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Differential exposure to second-generation anticoagulant rodenticides in raptors from continental and insular regions of the Iberian Peninsula☆

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ABSTRACT

The global impact of anticoagulant rodenticides (ARs) on non-target species is well-recognized. Birds of prey, as apex predators, are highly vulnerable to AR exposure and are widely used as biomonitors for priority pollutants in Europe. This study investigates differential SGAR exposure in raptors from insular versus continental regions, hypothesizing greater exposure in insular areas due to ecological factors like reduced prey diversity, intensive rodenticide use, and resistant rodent populations. We analyzed the livers of 190 common kestrels (*Falco tinnunculus*) and 104 common buzzards (*Buteo buteo*) across the Iberian Peninsula and its archipelagos using LC-MS/MS to assess their role as AR sentinels and the differences between insular and continental areas. Results revealed a high prevalence (>80%) of second-generation anticoagulant rodenticides (SGARs), with brodifacoum and bromadiolone, being the most frequent. Multiple SGAR detections were also common (≈50%). A binomial logistic regression showed that species and region significantly influence the likelihood of SGAR exposure. Kestrels had a greater probability of exceeding 100 ng/g wet weight (ww) compared to buzzards. Raptors from insular territories were ten times more likely to have higher SGAR concentrations than those from continental areas. However, the legal restriction on SGAR bait concentrations that came into effect in 2018 did not significantly impact exposure levels. This study highlights the need for targeted conservation efforts to mitigate AR exposure risk in vulnerable island ecosystems.

1. Introduction

Pest management, particularly concerning rodents, remains essential for public health, food safety, and resource conservation. Despite the availability of mechanical and biological approaches, chemical control methods remain predominant due to their large-scale effectiveness (Jacob et al., 2020; Labuschagne et al., 2016; Luna et al., 2020; Memmott et al., 2017; Walther et al., 2024). Among these, second-generation anticoagulant rodenticides (SGARs) emerge as the primary option to address this issue. These compounds act by inhibiting the Vitamin K epoxide reductase complex (VKORC), interrupting the vitamin K cycle and altering the coagulation cascade (Ishizuka et al., 2008; Nakayama et al., 2019). However, the symptoms associated with coagulopathy are not always evident and several animals may be asymptomatic (Rached et al., 2020). Moreover, exposure to these biocides has been linked to possible physiological and behavioral alterations that, while not lethal, pose a risk to the survival of both target and non-target species (Martín-Cruz et al., 2024; Martínez-Padilla et al., 2016; Murray, 2018; Rattner et al., 2014; Sánchez-Barbudo et al., 2012; Serieys et al., 2018).

Furthermore, recent evidence has shown resistance to these products in target species across Europe (Carromeu-Santos et al., 2023; Damin-Pernik et al., 2022; Krijger et al., 2022), as well as the ineffectiveness of regulatory measures for wildlife protection, leading to higher risks for non-target wildlife including birds of prey (Carrillo-Hidalgo et al., 2024;

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George et al., 2024; Moriceau et al., 2022) and mammals (Campbell et al., 2024). These findings highlight the persistent challenge of controlling rodenticide use and protecting wildlife from secondary poisoning. Studies from Spain, the UK, and France have demonstrated the limited effectiveness of existing regulatory frameworks on wildlife. These studies examined the impact of public policy measures, including European Union regulations (EU) 528/2012 and (EU) 2016/1179, which mandate the use of bait stations, outdoor use restrictions, and lower anticoagulant concentrations in baits (Carrillo-Hidalgo et al., 2024; Moriceau et al., 2022). Similarly, studies on industry-led stewardship schemes implementing new rodenticide regulations also reported limited effectiveness (Campbell et al., 2024; George et al., 2024).

These inefficiencies stem from the difficulty of managing widespread use of SGARs in agricultural and urban environments, where resistance in target species drives the continuous use of baits, exacerbating the exposure risk for non-target wildlife. Specifically, in the Iberian Peninsula and the Azores and Madeira archipelagos, the widespread distribution of resistance-conferring mutations in the Vkorc1 gene among house mouse populations has severely diminished the effectiveness of first-generation, and some second-generation anticoagulant rodenticides (Bermejo-Nogales et al., 2022; Carromeu-Santos et al., 2023). This resistance leads to greater environmental contamination, as rodenticides persist in ecosystems and bioaccumulate in non-target species like birds of prey and mammals, exacerbating the risks of secondary poisoning and biomagnification in the food chain (Carromeu-Santos et al., 2023). The use of rodenticides in areas where resistance has been documented potentiates the exposure risks to wildlife due to the continuous selection of non-susceptible rodent populations.

Among the non-target species exposed to these compounds, birds of prey stand out significantly. As apex predators with long lifespans and wide-ranging territories, they are highly vulnerable to AR exposure and serve as invaluable environmental sentinels (Gomez et al., 2022; Moriceau et al., 2022; Nakayama et al., 2019; Pay et al., 2021; Rial-Berriel et al., 2021a). In Europe, the use of raptors for this purpose is widespread, with the European Raptors Biomonitoring Facility currently coordinating pan-European monitoring efforts to track priority pollutants (ERBFacility, 2024). However, despite these efforts, limitations in the data collected through official wildlife poisoning databases may hinder our full understanding of the extent of contamination in non-target species (Fernández-García et al., 2024). Strengthening the role of sentinel species, such as raptors, is critical to overcoming these data limitations and enhancing our capacity to monitor environmental contaminants effectively. In this regard, the initial step towards achieving this goal involves selecting the most suitable sample and candidate species based on their ecological traits (Badry et al., 2020; Espín et al., 2016; Gómez-Ramírez et al., 2014; Ramello et al., 2022).

