persistence and capacity to bioaccumulate, combined with extensive and often regular prophylactic use in agricultural,

Table 1

any control birds. Symptoms of AR poisoning observed during necropsy are summarized by group in Table 1, with

Japanese Quail Hb and Hct

The concentration of Hb and the Hct varied signiÞcantly with dose and time ($p < 0.001$; Fig.6

7 days after exposure, respectively. The diagnostic speciÞcity and sensitivity of the ACT test were calculated as 100 and 75 %, respectively.

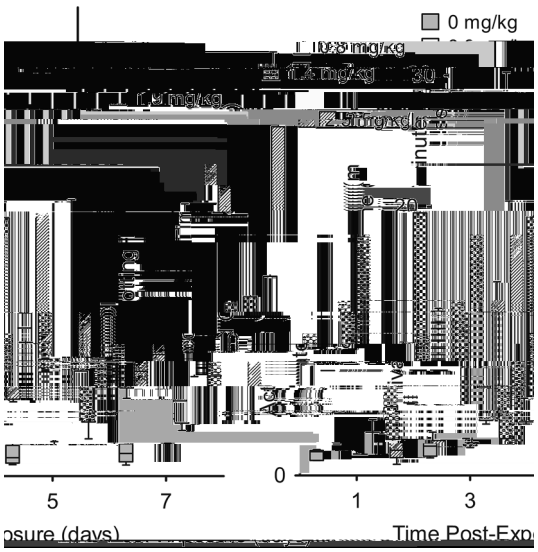

Fig. 5 Activated clotting time (min) measured in Japanese quail (Coturnix japonica) after oral exposure to brodifacoum. Median presented with error bars showing the 5th to 95th percentile. See Table 2 for sample sizes for all 20 dose groups

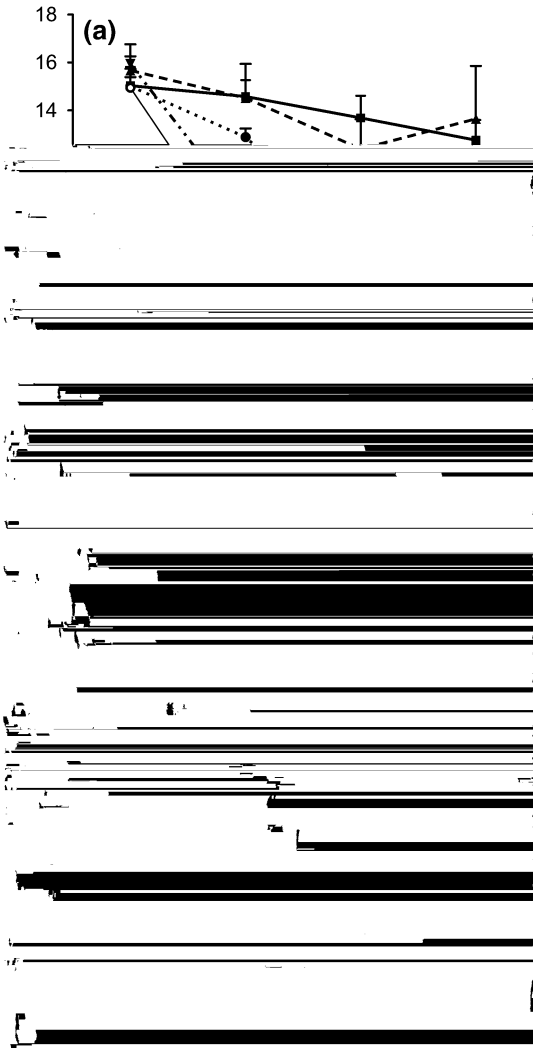

Fig. 6 a Hemoglobin (g/dl) measured in Japanese quail (Coturnix japonica) after oral exposure to brodifacoum. Significant differences ($p < 0.05$) occurred between dose groups in comparison to same-day control 1, 3, 5, and 7 days post-exposure (*), and within each dose group after 3, 5, and 7 days post-exposure when compared with 1 day post-exposure (#). b) Hematocrit measured in Japanese quail (Coturnix japonica) after oral exposure to brodifacoum. Significant differences ($p < 0.05$) occurred between dose groups in comparison to same-day control 1, 3, 5, and 7 days post-exposure (*), and within each dose group after 3, 5, and 7 days post-exposure when compared with 1 day post-exposure (#). See Table 3 for sample sizes for all 20 dose groups

