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# High Exposure Rates of Anticoagulant Rodenticides in Predatory Bird Species in Intensively Managed Landscapes in Denmark

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**Abstract** The extensive use of anticoagulant rodenticides (ARs) for rodent control has led to widespread secondary exposure in nontarget predatory wildlife species. We investigated exposure rates and concentrations of five ARs in liver samples from five raptors and six owls from Denmark. A total of 430 birds were analysed. ARs were detected in 84–100 % of individual birds within each species. Multiple AR exposure was detected in 73 % of all birds. Average number of substances detected in individual birds was 2.2 with no differences between owls and raptors. Difenacoum, bromadiolone, and brodifacoum were the most prevalent substances and occurred in the highest concentrations. Second-generation ARs made up 96 % of the summed AR burden. Among the six core species (sample size >30), summed AR concentrations were lower in rough-legged buzzard (*Buteo lagopus*) and long-eared owl (*Asio otus*) than in barn owl (*Tyto alba*), buzzard (*B. buteo*), kestrel (*Falco tinnunculus*), and tawny owl (*Strix aluco*). There was a strong tendency for seasonal variations in the summed AR concentration with levels being lowest during autumn, which is probably related to an influx of less-exposed migrating birds from northern Scandinavia during autumn. High hepatic AR residue concentrations (>100 ng/g wet weight), which have been associated with symptoms of rodenticide poisoning and increased mortality, were recorded high frequencies (12.9–37.4 %) in five of the six core species. The results suggest that the present use of ARs in Denmark, at least locally, may have adverse effects on reproduction and, ultimately, population status in some raptors and owls.

Anticoagulant rodenticides (ARs) are widely used to control rodent populations (World Health Organization 1995). ARs are vitamin K antagonists that disrupt normal blood-clotting mechanisms causing lethal haemorrhage (Erickson and Urban 2004; Vandenbrough et al. 2008). Resistance in rodents to the first types of ARs led to the development of more toxic and persistent second-generation ARs in the 1970s and 1980s (Erickson and Urban 2004; Laakso et al. 2010). These second-generation ARs pose a greater risk to nontarget species because accumulation with repeated sublethal exposures may lead to secondary poisoning of predators feeding on poisoned rodents (Eason et al. 2002; Fisher et al. 2003; Hoare and Hare 2006). The toxic action of ARs is slow, and rodents may not die for several days after consuming a lethal dose. Furthermore, ARs can be found in rodents for several months after a rodent-control campaign (Murphy et al. 1998; Giraudoux et al. 2006).

The use of ARs to control rodents have led to multiple cases of documented exposures and the poisoning of many nontarget wildlife species (e.g., Berny et al. 1997; de Snoo et al. 1999; Dowding et al. 2010; Elmeros et al. 2011), including raptors and owls (Mendenhall and Pank 1980; Merson and Byers 1984; Stone et al. 1999, 2003; Shore et al. 2000, 2001; Walker et al. 2008a, b; Albert et al. 2010; Walker et al. 2010a, b). Fatal incidences of secondary AR poisoning of predatory birds have been documented in both free-ranging birds and in laboratory tests (Grolleau et al. 1989; Newton et al. 1999). From these studies, a hepatic

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AR concentration of 100–200 ng/g wet weight (ww) has been suggested as level of concern, and concentrations >200 ng/g ww as are considered critical for predatory birds. The sensitivity to ARs is known to vary between species and individuals, and a more recent study has suggested that toxic effects in predatory birds may occur at lower AR concentrations than previously recognized (Thomas et al. 2011).

In Denmark, municipalities and landowners are legally obligated to control rats (*Rattus* sp.). Rat infestations are primarily controlled with the most toxic and persistent second-generation ARs because resistance to first-generation and the least toxic second-generations AR in rats is widespread (Lodal 2010). ARs are used for rat control in urban areas and in rural areas in and near buildings as well as for chemical control of rats away from buildings at, e.g., game feeding stations and fish farms (Miljøstyrelsen 2005; Lodal 2010). Furthermore, ARs are the preferred method to control mice and voles in and near buildings and in agriculture, orchards, and forestry. Products based on second-generation ARs are also available to private householders and landowners for mice and vole control.

