EXPOSICIÓN TRÓFICA A RODENTICIDAS ANTICOAGULANTES EN LA FAUNA SILVESTRE DE CANARIAS. BIOMONITORIZACIÓN MULTIESPECIE, FACTORES DE RIESGO Y ESPECIES CENTINELA

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CONTENIDOS

RESUMEN/ABSTRACT

RESUMEN

El uso generalizado de rodenticidas anticoagulantes (RAs), en particular los compuestos de segunda generación (SGARs), representa una amenaza creciente para la fauna silvestre, especialmente en ecosistemas insulares, donde convergen una elevada biodiversidad endémica y una sensibilidad ecológica particular. Esta tesis evalúa la exposición a RAs en distintos niveles tróficos de las Islas Canarias, abarcando especies no objetivo de diferentes taxones, y analiza su papel potencial como centinelas ecológicos de la contaminación ambiental. Con este fin, se analizaron más de 1.000 muestras hepáticas de culebras reales de California (*Lampropeltis californiae*), cernícalos vulgares (*Falco tinnunculus canariensis*), ratoneros comunes (*Buteo buteo*), camaleones de Yemen (*Chamaeleo calyptratus*) y diversas especies de aves no rapaces. Los análisis mediante cromatografía líquida acoplada a espectrometría de masas en tándem (HPLC-MS/MS) revelaron una contaminación generalizada por SGARs. La frecuencia de detección fue especialmente elevada en depredadores del ecosistema terrestre canario, superando el 90% en cernícalos y serpientes, pero también fue significativa en especies de niveles tróficos bajos, con hasta un 80% de reptiles insectívoros y un 40% de aves no rapaces expuestas. El brodifacum fue, de forma consistente, el compuesto más prevalente en todos los grupos y los rodenticidas anticoagulantes de primera generación (FGARs) se detectaron anecdóticamente. El análisis de variables biológicas, antrópicas, geográficas y legislativas se realizó con el fin de identificar los predictores más relevantes en relación con la exposición de estos compuestos. Entre ellos, el mayor tamaño corporal y la edad destacaron como factores biológicos asociados a una mayor concentración de SGARs. Asimismo, la localización geográfica resultó determinante en el estudio comparativo entre regiones continentales e insulares mostrando una mayor carga de RAs en rapaces insulares. Finalmente, la evaluación de actividades antrópicas y uso del suelo en las islas revelaron que la actividad ganadera era un predictor significativo del riesgo de exposición, mientras que las restricciones legales sobre el uso de SGARs no mostraron un efecto reductor en los niveles detectados. Un hallazgo especialmente relevante fue la presencia de SGARs en especies insectívoras, lo que sugiere una posible transferencia secundaria a través del consumo de invertebrados contaminados. Esta vía de exposición, poco estudiada hasta la fecha, podría desempeñar un papel importante en la integración de rodenticidas en la red trófica terrestre, particularmente en ecosistemas insulares. En conjunto, los resultados de esta tesis demuestran que la exposición a rodenticidas no se limita a los grandes depredadores, sino que afectan a una amplia gama de especies y niveles tróficos, lo que refleja su persistencia y capacidad de bioacumulación en el medio ambiente. Estos hallazgos, obtenidos gracias a las tareas de vigilancia ecotoxicológica como las de la Red Canaria de Vigilancia Sanitaria de Fauna Silvestre, subrayan la urgencia de reforzar la regulación del uso de rodenticidas e implementar estrategias de gestión integrada de plagas con el objetivo de conservar la biodiversidad insular de nuestro archipiélago.

ABSTRACT

The widespread use of anticoagulant rodenticides (ARs), particularly second-generation compounds (SGARs), poses a growing threat to wildlife, especially in island ecosystems, which are characterized by high levels of endemic biodiversity and particular ecological sensitivity. This thesis investigates AR exposure across multiple trophic levels in the Canary Islands, encompassing non-target species from diverse taxa, and evaluates their potential role as ecological sentinels of environmental contamination. More than 1,000 liver samples were analyzed from California kingsnakes (*Lampropeltis californiae*), common kestrels (*Falco tinnunculus canariensis*), common buzzards (*Buteo buteo*), veiled chameleons (*Chamaeleo calyptratus*), and various species of non-raptorial birds. Analyses using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) revealed widespread SGAR contamination. Detection frequencies were especially high among terrestrial predators, exceeding 90% in kestrels and snakes, but also notable in species from lower trophic levels, with exposure detected in up to 80% of insectivorous reptiles and 40% of non-raptorial birds. Brodifacoum was consistently the most prevalent compound across all groups, whereas first-generation anticoagulant rodenticides (FGARs) were detected only sporadically. Biological, anthropogenic, geographic, and regulatory variables were examined to identify the strongest predictors of exposure. Larger body size and older age were associated with higher SGAR concentrations. Geographical location emerged as a key factor, with insular raptors exhibiting higher AR burdens than their continental counterparts. In addition, land use and human activity—specifically livestock—was a significant predictor of exposure risk, while legal restrictions on SGAR use did not appear to effectively reduce contamination levels. A particularly significant finding was the detection of SGARs in insectivorous species, suggesting the potential for secondary exposure through the consumption of contaminated invertebrates. This pathway, which remains poorly understood, could play an important role in the incorporation of rodenticides into terrestrial food webs, particularly in insular environments. Collectively, the findings of this thesis demonstrate that ARs affect a broad spectrum of species and trophic levels, reflecting their environmental persistence and bioaccumulation potential. These results, obtained through ecotoxicological surveillance efforts such as those of the Canary Islands Wildlife Health Surveillance Network, underscore the urgent need to reinforce rodenticide regulation and implement integrated pest management strategies to safeguard island biodiversity.

CONTEXTUALIZACIÓN

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Los roedores comensales viven en hábitats humanos donde pueden encontrar elementos esenciales para su supervivencia como el alimento, agua o refugio. El ratón doméstico (*Mus musculus* o *M. domesticus*), la rata parda (*Rattus norvegicus*) y la rata negra (*Rattus rattus*) son las principales especies de roedores categorizadas como comensales. Su desarrollo en torno a la vida humana, ha supuesto históricamente graves problemas para la salud pública, vinculándose como vectores de grandes pandemias en la historia o de actuales transmisiones de bacterias, virus y parásitos que suponen un riesgo para la ciudadanía [1–3]. No obstante, su impacto es multifactorial, pues también es conocida su interacción con la ganadería, la agricultura o las infraestructuras modernas. En estos entornos, los roedores provocan pérdidas, daños y contaminación de las materias primas y pueden actuar como reservorio de enfermedades para el ganado [4–6]. De la misma manera, se ven afectados la biodiversidad y el medioambiente, especialmente en aquellos ecosistemas de gran vulnerabilidad como las islas, donde estos roedores invaden el territorio a un ritmo acelerado y pueden causar efectos devastadores mediante la depredación y el desplazamiento de especies locales [7].

En base a este conflicto multifactorial, la necesidad de controlar la presencia masiva de estos micromamíferos se presenta como un imperativo pasado, presente y futuro. Los métodos empleados para tal fin son de diversa índole: biológicos [8,9], mecánicos [10] y químicos. No obstante, y aunque la tendencia actual se orienta hacia una gestión integrada de las plagas (GIP), los productos químicos representan la metodología predominante en el control a gran escala de estos animales, y entre ellos destacan los rodenticidas anticoagulantes [11]. Este tipo de compuestos fue descubierto de manera fortuita en los años 20, en Canadá y EE.UU., al asociarse un síndrome hemorrágico por deficiencia de protrombina en el ganado vacuno con el consumo de heno de trébol dulce enmohecido. Más tarde, se descubrió que ciertos hongos transformaban compuestos del trébol en dicumarol. A raíz de este descubrimiento, se creó la warfarina, el primer rodenticida anticoagulante de primera generación (FGARs: First Generation Anticoagulant Rodenticides) que resultó exitoso durante tres décadas hasta que aparecieron las primeras resistencias entre los roedores [12–14]. Fue entonces cuando surgió la necesidad de modificar molecularmente esta sustancia y crear las "*súperwarfarinas*", actualmente conocidas como rodenticidas de segunda generación (SGARs: Second Generation Anticoagulant Rodenticides). A este grupo pertenecen los compuestos mayormente detectados en la actualidad en estudios de biomonitorización silvestre (brodifacum, bromadiolona, difenacum, flocumafeno y difetialona) [15–18]. No obstante, se sigue registrando cierta prevalencia entre los FGARs (warfarina, coumatetralil, difacinona, clorofacinona) que actualmente representan una fracción muy pequeña (menos del 3,5%) de los rodenticidas anticoagulantes disponibles en el mercado en Europa [19].

Los rodenticidas anticoagulantes más recientes, SGARs, fueron dotados de una mayor capacidad de bioacumulación en el tejido hepático, de mayor toxicidad con una sola ingesta y de mayor liposolubilidad, incluyéndose junto con algunos FGARs en el grupo de compuestos persistentes, bioacumulativos y tóxicos (PBT) [20,21]. Estas características han permitido que su efectividad sea elevada, aunque en los últimos años ya se han detectado poblaciones de roedores resistentes a ellos en múltiples países [22–27]. Sin embargo, este no es el único problema que se asocia a estos compuestos. En el balance entre su efectividad y daño colateral al medio natural, la fauna silvestre se ha visto gravemente perjudicada debido a su capacidad para bioacumularse en los seres vivos que los consumen de manera directa o indirecta [28].

En este sentido, los animales que ocupan las posiciones más elevadas de la cadena trófica se ven alarmantemente afectados, pues en ellos se dan dos acontecimientos simultáneamente; la bioacumulación y la biomagnificación. No obstante, su presencia también ha sido confirmada en el sustrato y el agua [29,30], así como en otros seres vivos como los invertebrados que forman la base de sus ecosistemas [31,32]. Esta problemática ambiental, que en algunas especies podría ser un factor antropogénico más para el detrimento de sus poblaciones, ha llevado a elaborar estrategias y normativas autonómicas y europeas que regulen el uso de estos compuestos tanto como fitosanitarios como biocidas, estableciendo la protección de la biodiversidad entre sus principales objetivos [20,33].

En este contexto, en la Comunidad Autónoma de Canarias, hace once años, entró en vigor la Orden de 28 de marzo de 2014, por la que se aprobó la estrategia para la erradicación del uso ilegal de veneno en el medio no urbano de Canarias [34]. Esta estrategia basó su diagnóstico en los resultados de varios años facilitados por el Servicio de Toxicología de la Universidad de Las Palmas de Gran Canaria (ULPGC), que referían tasas de exposición de la fauna silvestre a diversos compuestos potencialmente tóxicos, entre los que se encontraban los rodenticidas anticoagulantes, que, junto con el carbofurano, eran los principales agentes responsables de envenenamiento en las islas.

Posteriormente, la puesta en marcha desde el año 2018 de la Red Canaria de Vigilancia Sanitaria de Fauna Silvestre (Red Vigía Canarias) [35], ha permitido el estudio continuado de estos compuestos aplicando una visión integradora de la toxicología, la anatomía patológica, la ecología y el bienestar animal de las especies afectadas, aportando información valiosa sobre el estado de amenaza de nuestros ecosistemas y contribuyendo a un desarrollo más sostenible de las prácticas antropogénicas equilibrando la salud pública y la conservación ambiental.

INTRODUCCIÓN

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1. RODENTICIDAS ANTICOAGULANTES

1.1. Definición y Clasificación de los Rodenticidas Anticoagulantes

Los rodenticidas anticoagulantes son un grupo de agentes químicos empleados globalmente para el control de plagas de roedores. Como bien se comentó en el anterior apartado de este documento, el primer rodenticida anticoagulante sintético fue la warfarina, un producto derivado del dicumarol. Más tarde, surgieron otras sustancias conocidas comúnmente como rodenticidas de primera generación o FGARs. Estos compuestos fueron creados a partir de modificaciones en la estructura molecular central, existiendo los derivados de la 4-hidroxicumarina (coumatetralil, coumaclor) y los derivados de indandiona (clorofacinona y difacinona). Dichos compuestos conseguían de manera exitosa la muerte de los roedores tras varias ingestas, no obstante, la aparición de resistencias llevó al desarrollo de nuevos agentes químicos [12,14].

En este contexto de necesidad, nacen los rodenticidas de segunda generación (SGARs), también derivados de cumarina e indandiona, como la bromadiolona, difenacum, brodifacum, flocumafeno y difetialona. La modificación molecular de este grupo de compuestos le atribuyó una $T_{1/2}$ mayor y un valor de LD_{50} menor, es decir, los compuestos al ser más liposolubles y tener mayor afinidad hepática y enzimática, permanecen más tiempo en el organismo y son capaces de producir la muerte de los roedores con una sola ingesta [14,21,36]. Sin embargo, la toxicidad, la persistencia ambiental y la resistencia en las especies no objetivo varía dentro de ambos grupos e incluso entre compuestos del mismo grupo [21,37].

1.2. Vías de Exposición de la Fauna Silvestre a Rodenticidas Anticoagulantes

Atendiendo a la dotación de estas características, los rodenticidas anticoagulantes, y, en particular, los SGARs, han resultado exitosos durante décadas para el control de roedores comensales. No obstante, en términos de protección a la biodiversidad, el balance no ha sido beneficioso y son numerosos los estudios que confirman la integración de estos compuestos en los ecosistemas a través de diferentes vías de exposición [28].

Cuando hablamos de vías de exposición, nos referimos a las múltiples formas en las que los seres vivos llegan a exponerse a estos agentes químicos. En el caso de las especies objetivo, esta exposición es primaria, por el consumo directo del cebo. No obstante, para la fauna silvestre se registran al menos dos.

En primer lugar, el consumo directo o vía primaria confirmado en observaciones directas de pequeños individuos como insectos, aves granívoras, pequeños reptiles o micromamíferos no objetivo, que ingieren el cebo en grano o son capaces de entrar en las estaciones de cebo atraídos por la palatabilidad de este [28,38,39]. En segundo lugar, y la más estudiada en el área de biomonitorización silvestre, la vía secundaria o consumo de presas contaminadas. Este tipo de exposición es muy común en aves rapaces y mamíferos carnívoros cuya dieta se basa principalmente en micromamíferos. Sin embargo, también es propia de depredadores menores que ingieren una amplia gama de presas previamente contaminadas [17,40–42]. Adicionalmente, algunos autores hacen referencia a una tercera vía de exposición, especialmente en animales carroñeros, que incluye la ingesta de cadáveres de depredadores o grandes presas envenenados secundariamente [43]. Finalmente, en el estudio de la transferencia trófica de estos compuestos, se incentiva la investigación de rutas alternativas como la exposición dérmica en reptiles [44], la posible transferencia transplacentaria en mamíferos o la transmisión *in ovo* en gallinas [45].

1.3. Toxicocinética y Mecanismo de Acción de Rodenticidas Anticoagulantes

Independientemente del grupo al que pertenezcan, FGARs o SGARs, la ruta principal de exposición de estos compuestos es la vía oral y su absorción es buena a nivel gastrointestinal. Tras su consumo, los rodenticidas anticoagulantes son metabolizados a nivel hepático por el complejo enzimático citocromo P450 y en función del compuesto y del grado de biotransformación que sufran, su excreción se produce a través de la orina y las heces en forma de metabolitos (FGARs) o mayoritariamente como compuesto original (SGARs) [45,46]. Sin embargo, existen diferencias sustanciales en estos procesos entre ejemplares de diferentes taxones, como es el caso de las aves, que, a diferencia de los mamíferos, cuentan con una vena coccigomesentérica que desvía parte del flujo sanguíneo gastrointestinal pudiendo variar su susceptibilidad a la intoxicación y los niveles residuales acumulados [45].

En el organismo, estas sustancias, actúan interfiriendo en el ciclo de la vitamina K, una coenzima esencial para la activación de los factores de coagulación sanguínea II (protrombina), VII, IX y X. Su mecanismo de acción se basa en la inhibición de la enzima vitamina K 2,3-epóxido reductasa (VKOR), lo que impide la regeneración de la vitamina K reducida, esencial para la carboxilación de los factores de coagulación. Esto provoca la acumulación de formas subcarboxiladas inactivas, incapaces de unirse al calcio y a las membranas celulares para formar complejos de coagulación activos. En última instancia, la deficiencia de estos factores impide la coagulación sanguínea, causando hemorragias espontáneas y, finalmente, la muerte del animal afectado [45–47].

1.4. Sintomatología Clínica, Tratamiento y Diagnóstico

Vinculado a este mecanismo de acción, la sintomatología esperada ante la intoxicación de estos compuestos estaría relacionada con alteraciones visibles de la coagulación. No obstante, y aunque también se dan estos síntomas, las hemorragias no siempre son visibles y la sintomatología presente puede resultar inespecífica especialmente en animales silvestres [48,49].

A diferencia de los animales domésticos, los casos de intoxicación en fauna silvestre son mucho más complejos de resolver, pues la información aportada por los dueños de las mascotas no existe, y en su lugar disponemos de descripciones muy limitadas del ciudadano o agente que se lo encuentre. Tanto en estudios controlados en centros de recuperación como en fauna silvestre libre expuestos a estos compuestos, la sintomatología principalmente descrita es la depresión (disminución del estado mental ante respuesta a los estímulos del entorno y letargia), debilidad y palidez de mucosas, sintomatología que puede confundirse con otras patologías [48–50].

De manera más severa, también pueden llegar ejemplares con hemorragias intramusculares y subcutáneas de diferente severidad, muchas veces asociadas a traumatismos. Además, pueden detectarse hemorragias internas asociadas a diversos órganos con sus afecciones específicas [48–52]. Asimismo, a nivel microscópico se pueden observar focos hemorrágicos en diferentes órganos o tejidos [51]. Por tanto, y como bien se comentaba anteriormente, la ausencia de hemorragias externas no exime de un posible diagnóstico de intoxicación por rodenticidas.

Mientras el animal está vivo se puede obtener una muestra de sangre, en la que los tiempos de coagulación y el hematocrito pueden estar alterados, aunque estos cambios dependen en gran medida de la dosis a la que se expongan [48,51,53]. Este hallazgo, en combinación con la sintomatología anteriormente descrita lleva al profesional veterinario a instaurar un tratamiento basado en vitamina K, restauración de la volemia y tratamiento de soporte [54].

En última instancia, el diagnóstico definitivo precisa de la confirmación laboratorial mediante análisis cromatográfico de los diferentes compuestos. Para ello, las muestras de elección son la sangre y el hígado. No obstante, es importante destacar el riesgo de un resultado falso negativo al emplear la sangre como matriz debido al menor tiempo de permanencia de los rodenticidas anticoagulantes en el torrente sanguíneo. Por tanto, el hígado se presenta como la opción idónea para su evaluación, aunque no nos permite la evaluación en individuos vivos [55–57].

1.4.1. Efectos Subletales en la Fauna Silvestre

En ocasiones, las especies no diana se exponen a concentraciones no letales de estos compuestos complicando aún más el diagnóstico toxicológico. Los efectos subletales de los rodenticidas anticoagulantes en la fauna silvestre no están completamente caracterizados y los intentos de recrear dicha exposición en cautividad no son un retrato real de la naturaleza en el que otras muchas variables pueden influir [37,38,50]. No obstante, y a pesar de no tener resultados concluyentes, es creciente la idea de que la exposición subletal y crónica podría provocar alteraciones fisiológicas y clínicas que comprometen su supervivencia.

En aves rapaces, la exposición subletal de RAs puede prolongar los tiempos de coagulación, conduciendo a una coagulopatía leve y consecuentemente aumentar el riesgo de hemorragias internas haciéndoles más vulnerables a lesiones u otros factores estresantes [53,58,59]. Asimismo, se ha observado disfunción en la termorregulación en algunas especies que muestran piloerección al exponerse a dosis subletales [58], aumento de eritrocitos inmaduros [60] y daño tisular incluso a dosis bajas con evidencia histopatológica de hemorragias en tejidos, evidenciando que se pueden producir sangrados internos sin signos externos evidentes [53]. Por tanto, estas alteraciones podrían reducir la capacidad de una rapaz para sobrevivir a lesiones, cazar eficazmente, ser más vulnerable a hemorragias por traumatismos o hacer frente a factores estresantes, lo que podría afectar a la salud de las poblaciones. No obstante, la alteración de otros parámetros como la condición corporal solo ha sido sugerido por algunos autores [61] sin obtener el respaldo de otros estudios que incluyen valoraciones similares [48,50,51].

Por su parte, en mamíferos silvestres existe una creciente evidencia sobre el potencial de amenaza que los agentes químicos pueden suponer para ellos [62]. Algunos autores han documentado cambios en la expresión génica que afectan la función inmunitaria, el metabolismo y la integridad tisular, lo que podría reducir la resistencia a enfermedades y la aptitud general [63,64]. No obstante, no se ha demostrado de forma concluyente que causen declive generalizado en las poblaciones y tampoco se ha logrado encontrar una relación directa entre la respuesta inmune y la exposición a RAs [38,65–67].

En otros taxones como los reptiles o invertebrados, estos estudios son aún más escasos, aunque ya se han sugerido alteraciones de comportamiento en algunas especies mediante estudios de experimentación [68,69]. Por tanto, se requiere de un mayor desarrollo investigador en este campo para esclarecer el espectro completo de efectos subletales, su impacto en la dinámica poblacional y los riesgos derivados de exposiciones crónicas a dosis bajas en todos los taxones.

1.5. Normativa Europea de Rodenticidas Anticoagulantes

A pesar de su impacto sobre los seres vivos no diana, justificar la aplicación de los rodenticidas anticoagulantes es tarea fácil, ya que como se ha descrito en apartados anteriores, son la solución más factible para el control de roedores que protagonizan la transmisión de enfermedades y pérdidas económicas asociadas a la agricultura y la ganadería. No obstante, la creciente preocupación por la integridad de la biodiversidad expuesta a estas sustancias ha marcado como objetivo minimizar los riesgos para la salud humana, el medio ambiente y la fauna silvestre mediante la regulación de su uso.

En este contexto, desde la Unión Europea, se ha llevado a cabo una diferenciación normativa en función del ámbito en el que se empleen, diferenciándolos en biocidas y fitosanitarios. Para comprenderlo con mayor claridad, según el Ministerio para la Transición Ecológica y el Reto Demográfico:

"Los productos fitosanitarios son mezclas químicas que contienen una o varias sustancias activas y otros ingredientes, y cuyo objetivo es proteger los vegetales y sus productos de organismos nocivos. También se consideran productos fitosanitarios a las sustancias que destruyen las plantas, regulan o inhiben la germinación [70]."

Por su parte, un producto biocida se define como "las sustancias o mezclas que están compuestas por, o generan, una o más sustancias activas (incluidos los microorganismos) cuyo objetivo es destruir, contrarrestar, neutralizar, impedir la acción o ejercer un control de otro tipo sobre cualquier organismo nocivo por cualquier medio que no sea una mera acción física o mecánica". Y existen cuatro grupos principales: desinfectantes, conservantes, plaguicidas y otros biocidas [71].

Teniendo en cuenta estas definiciones y la división normativa europea en cuanto al uso de los rodenticidas anticoagulantes, todos los países miembros deben actuar en base a la siguiente legislación.

1.5.1. Rodenticidas Anticoagulantes como Fitosanitarios en Europa

Para que un producto pueda comercializarse como fitosanitario en Europa, debe estar registrado en el Registro Oficial de Productos Fitosanitarios y estar catalogado como *"aprobado"* en la Base de Datos de Pesticidas Europeo [72].

Actualmente, según el Reglamento (UE) 1107/2009, los rodenticidas anticoagulantes no están autorizados para este fin en ninguno de los países europeos registrados [72]. No obstante, el artículo 53 de dicha normativa recoge la posibilidad de que los Estados Miembros soliciten autorizaciones de emergencia para situaciones en las que no sea posible controlar las plagas con otros métodos o compuestos [33]. La última autorización de emergencia en España fue solicitada por la Dirección General de Producción Agropecuaria e Infraestructuras Agrarias de la Junta de Castilla y León entre 2016-2017, coincidiendo con la plaga de topillo campesino de la zona [73]. España, al igual que otros países europeos, sufre periódicamente las plagas del topillo campesino, que tienen lugar estacional y periódicamente cada 5 años aproximadamente y son catalogadas por muchos agricultores como un desastre económico [74,75].

Como alternativas químicas, en nuestro país está aprobado como pesticida el fosfuro de aluminio y el fosfuro de magnesio [73]. Además, se están empleando otras alternativas biológicas como el uso de depredadores naturales [8,9] y trampas mecánicas [10] como parte de la tendencia global hacia la gestión integrada de las plagas. No obstante, las medidas alternativas son catalogadas por muchos agricultores y profesionales como insuficientes y generan una gran brecha en el sector agrario del país [74].

1.5.2. Rodenticidas Anticoagulantes como Biocidas en Europa

Como biocidas, los rodenticidas están regulados a nivel europeo por el Reglamento (UE) 528/2012, por el que se regula la comercialización y el uso de estos. Según este reglamento, los rodenticidas anticoagulantes cumplen diversos criterios de exclusión detallados en el artículo 5 de su desarrollo normativo y están catalogados como persistentes, bioacumulables y tóxicos (PBT). Por este motivo, la renovación de autorización de estas sustancias activas no puede ser superior a cinco años [20].

No obstante, hoy en día, estas sustancias cuentan con la aprobación hasta el 31 de diciembre del año 2026 en base a la emisión de la Decisión de Ejecución (UE) 2024/734 por la que se retrasa la fecha de expiración de la aprobación del brodifacum, la bromadiolona, la clorofacinona, el coumatetralil, el difenacum, la difetialona y el flocumafeno para su uso como biocidas [76].

Con esta premisa, en España, existen actualmente 371 productos biocidas del tipo TP14 (rodenticidas) registrados en el Registro Oficial de Biocidas, siendo los más abundantes aquellos que contienen brodifacum, bromadiolona y difenacum (***Figura 1***) y cuya presentación comercial más frecuente es el cebo sólido en bloque seguido del pellet o grano [77]. En este listado se puede comprobar que todos los productos formulados con concentraciones superiores a 0,003% están destinados exclusivamente a personal profesional especializado. Por el contrario, las formulaciones con concentraciones comprendidas entre el 0,001% y el 0,0029% presentan una mayor diversidad de usos, incluyendo su disponibilidad para el público general (***Figura 1***). Esta clasificación está estrechamente relacionada con la aplicación del Reglamento (UE) 2016/1179, que impulsó la fabricación de rodenticidas con dosis significativamente reducidas (<30 ppm). Esta medida llevó a la reclasificación de aquellos productos con concentraciones superiores a dicho umbral como sustancias reprotóxicas, lo que conllevó la restricción de su uso al ámbito estrictamente profesional y la prohibición de su acceso por parte del público en general [78,79]. Asimismo, la tendencia hacia la protección de las intoxicaciones accidentales ha llevado a la protección de los cebos con estaciones que lo recubran o con localizaciones en lugares catalogados como inaccesibles para las especies no objetivo como recoge el Reglamento (UE) 528/2012 [20] y las guías para personal profesional en Europa [80].

Estos cambios legislativos entre otras medidas han intentado disminuir la exposición de la fauna silvestre, no obstante, son varios los estudios que tomando dichas modificaciones regulatorias como punto de inflexión no han detectado mejoras en la exposición de la fauna a estos compuestos o incluso han visto incrementada la exposición a determinadas sustancias [81–84].

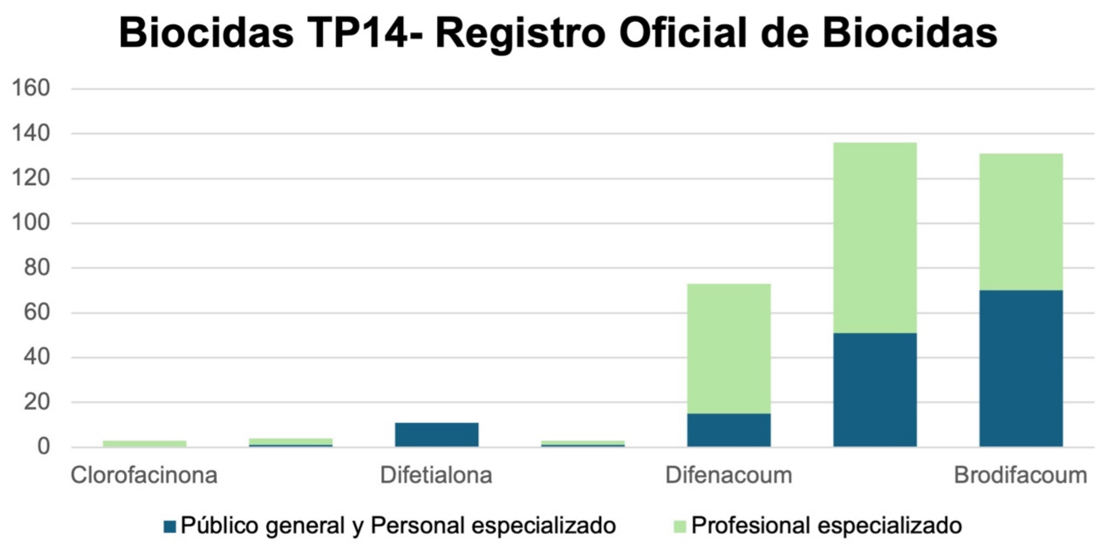

Figura 1. Gráfico de barras apiladas que muestra el número de productos rodenticidas anticoagulantes (TP14) registrados en el Registro Oficial de Biocidas del Ministerio de Sanidad en España, desglosado por principio activo [77]. Para cada compuesto se representa el total de productos disponibles, diferenciando aquellos autorizados exclusivamente para uso por personal profesional especializado (verde) de los que también pueden ser utilizados por el público en general (azul).

1.5.3. <u>Alternativas Químicas a los Rodenticidas Anticoagulantes</u>

En la actualidad, las únicas sustancias activas alternativas a los rodenticidas anticoagulantes como biocidas en Europa son los productos que contienen alfacloralosa, fosfuro de aluminio generador de fosfina, dióxido de carbono, cianuro de hidrógeno y colecalciferol [19]. Estas sustancias en usos determinados presentan un perfil de riesgo más bajo para la salud pública, animal y medioambiental como es el caso del dióxido de carbono. No obstante, en términos de medio ambiente, el colecalciferol y la alfacloralosa muestran riesgos significativos de intoxicación que en principio no difieren de los anticoagulantes y se han visto involucrados en numerosos casos de envenenamiento de rapaces a nivel europeo [19,85]. Por tanto, actualmente, los rodenticidas, se presentan como la opción más factible debido a su acción retardada, su eficacia, bajo coste y la disponibilidad de un "*antídoto*" [19].

Concretamente en España contamos con 12 productos incluidos en el Registro Oficial de Biocidas con estos principios activos (6 de alfacloralosa, 2 colecalciferol, 1 cianuro de hidrógeno, 2 dióxido de carbono y 1 generador de fosfina), siendo la alfacloralosa el único compuesto que permite el uso del público general para la mayoría de sus productos aprobados a pesar de la toxicidad registrada en la fauna silvestre [77].

1.6. Resistencia de Roedores Comensales a Rodenticidas Anticoagulantes

Tanto a nivel científico como social e industrial, la aparición de resistencias en roedores se presenta como un conflicto de vital importancia y ha llevado a la Comisión Europea a solicitar recientemente a la Agencia Europea de Sustancias y Mezclas Químicas (ECHA) la resolución de una serie de cuestiones relacionadas con las alternativas eficaces a los rodenticidas. No obstante, dichos resultados no han sido muy aulagueños, pues solo existen alternativas para el *Mus musculus* y no en todos los escenarios. [19].

Este fenómeno en roedores comensales como *Rattus norvegicus*, *Rattus rattus* y *Mus musculus*, se ha vinculado a un uso prolongado de rodenticidas anticoagulantes, lo que ha favorecido la aparición y expansión de cepas resistentes comprometiendo la eficacia de los tratamientos, especialmente en *Rattus norvegicus* y *Mus musculus.* Esta resistencia, de origen genético o conductual, permite la supervivencia frente a tratamientos previamente eficaces y complica el control poblacional [86].

El mecanismo principal de resistencia identificado se relaciona con mutaciones en el gen VKORC1, que codifica la subunidad 1 del complejo de la vitamina K epóxido reductasa, enzima diana de los rodenticidas anticoagulantes [22,87–89]. Estas mutaciones, comúnmente polimorfismos de un solo nucleótido (SNP), alteran la estructura de la enzima, reduciendo su afinidad por los anticoagulantes y permitiendo la síntesis continua de factores de coagulación dependientes de vitamina K, incluso en presencia del tóxico [86].