The common kestrel (*Falco tinnunculus*) and the common buzzard (*Buteo buteo*), both diurnal raptors belonging to the orders Falconiformes and Accipitriformes respectively, have been extensively studied across Europe as biomonitoring subjects, indicating their potential as indicators of exposure to ARs (Badry et al., 2022; Carrillo-Hidalgo et al., 2024; Gómez-Ramírez et al., 2014; Ozaki et al., 2024; Roos et al., 2021). Widely distributed in the Iberian Peninsula and its archipelagos, these birds of prey are particularly suitable as biomonitoring agents due to their adaptability to diverse environments, including urban and agricultural areas, and their generalist predator diet, which includes invertebrates, small mammals, reptiles, birds, and amphibians (Carrillo et al., 2017; Orihuela-Torres et al., 2017; Rodríguez et al., 2010; Tapia et al., 2007; Zuberogoitia et al., 2006). However, they face significant threats to their conservation such as habitat destruction or modification, intentional killing, power line collisions or nest poaching among others (Butet et al., 2022; McClure et al., 2018; Tapia et al., 2017). Their ecological relevance, combined with their position at the top of the food chain, makes them highly vulnerable to bioaccumulation of SGARs.

These challenges become particularly relevant in insular territories such as the Macaronesian islands. These islands, like other isolated regions, face unique challenges in managing invasive species and pest control, where SGARs are widely used. This situation is exacerbated by the inherent vulnerability of island ecosystems, characterized by lower prey diversity, which increases the risks of bioaccumulation and poses serious ecological risks (Carromeu-Santos et al., 2023; Fisher et al., 2019; Martín-Cruz et al., 2024). In the Macaronesian region, previous studies conducted in the Canary Islands have demonstrated widespread exposure to anticoagulant rodenticides (ARs) among various wildlife species, such as native birds of prey, mammals, and invasive reptiles (Carrillo-Hidalgo et al., 2024; Martín-Cruz et al., 2024; Rial-Berriel et al., 2021a, 2021c; Ruiz-Suárez et al., 2014). However, raptor exposure to ARs has been sparsely documented in mainland Portugal, and to our knowledge, no data is available on this issue in its archipelagos (Grilo et al., 2021). Nevertheless, AR resistance has been observed in rodent populations in the archipelagos of Madeira and the Azores, leading us to hypothesize that this could increase the risk of bioaccumulation and biomagnification in raptors on the Portuguese islands which feed on these preys (Carromeu-Santos et al., 2023). The exposure of native raptors subspecies - such *F. tinnunculus canariensis, F. tinnunculus dacotiae*, *B. buteo insularum* in the Canary Islands and *B. buteo harterti* in Madeira-to SGARs is of critical concern given their role in maintaining the balance of fragile island ecosystems and highlights the considerable ecological risks posed by these compounds in the archipelago. Protecting this biodiversity from anthropogenic threats, such as the use of chemical products, is of paramount importance.

Following European monitoring efforts for wildlife conservation, the objectives of this study were: (i) to evaluate the exposure of common kestrels and common buzzards to ARs in the Iberian Peninsula and its Atlantic islands; (ii) to investigate their potential as sentinels of AR exposure in these territories; (iii) to assess the difference in AR exposure between insular and mainland regions.

2. Material and methods

2.1. Study area

The Iberian Peninsula, encompassing continental Spain and Portugal, is situated in southwestern Europe, spanning 583,254 km^2 between the Mediterranean Sea and the Atlantic Ocean. This region's strategic position, coupled with its diverse climates and landscapes, supports a wide range of habitats and species (Araújo et al., 2007). The natural richness of the Iberian Peninsula is particularly evident in its archipelagos - Azores, Madeira, Canary Islands, and Selvagens Islands-located in the eastern North Atlantic. These islands, which are part of the Macaronesian region within the European Union, are known for hosting a significant number of endemic species (Florencio et al., 2021). Approximately 30% of the land area is designated as Special Protection Areas for Birds (SPABs) and/or Community Interest Sites (CISs) (Sundseth et al., 2010).

To collaborate in the protection of this biodiversity, livers from kestrels and buzzards across the Iberian Peninsula and some of its Atlantic islands were analyzed as the most suitable organ for detecting ARs (Espín et al., 2016). The territorial representation of the studied animals included six districts of mainland Portugal (Faro, Beja, Portalegre, Setúbal, Évora, Castelo Branco), the Community of Madrid in Central Spain, and some neighboring provinces (Toledo, Segovia, Guadalajara, and Palencia), the island of Madeira, and seven out of the eight Canary Islands (Gran Canaria, Tenerife, Fuerteventura, Lanzarote, La Palma, La Gomera and La Graciosa) (Fig. 1).

2.2. Sampling and ethical statements

Liver samples were collected during necropsies of 190 kestrels and 104 buzzards from seven different recovery centers (*Centro de Estudos e Recuperação de Animais Selvagens* (CERAS), *Centro de Recuperação e Investigação de Animais Selvagens* (RIAS), *Centro de Recuperação de*