For rodent control operations outside buildings, the authorities' guidelines and the product labels state that AR baits should be placed in bait boxes or in rodent holts to minimize the risk of exposure to nontarget wildlife species, humans, and livestock (Miljøstyrelsen 2005). The guidelines for commercial rat-control operators also stresses that the most toxic second-generation ARs should only be used when control with less toxic ARs has failed. During the past two decades, the total sale of ARs has decreased, but sales of second-generation AR have remained constant (Miljøstyrelsen 2011). The temporal and spatial patterns of AR use and application methods by commercial and private operators are not monitored. Incidents of suspected AR poisoning of nontarget birds have been recorded (Anonymous 2007), but the exposure of nontarget wildlife are likely to be more widespread than incident reports suggest (Erickson and Urban 2004; Laakso et al. 2010).

The aim of the present study was to examine AR exposure rates and residue concentrations in the most common nontarget species in the predatory bird guild in Denmark in relation to the species' habitat use, feeding preferences, and migratory behaviour.

## Materials and Methods

### Sample Collection

Raptors and owls were collected throughout Denmark in northern Europe (55°–57°N, 8°–15°E). All raptors and owls are protected. Consequently, liver samples or whole carcasses of raptors and owls were collected from birds delivered by the public to private taxidermists, zoological museums, and wildlife rescue stations as well as birds submitted to the Danish National Veterinary Institute for postmortem examinations. Birds were also collected from wildlife control units in airports where a small number of birds are in culled to decrease risk of bird strikes when nonlethal measures to scare off birds have been unsuccessful. Date and immediate cause of death reported by the collector were recorded on collection. Systematic detailed postmortem analysis and detection of potential effects of AR exposure, e.g., haemorrhaging from heart, lungs, liver, brain, or subcutaneous areas (Newton et al. 1999), were not possible with the diverse sampling sources. No symptoms of AR poisoning were noted in birds examined at the veterinary institute. Haemorrhaging caused by traumas in road-killed and culled birds may have hampered detection of symptoms of AR poisoning. Age and sex were recorded by some taxidermist, museums, or in our laboratory. Birds were aged as juveniles or adults based on morphological and plumage characteristics (Baker 1993; Forsman 1998), and grouped into season (spring = March through May; summer = June through August; autumn = September through November; winter = December through February). Most carcasses (>95 %) were collected in 2000 to 2009. Fifty-eight percent of the examined birds were violently killed by road traffic, collision with windows or power lines, bird strikes, or shot at airports by bird control teams; 24 % were reported found dead with no obvious cause of death; and 3 % were killed at wildlife rescue stations or by veterinarians. For 15 % of the birds, no data on cause of death were available. Liver tissues were kept frozen at –18 °C until chemical analysis.

### Chemical Analysis

Liver tissues were analysed for residues of brodifacoum, bromadiolone, coumatetralyl, difenacoum, and flocoumafen by high-performance liquid chromatography coupled with a fluorescence and photodiode array detector (Jones 1996; Palazoglu et al. 1998; Guan et al. 1999; Meiser 2005). Liver samples were dried with 2 g diatomaceous earth (Hydromatrix, Varian, USA) and homogenized by grinding. Rodenticides were extracted with acetone and dichloromethane [30:70 vol:vol (v:v)]. Extraction solvent, 10 mL, was mixed thoroughly with the homogenized tissue and shaken mechanically for 1 h and left to settle for approximately half an hour. The supernatant was transferred and collected through a funnel containing glass wool with anhydrous sodium sulphate on top (pro analysis, Merck, Germany). The homogenized tissue was re-extracted with an additional 10 mL acetone-and-dichloromethane aliquot. The combined extracts were evaporated

to dryness and subsequently redissolved in 1 mL methanol and analyzed by HPLC analysis (Waters$^{TM}$ HPLC system: 616 pump, 600S controller and 717 plus autosampler) using a Hypersil 5 $\mu$ C18 (octadecylsilane) 250 × 4.6 mm column equipped with an Analytical Guard Cartridge System (Phenomenex, France) at room temperature, and 10 µL was injected onto the column. The mobile phase consisted of a gradient of 0–9 min. methanol:ammonium acetate:acetonitrile (30:45:25 v:v); 9–45 min. ammonium acetate:acetonitrile (45:55 v:v); and 45–55 min. water:acetonitrile (45:55 v:v). Flow rate was 0.5 mL/min. Rodenticides were detected by fluorescence spectrometry (Waters$^{TM}$ 474 Scanning Fluorescence detector and Waters$^{TM}$ 996 Photodiode Array detector) with excitation wavelength at 310 nm and emission wavelength at 390 nm. Acetonitrile and methanol were HPLC grade from Merck, Germany. Acetone and dichloromethane were glass-distilled from Rathburn, Scotland.