En *Rattus norvegicus*, las mutaciones más frecuentes incluyen Tyr139Cys (Y139C), Tyr139Phe (Y139F) y Leu120Gln (L120Q), cada una con distribución geográfica distinta en Europa [25,26,89–92]. Estas variantes confieren distintos niveles de resistencia a anticoagulantes de primera generación (FGARs) y a algunos de segunda generación (SGARs) como bromadiolona y difenacum, aunque no se ha documentado resistencia práctica en el control de estos roedores en campo a los compuestos más potentes como brodifacum, difetialona y flocumafeno [86]. Por su parte, en *Rattus rattus*, algunas variantes del gen VKORC1 podrían conferir cierta resistencia, aunque estudios previos sugieren que este rasgo es multifactorial e inestable y necesita de mayor atención [86,91,93].

En el caso de *Mus musculus*, se han detectado mutaciones como Leu128Ser (L128S), Tyr139Cys (Y139C) [24,27,89,94] y combinaciones de SNPs denominadas como cepa "*spretus introgression*", resultado de hibridación con *Mus spretus* [95,96]. Estas variantes confieren resistencia a FGARs e incluso a SGARs como bromadiolona y posiblemente a difenacum, limitando considerablemente las opciones terapéuticas [86].

Concretamente en España, se han descrito casos de resistencia a rodenticidas en roedores en diversos entornos, incluidos medios rurales, urbanos y costeros [87], aunque solo algunas comunidades autónomas han llevado a cabo estudios específicos al respecto. En el caso de la rata negra (*Rattus rattus*), se han identificado resistencias en provincias como Zaragoza, Segovia, Toledo, Alicante, Barcelona, Cádiz y Valladolid, asociadas a una amplia variedad de mutaciones en el exón 3 del gen VKORC1 [87,88,95]. Por su parte, en la rata parda (*Rattus norvegicus*), se han detectado mutaciones del tipo S149I en Gipuzkoa y Madrid [87]. Asimismo, se han descrito resistencias en el ratón doméstico en Barcelona [23]. No obstante, otros estudios realizados en esta misma provincia analizando ratas no han encontrado evidencias de resistencia en los individuos estudiados, lo que pone de manifiesto la variabilidad espacial del fenómeno y las diferencias ecológicas y comportamentales entre ratas y ratones, así como las diferencias de manejo del proceso de erradicación en función de la especie objetivo [97].

Por último, llama la atención la ausencia de estudios en las islas españolas, un territorio especialmente sensible por su aislamiento geográfico, su elevada biodiversidad y la intensa presión sobre los ecosistemas insulares. Esta carencia contrasta con los datos disponibles en otras islas del mediterráneo, como las portuguesas e italianas, donde se han detectado resistencias, especialmente en *M. musculus* [22,98,99].

2. BIOMONITORIZACIÓN DE RODENTICIDAS ANTICOAGULANTES EN FAUNA SILVESTRE

2.1. Biomomitorización y Especies Centinela

El biomonitoreo ambiental se define a grandes rasgos como la evaluación de la calidad del entorno utilizando organismos vivos como indicadores de la salud del ecosistema. Este enfoque es de especial importancia en la actualidad, donde el concepto "*One Health*" impulsa el uso de especies centinela en la ciencia, dejando atrás la visión fragmentada de la salud humana y animal, para dar paso a una visión compartida de la misma [100,101].

Los organismos empleados para tal fin se conocen como especies centinelas, bioinidicadores o biomonitores y actúan como alertas tempranas sobre posibles riesgos para la salud humana o la biodiversidad señalando la presencia de los contaminantes en sus hábitats. No obstante, y aunque estos términos se empleen de manera indistinta en estudios de biomonitorización, sus definiciones difieren en algunos puntos [102].

La elección de estos organismos debe realizarse en función del contexto de investigación y se basa en una serie de características que los hacen más idóneos para cada objetivo de estudio. Entre estas características se encuentra su distribución, la posición en la cadena trófica, la capacidad para bioacumular contaminantes, el conocimiento de la especie en libertad y cautiverio, la capacidad para ser capturado en cantidad suficiente, el área de distribución restringida o la sensibilidad a contaminantes [103].

Basándonos en las características anteriormente citadas, y a pesar de que algunos autores reiteren la importancia de la residencia como condición indispensable para ser especie centinela [104], la aparición y establecimiento de ciertas especies invasoras, aunque suponen un grave riesgo ecológico por su competencia con especies nativas, también pueden ofrecer información relevante sobre la presencia de contaminantes ambientales. Varios estudios han documentado hasta la fecha el uso de especies invasoras como centinelas de diversos contaminantes tanto en entornos acuáticos [105,106] como terrestres [107] siendo en muchas ocasiones un manejo más fácil al formar parte de programas de erradicación o control poblacional. Estas especies, al ocupar nichos similares o compartir recursos con fauna endémica, permiten evaluar la exposición a tóxicos sin comprometer poblaciones protegidas de difícil acceso, facilitando una vigilancia ambiental más eficaz y menos invasiva. Basándonos en esta premisa, la presente tesis doctoral pretende evaluar la salud del ecosistema canario con relación a los rodenticidas anticoagulantes empleando especies residentes e invasoras de medio-largo establecimiento.

2.2. Factores Antropogénicos como Predictores de Exposición a Rodenticidas

La necesidad de biomonitorizar estos compuestos nace del uso del suelo asociado a actividades antropogénicas —en particular, el desarrollo urbano, la agricultura y la ganadería— las cuales desempeñan un papel clave en la exposición de la fauna silvestre a rodenticidas anticoagulantes (RAs). Diversos estudios han demostrado que las zonas urbanas y las explotaciones ganaderas son algunos de los predictores más fuertes de contaminación por RAs en especies no diana.

Los entornos urbanos, caracterizados por una alta densidad de población humana y un uso intensivo de rodenticidas como biocidas, se asocian estrechamente con concentraciones elevadas de RAs en varios taxones [18,41,108–111]. De forma similar, la presencia de explotaciones ganaderas, donde los RAs se utilizan frecuentemente para el control de roedores, se ha relacionado con una mayor carga de residuos [112–115]. Asimismo, los vertederos asociados a estas actividades pueden aumentar la disponibilidad de presas contaminadas convirtiéndose en trampas ecotoxicológicas para los depredadores que los consumen y se ven atraídos por la abundancia de presas [108,112]. Aunque las zonas agrícolas tienden a ser un predictor menos potente de exposición a RAs, ciertos paisajes agrícolas o intensificación de la actividad también representan un riesgo, especialmente en áreas donde las especies cazan o forrajean cerca de asentamientos humanos [28,109,116].

No obstante, la bibliografía disponible es variada y esta correlación no se observa en todas las regiones o especies de la misma manera. Asimismo, cabe destacar que la contaminación por RAs no se limita a especies objetivo o especies especializadas en la depredación de roedores, sino que, tal y como se señala en las referencias citadas en esta sección, afecta a un amplio espectro de depredadores y presas, lo que evidencia una exposición crónica y generalizada en la red trófica.

2.3. Diferencias de Exposición a Rodenticidas Anticoagulantes entre Taxones

Considerando las definiciones y criterios establecidos al inicio de este apartado, la biomonitorización de los rodenticidas anticoagulantes y otros contaminantes en el medio natural se ha centrado en las últimas décadas en la exposición de los grandes depredadores de los ecosistemas [117] como las aves rapaces [118], los mamíferos carnívoros [119] y las aves carroñeras [120] dejando una distribución desequilibrada que dificulta la visión global e integrada de estos contaminantes en el medio ambiente.

Esta predisposición investigadora queda reflejada en algunas revisiones bibliográficas recientes, en las que otros taxones como los reptiles, anfibios, aves no rapaces, animales acuáticos o invertebrados han pasado inadvertidos para gran parte de la comunidad científica a pesar de su gran valor para el entendimiento de la dinámica de estos compuestos en el medio natural [21].

No obstante, en los últimos años, son varios los estudios que intentan dar respuesta a estas lagunas de conocimiento en el intento de valorar el riesgo real de los rodenticidas en especies no objetivo, considerando sus diferencias ecológicas y fisiológicas que influyen tanto en el grado de exposición, como en la toxicidad o bioacumulación en la cadena trófica.

2.3.1. Aves

2.3.1.1. Aves rapaces

Es el taxón más abundante en las publicaciones de biomonitorización para rodenticidas anticoagulantes y otros compuestos catalogados como persistentes, bioacumulativos y tóxicos (PBT). A nivel europeo existe una iniciativa que emplea las aves rapaces como centinelas ambientales y busca el objetivo de mejorar el seguimiento del cumplimiento normativo, detectar riesgos emergentes y fortalecer la biomonitorización en Europa mediante una red coordinada de análisis, bancos de muestras y recolección de datos en campo [121,122].

Entre las especies más empleadas para tal fin en Europa, destacan el cárabo común (*Strix aluco*), busardo ratonero (*Buteo buteo*), cernícalo vulgar (*Falco tinnunculus*), búho real (*Bubo bubo*), lechuza común (*Tyto alba*), mochuelo europeo (*Athene noctua*) y búho campestre (*Asio otus*). Estas aves se caracterizan por tener una distribución amplia, contar con un porcentaje elevado de roedores en su ingesta, ser carroñeros o frecuentar entornos antropizados [118].

No obstante, la bibliografía es variada en cuanto a las diferencias de exposición entre especies de diferentes grupos, hábitos y dieta. En términos generales, se espera que las aves rapaces nocturnas especialistas predadoras de pequeños mamíferos muestren mayor grado de exposición a rodenticidas anticoagulantes. Sin embargo, este patrón no se verifica en todos los estudios, describiéndose frecuencias de detección muy similares en aves rapaces especialistas y generalistas, y mayores concentraciones hepáticas en las segundas [43,123], lo que refuerza la idea de que existe una dispersión amplia de estos compuestos en toda la cadena trófica y tipo de presas [15,124].

Con respecto a la sensibilidad, las aves rapaces son consideradas más sensibles a estos compuestos en comparación con otras aves, lo cual se atribuye principalmente a una menor actividad enzimática y a una mayor susceptibilidad a la inhibición de la enzima vitamina K 2,3-epóxido reductasa (VKOR)[125]. No obstante, también son conocidas las diferencias de sensibilidad entre las diferentes especies de aves rapaces. Un estudio a gran escala realizado en América del Norte reveló que las rapaces pertenecientes a la familia Tytonidae presentan una sensibilidad significativamente mayor a los rodenticidas anticoagulantes (RAs) en comparación con otras familias de rapaces, evidenciada por umbrales de toxicidad notablemente más bajos [126]. A partir de estos resultados, se propusieron nuevos valores de referencia para la interpretación toxicológica, recomendando un umbral promedio de 80 ng/g con una probabilidad del 50% de toxicidad para las familias Accipitridae, Falconidae y Strigidae. En contraste, para las Tytonidae, como las lechuzas, se sugiere aplicar un umbral de 40 ng/g para alcanzar la misma probabilidad debido a su mayor vulnerabilidad, e incluso considerar valores más bajos en el caso de especies endémicas o en peligro, como medida de precaución [126]. Asimismo, los autores de este estudio sugieren que la difetialona y el brodifacum tienen una toxicidad comparable, mientras que la bromadiolona es menos tóxica para estos animales, pero no lo suficiente como para justificar su uso exterior sin restricciones.

A pesar de estas diferencias, la bibliografía global apunta a la presencia generalizada de SGARs, concretamente brodifacum, bromadiolona. Asimismo, la coexistencia de múltiples rodenticidas ha sido documentada en rapaces de América del Norte, Europa y Asia, lo que indica que se trata de un fenómeno generalizado, no limitado a una región o especie específica e incluso en edades tempranas [109,116,123,127–129].

Finalmente, cabe destacar que el uso de estos compuestos, aunque presentes en los eventos de envenenamiento de rapaces, no han sido las principales sustancias detectadas en Europa. En su lugar, el aldicarb, el carbofurano y la alfacloralosa son los principales protagonistas de estos eventos. No obstante, los pesticidas, entre los que se incluyen los rodenticidas anticoagulantes son una de las principales amenazas para la supervivencia de las aves rapaces y carroñeras [85].

2.3.1.2. Aves carroñeras

Las aves carroñeras, facultativas y obligadas, enfrentan una amenaza global por la exposición a pesticidas, tanto accidental como intencionada, que afecta a cerca del 70% de las especies. Aunque la mayoría de los datos se refieren a exposiciones no letales a organoclorados, se han documentado en todo el mundo casos de intoxicaciones con rodenticidas anticoagulantes, fármacos veterinarios, carbamatos y organofosforados, incluso en áreas protegidas, con eventos de mortalidad masiva [120]. Un ejemplo de ello es la ruta del Mediterráneo Oriental, donde la causa de mortalidad por envenenamiento involuntario es común a 13 países de toda la zona geográfica para aves migratorias como el alimoche común [130].

La exposición de este grupo a rodenticidas anticoagulantes se produce principalmente por la vía secundaria a partir del consumo de carcasas o tejidos de roedores u otras carcasas de animales mayores previamente intoxicados [28,38,112,131–133]. Las aves también pueden estar expuestas al consumir otros depredadores o carroñeros que, a su vez, han acumulado RAs, amplificando así la contaminación a lo largo de la cadena trófica a través de la exposición terciaria [38].

Diversas especies de aves carroñeras presentan una alta vulnerabilidad a la exposición a rodenticidas anticoagulantes, especialmente aquellas con hábitos tróficos estrechamente ligados a la carroña en paisajes antropizados. El cóndor de California (*Gymnogyps californianus*) es especialmente susceptible debido a su comportamiento carroñero obligado y su dependencia de grandes carroñas, las cuales pueden estar contaminadas con RAs; casi la mitad de los individuos analizados mostraron niveles compatibles con toxicidad, lo que representa una amenaza significativa para los esfuerzos de conservación de esta especie en peligro de extinción [131,132]. De manera similar, el buitre americano (*Cathartes aura*) y el cuervo común (*Corvus corax*) han mostrado elevadas tasas de exposición (hasta un 93% en buitres americanos), con efectos fisiológicos adversos como deterioro del estado corporal e incremento de los niveles de estrés, particularmente en regiones con uso intensivo de RAs [131,132]. En España, el 39,1% de los buitres y milanos de vida libre estaban expuestos a SGARs, registrándose para el milano negro (*Milvus migrans*) y el milano real (*Milvus milvus*) un 100% y un 66,7% respectivamente, especialmente en áreas con intensa actividad humana donde consumen carroña de tamaño pequeño o mediano [112].

La exposición de este grupo de aves a rodenticidas anticoagulantes, aunque no sean los protagonistas de los escenarios de envenenamiento, suponen una amenaza indirecta más para la supervivencia de estas especies emblemáticas en Europa y requieren de un biomonitoreo constante de las poblaciones de vida libre.

2.3.1.3. Aves no rapaces

Se han documentado casos de intoxicación por RAs en al menos 190 especies de aves no rapaces pertenecientes a 17 órdenes y 58 familias, que incluyen especies granívoras, omnívoras e insectívoras [42].

Estas aves pueden ingerir RAs tanto por rutas de exposición primaria como secundaria. Muchas de ellas debido a su pequeño tamaño pueden entrar en las estaciones de cebo o ingerirlos del suelo, especialmente aquellos formulados a base de cereales dirigidos a roedores. Esta vía es especialmente relevante para especies granívoras y omnívoras que pueden confundir los cebos granulados con alimento [42,134]. No obstante, también pueden exponerse al consumir presas contaminadas, como invertebrados, pequeños mamíferos u otras aves que hayan ingerido RAs, aunque esta vía está menos caracterizada [28,42,134].

Entre los factores que influyen en esta exposición destacan la morfología, el tipo de forrajeo y su dieta. Las aves que son más propensas a alimentarse en el suelo, ya sea de forma habitual u oportunista, y que muestran comportamientos curiosos o una mayor disposición a probar alimentos nuevos. La habituación a la presencia humana, la capacidad de forrajear en zonas extensas, el consumo directo de la plaga objetivo (como los roedores) y la tendencia a aprovechar recursos alimenticios efímeros o la escasez de alimento en el entorno se relacionan con una mayor exposición [42,135]. A nivel antropogénico, también se ha observado una mayor exposición en aves cerca de estaciones de cebo usadas en regiones agrícolas-ganaderas que aquellas analizadas a mayor distancia tal y como ocurre con los pequeños mamíferos [135,136].

Las aves granívoras, especialmente en regiones tratadas por plagas de topillos, han mostrado tasas de exposición particularmente elevadas (hasta el 51%) [134] y ciertos paseriformes recolectados cerca de granjas tratadas o en entornos de erradicación de islas han presentado residuos en el 30-50% de los casos incluyendo mortalidad en polluelos con concentraciones moderadas de brodifacum [137]. Asimismo, las aves acuáticas o límicolas de zonas donde se utilizan RAs para el control de plagas también se han visto afectadas [138,139].

En cuanto al riesgo y prevalencia de exposición, existe una gran variabilidad entre la bibliografía disponible y depende de todos los factores anteriormente citados. La variabilidad de las concentraciones hepáticas entre individuos sugiere una combinación de exposición primaria y secundaria, ya que en un mismo estudio se pueden hallar rangos de concentraciones que oscilan entre 4 y 7.809 ng/g en hígado [135]. Finalmente, evaluando el riesgo de exposición, algunos autores han catalogado a las aves granívoras como de alto riesgo de exposición primaria, mientras que las aves insectívoras se encuentran dentro del grupo de riesgo moderado de exposición secundaria a rodenticidas anticoagulantes (RAs) [140,141]. No obstante, la escasez de datos sistemáticos y la invisibilización de la exposición en especies no emblemáticas de este grupo dificultan comprender el alcance real del impacto ecológico de estos compuestos en aves no rapaces.

2.3.2. Mamíferos

Los residuos de rodenticidas anticoagulantes se detectan con frecuencia en mamíferos silvestres de todo el mundo, con tasas de prevalencia variables en función de la región, la especie y la proximidad a actividades humanas. En Europa, contamos con una representación creciente de estudios en este grupo de animales en el que destaca la vulnerabilidad del zorro (*Vulpes vulpes*) entre el resto de las especies [16,17,111,142–145]. La evidencia disponible a nivel global indica una exposición generalizada, y en muchos casos elevada, tanto en carnívoros [66,119] como en otros pequeños y medianos mamíferos piscívoros, insectívoros, omnívoros o herbívoros [17,40,136,142,146], ya sea por exposición primaria o secundaria según el grupo. Además, también se han documentado con regularidad intoxicaciones en mamíferos domésticos como perros y gatos [147,148].

A nivel global, dos recientes revisiones bibliográficas han registrado exposición a RAs en especies de carnívoros, concluyendo que las familias Mustelidae, Canidae, Felidae son las más representadas e identificándose al menos 11 compuestos diferentes de RAs, en los que fueron identificados como causa de mortalidad en el 33,9% de los individuos [66]. De forma similar, aunque centrando su atención en la dieta y hábitos exclusivamente de los mesocarnívoros, se registró un 63,78% de individuos positivos a dichas sustancias [119]. Además, estos autores señalan que la dieta basada en roedores y micromamíferos, así como el desarrollo de hábitos oportunistas y carroñeros relacionados con áreas antropizadas podrían ser los causantes de su exposición, no obstante, no existe un consenso general al respecto [66].

Así se recoge en el capítulo del libro sobre rodenticidas anticoagulantes en fauna silvestre, donde se indica que, aunque la frecuencia de exposición es ligeramente mayor en los depredadores especialistas, las concentraciones hepáticas resultan superiores en los mamíferos generalistas [43]. Además, ambas revisiones coindicen en la frecuencia de presentación simultánea de compuestos y en que los compuestos de segunda generación (SGARs), especialmente brodifacum y bromadiolona, se detectan con mucha más frecuencia que los de primera generación (FGARs), lo que refleja su persistencia y potencial bioacumulativo [66,119]. Finalmente, ponen de manifiesto que en la mayoría de los casos estos animales están expuestos a concentraciones que superan los umbrales de toxicidad establecidos en algunos estudios de rapaces entre los 100-200 ng/g y refieren la problemática de la falta de estudios o conclusiones referentes a los efectos subletales en el grupo [119].

Esta laguna de conocimiento, ya señalada en la sección 1.4.1 de la presente tesis doctoral, pone de relieve que, aunque ha aumentado el número de estudios sobre los efectos de los rodenticidas anticoagulantes en este taxón, aún no se ha logrado una caracterización precisa de la magnitud y complejidad del problema. Sin embargo, existe una base sólida de evidencia que demuestra que estos compuestos, especialmente los SGARs, se bioacumulan y biomagnifican en las redes tróficas de mamíferos, afectando no solo a grandes depredadores, sino también a micromamíferos no diana en los que se ha comprobado una relación directa entre la exposición a rodenticidas y la cercanía a los cebos [115,136]. Asimismo, se ha confirmado la exposición de mamíferos de pequeño- mediano tamaño de hábitos herbívoros, frugívoros o insectívoros señalando nuevas rutas de contaminación [40,115,136,146]. En consecuencia, la diversidad de especies expuestas pone de manifiesto la amplia dispersión de estos compuestos en la red trófica y acentúa significativamente el riesgo de intoxicación secundaria en especies predadoras y carroñeras, muchas de ellas con un importante valor ecológico y cultural en distintas regiones.

2.3.3. Reptiles

El conocimiento sobre la exposición de reptiles a rodenticidas anticoagulantes ha avanzado considerablemente en los últimos años, aunque sigue existiendo una notable brecha respecto a otras clases taxonómicas como aves o mamíferos.

La mayoría de los estudios publicados sobre biomonitorización en este taxón proceden de Australia, Nueva Zelanda y otras islas del Pacífico donde los reptiles son víctimas de las abundantes campañas de erradicación en islas remotas o forman parte del entorno urbano y silvestre del lugar [41,149,150]. Por tanto, casi la totalidad de los estudios se basa en tomas de muestras oportunistas que intentan evaluar el daño colateral de las campañas de erradicación en las especies no diana del espacio natural tratado.

Hasta la fecha de esta tesis doctoral, y bajo nuestro conocimiento, en España, contamos con escasos estudios en este sector y muchas veces están asociadas a bases de datos de servicios de toxicología donde los ejemplares han estado vinculados a episodios de envenenamiento intencionado o a campañas de erradicación [114,134,151].

En líneas generales, la bibliografía disponible los coloca en rutas de exposición directa e indirecta, puesto que en la naturaleza se les ha observado consumir directamente el cebo e ingiriendo presas como roedores, aves o invertebrados [41,139,149,150,152]. En estos entornos, tanto urbanos como salvajes, se han reportado elevadas concentraciones y frecuencias, pudiendo confirmar el proceso de bioacumulación y biomagnificación en función de la dieta de estos animales y su posición en la cadena trófica. Asimismo, la valoración del riesgo de este taxón los incluye dentro del grupo de mayor riesgo de exposición primaria [140] y en el grupo de riesgo bajo para reptiles insectívoros en exposición secundaria [141].

En cuanto a su sensibilidad, se cree que este grupo, a diferencia de los mamíferos o las aves, podrían tener un nivel de tolerancia mayor a estos compuestos debido a su metabolismo más lento, la falta de ciertos factores de coagulación o su condición como ectotermos [149,153]. Así lo demuestran algunos estudios experimentales y observaciones realizadas en ambientes de erradicación donde no se ha detectado mortalidad o signos de coagulopatía [68,139,152,154]. No obstante, estos resultados son variables en función de la especie de estudio, ya que, en tortuga verde, iguanas y amebas sí se han observado efectos adversos observables [68,155]. Asimismo, en el medio natural se ha hipotetizado la posibilidad de un efecto retardado de mortalidad.

La suma de la posible tolerancia a los compuestos y su interacción con ellos, le ha otorgado las condiciones idóneas para ser un posible vector para otros animales que preden sobre ellos, o incluso para seres humanos que los consuman en su dieta en algunas regiones del planeta.

2.3.4. Anfibios

De todos los taxones incluidos, los anfibios son los menos representados en la literatura. No obstante, una publicación reciente detectó la presencia de varios pesticidas, incluidos rodenticidas anticoagulantes, en cinco especies de ranas silvestres en Australia [156]. Previamente, se había documentado la exposición a rodenticidas en serpientes cuya dieta se basaba en gran medida en anfibios [41], aunque hasta entonces no se había confirmado la exposición directa en sus presas. En particular, esta publicación detectó el SGAR brodifacum en el 66,6% de los ejemplares analizados, lo que evidencia la incorporación de estos compuestos en otro grupo taxonómico y su potencial papel como fuente de contaminación para sus depredadores. Entre las posibles vías de exposición, los autores sugieren el contacto con agua contaminada, la ingestión de invertebrados, o, en el caso de individuos de mayor tamaño, el consumo ocasional de pequeños roedores [156].

2.3.5. Animales acuáticos

La relación entre el medio acuático y los rodenticidas anticoagulantes ha sido tradicionalmente subestimada. No obstante, en los últimos años ha emergido una creciente evidencia sobre la bioacumulación y el efecto de estos compuestos en organismos acuáticos, incluyendo peces, invertebrados y aves acuáticas [157–163]. Esta contaminación proviene principalmente de fuentes como las aguas residuales [29,159,161], que a pesar de ser tratadas no consiguen eliminar de manera absoluta este tipo de residuos, o del agua de escorrentía contaminada durante las campañas de erradicación [163].

En cuanto a la fauna silvestre expuesta, diversos estudios han documentado la presencia de rodenticidas anticoagulantes con elevadas prevalencias en el hígado de peces silvestres de agua dulce, lo que indica una bioacumulación considerable y su papel como vectores de estos compuestos en la cadena trófica acuática [157–159,161]. Este fenómeno ha afectado también a aves piscívoras como cormoranes y ánades y a mamíferos como las nutrias que se ven expuestos al consumir peces contaminados. En estas especies, destaca la presencia predominante de compuestos de segunda generación, tales como brodifacum, difenacum y bromadiolona [142,158]. Además, se han registrado casos de aves limícolas halladas muertas tras campañas de erradicación de roedores en islas [139]. Asimismo, algunos estudios han analizado los efectos de estos compuestos en organismos acuáticos inferiores, observando impactos negativos en gametos y larvas de coral a concentraciones elevadas (100 ppb), aunque se han catalogado como escenarios extremos y poco probables en condiciones naturales [164].

Cabe destacar, que no solo los seres vivos eminentemente acuáticos se ven afectados por las aguas contaminadas, la proximidad a los lechos de los ríos y los ecosistemas acuáticos también se ha vinculado con una mayor prevalencia de rodenticidas anticoagulantes en algunas aves rapaces [116]. En conjunto, estos hallazgos destacan la transferencia de rodenticidas anticoagulantes a lo largo de la red trófica acuática, lo que refuerza la necesidad de intensificar las investigaciones y fortalecer las regulaciones en este ámbito, adoptando un enfoque "*One Health*" para salvaguardar tanto la salud animal como humana.

2.3.6. Invertebrados

Por último, en la base de la cadena trófica, la exposición de los invertebrados a rodenticidas anticoagulantes ha sido poco investigada. Sin embargo, cada vez son más los estudios que apuntan a su posible papel como vectores de contaminación para depredadores insectívoros, lo que pone de manifiesto la necesidad de evaluar con mayor detalle el riesgo que pueden representar.

Entre los grupos de invertebrados con mayor capacidad de absorción de estos compuestos encontramos los gasterópodos, resultando más anecdótico en insectos [31,32,165]. Si bien es cierto que la literatura incluye una amplia gama de invertebrados con residuos detectables tanto en campo como en estudios experimentales [28,139,164,166–168].

Aunque aún no se conoce con precisión el riesgo real que estos organismos suponen para sus depredadores, se ha demostrado que los invertebrados pueden ingerir y bioacumular rodenticidas anticoagulantes, sin mostrar, en la mayoría de los casos, signos evidentes de toxicidad aguda [31,165]. Esta aparente tolerancia podría deberse a diferencias fisiológicas clave, como su sistema de coagulación, que difiere notablemente del de los vertebrados [169]. No obstante, estudios recientes han comenzado a revelar efectos subletales en estos organismos relacionados con el comportamiento [69] o con alteraciones fisiológicas asociadas al crecimiento y el estrés oxidativo [167].

Por tanto, el hecho de que estos seres vivos puedan actuar como vectores supone un riesgo para aquellos mamíferos, reptiles, anfibios y aves incluidas algunas rapaces como los cernícalos (*Falco tinnunculus*), los *moreporks* (*Ninox novaeseelandiae*) o los mochuelos (*Athene noctua*) cuya dieta puede incluir un porcentaje significativo de ellos [21].

En este sentido, diversos autores señalan que las aves insectívoras podrían presentar un riesgo moderado de exposición secundaria, mientras que los reptiles y las rapaces con dietas parcialmente insectívoras se encontrarían en un nivel de riesgo más bajo [141]. Paralelamente, otros autores afirman que el consumo de estos invertebrados tendría que ser como mínimo el de su peso corporal para estar en una situación de riesgo del 50% de intoxicación aguda, lo que resulta en una preocupación menor [166]. No obstante, otros estudios han advertido de posibles riesgos agudos, acumulativos o crónicos para ciertos depredadores como la musaraña (*Sorex araneus*), el estornino (*Sturnus vulgaris*) o el erizo *(Erinaceus europaeus),* especialmente tras el consumo de babosas contaminadas [31]. Asimismo, el desarrollo de modelos probabilísticos de riesgo de mortalidad y coagulopatía subaguda en aves insectívoras que se alimentan de caracoles revela rangos de riesgo de mortalidad (3-8%) y de coagulopatía subaguda (0,42-11%) variables. En contraste, especies como la codorniz *(Coturnix coturnix)* o el ánade real *(Anas platyrhynchos)* se situaron dentro de rangos de riesgo aceptables en el mismo estudio [165].

Por tanto, estos hallazgos evidencian la necesidad de profundizar en el estudio tanto de los efectos directos de los RAs sobre los invertebrados como de las implicaciones ecológicas de su papel como eslabones en la transferencia trófica de estos compuestos. Ello permitirá comprender mejor la dinámica de movimiento de los rodenticidas anticoagulantes en los ecosistemas y su verdadero impacto en la fauna silvestre.

2.4. Biomonitorización de Rodenticidas Anticoagulantes en España

Se realizó una búsqueda no sistemática de literatura científica para identificar estudios realizados en la última década sobre biomonitorizacion de rodenticidas anticoagulantes en fauna silvestre en España. Las bases de datos consultadas fueron PubMed, Scopus y Google Scholar. La búsqueda incluyó combinaciones de palabras clave en inglés, tales como: *Spain Rodenticides*, *Rodenticides Wildlife Spain*, *Rodenticides Reptiles Spain*, *Rodenticides Mammals Spain*, *Rodenticides Birds Spain*, *Rodenticides Raptors Spain*, *Rodenticides Invertebrate Spain*, *Rodenticides Biomonitoring Spain*, *Rodenticides Poisoning Spain*, *Poisoning Wildlife Spain*, así como las mismas combinaciones sustituyendo *"Spain"* por *"Mediterranean"*. Se revisaron artículos en inglés y español publicados hasta abril de 2025.

Como se puede observar en la tabla y mapa adjuntos (***Tabla 1***, ***Figura 2***), el grupo más estudiado en términos de biomonitorización en nuestro país fue el de las aves rapaces seguido de los mamíferos. No obstante, si ampliamos la búsqueda a estudios de envenenamiento de fauna silvestre podemos encontrar una variedad más amplia de grupos taxonómicos debido al carácter oportunista de estas investigaciones en el medio natural asociados a muestras recibidas en los laboratorios de toxicología o cuerpos oficiales del Estado.

El estudio más reciente de uso de cebos en contra de la fauna silvestre incluye una vista retrospectiva de 17 años en la región de Extremadura, en la que destacan como principales agentes los organofosforados y carbamatos, siendo los responsables del 85,3% de los cebos. No obstante, el uso de cebos comerciales a base de rodenticidas anticoagulantes está presente en un 10,1% de los casos, destacando el brodifacum como el compuesto más detectado seguido de la bromadiolona y el difenacum [170]. Patrones similares se observaron en el estudio de envenenamiento de la fauna silvestre canaria [151], coincidiendo con los productos más abundantes permitidos en nuestro país y los que más variedad tienen para el público general (***Figura 1***; [77]). En este sentido, la información sobre los delitos de la fauna silvestre queda reflejada mayormente en las bases de datos oficiales, aunque su interpretación no siempre refleja el escenario preciso de los eventos de envenenamiento [171].

