Science of the Total Environment 485–486 (2014) 371–376

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

# Assessment of anticoagulant rodenticide exposure in six raptor species from the Canary Islands (Spain)

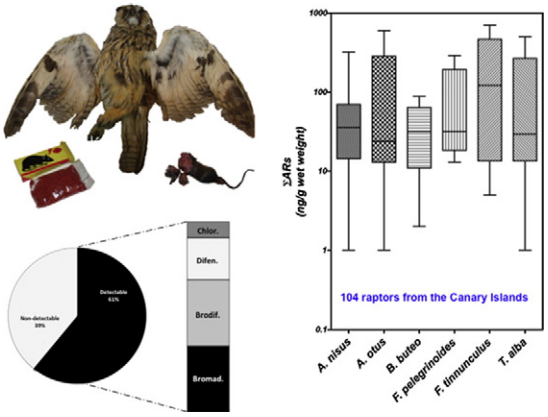
Norberto Ruiz-Suárez, Luis A. Henríquez-Hernández, Pilar F. Valerón, Luis D. Boada, Manuel Zumbado, María Camacho, Maira Almeida-González, Octavio P. Luzardo *

*Unidad de Toxicología, Departamento de Ciencias Clínicas, Facultad de Veterinaria/Facultad de Ciencias de la Salud, Universidad de Las Palmas de Gran Canaria, Apartado de correos 550, 35080 Las Palmas de Gran Canaria, Spain*

## HIGHLIGHTS

- Monitoring of seven anticoagulant rodenticides in six species of birds of prey
- 35% of raptors exceeded the toxicity threshold.
- Higher levels in nocturnal and mammal-eater birds of prey
- High levels in birds of prey that feed on other birds

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 25 February 2014
Received in revised form 19 March 2014
Accepted 20 March 2014
Available online 16 April 2014

Editor: D. Barcelo

*Keywords:*
Exposure assessment
Anticoagulant rodenticides
Raptors
Wildlife
Brodifacoum
Bromadiolone

## ABSTRACT

Anticoagulant rodenticides are highly toxic compounds that are widely used for pest control of rodents, but that also may threaten the wildlife's health. This work aimed to assess the exposure to first- and second-generation anticoagulant rodenticides (ARs) in six birds of prey species from the Canary Islands (Spain). The concentrations of seven widely used ARs were determined by LC–MS/MS in 104 liver samples of six species of birds of prey (*Buteo buteo*, *Accipiter nisus*, *Falco pelegrinoides*, *Falco tinnunculus*, *Asio otus*, and *Tyto alba*). We determined that 61% of the livers had detectable residues of at least one AR. The most frequently detected AR was bromadiolone, which was detected in 60.3% of the positive cases. The detection frequencies of these compounds varied widely, depending on the species. More than 75% of the *A. nisus*, *T. alba*, and *A. otus* individuals had detectable rodenticide residues in the liver. However, *F. tinnunculus* exhibited the highest concentrations of AR, with median values above 100 ng/g w.w. We did not detect first-generation ARs in any of the samples. When grouped, nocturnal species exhibited higher AR concentrations than diurnal species ($P < 0.001$). The residue levels were higher among small mammal-eaters than bird-eaters ($P < 0.01$). While most animals exhibited no macroscopic signs of coagulation disorders, approximately 35% exceeded the threshold levels of toxicity, which suggests that these compounds could weaken these animals in their natural environment. In conclusion, the control of rodent populations by ARs suggests that these compounds will enter the food chain and thus threaten the vulnerable

* Corresponding author at: Toxicology Unit, Department of Clinical Sciences, University of Las Palmas de Gran Canaria, Plaza Dr. Pasteur s/n, 35016 Las Palmas de Gran Canaria, Spain. Tel.: +34 928 451 424; fax: +34 928 451 461.
*E-mail address:* operez@dcc.ulpgc.es (O.P. Luzardo).

http://dx.doi.org/10.1016/j.scitotenv.2014.03.094

populations of raptors on the Canary Islands. Our findings require authorities to ban or strictly control the use of these rodenticides in the natural environment for the conservation of raptors and other predatory species.


## 1. Introduction

In the agricultural sector, rodent populations remain one of the primary causes of economic losses in crops not only prior to harvesting but also during storage (Colazo, 1997). Public health authorities also target rodent populations because these animals can transmit zoonotic diseases, such as leptospirosis and plague (Bharti et al., 2003; Collins et al., 1996; Schelotto et al., 2012).

The most preferred and widely used method for rodent population control is the use of anticoagulant rodenticides (ARs), which are chemical products that interfere with normal blood clotting and cause death by inducing diffuse hemorrhages. The first rodenticide anticoagulants started being used in the 1940s, and these chemicals are currently referred to as the first-generation anticoagulants. Because of widespread use and the continuous exposure to these products, resistance to first-generation ARs developed in rodents. This motivated the development of new chemical formulas, and second generation rodenticides (SGARs) appeared in the market in the 1970s. These new chemicals include bromadiolone, brodifacoum, difenacoum, flocoumafen, chlorophacinone, and diphacinone (Murphy, 2007; Pelfrène, 2010). The SGARs are much more powerful and persistent than the first-generation ARs and are considered toxic after a single dose (Pelfrène, 2010). The primary mechanism of toxicity for these substances is the inhibition of vitamin K epoxide reductase. This enzymatic inhibition blocks vitamin K regeneration, and as a result, the vitamin K-dependent coagulation factors II, VII, IX and X are incorrectly synthesized and do not bear the post-translational carboxylation required for activation. This impairs normal blood coagulation and predisposes the animal to death due to bleeding (Murphy, 2007; Pelfrène, 2010).