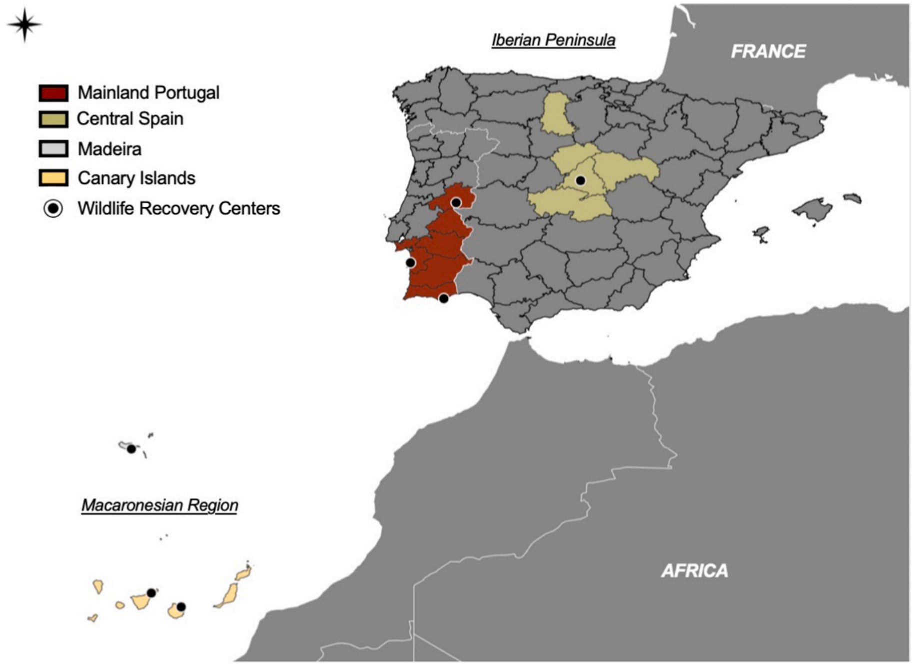

Fig. 1. Map of the Iberian Peninsula (Mainland Spain and Portugal) and the Macaronesian islands involved in the study. Each territory is represented with a specific color as shown in the legend. The wildlife recovery centers participating in the sampling are marked by black circles, also detailed in the legend. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
Fig. 1General map of the Iberian Peninsula, Canary Islands and Madeira, indicating each island and continental territory with a specific color in the legend ***(Left).*** Enlarged map of the continental and island regions with the location of the recovery centers participating in the sampling represented with a black circle included in the legend ***(Right).***

Animais Selvagens de Santo André (CRASSA) – in mainland Portugal; *Grupo de Rehabilitación de la Fauna Autóctona y su Hábitat* (GREFA) - in peninsular Spain; *Centro de Recuperação de Animais Selvagens* (CRAS) - in Madeira Island and *Centro de Recuperación de Fauna Silvestre La Tahonilla and Tafira* - in the Canary Islands), between 2014 and 2024 (Fig. 1). No animals were sacrificed for the purpose of this study. Instead, birds were incidentally found in nature, died after hospitalization, or were euthanized due to irreversible injuries at rehabilitation centers. Necropsies were performed by veterinary professionals in all cases. Additionally, all carcasses and the livers extracted during necropsies were stored frozen at −20 °C until chemical analyses at the Toxicology Service (SERTOX), Department of Toxicology, University of Las Palmas de Gran Canaria.

Given the diverse origin of raptors and the extensive spatiotemporal range of the study, it was not feasible to systematically collect data or conduct complete post-mortem analysis on all birds of prey. Therefore, the exact GPS location of individuals and valuable biological or anatomopathological variables remain unknown.

2.3. Analysis of anticoagulant rodenticides in liver tissue

For the analysis of anticoagulant rodenticides in liver tissue, procedural-internal standards (P-IS, (±)- Warfarin-d5) and certified ARs standards, including warfarin, diphacinone, chlorophacinone, coumachlor, coumatetralyl, brodifacoum, bromadiolone, difethialone, difenacoum, and flocoumafen, with purity levels ranging from 98.0% to 99.8%, were sourced from Dr. Ehrenstorfer in Augsburg, Germany. Mass spectrometry grade acetonitrile (ACN), methanol (MeOH) and formic acid (FA) with 98% purity, were procured from Honeywell in Morristown, NJ, USA. Water for the study was produced in our facilities through a MilliQ A10 water purification system by Millipore in Molsheim, France. The QuEChERS Extract Pouch, AOAC Method, containing 6 g of magnesium sulfate and 1.5 g of sodium acetate, was obtained as commercial premixes from Agilent Technologies in Palo Alto, CA, USA.

Liver sample extraction followed a methodology previously validated by our research team (Rial-Berriel et al., 2021b, 2020). Briefly, 1 g of liver tissue was initially disaggregated and homogenized with 4 mL of MilliQ water at 6,500 rpm for 2 sets of 30 s using a Precellys Evolution homogenizer from Bertin Technologies in Rockville, Maryland, USA. Subsequently, 1 g of the homogenate was manually shaken with 2 mL of ACN 0.5% FA in a 5 mL Eppendorf tube and sonicated for 20 min using equipment from VWR (Selecta, Barcelona, Spain). Further processing involved adding 480 mg of anhydrous magnesium sulfate and 120 mg of sodium acetate to each sample tube, followed by vortex mixing for 30 s and manual shaking for 1 min. After centrifugation at 3,125 g for 5 min at 2 °C, the supernatant was filtered through a 0.2 μm Chromafil PET-20/15 filter into glass amber vials.

Quality Control samples (QCs) were prepared using a blank chicken liver matrix to ensure methodological consistency. A ten-point calibration curve covering a concentration range of 0.195–100 ng/g was meticulously constructed following the same extraction protocol outlined earlier. Similarly, QCs were established at a single concentration of 5 ng/g (with RSD $\leq$20% and REC = 70–120% for QCs, LODs and LOQs; Supplementary Table 1). All samples, QCs, calibration points, and blanks were spiked with the P-IS solution before extraction, and concentrations were expressed as wet weights (ww).

For the detection and quantification of ARs, an Agilent 1290 UHPLC system coupled with an Agilent 6460 triple quadrupole mass spectrometer was employed. The chromatographic setup included a heated InfinityLab Poroshell 120 column with an inline filter and UHPLC guard column. A gradient mobile phase consisting of 0.1% FA and 2 mM ammonium acetate in water (Phase A) and 2 mM ammonium acetate in MeOH (Phase B) was employed. Injection volume and flow rate were set at 8 μL and 0.4 mL/min, respectively. The mass spectrometer operated in dynamic multiple reaction monitoring (dMRM) mode across both polarities, with specific cycle, dwell, and run times. Detailed operational parameters for the Agilent Jet Stream Electrospray Ionization Source (AJS-ESI) and the gases used can be found in the referenced methods for further validation parameters.

2.4. Statistical analyses

R software v4.1 and JAMOVI® v.2.4.7 (R Core Team, 2022; The Jamovi Project, 2023) were used to conduct descriptive and inferential analysis in this study. All the analysis conducted were centered on SGARS, given the non-detection of FGARs in the studied raptors.