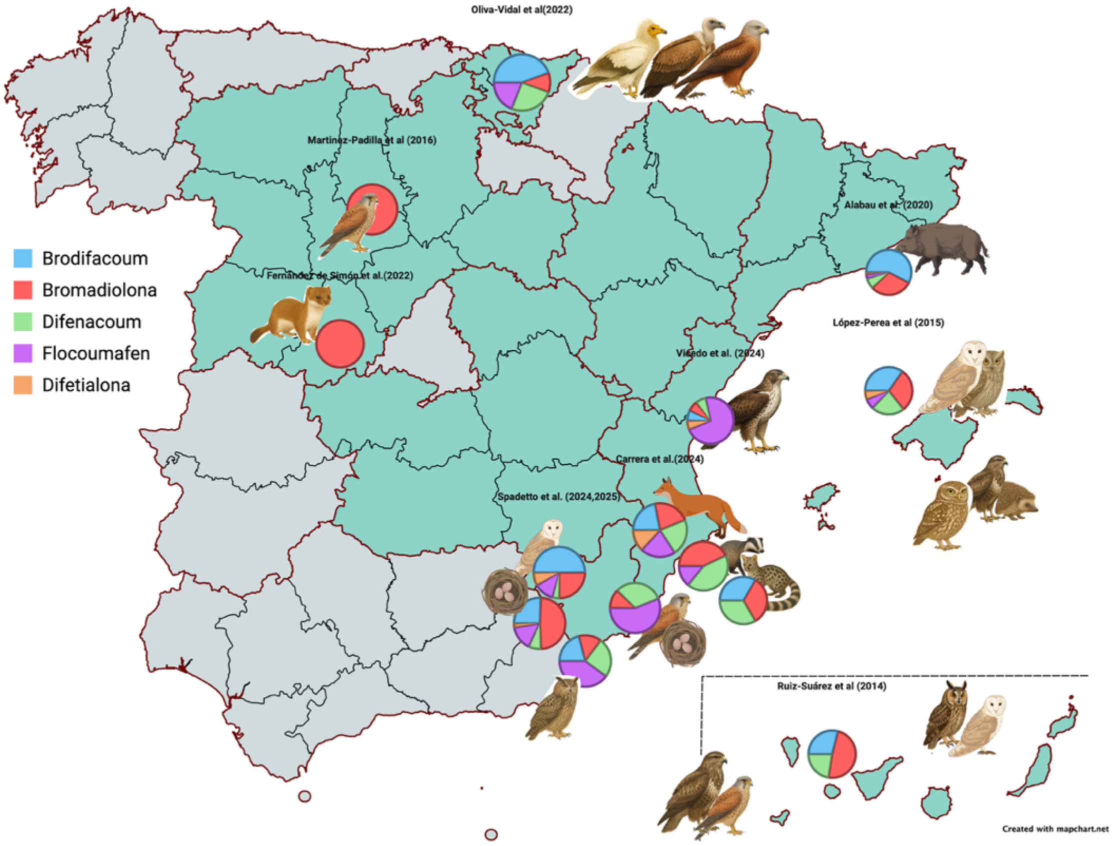

Figura 2. Regiones de España con estudios de biomonitorización de rodenticidas anticoagulantes realizados en los últimos diez años. Los gráficos de tipo donut, específicos para cada región, muestran los compuestos más frecuentemente detectados en cada área, diferenciados por colores según la leyenda. Mapa creado por mapchart.net y editado en biorender.

Adicionalmente a estos estudios, en la última década se han publicado varios trabajos que combinan el biomonitoring con el estudio de episodios de envenenamiento en el que podemos observar una gama más amplia de especies y taxones incluyendo reptiles, aves y mamíferos [113,114,134,151]. En ellos destaca el protagonismo de los rodenticidas como agentes confirmados del envenenamiento de la fauna en un 21,1% de los casos, aunque en este trabajo hay que considerar que los resultados podrían no representar la situación real del medio natural al ser una zona previamente tratada por la plaga de topillo campesino con amplio uso de bromadiolona y clorofacinona [134]. En el resto de los estudios citados, el brodifacum se presentó como el compuesto más prevalente, no obstante, también se achaca la causa de muerte por estos compuestos en el 29% de los casos en Canarias sin estar relacionado con campañas de erradicación [114,151] y se observaron concentraciones potencialmente letales >200 ng/g frecuentemente en el nordeste de España [113].

Tabla 1. Síntesis retrospectiva de estudios de biomonitorización de rodenticidas anticoagulantes en fauna silvestre en España durante la última década.

Autor (Año)	Taxón	Región	Especies	Variables	Matriz	FGARs detectados	SGARs detectados	Múltiples compuestos	Prevalencia (%), n total	Umbral toxicidad
Ruiz-Suárez *et al.* (2014) [172]	Rapaces	Islas Canarias	Rapaces nocturnas y diurnas	Hábitos alimenticios	Hígado	Clorofacinona	Bromadiolona > Brodifacum > Difenacum	36,5%	61% (n=104)	35% > 100 ng/g
López-Perea *et al.* (2015) [110]	Aves rapaces y mamíferos	Cataluña y Mallorca	Rapaces nocturnas y erizo	Especie, región, actividad antropogénica nivel de toxicidad	Hígado	Warfarina	Brodifacum > Bromadiolona > Difenacum > Flocumafeno y Difethialona	34,6%	62,8% (n=344)	23,3% >200 ng/g
Martínez-Padilla *et al.* (2016) [61]	Rapaces	Palencia y Valladolid	Cernícalo (pollos)	Peso, condición corporal, sexo, edad, región y año	Sangre	No se analizaron	Bromadiolona	No aplica	16,9%	Datos no disponibles
Alabau *et al.* (2020) [111]	Mamíferos	Barcelona, Ciudad Real, Castilla-La Mancha	Jabalí	Edad, sexo estereoquímica (cis/trans) y región	Hígado	Difacinona (Solo detectado en músculo)	Brodifacum > Bromadiolona > Difenacum > Flocumafeno > Difethialona	20,2%	45,2% (n=84)	9,5% >200 ng/g
Fernández de Simón *et al.* (2022) [173]	Mamíferos	Castilla y León	Comadreja	Sexo, año, factores de riesgo (brote plaga)	Hígado	0%	Bromadiolona (solo isómero trans)	0%	21,9% (n=32)	6,3% > 100 ng/g

Autor (Año)	Taxón	Región	Especies	Variables	Matriz	FGARs detectados	SGARs detectados	Múltiples compuestos	Prevalencia (%), n total	Umbral toxicidad
Oliva-Vidal *et al.* (2022) [112]	Carroñeras	Pirineos	Milano real y negro, alimoche y buitres	Especie (facultativa/ obligada), edad y sexo	Sangre	No se incluyeron	Brodifacum > Difenacum > Flocumafeno > Bromadiolona	18,4%	39,1% (n=261)	Datos no disponibles
Vicedo *et al.* (2024) [109]	Rapaces	Comunidad Valenciana	Águila de Bonelli	Antropogénicas, causa de muerte	Hígado	Clorofacinona > Difacinona, Warfarina Coumatetralil Coumafuril (< 25%)	Difenacum, Bromadiolona, Flocumafeno, Brodifacum (100%) > Difethialona (88,2%)	100%	100% (n=17)	35% > 200 ng/g
Carrera *et al.* (2024) [145]	Mamíferos	Alicante	Zorro, tejón, jineta	Causa de muerte, edad, sexo y peso	Hígado	Coumatetralil, Coumafuril Warfarina, Clorofacinona Difacinona	Difenacum, Bromadiolona y Brodifacum (100%) > Flocumafeno (96%), > Difethialona (60%)	100%	100% (n=30)	53% > 200 ng/g
Spadetto *et al.* (2024) [18]	Rapaces	Murcia	Cernícalos y lechuzas Pollos y adultos	Antropogénicas edad y parámetros de coagulación	Sangre	Coumatetralil, Difacinona, Clorofacinona	Brodifacum, Bromadiolona, Flocumafeno, Difenacum, Difetialona (varía pollo vs adulto y especie)	16% lechuzas y 32,9% cernícalos	Pollos Cernícalos: 68,6%; Pollos lechuza (50%) Lechuzas adultas: 100% (n=136)	Datos no disponibles

Autor (Año)	Taxón	Región	Especies	Variables	Matriz	FGARs detectados	SGARs detectados	Múltiples compuestos	Prevalencia (%), n total	Umbral toxicidad
Spadetto *et al.* (2024) [116]	Rapaces	Murcia	Búho chico (pollos)	Uso del suelo, año, lugar y parámetros de coagulación	Sangre	Clorofacinona > Coumatetralil	Flocumafeno > Brodifacum > Difenacum > Bromadiolona	82,6%	98,6% (n=69)	Datos no disponibles
Spadetto *et al.* (2025) [108]	Rapaces	Murcia	Búho real (pollos)	Antropogénicas ambientales biomarcadores y parámetros de coagulación	Sangre	Warfarina > Coumatetralil > Clorofacinona, Difacinona	Flocumafeno > Difenacum > Brodifacum > Bromadiolona > Difethialona	70,8%	91,5% (n=106)	Datos no disponibles

2.4.1. Limitaciones de los Estudios de Biomonitorización y Epidemiología de Envenenamiento

Es fundamental tener en cuenta que muchos estudios de biomonitorización se basan en muestras hepáticas obtenidas de forma oportunista. Estas suelen proceder de individuos ingresados en centros de recuperación de fauna silvestre o recuperados por los servicios ambientales de los gobiernos autonómicos. Este enfoque plantea varias consideraciones importantes. En primer lugar, las muestras analizadas no siempre son representativas de las poblaciones silvestres en su conjunto. En segundo lugar, la mayoría de los ejemplares presentan un estado de salud comprometido —por lesiones, enfermedades o estrés— y mueren o son tratados en instalaciones veterinarias, lo que puede sesgar los resultados hacia una mayor detección de contaminantes. Asimismo, la heterogeneidad en los protocolos de necropsia y en la recogida de muestras entre centros puede dificultar la comparación sistemática entre especies o regiones impidiendo una caracterización completa del estado de los individuos evaluados. No obstante, recientes estudios en los que se ha conseguido evaluar la presencia de estos compuestos en sangre de individuos de vida libre [18,112], indican una exposición generalizada de las poblaciones incluso a edades tempranas [108,116], lo que refuerza el valor de la vigilancia ambiental a nivel global.

Finalmente, es importante destacar el abordaje generalizado de la literatura disponible y de la presente tesis doctoral en cuanto al uso de la sumatoria de compuestos para realizar los modelos probabilísticos de riesgo de las poblaciones estudiadas. En este sentido, algunos autores ponen en duda esta aproximación debido a las diferencias en la toxicocinética y potencia entre compuestos [37]. No obstante, las mayores diferencias se observan entre compuestos del grupo FGARs vs SGARs, y en este sentido, la mayoría de las publicaciones se centra en el segundo grupo para realizar los análisis estadísticos correspondientes.

3. RODENTICIDAS ANTICOAGULANTES EN LAS ISLAS CANARIAS

3.1. Canarias: Protección y Vigilancia Ecológica

El archipiélago canario se localiza en el océano Atlántico nororiental cercano al continente africano. Políticamente forma parte de España y constituye una de sus comunidades autónomas. Está integrado por ocho islas principales y varios islotes menores, con una superficie total aproximada de 7.500 km². La combinación de su origen volcánico, su aislamiento geográfico y la diversidad de microclimas ha favorecido el desarrollo de una notable riqueza biológica, con elevadas tasas de endemicidad en numerosos grupos taxonómicos, como los invertebrados y los reptiles [174]. Estas características hacen de Canarias un enclave de gran valor ecológico y estratégico para la conservación de la biodiversidad. Sin embargo, también la convierten en un territorio especialmente vulnerable a las perturbaciones externas, siendo la introducción de especies exóticas invasoras una de las principales amenazas para sus ecosistemas nativos [175].

Junto con Madeira, Azores, Cabo Verde e Islas Salvajes, Canarias forma parte de la región biogeográfica macaronésica, reconocida por su singularidad ecológica. En este contexto, una parte significativa del territorio insular se encuentra incluida en la Red Natura 2000, una iniciativa europea destinada a preservar los hábitats naturales y las especies de fauna y flora silvestres [176]. Esta red contempla dos figuras principales de protección: las Zonas Especiales de Conservación (ZEC), anteriormente denominadas Lugares de Importancia Comunitaria (LIC), y las Zonas de Especial Protección para las Aves (ZEPA). En la actualidad, el archipiélago canario alberga 154 ZEC y 45 ZEPA, lo que refleja su relevancia como área prioritaria para la conservación a nivel europeo [177].

La elevada biodiversidad del archipiélago, junto con las amenazas a las se que enfrenta, hace que el seguimiento sanitario de la fauna silvestre resulte clave para su conservación. Bajo esta premisa, en 2018 se creó la Red Vigía de Canarias, un sistema de vigilancia sanitaria orientado a la detección y análisis de las principales causas de mortalidad en la fauna silvestre [35]. Dentro de esta red, el Servicio de Toxicología de la Universidad de Las Palmas de Gran Canaria ha desempeñado un papel esencial como laboratorio de referencia durante más de una década. De hecho, sus primeros informes sentaron las bases científicas para el desarrollo de la normativa autonómica sobre el uso de venenos en zonas no urbanas del archipiélago, consolidando así su contribución a la gestión y conservación de la biodiversidad canaria [34].

Desde entonces, se han detectado 133 sustancias tóxicas en los análisis realizados, tras examinar casi 400 compuestos diferentes por caso, lo que facilita la identificación de amenazas concretas con el fin de avanzar hacia un análisis predictivo que permita identificar patrones recurrentes y ayuden a la anticipación de riesgos y la implementación de medidas preventivas orientadas a la conservación [35].

3.2. Exposición de la Fauna Silvestre en Canarias a Rodenticidas Anticoagulantes

Hasta la fecha del comienzo de esta tesis doctoral, nuestro equipo de investigación llevó a cabo el desarrollo de métodos de extracción multirresiduo basados en QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) adaptados y validados para el suelo y diversas matrices biológicas como la sangre o hígado con los que se ha conseguido obtener una visión aproximada de la exposición de la fauna silvestre y los suelos de nuestro archipiélago a una amplia gama de agentes químicos [56,57,178,179]. Algunos de estos métodos han sido evaluados por la comunidad científica, situándolos entre los mejor posicionados en los rankings de técnicas validadas para la determinación de rodenticidas anticoagulantes en determinadas matrices biológicas [55].

Entre los compuestos más frecuentemente detectados en la fauna silvestre canaria se encuentran los rodenticidas anticoagulantes (RAs), caracterizados por su alta toxicidad y persistencia ambiental e identificados en especies no diana, principalmente en aves rapaces, debido a la exposición secundaria tras la ingestión de presas contaminadas y a su posición como máximos depredadores terrestres en el ecosistema insular.

Cronológicamente y sirviendo de antecedente a los estudios realizados en la presente tesis doctoral, en el archipiélago canario se han publicado tres artículos de biomonitorización y/o evaluación de causas de envenenamiento.

El primero de ellos se llevó a cabo entre 2009 y 2012 en seis especies de rapaces y reveló que el 61% de los individuos analizados contenían residuos de al menos un AR, siendo bromadiolona el más frecuente. Un 35% de las aves presentaba niveles por encima del umbral tóxico, con especial incidencia en especies nocturnas y consumidoras de micromamíferos, como *Tyto alba* y *Asio otus*. La ausencia de RAs de primera generación indicó un uso predominante de SGARs en el archipiélago [172].

Estos hallazgos se vieron reforzados por un análisis posterior (2011–2020) que incluyó 831 animales. En este estudio, la prevalencia de exposición a RAs en rapaces fue del 60%, con una media superior a dos compuestos detectados por ejemplar. El cernícalo común (*Falco tinnunculus*) mostró las mayores concentraciones, y se documentó por primera vez la presencia de RAs en especies como el halcón de Eleonor (*Falco eleonorae*) y el halcón tagarote (*Falco pelegrinoides*). Además, el análisis geoespacial reveló una estrecha relación entre la presencia de explotaciones ganaderas intensivas y el grado de exposición a estos tóxicos [114].

Sin embargo, la exposición no se limita a aves rapaces. La epidemiología de los envenenamientos (2014–2021) evidenció que los RAs siguen siendo causa frecuente de mortalidad en fauna silvestre, tanto por exposición intencional como secundaria. Se detectaron residuos en el 7,2% de los cuervos canarios (*Corvus corax canariensis*), especie en peligro crítico, así como en otras aves no rapaces y reptiles. Estos hallazgos sugieren posibles vías de exposición no tradicionales, como la ingestión directa de cebos o la bioacumulación a través de invertebrados contaminados [151].

En conjunto, estos datos reflejan un uso generalizado e inapropiado de estos compuestos en los entornos naturales de Canarias, y marca un precedente esencial para la continuidad de su monitoreo en nuestro archipiélago.

4. FUTURAS LÍNEAS DE MANEJO SOSTENIBLE

4.1. Estereoquímica de Rodenticidas Anticoagulantes: Isómeros *cis- trans*

Investigaciones recientes destacan que los isómeros *cis* y *trans* (diasteroisómeros) de los rodenticidas anticoagulantes de segunda generación (SGARs) difieren significativamente en su persistencia ambiental y capacidad de bioacumulación, lo que influye directamente en la exposición y toxicidad para la fauna silvestre [180].

Un ejemplo de ello es la larga semivida hepática de entre 10-30 días descrita para la bromadiolona en topillos asociada a la elevada carga del isómero trans en la composición del producto [181]. Esta diferencia en la persistencia también se observa en fauna silvestre, donde los isómeros menos persistentes, como *cis*-bromadiolona y *trans*-difenacum, rara vez se detectan o lo hacen en concentraciones muy bajas en hígados de depredadores, mientras que los más persistentes (*trans*-bromadiolona, *cis*-difenacum y *cis*-brodifacum) predominan en los perfiles de residuos alcanzando concentraciones tóxicas en especies carroñeras y rapaces, y contribuyendo así al envenenamiento secundario [182–184].

Esta tendencia se ha podido comprobar también en España, en estudios de mamíferos como la comadreja *(Mustela nivalis)* [173] y en aves carroñeras [112]. No obstante, los resultados obtenidos en estudios de jabalí *(Sus scrofa)* mostraron dicha tendencia para el difenacum, pero no para la bromadiolona y el brodifacum en la que ambos isómeros se detectaron en proporciones similares [111].

Si bien ambos isómeros presentan potencias anticoagulantes similares, la persistencia prolongada de algunos de ellos aumenta el riesgo de exposición crónica y toxicidad acumulativa [180,182]. En este contexto, la modificación de las formulaciones comerciales para incluir preferentemente isómeros de rápida eliminación se plantea como una estrategia prometedora para reducir los efectos ecotoxicológicos sobre especies no diana, sin comprometer la eficacia del control de roedores [180–182].

4.2. Gestión Integrada de Plagas (GIP)

La Gestión Integrada de Plagas (GIP) nace como respuesta a la creciente preocupación por las resistencias, los envenenamientos de especies no diana y la persistencia en el medio de los rodenticidas anticoagulantes.

El enfoque de esta gestión busca manejar y prevenir problemas causados por diferentes seres vivos de una forma más segura, efectiva y sostenible, aplicando diversas estrategias de prevención, monitoreo o métodos biológicos que no hagan depender únicamente de los productos químicos [185,186]. Entre estas alternativas destaca la vital importancia de realizar un correcto diagnóstico de la situación inicial para desarrollar una estrategia efectiva conociendo el hábitat y la especie involucrada en el área infestada. Así como el monitoreo y evaluación continua durante y después del tratamiento. No obstante, es sabido por los profesionales del sector que existen infestaciones masivas imposibles de controlar únicamente con otros métodos y precisan de anticoagulantes para su completa erradicación [11].

De manera preventiva, la mejora de la higiene, la limitación de las fuentes de alimento y la gestión del hábitat ha resultado tener un mayor éxito en el control y retrasa la reaparición de ratas en comparación con las granjas que dependen únicamente de rodenticidas. Estas medidas también parecen reducir la necesidad de utilizar rodenticidas y limitan indirectamente la propagación de poblaciones de roedores resistentes [187]. En segundo lugar, el uso de métodos biológicos, especialmente el uso de aves rapaces como depredadores, tiende a reducir las poblaciones de roedores plaga, aunque la mayoría de las investigaciones son a corto plazo y carecen de datos poblacionales a largo plazo para cuantificar completamente su impacto [188–190]. Concretamente en España, la instalación de cajas nido para cernícalos y lechuzas ha resultado en un aumento de sus poblaciones, y una reducción local del topillo campesino en las zonas más cercanas a dichas áreas de cría [8,9]. Asimismo, se ha podido comprobar que su efectividad depende de la especie de depredador, el contexto del paisaje y las prácticas de manejo, siendo especialmente eficaces cuando se integran en estrategias más amplias de manejo de plagas. Finalmente, el uso combinado de trampas mecánicas —actualmente equipadas con tecnologías avanzadas que permiten la monitorización remota y la videovigilancia— ha demostrado ser eficaz, especialmente cuando se aplica siguiendo las directrices de la estrategia NoCheRo (Non-Chemical alternatives of Rodent control). Esta alternativa representa un riesgo global significativamente menor para la salud humana, la salud animal y el medio ambiente [10,19].

4.3. Uso de Matrices Biológicas Menos Invasivas

El diagnóstico de rodenticidas anticoagulantes puede realizarse a partir de diversas matrices biológicas, si bien es cierto, que las más elegidas son el hígado y la sangre debido al movimiento y la bioacumulación de estos compuestos hacia dichas matrices biológicas [55]. No obstante, son muestras poco accesibles debido a la necesidad de que los ejemplares estén muertos o haya que capturarlos en el medio natural. Por ello, el contar con alternativas menos invasivas se presenta como una línea de estudio de gran interés, especialmente en el medio natural.

Estudios recientes han evaluado diversas matrices biológicas no invasivas para la detección de RAs obteniendo resultados prometedores como el uso del pelo en la detección de FGARs y SGARs en mamíferos. Un ejemplo de ello es la detección en zorros rojos observando que el 50% de las muestras de pelo resultaron positivas para al menos un RA, siendo los compuestos de segunda generación los más detectados, lo que demuestra que esta matriz puede reflejar eficazmente la exposición a RAs en fauna silvestre [16]. De igual forma se ha evaluado el potencial de las heces como método práctico y no letal para evaluar la exposición en animales vivos como los zorros rojos. En estos mamíferos se detectaron residuos de RA en el 53% de las muestras fecales, con buena concordancia para ciertos compuestos (como coumatetralil, difenacum y difetialona) entre las heces y el hígado [191]. No obstante, estudios realizados en ratas con bromadiolona concluyen que las concentraciones en hígado no son extrapolables a las halladas en las heces [192]. Además, se debe tener en cuenta que los residuos de RA en heces se degradan relativamente rápido tras la exposición ambiental, con semividas que oscilan entre aproximadamente 5 y 8 días, según el compuesto. Por ello, se recomienda recolectar excrementos frescos [193]. En cuanto a las plumas, no se ha encontrado evidencia directa en los estudios revisados que respalden su uso como matriz fiable para la detección de RAs en fauna silvestre. Por tanto, la investigación y validación se centran en pelo y heces como matrices no invasivas.

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JUSTIFICACIÓN

JUSTIFICACIÓN

El archipiélago canario forma parte de la región biogeográfica macaronésica, reconocida por su alto nivel de biodiversidad y endemismo. Este patrimonio natural singular ha motivado que una parte significativa del territorio insular esté integrada en la Red Natura 2000, la principal herramienta de conservación de la Unión Europea, lo que subraya su importancia estratégica en la conservación de hábitats y especies a nivel comunitario.

Sin embargo, esta riqueza biológica se enfrenta a múltiples amenazas derivadas de la actividad humana, entre las que destaca el uso generalizado de rodenticidas anticoagulantes (RAs), especialmente en entornos rurales y periurbanos. Estos compuestos, empleados para el control de roedores, tienen un elevado potencial de toxicidad no solo para las especies objetivo, sino también para numerosos organismos no diana a través de procesos de exposición directa o indirecta. A pesar de su relevancia ecológica, la información sobre la presencia y el impacto de los RAs en la fauna silvestre del archipiélago sigue siendo limitada, especialmente en especies de niveles tróficos bajos o con rutas de exposición cuya caracterización no está completamente definida.

En este contexto, el seguimiento sanitario de la fauna silvestre se convierte en una herramienta clave para la detección temprana de amenazas toxicológicas. Con este fin, se creó la Red Vigía de Canarias, una red de vigilancia sanitaria orientada al estudio de las principales causas de mortalidad en la fauna silvestre. Dentro de este sistema, el Servicio de Toxicología de la Universidad de Las Palmas de Gran Canaria ha desempeñado un papel fundamental como laboratorio de referencia, aportando datos que han sido esenciales para la elaboración de normativa autonómica sobre el uso de venenos y productos tóxicos en el medio natural entre los que destacan como protagonistas los rodenticidas anticoagulantes.

Asimismo, otro fenómeno que afecta al archipiélago es la llegada y establecimiento de ciertas especies exóticas invasoras como el camaleón de Yemen (*Chamaeleo calyptratus*) o la culebra real de California (*Lampropeltis californiae*). Dichas especies pueden desempeñar un papel relevante como centinelas ecológicos en la detección de contaminantes ambientales al ocupar nichos tróficos similares a los de especies residentes, pero con poblaciones más abundantes o accesibles, permitiendo obtener información valiosa sobre los riesgos ambientales sin comprometer directamente a especies emblemáticas. Su estudio puede, por tanto, servir como modelo para entender rutas de exposición, bioacumulación y posibles efectos ecotoxicológicos en la biota nativa.

Esta tesis se justifica en la necesidad urgente de ampliar el conocimiento sobre la exposición a rodenticidas anticoagulantes en la fauna silvestre canaria, incluyendo especies residentes e invasoras de medio-largo establecimiento que hasta ahora han sido escasamente consideradas. Los resultados de este trabajo contribuirán no solo a mejorar la comprensión de los riesgos ecológicos asociados a estos contaminantes, sino también a fortalecer las bases científicas para la toma de decisiones en materia de conservación y gestión de la biodiversidad en un territorio tan frágil y valioso como el archipiélago canario.

HIPÓTESIS Y OBJETIVOS

HIÓTESIS GENERAL

La exposición a rodenticidas anticoagulantes de segunda generación (SGARs) está ampliamente extendida en la fauna silvestre de Canarias, afectando a múltiples niveles tróficos y taxones, incluidos aquellos de bajo nivel trófico, debido a su persistencia ambiental y capacidad de bioacumulación. Esta exposición no se explica únicamente por la proximidad a zonas urbanas, sino que depende de factores biológicos, geográficos y antrópicos como el uso del suelo. Algunas especies, incluyendo ciertos invasores, pueden actuar como centinelas eficaces para el monitoreo de esta contaminación en ecosistemas insulares.

OBJETIVOS

1. Cuantificar la exposición a rodenticidas anticoagulantes de segunda generación (SGARs) en diferentes especies silvestres representativas de los ecosistemas insulares canarios, abarcando múltiples niveles tróficos y estrategias alimentarias.

2. Explorar las vías de entrada de SGARs en la red trófica terrestre, incluyendo posibles rutas de exposición secundaria a través del consumo de invertebrados contaminados por parte de especies insectívoras.

3. Analizar los factores biológicos (edad, sexo, tamaño), espaciales (región, hábitat), antrópicos (uso del suelo, ganadería) y regulatorios que influyen en la prevalencia y concentración de estos compuestos en fauna no diana.

4. Comparar la carga contaminante de SGARs entre regiones insulares y continentales, con el fin de valorar el grado de vulnerabilidad ecológica asociado a las características propias de los ecosistemas insulares.

5. Evaluar la idoneidad de determinadas especies silvestres, incluyendo especies invasoras, como centinelas ecológicos, en función de su representatividad trófica, sensibilidad toxicológica, accesibilidad para el muestreo y utilidad para la vigilancia integrada.

PUBLICACIONES

PUBLICACIÓN 1

SCIENCE OF THE TOTAL ENVIRONMENT

"Potential exposure of native wildlife to anticoagulant rodenticides in Gran Canaria (Canary Islands, Spain): Evidence from residue analysis of the invasive California Kingsnake (Lampropeltis californiae)"

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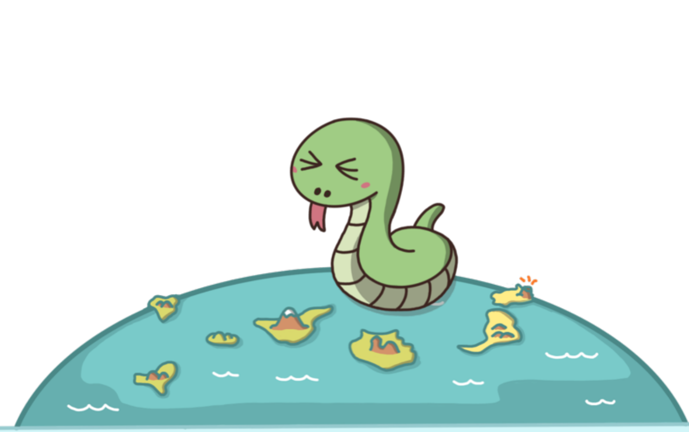

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Potential exposure of native wildlife to anticoagulant rodenticides in Gran Canaria (Canary Islands, Spain): Evidence from residue analysis of the invasive California Kingsnake (*Lampropeltis californiae*)

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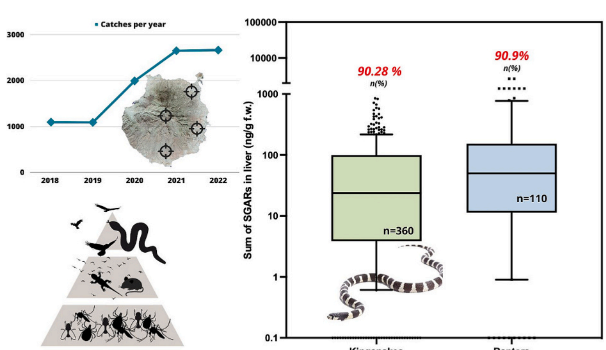
HIGHLIGHTS

- *Lampropeltis californiae* is a good indicator of wildlife exposure to ARs in Gran Canaria.
- High AR frequency of detection and concentration observed in California kingsnakes
- Higher concentrations of ARs are influenced by snakes' body condition and geographic area.
- California kingsnakes seem to tolerate a high degree of ARs exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Anticoagulant rodenticides (ARs), particularly second-generation compounds (SGARs), are extensively used in pest management, impacting non-target wildlife. The California kingsnake (*Lampropeltis californiae*), an invasive species in Gran Canaria, is under a control plan involving capture and euthanasia. This research aimed to detect 10 different ARs in these snakes, explore geographical and biometrical factors influencing AR exposure, and assess their potential as sentinel species for raptors, sharing similar foraging habits. Liver samples from 360

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Non-target animals
Brodifacoum
Bromadiolone

snakes, euthanized between 2021 and 2022, were analysed for ARs using LC-MS/MS. Results showed all detected rodenticides were SGARs, except for one instance of diphacinone. Remarkably, 90 % of the snakes tested positive for ARs, with over half exposed to multiple compounds. Brodifacoum was predominant, found in over 90 % of AR-positive snakes, while bromadiolone and difenacoum were also frequently detected but at lower levels. The study revealed that larger snakes and those in certain geographic areas had higher AR concentrations. Snakes in less central or more peripheral areas showed lower levels of these compounds. This suggests a correlation between the snakes' size and distribution with the concentration of ARs in their bodies. The findings indicate that the types and prevalence of ARs in California kingsnakes on Gran Canaria mirror those in the island's raptors. This similarity suggests that the kingsnake could serve as a potential sentinel species for monitoring ARs in the ecosystem. However, further research is necessary to confirm their effectiveness in this role.