## Quality Assurance

Rodenticide residue concentrations were quantified by comparison with analysis of standards of individual substances (Ehrenstorfer GMBH, Germany). Linear calibration range was brodifacoum 0.7–390 ng/mL, bromadiolone 1.5–400 ng/mL, coumatetralyl 0.2–123 ng/mL, difenacoum 0.7–370 ng/mL, and flocoumafen 0.5–56 ng/mL. Detection limits for each rodenticide were assessed from calibration standards using statistic regression. Detection limits were equivalent to 2 ng/g brodifacoum, 3 ng/g bromadiolone, 2 ng/g coumatetralyl, 2 ng/g difenacoum, and 1 ng/g flocoumafen for 1-g samples. Mean wet weight (±SD) of the samples was 1.15 ± 0.24 g.

Procedural blanks were analysed alongside samples to detect possible contamination during sample preparation. Recovery rates were assessed from spiked control samples of chicken liver with known rodenticide concentrations. Recovery rates ranged between 75 % and 82 % for brodifacoum, bromadiolone, difenacoum, and flocoumafen but only 51 % for coumatetralyl. Concentrations were not corrected for recovery rates.

## Statistical Analysis

Numbers of rodenticides in birds were compared by non-parametric analysis, including all sampled birds. However, species with an inadequate sample size (<30 individuals) was excluded from further analysis to warrant statistical power. Thus, differences in prevalence of rodenticides among species, season, age, sex, and cause of death was compared using G-tests in six core species only: barn owl (*Tyto alba*), buzzard (*Buteo buteo*), kestrel (*Falco tinnunculus*), long-eared owl (*Asio otus*), rough-legged buzzard (*B. lagopus*), and tawny owl (*Strix aluco*) (Table 1). Negative binomial regression analyses were used to determine factors (species, season, cause of death, sex, and age) influencing the concentrations of each rodenticide and the summed AR concentration in the core species. For statistical analyses, the cause of death was categorized as traffic

**Table 1** Distribution between season, sex, age, and cause of death of the six core species with sample size >30 birds

| Season, sex, age, and cause of death | Barn owl | Buzzard | Kestrel | Long-eared owl | Rough-legged buzzard | Tawny owl |
|---|---|---|---|---|---|---|
| Season | | | | | | |
| Winter | 32 | 53 | 8 | 14 | 2 | 11 |
| Spring | 13 | 32 | 6 | 9 | 8 | 7 |
| Summer | 9 | 19 | 26 | 4 | 0 | 7 |
| Autumn | 20 | 28 | 18 | 7 | 21 | 17 |
| Not recorded | 6 | 9 | 8 | 4 | 0 | 2 |
| Age | | | | | | |
| Female | 32 | 59 | 20 | 20 | 12 | 20 |
| Male | 35 | 53 | 27 | 11 | 12 | 14 |
| Not recorded | 13 | 29 | 19 | 7 | 7 | 10 |
| Age | | | | | | |
| Adult | 27 | 66 | 28 | 20 | 7 | 22 |
| Juvenile | 21 | 44 | 26 | 5 | 23 | 9 |
| Not recorded | 32 | 31 | 12 | 13 | 1 | 13 |
| Cause of death | | | | | | |
| Traffic | 24 | 47 | 13 | 19 | 1 | 29 |
| Culled | 0 | 31 | 39 | 7 | 29 | 0 |
| Undetermined | 42 | 43 | 8 | 7 | 0 | 3 |
| Not recorded | 14 | 20 | 6 | 5 | 1 | 12 |

(road kills, bird strikes, and window collisions), shot, or undetermined. Birds were excluded from these statistical analyses if no data on cause of death, age and sex had been recorded. Because cause of death, age, and sex were only recorded for approximately 60 % of the birds, the sample size in the statistical analyses decreased markedly when these parameters were included in the statistical analysis. Hence, negative binomial regression analyses first included species and season as the only explanatory variables. Cause of death, age, and sex were subsequently included in the analyses. The negative binomial regression analyses also included animals in which no AR residues were detected. Differences of least squares means ($\alpha = 0.05$) were used in pairwise comparisons between species or seasons. Statistical analyses were performed using SAS® 9.2 and SAS Enterprise Guide® 4.1 software (SAS Institute, Cary, USA).

