying impacts of wastewater treatment plant effluents at local foraging habitats, but further studies are needed to verify this assumption. Sorption of ibuprofen to sewage sludge is lower compared to e.g. diclofenac (Bergmann et al., 2011; Ternes et al., 2004) indicating that sewage sludge fertilisation did not represent a major exposure source. Furthermore, foraging on treated livestock can be excluded as a potential exposure source as ibuprofen is not registered as VMP. However, terrestrial exposures might still have occurred through agricultural applications of contaminated wastewater as ibuprofen had one of the highest environmental risk scores in wastewater samples intended for agricultural reuse (Alygizakis et al., 2020). Thus, the detection of ibuprofen in two northern goshawks might be attributed to exposures through agricultural wastewater reuse.

Sales of antibiotics for veterinary use has been registered in Germany since 2011 and fluoroquinolones have, in contrast to other antibiotics, constant sales of ~10 t per year (Wallmann et al., 2018). Fluoroquinolones are known to alter the normal microbiome and cause adverse effects on the embryonic development of birds (Hruba et al., 2019). The absorption of fluoroquinolones in organisms is driven by lipophilicity (Cabrera Pérez et al., 2002) and the liver represents a target tissue for metabolization after enrofloxacin administration (EMA, 2002). Enrofloxacin was previously detected in the plasma of griffon vulture nestlings (*Gyps fulvus*) in Spain, which was suggested to be related to foraging on

livestock prior to sampling as the half-live in bird tissues are short (<10 h) (Cox et al., 2004; Gómez-Ramírez et al., 2020). Furthermore, all investigated fluoroquinolones were detected in the plasma of griffon vulture nestlings, which was suggested to be associated with scavenging on livestock as well (Blanco et al., 2016). Marbofloxacin was not detected by the current study, which might reflect the lack of readily available livestock carcasses in Germany, different antibiotic use patterns as well as matrix specific differences. Exposure of one white-tailed sea eagle to ciprofloxacin but not enrofloxacin might be related to aquatic exposures as ciprofloxacin was detected in 90% of wastewater treatment effluents across Europe (Loos et al., 2013). However, transformations of enrofloxacin to ciprofloxacin are known to occur in mammals (Martinez et al., 2006) but correlations between both are not always observed in raptors (Gómez-Ramírez et al., 2020). Furthermore, ciprofloxacin sorbs to solids (Golet et al., 2003) and shows considerable concentrations in sewage sludge from Germany (max. 3500 μ g g⁻¹; Bergmann et al., 2011), which indicates that sewage sludge fertilisation may contribute to environmental exposures. In contrast to ciprofloxacin, enrofloxacin is used as VMP and exposures might rather be related to manure fertilisation as enrofloxacin (max. 8300 μ g l⁻¹) shows considerably higher concentrations in manure from Germany than ciprofloxacin (max. 28 µg l⁻¹; Bergmann et al., 2011).

As Germany prohibits the provision of livestock carcasses to scavengers, we assume that the detection of certain MPs in raptors in the present study might be explained by (i) the wide dispersive use in large quantities of MPs in both humans and livestock, and by (ii) the insufficient elimination capacity of wastewater treatment plants (Van Doorslaer et al., 2014). The latter potentially leads to high concentrations in water and sewage sludge intended for agricultural reuse. However, it cannot be ruled out that carcasses of treated companion and farm animals are available to scavengers. The fact that we found concentrations of both fluoroquinolones in one red kite at similar concentrations to pigs under treatment (5 mg kg⁻¹, 1 day post-dose; Garcia et al., 2005) might most likely be explained by the recent uptake of treated prey items as red kites frequently patrol dunghills on farms and settlements in the search of small carcasses. Alternatively, the red kite might have been treated, set free and later collided with a wind power plant, as the bird died from trauma next to a wind park. However, birds treated in captivity are normally marked with a ring prior to release and reported to the ringing centre to provide this type of information. Nevertheless, based on the frequency of detection and the exclusion of species treated in veterinary clinics, the results indicate that environmental exposures of raptors to ibuprofen and fluoroquinolone antibiotics prevails.