1. Introduction

Anticoagulant rodenticides (ARs) are widely used to control rodent populations, but they also pose a threat to non-target wildlife. ARs can be classified into first-generation anticoagulants (FGARs) and second-generation anticoagulants (SGARs), which differ in toxicity, half-life, and resistance. Both types of ARs cause coagulopathy by inhibiting vitamin K1 and clotting factors. SGARs are more effective and persistent than FGARs, but also more hazardous to the environment (Bermejo-Nogales et al., 2022; Nakayama et al., 2019; Rattner et al., 2014; Ravindran et al., 2022). Many non-target species, such as mammals, birds, and reptiles, are exposed to ARs through various pathways, resulting in adverse effects on their health and survival (Lohr and Davis, 2018; Nakayama et al., 2019; Ravindran et al., 2022; Sánchez-Barbudo et al., 2012; Shore and Coeurdassier, 2018). The European Commission has implemented regulations to limit the use and concentration of ARs in baits, and to promote sustainable alternatives for pest management (EC, 2019, 2017). Wildlife biomonitoring studies are essential to assess the presence and impact of ARs in the environment, and to protect both biodiversity and human health (García-Fernández et al., 2020; Gómez-Ramírez et al., 2014; Grove et al., 2009).

Sentinel species are used in wildlife toxicology to assess the risks of contaminants for specific or similar species (Badry et al., 2021; Chumchal et al., 2022; Grove et al., 2009; Ruiz-Suárez et al., 2016; Sonne et al., 2020). To be an effective sentinel, a species must meet criteria such as exposure potential, geographic distribution, ease of collection, and susceptibility to contaminants (Basu et al., 2007; Golden and Rattner, 2003). Invasive snakes, like the California kingsnake (*Lampropeltis californiae*), the horseshoe whip snake (*Hemorrhois hippocrepis*) or the brown tree snake (*Boiga irregularis*), are generalist predators that have impacted endemic fauna, especially on islands (McElderry et al., 2022; Montes et al., 2021; Wiseman et al., 2019). They are also subject to governmental management (Gallo Barneto et al., 2018; Soto et al., 2022), which facilitates their collection over time. Therefore, they are promising sentinels for studying wildlife contaminant exposure in regions where they are established. Recent research support the suitability of snakes as indicators of environmental and food web contamination (Hoang et al., 2021; Lettoof et al., 2020; Lohr and Davis, 2018).

The California kingsnake (*Lampropeltis californiae*) is a nonvenomous constrictor and a generalist predator native to the southwestern North

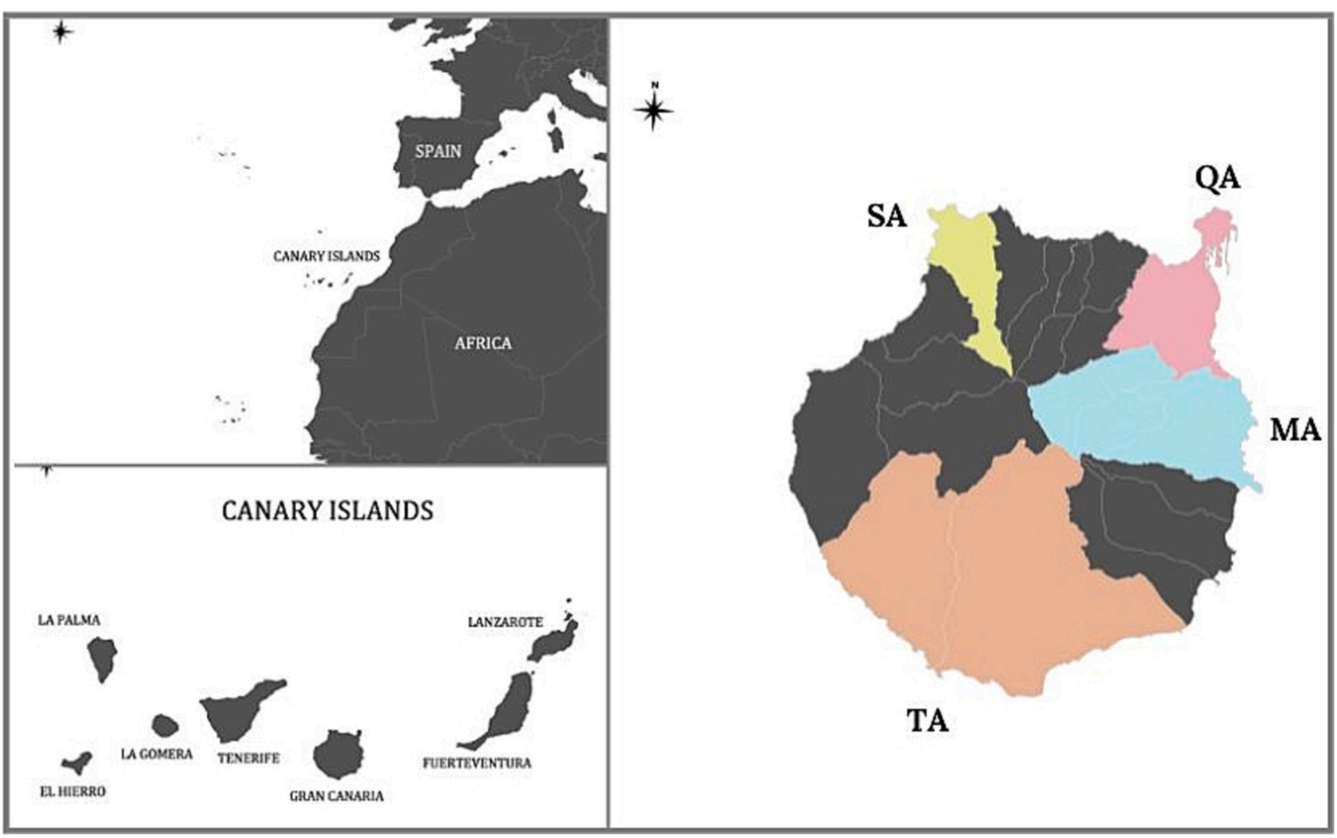

Fig. 1. Geographical location of the Canary Islands archipelago on the left; and Gran Canaria Island with the four distribution areas of the California kingsnake (MA = Main Area, SA = Secondary Area, TA = Tertiary Area, QA = Quaternary Area) on the right.

America and northwestern Mexico (Pyron and Burbrink, 2009; Wiseman et al., 2019). In Gran Canaria (Canary Islands, Spain) it has been classified as invasive, and it preys on various species, especially rodents and three endemic reptiles: the giant lizard (*Gallotia stehlini*), the skink (*Chalcides sexlineatus*) and the Boettger's wall gecko (*Tarentola boettgeri*) (Gallo and Mateo, 2020; Monzón-Argüello et al., 2015; Piquet and López-Darias, 2021). The snake was introduced to the island over two decades ago, probably by accidental or intentional releases of captive individuals. It naturalized in 2007 and has rapidly expanded despite control efforts (Cabrera-Pérez et al., 2012; Gallo and Mateo, 2020; Piquet et al., 2021). It is now established in four main areas of the island the island (Fig. 1) and >2000 snakes have been removed in the past year (BOE, 2022; GESPLAN, 2023a). The island's isolation, climate and lack of predators favour this adaptable and opportunistic snake (Gallo and Mateo, 2020; Monzón-Argüello et al., 2015; Piquet et al., 2021). If not contained, it could colonize other islands in the archipelago (Fisher et al., 2021; Piquet et al., 2021).

These characteristics makes it a suitable sentinel species for assessing the exposure of non-target wildlife species to ARs in the Island of Gran Canaria, particularly for raptor species such as Common buzzards (*Buteo buteo*), European sparrowhawks (*Accipiter nisus*), Barbary falcon (*Falco pelegrinoides*), Common kestrels (*Falco tinnunculus*), Long-eared owls (*Asio otus*), and Barn owls (*Tyto alba*). These birds of prey have exhibited elevated exposure to these contaminants in the archipelago, given their dietary habits centered around rodents, reptiles, and birds, which closely resemble those of snakes (Rial-Berriel et al., 2021a, 2021c; Ruiz-Suárez et al., 2014).

The aims of this study were: a) to characterize the exposure of the California kingsnake to ARs; b) to identify the geographical and biometrical factors influencing snakes' exposure to ARs; c) to investigate the potential suitability of kingsnakes as a sentinel species by comparing their AR exposure to that of raptors.

2. Material and methods

2.1. Study area

This research was conducted on Gran Canaria, belonging to the Canary Islands archipelago, situated off the northwest coast of Africa. These oceanic islands are notable for their geographical isolation and subtropical climatic conditions. Notably, 43 % of the island's central and southwestern landmass is a Biosphere Reserve. This region has an average annual temperature of 22 °C, a variety of ecosystems, and a remarkable prevalence of endemic invertebrate, avian, and reptilian species (Biota, 2023; UNESCO, 2023).

The invasive California kingsnake is predominantly located in four established areas, which are associated with the following municipalities (Fig. 1): main area (MA: municipalities of Telde, Santa Brígida, Valsequillo and San Mateo), secondary area (SA: municipalities of Gáldar and Agaete), tertiary area (TA: municipalities of San Bartolomé de Tirajana and Mogán) and quaternary area (QA: municipality of Las Palmas de Gran Canaria) in addition to other independent dispersal areas (BOE, 2022; Gallo and Mateo, 2020; GESPLAN, 2023a).

2.2. Sampling of snakes and raptors

Snakes were collected randomly by hand or using traps throughout the island, euthanized and consequently frozen at the GESPLAN facility in Gran Canaria between 2021 and 2022 as part of the eradication program *Strategic Plan for the control of California kingsnake in the Canary Island* #STOPCULEBRAREAL (GESPLAN, 2023b). All snakes were alive when they were retrieved from the traps. The frozen snakes were necropsied following the procedures described by the Association for Sustainable Development and Conservation of Biodiversity (ADS) guidelines (GESPLAN, 2023c) at SERTOX facilities, Department of Toxicology, University of Las Palmas. Livers, recognized as the primary organs for rodenticide accumulation (Vudathala et al., 2010), were collected and subsequently stored at −20 °C, until chemical analysis. Snake characteristics including sex (female, male or unknown sex-lack of developed sexual organs), design pattern (banded, striped, or aberrant), coloration pattern (albino, normal: brown/black), weight, fat weight, snout-vent length (SVL in cm), total length (TL in cm) and tail length (in cm) were noted. Additionally, the presence or absence of observed haemorrhagic lesions was also recorded. This involved the identification of external or internal haemorrhagic wounds (superficial or deep), bleeding within body cavities, and generalized haemorrhages.

In parallel, data regarding raptors, including species identification and location, were collected as part of the Poisoning Control and Prevention Strategy in the Canary Islands (BOC, 2014). The study included six raptors endemic to the Canary Islands: *Tyto alba, Asio otus, Falco tinnunculus, Accipiter nisus, Falco pelegrinoides* and *Buteo buteo insularum.* These raptors were either found dead in the wild, or in the recovery centres of Gran Canaria between 2020 and 2022. Necropsies, including the collection of livers and storage, were carried out by the Anatomical Pathology Department, at the University of Las Palmas.

2.3. Analysis of anticoagulant rodenticides in snake and raptor livers'

Certified AR standards (warfarin, diphacinone, chlorophacinone, coumachlor, coumatetralyl, brodifacoum, bromadiolone, difethialone, difenacoum and flocoumafen) and procedural-internal standard (P-IS, (±)-Warfarin-d5) of maximum purity (93.1 %–99.8 %) from Dr. Ehrenstorfer (Augsburg, Germany) were used. The solvents used were acetonitrile, methanol (ACN and MeOH, >99.9 % purity) and formic acid (FA, 98 % purity) from Honeywell (Morristown, NJ, USA). While water was produced in the laboratory using a MilliQ A10 water purification system (Millipore, Molsheim, France). QuEChERS Extract Pouch, AOAC Method (6 g de magnesium sulphate and 1.5 g sodium acetate), were purchased in commercial premixes from Agilent Technologies (Palo Alto, CA, USA).

Liver samples were extracted by the methodology previously validated in our research team (Rial-Berriel et al., 2021b, 2020b, 2020a). Liver tissue (1 g) was disaggregated and homogenized with 4 ml of miliQ water at 6500 rpm, 2 × 30 s in a Precellys Evolution homogenizer (Bertin Technologies, Rockville, Maryland., USA). Then, 1 g of the homogenate was manually shaken for 1 min with 2 mL of ACN 1 % FA in a 5 mL Eppendorf tube and submitted to ultrasonication for 20 min (VWR, 50/60 Hz, 120 W). Next, 480 mg of anhydrous magnesium sulphate and 120 mg sodium acetate were added to each sample tube, mix with a vortex for 30 s, and manually shaken for 1 min. Samples were then centrifuged at 3125.16 xg (4200 rpm) and 2 °C for 5 min (5804 R, Eppendorf, Hamburg, Germany). The supernatant was then filtered through a 0.2 μm Chromafil PET-20/15 (Macherey-Nagel, Düren, Germany) into glass amber vials.

Quality Control samples (QCs) were generated utilizing a blank chicken liver matrix to ensure methodological consistency. A comprehensive ten-point calibration curve was constructed, spanning a concentration range of 0.195 to 100 ng/g. This curve was prepared following the same extraction protocol delineated in the preceding section, thereby facilitating more accurate and reliable comparative analyses. Similarly, QCs were prepared at a single concentration of 2 ng/g (with RSD ≤ 20 % and REC = 70–120 % for QCs, LOD/LOQ) (Supplementary Tables 1 and 2). All samples, QCs, calibration points, and blanks were spiked with the P-IS solution prior to extraction and concentrations were presented as wet weights (ww).

To detect and quantify ARs, an Agilent 1290 UHPLC linked to an Agilent 6460 triple quadrupole mass spectrometer was utilized. A heated InfinityLab Poroshell 120 column was used in tandem with an inline filter and UHPLC guard column. The mobile phase employed a gradient of 0.1 % FA and 2 mM ammonium acetate in water (Phase A) and 2 mM ammonium acetate in MeOH (Phase B). Injection volume and flow rate were set at 8 μL and 0.4 mL/min, respectively. The mass spectrometer ran in dynamic multiple reaction monitoring (dMRM) mode across both polarities, with specific cycle, dwell, and run times. Operational parameters for the Agilent Jet Stream Electrospray Ionization Source (AJS-ESI) and the gases used are detailed in the methods. Further validation parameters are available in (Rial-Berriel et al., 2020a, 2020b).

2.4. Statistical analyses

All statistical analyses were conducted in R (R Core Team, 2021). Frequency of detection was determined as the percentage of animals presenting at least one AR in their livers. All the analyses conducted were centered on SGARs. This decision was based on the substantial differences in molecular weights between FGARs and SGARs, as well as the fact that we only detected one FGAR in a single snake (Herring et al., 2022).

To better understand the factors influencing exposure to second-generation anticoagulant rodenticides (SGARs) in California kingsnakes, we utilized a Generalized Linear Model (GLM) with binomial error distribution and logit link function. Given the high prevalence of SGARs in our samples (90 % positive) and aiming to assess kingsnakes as potential sentinel species for wildlife, particularly raptors, we adopted a threshold-based approach. We dichotomized our data at 100 ng/g wet weight (ww), aligning with the 75th percentile of our data (99.1 ng/g ww) and near levels deemed potentially toxic to certain raptors (Newton et al., 1999; Thomas et al., 2011). However, we recognize the limitations of direct comparisons, as our focus is on reptiles, and raptor species vary. Recent literature, including Herring et al. (2022), suggests nuanced interpretations of toxicosis probabilities at different concentration thresholds. Our model included sex, age class, weight, snout-vent length, and distribution areas, analyzing data from 356 snakes, excluding four with missing data.

Furthermore, we examined predictors of variation in the sum of AR concentrations (ΣARs) in the snakes. The response variable, ΣARs, was log-transformed due to its non-normal distribution. The explanatory variables for this model were similar: sex, age class, weight (g), snout-vent length (cm), distribution areas, and necropsy findings (0 = absence, 1 = presence). We also considered including the capture method (manual/trap) as a variable. This decision was influenced by Lettoof et al. (2020), who suggested that AR exposure might alter normal reptilian behavior, potentially leading to a bias in which easily hand-caught snakes exhibit higher AR concentrations.

Given the low limits of quantification of our method (below 1 ng/g), we treated snakes with non-detectable ARs as true negatives. Consequently, we excluded these 35 individuals, in addition to the four with missing data, from the model ($n = 39$).

Explanatory variables were checked for correlations using the Spearman's correlation test before being inserted in both models, and those presenting high correlations (Spearman's rho >0.7) were prevented to appear in the models. Given the high correlation between age class, weight, and SVL, we inserted just SVL in both global models and excluded the other overmentioned explanatory variables.

For model refinement, we performed a multi-model inference using the 'MuMIn' R package (v.1.46.0) (Bartoń, 2022) ranking the best models through the corrected Akaike's Information Criterion (AICc) and ΔAICc <2 (those within two units) (Burnham and Anderson, 2002). Best models were checked for general fit using the 'performance' R package (v.0.10.4) (Lüdecke et al., 2021).

Concentrations of ARs between raptors and snakes were compared using a Mann-Whitney test. Finally, figures were generated using Microsoft® Excel v16.77.1 and GraphPad Prism v9.4.3 (GraphPad Software, CA, USA).

3. Results and discussion

A total of 360 snakes, with the majority captured in 2021 ($n = 338$), was sampled across the different distribution areas (Fig. 2). Of these, 51.1 % ($n = 184$) were females, 41.7 % ($n = 150$) were males, and 7.2 % had unknown sex ($n = 26$). While 52.2 % ($n = 188$) were adults, 32.8 % subadults ($n = 118$), and 15 % ($n = 54$) juveniles, according to the classification used by Wiseman et al. (2019). The most prevalent coloration pattern was normal (79.7 %, $n = 287$), while the most common design pattern was striped (84.7 %, $n = 305$).

3.1. Descriptive analysis of ARs found in California kingsnakes from Gran Canaria

Among the 10 ARs tested, all of them were SGARs (brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen). However, diphacinone (8.13 ng/g ww) was an exception as it belongs to FGARs and was detected in just one individual (Fig. 4). This trend appears to be widespread across diverse geographical regions and taxa, with SGARs clearly prevailing in food chains (Lohr and Davis, 2018; López-Perea and Mateo, 2018; Nakayama et al., 2019; Sánchez-Barbudo et al., 2012).

In this study, only 9.7 % ($n = 35$) of the examined snakes exhibited undetectable concentrations of ARs in their liver (Fig. 3). This noteworthy frequency of AR detection in kingsnakes surpasses that observed in the native lizard species across the archipelago (62.7 %), as reported previously (Rial-Berriel et al., 2021a). Additionally, over half of the analysed snakes showed two or more ARs (Fig. 3). Exposure to multiple ARs within the same animal has been extensively described in birds and mammals (Lohr, 2018; López-Perea and Mateo, 2018; Luzardo et al., 2014; Ruiz-Suárez et al., 2016, 2014). Although reports on reptiles are less common, Lettoof et al. (2020) have documented that certain snakes *(Pseudonaja affinis)* and lizards *(Tiliqua rugosa)* were exposed to multiple ARs, with a maximum of three compounds. In our study, the most frequently detected pairwise combination of ARs consisted of brodifacoum-bromadiolone, while the trio with the highest frequency of detection included brodifacoum-bromadiolone-difenacoum. These findings raise concerns, as others have previously suggested the possibility of synergistic effects (Lettoof et al., 2020; Lohr, 2018).

Focusing on brodifacoum, this compound exhibited the greatest concentrations among SGARs (max = 790.6 ng/g ww) and the highest frequency of detection (Table 1, Fig. 4). Either alone or in combination with other rodenticides, brodifacoum was present in 99 % of the positive samples. In contrast, difethialone, another SGAR, was detected least frequently (8.6 %, $n = 31$) (Fig. 4). Brodifacoum was previously detected in 23 carcasses of endemic giant lizards on the Canary Islands (Rial-Berriel et al., 2021a). Notably, a study of three urban reptile species in Australia revealed an AR detection frequency of 91 % of snakes *(Pseudonaja affinis)*, 60 % of lizards *(Tiliqua rugosa)* and 45 % of tiger snakes *(Notechis scutatus),* in which brodifacoum was the most frequently detected compound (Lettoof et al., 2020). This pattern is consistent with the findings of Nakayama et al. (2019), who conducted a global literature review and reported that brodifacoum is the most frequently detected AR in wildlife, followed by bromadiolone and difenacoum. The high detection frequency of brodifacoum could be attributed, in part, to its longer half-life compared to others. This is a troublesome finding, considering that it is one of the most toxic rodenticides in wildlife (Nakayama et al., 2019).

3.2. Study of the factors of the exposure of California kingsnake to anticoagulant rodenticides

This study allowed us to identify the biometric and geographical factors influencing snakes' exposure to SGARs.

Regarding SGARs concentration above 100 ng/g ww, the best GLM model was the one including snout-vent length (SVL) and distribution areas (MA, SA, TA, QA) as fixed factors (Table 2). In detail, for each additional unit increase in snake length, there was a 3.6 % rise in the likelihood of detecting SGARs at these concentrations [OR (95 % CI) = 1.036 (1.021–1.052), $p < 0.001$] (Table 2). The distribution areas QA

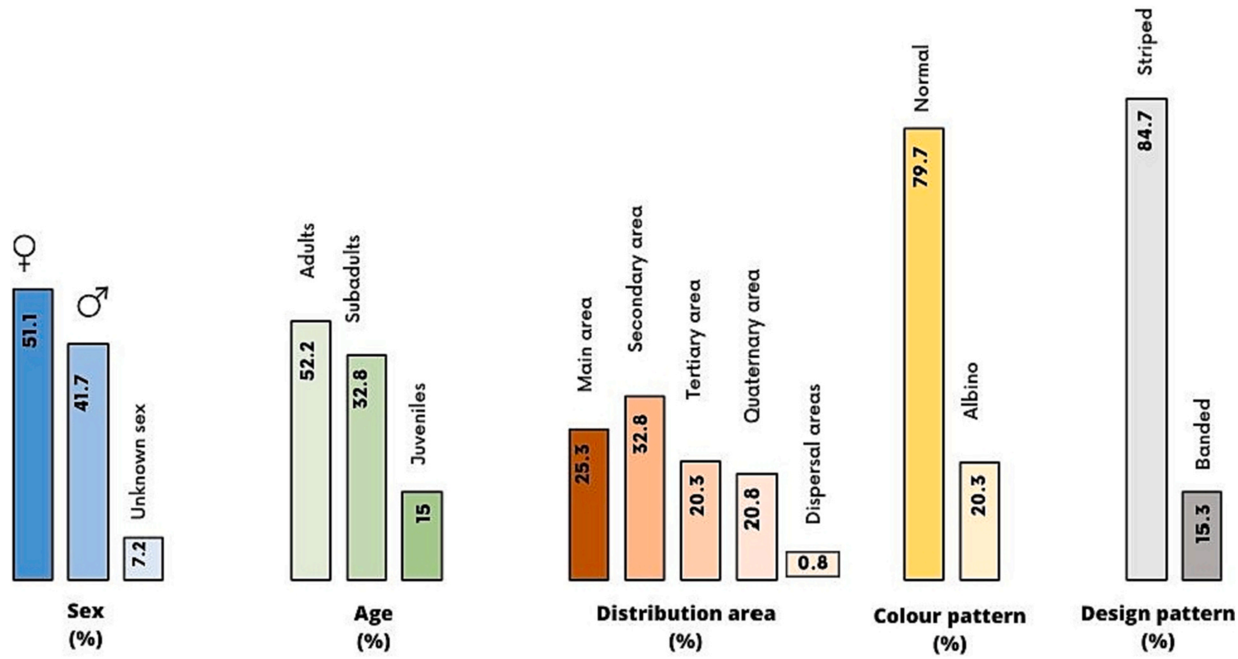

Fig. 2. Characteristics of the series of 360 California kingsnakes (*Lampropeltis californiae*) of Gran Canaria (Canary Islands).

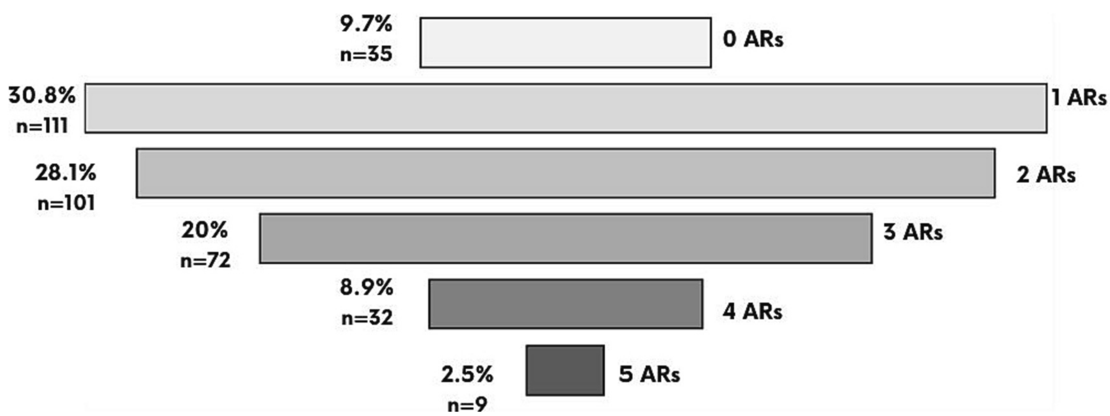

Fig. 3. Funnel plot showing the percentage of snakes exposed to different anticoagulant combinations.

Table 1
Descriptive statistics for the identified SGARs in the snakes of Gran Canaria.

	Brodifacoum	Bromadiolone	Difenacoum	Difetihalone	Flocoumafen
Minimum	0.57	0.34	1.20	1.52	0.34
Maximum	790.6	673.4	168.0	101.4	141.7
Geometric mean	17.77	7.06	5.01	7.21	1.36
SE	5.87	6.72	1.99	4.97	2.40

Note: Descriptive statistics of the SGARs detected in the snakes, including the maximum and minimum values, geometric mean, and standard error of the mean (SE), reported in ng/g ww. in liver. Number of snakes tested positive for SGARs = 325.

and SA exhibited a significant negative effect, compared to MA, indicating that snakes residing in these areas are less likely to exhibit presence of SGARs above the established threshold [(QA = OR (95 % CI): 0.364 (0.169–0.754), p = 0.008); (SA = OR (95 % CI): 0.257 (0.128–0.502), $p < 0.001$); (TA = OR (95 % CI): 0.490 (0.237–0.987), p = 0.049)] (Table 2).

When considering the sum of SGARs (ΣSGARs) as the response variable, the best model was the one that included SVL ($p < 0.001$), distribution area [(QA; $p = 0.001$), (SA; $p < 0.001$), (TA; $p = 0.118$)] and capture method. Specifically for one unit increase in snake length the probability of finding higher concentrations of SGARs was 4 % (Table 3). Snakes inhabiting SA and QA, had 73 % and 55 % lower probability, respectively, of presenting high concentrations of these compounds. (Table 3).

Based on these findings, it is apparent that snake size is an important biometric factor in predicting both variations and higher concentrations of SGARs (Tables 2 & 3), likely to be related to the process of bioaccumulation and biomagnification that occurs in larger animals when they consume greater quantities and larger prey items (Hoang et al., 2021). However, the same study has reported contrasting trends related

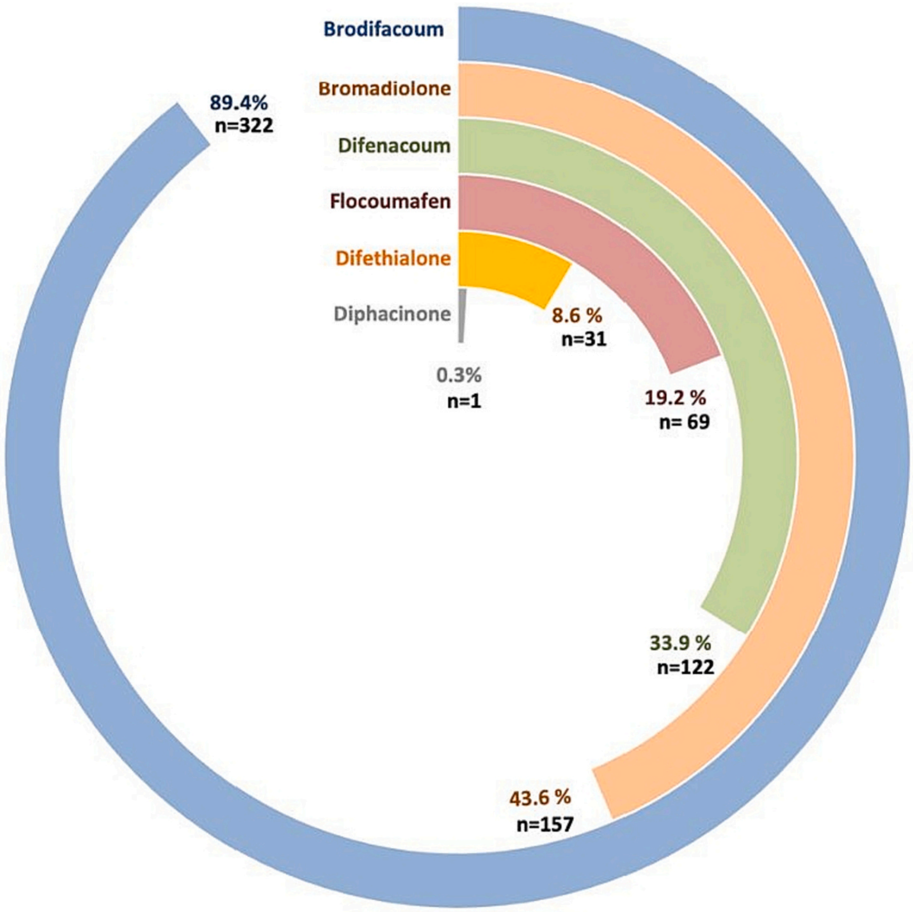

Fig. 4. Doughnut chart showing the frequencies of rodenticide detection found in the total number of snakes sampled ($n = 360$) in Gran Canaria (Canary Islands).

Table 2
Summary of the analysis of exposure to higher concentrations of SGARs (above the threshold set at 99.1 ng/g) in the California kingsnake.

	Est.	SE	OR (95 % CI)	*p*	AICc
Intercept	−3.341	0.660	0.035 (0.009–0.125)	<0.001	
SVL	0.036	0.008	1.036 (1.021–1.052)	<0.001	
Distribution area QA	−1.010	0.380	0.364 (0.169–0.754)	0.008	370.9
Distribution area SA	−1.358	0.348	0.257 (0.128–0.502)	<0.001	
Distribution area TA	−0.713	0.363	0.490 (0.237–0.987)	0.049	

Note: Model outcomes are summarized as the estimated regression parameters (Est.) with standard errors (SE), odds ratio (OR- exponential of estimates) and correspondent 95 % confidence interval (95 % CI), and *p*-values from a Generalized Linear Model with binomial distribution. The corrected Akaike's Information Criterion for the model is also reported. Response variable: 75th percentile (threshold set at 99.1 ng/g). Number of snakes in the analysis = 356. The comparative table, containing selected models based on the corrected Akaike's Information Criterion (AICc) and ΔAICc <2 (those within two units), can be found in Supplementary Table 4.

Table 3
The best linear model explaining the variation in rodenticide concentrations (ΣSGARs) in the California kingsnake.

	Estimates	SE	Effect size 95%CI	*p*	AICc
Intercept	0.815	0.426	2.26 (0.976–5.228)	0.057	1195.9
SVL	0.038	0.005	1.04 (1.029–1.049)	<0.001	
Distribution area QA	−0.800	0.248	0.45 (0.275–0.733)	0.001	
Distribution area SA	−1.315	0.231	0.27 (0.170–0.423)	<0.001	
Distribution area TA	−0.394	0.251	0.67 (0.412–1.105)	0.118	
Capture method TRAP	−0.326	0.212	0.72 (0.476–1.095)	0.125	

Note: Models' outcomes are summarized as the estimated regression parameters (Est.) with standard errors (SE), 95 % confidence interval (95 % CI), and p-values from a linear model. The corrected Akaike's Information Criterions for the model is also reported. Number of snakes in the analysis equals to 321. Response variable (ΣSGARs) was log-transformed for analysis. Effect sizes (95 % CI) are proportional changes in the response variable, derived by exponentiating the estimates and 95 % confidence interval.

to size in other organic pollutants, attributing such correlations to growth dilution. Furthermore, given that the categorization into age groups (juvenile, subadult, and adult) relies on this biometric measurement, and since such variables are highly correlated, it is reasonable to anticipate that older individuals will exhibit elevated concentrations of these compounds compared to younger ones.