## Results

### Prevalence of ARs

Overall, 92 % of all birds contained detectable hepatic AR residue concentrations. Within species, between 84 and 100 % of individual birds had detectable AR concentrations (Table 2). Second-generation ARs were detected in 91 % of the birds; 73 % of all birds contained detectable levels of more than one rodenticide; and all five substances were detected in 3 % of all birds. Mean numbers (±SD) of rodenticides in individual birds indicated no differences in exposure patterns between owls and raptors [owls 2.2 ± 1.1, raptors 2.2 ± 1.2 (Wilcoxon $z = 0.548$, $P = 0.548$)] or between the six core species of the study (Krushal–Wallis $K = 0.78$, $P = 0.98$) (Fig. 1). Second-generation ARs comprised 95 % of the summed AR burden. Difenacoum, brodifacoum, and bromadiolone were the most prevalent substance. These substances were also detected in the highest concentrations in all species (Table 3).

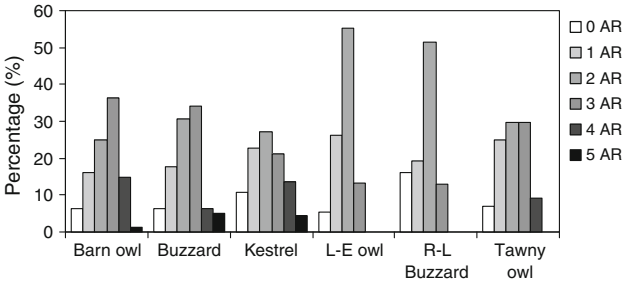


**Fig. 1** Frequency distribution of numbers of ARs detected in individual birds of the six core species

For the six core species prevalence of one or more ARs, prevalence of second-generation AR and multiple AR exposure of rodenticide did not differ ($G = 3.94$, $df = 5$, $P = 0.56$; $G = 4.53$, $df = 5$, $P = 0.48$; $G = 4.00$, and $df = 5$, $P = 0.55$, respectively). During autumn, multiple exposures to AR tended to be less prevalent than in other seasons ($G = 7.21$, $df = 3$, $P = 0.065$). Multiple exposure was also less prevalent in juvenile than in adult birds ($G = 5.70$, $df = 1$, $P < 0.05$), and similar tendencies were seen in the prevalence of exposure to one or more ARs ($G = 3.13$, $df = 1$, $P = 0.077$).

### AR Concentrations

The majority of the AR residue concentrations were low, but among the six core species 12.9–37.4 % of individual birds had summed hepatic AR concentrations >100 ng/g ww, which may cause haemorrhaging in predatory birds (Newton et al. 1999; Thomas et al. 2011). Potentially lethal

**Table 2** Sample sizes, prevalence of any ARs, mean and median wet weight in birds with detectable AR levels, and maximum summed AR concentration in all analysed species

| Species | N | Prevalence (%) | Mean (ng/g ww) | Median (ng/g ww) | Maximum (ng/g ww) | 100–200 ng/g (%) | >200 ng/g (%) |
|---|---|---|---|---|---|---|---|
| Barn owl (*T. alba*) | 80 | 94 | 114.1 | 71.0 | 1092 | 23.7 | 13.7 |
| Buzzard (*B. buteo*) | 141 | 94 | 74.5 | 50.0 | 721 | 14.9 | 5.7 |
| Eagle owl (*B. bubo*) | 10 | 100 | 193.1 | 241.0 | 313 | 0.0 | 70.0 |
| Kestrel (*F. tinnunculus*) | 66 | 89 | 99.0 | 46.0 | 690 | 13.6 | 13.6 |
| Little owl (*A. noctua*) | 9 | 100 | 118.6 | 39.0 | 411 | 11.1 | 22.2 |
| Long-eared owl (*A. otus*) | 38 | 95 | 19.4 | 13.5 | 84 | 0.0 | 0.0 |
| Marsh harrier (*C. aeruginosus*) | 3 | 100 | 12.3 | 15.0 | 21 | 0.0 | 0.0 |
| Red kite (*M. milvus*) | 3 | 100 | 413.0 | 260.0 | 962 | 0.0 | 66.7 |
| Rough-legged Buzzard (*B. lagopus*) | 31 | 84 | 40.8 | 26.5 | 139 | 12.9 | 0.0 |
| Short-eared owl (*A. flammeus*) | 5 | 100 | 15.0 | 18.0 | 37 | 0.0 | 0.0 |
| Tawny owl (S. *aluco*) | 44 | 93 | 78.4 | 39.0 | 534 | 11.4 | 9.1 |