The initial step involved a comprehensive evaluation of variable distributions. The Shapiro- Wilk test revealed that the concentrations of SGARs did not follow a normal distribution even after a logarithmic transformation. As a result, descriptive statistics were represented using the median and interquartile range ($p25^{th}$ - $p75^{th}$), and the frequency of detection was determined as the percentage of raptors with at least one detected SGAR in their livers (Supplementary Tables 2 and 3).

For statistical analysis, raptors with concentrations below the limit of quantification (LOQ) but above the limit of detection (LOD) were assigned a random value between these two limits. Concentrations below LOD were considered non-detected and were assigned a random value between zero and half of the LOD.

To better understand the factors influencing the likelihood of higher concentrations of SGARs in raptors, a Binomial Logistic Regression model was constructed. The data were dichotomized at 100 ng/g ww, guided by thresholds often considered indicative of possible/likely toxicity in raptors (Lohr, 2018; Pay et al., 2021), with a 50% probability of ΣSGARs toxicity reported in species within the Falconidae and Accipitridae families (Elliott et al., 2024). The forward selection procedure was used for model construction, with Akaike's Information Criteria guiding the selection process. The independent variables included *species* (*Falco tinnunculus* and *Buteo buteo*), *region* (Continental: central Spain and mainland Portugal *vs*. Insular: Canary Islands and Madeira Island), and *legal modification* in baits concentrations in 2018 (Before or after legal modification) which resulted in a significant reduction from the traditional concentration of 50 ppm to <30 ppm of biocidal agent in baits (Commission Regulation, 2016; Frankova et al., 2019). No other variables could be explored due to lack of information.

For comparative analyses between continental and insular regions of each country, nonparametric tests were employed due to the non-normal data distribution of the data. Specifically, a Mann-Whitney *U* test was employed for pairwise comparisons to assess the exposure of ΣSGARs in continental and insular territories from Spain (Central Spain vs Canary Islands) and Portugal (Mainland Portugal vs Madeira Island).

Statistical significance was set at $p \leq 0.05$ for all analyses in this study. Finally, figures were generated using GraphPad Prism v10.2.3 (GraphPad Software, CA, USA).

3. Results

This study analyzed a total of 294 livers collected between 2014 and 2024 from two raptor species: *Falco tinnunculus* (65%, n = 190) and *Buteo buteo* (35%, n = 104). The distribution of the samples across different geographic regions was as follows: 39% (n = 115) from Central Spain, 20% (n = 58) from mainland Portugal, 38% (n = 113) from the Canary Islands, and 3% (n = 8) from Madeira Island (Fig. 2). Regarding

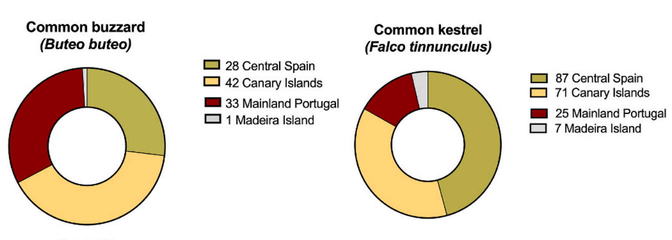

Fig. 2. Doughnut chart showing the distribution of common buzzard ***(Left)*** and common kestrel ***(Right)*** samples by regions (Central Spain, Canary Islands, Mainland Portugal, and Madeira Island).

the spatiotemporal sampling period, 9% of the samples were obtained before the 2018 legal restriction on SGAR bait concentrations (Frankova et al., 2019), while 89% were collected after this legal modification. The year of admission to the wildlife recovery center was unknown for the remaining 6 specimens.

3.1. Descriptive analyses of ARs in raptors' livers

Among the 10 rodenticides analyzed, only second-generation anticoagulant rodenticides (SGARs) were detected, including brodifacoum, bromadiolone, difenacoum, flocoumafen, and difethialone. In the study, 81.7 % (n = 85) of common buzzards and 84.7% (n = 161) of common kestrels tested positive for at least one SGAR (Fig. 3). The highest frequencies of detection were recorded on island territories of both countries (>95%). Additionally, around half of the positive animals (45.2% of buzzards and 58.9 % of kestrels) were simultaneously exposed to two or more rodenticides. (Fig. 3).

In detail, buzzards were primarily exposed to brodifacoum, bromadiolone, and difenacoum in decreasing order and across all regions, except in Madeira Island where the only buzzard sampled was exposed exclusively to bromadiolone (Supplementary Table 3). Similarly, kestrels in the Canary Islands and mainland Portugal followed this pattern, although in Central Spain and Madeira Island, the third most prevalent compound was difethialone (Supplementary Table 2). Moreover, the highest concentrations of the study were recorded in kestrels from Madeira Island with a maximum value of 602.1 ng/g ww of bromadiolone.

Bromadiolone and brodifacoum were the most frequently detected SGARs, reflecting their widespread use and persistence in ecosystems, which contribute significantly to AR contamination in raptors. The most common pairwise combination for both species was brodifacoum-bromadiolone, while the most frequent triple combinations were brodifacoum-bromadiolone-difenacoum and brodifacoum-bromadiolone-difethialone, the latter especially noted in kestrels from central Spain and Madeira Island.

Specifically, brodifacoum was detected in 95.8% of kestrels from the Canary Islands (median: 19.03 ng/g ww), 100% from Madeira (median: 60.96 ng/g ww), 59.8% from Central Spain (median: 1.24 ng/g ww), and 92% from mainland Portugal (median: 3.86 ng/g ww) (Supplementary Table 2). Similarly, bromadiolone was detected in 78.9% of kestrels from the Canary Islands (median: 15.66 ng/g ww), 100% from Madeira (median: 106.75 ng/g ww), 43.7% from Central Spain (median: 8.38 ng/g ww), and 52% from mainland Portugal (median: 14.90 ng/g ww). In buzzards, brodifacoum was the most prevalent SGAR in the Canary Islands (95.2%, median: 32.35 ng/g ww), followed by Central Spain (67.9%, median: 0.92 ng/g ww) and mainland Portugal (54.5%, median: 1.58 ng/g ww) (Supplementary Table 3). Furthermore, bromadiolone was detected in 50% of buzzards from the Canary Islands (median: 3.22 ng/g ww), 32.1% from Central Spain (median: 3.79 ng/g ww), and 39.4% from mainland Portugal (median: 6.46 ng/g ww).