Regarding the geographical factor, it was found that certain distribution areas had a negative significant association with respect to both response variables, particularly, the second and forth areas of distribution (SA and QA). This is an interesting finding because these areas have a lower density of livestock and crops (ISTAC, 2023) compared to MA. It is well documented that wildlife populations near livestock and agricultural farms have an increased susceptibility to AR exposure (Lohr, 2018; López-Perea et al., 2019; Rial-Berriel et al., 2021a). Therefore, future studies could benefit from more detailed georeferencing the location of snake captures to enable a more accurate assessment.

Finally, the observation of hemorrhagic lesions at necropsy and the capture method were considered factors of interest for the study due to the variability in sensitivity and tolerance of reptiles to these compounds (Lettoof et al., 2020; Lohr and Davis, 2018; Mauldin et al., 2020; Ravindran et al., 2022; Sánchez-Barbudo et al., 2012). However, while they were included in the analysis, there was no discernible effects of these variables on ΣSGAR concentrations (Table 3, Supplementary Table 4).

3.3. Potential role of snakes as a sentinel of raptors' exposure to ARs

The frequencies of ARs detection and detection patterns of the primary compounds were similar in raptors form Gran Canaria (2020–2022) and California kingsnakes (2021–2022). Brodifacoum, bromadiolone and difenacoum emerged as the predominant rodenticides in both groups (Table 4). This consistency aligns with previous studies involving archipelago birds of prey (Rial-Berriel et al., 2021a; Ruiz-Suárez et al., 2014). Similarly, raptors inhabiting the island showed rodenticide combinations in a high percentage of individuals (almost 70 %). In both series, combinations of 2–3 rodenticides were the most prevalent. Nonetheless, a substantial disparity in median values between the two groups was evident, as indicated in Table 4. Based on physiological and behavioural effects that can result from exposure to ARs, it becomes conceivable livers of these raptors, whether found dead in the wild or within wildlife recovery centres, may exhibit a tendency towards higher levels of ARs (Lettoof et al., 2020). Furthermore, it is important to clarify that we only analysed liver samples that appeared fresh, excluding any that were in a state of putrefaction or mummification. However, it must be acknowledged that the exact degree of freshness for some samples was uncertain, which could potentially introduce a bias in the observed concentrations. (Herring et al., 2022).

Considering these results, both species exhibit a high probability of exposure to ARs in Gran Canaria. However, these data alone do not provide conclusive evidence regarding their role as sentinels of raptors on the island. Nevertheless, further in-depth research should be conducted to explore their potential sentinel role, as they have certain characteristics that make them a subject of interest for this purpose.

Firstly, these snakes have been extensively documented throughout the island of Gran Canaria, with established populations found in four distinct areas encompassing eight municipalities, in addition to sporadic occurrences in non-established zones. Moreover, their limited home range, in conjunction with their prevalence make them good indicators of local pollution (Gallo and Mateo, 2020; Hoang et al., 2021). Secondly, they occupy a medium-high trophic level in the Canarian ecosystem and are rarely preyed upon, with only occasional attacks recorded (Gallo and Mateo, 2020; Monzón-Argüello et al., 2015). Thirdly, these snakes exhibit the capacity to bioaccumulate pollutants, as evidenced in this study. This is reinforced by prior research suggesting that snakes preying on rats/mice or smaller reptiles may directly bioaccumulate SGARs due to their persistence in liver tissue (Lettoof et al., 2020). Additionally, there are indications of AR biomagnification, akin to observations with other compounds like persistent organic pollutants, where concentrations surpass those found in their prey (Hoang et al., 2021). Lastly, kingsnake exhibit a substantial annual capture rate, with over 2000 specimens captured in the past year (GESPLAN, 2023a). This species has been the subject of research in both its native habitat and within our archipelago region, with ongoing studies continuing in the area.

In summary, it is important to continue studying the presence of these compounds in Gran Canaria's reptiles, not only considering their role as sentinels but also as potential risks to the local raptor population. These raptors might eventually specialize in snake hunting in the future, which could introduce a new source of high exposure to rodenticides, as evidenced in previous studies involving raptors and high-trophic-level mammals that prey upon snakes or similar reptiles (Hong et al., 2019; Lettoof et al., 2020; Nakayama et al., 2019).

Table 4
Comparison of ARs levels in kingsnakes and raptors of Gran Canaria.

	Kingsnakes n = 360		Raptors *n* = 110		
	n (%)	Median (ng/g ww)	n (%)	Median (ng/g ww)	*p*-value
Brodifacoum	322 (89.4)	19.2	96 (87.3)	32.3	0.014
Bromadiolone	157 (43.6)	6.8	57 (51.8)	9.5	0.129
Difenacoum	122 (33.9)	3.7	44 (40.0)	3.9	0.551
Difetihalone	31 (8.6)	4.7	21 (19.1)	5.7	0.956
Flocoumafen	69 (19.2)	0.8	19 (17.3)	1.8	0.007
Diphacinone	1 (0.3)	–	1 (0.9)	–	–
ΣARs	90.3	23.8	90.9	49.7	0.003

Note: n (%): Frequency of detection for each compound and ΣARs in both species; Median: Median value in ng/g ww.

4. Conclusions

The examination of 360 snake livers from Gran Canaria showed a strikingly high prevalence (>90 %) of anticoagulant rodenticides, with brodifacoum being the most frequent and at highest concentrations. Moreover, over a half of the individuals exhibited the presence of at least two or more rodenticides.

Explanatory factors associated to the presence of higher SGAR concentrations were the snake size, with bigger snakes more likely to be exposed to rodenticides compared to smaller ones and the distribution area, with those inhabiting the Main Area showing higher prevalence of elevated concentrations of rodenticides compared to those in the other three areas.

In the context of its potential as an environmental sentinel species, the California kingsnake has exhibited remarkable effectiveness in indicating contamination by these compounds. Moreover, when compared to raptors, with whom they share certain feeding habits, both species showed elevated frequencies of ARs detection and exhibit similar detection patterns. Nonetheless, advanced investigations are imperative to ascertain its role as a sentinel for exposure in other wildlife species, such as birds of prey.

Consequently, it is of paramount importance to persist in the exploration of these compounds in reptilian populations within the Canary Islands and to contemplate their examination in other regions dealing with invasive snake species. Legislative measures and educational initiatives assume pivotal roles in advocating for the responsible application of these biocides, particularly considering worrying wildlife discoveries.

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CRediT authorship contribution statement

1. **Guarantor of integrity of the entire study:** OPL, BMC.
2. **Study concepts and design:** OPL, BMC.
3. **Sample providing:** RGB, M.A. C-P, AMM.
4. **Literature research:** OPL, BMC, RGM, AMM, CRB.
5. **Laboratory work:** CRB, BMC, AAD.
6. **Data analysis:** OPL, BMC.
7. **Statistical analysis:** OPL, BMC, LAH, KSR, MC.
8. **Manuscript preparation (original draft):** OPL, BMC, MZ.
9. **Manuscript editing:** OPL, AAD, BMC, MZ, M.A.C-P, RGB, ASP, KRS, MC.
10. **Project administration:** OPL.
11. **Funding acquisition:** OPL, MZ, LAH.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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PUBLICACIÓN 2

ENVIRONMENTAL POLLUTION

“Differential exposure to secondgeneration anticoagulant rodenticides in raptors from continental and insular regions of the Iberian Peninsula”

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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*

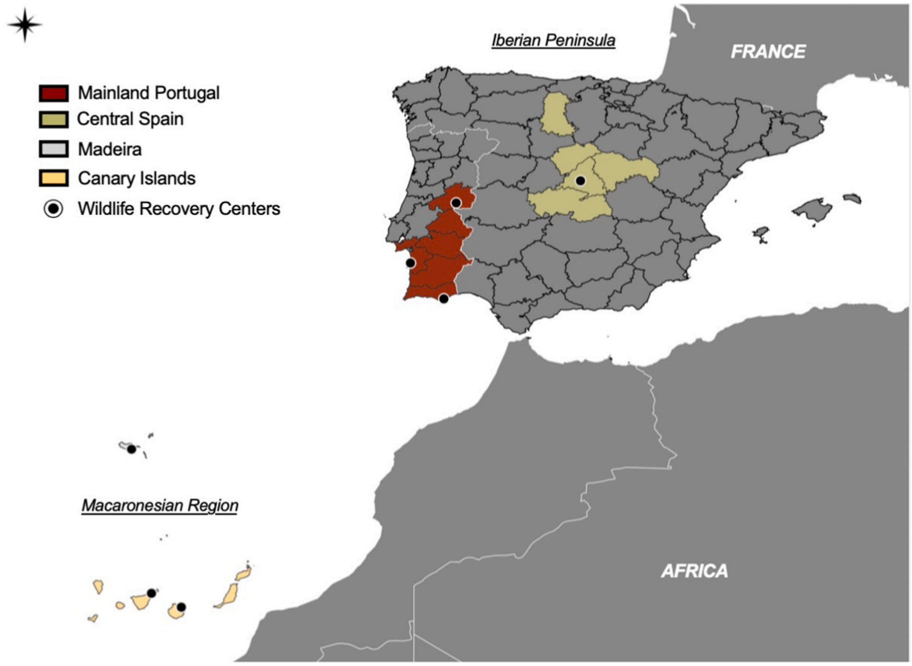

Fig. 1. Map of the Iberian Peninsula (Mainland Spain and Portugal) and the Macaronesian islands involved in the study. Each territory is represented with a specific color as shown in the legend. The wildlife recovery centers participating in the sampling are marked by black circles, also detailed in 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 ≤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 (p25th - p75th), 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 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.

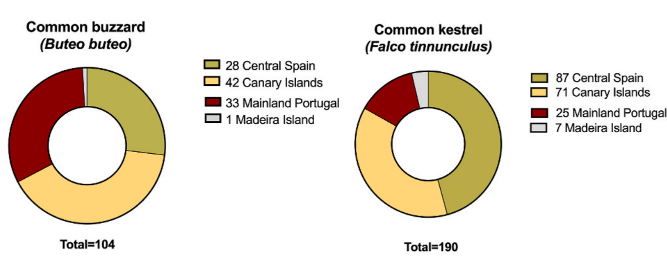

Fig. 2. Doughnut chart showing the distribution of common buzzard ***(Left)*** and common kestrel ***(Right)*** samples by regions (Central Spain, Canary Islands, Mainland Portugal, and Madeira Island).

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/

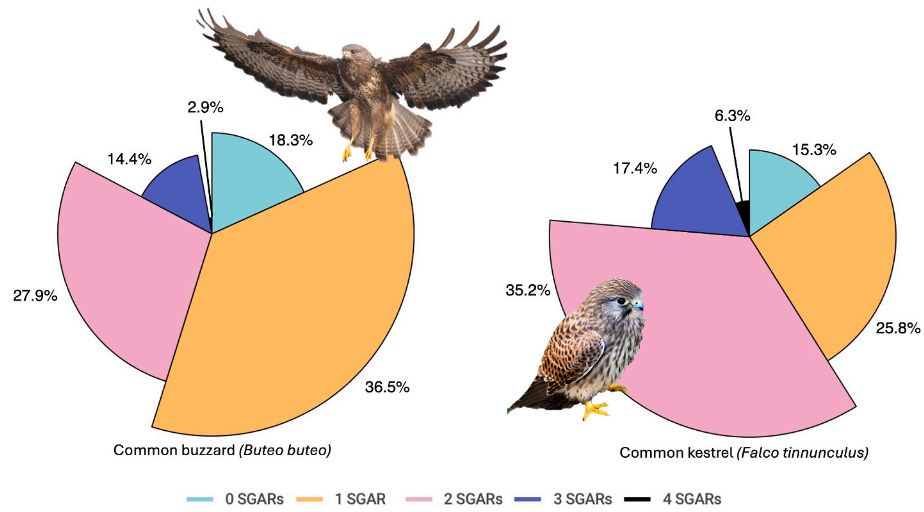

Fig. 3. ***Left.*** Number of SGARs per animal, expressed as percentage in the common buzzard *(Buteo buteo;* n = 104). ***Right.*** Number of SGARs detected per animal, expressed as percentage in the common kestrel *(Falco tinnunculus;* n = 190).

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 *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

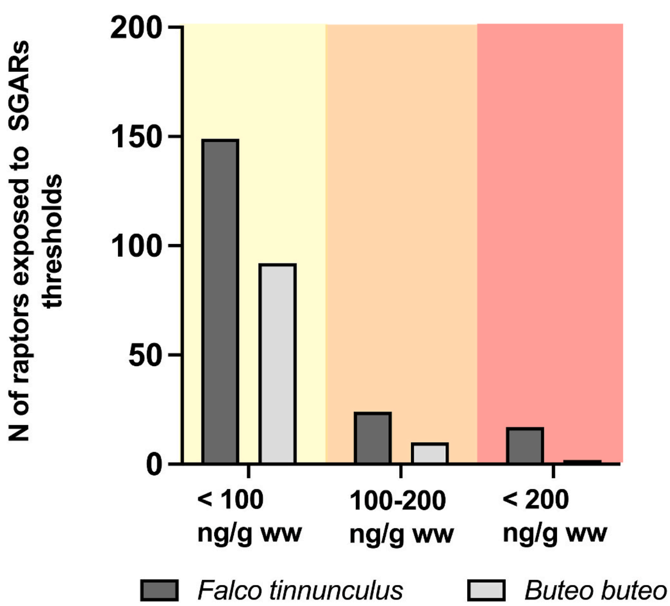

Fig. 4. Number of raptors' livers with ΣSGARs concentration within each toxicity threshold (<100 ng/g ww, 100–200 ng/g ww, >200 ng/g ww) for both raptor species, *Falco tinnunculus* and *Buteo buteo*.

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.

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,

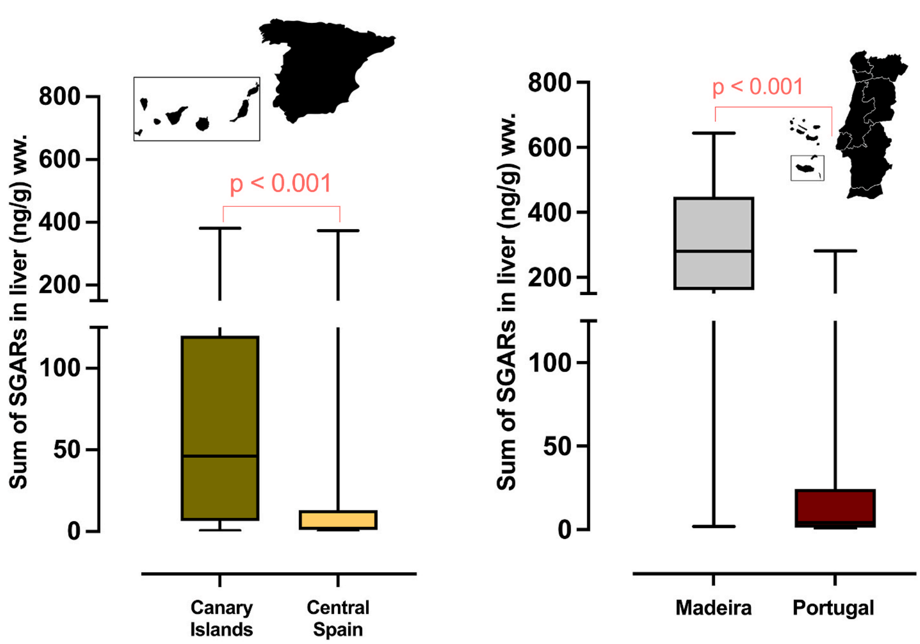

Fig. 5. Box and whisker plot showing the comparison of ΣSGARs in both countries between their mainland and insular regions (***Left.*** Mainland and insular comparison in Spain; ***Right.*** Mainland and insular comparison in Portugal). The lines represent the medians, the boxes the 25th to 75th percentiles,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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.125034.

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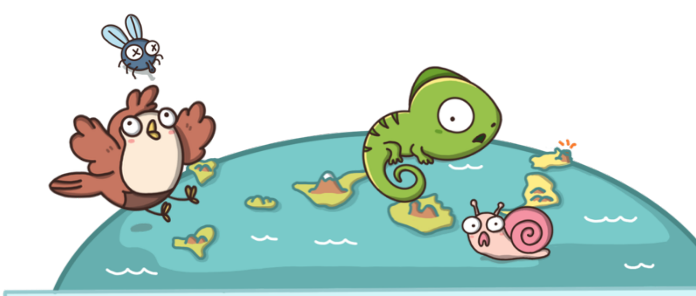

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Widespread Contamination by Anticoagulant Rodenticides in Insectivorous Wildlife from the Canary Islands: Exploring Alternative Routes of Exposure

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Abstract: Research on anticoagulant rodenticides (ARs) in wildlife has primarily focused on apex predators, with less attention given to their potential integration into lower trophic levels and the associated exposure pathways. At the base of the terrestrial food web, invertebrates have been suggested as potential vectors of ARs to insectivorous species such as small mammals, reptiles, and birds. To explore this hypothesis, we analyzed the presence of nine anticoagulant rodenticides—including both first-generation (FGARs) and second-generation (SGARs) rodenticides—in 36 liver samples from Yemen chameleons (*Chamaeleo calyptratus*) and 98 liver samples from six non-raptorial, predominantly insectivorous bird species from the Canary Islands. Through HPLC-MS/MS analysis, only SGARs were detected in both animal groups collected between 2021 and 2024. Approximately 80% of reptiles and 40% of birds tested positive for at least one SGAR, with brodifacoum being the most frequently detected compound. In more than 90% of positive cases, it was found as the sole contaminant, while co-occurrence with other SGARs was uncommon. Additionally, most concentrations were below 50 ng/g wet weight, except for two bird specimens, suggesting heterogeneous exposure scenarios and potential variability in contamination sources across individuals. These findings provide evidence of AR integration at the base of the terrestrial food web in the Canary Islands and suggest secondary exposure via invertebrates as a plausible route of contamination. Further research directly analyzing invertebrate samples is needed to confirm their role as vectors of ARs to insectivorous wildlife in insular ecosystems.

Keywords: insects; biomonitoring; food chain; brodifacoum; non-raptor birds; reptiles

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1. Introduction

Anticoagulant rodenticides (ARs), particularly second-generation compounds (SGARs), are widely used across urban and agricultural environments to control rodent populations [1,2]. Although highly effective, these substances pose substantial risks to non-target wildlife due to their high toxicity, environmental persistence, and bioaccumulation [3,4]. SGARs inhibit vitamin K epoxide reductase (VKORC), a key enzyme in the blood clotting cascade, causing fatal hemorrhaging in exposed animals [5,6]. Beyond lethal effects, there is growing concern over sublethal effects on the health and behavior of non-target species [7–10], prompting regulatory efforts to mitigate their impact—although these measures have shown limited success [3,11–14].

Most research to date has focused on top predators—such as birds of prey, scavengers [13,15–19], and carnivorous mammals [20–22]—due to their trophic position and high susceptibility to secondary poisoning. However, knowledge on how SGARs affect lower trophic levels remains limited, despite the essential ecological roles of these species [23]. While direct consumption of bait (primary exposure) and ingestion of contaminated prey (secondary exposure) are well-documented for target species and apex predators, the exposure of non-target prey species—such as small mammals, reptiles, and non-raptorial birds—remains underexplored [24–26]. These animals may consume bait directly due to its attractive formulation or indirectly through ingestion of contaminated invertebrates, plants, soil, or even water sources [10,25,27–30].

Factors such as habitat, diet, and foraging behavior play key roles in shaping AR exposure risk across trophic groups [31,32]. Insectivorous predators, in particular, may represent an interesting secondary exposure route, given the potential role of invertebrates as AR vectors. Experimental studies have shown that various invertebrate species can consume rodenticide bait without exhibiting acute toxicity [33–35]—likely due to fundamental differences in their hemostatic systems compared to vertebrates [36]. However emerging evidence indicates that invertebrates may also experience toxic or sublethal effects [37–39]. Likewise, field observations from island eradication campaigns and rodenticide-treated agricultural areas have documented invertebrates feeding on bait, rodent feces, and animal carcasses [10,25,40–42], further supporting their potential role as contamination vectors.

Their capacity to accumulate residues, coupled with apparent physiological resilience, raises significant concerns about trophic transfer to insectivorous vertebrates. This indirect pathway has been proposed in mammals [43,44], reptiles [32,45], and birds [31,46], although causal links between AR residues in invertebrates and predator exposure remain unclear. In the Canary Islands, recent studies confirm the widespread presence of SGARs in top predators, both native and invasive, suggesting broad environmental contamination [11,47–51]. However, biomonitoring efforts targeting lower trophic levels—particularly non-raptorial birds and reptiles—are virtually nonexistent.

Intentional poisoning has been reported in some endemic reptile species within the Canary Islands [47,49], and the invasive California kingsnake (*Lampropeltis californiae*) has been proposed as a potential sentinel species for AR exposure due to its predatory habits and position at the top of the island food chain [48]. Nevertheless, no previous studies have assessed AR exposure in insectivorous reptiles. For this purpose, we selected the veiled chameleon (*Chamaeleo calyptratus*), an invasive species with an insectivorous diet [52,53], as a sentinel candidate. Its trophic position closely mirrors that of several protected endemic reptiles such as *Gallotia stehlini*, *Chalcides sexlineatus*, and *Tarentola boettgeri* [54–57]—for which biomonitoring is challenging due to their high conservation status in the archipelago.

Similarly, research on AR exposure in Canary Island birds has primarily focused on raptors [11,47,49–51], reporting both high prevalence and alarming residue concentrations in species such as the Eurasian kestrel (*Falco tinnunculus*) [11,51]. However, studies in non-raptorial birds are scarce and typically limited to cases of confirmed poisoning [47]. To date, no investigations have evaluated AR exposure in non-raptors from an ecotoxicological perspective, particularly in relation to dietary habits. To address this gap, we evaluated six non-raptorial bird species—some of which are endemic subspecies of the Canary Islands (*Upupa epops*, *Burhinus oedicnemus*, *Chlamydotis undulata*, *Turdus merula*, *Dendrocopos major*, *Apus apus*)—whose diets consist primarily or substantially of invertebrates [58–67]. These species could offer a valuable perspective on the potential exposure of insectivorous predators within the archipelago.

Based on the hypothesis that invertebrates could act as vectors for ARs in insectivorous species, the aims of this study were as follows:

(i) Assess the integration of ARs into lower trophic levels of the Canary Islands' terrestrial ecosystem by analyzing liver samples from six insectivorous non-raptor bird species and one invasive insectivorous reptile (*Chamaeleo calyptratus*);
(ii) Evaluate the potential role of invertebrates as vectors of ARs for these lower-level predators.

2. Materials and Methods

2.1. Study Area, Sampling, and Ethical Statements

This study was conducted in the Canary Islands, an archipelago of eight inhabited islands and several islets located in the Atlantic Ocean and part of the Natura 2000 network within the Macaronesian biogeographic region [68]. Covering approximately 7000 km^2 and home to around 2.2 million people [69], the archipelago includes 154 Sites of Community Importance (SCIs) and 45 Special Protection Areas for Birds (SPAs) [70]. The Canary Islands are considered one of Europe's biodiversity hotspots, hosting a high proportion of endemic species—about 45% of the fauna and 25% of the flora [68]. In contrast, 48 species are currently classified as invasive alien species of concern in the region [71].

To contribute to the conservation of native biodiversity, liver samples were collected between 2021 and 2024 during necropsies of 36 Veiled chameleons (*Chamaeleo calyptratus*) and 98 non-raptorial birds: 22 endemic Canary bustards (*Chlamydotis undulata fuertaventurae*), 44 stone-curlews (*Burhinus oedicnemus insularum*, endemic to Fuerteventura, Lanzarote, and La Graciosa, and *B. oedicnemus distinctus* in the rest of the archipelago), 5 hoopoes (*Upupa epops*), 5 woodpeckers (*Dendrocopos major canariensis*, endemic to Tenerife, and *D. major thanneri*, endemic to Gran Canaria), 4 common swifts (*Apus apus*), and 18 blackbirds (*Turdus merula*).

Bird samples were collected from five of the eight islands (Fuerteventura, Gran Canaria, Tenerife, Lanzarote, and La Palma), while all chameleon samples were collected from Gran Canaria, where this species has established. No animals were sacrificed for the purpose of this study. Instead, bird specimens were either found dead in the wild, died during veterinary care, or were humanely euthanized by veterinarians due to irreversible conditions incompatible with recovery. The chameleons were captured and euthanized within the framework of an eradication program, as this species is officially recognized as an invasive alien species of concern in the outermost regions of the Canary Islands [72]. All necropsies were performed by veterinary personnel, and both carcasses and extracted livers were stored at –20 °C until toxicological analysis. Chemical analyses were conducted at the Toxicology Service (SERTOX) of the University of Las Palmas de Gran Canaria as part of the toxicological monitoring efforts coordinated by the Canary Islands Wildlife Health Surveillance Network (Red VIGIA) [73], aimed at assessing environmental threats to wildlife health.

Biological data for chameleons were recorded during necropsy, and geolocation data were provided by personnel from the eradication program. Recorded variables included sex, body weight, and snout-vent length (SVL). All chameleons were collected in Gran Canaria (Arucas municipality). Of the 36 individuals analyzed, 38.8% were female (n = 14), 55.6% were male (n = 20), and 5.6% were undetermined due to underdeveloped sexual traits (n = 2) (Table S2). No macroscopic signs of coagulopathy consistent with rodenticide poisoning were observed during necropsy.

Biological and geographical data were incomplete for all bird specimens due to variable preservation status and diverse origins. For those with available information, geographic data were restricted to the island of origin. The sex ratio was nearly balanced (n = 21 females, n = 20 males), with adults representing the most frequent age group (n = 33, 33.7%). Most individuals were in optimal body condition (n = 32, 32.7%). Trauma, including collisions with vehicles, power lines, wind turbines, or unspecified traumatic events, was the confirmed cause of death in 33.7% of cases. Natural causes, such as infectious or parasitic diseases, accounted for 15.3% of deaths (n = 15). Rodenticide poisoning was not identified as the direct cause of death in any specimen.

2.2. Analysis of Anticoagulant Rodenticides in Liver Tissue and Sample Preparation

Anticoagulant rodenticides were analyzed using certified standards and internal procedural standards (P-IS, (±)-Warfarin-d5) of maximum purity (98–99.8%) from Dr. Ehren-Storfer® (Augsburg, Germany). Among the ARs included in the panel, four were first-generation (chlorophacinone, coumachlor, coumatetralyl, and diphacinone), and five were second-generation (brodifacoum, bromadiolone, difenacoum, difethialone, and flocoumafen). As for solvents, acetonitrile, methanol (ACN and MeOH, >99.9% purity), and formic acid (FA, 98% purity), all LC-MS grade and from Honeywell® (Morristown, NJ, USA), were used. Additionally, the water required for the analyses was produced in-house using a MilliQ® A10 system (Millipore, Molsheim, France). The QuEChERS extraction salts, AOAC method (6 g of magnesium sulfate and 1.5 g of sodium acetate), were obtained from Agilent Technologies® (Palo Alto, CA, USA).

To ensure accurate analysis, quality control (QC) samples were incorporated in the series at a concentration of 5 ng/g, ensuring relative standard deviation (RSD) values ≤20% and recovery (REC) rates between 70–120%. In addition, a ten-point calibration curve was prepared using a blank chicken liver matrix, covering concentrations from 0.195 to 100 ng/g. Prior to extraction, all samples, calibration curve, blanks, and QCs were fortified with the P-IS solution, and the resulting concentrations were reported on a wet weight (ww) basis.

The extraction of analytes from samples, QCs, and calibration curve was performed following the method described by our research team [74,75]. The extraction process was carried out using a micro-QuEChERS approach, as follows. For this procedure, 1 g of liver was homogenized using a Precellys Evolution® system (Bertin Technologies, Rockville, MD, USA). Subsequently, the homogenized matrix was diluted with 4 mL of ultrapure water, and 1 g of this solution was transferred to a 5 mL Eppendorf® tube, to which 20 µL of P-IS at 100 ppb and 2 mL of ACN (0.5% FA) were added. Then, the solution was subjected to ultrasound (VWR®, 50/60 Hz, 120 W), and 480 mg of magnesium sulfate and 120 mg of sodium acetate were added to each sample tube, vortexed, and manually shaken. Finally, they were centrifuged at 4200 rpm and 2 °C for 15 min (5804 R, Eppendorf, Hamburg, Germany), and the supernatant was filtered through a Chromafil® PET-20/15 filter with a 0.2 µm pore size (Macherey-Nagel, Düren, Germany).

Finally, quantitative analysis was performed using an Agilent 1290 UHPLC system (Agilent Technologies, Palo Alto, CA, USA) coupled with an Agilent 6460 triple quadrupole mass spectrometer. The chromatographic configuration incorporated a heated InfinityLab Poroshell 120 column, equipped with an inline filter and a UHPLC guard column. A gradient elution method was applied, using a mobile phase composed of 0.1% formic acid and 2 mM ammonium acetate in water (Phase A) and 2 mM ammonium acetate in methanol (Phase B). The injection volume was set to 8 µL, with a flow rate of 0.4 mL/min. The mass spectrometer operated in dynamic multiple reaction monitoring (dMRM) mode, covering both positive and negative ionization polarities, with precisely defined cycle, dwell, and run times. Additional operational parameters for the Agilent Jet Stream Electrospray Ionization Source (AJS-ESI) and the gas settings used are detailed in the referenced methodologies, which also include the parameters for method validation [74,76].

2.3. Statistical Analyses

To perform descriptive statistical analyses, JAMOVI® v.2.4.7 (The Jamovi Project, 2023; available at https://www.jamovi.org (accessed on 9 June 2025)) was employed.

Descriptive data were expressed as mean, medians, standard deviation, and interquartile ranges (25th–75th percentiles). Additionally, detection frequency was determined as the percentage of animals with at least one SGAR detected in their liver tissues. Animals with concentrations above the limit of quantification (LOQ), as well as those with concentrations below the LOQ but above the limit of detection (LOD), were considered as positive detections; in the latter case, random values within the LOQ–LOD range (Table S1) were assigned. Concentrations below the LOD were classified as non-detected and assigned a random value between zero and half the LOD for statistical analysis purposes.

The graphical resources and illustrations were created using BioRender and Infogram.

3. Results and Discussion

Biomonitoring of anticoagulant rodenticides (ARs) has primarily focused on top predators [13,15–22], while exposure routes in prey species like insectivorous reptiles and birds have received less attention [23]. However, recent evidence suggests that ARs may also affect insectivores, possibly through invertebrate-mediated exposure [31,32,43–46]. To explore this, we analyzed liver samples from veiled chameleons (*Chamaeleo calyptratus*) and six non-raptorial bird species with predominantly insectivorous diets in the Canary Islands for nine AR compounds, including first-generation rodenticides (FGARs: coumatetralyl, diphacinone, warfarin, and chlorophacinone) and second-generation rodenticides (SGARs: brodifacoum, bromadiolone, difenacoum, flocoumafen, and difethialone).

3.1. SGARs Exposure in Reptiles: Veiled Chamaeleon as a Case Study

Although the study of anticoagulant rodenticides (ARs) in reptiles has historically been limited compared to other taxa, several authors have begun to recognize both their direct and indirect interactions with these compounds, as well as their potential role as vectors [32,45].

In the present study, the livers of 36 individuals of veiled chameleon (*Chamaeleo calyptratus*), an arboreal insectivore reptile, were analyzed [52,53]. Of the nine ARs investigated, including both SGARs and FGARs, only two SGARs were detected: brodifacoum and bromadiolone. In total, 77.8% (n = 28) of the chameleons tested positive, suggesting their potential use as a sentinel species at this trophic level. Brodifacoum was the predominant compound, found in all the positive individuals (n = 28), reaching a maximum concentration of 33.11 ng/g wet weight (ww) (Table 1, Figure 1). Bromadiolone, on the other hand, was detected in only one individual, in combination with brodifacoum, at 0.47 ng/g ww.

Table 1. Descriptive statistics of brodifacoum (ng/g ww.) concentrations in *Chamaeleo calyptratus* specimens from Gran Canaria (n = 36).

Brodifacoum (ng/g ww.)								
n+ (%)	**Mean Total**	**Mean Detected**	**Median Total**	**Median Detected**	**SD**	**Max**	**P25**	**P75**
28 (77.8)	6.82	8.73	3.13	4.36	8.94	33.11	0.55	8.57

Values are presented for brodifacoum in detected individuals and for the total series, including mean, median, standard deviation (SD), maximum, and the 25th and 75th percentiles (P25, P75). All data are presented in ng/g ww.