In the European Union (EU), these products are freely sold and distributed. Even more, governmental organizations encourage their use and finance the purchase of these products to farmers and ranchers. This situation leads to an extensive use of these products by unqualified personnel that may apply the rodenticides directly to open spaces. This has been reported as a usual practice and facilitates free access to these chemicals for many animals (SEO/Birdlife, 2012). It should also be noted that after rodents have consumed a lethal dose of ARs, they do not get sick or die instantly but do so over the course of several days (generally 2 to 4 days), experiencing a gradual change in their habits that can include erratic behavior or spending more time in open spaces; thus, they become easy prey for raptors (Cox and Smith, 1992; Stansley et al., 2013). During the period when rodents feed on the baits, they can consume approximately 8–10 times the LD50 of the products most commonly used in rodent control campaigns (Stansley et al., 2013). All of these factors lead to AR exposure in many non-target species, and this has been documented for various raptor species worldwide (Albert et al., 2010; Dowding et al., 2010; Elmeros et al., 2011; Guitart et al., 2010; Hughes et al., 2013; Lambert et al., 2007; Stansley et al., 2013; Stone et al., 2003). In some cases, raptors feed on the rodents against which these substances are used, but some species also feed on granivorous birds that sometimes have accidentally ingested cereal baits (Sanchez-Barbudo et al., 2012). As a result, several studies confirm the presence of AR residues in the tissues of raptors (Albert et al., 2010; Hughes et al., 2013; Rattner et al., 2011, 2012; Sanchez-Barbudo et al., 2012; Thomas et al., 2011; Walker et al., 2008), and it appears that in many cases, this exposure leads the birds to a secondary poisoning that can cause them to weaken or die (Hughes et al., 2013; Sanchez-Barbudo et al., 2012; Stone et al., 1999; Thomas et al., 2011).

Due to the relative isolation and climate of the Canary Islands, the flora and fauna of the islands are completely unique from those of the European and African continents. On this archipelago, many endemic species and subspecies are found in areas of high ecological value. There are 7 species of diurnal birds of prey and 2 nocturnal nesting birds of prey on the Canary Islands. Four of these are subspecies that are endemic to the Canary Islands, and two other species are endemic to the Macaronesian region (which includes the Azores, Madeira, Canaries and Cape Verde regions) (Lorenzo et al., 2012). Several anthropogenic circumstances have provoked a population decline of some of these species in recent decades which, along with their characteristic slow reproductive rates, are threatening their survival: power lines, malicious or accidental poisonings, and high tourist pressure on the territory (the archipelago has four national parks that receive 5.5 million visitors a year (MAGRAMA, 2013)), as well as the extensive past and present uses of pesticides in agriculture, among others. In particular, the rodenticides have been widely used in these islands in recent years because the local public administration has provided these products to the farmers for free (BOP, 2011). Although it has been demonstrated that the exposure of raptors to these chemicals is related to potential risks to their health and that this exposure could be threatening the raptor populations of these islands, there are no data documenting the rodenticide exposure for the populations of birds of prey from this region. To address this gap, we have designed this study with the aim of assessing first- and second-generation AR exposures in six species of raptors on the Canary Islands to determine if raptor species of the archipelago are exposed to toxic quantities of these substances, which could potentially represent a threat to their conservation.

## 2. Material and methods

### 2.1. Sample collection and ethics statement

Liver samples were obtained from necropsies of 104 birds of prey from 6 species that were admitted to the Wildlife Recovery Centers (WRCs) of Tafira (Gran Canaria, Spain) and La Tahonilla (Tenerife, Spain) between October 2009 and December 2012. The series included 9 common buzzards (*Buteo buteo*), 14 European sparrowhawks (*Accipiter nisus*), 16 Barbary falcons (*Falco pelegrinoides*), 21 common kestrels (*Falco tinnunculus*), 23 long-eared owls (*Asio otus*), and 21 barn owls (*Tyto alba*). The birds died naturally or were euthanized within one week of admission. Dead animals were kept frozen until the moment of the necropsy. No animal was killed for the purposes of this study. The main cause of death was determined by examining the birds macroscopically at the WRCs, and, when necessary, radiological or toxicological analyses were performed. The causes of death for all of the animals that were included in this study consisted of different types of trauma. The whole livers, the primary organ for the accumulation of rodenticides (Dowding et al., 2010), were excised and stored at −20 °C until sample preparation. Part of the liver samples used in this study was retained from a previous study of anthropogenic persistent pollutant exposure in raptors (Luzardo et al., 2014a).

### 2.2. Chemicals and reagents

Dichloromethane, hexane, ethyl acetate and cyclohexane were of the highest purity available (>99.9%) and were purchased from Fisher Scientific (Leicestershire, United Kingdom). Ultrapure (UP) water was produced from a Milli-Q Gradient A10 (Millipore, Molsheim, France). Diatomaceous earth was purchased from Sigma-Aldrich (St. Louis, USA). Bio-Beads SX-3 was purchased

from BioRad Laboratories (Hercules, USA). Standards for ARs (warfarin, coumatetralyl, bromadiolone, brodifacoum, difenacoum, difethialone, and chlorophacinone) and an internal standard (IS, (±)-Warfarin-$d_5$) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). All standards were pure compounds (purity from 98% to 99.5%). Stock solutions of each compound at 1 mg/mL were prepared in cyclohexane and stored at −20 °C. Diluted solutions from 0.1 ng/mL to 500 ng/mL were used for calibration curves.