While brodifacoum and bromadiolone were the predominant SGARs, difenacoum, difethialone, and flocoumafen were also detected, though with lower frequencies and concentrations. Difenacoum was identified mainly in kestrels from Madeira Island and the Canary Islands, as well as in buzzards from Central Spain and the Canary Islands, while difethialone was more frequently found in kestrels from Central Spain and Madeira, and in buzzards from the Canary Islands. Flocoumafen was the least detected SGAR, present sporadically across regions. These results highlight the predominance of brodifacoum and bromadiolone as the key contributors to SGAR contamination in these regions, with considerable variability in concentrations between different geographic locations and less frequent exposure to other SGARs.

Finally, the use of ΣSGARs for the estimation of the potential toxicological risk levels showed that 82% (n = 241) of the animals were exposed to concentrations below 100 ng/g ww, 12% (n = 34) between 100 and 200 ng/g ww, and 6% (n = 19) at concentrations above 200 ng/

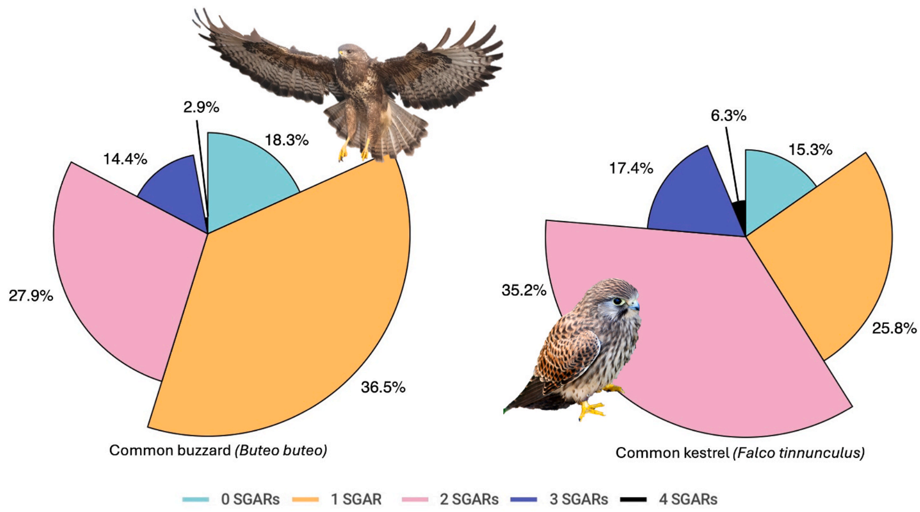

Fig. 3. ***Left.*** Number of SGARs per animal, expressed as percentage in the common buzzard *(Buteo buteo;* n = 104). ***Right.*** Number of SGARs detected per animal, expressed as percentage in the common kestrel *(Falco tinnunculus;* n = 190).

g ww (Fig. 4). Additionally, it highlights the percentage of kestrels exposed to concentrations above 100 ng/g compared to buzzards, being nearly twice as high (Table 2).

3.2. Influence of species, region, and legislative changes on SGAR concentrations

A binomial logistic regression, including the variables species, region, and legislative changes, was conducted to assess the likelihood of the animals presenting ΣSGARs concentrations above 100 ng/g ww. The analysis showed that legislative changes did not significantly impact ΣSGARs concentrations (p = 0.979), either positively or negatively. However, raptors from island territories were ten times more likely to present concentrations above 100 ng/g ww. When compared to animals from continental regions [OR (95% CI) = 10.74 (4.86–23.72); p < 0.001]. Additionally, the species *Falco tinnunculus* had more than twice the probability of having high ΣSGARs concentrations compared to

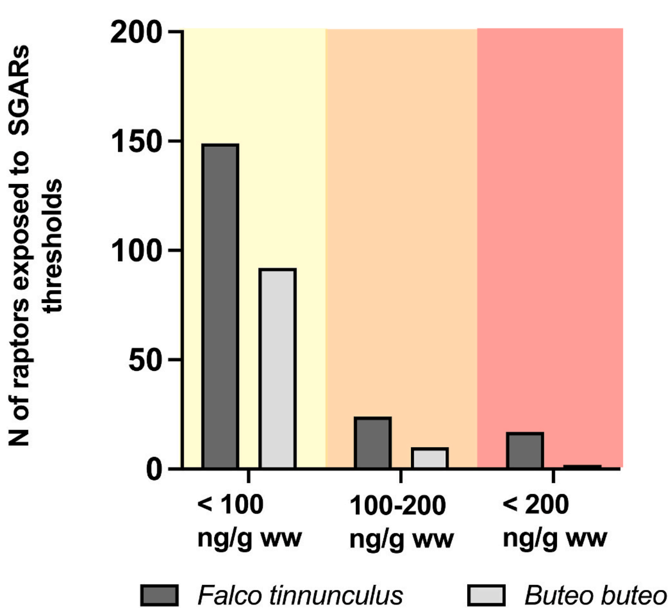

Fig. 4. Number of raptors' livers with ΣSGARs concentration within each toxicity threshold (<100 ng/g ww, 100–200 ng/g ww, >200 ng/g ww) for both raptor species, *Falco tinnunculus* and *Buteo buteo.*

Table 1
Best fitting model explaining the presence (threshold set at 100 ng/g ww.) of SGARs in the studied raptors (n = 294).