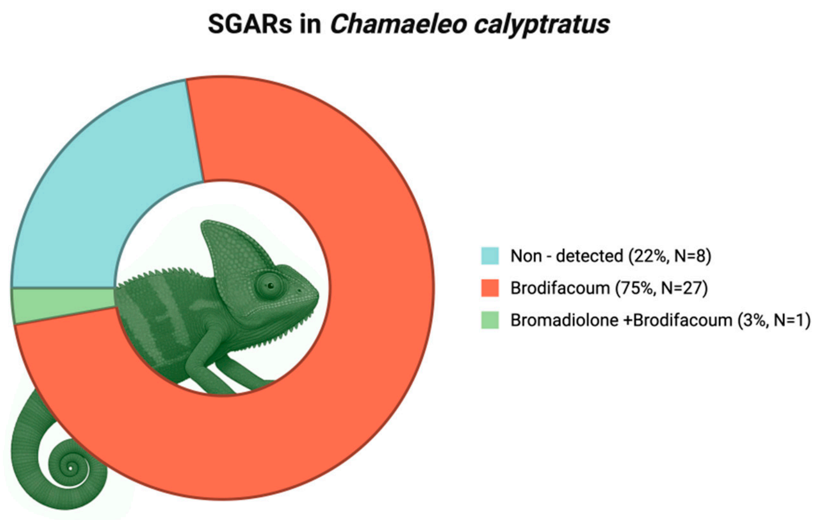

Figure 1. Donut chart showing the proportion and sample size of *Chamaeleo calyptratus* individuals analyzed for anticoagulant rodenticides (ARs), differentiating between specimens with non-detected compounds and those that tested positive. Created in BioRender: https://BioRender.com/dmusrqq (accessed on 9 June 2025).

These frequencies partially align with a previous study in the Canary Islands, which found brodifacoum in 62.7% (n = 23) of giant lizards *(Gallotia gallotia)* [47]. However, the median concentrations were much higher in the lizards (562.3 ng/g ww.) compared to the chameleons (3.13 ng/g ww.), possibly due to their association with intentional poisoning events, leading to a bias toward higher levels. In both studies, brodifacoum was the only compound detected, except for one isolated case of bromadiolone in a chameleon, reinforcing the prevalence of this compound as the main rodenticide used on the islands [11,48,51]. ARs have also been linked to poisoning incidents in other endemic reptiles of the Canary Islands, such as the skink (*Chalcides simonyi*, n = 1), although concentrations were not detailed [49]. Furthermore, a recent study in Gran Canaria by our team identified high exposure to ARs in the invasive and generalist predator California kingsnake (*Lampropeltis californiae*). Over 90% of the analyzed specimens contained ARs, with brodifacoum as the most frequently observed compound. In contrast to chameleons, up to five different SGARs were detected in the snakes, and more than 50% were exposed to combinations of these compounds [48]. These differences could be explained by their distinct trophic positions: while chameleons are strictly insectivorous, snakes primarily feed on rodents and reptiles [77,78], which increases their risk of exposure, as also suggested by other authors [32,79].

In other regions of Spain, a positive case of flocoumafen was reported in a horseshoe whip snake (*Hemorrhois hippocrepis*), linked to the use of this compound during a seabird protection campaign in the Chafarinas Islands [80]. However, no ARs were found in the sole individual of *Testudo hermanni* in Aragón [81]. Internationally, three species of urban reptiles were evaluated in Australia, observing varying levels of exposure based on diet and trophic level. They reported 91% in rodent-predating snakes, 60% in omnivorous dugites, and 45% in tiger snakes (amphibian specialists) [32]. In our study, 77.8% of the chameleons, captured in an urban environment, tested positive, representing an intermediate value, which is relevant given their predominantly insectivorous diet. However, the average concentration in chameleons (6.83 ng/g ww) was lower than that of the Australian species at 178 ng/g, 40 ng/g, and 9 ng/g, respectively. This may be related to differences in trophic behavior: while in Australia, direct consumption of baits and garbage has been observed, the chameleons in Gran Canaria have not shown such anthropogenic interactions. However, consistent with our findings, brodifacoum was identified as the most prevalent compound, along with bromadiolone and difenacoum [32].

In contrast, other authors analyzed 185 pit vipers and 89 green anoles on a Pacific island, detecting only FGARs (warfarin, diphacinone, and coumatetralyl). The prevalence was 9% in pit vipers (max. 436.5 ng/g), and there was only one positive case in anoles (51.9 ng/g diphacinone) [79]. In comparison, the chameleons in our study showed similar maximum concentrations to the anoles with a similar diet but with a much higher prevalence. The exclusive detection of FGARs could be due to the compound used in each area, with diphacinone being the reference product on that island in previous years.

Regarding toxicological risk, there are no established toxicity thresholds for reptiles. However, lower susceptibility compared to birds and mammals has been suggested, along with a possible capacity for tolerance and biomagnification [42,45,82]. The concentrations observed in the chameleons (<50 ng/g ww) are considered to have a low probability of toxicity in other vertebrates [83]. Nevertheless, information on this species is scarce, and susceptibility may vary significantly among reptiles. Apparently, resistance has been suggested in lava lizards [84], geckos [27], monitors [85], boas, and tortoises [86], which were exposed to rodenticides through eradication programs and/or laboratory experiments, and they rarely experienced mortality. However, other authors suggest greater sensitivity in species such as green turtles [87], iguanas, and ameivas [86].

Finally, it is worth noting that all the chameleons were alive at the time of capture and showed no macroscopic signs of coagulopathy, consistent with other studies [79,86]. No direct interaction with baits has been documented either, suggesting that insects may act as vectors, as proposed by other authors [32,45]. As a result, endemic insectivorous species in the Canary Islands, such as *Gallotia stehlini*, *Chalcides sexlineatus*, and *Tarentola boettgeri*, may also be at risk of exposure. Further studies should include stomach content analysis and should expand to other reptile species in the archipelago to better assess the real threat that ARs pose to the local herpetofauna.

3.2. SGARs Exposure in Non-Raptor Birds: Insectivorous Birds

Non-raptor birds may be exposed to anticoagulant rodenticides (ARs) through primary or secondary pathways [26,28,88,89]. Over 600 incidents of rodenticide poisoning have been documented across 15 countries, with exposure levels influenced by species' diets, foraging behavior, and even morphology [31]. Granivorous birds are categorized at high risk of primary exposure, while insectivorous birds are classified as having a moderate risk of secondary exposure to ARs [26,82].

In this study, we analyzed six species of non-raptor birds with diets consisting predominantly or substantially of insects [58–67]. These species differ in habitat use, foraging strategies, and morphology. Of the total birds analyzed (n = 98), 39.8% (n = 39) tested positive for at least one SGAR, with no detection of first-generation compounds (Figure 2). Among the SGARs detected—brodifacoum, bromadiolone, difenacoum, and difethialone—brodifacoum was the most detected, found in 92.3% (n = 36) of positive individuals, with a maximum concentration of 451.2 ng/g ww. It was generally detected alone (91.6%, n = 33), but it was also present in combination with other SGARs in three individuals (brodifacoum + bromadiolone, n = 1; brodifacoum + bromadiolone + difethialone, n = 2). Bromadiolone (n = 2) and difenacoum (n = 1) were also detected individually, at lower concentrations (1.35 ng/g ww, 0.87 ng/g ww and 3.16 ng/g ww, respectively). Difethialone was only detected in two individuals (11.9 ng/g ww and 2.94 ng/g ww), both in combination with other SGARs (Table S3).

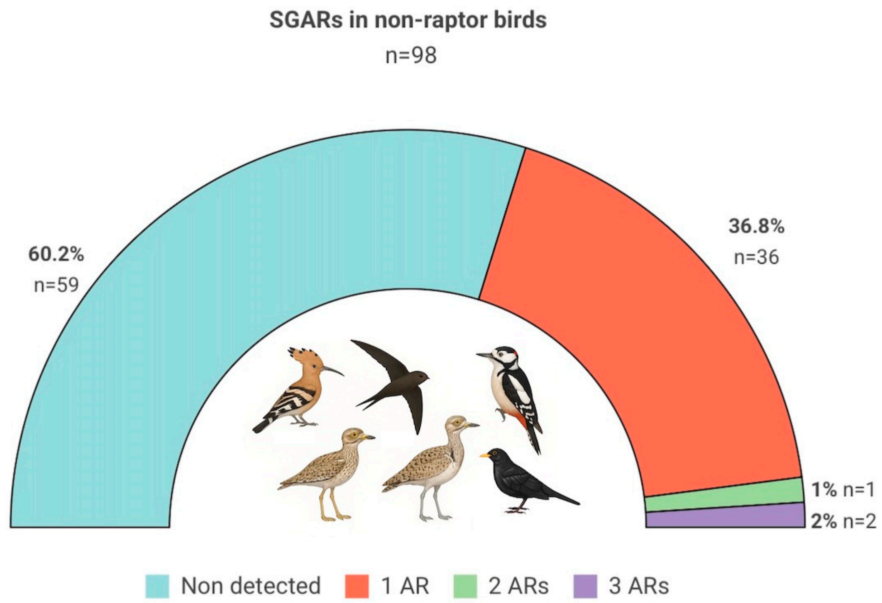

Figure 2. Semi-circle chart showing the proportion and sample size of six non-raptorial bird species—hoopoe (*Upupa epops*), stone-curlew (*Burhinus oedicnemus*), houbara bustard (*Chlamydotis undulata*), blackbird (*Turdus merula*), woodpecker (*Dendrocopos major*), and common swift (*Apus apus*)—analyzed for anticoagulant rodenticides (ARs), distinguishing between individuals with no detected compounds and those testing positive for one or more second-generation anticoagulant rodenticides (SGARs). Created in BioRender. https://BioRender.com/i16zaee (accessed on 9 June 2025).

Although there are no previous studies focused exclusively on insectivorous birds in the Canary Islands, AR exposure in non-raptor birds has been previously documented. However, many of these cases were linked to intentional poisoning incidents [47]. In a previous study involving 343 birds, 4.7% (n = 16) tested positive, with the highest exposure found in the common raven (n = 7/97, 7.2%). Additional positive detections were reported in grey herons (n = 2), stone-curlews (n = 3), European turtle doves (n = 2), Eurasian blackbirds (n = 2), and one red-legged partridge (n = 1) in another insular study [49]. While these species differ from those included in our sample, the overall detection rate was significantly lower. In contrast, our results are more comparable to those reported elsewhere, with detection rates of 52.1% in non-raptor birds [23] and 28% in passerines, with the highest rates reported in insectivores such as the European robin (44.4%, n = 22) [28]. In addition, other studies conducted in Spain documented eight positive cases in non-raptor birds [81] and found a 50.7% detection rate in granivorous birds, although many of the animals included in that study also showed signs of intentional poisoning [80].

Regarding the pattern of SGARs detected, the results of our study mirror those reported for non-raptor birds in the Canary Islands and mainland Spain, with brodifacoum, bromadiolone, difenacoum, and flocoumafen being the most detected compounds [47,81]. Similar detection patterns have been reported in raptors from the archipelago [11,47,48,51]. However, the prevalence of brodifacoum in our study (36.7%) is approximately ten times higher than previous findings in non-raptor birds from the Canary Islands at 3.9% [47]. The increase in brodifacoum detection over time has been previously observed in kestrels in Tenerife, highlighting both its persistence and the limited effectiveness of regulatory measures [11]. In contrast to a previous study that reported widespread detection of chlorophacinone (n = 71) in granivorous birds during a vole outbreak control campaign in 2007, our study did not detect any first-generation ARs [80]. However, their study also recorded brodifacoum (n = 3), bromadiolone (n = 3), and flocoumafen (n = 1), confirming the environmental persistence of SGARs even after their use had discontinued.

Likewise, only 3% of birds in our study were exposed to multiple SGARs (Figure 2), a much lower proportion than reported in raptors from the Canary Islands, where >50% carry multiple residues [11,48,51]. This difference is likely due to bioaccumulation and biomagnification in apex predators. Additionally, only two birds in our sample exceeded the 100–200 ng/g ww thresholds used in some raptors for assessing toxicity or sublethal effects [83,90,91]: one Eurasian hoopoe (451.2 ng/g ww) and one stone-curlew (235.47 ng/g ww). These values approach the maximum concentration reported in insectivorous birds, such as the dunnock (*Prunella modularis*) at 348 ng/g ww sampled in eradication areas [28]. One additional stone-curlew fell within the 50–100 ng/g ww range (50.25 ng/g ww), while the remaining individuals were below 50 ng/g ww. Importantly, none of the birds in our sample were confirmed as victims of intentional poisoning, and hemorrhages were only observed in one individual, whose cause of death was attributed to trauma. The absence of macroscopic signs of coagulopathy, even in birds with high AR levels, has been previously reported [46,80].

Additionally, the differences in concentrations observed among certain individuals may be attributed to distinct exposure pathways. The two individuals with the highest concentrations may have been exposed via primary ingestion of bait, as seen in sparrows (0.073 μg/g brodifacoum) [25] or in *Petroica australis*, where 50% of pairs were exposed to coumatetralyl after rat eradication [88]. However, it is important to recognize that this risk is much higher in granivorous birds. In contrast, the lower residual concentrations observed in most birds suggest secondary exposure through consumption of contaminated invertebrates [28]. Nevertheless, high concentrations have also been reported in nestling robins exposed secondarily through parental feeding on contaminated invertebrates (0.08 ± 0.02 μg/g brodifacoum) [46]. Mortality associated with coagulopathy has also been documented in the poouli, which consumes snails in areas where chlorophacinone was used [33]. Moreover, brodifacoum has been detected in shorebirds found dead after eradication programs, suggesting potential secondary exposure [27]. However, due to the lack of species-specific toxicity reference values for many bird species, it remains difficult to draw firm conclusions about toxicological risk.

Bird Exposure to Anticoagulant Rodenticides: Foraging and Habitat Considerations

As previously described, factors such as diet, habitat, and foraging behavior may influence the risk of exposure to these compounds across different trophic groups [31,32]. In our study, detection rates were highest on the two most urbanized islands (Gran Canaria: 46.2%, and Tenerife: 57.1%; Table S3), consistent with studies showing positive correlations between urban development and AR exposure [24,81,92]. However, our sample size and lack of geolocation data prevent robust spatial analyses. Future studies incorporating precise location data could provide more insight into urban–rural exposure gradients.

Among species, the houbara bustard was the least exposed (Table 2 and Figure 3), likely due to its preference for arid and steppe habitats—many within protected areas on Lanzarote, Fuerteventura, and La Graciosa—far from urban or intensive agricultural zones [62,93]. Similarly, the Canary woodpecker (n = 1, 20%) also showed low exposure, probably due to its restricted distribution in pine forests on Gran Canaria and Tenerife far from anthropogenic activities [66,67]. However, the sample representation of this species is too limited to draw conclusions.

Table 2. Descriptive statistics of ARs in non-raptor birds from the Canary Islands.

	Sum of ARs (ng/g ww.)					Brodifacoum (ng/g ww.)				
Species	**n+ (%)**	**Mean (+) ± SD**	**Median (+)**	**Mean Total ± SD**	**Median Total**	**n+ (%)**	**Mean (+) ± SD**	**Median (+)**	**Mean Total ± SD**	**Median Total**
Hoopoe (n = 5)	5 (100)	102.65 ± 195.18	26.24	102.65 ± 195.18	26.24	5 (100)	102.65 ± 195.18	26.24	102.65 ± 195.18	26.24
Stone-curlew (n = 44)	22 (50)	19.30 ± 49.85	4.79	9.65 ± 36.18	0.17	21 (47.8)	19.87 ± 50.97	4.86	9.48 ± 36.18	0
Houbara bustard (n = 22)	2 (9.1)	0.72 ± 0.09	0.72	0.07 ± 0.21	0	2 (9.1)	0.72 ± 0.09	0.72	0.07 ± 0.21	0
Blackbird (n = 18)	6 (33.3)	3.24 ± 4.64	1.65	1.08 ± 2.97	0	6 (33.3)	3.24 ± 4.64	1.65	1.08 ± 2.97	0
Woodpecker (n = 5)	1 (20)	3.16	3.16	0.63 ± 1.41	0	0	-	-	-	-
Common swift (n = 4)	3 (75)	5.89 ± 7.19	2.68	4.42 ± 6.57	1.78	2 (50)	1.73 ± 1.35	1.73	0.86 ± 1.26	0.39

Values are presented for the sum of detected compounds and for brodifacoum individually, including frequency of detection, mean, median, and standard deviation (SD), for positive individuals and for the total series.

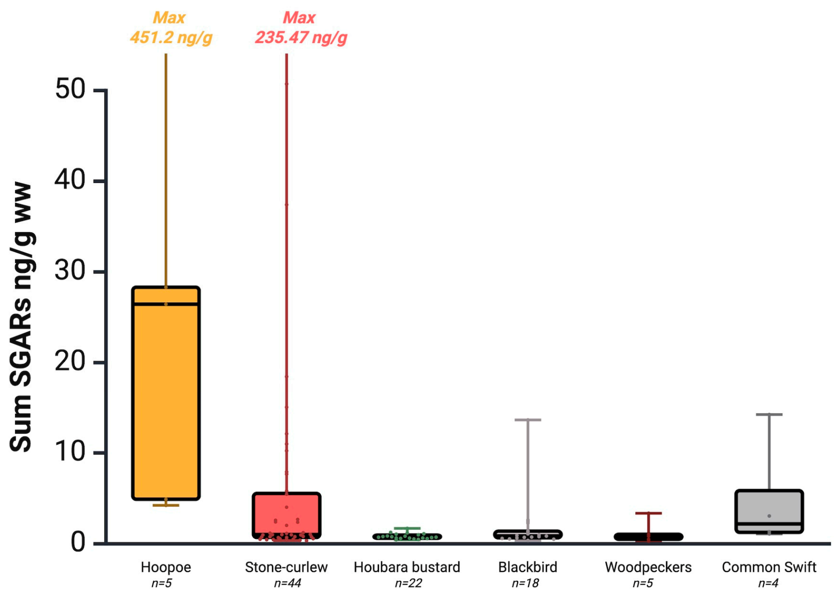

Figure 3. Box and whisker plot showing the comparison of ΣSGARs between six non-raptor birds (hoopoe, stone-curlew, houbara bustard, blackbird, woodpecker, and common swift). The lines represent the medians, the boxes represent the 25th to 75th percentiles, and the minimal and maximal values are shown at the ends of the bars with lines or text. Created in https://BioRender.com (accessed on 9 June 2025).

In contrast, all hoopoes (n = 5) tested positive, including the individual with the highest concentration (451.2 ng/g ww.). This may be linked to their foraging behavior of insects on bare ground [58,59,94], a trait associated with higher AR exposure risk [31]. Nonetheless, the small sample size precludes firm conclusions. Stone-curlews and blackbirds were the following most exposed species, and they have been previously reported in the islands [47]. Exposure in stone-curlews could be related to their broad surface-feeding behavior and preference for traditional agricultural landscapes, generally abandoned, and semi-natural grassland [64,95]. Likewise, their potential interaction with agricultural areas has also been linked to higher exposure levels in other animals [24,96]. Similarly, the fact that the blackbird is one of Europe's most common urban birds [97] may also contribute to its AR exposure, as habituation to human presence and the possible opportunistic consumption of novel food items have been suggested as potential risk factors [31]. In this species, the role of diet, based on insects and earthworms, in relation to heavy metal contamination has already been assessed, suggesting that the influence of landscape on pollutant transfer may be related to dietary variations [65]. A similar influence could occur with other compounds such as anticoagulant rodenticides.

Finally, the detection of AR residues in swifts (n = 3, 75%) was unexpected, as these birds spend most of their lives flying and feed exclusively on aerial invertebrates. Their exposure is therefore most likely attributable to the ingestion of contaminated prey. Swifts have previously been used as bioindicators of atmospheric contamination with pesticides and PCBs, and microplastics have even been found in their digestive tracts, likely ingested via contaminated insects [60,61,98].

Overall, the individual and interspecific variability in AR exposure is consistent with previous findings, though conclusive associations remain elusive due to variation in ecology, metabolism, susceptibility, and other factors [28,31]. In our study, the limited sample sizes for many species and islands precluded robust statistical analyses. Future studies with larger, geographically stratified samples and stomach content analyses would be valuable for confirming invertebrate predation as the primary exposure route.

3.3. Role of Invertebrates as a Potential Vector of Anticoagulant Rodenticides

The interaction between invertebrates and anticoagulant rodenticides (ARs) has been documented both in laboratory experiments and field observations, including coastal environments following eradication campaigns [99]. Some authors have demonstrated that invertebrates are capable of directly ingesting bait [40–42,44], while others have provided evidence of exposure by analyzing the movement of ARs through the food web, including ingestion of feces from intoxicated small mammals, rodent carcasses, or soil-bound AR residues [10,25].

In general, invertebrates appear more resistant than vertebrates due to physiological differences in their coagulation systems [36]. Nevertheless, sublethal effects have been observed, such as increased shelter-seeking behavior and reduced activity, boldness, and aggressiveness [37]. A concentration of 1 mg/kg of bromadiolone in soil has also been reported to inhibit earthworm growth and elevate biomarkers of oxidative stress [38]. Furthermore, brodifacoum at 100 ppb has been shown to reduce fertilization success in coral gametes and larvae by up to 15%, although this concentration does not reflect a likely environmental scenario [39].

Given that ingestion is the primary route of exposure, the potential role of insects as AR vectors highlights the need to assess the risk they may pose to their predators such as mammals, reptiles, and birds, including raptors that feed on invertebrates such as kestrels, little owls, and moreporks [23]. However, additional exposure routes, such as the direct ingestion of baits, should also be considered in light of the high detection rates recorded in this study. Likewise, other factors such as foraging behavior in high-risk habitats or a more generalist diet may further influence exposure levels.

Insectivorous predators could therefore be secondarily exposed to these compounds, but the actual risk remains uncertain. Insectivorous birds have been classified as having a moderate risk and insectivorous reptiles and raptors as having a low risk of secondary exposure in Southeast Asian oil palm plantations [82]. It has been estimated that a bird would need to consume its body weight in contaminated cockroaches to reach a 50% risk of acute intoxication, suggesting a relatively low risk compared to primary exposure. The same study also reported that AR concentrations in invertebrates decline rapidly within the first two weeks and more slowly over the following four weeks [100]. However, a potential acute, repeated, or chronic risk to species such as shrews, starlings, and hedgehogs following the ingestion of contaminated slugs had been suggested [34]. Similarly, a 3–8% mortality risk and a 0.42–11% sublethal coagulopathy risk have been predicted for pooulis feeding on contaminated snails, with acceptable risk levels for quail and mallards [33].

Therefore, it is reasonable to suggest that invertebrates in the Canary Islands' insular ecosystem may act as vectors of ARs to insectivorous predators considering diet as the primary exposure route and the concentrations found in most of the studied animals (<50 ng/g ww). However, targeted studies analyzing AR residues directly in invertebrate prey would be necessary to confirm this exposure pathway and better evaluate the potential ecological risk involved.

4. Limitations and Strengths

This study has several limitations that should be acknowledged. First, the overall sample size, although considerable for a wildlife toxicology investigation, remains modest—particularly within the avian group, where the number of individuals per species was relatively low. This limitation is largely attributable to the opportunistic nature of sample collection, which relied on animals received through the Canary Islands' wildlife health surveillance system (Red Vigía) for diagnosis of suspected poisonings or post-mortem investigations. Consequently, sampling was constrained by the availability of deceased animals and did not allow for targeted collection to equalize group sizes.

Despite the limited number of individuals per species, we deliberately opted for a broader representation of insectivorous taxa, prioritizing ecological diversity over statistical robustness. This approach enabled a more comprehensive assessment of AR exposure risk across species with different diets, foraging behaviors, and habitat preferences, thereby improving the ecological relevance of the findings.

Secondly, there are biogeographic constraints that affect interpretation. While the bird samples came from five of the eight main Canary Islands, offering a relatively broad regional scope, all the reptile samples (veiled chameleons) were limited to the island of Gran Canaria, and even within this island, they were restricted to a few municipalities. This discrepancy reflects the limited geographic distribution of *Chamaeleo calyptratus* in the archipelago, currently confined to a small area in Gran Canaria. The species was first detected in the wild in 2017 by the Canary Islands' early warning system for invasive species, and since then, the invasion has remained geographically contained. Nevertheless, *C. calyptratus* has been present on the island long enough to act as a useful sentinel for long-term environmental exposure to anticoagulant rodenticides, particularly due to its insectivorous diet and terrestrial habits. This situation underscores the need to incorporate additional sentinel species with broader distributions across the archipelago to more accurately assess spatial variability in AR exposure.

Thirdly, a key limitation of the present study is the lack of direct analysis of invertebrate prey. Although our findings strongly suggest that secondary exposure to ARs may occur through the consumption of contaminated invertebrates, we were not able to test this pathway directly. The detection of SGAR residues in insectivorous wildlife is consistent with this hypothesis, but confirmation will require future studies that include the analysis of potential invertebrate vectors. Such studies would provide a crucial link in understanding the transmission of rodenticides through the terrestrial food web and further validate the role of invertebrates as vectors of toxicants.

Finally, statistical comparisons between birds and reptiles were deliberately avoided, given the taxonomic and ecological differences between the two groups. Their distinct metabolic rates, susceptibility to toxicants, and exposure pathways preclude meaningful quantitative comparisons. Therefore, the study's strength lies not in inferential statistics but in the qualitative and descriptive evidence it provides.

Despite these limitations, this study presents several key strengths. Most notably, it offers robust evidence that second-generation anticoagulant rodenticides (SGARs) have penetrated the lowest trophic levels of the terrestrial food web in the Canary Islands. The detection of SGARs in nearly 80% of the insectivorous reptiles and 40% of the non-raptorial insectivorous birds underscores the pervasive environmental contamination by these compounds. By focusing on species at the base of the food chain, the study sheds light on a largely overlooked exposure pathway—namely, secondary exposure via invertebrate prey—which may be of particular concern in insular ecosystems characterized by high endemism and ecological sensitivity.

In this context, our findings contribute to the growing body of evidence that highlights the widespread and insidious presence of SGARs in island environments. They emphasize the need to expand biomonitoring efforts beyond apex predators and to consider the full spectrum of ecological interactions when assessing the risks of rodenticide use in biodiversity hotspots.

5. Conclusions

Tracking the movement of anticoagulant rodenticides (ARs) through food webs is essential to assessing their ecological impact. Invertebrates occupy a foundational position in terrestrial trophic networks and serve as key prey for a variety of vertebrate species. This study provides valuable information on SGAR contamination in the lower trophic levels of the Canary Islands' terrestrial ecosystems, with detections in 77.8% of sampled reptiles and 39.4% of non-raptorial insectivorous birds. The prevalence of low AR concentrations (<50 ng/g ww.) supports the hypothesis that secondary exposure via the ingestion of contaminated invertebrates is a likely pathway. Our findings highlight the potential of the veiled chameleon as a valuable sentinel for AR exposure in insular reptile communities. Additionally, the observed variation in exposure among birds suggests that habitat preferences and foraging strategies may modulate individual risk.

However, a key limitation of this study is the small sample size for some studied species and the absence of direct data on AR residues in invertebrate prey. Without empirical evidence of accumulation in these organisms, their role in trophic transfer remains unconfirmed. Future studies should include more individuals and prioritize the analysis of invertebrate taxa commonly consumed by insectivorous vertebrates to clarify their involvement in AR transmission and to enhance environmental risk assessments, particularly in insular ecosystems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/toxics13060505/s1, Table S1: Limits of detection (LODs) and quantification (LOQs) in ng/mL of the extraction method; Table S2: Descriptive table of *Chamaeleo calyptratus* specimens from Gran Canaria; Table S3: Dataset of non-raptor bird species from the Canary Islands.

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Institutional Review Board Statement: Ethical review and approval were waived for this study as no animals were sacrificed for research purposes. All specimens analyzed were deceased at the time of collection, either found dead in the wild, euthanized for veterinary reasons, or collected within official control programs for invasive species under governmental mandate. The Toxicology Unit of the University of Las Palmas de Gran Canaria (SERTOX) serves as the official reference laboratory for wildlife toxicology under the Government of the Canary Islands and routinely receives animal carcasses for diagnostic purposes as part of the Red Vigia surveillance program. The use of data generated from these necropsies for scientific purposes is embedded in this institutional collaboration and fully complies with applicable regulations. No personal or sensitive data were used, and all animals studied were either wild or invasive species with no individual ownership.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: Author Ramón Gallo-Barneto was employed by the Gestión y Planeamiento Territorial y Medioambiental, S.A. (GESPLAN), Canary Islands Government. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

SGAR	Second-Generation Anticoagulant Rodenticide
FGAR	First-Generation Anticoagulant Rodenticide
AR	Anticoagulant Rodenticide
LOD	Limit of Detection
LOQ	Limit of Quantification
QCs	Quality Control
SERTOX	Toxicology Service
VKORC	Vitamin K Epoxide Reductase
SVL	Snout-vent length
SCIs	Sites of Community Importance
SPAs	Special Protection Areas for Birds
RSD	Relative Standard Deviation
P-IS	Internal Standard Procedural
ACN	Acetonitrile
FA	Formic Acid

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PUBLICACIÓN 4

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"Intraspecific and geographical variation in rodenticide exposure among common kestrels in Tenerife (Canary Islands)"

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Intraspecific and geographical variation in rodenticide exposure among common kestrels in Tenerife (Canary Islands)

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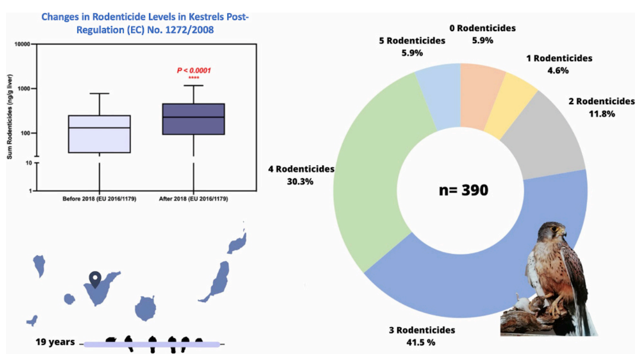
HIGHLIGHTS

- Monitoring of SGARs in a series of 390 kestrels from 11 years from Tenerife
- 93.1 % of kestrels had detectable residues of SGARs.
- 46.9 % of animals had >200 ng/g, with a maximum of 1107.07 ng/g.
- Brodifacoum is increasing its presence and concentration over the years despite current legislation.
- Age, high human density and high livestock development, were determining factors of exposure.

GRAPHICAL ABSTRACT



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Raptor biomonitoring

ABSTRACT

This study assesses the impact of second-generation anticoagulant rodenticides (SGARs) on the common kestrel (*Falco tinnunculus canariensis*) in Tenerife, Canary Islands. The analysis of 390 liver samples over 19 years using HPLC-MS/MS showed that 93.1 % of kestrels were exposed to SGARs in this island. A notable shift in SGAR profiles was observed, with bromadiolone and flocoumafen decreasing, while brodifacoum levels increased sharply from 2018 onwards. Comparatively, Tenerife kestrels had a higher detection frequency of SGARs (93.1 %) than those in the rest of the islands of the archipelago (68.2 %), with median concentrations nearly double ($\sum$AR = 180.9 vs 102.4 ng/g liver, $P < 0.0001$). Furthermore, on average, kestrels from Tenerife were found to have a higher number of different rodenticide compounds per individual. A Generalized Linear Model (GLM) analysis revealed that several factors contribute to the likelihood of SGAR exposure: being an adult kestrel, the enactment of legal restrictions on SGAR bait concentrations in 2018, higher livestock density, and greater human population density. These findings suggest that both bioaccumulation over the birds' lifespans and environmental

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factors related to human and agricultural activity are influencing the levels of SGARs detected. Alarmingly, 44.7 % of kestrels had SGAR levels above the toxicity threshold established for other raptor species (200 ng/g liver), signaling a high poisoning risk. This is despite EU regulations to protect wildlife, with our findings indicating an increase in both exposure rates and SGAR concentrations since these laws were enacted. The data highlight a critical environmental threat to endemic species on islands like Tenerife. The common kestrel, not considered globally endangered, is nonetheless facing regional threats from SGAR contamination. These results emphasize the urgent need for effective regulations to address the persistent and growing impact of SGARs on island biodiversity.