reference intervals of 9.6–18.4 g/dl for Hb and 34–55 % for Hct. There were no significant differences in Hct or Hb over time within the control or 0.8 mg/kg dose groups after any exposure period, or 1 day after dose administration between dose groups. In contrast, there were significant decreases in Hct and Hb over time within the 1.9 and 2.5 mg/kg dose groups on days 3 and 5, and within the 1.4 mg/kg dose group on days 5 and 7 when compared to same-dose group results on day 1. At 1.9 mg/kg, both Hct and Hb were significantly reduced 5 days post exposure and at 1.4 mg/kg, Hct and Hb remained significantly reduced 7 days post-exposure.

Japanese quail liver residues

A maximum liver concentration of 0.84 μg/g wet weight was observed in the 1.4 mg/kg dose group, 3 days post-exposure, and the 97.5 % upper confidence limit of the mean (UCLM) for all dose groups was 0.51 μg/g wet weight. Liver residues are presented in Table 3 by treatment group. As shown in Fig. 7, liver residue data for individual birds in the 0.8 and 1.4 mg/kg dose groups corresponds to a one-phase exponential decay model ($R^2 = 0.141$ and $R^2 = 0.558$, respectively). However, sampling intervals were not prolonged enough to calculate a tissue half-life. Brodifacoum residues in the liver were not correlated with liver somatic index, Hb concentration, Hct, ACT, PT, or sex. No significant difference in brodifacoum liver residue was determined between quail with or without gross pathological signs of toxicosis; nor was a correlation determined between the mean hepatic residue and the proportion of quail with these signs in each treatment group ($R^2 = 0.0006$, $p = 0.925$; Fig. 8).

Barn owl PT

All Barn Owl PTs were adjusted using the freezing storage factor based on data for quail. After adjustment for storage

Table 3 Mean (SEM) results for hemoglobin, hematocrit and liver residues for each treatment group

Treatment group	Hb (g/dl)	Hct	Liver residues (µg/g)
0 mg/kg			
Day 1	15.0 (1.17, n= 6)	0.465 (0.032, n= 6)	Ð
Day 3	14.6 (1.17, n= 6)	0.449 (0.032, n= 6)	Ð
Day 5	13.7 (1.17, n= 6)	0.462 (0.032, n= 6)	Ð
Day 7	12.8 (1.17, n= 6)	0.407 (0.032, n= 6)	Ð
All controls	14.0 (0.42, n= 6)	0.446 (0.011, n= 6)	0 (pooled, n= 24)
0.8 mg/kg			
Day 1	15.7 (1.17, n= 6)	0.462 (0.032, n= 6)	0.35 (0.089, n= 6) range 0.24Ð0.5
Day 3	14.5 (1.17, n= 6)	0.417 (0.032, n= 6)	0.31 (0.093, n= 6) range 0.21Ð0.43
Day 5	12.4 (1.17, n= 6)	0.418 (0.032, n= 6)	0.27 (0.073, n= 6) range 0.19Ð0.38
Day 7	13.7 (1.43, n= 4)	0.424 (0.040, n= 4)	0.24 (0.074, n= 4) range 0.15Ð0.33
1.4 mg/kg			
Day 1	15.0 (1.17, n= 6)	0.443 (0.032, n= 6)	0.50 (0.17, n= 6) range 0.4Ð0.84
Day 3	12.9 (1.17, n= 6)	0.426 (0.032, n= 6)	0.27 (0.080, n= 5) range 0.17Ð0.37
Day 5	8.5 (1.28, n= 5)	0.289 (0.035, n= 5)	Day 50.25 (0.065,8n5 (1.28, n

time, the median PT of barn owl chicks (aged 40Ð55 days) sampled directly from nest sites, and adults was 22.1 s (n = 61, reference interval= 17Ð29 s; Fig9). The mean PT of two captive barn owls was 22.6 s, and the PT of one Barn Owl admitted to a local wildlife rehabilitation centre was 103.6 s (Fig9).