Hepatic AR concentration >100 ng/g ww has been suggested as the level of concern and >200 ng/g ww as a critical level for raptors and owls

**Table 3** Prevalence (%), median, and maximum concentrations (ng/g ww) of individual ARs in the six core species

| Species [sample size (*n*)] | BRD | BRM | COU | DIF | FLO |
|---|---|---|---|---|---|
| Barn owl (80) | | | | | |
| Prevalence | 62.5 | 68.4 | 15.0 | 83.1 | 16.3 |
| Median | 4.0 | 16.0 | 0.0 | 11.0 | 0.0 |
| Maximum | 957.0 | 252.0 | 18.0 | 223.0 | 34.0 |
| Barn owl (80) | | | | | |
| Prevalence | 55.4 | 60.0 | 22.3 | 77.9 | 19.9 |
| Median | 2.0 | 7.5 | 0.0 | 10.0 | 0.0 |
| Maximum | 613.0 | 282.0 | 435.0 | 170.0 | 115.0 |
| Kestrel (66) | | | | | |
| Prevalence | 59.1 | 48.5 | 18.8 | 65.2 | 27.3 |
| Median | 2.0 | 0.0 | 0.0 | 6.5 | 0.0 |
| Maximum | 298.0 | 679.0 | 64.0 | 450.0 | 20.0 |
| Long-eared owl (38) | | | | | |
| Prevalence | 63.2 | 26.3 | 10.5 | 72.2 | 7.9 |
| Median | 3.0 | 0.0 | 0.0 | 7.0 | 0.0 |
| Maximum | 40.0 | 33.0 | 29.0 | 52.0 | 2.0 |
| Rough-legged buzzard (31) | | | | | |
| Prevalence | 61.3 | 16.1 | 9.7 | 74.2 | ND |
| Median | 3.0 | 0.0 | 0.0 | 14.0 | – |
| Maximum | 34.0 | 130.0 | 3.0 | 105.0 | – |
| Tawny owl (44) | | | | | |
| Prevalence | 53.5 | 61.4 | 14.0 | 72.1 | 11.4 |
| Median | 3.0 | 8.0 | 0.0 | 7.0 | 0.0 |
| Maximum | 220.0 | 496.0 | 39.0 | 90.0 | 42.0 |

*BRD* brodifacoum, *BRM* bromadiolone, *COU* coumatetralyl, *DIF* difenacoum, *FLO* flocoumafen, *ND* not detected

**Table 4** Relationship between species, season, and concentrations of individual ARs, and summed AR concentrations in the six core species, as determined by negative binomial regression

| Species | Sample size (*n*) | Parameter | DF | $\chi^2$ | *P* |
|---|---|---|---|---|---|
| Summed AR | 371 | Species | 5 | 40.82 | <0.0001 |
| | | Season | 3 | 7.27 | 0.0639 |
| Brodifacoum | 368 | Species | 5 | 31.06 | <0.0001 |
| | | Season | 3 | 3.16 | 0.3678 |
| Bromadiolone | 369 | Species | 5 | 23.82 | 0.0002 |
| | | Season | 3 | 8.93 | 0.0302 |
| Coumatetralyl | 366 | Species | 5 | 14.54 | 0.0125 |
| | | Season | 3 | 4.23 | 0.2377 |
| Difenacoum | 360 | Species | 5 | 13.67 | 0.0179 |
| | | Season | 3 | 1.50 | 0.6823 |
| Flocoumafen | 371 | Species | 5 | 19.65 | 0.0015 |
| | | Season | 3 | 5.98 | 0.1126 |

AR concentrations (>200 ng/g ww) were detected in 13.6 % of the kestrels and 13.7 % of the barn owls (Table 2). Critical AR residue levels were detected in an even higher percentage of the small sample of red kite (*Milvus milvus*) and eagle owl (*Bubo bubo*) analysed, whereas no marsh harrier (*Circus aeruginosus*), long-eared owl (*A. otus*), and short-eared owl (*A. flammeus*) had critical AR residue burdens.