1. Introduction

The use of artificial or synthetic chemical substances with biocidal properties to control pest populations of some organisms became increasingly widespread after World War II (Carson, 1962). Raptors (i.e., *Accipitriformes, Cathartiformes, Falconiformes, Strigiformes*) are among wildlife organisms adversely affected by anthropogenic contaminants (e.g., biocides). As a result of biomagnification in food webs, the bioaccumulative transfer of contaminants can result in elevated levels of contaminants in these apex predators. Therefore, raptors can be used as powerful sentinels in environmental monitoring programs due to their position at the top of food webs, their long lifespan, low reproductive rate, and extensive home-ranges (Newton, 1998). The value of raptors as efficient biodiversity indicators (Buechley et al., 2019; Sergio et al., 2005) led to the creation of EURAPMON (Research and Monitoring for and with Raptors in Europe), a Research Networking Programme of the European Science Foundation. One of the main objectives of EURAPMON is to link raptor conservation monitoring studies with those using raptors as biological indicators of environmental change (Derlink et al., 2018). Similarly, European environmental monitoring programs (e.g., Finland, Norway, Sweden, UK) use raptors as sentinels to monitor the detrimental effects of environmental pollutants on wildlife and human health (Badry et al., 2019, 2020; Gómez-Ramírez et al., 2014; Helander et al., 2008; Ramello et al., 2022). The use of raptors for the monitoring of environmental pollutants has also been reported in some published works and studies in other European countries, including Germany, Spain, and the Netherlands (Gómez-Ramírez et al., 2014).

The common kestrel (*Falco tinnunculus,* hereafter, kestrel) is considered an effective model for studying behavioral (such as reduced activity, reduced hunting capability, altered intraspecific and interspecific interactions) as well as physiological effects (such as poor health, starvation, impaired motor coordination, low fertilization rate) caused by biocides (Constantini and del Olmo, 2020; Dumonceaux and Harrison, 1994; Martínez-Padilla et al., 2021). Thus, this is one of the most frequently studied raptors in European environmental monitoring programs, even since the early 1960s (Cooke et al., 1982). Kestrels, as well as other raptor species, have been shown to be sensitive to biocides (Costantini and Dell'Omo, 2020). Kestrels, as one of the most common and geographically widespread raptors in the Canary Islands, serve as a key indicator species due to their diurnal habits and presence across diverse habitats, from sea level to 2400 m, excluding only the highest peaks and densest forests (Carrillo, 2007; Carrillo and González-Dávila, 2005; Kangas et al., 2018). Their adaptability to both urban and rural environments, along with their varied diet, positions them as an excellent subject for studying the impact of biocides within the archipelago. The kestrel's role as a bioindicator is underscored by its high admission rates to the Wildlife Recovery Centre in Tenerife, representing a significant proportion of raptor admissions over two decades. Juvenile kestrels, which experience high mortality rates during their dispersal from natal territories, are particularly vulnerable to a range of anthropogenic threats, including poisoning and habitat destruction (Carrillo, 1991; Rodríguez et al., 2010; A. Village, 1990b).

SGARs are a major concern for wildlife conservation, particularly for predatory birds such as raptors (Newton, 1998). A pivotal change occurred in May 2018 when new regulations mandated the reclassification of anticoagulant substances exceeding 30 μg/g as reprotoxic, leading to stricter controls on their use and availability (Frankova et al., 2019). This legal adjustment has resulted in most rodenticide baits being produced with concentrations at or below 30 μg/g, aiming to reduce the risk of secondary poisoning in non-target species.

The accumulation of anticoagulant rodenticides (SGARs) in kestrels has been well-documented, highlighting significant health risks to these raptors in the Canary Islands (Luzardo et al., 2014; Rial-Berriel et al., 2021a). These findings are particularly relevant in light of the Poisoning Control and Prevention Strategy implemented in the Canary Islands since 2014 (BOC, 2014), which has enhanced the detection of environmental pollutants affecting wildlife. Despite the strategy's effectiveness, there is still a lack of comprehensive data on the impact of these substances on the kestrel population of Tenerife, an island with a rich ecological diversity and the highest human population density in the archipelago (Fernández-Palacios and Andersson, 2000).

The objectives of this long-term study were: i) to assess the incidence and concentration of SGARs in the common kestrel population of Tenerife, providing a detailed analysis of the prevalence and patterns of these rodenticides over an 19-year study period; ii) to investigate the relationship between SGAR exposure and various demographic factors, including age and sex, as well as the physiological health as indicated by body condition scores in the kestrel population; iii) to explore the spatial distribution of SGAR concentrations within the kestrel population, examining potential variances across different regions of Tenerife and the implications of land use and anthropogenic impact; and iv) to evaluate the temporal trends of SGAR exposure in kestrels in relation to regulatory changes, particularly the impact of amended bait dosages on the occurrence and levels of these compounds over time.

2. Material and methods

2.1. Study area, sampling, and ethical statement

The volcanic island Tenerife (27° 55′ and 28° 40′ N, 16–17° W) is in the Atlantic Ocean, 292 km from Morocco. Its most diverse habitats and vegetation units make it the largest (2034 km^2) and the highest (3715 m a.s.l.) of the Canary Islands (del Arco Aguilar et al., 2010). Urban areas occupy up to 24.2 % of its territory, with a population of 928,604 inhabitants in 2020. The island is the most densely populated in the Canary Islands (ISTAC, 2022). This island has 43 protected areas covering 48.6 % of its surface, as well as private properties with gardens or orchards and cultivation areas (vineyards, potatoes, tomatoes, banana plantations, orchards, greenhouses) particularly on the windward slopes (Beltrán, 2001; Carralero, 2001). Therefore, the common distribution of private properties and cultivation areas facilitate the arbitrary application of rodenticides by owners rather than specialized personnel.

Our study examined the liver of kestrels as the primary organ for accumulation of rodenticides (Thomas et al., 2011a, 2011b). Liver samples were obtained from necropsies of 390 kestrels. Most birds were admitted to the Wildlife Recovery Centre (WRC) of “La Tahonilla” (Tenerife, Canary Islands) between 2003 and 2009 ($n = 130$) and between 2017 and 2021 ($n = 260$). Additionally, birds were also collected from airport wildlife control units that had been involved in various types of aircrafts collisions. Our database does not contain georeferenced information regarding the exact location of all birds found, but in all cases, we collected a description of the location where the bird was found. The date and immediate cause of death were noted at the time of collection. Birds were either dead (20.8 %) or dead after hospitalization (68.5 %) or were euthanised (10.5 %) when their clinical signs and injuries were irreversible when brought to the WRC. No birds were sacrificed for the purpose of this study. Dead animals were kept frozen at −18 °C until necropsy. Livers were also frozen at −18 °C until the preparation of the extraction and chemical analysis.

An extensive and systematic post-mortem analysis was performed to detect potential effects of SGAR, such as weakness, anaemia, subcutaneous haemorrhage, lethargy, anorexia, and dyspnoea (Dumonceaux and Harrison, 1994; Murray, 2018). These signs were observed in 18.5 % of kestrels of our study. Several other causes of death in kestrels, were also found in this study, including trauma (21.0 %), blindness, drowning, starvation, shooting, lung disease and glue trapping. Despite performing necropsies on all individuals, the cause of death could not be determined with certainty in some individuals. We used the size and thickness of the pectoral muscle as an indicator of the body condition (i.e., the amount of protein reserves) of the examined kestrel (Dumonceaux and Harrison, 1994). By examining the gonads of the carcasses, we were able to identify the sex of the birds (180 females, 206 males, 4 indeterminate). We determined the age of the corpses according to the chromatic characteristics of the plumage using the code of age classification of birds (EURING codification; (EURING, 2020)) and the descriptions by Forsman (1999). In accordance with EURING coding scheme, we identified five ages, namely age 1: chick ($n = 7$), unable to fly freely (age < 35 days for kestrels); age 3: juvenile ($n = 252$), first-year bird, able to fly, born in the breeding season of this calendar year; age 5: adult ($n = 43$), bird in its second year, born last calendar year and now in its second calendar year; age 6: adult ($n = 53$), full-grown bird, born before last calendar year but year of birth unknown; age 8: adult ($n = 35$), bird after the third year, born more than three calendar years ago (including current year) and year of birth unknown.

2.2. Analysis of anticoagulant rodenticides in liver

LC-MS grade formic acid (FA), acetonitrile (ACN) and methanol, were purchased from Honeywell, (Morristown, NJ, USA), while ultrapure water was produced in our laboratory (Gradient A10 Milli-Q, Millipore, Molsheim, France). Standards for 9 ARs (5 SGARs: Brodifacoum, Bromadiolone, Difenacoum, Difethialone, Flocoumafen; and 4 FGARs: Chlorophacinone, Coumatetralyl, Diphacinone, Coumachlor) and an internal procedural standard (P-IS, Warfarin) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). All standards were pure compounds (98 %–99.5 % purity), and stock solutions were prepared at 1 mg/ml in ACN and stored at −20 °C until use. A matrix-matched calibration curve containing all rodenticides studied was prepared from the stock solutions.

2.2.1. Sample preparation

Post-mortem analysis of all kestrel livers was performed, and 1 g of liver was used for extraction of the analytes. After adding P-IS to the sample (or blank matrix for calibration points and quality controls) the mixture was then diluted with ultrapure water (4 ml) for extraction using the modified micro-QuEChERs method, as previously described (Rial-Berriel et al., 2020a, 2020b). Validation of the method indicated the presence of a strong matrix effect. Therefore, calibration curves were prepared with chicken liver for human consumption that tested negative for the analytes of interest. In the same way as the samples, each curve point was extracted using 2 ml of acidified ACN (0.5 % FA). Similarly, QC samples were prepared at 1 ng/g, and analyzed every 30 samples.

2.2.2. UHPLC-QqQ quantitative analysis

An Agilent 1290 UHPLC (Agilent Technologies, Palo Alto, USA) coupled with an Agilent 6460 triple quadrupole mass spectrometer was used to separate and detect the analytes. The method was previously fully validated for liver tissue according to the Standard Practice Guidelines for Method Validation in Forensic Toxicology (SWGFT, 2013) and the SANTE analytical guide (CE, 2019). Recovery rates ranged between 80 and 120 % for all analytes with good linearity ($R^2 > 0.99$) and LOQs ranged from 0.4 to 1.6 ng/ml. A detailed description of the chromatographic and acquisition conditions as well as basic details of the procedure can be found in previous publications (Acosta-Dacal et al., 2021; Rial-Berriel et al., 2020b).

2.3. Data acquisition for the study of exposure determinants

Despite the absence of GPS coordinates for each kestrel, we had access to detailed descriptions of the collection sites, including the specific locality and municipality. Leveraging this data, along with statistics from the Canary Islands Institute of Statistics (ISTAC, 2022), and additional information from the Canary Islands Government, we generated a suite of locality-related variables. We conducted a descriptive analysis for each variable, determining median values which were then employed as thresholds to dichotomize the dataset into two categories for statistical comparison: values below and those at or above the median.

The variables analyzed encompassed a range of ecological and socioeconomic factors, including the area of the municipality (cut-off = 88.8 km^2), population size (36,727 inhabitants), population density (577 inhabitants per km^2), adjusted population density considering buildable space (873 inhabitants per km^2), per capita income (€22,663/year), income per square kilometer (€13,807,774), hectares of protected natural areas within the municipality (2465 Ha), percentage of municipal land designated as protected (33.9 %), hectares under cultivation (621.7 Ha), per capita cultivated area (201.9 m^2), percentage of land cultivated (11.4 %), and specific agricultural metrics such as banana plantation density (8333 m^2/km^2), vineyard (123 m^2/km^2), family orchards (5546 m^2/km^2), vegetable and tuber cultivation (17,162 m^2/km^2), fruit cultivation (9355 m^2/km^2), cereal (2022 m^2/km^2), and greenhouse crops (3172 m^2/km^2), along with total livestock numbers (359 animals) and livestock density (4 heads/km^2).

Furthermore, the year 2018 was a significant temporal marker, delineating the pre- and post-enactment phases of the regulatory change that mandated the reduction of SGAR concentrations in baits from 50 to 30 mg/kg. This dichotomization allowed for an assessment of the regulation's impact on SGAR levels detected in the kestrels.

2.4. Statistical analysis

All statistical analyses were conducted using R software (R Core Team, 2021). The initial step involved a thorough assessment of the distribution of variables. The Kolmogorov-Smirnov test revealed that the concentrations of Second-Generation Anticoagulant Rodenticides (SGARs) and distances did not follow a normal distribution, even after log transformation of the data. Therefore, these variables were represented using the median and interquartile range (p25th - p75th), in addition to the mean ± SD for descriptive purposes.

For comparative analyses between common kestrels from Tenerife and those from previous studies in other Canary Islands, nonparametric tests were employed due to the non-normal distribution of the data. Specifically, the Mann-Whitney U test was used for pairwise comparisons. To control for Type I error inflation due to multiple comparisons, a Bonferroni correction was applied to the p-values.

A Generalized Linear Model (GLM) with a binomial error distribution and logit link function was fitted to explore predictors of kestrel exposure to SGARs. We included 376 common kestrels in the analysis, as 13 had missing data. This method was chosen to analyze the presence or absence of ARs as the binary outcome of having a concentration above or below the threshold set at 200 ng/g in liver tissue, as suggested by various authors for potentially lethal effects in raptor species (Newton et al., 1999; Rattner et al., 2020; Thomas et al., 2011a, 2011b). All potential explanatory variables were dichotomized based on median values (coded as 0/1). The explanatory variables ultimately included in the model were age class (adult/juvenile), legal modification (before and after 2018, the date of its enactment), cattle density, and population density. Additionally, the sum of SGAR concentrations in liver was explored as a continuous dependent variable (continuous outcome), but the explanatory variables for this outcome were the same, hence we decided not to include these analyses in the Results and Discussion section. The forward selection procedure was utilized for model construction, with the Akaike Information Criterion (AIC) guiding the selection process. Prior to the inclusion of explanatory variables in the GLM, potential correlations were assessed using Spearman's correlation test, and highly correlated variables were excluded from the model to prevent multicollinearity. The level of statistical significance was set at $p \leq 0.05$ for all tests.

3. Results and discussion

3.1. Characteristics of the sampled population

This study involved sampling 390 common kestrels during 2 sampling phases (2004–2009 and 2017–2021), an 11-year period over 19-years. Fig. 1 shows the characteristics of the sampled population, including the causes of admission. The proportion of males ($n = 180$; 46.1 %) and females ($n = 206$; 52.8 %) was very similar. However, about two-thirds of the sampled group of kestrels were juveniles (EURING 3; $n = 252$, 64.0 %). The mortality rate of birds, including birds of prey, has been reported to be high within the first year of life due to inexperience in hunting, competition with other adults for occupied territories, and interspecific inexperience (identifying humans and enemy predators as well as factors associated with human activity is crucial to their survival). It has been established that for different species of raptors, death rate can range between 30 and 40 % during the first year of life (Newton et al., 2016), although it varies greatly between the species (Village, 1990a). In kestrels survival to adulthood has been reported to be even lower, with mortality rates ranging from 60 to 70 % (Hiraldo et al., 1996; Newton, 1979). The representation of kestrels sampled from southern ($n = 121$, 31 %) and northern ($n = 269$, 69 %) parts of the island showed a noticeable disparity, as did the proportion between individuals from urban ($n = 94$, 24 %) and rural areas ($n = 296$, 76 %). This imbalance is likely attributable to anthropogenic factors. Particularly, the island's northern slopes, especially the coastal strip, house the densest human populations and the highest levels of agricultural, livestock, and industrial activities. Therefore, kestrels in these areas encounter a more diverse range of threats, potentially increasing mortality rates. Additionally, areas with a higher density of human population correlate with an increased chance of discovering deceased, injured, or malnourished birds (Carrillo, 1991, 2007; Carrillo and González-Dávila, 2005).

3.2. Descriptive analysis of the levels of anticoagulant rodenticides

The descriptive results and comparisons between the incidences and concentrations of the rodenticides in the livers of the common kestrels in Tenerife and the rest of the Canary Islands, over the respective study periods, are presented in Table 1. This table demonstrates the frequency and mean concentrations of each SGAR (brodifacoum, bromadiolone, difenacoum, difethialone, and flocoumafen) found in the two groups. In addition to the five SGARs identified, our analytical method also incorporated testing for four First Generation Anticoagulant Rodenticides (FGARs). However, none of these were detected in our study.

The detection pattern we found fully coincides with that described previously in the Canary Islands for birds of prey, including kestrels, as well as for other birds, reptiles and mammals found in the Canary Islands (Rial-Berriel et al., 2021a).

There is no doubt that the widespread use of SGARs for rodent control leads to the exposure of non-target species, with raptors being among the animals exposed at the highest rate (Nakayama et al., 2019; Sánchez-Barbudo et al., 2012; Van den Brink et al., 2018). However, the very high frequency and concentrations of SGARs found in kestrels on the island of Tenerife were striking. Only 27 kestrels were negative for anticoagulant rodenticides (17 juveniles, 8 adults, 2 chicks; 19 females, 8 males) (Fig. 1), giving a positivity percentage of 93.1 % ($n = 363$). Furthermore, 68 individuals (17.4 %) showed clear signs of internal and external bleeding. However, it is important to note that not all adverse

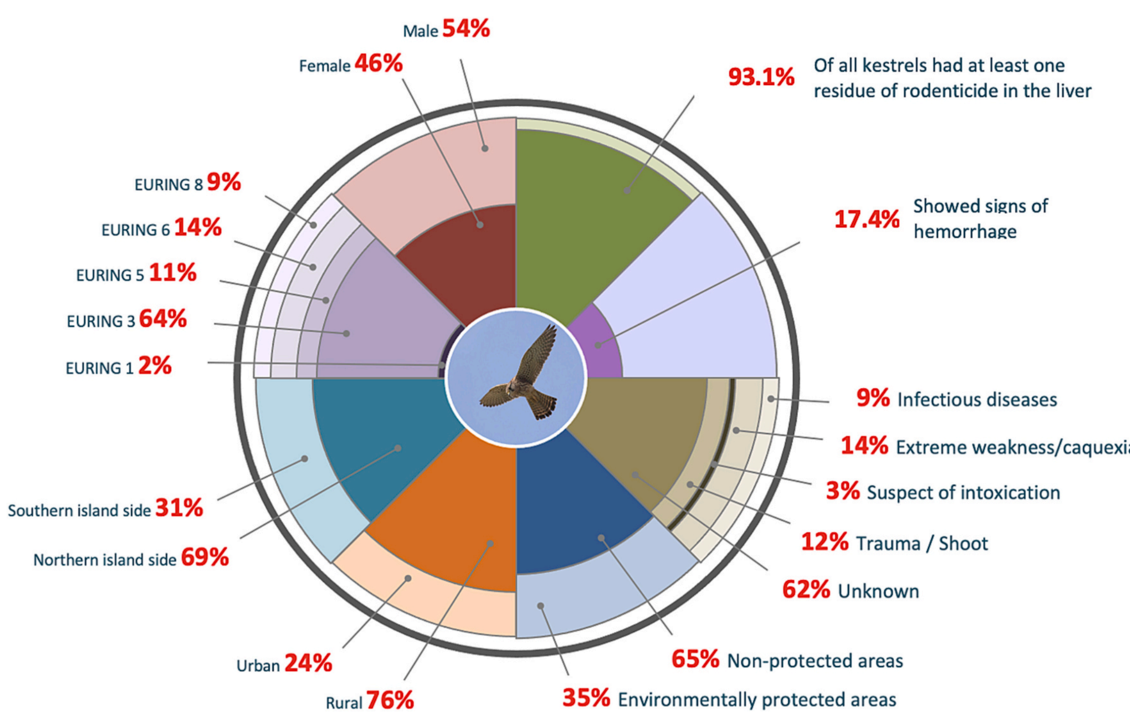

Fig. 1. Characteristics of the sample population of 390 common kestrels (*Falco tinnunculus*) of Tenerife (Canary Islands).

Table 1
Incidence and concentrations of the rodenticides in liver of the common kestrels in the Canary Islands.

	Tenerife (n = 390) 2003–2009 and 2017–2021				Rest of the Canary Islands (n = 86) 2009–2021					
	Freq (%)	Mean ± SD	Med (ng/g)	P25-P75	Freq (%)	Mean ± SD	Med (ng/g)	P25-P75	*P1*	*P2*
Brodifacoum	89.7 **	189.3 ± 229.8	97.6 *	18.1–289.5	58.7	133.0 ± 263.4	34.8	12.7–88.9	0.0046	0.0119
Bromadiolone	85.6 *	67.9 ± 88.8	33.1	12.5–89.9	61.3	232.5 ± 745.2	30.6	5.1–67.1	0.0326	–
Difenacoum	52.8 **	15.8 ± 26.3	5.5 *	3.1–16.7	29.0	12.2 ± 16.1	4.1	1.4–25.0	0.0087	0.0432
Difethialone	19.2 *	65.8 ± 149.8	7.1 **	3.6–38.3	8.0	3.4 ± 2.9	2.5	1.0–6.7	0.0404	0.0045
Flocoumafen	30.5 **	17.4 ± 36.2	5.1	2.9–13.7	4.8	5.6 ± 7.3	5.6	0.5–10.8	0.0018	–

effects of SGARs are related to blood coagulation disorders; rather, recent studies have shown that SGAR exposure can influence disease susceptibility, immune function, and several other effects independently of coagulopathy (Rattner et al., 2018; Van den Brink et al., 2018).

Previous studies reported high concentrations of SGARs in birds of prey in the Canary Islands (Rial-Berriel et al., 2021a). To highlight the significant exposure observed, we compared the current SGAR detection frequency and concentrations in Tenerife's kestrels with our previously published data for the species in the other Canary Islands. Instead of combining the datasets, these earlier results served as a benchmark for comparison. In Tenerife, we found that 93.1 % of kestrels (n = 363) had detectable levels of SGARs, marking a notable increase from the 68.2 % detection rate reported for the species on the other islands (Rial-Berriel et al., 2021c). The median value for the sum of ARs in Tenerife was practically double the value previously reported for the entire Canary archipelago ($\sum$AR = 180.9 vs 102.4 ng/g liver for Tenerife and the rest of the Canary Islands respectively; $P < 0.0001$) (Fig. 2). Additionally, in Tenerife, kestrels not only showed significantly higher values than in the rest of the archipelago, but the average number of rodenticides per animal was also significantly higher (Fig. 2, inset).

Furthermore, it's noteworthy that the median SGARs value for this group of 390 individuals is remarkably close to the toxicity/lethality cut-off values established for certain raptor species (200 ng/g liver). While these cut-offs are not universal and are based on probabilistic data derived from species different than kestrels, they are used as references due to the lack of other reference values (Thomas et al., 2011a, 2011b).

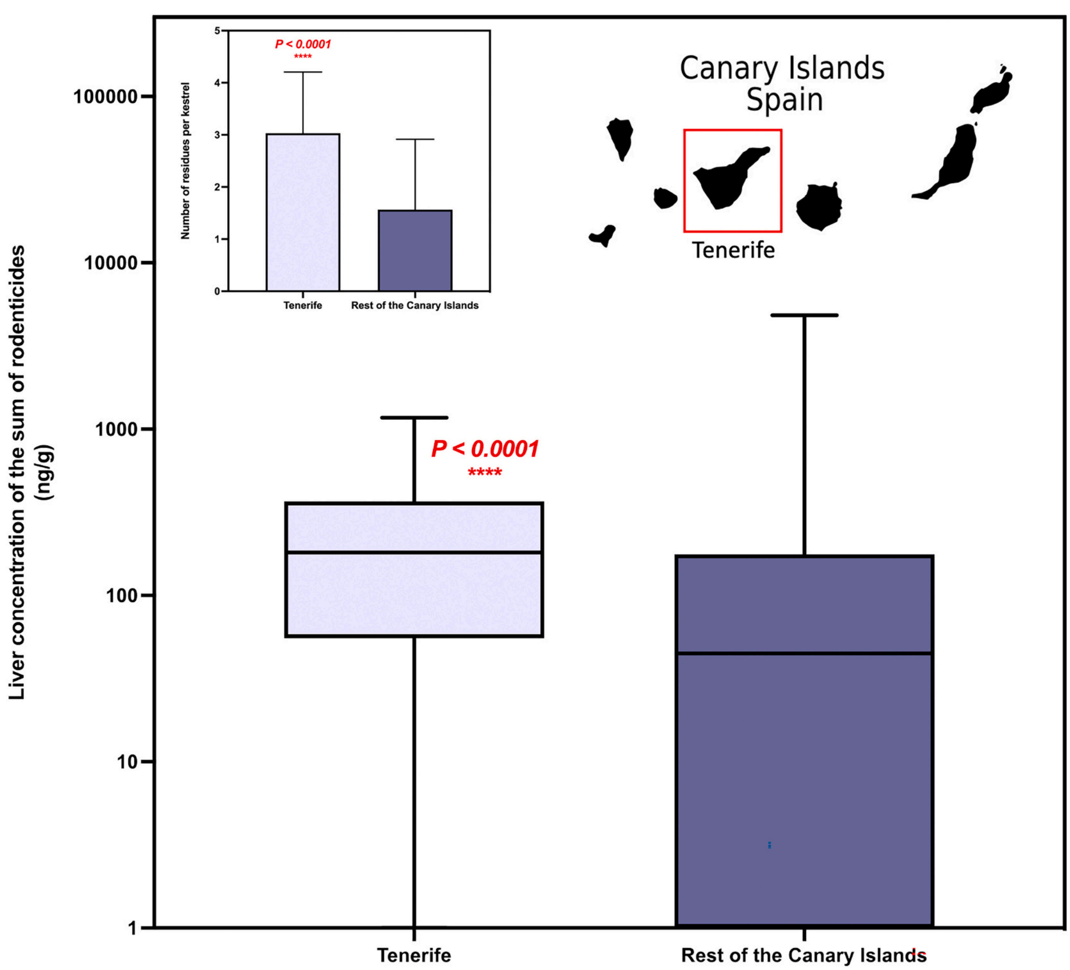

Fig. 2. Box-and-whisker plot showing the comparison of anticoagulant rodenticides in kestrel livers on Tenerife with those in the rest of the Canary Islands. The lines represent the medians, the boxes indicate the 25th to 75th percentiles, and the minimal and maximal values are shown at the ends of the bars. (Inset graphs) *Left.* Average number of ARs found per animal, comparing both territories; *Right.* Location of Tenerife in the Canary Islands archipelago.

Remarkably, a total of 185 individuals (46.9 %) exceeded the highest cut-off value (200 ng/g liver). Brodifacoum stands out due to its high concentrations and frequent detection. Out of a total of 363 kestrels showing rodenticides in the liver, 350 (96.4 %) contained brodifacoum, either alone or in combination with other rodenticides. These concentrations and frequency are significantly higher than those previously reported for the rest of the Canary Islands (Rial-Berriel et al., 2021a, 2021b; Ruiz-Suárez et al., 2014). Brodifacoum, being the most toxic rodenticide for birds and mammals, is of particular concern, and recently its use has been restricted to indoor and immediate outdoor areas of buildings, with its application in open spaces prohibited (EC, 2022). The mean value for brodifacoum in our study was 189.3 ng/g liver (Table 1), which is a very high value, akin to what would be achieved by consuming 1–3 μg of brodifacoum per gram of feed along a 24 h-period, as found in experimental studies with American kestrels (Rattner et al., 2020). Furthermore, liver concentrations above 200 ng/g liver were found in 110 kestrels, which, in the literature, correlate with a variety of clinical manifestations in kestrels, ranging from visible bruising in non-feathered areas to frank bleeding in the oral cavity (Rattner et al., 2020). Detection frequencies and concentrations of difenacoum and difethialone were also significantly higher in this group of Tenerife kestrels (Table 1) compared to the rest of the Canary Islands, and published previously (Rial-Berriel et al., 2021a, 2021b). According to the US Environmental Protection Agency, brodifacoum and difethialone pose the greatest overall potential risk to birds and non-target mammals (Erickson and Urban, 2004). Even though bromadiolone and flocoumafen levels were similar in kestrels from other Canary Islands in kestrels, the frequency of detection on Tenerife was significantly higher, with bromadiolone detected in 334 individuals and flocoumafen detected in 119.

It is also important to note that the average number of rodenticides per animal were also high, and they were significantly higher than those previously reported for kestrels in the Canary Islands (median 2.98 vs. 1.42 rodenticides per animal; $P < 0.0001$) (Fig. 2, inset). A total of 85.6 % ($n = 334$) of the positive cases had >1 rodenticide, and most animals had 3 (41.5 %, $n = 162$)) or 4 (30.3 %, $n = 122$)) different rodenticides simultaneously (Fig. 3, left). Combinations of >1 rodenticide are commonly found in raptors, but usually at a much lower percentage (40–60 %), as reported in recent literature, including the Canary Islands (Coeurdassier et al., 2019; Hong et al., 2019; Huang et al., 2016a; Murray, 2011; Rial-Berriel et al., 2021a; Thornton et al., 2022). Brodifacoum in combination with bromadiolone and difenacoum was the most encountered combination followed by the same combination with flocoumafen (Fig. 3, right). Given its predominance in this group of kestrels, brodifacoum can be found in 99.4 % of combinations containing two or more rodenticides.

3.3. Temporal trends of the exposure of kestrels to SGARs

Our longitudinal study spans two distinct periods, 2004–2008 and 2017–2021, totaling 11 years within a 19-year framework. This design facilitated an analysis of the temporal dynamics of SGAR exposure among common kestrels in Tenerife.

Previous research by Rial-Berriel et al. (2021a) indicated an uptick in certain SGARs, notably brodifacoum, within Canary Islands wildlife. Our findings mirror this trend in Tenerife kestrels, as depicted in Fig. 4. Notably, since 2018, the annual average SGAR burden per bird has frequently exceeded the conservative benchmark of 200 ng/g liver tissue. Thus, Post-2018, we observed a surge in both the concentrations of SGARs and the proportion of samples surpassing this threshold (Fig. 5). This period coincides with amendments to Regulation (EC) No. 1272/2008, mandating reprotoxic labelling for anticoagulant baits exceeding 30 μg/g. Despite the regulation's intent to reduce bait concentrations, our data indicate an increase in brodifacoum levels post-regulation, suggesting compensatory usage patterns by end-users (Table 2) (Frankova et al., 2019; Rial-Berriel et al., 2021a).

Moreover, the regulatory changes coincide with intensified efforts to manage feral cat populations on the islands, potentially influencing rodenticide application rates due to altered rodent-predator dynamics (Mahlaba et al., 2017). The implications of such ecological shifts are evident in the heightened rodenticide residues we detected.

The pattern of increased SGAR exposure is not unique to Tenerife. Parallel increases in brodifacoum in raptors have been documented globally, with regional studies reporting similar trends. For example, recent findings from south-eastern France indicate an increase when compared to previous periods (Moriceau et al., 2022). In Western Canada, the mean concentrations of bromadiolone have markedly increase, likely due to regulatory changes that permit only bromadiolone for

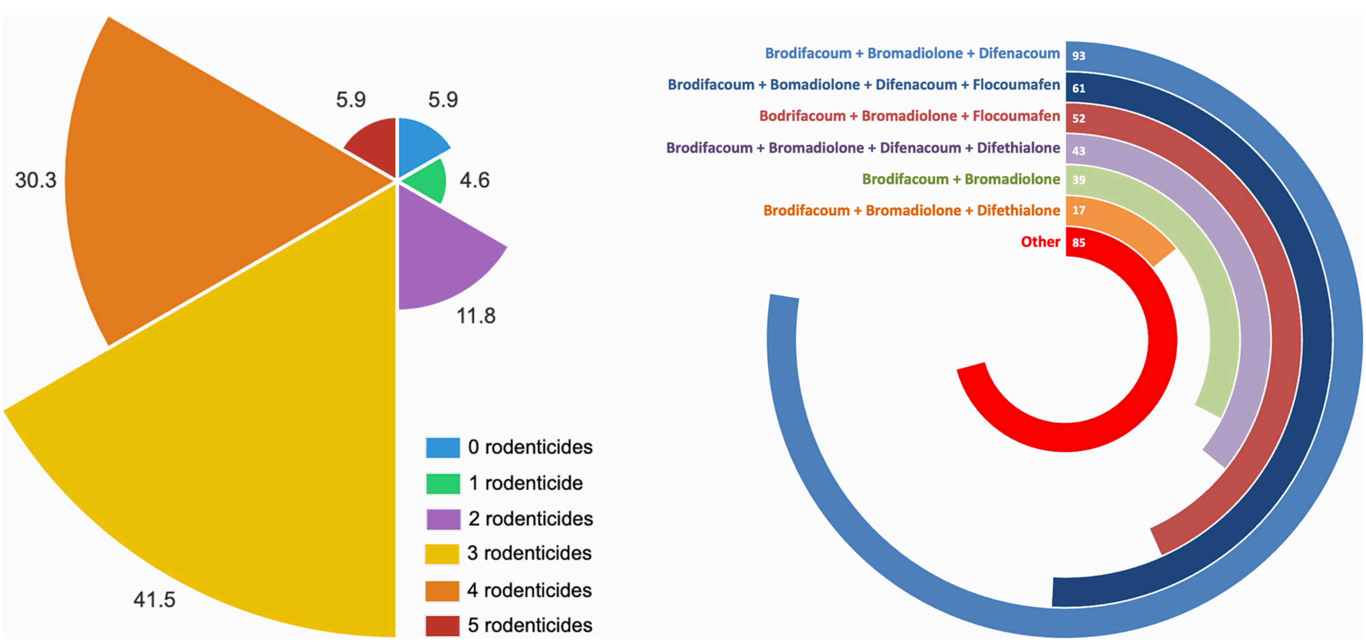

Fig. 3. Left. Number of anticoagulant rodenticides per animal, expressed as a percentage. Right. Most frequent combinations of rodenticides found in kestrels with more than one AR. Figures indicate the number of individuals with that combination.