### 2.3. Sample preparation and chemical analysis

The procedures for AR extraction and chemical analysis have been previously validated in our laboratory (Luzardo et al., 2014b). Briefly, 2 g of liver was homogenized in 5 mL of ultrapure water. Ten microliters of the IS (50 μg/mL in acetone) was added to each vial to reach a final concentration of 500 ng/mL. Ten grams of diatomaceous earth was added to absorb the moisture in the sample, 10 mL of dichloromethane/ethyl acetate/acetone (50/30/20) was added, and the sample was left in agitation for 5 min. The sample was centrifuged (4000 *g*), and the supernatant was concentrated, redissolved in acetonitrile, filtrated, and subjected to a purification step by freezing centrifugation. The resulting supernatant was used for the quantification of ARs.

The separation and identification of extracted analytes were performed by LC–MS/MS using a Thermo LC Accela Ultra instrument (Thermo Fisher Scientific Inc., USA) equipped with an analytic Accucore C18 column (2.6 μm, 150×3 mm; Thermo Fisher Scientific Inc., USA) as the stationary phase. The mobile phases were (A) ultrapure water as the aqueous phase and (B) methanol (HPLC–MS grade) as the organic phase. The flow was set at 800 μL/min. The injection volume was 25 μL, and the total run time was 5 min. The gradient was programmed as follows: 0–1 min: 50% A; 1–1.5 min: 50% A → 5% A; 1.5–3.5 min: 5% A; 3.5–3.7 min: 5% A → 50% A; 3.7–10 min: 50% A (for equilibration). The ARs were detected using a TSQ Quantum Max QqQ mass spectrometer equipped with the H-ESI II heated electrospray ionization source (Thermo Fisher Scientific Inc., USA). The mass spectrometer and the ionization source were programmed according to the following parameters: skimmer offset (4 V), sheath gas pressure (10 arbitrary units, a.u.), ion sweep gas pressure (8 a.u.), capillary temperature (250 °C), spray voltage (3500 V), and vaporization temperature (200 °C). The spectrometer was programmed in negative ionization mode. We initially determined the retention times of each compound in the full scan mode (range: m/z 45–500), and then we constructed a time-selected reaction monitoring (SRM) method by directly infusing pure standard methanolic solutions into the source. The gas in the collision cell was argon (99.99%) at a pressure of 0.25 Pa. The mass spectrometry settings, validation parameters, and toxicity values (Mineau et al., 2001) for the analytes included in the method are shown in Table 1.

### 2.4. Statistical analyses

Database management and statistical analysis were performed with the PASW Statistics v 17.0 statistical software (SPSS Inc., Chicago, IL, USA). Using non-parametric statistics as data lacked normality and homogeneity of variances, mean rodenticide concentration was compared between raptor species using Kruskal–Wallis and Mann–Whitney U tests for general and pair-wise comparisons, respectively. The categorical variables were presented as percentages of detection, and differences in percentages were evaluated using the Chi-square test. $P$ values $<0.05$ (two-tailed) were considered to be statistically significant.

## 3. Results and discussion

Of the 104 liver samples analyzed, 61% had detectable residues of at least one AR (Fig. 1A), and 36.5% contained more than one compound (six livers had detectable residues of three ARs and one liver had residues of four different compounds). None of the two first-generation ARs included in the study (warfarin and coumatetralyl), nor the diphacinone and difethialone, were detected in any of the samples (Fig. 1A). Bromadiolone, brodifacoum, difenacoum, and chlorophacinone were commonly detected in all six species (Table 2). The most frequently detected compound was bromadiolone (60.3% of positive cases). Conversely, the least common substance was chlorophacinone (14.7% of the positive cases). However, as shown in Table 2, the detection frequencies of these compounds varied widely depending on the species. Considering the sum of all residues, more than 75% of the individuals belonging to *A. nisus*, *T. alba*, and *A. otus* showed rodenticide residues in the liver, whereas the detection frequencies were lower in *B. buteo* and *F. pelegrinoides* (approximately 30%, Table 2). The frequencies of AR occurrence in the Canary Islands raptors were similar to those previously reported for *T. alba*, *B. buteo*, *A. nisus*, and *F. tinnunculus* in Britain (Dowding et al., 2010; Hughes et al., 2013) and for *T. alba*, *B. buteo*, and *F. tinnunculus* in France (Lambert et al., 2007). The occurrence in the Canary Islands raptors was also similar to the detection frequencies of rodenticide residues in other species of raptors from the European and American continents (Albert et al., 2010; Stansley et al., 2013; Thomas et al., 2011; Walker et al., 2008). However, it should be noted that the pattern of AR detection in raptors from the Canary Islands was more similar to that found in European countries other than Spain (mainland), where the most frequently detected compound was chlorophacinone (70%), while brodifacoum and difenacoum were only marginally detected (Sanchez-Barbudo et al., 2012). In our