Variables	Estimate	SD	OR (95% CI)	p	AIC
Intercept	−3.48	0.83	0.03 (0.01–0.16)	<0.001	230.54
Species:					
Falco tinnunculus – Buteo buteo	0.85	0.38	2.35 (1.10–4.99)	0.027	
Region:					
Insular – Continental	2.37	0.40	10.74 (4.86–23.72)	<0.001	
Legal modification (EU 2016/1179):					
After – Before	−0.02	0.82	0.98 (0.19–4.92)	0.979	

Note: Model outcomes are summarized as the estimated regression parameters (Est.) with standard errors (SE), odds ratio (OR) and correspondent 95% confidence interval (95% CI), and p-values from a Binomial Logistic Regression model. The Akaike's Information for the model is also reported. Response variable: threshold at 100 ng/g ww.

Table 2
Summary of the variables considered for inclusion in the model categorized based on the threshold set at 100 ng/g ww.

Variables	Categories	N total	<100 ng/g ww. N (%)	>100 ng/g ww. N (%)
Species	*Buteo buteo*	104	92 (88.5)	12 (11.5)
	Falco tinnunculus	190	149 (78.4)	41 (21.6)
Region	Continental	173	164 (94.8)	9 (5.2)
	Insular	121	77 (63.6)	44 (36.4)
Legal modification (EU 2016/1179)	Before modification	26	24 (92.3)	2 (7.7)
	After modification	262	211 (80.6)	51 (19.4)

Note: The 6 individuals missing for the "*legal modification*" variable could not be included due to lack of information regarding the year of admission to the Wildlife Recovery Center.

Buteo buteo [OR (95% CI) = 2.35 (1.10–4.99); p = 0.027] (Table 1). Additionally, as illustrated in Fig. 3, differences in SGAR exposure between species were evident. A higher percentage of common kestrels were exposed to two or more SGARs compared to common buzzards, which were predominantly exposed to one SGAR. This difference suggests a variation in SGAR exposure and accumulation patterns between the two species.

Moreover, nonparametric tests comparing the insular and continental territories by countries revealed a significant difference in their ΣSGARs concentrations, with significantly higher levels detected in both the Canary Islands and Madeira ($p < 0.001$) (Fig. 5). However, it is important to acknowledge the limitations in comparing Portugal territories due to the small sample size of the Madeira Island group (n = 8). Nevertheless, despite these limitations, the data obtained from the descriptive analysis of this group are alarming. Kestrels from this island exhibited the highest ΣSGARs concentrations within the overall series (max = 643.5 ng/g ww), and the disparity between their medians values is substantial (Madeira Island = 298.04 ng/g ww; mainland Portugal = 16.56 ng/g ww) (Supplementary Table 2).

4. Discussion

The results of this study provide new evidence supporting the suitability of common kestrels and common buzzards as effective sentinels for AR exposure in the Iberian Peninsula and its islands. These findings reinforce their status as reliable biomonitors for these compounds across Europe (Badry et al., 2020; Gómez-Ramírez et al., 2014). Furthermore, the study introduces a novel line of research by highlighting the variations in AR exposure between insular and continental European regions. This distinction highlights the importance of protecting island biodiversity, where endemic species, such as the kestrel subspecies F. *tinnunculus canariensis and F. tinnunculus dacotiae*, as well as buzzards like *B. buteo insularum* and *B. buteo harterti*, play a pivotal role in island ecosystems (Sundseth et al., 2010). Their exposure to SGARs, alongside other species, further underscores the far-reaching impacts of rodenticides on biodiversity and ecosystem health (Fisher et al., 2019).

4.1. Descriptive analysis of ARs in raptor's livers

The presence of anticoagulant rodenticides at high frequencies indicates a significant level of exposure of non-target wildlife to these biocides within the study areas. The prevalence of second-generation anticoagulant rodenticides (SGARs) in both raptor species analyzed - exceeding 80% in each - is consistent with recent findings from the Canary Islands, where raptors species, including kestrels and buzzards, displayed alarming exposure rates, with over 90% of the analyzed birds testing positive for ARs (Carrillo-Hidalgo et al., 2024; Martín-Cruz et al., 2024). Moreover, these findings align with studies conducted in the UK and Denmark, where detection rates for these species also surpass 80% (Christensen et al., 2012; Ozaki et al., 2024). However, within the same regions, other UK- based studies have reported lower detection rates, ranging between 50 and 70% (George et al., 2024; Roos et al., 2021). Similarly, lower detection frequencies have been observed across other European regions, including Scotland, mainland Spain, Germany, and France (Badry et al., 2022; Fourel et al., 2024; Hughes et al., 2013; Moriceau et al., 2022; Ruiz-Suárez et al., 2014; Sánchez-Barbudo et al., 2012). In these studies, exposure levels in kestrels and buzzards often vary due to differences in monitoring periods, species susceptibility, and the types of rodenticides employed. Furthermore, the variation in the biological matrix and sample size may account for the inconsistencies observed across different regions and studies (Badry et al., 2022; Moriceau et al., 2022; Ruiz-Suárez et al., 2014).

Moreover, the absence or minimal detection of first-generation anticoagulant rodenticides (FGARs) in this study is consistent with recent global findings (Carrillo-Hidalgo et al., 2024; Martín-Cruz et al., 2024; Moriceau et al., 2022; Pay et al., 2021). This trend may be attributed to the chemical properties of SGARs, which are more potent and persistent, as well as to regulatory restrictions and the genetic resistances, which have contributed to a feeling of inefficacy of FGARs on the users' perspective (López-Perea and Mateo, 2018; Rattner et al., 2014). In particular, both Carrillo-Hidalgo et al. (2024) and Martín-Cruz et al. (2024), reported similar results in insular environments such as the Canary Islands, where SGARs dominated the detected compounds, and FGARs were almost absent. These studies also highlighted the high frequencies of SGARs (>90%) and the frequent detection of multiple SGARs (>50%), possibly due to the intense use of these compounds for pest control, corroborating our findings of significant contamination in wildlife populations in island territories (Carrillo-Hidalgo et al., 2024; Martín-Cruz et al., 2024; Ruiz-Suárez et al., 2014). Similar trends have been observed globally with other raptor species mainly exposed to SGARs and showing high levels of exposure to multiple rodenticides,