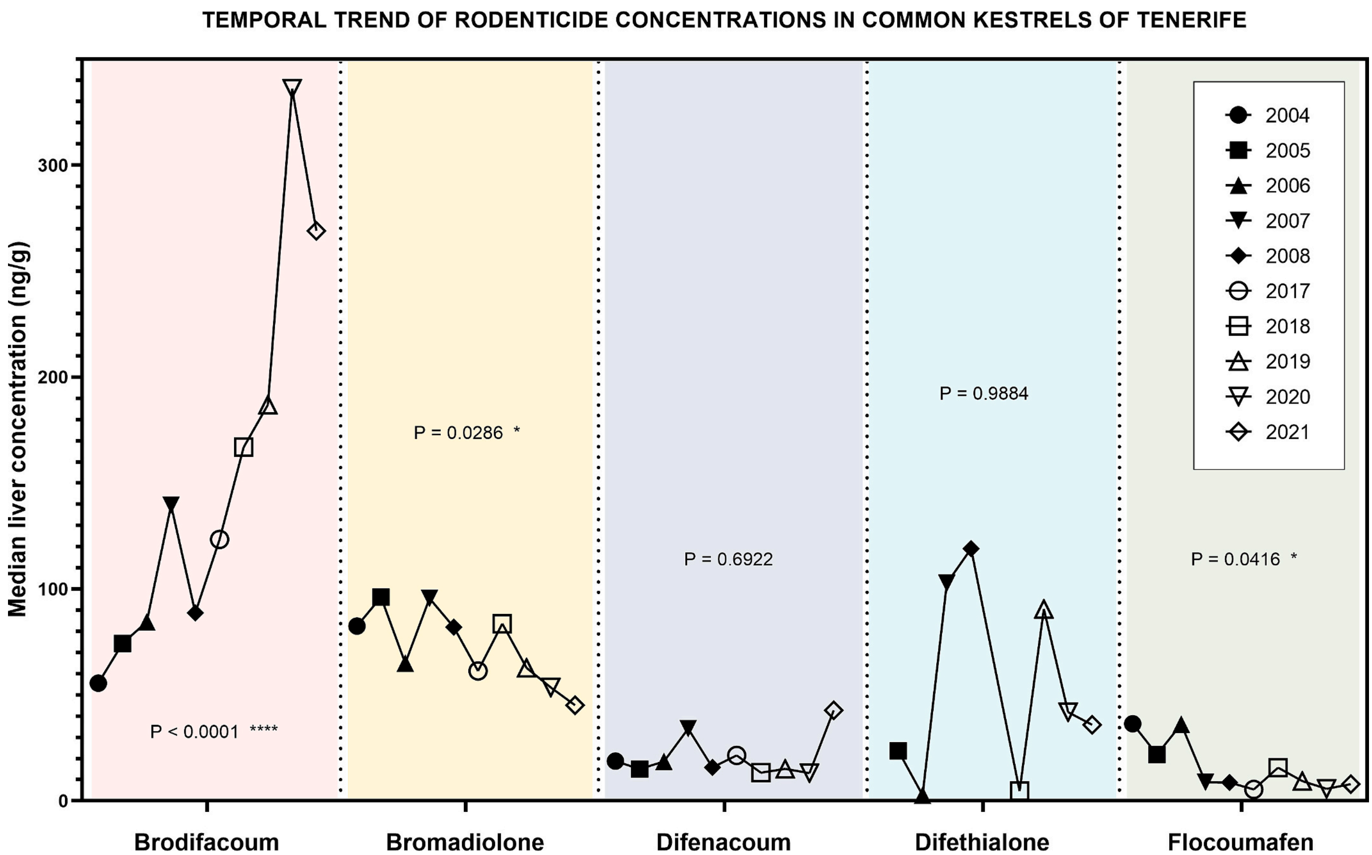

Fig. 4. Temporal trend of rodenticide concentrations in common kestrels from Tenerife.

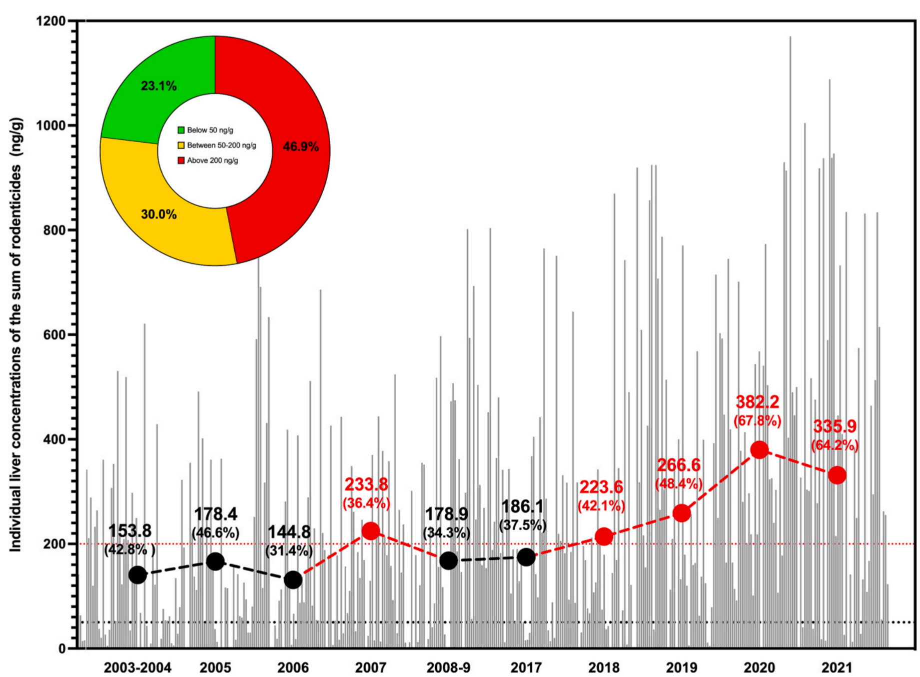

Fig. 5. Individual values of the sum of anticoagulant rodenticides of all kestrels sampled. The dots indicate the median value of all the animals sampled in the specified year or period, as well as the percentage of individuals with values above 200 ng/g liver (in brackets). The dashed red line indicated the threshold value of 200 ng/g liver. When the median value for all birds exceeds the threshold value, the dots and numbers appear in red. Inset graph. Chart indicating the percentage of individuals with liver rodenticide sum values below 50 ng/g liver; from 50 to 200 ng/g liver; and above 200 ng/g liver.

Table 2
Analyses of anticoagulant rodenticides concentrations detected in the liver of common kestrels prior and following the application of the new UE regulation regarding the commercialization of rodenticide baits.

	Before March 2018 (EU 2016/1179)				After March 2018 (EU 2016/1179)				
	Freq (%)	Mean ± SD	Med (ng/g)	P25-P75	Freq (%)	Mean ± SD	Med (ng/g)	P25-P75	P
Brodifacoum	85.8	98.5 ± 142.7	23.7	8.2–123.9	91.5	246.2 ± 255.3	147.2 ****	42.7–378.2	< 0.0001
Bromadiolone	84.5	77.4 ± 102.7	39.8 *	15.0–730.0	78.8	61.7 ± 78.4	25.2	11.5–376.3	0.0482
Difenacoum	32.2	19.6 ± 33.7	5.1	3.0–16.1	66.8	14.7 ± 23.5	6.3	3.1–16.8	0.9423
Difethialone	9.0	84.1 ± 198.7	4.7	2.6–28.1	23.9	61.7 ± 137.9	7.2	3.9–39.6	0.2608
Flocoumafen	49.7	21 ± 42.8	6.5 *	3.4–17.2	16.1	9.9 ± 16.9	4.3	2.6–9.9	0.0307

outdoor applications (Elliott et al., 2022). In contrast, brodifacoum levels decreased over the same period, likely in line with risk mitigation measures. A similar pattern has been observed on Reunion Island (Coeurdassier et al., 2019). These trends extend beyond Tenerife and underscore a broader issue. This is of special concern, especially since various studies have suggested that SGARs may contribute to a significant population decline in kestrels across Europe (Harris et al., 2020; PECBMS, 2021; Roos et al., 2021a, 2021b). Our data, coupled with recent population assessments (Martínez-Padilla et al., 2021; Carrillo-Hidalgo, unpubl. data), suggest that the kestrel population may also be experiencing a decline in Tenerife.

3.4. Integrated analysis of kestrel exposure to anticoagulant rodenticides

Our research has elucidated a complex interplay of factors influencing the presence of anticoagulant rodenticides (ARs) in the common kestrel population on Tenerife. The statistical models we refined reveal that both biological traits and human-induced changes significantly determine AR levels in these raptors. The logistic regression models (Tables 3 and 4) reveal that age and recent legal modifications are prominent determinants of AR presence in kestrels.

Adult kestrels are more likely to exhibit detectable levels of ARs

Table 3
Best adjusted models explaining the presence (threshold set at 200 ng/g) of anticoagulant rodenticides in the common kestrels from Tenerife.

		Estimates	SE	OR (95%CI)	p	AIC
M1	Intercept	−1.19	0.23	0.30 (0.19–0.48)	<0.001	498.60
	Legal modifications Yes-No	0.77	0.22	2.15 (1.39–3.33)	<0.001	
	Age Adult-juvenile	0.66	0.23	1.93 (1.23–3.03)	0.004	
	Cattle density 1–0	0.46	0.22	1.59 (1.03–2.45)	0.036	
	Population density 1–0	0.36	0.22	1.44 (0.94–2.20)	0.093	
M2	Intercept	−1.04	0.21	0.35 (0.23–0.54)	<0.001	499.42
	Legal modifications Yes-No	0.78	0.22	2.17 (1.40–3.36)	<0.001	
	Age Adult-juvenile	0.68	0.23	1.97 (1.26–3.09)	0.003	
	Cattle density 1–0	0.49	0.22	1.63 (1.06–2.50)	0.027	

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 Criterion for the model is also reported. Response variable: threshold set at 200 ng/g. Number of kestrels in the analysis = 376.

Table 4
Summary information of the variables considered for inclusion in the models categorized based on the threshold set at 200 ng/g.

Variables	<200 ng/g N (%)	>200 ng/g N (%)
Age		
Juvenile	145(38.6)	102(27.1)
Adult	54(14.4)	75(19.9)
Law implementation		
Yes (after 2018)	103(27.4)	127(33.8)
No (before 2018)	96(25.5)	50(13.3)
Cattle density		
0 (< median)	126(33.5)	92(24.5)
1 (>median)	73(19.4)	85(22.6)
Population density		
0 (< median)	113(30.1)	81(21.5)
1 (> median)	86(22.9)	96(25.5)

compared to juveniles, with a 93 % increased likelihood [OR: 1.93, $P = 0.004$]. This finding aligns with previous observations of age-related variations in SGAR levels among raptors and suggests that adult kestrels, due to their foraging behavior and longer exposure times, accumulate higher concentrations of these compounds. For example, Roos et al. (2021b) found that juvenile kestrels possess higher levels of difenacoum than adults, whereas Huang et al. (2016b) reported higher levels in adult owls (*Tyto alba*). Despite these variable findings, the prevalence of SGARs remains high, with 88–93.8 % detected for brodifacoum and bromadiolone, and 19.7–51.4 % for other rodenticides. Given this widespread presence, concerns have been raised about the use of these rodenticides, even when their usage is restricted to interior or perimeter areas of buildings, or when bait concentrations are limited.

As previously mentioned, the implementation of legal modifications has also had a significant impact, though not in the desired manner. Kestrels found post-regulation exhibit more than twice the likelihood of AR presence [OR: 2.15, $P < 0.001$]. This increase in detection frequency and concentration of brodifacoum post-2018 is a critical finding, considering the regulatory intent to mitigate the risks associated with SGARs by mandating reprotoxic labeling for higher concentration baits.

In our study, we expanded the scope of investigation to consider the impact of socio-demographic factors and land use patterns on the presence of SGARs in the common kestrel population. Our findings reveal that among the variables considered, cattle density emerged as a significant factor. Specifically, kestrels found in areas with higher cattle density are 59 % more likely to have detectable levels of SGARs [Odds Ratio (OR): 1.59, $P = 0.036$]. This relationship indicates that practices associated with livestock farming, such as the use of rodenticides to safeguard feed or control rodent populations, could inadvertently increase the levels of SGARs found in kestrels. Furthermore, our analysis has shown a direct link between the density of agricultural cultivation and livestock presence on the island. However, it is noteworthy that the density of crops alone was not a significant predictor for the presence of

SGARs when considering the established threshold of 200 ng/g. This finding implies that while agricultural activities are related to the presence of SGARs in the environment, it is specifically the practices related to livestock management that are more influential in determining the exposure of kestrels to these rodenticides.

Contrary to expectations, human density did not show a statistically significant correlation in our models, despite being a factor in model performance. This nuance highlights the complexity of the relationship between human density and wildlife exposure to contaminants and suggests that local factors may modulate this relationship in Tenerife. The results of several studies indicate that urban environment and human density are clear determinants of wildlife exposure to SGARs (Alabau et al., 2020; Burke et al., 2021; Lettoof et al., 2020; Lohr, 2018; López-Perea et al., 2019). However, it is crucial to note that the effects of these variables may be shaped by specific local factors.

When considering multiple variables simultaneously, age remains the sole significant predictor of high AR levels in kestrels. This finding underscores the importance of considering life history traits when assessing the risk of contaminant exposure in wildlife. While agricultural and livestock factors appeared influential in univariate analyses, their significance diminished when age was accounted for. This suggests that the accumulation of ARs over time is a critical factor, and age-related bioaccumulation may be a more significant determinant of AR levels than previously understood.

However, our study's interpretive power is subject to certain limitations. Notably, we cannot definitively ascertain whether the rodenticides found in juvenile kestrels (EURING code 3) were accumulated in the areas where these individuals were located at the time of discovery. Given that most juveniles of this species disperse around the island of Tenerife after a variable period of 20–40 days in their parents' territory, the exact localities, habitats, and feeding sites during their dispersal are not known. This uncertainty means that SGAR concentrations in these juvenile kestrels could have been accumulated throughout the dispersal period, rather than being indicative of the contamination levels in the specific areas where they were found. Moreover, the adults (EURING codes 5, 6, and 8) found before the dispersal dates of juveniles may provide insights into geographical differences in SGAR exposure across Tenerife. Our study did not find significant differences between areas, suggesting that AR contamination is widespread across the island. This pervasive presence of ARs, despite recent regulatory changes aimed at protecting wildlife, indicates that these measures are not effectively curtailing the environmental impact of these substances.

Our integrated analysis has delineated the multifaceted nature of AR exposure in kestrels, highlighting the interplay between regulatory changes, biological traits, and anthropogenic land use. These insights are crucial for informing future conservation strategies and regulatory decisions aimed at mitigating the impact of these environmental contaminants on wildlife. Nonetheless, the absence of spatial clustering in exposed individuals and the widespread contamination revealed by our study call into question the efficacy of current regulations and underscore the need for a re-evaluation of wildlife protection policies in the face of persistent environmental contaminants.

4. Conclusions

Our study has revealed a concerning prevalence of anticoagulant rodenticides (ARs) in Tenerife's kestrel population, with most individuals showing levels near or above lethal thresholds, especially concerning given the high toxicity of compounds like brodifacoum and bromadiolone. An upward trend in AR detection has been observed, particularly following regulatory changes intended to reduce bait concentrations, suggesting these efforts have not been fully successful.

The research underscores livestock farming as a significant factor in AR exposure for kestrels, more so than general agricultural activities. Adult kestrels are particularly at risk, likely due to bioaccumulation effects over time. Interestingly, human population density does not correlate strongly with AR exposure, indicating that other local factors may be influencing the risk.

The current regulatory framework appears inadequate in protecting kestrels and potentially other wildlife from AR exposure. This calls for more robust and effectively enforced regulations, with a need for conservation strategies and policies that reflect the complex nature of AR exposure, integrating biological and socio-economic considerations. Further studies are essential to deepen our understanding of the interplay between land use, rodent control practices, and wildlife exposure to ARs.

CRediT authorship contribution statement

Guarantor of integrity of the entire study: OPL, JCH
Study concepts and design: OPL, JCH
Sample providing: JCH
Literature research: OPL, BMC, JCH, CRB, AAD
Laboratory work: CRB, AAD, BMC, MZ, OPL
Data analysis: OPL, BMC, CRB, JCH
Statistical analysis: OPL, BMC, LAHH, MZ
Manuscript preparation (original draft): OPL, JCH, BMC, AAD, CRB
Manuscript editing: OPL, BMC, JCH, CRB, AAD, BMC, LAHH, MZ
Project administration: OPL
Funding acquisition: OPL, MZ, LAHH

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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CONCLUSIONES/ CONCLUSIONS

CONCLUSIONES

1. La exposición a rodenticidas anticoagulantes de segunda generación (SGARs) es alta y generalizada en la fauna silvestre terrestre de Canarias, con detecciones en especies tanto depredadoras como presas, abarcando distintos niveles tróficos.

2. El brodifacum fue el compuesto más prevalente en todos los grupos, mientras que los rodenticidas de primera generación (FGARs) se detectaron de forma anecdótica.

3. Se ha documentado por primera vez la presencia significativa de SGARs en reptiles y aves insectívoras, lo que sugiere una vía de exposición secundaria a través de invertebrados contaminados, hipótesis que merece ser investigada en estudios futuros.

4. La concentración de SGARs en fauna silvestre está influida por múltiples variables, siendo especialmente relevantes el tamaño corporal, la edad, la actividad ganadera y la localización geográfica.

5. Las restricciones legales sobre el uso de SGARs no han logrado reducir eficazmente los niveles de exposición ambiental, lo que pone en cuestión la efectividad de las medidas regulatorias vigentes.

6. Las especies rapaces de las islas presentan niveles significativamente superiores de SGARs frente a sus homólogas continentales, confirmando una mayor vulnerabilidad ecológica de los ecosistemas insulares frente a estos compuestos.

7. Algunas especies, como la culebra real de California y el camaleón de Yemen, han demostrado su utilidad como centinelas ecológicos, al reflejar con fidelidad la contaminación por SGARs presente en el ecosistema.

8. Las especies centinelas, tanto nativas como invasoras, ofrecen una herramienta eficaz para la vigilancia ecotoxicológica en islas y deberían incorporarse a los programas de monitoreo ambiental de forma estructurada.

CONCLUSIONS

1. Exposure to second-generation anticoagulant rodenticides (SGARs) is high and widespread in the terrestrial wildlife of the Canary Islands, with detections in both predatory and prey species, spanning multiple trophic levels.

2. Brodifacoum was the most prevalent compound across all groups, whereas first-generation anticoagulant rodenticides (FGARs) were detected only sporadically.

3. This study documents for the first time the significant presence of SGARs in reptiles and insectivorous birds, suggesting a secondary exposure pathway through contaminated invertebrates—a hypothesis that warrants further investigation in future research.

4. SGAR concentrations in wildlife are influenced by multiple variables, with body size, age, livestock activity, and geographic location being particularly relevant.

5. Legal restrictions on SGAR use have not effectively reduced environmental exposure levels, calling into question the efficacy of current regulatory measures.

6. Raptors from the islands exhibit significantly higher SGAR levels compared to their continental counterparts, confirming an increased ecological vulnerability of insular ecosystems to these compounds.

7. Certain species, such as the California kingsnake and the Yemen chameleon, have demonstrated their utility as ecological sentinels by reliably reflecting SGAR contamination present in the ecosystem.

8. Sentinel species, both native and invasive, offer an effective tool for ecotoxicological monitoring on islands and should be incorporated into environmental surveillance programs in a structured manner.

ANEXOS

PUBLICACIÓN 5

DATA IN BRIEF

"An open dataset of anticoagulant rodenticides in liver samples from California kingsnakes and raptors in Gran Canaria (Canary Islands, Spain)"

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Data Article

An open dataset of anticoagulant rodenticides in liver samples from California kingsnakes and raptors in Gran Canaria (Canary Islands, Spain)

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A R T I C L E   I N F O

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Dataset link: Table 1. Snakes data: concentration of anticoagulant rodenticides detected in liver (ng/g), total number of rodenticides, biometric, geographical and necropsy findings). (Original data)
Dataset link: Table 2. Raptors data: concentration of anticoagulant rodenticides detected in liver (ng/g), total number of rodenticides per animal and geographical information. (Original data)

A B S T R A C T

It is well known that rodenticides are widely used, and there are multiple routes by which they can reach non-target wildlife species. Specifically, in the Canary Islands, a high and concerning incidence of these compounds has been reported. However, in this scenario, reptiles remain one of the least studied taxa, despite their potential suitability as indicators of the food chain and environmental pollution has been noted on several occasions. In this context, the California Kingsnake (*Lampropeltis Californiae*), widely distributed on the island of Gran Canaria, occupies a medium trophic level and exhibits feeding habits that expose it to these pollutants, could be studied as a potential sentinel of exposure to these compounds. For this reason, 360 snake livers were analyzed by LC-MS/MS. Similarly, 110 livers of birds of prey were sampled. Thus, we present the analysis of 10

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anticoagulant rodenticides (warfarin, diphacinone, chlorophacinone, coumachlor, coumatetralyl, brodifacoum, bromadiolone, difethialone, difenacoum and flocoumafen) in both data series; snakes, and raptors. Furthermore, this dataset includes biological data (weight, length, sex, colour, and design pattern), geographic data (distribution area and municipalities) and necropsy findings that could be of interest for a better understanding of this snake species and for future studies.

Specifications Table

Subject	Environmental Science: Health, Toxicology and Mutagenesis
Specific subject area	Relevant sampling data and analysis of anticoagulant rodenticides in the liver of the California kingsnakes and raptors from Gran Canaria (Canary Islands, Spain).
Data format	Raw data
Type of data	Tables
Data collection	Liver samples were collected during necropsy and subsequently stored at -20 ºC until the moment of their processing for analysis by Agilent 1290 UHPLC liquid chromatography coupled to Agilent 6460 triple quadrupole mass spectrometry (Agilent Technologies, Palo Alto, USA). Geographic data were recorded in the field and biometric data and relevant necropsy findings were taken in the laboratory facilities during necropsies. The testing period took place between 2021-2022 for snakes and 2020-2022 for raptors. All samples were analysed by the Toxicology Unit of the University of Las Palmas de Gran Canaria.
Data source location	Institution: Toxicology Unit, Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria City/Town/Region: Las Palmas de Gran Canaria Country: Spain
Data accessibility	Repository name: Mendeley Data Data identification number: DOI Table 1: 10.17632/gd884g8g7s.2 DOI Table 2: 10.17632/6g4ftskcsf.2 Direct URL to data: Table 1: https://data.mendeley.com/datasets/gd884g8g7s/2 Table 2: https://data.mendeley.com/datasets/6g4ftskcsf/2
Related research article	Beatriz Martín-Cruz, Martina Cecchetti, Katherine Simbaña-Rivera, Cristian Rial-Berriel, Andrea Acosta-Dacal, Manuel Zumbado-Peña, Luis Alberto Henríquez-Hernández, Ramón Gallo-Barneto, Miguel Ángel Cabrera-Pérez, Ayose Melián-Melián, Alejandro Suárez-Pérez, Octavio P. Luzardo. Potential exposure of native wildlife to anticoagulant rodenticides in Gran Canaria (Canary Islands, Spain): Evidence from residue analysis of the invasive California Kingsnake (Lampropeltis californiae), Science of The Total Environment, Volume 911, 2024,168761, ISSN 0048-9697, 10.1016/j.scitotenv.2023.168761 [1]

1. Value of the Data

- The dataset we present carries significance as it records the levels of key anticoagulant rodenticides found in a collection of 360 California kingsnakes (*Lampropeltis californiae*) in Gran Canaria. The values presented here can potentially serve as baseline concentrations for the snakes inhabiting this island.

- This dataset would be invaluable for researchers conducting biomonitoring studies on these pollutants in reptiles, a field that has seen fewer studied compared to other taxa [2–4].
- Beyond the raw data on contaminants, the inclusion of biometric data and potential signs of rodenticide exposure can be utilized for future investigations, providing comprehensive insights.
- Moreover, the raptor data offer further details about the considerable rodenticide exposure experienced by the endemic fauna in the Canary Islands [5–7].

2. Data Description

Snake dataset focuses on data related to 360 snakes captured on the island of Gran Canaria in the context of the eradication campaign during the years 2021-2022. In summary, it presents information on the analysis of anticoagulant rodenticides, along with biometric data, geographical distribution, and relevant necropsy findings for future studies. Analytical results include concentrations of each detected compound and the ΣARs in ng/g ww liver, along with the total number of rodenticides per animal. Biological values include sex (F = females; M= males and I= unknown sex), weight (in g), fat weight (in g), snout vent-length (SVL; in cm), color pattern (normal = black or brown color; albinos) and design pattern (striped or banded) are detailed. Geographical distribution data is defined by municipality and distribution area: main area (MA: municipalities of Telde, Santa Brígida, Valsequillo and San Mateo), secondary area (SA: municipality of Gáldar and Agaete), tertiary area (TA: municipalities of San Bartolomé de Tirajana and Mogán) and quaternary area (QA: municipality of Las Palmas de Gran Canaria). Additionally, the capture method (trap or manual) is shown, and the observation of lesions or relevant necropsy findings are detailed using the following numerical coding: 0= no lesions; 1=external and superficial wounds; 2=external and deep bleeding wounds; 3= bleeding in the oral cavity; 4=bloody feces; 5= generalized hemorrhages.

Raptor dataset presents data for the series of 110 raptors collected as part of the Poisoning Control and Prevention Strategy in the Canary Islands between 2020-2022 [8]. These birds of prey came from the wild or recovery centers where they were admitted and subsequently died. The series comprises six raptors endemic to the Canary Islands: *Tyto alba* ($n = 10$), *Asio otus* ($n = 36$), *Falco tinnunculus* ($n = 39$), *Accipiter nisus* ($n = 10$), *Falco pelegrinoides* ($n = 1$) and *Buteo buteo insularum* ($n = 14$). This table provides the analytical results of the rodenticides detected per animal, as well as their ΣARs expressed in ng/g ww liver. The total number of rodenticides per bird is also provided. Finally, geographical distribution by municipalities is detailed for those animals for which such information was available.

3. Experimental Design, Materials, and Methods

3.1. Sampling Area and Sample Collection

Snake carcasses and raptor livers were received at the Toxicology Unit of the ULPGC. For the sampling areas, only those birds of prey found on the island of Gran Canaria were admitted, representing a large proportion of the island. The admitted snakes came from the four regions of the island where they are established (MA: municipalities of Telde, Santa Brígida, Valsequillo and San Mateo), secondary area (SA: municipality of Gáldar and Agaete), tertiary area (TA: municipalities of San Bartolomé de Tirajana and Mogán) and quaternary area (QA: municipality of Las Palmas de Gran Canaria), collectively covering a significant part of the island's territory [9–11].

Only snakes in good states of conservation, allowing for the correct extraction of the livers, and correctly stored raptor livers were admitted to the study. The snake series comprised 360 individuals captured in 2021-2022, and the raptor series included 110 raptors of six different species: *Tyto alba* ($n = 10$), *Asio otus* ($n = 36$), *Falco tinnunculus* ($n = 39$), *Accipiter nisus* ($n = 10$), *Falco pelegrinoides* ($n = 1$) and *Buteo buteo insularum* ($n = 14$) found between 2020-2022. The raptor livers analyzed came from carcasses found by environmental officers in the wild or from recovery centers where they had been euthanized or had died after admission. These raptors were subsequently necropsied at the Department of Veterinary Pathology at the University of Las Palmas. The snakes came from captures made during the eradication campaign on the island of Gran Canaria and were subsequently euthanized by the entity in charge of this campaign. The carcasses were kept at -20 ºC until necropsy at the toxicology service facilities and the extracted livers were kept at the same temperature until analysis.

3.2. Standards and Elements

Certified AR standards and procedural-internal standard (P-IS, (±) – Warfarin -d5) of maximum purity (between 93.1% and 99.8% from Honeywell, Morristown, NJ, USA) were used to determine 10 analytes of interest: warfarin, diphacinone, cholorophacinone, coumachlor, coumatetralyl, brodifacoum, bromadiolone, difethialone, difenacoum and flocoumafen. All the solvents employed were of the highest purity available (ACN and MeOH >99,9%; FA 98%). The method employed consisted of an extraction by QuEChERS method previously validated [12–14], followed by a LC-MS/MS analysis using Agilent 1290 UHPLC (Agilent Technologies, Palo Alto, USA) coupled to an Agilent 6460 triple-quadrupole mass spectrometer.

3.3. Sample Preparation and Instrumental Analysis

Livers taken from necropsies were stored at -20 °C until analysis. 1 gram of liver representative of the whole organ was taken for extraction using the method already described by our team [12,14] In the same way, Quality Control samples (prepared at 2 ng/g), blanks and the ten calibration points of the curve (ranging from 0.195–100 ng/g) were prepared in chicken liver matrix. Before extraction, all samples including QCs, blanks and curve were spiked with P-IS. Finally, all concentrations were presented in wet weight basis. The quantitative analyses were performed by an Agilent 1290 UHPLC liquid chromatograph coupled with an Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, Palo Alto, USA).

Limitations

The collection of data on raptors has some limitations compared to the data collected for the snake series. In the case of these birds, as they were carcasses found in the wild or animals treated in recovery centers by external organizations, we did not have biometric data of the specimens or some geographical data, nor the exact freshness status of the cadaver. However, only livers from carcasses in good condition, not in an advanced state of decomposition, were admitted.

Ethics Statements

The authors had read and follow the ethical requirements established for the publication. No animals were euthanized for the purpose of the article. Specimens for the study were captured within the framework of a governmental eradication plan for the invasive exotic reptile and euthanized by a veterinarian following the procedure outlined in the aforementioned eradication plan [15]. Prior to their final disposal, the carcasses were donated to SERTOX for biometric study and liver extraction for chemical analysis. Similarly, raptor livers were provided by the government for toxicity analysis as part of the Poisoning Control and Prevention Strategy in the Canary Islands [8]. In accordance with RD53/2013, which establishes the basic standards applicable to the protection of animals used in experimentation and other scientific purposes, including teaching [16], additional approval from the animal experimentation ethics committee is not required.

Data Availability

Table 1. Snakes data: concentration of anticoagulant rodenticides detected in liver (ng/g), total number of rodenticides, biometric, geographical and necropsy findings). (Original data) (Mendeley Data).

Table 2. Raptors data: concentration of anticoagulant rodenticides detected in liver (ng/g), total number of rodenticides per animal and geographical information. (Original data) (Mendeley Data).

CRediT Author Statement

Beatriz Martín-Cruz: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft; **Cristian Rial-Berriel:** Validation, Methodology, Investigation; **Andrea Acosta-Dacal:** Validation, Methodology, Investigation; **Ramón Gallo-Barneto:** Investigation; **Miguel Ángel Cabrera-Pérez:** Investigation; **Octavio P. Luzardo:** Conceptualization, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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Declaration of Competing Interest

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

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