**Table 1**
Toxicities of the pesticides detected by LC–MS/MS, method settings and results from recovery experiments.

| | | | | Mass spectrometry settings | | | | | | | Validation parameters | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | Compound | Toxicity ($LD_{50}$, mg/kg)[a] in birds | Log kow[b] | RT (min) | CV (V) | First transition m/z ➔ m/z | CE (V) | Second transition m/z ➔ m/z | CE (V) | IPs | LOD (μg/mL) | LOQ (μg/mL) | Average recovery % (RSD) | IS |
| *LC–MS/MS method 1* | | | | | | | | | | | | | | |
| 1 | Coumatetralyl | 38.3 | 3.46 | 1.57 | 65 | 291.1 ➔ 140.9 | 28 | 291.1 ➔ 247.0 | 22 | 4 | 0.01 | 0.03 | 89.2 (13.6) | 1 |
| 2 | Warfarin | 942.0 | 2.70 | 1.71 | 56 | 307.1 ➔ 116.9 | 39 | 307.1 ➔ 250.0 | 24 | 4 | 0.005 | 0.02 | 92.7 (8.3) | 1 |
| 3 | Chlorophacinone | 430.0 | 5.50 | 1.76 | 123 | 373.1 ➔ 116.0 | 50 | 373.1 ➔ 200.9 | 25 | 4 | 0.01 | 0.03 | 87.9 (12.4) | 1 |
| 4 | Difenacoum | 50.0 | 7.62 | 1.83 | 90 | 443.2 ➔ 134.9 | 36 | 443.2 ➔ 293.0 | 33 | 4 | 0.005 | 0.01 | 91.3 (11.7) | 1 |
| 5 | Brodifacoum | 4.5 | 8.50 | 1.88 | 108 | 521.1 ➔ 135.0 | 44 | 521.1 ➔ 186.9 | 39 | 4 | 0.005 | 0.01 | 97.4 (5.8) | 1 |
| 6 | Bromadiolone | 138 | 7.02 | 2.02 | 96 | 525.1 ➔ 180.9 | 37 | 525.1 ➔ 249.9 | 37 | 4 | 0.005 | 0.01 | 94.3 (8.9) | 1 |
| 7 | Difethialone | 0.9 | 5.17 | 2.08 | 100 | 537.1 ➔ 150.9 | 45 | 537.1 ➔ 370.9 | 36 | 4 | 0.01 | 0.03 | 86.9 (13.4) | 1 |
| *Internal standard* | | | | | | | | | | | | | | |
| IS1 | (±)-Warfarin-$d_5$ | | | 1.71 | 56 | 312.1 ➔ 116.9 | 39 | 312.1 ➔ 250.0 | 24 | 4 | – | | – | – |

RT: retention time (min); CV: cone voltage; CE: collision energy; IPs: identification points; LOD: limit of detection; LOQ: limit of quantification; RSD: relative standard deviation.
[a] Average data from different species. These data have been taken from Mineau et al. (2001), the National Library of Medicine internet resources ChemIDplus (http://chem.sis.nlm.nih.gov/chemidplus/chemidheavy.jsp) and the Hazardous Substances Data Bank (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB).
[b] Octanol–water partition coefficients.

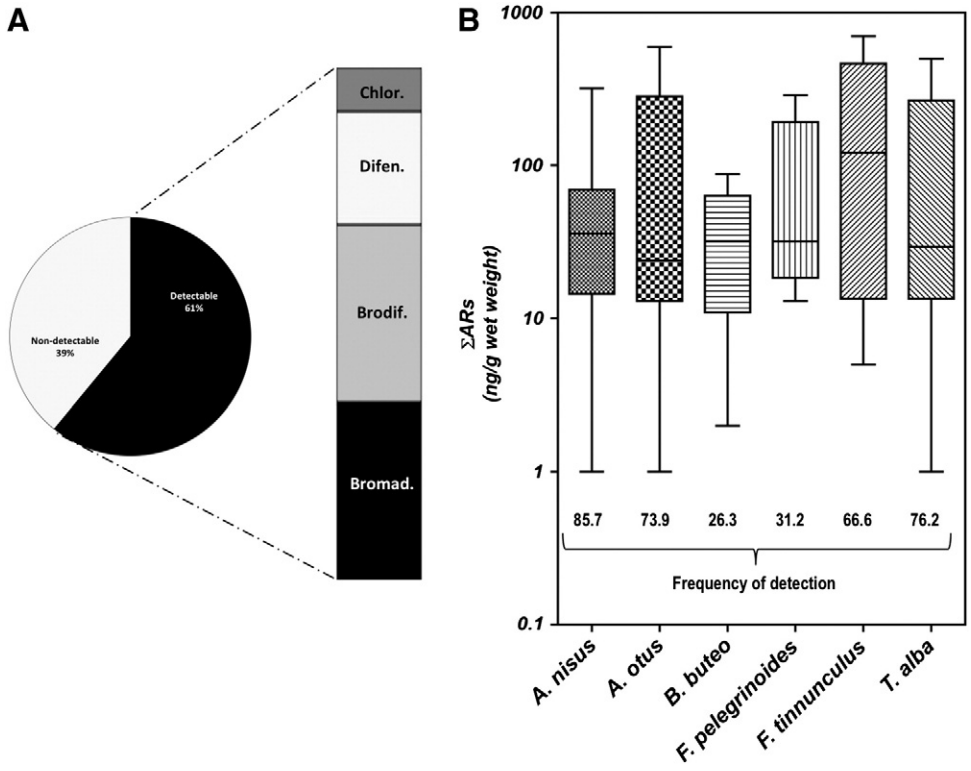



**Fig. 1.** Panel A. The percentages of animals with detectable and undetectable ARs and the distribution of the detected compounds. Panel B. The frequencies of detection and a box plot indicating the levels of ∑ ARs in the six species that were included in the study. The line inside the box represents the median, the bottom and top of the box are the first and third quartiles of the distribution, respectively, and the lines extending vertically from the boxes indicate the variability outside the upper and lower quartiles.

study, when more than one residue was detected, the most frequent combination was bromadiolone + brodifacoum (34.6%), followed by bromadiolone +diphenacoum (19.2%) (data not shown). Taken together, these results suggest that the marketing and use of these products in the Canary Islands are different from the rest of Spain, and our findings are consistent with the active ingredients of the products that are subsidized by the government (BOP, 2011).