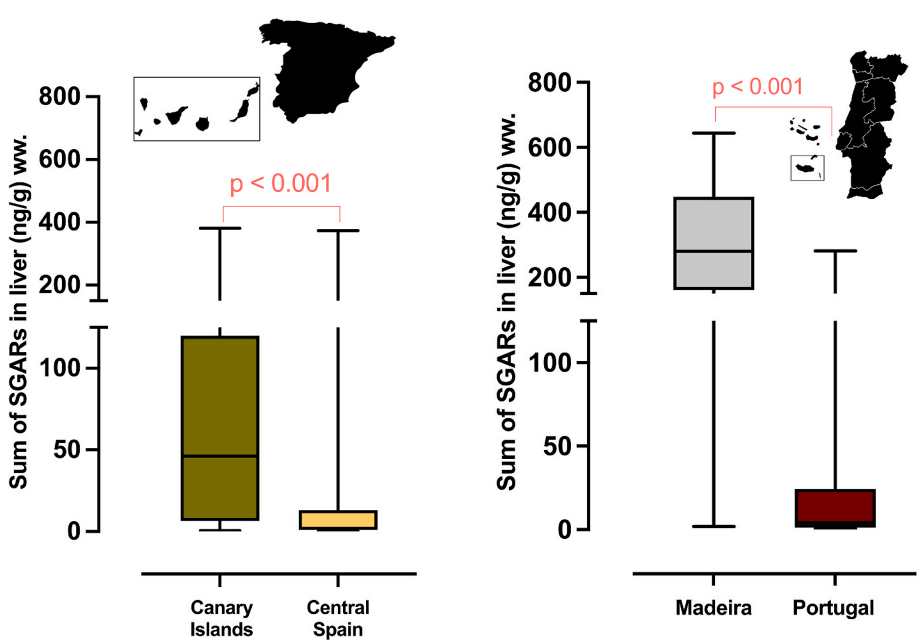

Fig. 5. Box and whisker plot showing the comparison of ΣSGARs in both countries between their mainland and insular regions (***Left.*** Mainland and insular comparison in Spain; ***Right.*** Mainland and insular comparison in Portugal). The lines represent the medians, the boxes the 25th to 75th percentiles, and the minimal and maximal values are shown at the ends of the bars.

ranging between 40 and 80%, even during early life stages (Cooke et al., 2023; Fourel et al., 2024; Christensen et al., 2012; Pay et al., 2021; Spadetto et al., 2024). Additionally, the identification of brodifacoum and bromadiolone as the predominant SGARs aligns with previous research on raptors in the Canary Islands (Carrillo-Hidalgo et al., 2024; Martín-Cruz et al., 2024; Rial-Berriel et al., 2021c). However, our results indicate a particularly high prevalence of brodifacoum, which differs with other European studies, such as those in the UK and Denmark, where difenacoum and bromadiolone were more commonly detected in kestrels and buzzards (George et al., 2024; Christensen et al., 2012; Ozaki et al., 2024; Roos et al., 2021). This variation may be related to differences in the commercial products available in each region (George et al., 2024; Christensen et al., 2012; Ozaki et al., 2023; Roos et al., 2021). Nevertheless, among SGARs, the high prevalence of brodifacoum in this study is concerning due to its toxicity in birds and its continued detection despite being strictly prohibited in open spaces (ECHA, 2016; European Commission, 2024). Additionally, although detected at lower frequencies, difenacoum, difethialone, and flocoumafen were also present, indicating a broader spectrum of SGAR contamination across regions.

4.2. *Variables influencing ARs exposure*

Published threshold values for interpreting SGAR hepatic concentrations vary considerably. Determining rodenticide concentrations in the environment relative to toxicological risk exposure is complex, due to individual and species-specific susceptibility differences, as well as exposure to multiple ARs, among other factors (Elliott et al., 2024; Fourel et al., 2024; Lohr, 2018; Rattner and Harvey, 2021; Thomas et al., 2011). Nonetheless, estimating the probable impacts on exposed animals is necessary to better understand the risks posed to wildlife by these compounds.

This study investigated the influence of legislative modifications, species and region on the likelihood of concentrations exceeding 100 ng/g ww ΣSGARs. This threshold seems appropriate given the widespread use of concentrations in the range of 100–200 ng/g in similar studies and appears suitable for both raptor species (Elliott et al., 2024; Lohr, 2018; Pay et al., 2021). This value is highly relevant to our study, given the exposure levels observed in our samples and the ecological sensitivity of island populations.

Considering these factors, the inclusion of the variable related to legislative modification (EU) 2016/1179 that took effect in 2018, which reduced SGAR concentrations in baits from 50 to 30 μg/g (Frankova et al., 2019) did not show significant effects on ΣSGAR concentrations. This suggests that regulatory measures may not be achieving the desired effect on wildlife protection. Recent studies from Spain and other European countries, including France and the United Kingdom, have reported similar findings evaluating the same legislative modification (Carrillo-Hidalgo et al., 2024) and other regulatory initiatives, such as new rodenticide regulations implemented through an industry-led stewardship scheme and the (EU) 528/2012 regulation, which mandates the use of bait stations and outdoors restrictions (Campbell et al., 2024; George et al., 2024; Moriceau et al., 2022). Moreover, they noted a significant increase in brodifacoum exposure post-stewardship (Campbell et al., 2024; Carrillo-Hidalgo et al., 2024; George et al., 2024; Moriceau et al., 2022; Ozaki et al., 2024). This fact could be due to the inappropriate use of this restricted compound or the longer half-life and persistence of the brodifacoum compared to other SGARs (George et al., 2024; Ozaki et al., 2024).