## Factors Influencing AR Concentrations

Negative binominal regressions of summed AR concentrations and concentrations of the individual substances showed significant effects of species ($\chi^2 = 40.82$, $df = 5$, $P < 0.001$) (Table 4). The summed AR concentrations were lower in rough-legged buzzard and long-eared owl than in buzzard, kestrel, barn owl, and tawny owl, and

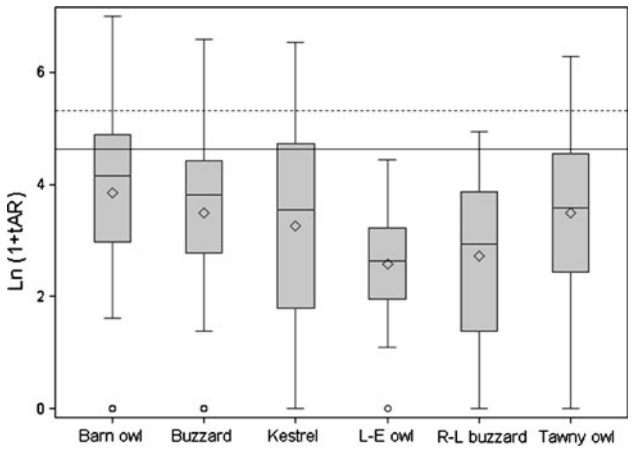


**Fig. 2** Summed concentrations of AR in the six core species examined, including specimens with no detectable levels of any AR. *Box* and *line* represents the upper and lower quartile and median; *diamond* is the mean value; and *whiskers* are the SD. *Horizontal lines* indicate the suggested threshold levels of 100 and 200 ng/g (*dotted*) (Newton et al. 1999)

buzzard had lower summed AR concentration than barn owl (Fig. 2). Similar effect of species was seen for the individual rodenticides. For the summed AR concentration, there was a strong tendency for seasonal differences ($\chi^2 = 7.27$, $df = 3$, $P = 0.064$). The seasonal variation was significant only for bromadiolone ($\chi^2 = 8.93$, $df = 3$, $P < 0.05$), which was detected in lower concentrations in autumn than in spring. There were no general effects of cause of death, age, and sex on the summed AR concentration (cause of death $\chi^2 = 3.04$, $df = 2$, $P = 0.22$; age $\chi^2 = 1.40$, $df = 1$, $P = 0.24$; and sex: $\chi^2 = 0.47$, $df = 1$, $P = 0.49$). However, adult birds had higher brodifacoum concentrations than juvenile birds ($\chi^2 = 3.85$, $df = 1$, $P < 0.05$), and difenacoum levels were lower in birds with unknown cause of death than in traffic-killed and culled birds collected in airports ($\chi^2 = 13.65$, $df = 2$, $P < 0.01$).

## Discussion

ARs were recorded in high frequencies in all species of raptors and owls, indicating a high general AR exposure level to nontarget wildlife species in Denmark. Human land-use is intensive in Denmark (62 % arable land and 10 % built-up area), and farm buildings are regularly dispersed in rural areas (Normander et al. 2009). A relatively high proportion of carnivorous species is likely to have access to AR-exposed rodents even if rodent control is only conducted around buildings. Rodent control in a specific habitat will also result in high-exposure risk for predators, e.g., semiaquatic carnivores when ARs are used along streams and lakes (Fournier-Chambrillon et al. 2004; Lemarchand et al. 2010).

Overall, the AR prevalence and concentration recorded in the present study reflected the bird species' feeding habits, habitat use, and migration patterns (Cramp and Simmons 1980; Bønløkke et al. 2006). The highest AR concentrations and prevalence were detected in scavengers, such as red kite and eagle owl, which may prey more significantly on rats, and in birds associated with agricultural landscapes and buildings in rural areas, such as buzzard, kestrel, barn owl, and little owl (*Athene noctua*). None of the examined migratory species and species inhabiting more natural habitats, such as rough-legged buzzard, marsh harrier, long-eared owl, and short-eared owl, had critical AR burdens (cf. Newton et al. 1999). The different AR exposure rates and levels among species with different feeding ecology correspond to studies from Great Britain and North America (Shore et al. 2000, 2001; Stone et al. 2003; Walker et al. 2008b, 2010a, b). Scavengers are presumed to be more exposed when preying on rodents that have died from the rodenticide poisoning.