Regarding the liver concentrations of ARs, *F. tinnunculus* showed the highest levels, with median values above 100 ng/g w.w. (Fig. 1B). Taken individually, bromadiolone was the substance that achieved the highest concentration in the entire series (geometric mean = 146.3 ng/g w.w., range = 0.2–516.1 ng/g w.w.) (Table 2). Brodifacoum was detected at high concentrations, especially among *F. tinnunculus* individuals (geometric mean = 57.4 ng/g w.w.). These results agree with other published studies showing that difenacoum, bromadiolone, and brodifacoum were the most prevalent substances and occurred in the highest concentrations (Christensen et al., 2012).

Because it has been previously reported that AR exposure levels can be determined by nocturnal or diurnal behavior (Sanchez-Barbudo et al., 2012), we explored the AR contamination profile in the Canary raptors according to their diurnal or nocturnal habits. We observed that the nocturnal species (*A. otus* and *T. alba*, n = 44 samples) showed higher concentrations of the sum of rodenticides (ΣARs) than the diurnal species ($P < 0.001$, Fig. 2A). These results agree with other studies that have shown the highest levels of AR exposure occurring in nocturnal raptors, especially with respect to second generation ARs (Sanchez-Barbudo et al., 2012). We also found that both the frequency of AR detection and the number of residues per animal were higher among nocturnal species (Fig. 2B and C, respectively). It should be highlighted that the percentage of AR detected in both nocturnal and

**Table 2**
Concentration (ng/g wet weight), 95% confidence interval, frequency of detection, and range of anticoagulant rodenticides in the livers of six raptor species from the Canary Islands, Spain.

| Species | ∑ARs[a] | | | Bromadiolone | | Brodifacoum | | Difenacoum | | Chlorophacinone | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean ± SD (range) | Freq. | No. res.[b] | Mean ± SD (95% confide.) | Freq. | Mean ± SD (95% confide.) | Freq. | Mean ± SD (95% confide.) | Freq. | Mean ± SD (95% confide.) | Freq. |
| *Accipiter nisus* (n = 14) | 57.7 ± 88.0 (n.d.–321.9) | 85.7% | 1.6 ± 0.7 | 31.9 ± 22.6 (−16.9–80.9) | 35.7% | 13.2 ± 8.1 (−4.2–112.0) | 35.7% | 3.6 ± 2.0 (n.d.–28.2) | 35.7% | 0.34 ± 0.57 (−0.6 ± 2.1) | 21.4% |
| *Asio otus* (n = 23) | 132.2 ± 177.6 (n.d.–598.2) | 73.9% | 1.6 ± 0.6 | 77.2 ± 29.6 (15.9–138.5) | 39.1% | 15.8 ± 5.4 (4.6–26.9) | 43.5% | 2.8 ± 1.3 (0.2–5.4) | 26.1% | 0.5 ± 0.4 (−0.5–1.5) | 4.3% |
| *Buteo buteo* (n = 9) | 36.8 ± 32.4 (n.d.–88.9) | 26.3% | 1.6 ± 0.6 | 2.6 ± 1.9 (−1.5–6.5) | 10.5% | 4.9 ± 4.1 (−3.7–13.5) | 10.5% | 2.9 ± 2.6 (−2.4–8.3) | 15.8% | 0.3 ± 0.2 (−0.3–0.8) | 5.3% |
| *Falco pelegrinoides* (n = 16) | 91.5 ± 115.9 (n.d. ± 298.8) | 31.2% | 1.2 ± 0.4 | 26.2 ± 18.6 (−13.5–66.0) | 18.8% | 0.8 – 0.8 (−0.9–2.6) | 6.3% | 1.4 ± 1.4 (−1.6–4.5) | 6.3% | 0.1 ± 0.1 (−0.1–0.2) | 6.3% |
| *Falco tinnunculus* (n = 21) | 219.0 ± 237.5 (n.d.–702.7) | 66.6% | 1.7 ± 0.6 | 79.8 ± 34.4 (8.0–151.4) | 42.9% | 57.4 ± 34.6 (−14.7–129.5) | 42.9% | 8.2 ± 6.9 (−6.2–22.6) | 23.8% | 0.6 ± 1.8 (−0.6–1.8) | 4.8% |
| *Tyto alba* (n = 21) | 134.4 ± 163.1 (n.d.–500.1) | 76.2% | 2.0 ± 0.8 | 75.8 ± 23.9 (25.9–125.6) | 61.9% | 12.5 ± 5.9 (0.1–24.9) | 38.1% | 12.6 ± 9.5 (−7.3–32.4) | 33.3% | 1.2 ± 1.0 (−0.9–3.3) | 14.3% |

[a] Sum of all anticoagulant rodenticides.
[b] Mean number of anticoagulant residues per sample.