Regarding species-specific differences in SGAR exposure identified in this study, kestrels were significantly more likely to present ΣSGAR concentrations exceeding 100 ng/g ww than buzzards, with kestrels showing more than double the prevalence of such concentrations. Similar findings have been reported, with nearly twice as many kestrels exhibiting concentrations over 200 ng/g compared to buzzards (Fourel et al., 2024; Hughes et al., 2013; Christensen et al., 2012). Additionally, our research team also found higher prevalence of concentrations (>200 ng/g ww) in kestrels from Tenerife, Canary Islands (Carrillo-Hidalgo et al., 2024), further highlighting the vulnerability of this species in insular environments and reinforce the high exposure of these birds of prey. Moreover, the common kestrels showed a greater prevalence of exposure to two or more SGARs compared to common buzzards (Fig. 3), reflecting their distinct ecological niches and dietary behaviors (Butet et al., 2010, 2022). Kestrels are generalist predators that inhabit more anthropogenic environments, making them more prone to ingesting contaminated prey (Carrillo et al., 2017; Orihuela-Torres et al., 2017). In contrast, buzzards, which tend to have more selective foraging strategies, were predominantly exposed to a single SGAR (Rodríguez et al., 2010; Tapia et al., 2007). These behavioral and ecological differences help explain the observed variability in SGAR exposure patterns across regions. Likewise, agricultural practices, livestock density, and urban development have all been linked to increased exposure to anticoagulants (Lohr, 2018; López-Perea et al., 2019; Pay et al., 2021; Rial-Berriel et al., 2021a). However, geolocation data were not available to assess the impact of these anthropogenic factors in this study.

Furthermore, the regional differences observed in this study, where island animals showed significantly higher ΣSGAR concentrations compared to those from mainland regions, emphasize the unique vulnerability of insular ecosystems to ARs bioaccumulation. As detailed in the results section (3.1), the highest prevalence (>95%) and median values were detected in insular territories (the Canary Islands and Madeira) compared to continental regions (central Spain and mainland Portugal). These findings underscore the urgent need for targeted conservation strategies in these highly sensitive ecosystems. Our results align with the initial hypothesis based on years of reporting concerning wildlife exposure to ARs in the Canary Islands (Carrillo-Hidalgo et al., 2024; Martín-Cruz et al., 2024; Rial-Berriel et al., 2021a, 2021c; Ruiz-Suárez et al., 2014), confirming that raptors inhabiting insular regions, such as the Canary Islands and Madeira, experience significantly higher exposure to SGARs compared to their continental counterparts. This heightened exposure can be explained by a combination of factors, including ecological isolation, the intensive use of SGARs for pest management, and the prevalence of rodenticide-resistant rodent populations, as highlighted in recent studies (Carrillo-Hidalgo et al., 2024; Carromeu-Santos et al., 2023). Additionally, the inherent vulnerability of island ecosystems—characterized by lower prey diversity and increased risks of bioaccumulation—further amplifies the impact of these toxicants (Goldwater et al., 2012). However, we acknowledge the limitations associated with the small sample size of animals from the Portuguese islands and the absence of necessary information on biological, anthropological, and environmental factors required to conduct a more robust statistical analysis.

Nevertheless, these findings should set a precedent for future research across Europe, aimed at unraveling why insular wildlife faces heightened exposure to these compounds compared to mainland areas. One plausible explanation could be the prevalence of rodenticide resistance in Spain and other European countries (Bermejo-Nogales et al., 2022; Damin-Pernik et al., 2022; Ruiz-López et al., 2022), especially in Portuguese Macaronesian Islands (Carromeu-Santos et al., 2023). Rodent populations resistant to rodenticides in island ecosystems present a unique threat, as their genetic traits proliferate more rapidly in isolated environments (Carromeu-Santos et al., 2023; Whitlock, 2003). These small mammals would behave like live baits, facilitating bioaccumulation and a riskier secondary toxicity in non-target wildlife (Carromeu-Santos et al., 2023; Ruiz-López et al., 2022). Moreover, the high use of rodenticides in these territories (BOC, 2014; Grilo et al., 2021; MITECO, 2004) could further exacerbate exposure levels.

Additionally, wildlife from insular environments, especially raptors, face heightened risks of exposure to ARs due to reduced rodent species diversity and increased population densities of invasive rodents (Goldwater et al., 2012). The reduced interspecific competition on islands allows rodent populations, the main target of pest control, to increase more rapidly, especially due to their higher resistance to rodenticides compared to their continental counterparts, which in turn raises the risk of SGAR contamination in raptors.

5. Conclusions

The use of raptors as biomonitoring agents for tracking priority pollutants is becoming increasingly prevalent. This study highlights a significant disparity in SGAR exposure between insular and continental regions, with raptors from insular areas showing substantially higher levels of exposure. Our study also reveals differences in SGAR exposure among raptors in the same territories. The common kestrel *(Falco tinnunculus)* shows notably higher exposure levels than the common buzzard *(Buteo buteo),* likely due to species-specific vulnerabilities that may be linked to differences in ecological behaviors, dietary preferences, and habitat use. Moreover, the regulatory changes implemented in 2018 with the legal modification (EU 2016/1179), aimed at reducing SGAR concentrations in baits, did not show the desired effect on wildlife protection. Overall, this study provides a valuable tool for assessing the potential toxicological risks in other insular regions of Europe, emphasizing the need to implement more effective protective measures focused on non-target wildlife in these vulnerable insular territories.

CRediT authorship contribution statement

Beatriz Martín Cruz: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Cristian Rial Berriel:** Investigation, Data curation. **Andrea Acosta Dacal:** Writing – review & editing, Visualization, Formal analysis. **Ana Carromeu-Santos:** Resources, Investigation, Data curation. **Katherine Simbaña-Rivera:** Formal analysis. **Sofia I. Gabriel:** Resources, Investigation. **Natalia Pastor Tiburón:** Resources, Data curation. **Fernando González González:** Resources. **Rocío Fernández Valeriano:** Investigation. **Luis Alberto Henríquez-Hernández:** Visualization, Writing – review & editing. **Manuel Zumbado-Peña:** Writing – review & editing, Validation. **Octavio P. Luzardo:** Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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