The seasonal differences in AR concentrations in raptors and owls differ from the seasonal pattern in small mustelids (*Mustela* sp.), the occurrence of which peaks during autumn and winter (Elmeros et al. 2011). The lower AR prevalence and concentrations in birds during autumn is probably caused by an influx of migratory buzzards, rough-legged buzzards, kestrels, and long-eared owls from northern Scandinavia (Bønløkke et al. 2006) where human density and land-use is less intense and AR use lower (Lodal and Hansen 2002).

The prevalence and pattern of rodenticides were similar among bird species and in small mustelids from Denmark (Elmeros et al. 2011), which may suggests that the AR exposure is relatively homogenous in all habitats represented by the examined birds and mammals. The long persistency and bioaccumulation, which is characteristics of second-generation rodenticides, is illustrated by the increased frequency of multiple exposures in adult birds. Compared with the annual sales of rodenticides (Miljøstyrelsen 2011), the relative prevalence of coumatetralyl in the predatory wildlife species was lower, whereas the prevalence of difenacoum and brodifacoum was higher than expected. The higher persistency of the second-generation rodenticides (Eason et al. 2002; Fisher et al. 2003) and lower recovery rates in the analytical process of coumatetralyl may explain some of the differences between sales and relative prevalence of individual rodenticides in wildlife species. The low prevalence in coumatetralyl may also be a result a lower exposure risk to wildlife because coumatetralyl-based products are only used by professional rodent-control operators for rat control.

AR exposure levels in raptors and owls in Denmark are comparable with AR prevalence reported in the most recent

studies in Great Britain, which applied analytical methods with similar detection limits (Walker et al. 2010a, b). Total use of ARs (kg active substance per area unit) and proportion of second-generation ARs in Denmark is comparable with use in southern and eastern England where most of the analyzed British birds were sampled (Garthwaite et al. 2000; Dawson et al. 2003; Dawson and Garthwaite 2004; Miljøstyrelsen 2011). However, the occurrence of multiple exposures were noticeably higher in Danish raptors and owls (this study) than recorded in the newest British studies on barn owl (78 vs. 54 %) and kestrel (67 vs. 50 %) (Walker et al. 2010a, b), suggesting that rodenticide application methods in Denmark result in higher exposure risk to predatory birds.

The populations of most raptors and owls have increased or been stable during the past decades in Denmark (Heldbjerg et al. 2011). Consequently, there is no indication that the recorded AR exposure rates and levels have a directly negative impact on the overall population development of raptors and owls. However, the recorded AR burdens associated with haemorrhage and mortalities (Grolleau et al. 1989; Newton et al. 1999; Thomas et al. 2011) were recorded in many individuals, particularly in kestrels, barn owls, and red kites. Exposure to lower levels of ARs may also result in decreased fitness due to greater susceptibility to infections, increased parasite burdens, and synergetic effects between ARs and other contaminants and pesticides (Fournier-Chambrillon et al. 2004; Lemus et al. 2011). Furthermore, the intensive use of ARs can cause decreased breeding success and population recruitment because young animals may be more susceptible to ARs and other toxins (Salmon and Marsh 1979; Moser 2011). In particular, the high prevalence and concentrations of ARs in little owls are concerning. The little owl is highly endangered and decreasing in population in Denmark (Thorup et al. 2010). The species typically nests in farm buildings, and increased chick mortality in the nests is the driver of the population decline.

The regulation of AR use in Denmark aims at minimising the risk of secondary poisoning of nontarget wildlife species. The widespread secondary exposure in predatory birds and small mustelids shown in this study and by Elmeros et al. (2011) indicates that the regulations fail to succeed in low secondary exposure of predatory birds and mammals, most probably as a result of extensive use of ARs in Denmark. Improved methods to assess temporal and spatial AR exposure on nontarget wildlife populations with different rodenticide-use scenarios, along with studies of the potential impact of ARs on individual fitness and the consequences for population status of nontarget birds and mammals, are urgently needed for more robust risk assessment.

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