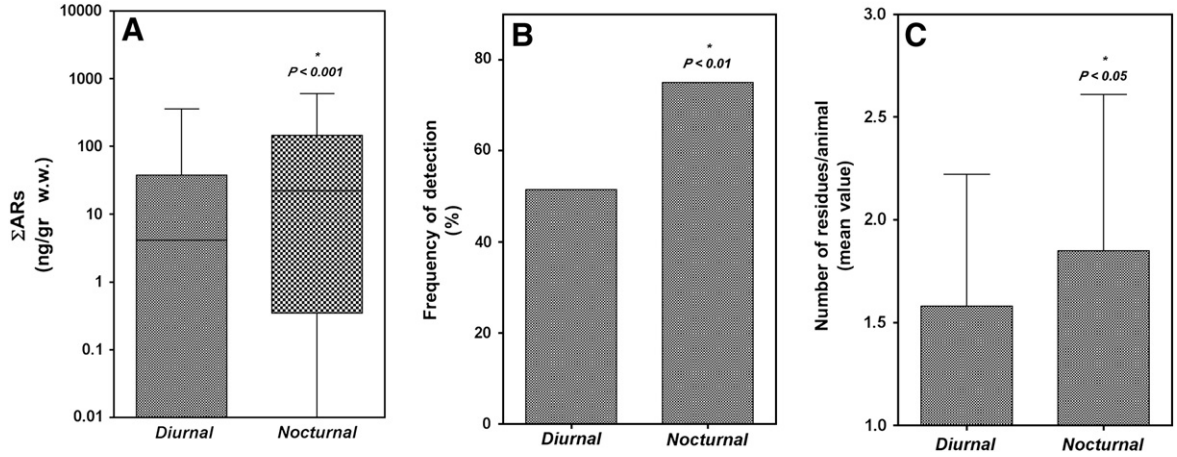



**Fig. 2.** A comparison between raptors grouped according to their diurnal and nocturnal behavior. Panel A. Liver concentration of ∑ARs. The line inside the box represents the median, the bottom and top of the box are the first and third quartiles of the distribution, respectively, and the lines extending vertically from the boxes indicate the variability outside the upper quartiles. Since the first quartile was 0 in all the cases, there are no lines extending outside the lower quartiles. Panel B. Frequencies of detection of ∑ARs. Panel C. Number of residues per animal (mean ± SD).

diurnal raptors from the Canary Islands was higher than that described in mainland Spain (Sanchez-Barbudo et al., 2012). The levels were double in the case of the diurnal raptors.

The profile of AR contamination was also analyzed with respect to the eating habits of the species under study. Notably, when we considered all of the birds that primarily feed on small mammals and reptiles as a group (*A. otus*, *T. alba*, *and B. buteo*), we found a higher median AR contamination level than that of the species whose diet was primarily composed of birds (*F. tinnunculus* > *F. pelegrinoides* > *A. nisus*) ($P < 0.01$, Fig. 3A). No significant differences were observed regarding the frequency of detection or the number of residues per animal detected in these two groups (Fig. 3B and C). Our results indicate that the contamination profile is dependent on the feeding habits of the raptors surveyed in this study. The fact that small mammal-eating birds accumulate AR residues is not a surprising result because ARs are designed to kill small mammals (such as mice and rats), and these small mammals can accumulate considerably high levels of the chemicals in their livers and digestive tract. However, it is remarkable that raptors that feed on other birds have relatively high levels of these contaminants. Surprisingly, it has been previously reported that some of these species, such as *A. nisus*, have similar exposure rates compared to species that prey on rodents (Hughes et al., 2013). Because birds are not the target species of ARs, this observation is an indicator of the penetration achieved by these contaminants in the food chain. Many granivorous birds may actually ingest the anticoagulant baits by cereal impregnation, in which the chemical is adhered to the grain with oil and dyed with a color, usually blue or red, to act as a deterrent to birds and for identification purposes. However, several studies have demonstrated that in spite of the deterrent measures, many granivorous birds feed on these baits and may suffer either a primary poisoning or a subtle but chronic exposure as a result (Borst and Counotte, 2002; Lemus et al., 2011; Sanchez-Barbudo et al., 2012). Thus, raptors that hunt these birds would also be exposed to significant amounts of these compounds. However, the insectivorous birds theoretically would represent a minor source of ARs for the raptors. Nonetheless, it has been shown that some of the invertebrates that are important parts of the diet for insectivorous birds may act as anticoagulant reservoirs because these invertebrates feed on the baits without experiencing adverse effects (Johnston et al., 2005a,b; Spurr and Drew, 1999). Thus, raptors that feed on insectivorous birds may also be exposed to considerable amounts of ARs.

Initially, all of the animals used in this study were diagnosed with trauma (mainly motor vehicle collisions, window collisions, electrocution, flight collision, and falling out of the nest). After the necropsy, we only had direct evidence of AR poisoning as the cause of the death in one case (diffuse hemorrhages evidencing a lack of blood clotting factors during the necropsy in one individual of *A. otus*). This finding was consistent with other studies that determined the AR levels in raptors:

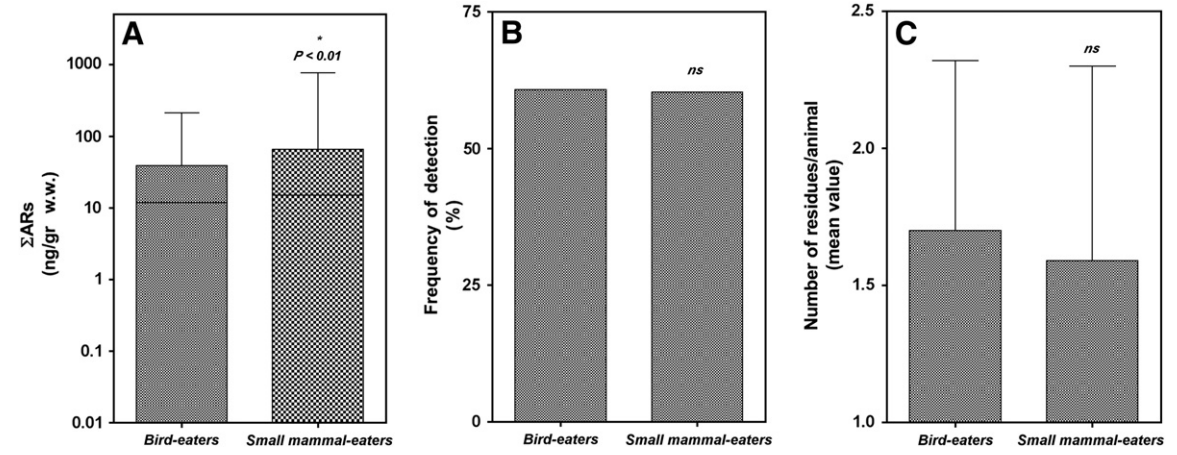



**Fig. 3.** A comparison between raptors grouped according to their feeding habits. Panel A. Liver concentration of ∑ARs. The line inside the box represents the median, the bottom and top of the box are the first and third quartiles of the distribution, respectively, and the lines extending vertically from the boxes indicate the variability outside the upper quartiles. Since the first quartile was 0 in all the cases, there are no lines extending outside the lower quartiles. Panel B. Frequencies of detection of ∑ARs. Panel C. Number of residues per animal (mean ± SD).

no direct relation between necropsy findings and ARs was found in most cases (Sanchez-Barbudo et al., 2012; Stansley et al., 2013). However, the lack of direct evidence for blood clotting disorders, which are the most obvious effects of these compounds, should not automatically be interpreted as a lack of toxicity in these animals. For example, chronic exposure to low levels of oral anticoagulants has been associated with reduced bone density and a higher frequency of bone fractures and osteoporosis due to a deficiency in the synthesis of osteocalcin, which is not carboxylated in the presence of these compounds (Pearson, 2007). In fact, low liver toxicity thresholds (0.1–0.2 mg/kg) have been established for these compounds in some species of raptors (Stansley et al., 2013; Thomas et al., 2011), which are the only available data that can be used for comparisons to date. It is remarkable that these thresholds were exceeded in a large percentage of the animals used in our study (34.8%). Moreover, it should be noted that a wide variation in AR sensitivity exists between different species (Erickson, 2004; Thomas et al., 2011). Although the diagnostic interpretation of liver AR residues in the absence of other clinical findings is problematic, the possibility exists that a chronic exposure to these compounds may be causing a change in the health status of these animals, which would predispose them to weakness, sickness and accidents (Albert et al., 2010).

In conclusion, our study shows that the use of ARs to control rodent populations in the environment implies that these products will enter the food chain, thus representing a relevant threat for wildlife, especially raptors. It is necessary for authorities to increase the management and regulation of these substances. Without addressing the problem of inadvertent rodenticide exposure in the Canary Islands wildlife, any wildlife recovery or reintroduction program may not be undertaken successfully.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgments

The authors would like to thank the Canary Islands Government (Dirección General de Protección de la Naturaleza, Gobierno de Canarias (Nº 104/2013) for their financial support. The authors are also thankful to the veterinarians of the Wildlife Recovery Centers of Gran Canaria (Pascual Calabuig and Dolores Estévez) and Tenerife (Santiago Mayans) for their collaboration in the necropsies and in the sampling of the animals, and also to Mrs. María de los Reyes Suárez Hanna for her technical assistance.