4.3. Plant protection products (PPPs)

The concentrations of the organophosphate insecticide dimethoate and its main metabolite omethoate in two red kites (Bra391, SH77; Tables SI–1) were a consequence of deliberate poisoning, which was confirmed as the cause of the death for the latter through the analysis of gut and gizzard content. Poisonings of raptors foraging in agricultural areas (such as the red kite) were reported as a frequent cause of death in Europe (Berny and Gaillet, 2008; Molenaar et al., 2017). As a consequence, poisonings of red kites in their winter grounds in Spain have shown to negatively impact the number of breeding pairs which even resulted in local extinctions (Mateo-Tomás et al., 2020). Together with AR poisonings, the current study adds evidence that poisonings represent a threat for red kites, which is expected to contribute declined survival rates in Germany (Katzenberger et al., 2019).

Both red kites with thiacloprid residues were found dead in agricultural habitats (NS57, S70; Table SI-8) where neonicotinoid (NNs) were commonly used as insecticidal seed dressings. The potential of NNs for persistency and bioaccumulation is low due to rapid metabolization and clearance (<24 h) in bird livers (Bean et al., 2019). Interestingly, NS57 was found dead in October (2009) and S70 in March (2015), which coincides with the timeframes at which sowing of winter and spring cereals occurs. During these timeframes, suspected NN poisoning of seed-eating farmland birds has shown to be most frequent (Millot et al., 2017). This is supported by a recent study showing that farmland birds have significantly higher concentrations of the NN clothianidin after sowing in autumn (Lennon et al., 2020). Therefore, facultative scavengers such as the red kite might be at risk for exposures shortly after NN application through foraging on acutely poisoned prey. Previous studies on NNs detected imidacloprid and thiacloprid in blood of European honey buzzards (Pernis apivorus) from Norway (Byholm et al., 2018) and imidacloprid in blood of an eagle owl nestling (Bubo bubo) from Spain (Taliansky-Chamudis et al., 2017). The authorisation of thiacloprid in the European Union was withdrawn in August 2020 due to its classification as being toxic for reproduction category 1 B as well as due to toxic groundwater exposures of metabolites (EC, 2019). Due to its low potential for bioaccumulation, persistency and potential rapid excretion from bird tissues, a long-term threat for raptors after its period of grace in the European Union (03/02/2021) is considered to be unlikely.

5. Conclusion

Our study demonstrates that AR contamination poses a threat to raptors in Germany. Whereas the use of urban habitats seems to determine exposure probability to ARs, species-specific

traits such as scavenging on smaller carcasses seem to explain the extent of exposure. The observed link between AR exposure probability and degree of urban land cover calls for further studies investigating terrestrial trophic transfers and associated risks in urban ecosystems. Among the investigated species, northern goshawks from urban habitats and red kites in general were at greatest risk for AR poisoning as levels exceeded thresholds associated with adverse effects. Together with deliberate insecticide poisoning, AR poisoning is considered to represent a threat to red kites and might ultimately contribute to decreasing survival rates in Germany. The detection of ARs in white-tailed sea eagles suggests that AR exposure might not be limited to terrestrial food webs but further studies including dietary proxies are needed to identify exposure sources. For MPs, the detection of ibuprofen and fluoroquinolone antibiotics suggests that their wide dispersive use in large quantities in combination with manure fertilization and incomplete wastewater removal results in environmental emissions. Most analysed and currently used PPPs were not detected, indicating that widespread contamination in the study region is unlikely. However, rapid metabolization of some PPPs in biological tissues indicates that other sample matrices such as blood from nestlings might be more adequate to assess spatiotemporal exposure scenarios in future. Taken together, the results of the current study demonstrate that ARs exposure represents a threat for facultative scavengers as well as for raptors living in urban habitats.

Funding

This work was supported by a grant through the World Wide Fund For Nature, Germany.

CRediT author statement

Alexander Badry: Conceptualization, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. Detlef Schenke: Methodology, Resources, Data curation, Validation, Writing – review & editing, Project administration. Gabriele Treu: Conceptualization, Writing – review & editing, Funding acquisition. Oliver Krone: Conceptualization, Investigation, Resources, 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.

Acknowledgements

We thank Susanne Auls for her assistance during necropsy, Alexandre Courtiol and Heribert Hofer for giving critical advice as well as Martina Hoffmann, Ramazan Koç, Christine Reichmann and Ina Stachewicz-Voigt for their support during residue analysis. We furthermore thank Lisa Giese and Nikolai Kraneis (https://nikolai-kraneis.com) for providing species illustrations for the graphical abstract.

Appendix A. Supplementary data

The following is the Supplementary data to this article:

Download : Download Acrobat PDF file (1MB)

Multimedia component 1.

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