## References

Albert CA, Wilson LK, Mineau P, Trudeau S, Elliott JE. Anticoagulant rodenticides in three owl species from Western Canada, 1988–2003. Arch Environ Contam Toxicol 2010; 58:451–9.
Bharti ARNJ, Ricaldi JN, Matthias MA, Diaz MM, Lovett MA, Levett PN, et al. Leptospirosis: a zoonotic disease of global importance. Lancet Infect Dis 2003;3:757–71.
BOP. Boletin Oficial de la Provincia de Las Palmas — 25 de febrero. Convocatoria para el año 2011 de entrega de productos raticidas por la Consejería de vivienda y arquitectura, agricultura, ganadería y pesca, y aguas del Cabildo de Gran Canaria; 2011.
Borst GH, Counotte GH. Shortfalls using second-generation anticoagulant rodenticides. J Zoo Wildl Med 2002;33:85.
Christensen TK, Lassen P, Elmeros M. High exposure rates of anticoagulant rodenticides in predatory bird species in intensively managed landscapes in Denmark. Arch Environ Contam Toxicol 2012;63:437–44.
Colazo RCJ. Los roedores dañinos: algunos aspectos del control químico y bacteriológico. Rev Invest Pecuarias 1997;8:1–9.
Collins FM. Pasteurella, Yersinia, and Francisella. In: Baron S, editor. Medical Microbiology, Vol. 4. Galveston, TX: The University of Texas Medical Branch at Galveston; 1996. p. 379.
Cox P, Smith RH. Rodenticide ecotoxicology pre-lethal effects of anticoagulants on rat behavior. Fifteenth Vertebrate Pest Conference, Nebraska, USA; 1992.
Dowding CV, Shore RF, Worgan A, Baker PJ, Harris S. Accumulation of anticoagulant rodenticides in a non-target insectivore, the European hedgehog (*Erinaceus europaeus*). Environ Pollut 2010;158:161–6.
Elmeros M, Christensen TK, Lassen P. Concentrations of anticoagulant rodenticides in stoats *Mustela erminea* and weasels *Mustela nivalis* from Denmark. Sci Total Environ 2011;409:2373–8.
Erickson W, Urban D. Potential risks of nine rodenticides to birds and nontarget mammals: a comparative approach. Environmental Protection Agency Office of Prevention. Washington, D.C., USA: Pesticides and Toxic Substances; 2004.
Guitart R, Sachana M, Caloni F, Croubels S, Vandenbroucke V, Berny P. Animal poisoning in Europe. Part 3: wildlife. Vet J 2010;183:260–5.
Hughes J, Sharp E, Taylor MJ, Melton L, Hartley G. Monitoring agricultural rodenticide use and secondary exposure of raptors in Scotland. Ecotoxicology 2013;22:974–84.
Johnston JJ, Holmes MJ, Hart A, Kohler DJ, Stahl RS. Probabilistic model for estimating field mortality of target and non-target bird populations when simultaneously exposed to avicide bait. Pest Manag Sci 2005a;61:649–59.
Johnston JJ, Pitt WC, Sugihara RT, Eisemann JD, Primus TM, Holmes MJ, et al. Probabilistic risk assessment for snails, slugs, and endangered honeycreepers in diphacinone rodenticide baited areas on Hawaii, USA. Environ Toxicol Chem 2005b;24:1557–67.
Lambert O, Pouliquen H, Larhantec M, Thorin C, L'Hostis M. Exposure of raptors and waterbirds to anticoagulant rodenticides (difenacoum, bromadiolone, coumatetralyl, coumafen, brodifacoum): epidemiological survey in Loire Atlantique (France). Bull Environ Contam Toxicol 2007;79:91–4.
Lemus JA, Bravo C, Garcia-Montijano M, Palacin C, Ponce C, Magana M, et al. Side effects of rodent control on non-target species: rodenticides increase parasite and pathogen burden in great bustards. Sci Total Environ 2011;409:4729–34.
Lorenzo JA, Alonso-Alonso JR, Barone-Tosco R, González-González C. Atlas de las aves nidificantes en el archipiélago canario. Tenerife. Islas Canarias: España: Ministerio de Medioambiente. Gobierno de España; 2012.
Luzardo OP, Ruiz-Suarez N, Henriquez-Hernandez LA, Valeron PF, Camacho M, Zumbado M, et al. Assessment of the exposure to organochlorine pesticides, PCBs and PAHs in six species of predatory birds of the Canary Islands, Spain. Sci Total Environ 2014a;472:146–53.
Luzardo OP, Ruiz-Suarez N, Valeron PF, Camacho M, Zumbado M, Henriquez-Hernandez LA, et al. Methodology for the identification of 117 pesticides commonly involved in the poisoning of wildlife using GC–MS–MS and LC–MS–MS. J Anal Toxicol 2014b;38(3):155–63.
MAGRAMA. Memoria 2011 - Red de Parques Nacionales. Ministerio de Agricultura, Alimentación y Medio Ambiente. Gobierno de España; 2013.
Mineau P, Baril A, Collins BT, Duffe J, Joerman G, Luttik R. Pesticide acute toxicity reference values for birds. Rev Environ Contam Toxicol 2001;170:13–74.
Murphy MJ. Anticoagulant rodenticides. In: Gupta RC, editor. Veterinary toxicology. New York: Academic Press; 2007.
Pearson DA. Bone health and osteoporosis: the role of vitamin K and potential antagonism by anticoagulants. Nutr Clin Pract 2007;22:517–44.
Pelfrène AF. Rodenticides. In: Krieger R, editor. Haye's handbook of pesticide toxicology. Elsevier Inc.; 2010.
Rattner BA, Horak KE, Lazarus RS, Eisenreich KM, Meteyer CU, Volker SF, et al. Assessment of toxicity and potential risk of the anticoagulant rodenticide diphacinone using Eastern screech-owls (*Megascops asio*). Ecotoxicology 2012;21:832–46.
Rattner BA, Horak KE, Warner SE, Day DD, Meteyer CU, Volker SF, et al. Acute toxicity, histopathology, and coagulopathy in American kestrels (*Falco sparverius*) following administration of the rodenticide diphacinone. Environ Toxicol Chem 2011;30: 1213–22.
Sanchez-Barbudo IS, Camarero PR, Mateo R. Primary and secondary poisoning by anticoagulant rodenticides of non-target animals in Spain. Sci Total Environ 2012;420:280–8.
Schelotto F, Hernandez E, Gonzalez S, Del Monte A, Ifran S, Flores K, et al. A ten-year follow-up of human leptospirosis in Uruguay: an unresolved health problem. Rev Inst Med Trop Sao Paulo 2012;54:69–75.
SEO/Birdlife. Correcto uso de productos rodenticidas en espacios abiertos. Journal 2012. Available at http://www.venenono.org/wp-content/uploads/2012/04/manual_manejo_raticidas.pdf, 2012.
Spurr EB, Drew KW. Invertebrates feeding on baits used for vertebrate pest control in New Zealand. N Z J Ecol 1999;23:167–73.
Stansley W, Cummings M, Vudathala D, Murphy LA. Anticoagulant rodenticides in red-tailed hawks, *Buteo jamaicensis*, and great horned owls, *Bubo virginianus*, from New Jersey, USA, 2008–2010. Bull Environ Contam Toxicol 2014;92(1):6–9.
Stone WB, Okoniewski JC, Stedelin JR. Poisoning of wildlife with anticoagulant rodenticides in New York. J Wildl Dis 1999;35:187–93.
Stone WB, Okoniewski JC, Stedelin JR. Anticoagulant rodenticides and raptors: recent findings from New York, 1998–2001. Bull Environ Contam Toxicol 2003;70:34–40.
Thomas PJ, Mineau P, Shore RF, Champoux L, Martin PA, Wilson LK, et al. Second generation anticoagulant rodenticides in predatory birds: probabilistic characterisation of toxic liver concentrations and implications for predatory bird populations in Canada. Environ Int 2011;37:914–20.
Walker LA, Turk A, Long SM, Wienburg CL, Best J, Shore RF. Second generation anticoagulant rodenticides in tawny owls (*Strix aluco*) from Great Britain. Sci Total Environ 2008;392:93–8.