Discussion

Acute toxicity to Japanese quail

Our study clearly shows that brodifacoum is a potent disruptor of coagulation in Japanese quail at 0.8 mg/kg or greater. Acute toxicity was observed in Japanese quail exposed to 0.8 mg/kg brodifacoum within 3 days of exposure, and severe coagulopathy was observed 5 days post-exposure. This dose is environmentally relevant for both primary consumption of brodifacoum in bait and secondary poisoning by consumption of brodifacoum-contaminated prey animals (US EPA 2011). Estimated daily intakes of brodifacoum by Passeriforms from bait was 7Ð13 mg/kg, and by secondary consumer birds was 0.82Ð19.14 mg/kg (US EPA2011). In this study at brodifacoum doses of 1.9 and 2.5 mg/kg, coagulopathy resulted in severe hemorrhage only 1 day after exposure. Overt signs of toxicity included ataxia

pathologic effect. In contrast, 1.4 mg/kg of brodifacoum resulted in signiÞcant reductions in Hb and Hct even 7 days post-exposure, from 12.8 to 3.8 g/dl (Fig. 6a) and 40.7Ð15.2 % (Fig. 6b), respectively, when PTs decreased due to apparent hepatic recovery (Fig. 3). This discrepancy at day 7 reßects faster hepatic recovery as a minimum of 3Ð5 days are required for the avian bone marrow to begin producing increased numbers of new red blood cells (Weiss and Wardrop 2011).

The Hct and Hb levels measured in brodifacoum-treated Japanese quail are similar to those reported in response to incidences of SGAR poisoning. Hct in the Red-tailed hawk (Buteo jamaicensis) and White-winged wood duck (Cairina scutulata) was reported at 9 and 16 %, respectively, after exposure to brodifacoum (James et al. 1998; Murray and Tseng 2008). Hct values such as those reported above are indicative of anemia (hematocrit < 34 % based on reference interval above) and correlated to acute vascular blood loss by necropsy Þndings.

Japanese quail liver residues

Hepatic concentrations were not measured within the Þrst 24 h in this study, however the decrease in hepatic brodifacoum concentration was assumed to be rapid during this period, followed by a slow decrease demonstrating brodifacoumÕs persistence in the liver (Fig. 7). In rats, a liver elimination half-life of up to 350 days has been reported (Batten and Bratt 1990). The persistence of brodifacoum in the liver and the delayed action of this toxin prior to death both contribute to secondary poisoning of wildlife which consume intoxicated prey animals. This delayed persistant action may be due to a reduced reserve capacity of VKOR synthesis after full or partial recovery of clotting time from brodifacoum exposure (Mosterd and Thijssen 1991).

The hepatic concentration of SGARs is a reliable indicator of exposure incidence, but does not provide adequate information about potential exposure impacts or risk to an individual or population. Although the present study found a liver saturation level of approximately 0.5 μg/g wet weight based on the 97.5 % UCLM across all treatment groups, death was observed at a hepatic concentration of 0.31 μg/g w/w, and severely prolonged PT associated with hemorrhagic effects occurred at a hepatic concentration of 0.23 μg/g wet weight. A recent study on raptors in Massachusetts discovered that although hepatic SGAR residues ranged from 0.012 to 0.269 μg/g wet weight (where 98 % contained brodifacoum as the only SGAR), only 6 % (n = 164) of all birds could be deÞnitively related to SGAR toxicosis (Murray 2011). A recent probabilistic risk assessment proposed threshold liver residue concentrations which would result in signs of toxicosis at 5Ð20 % effects levels for four raptor species, including a 5 % effects level at 0.05 mg/kg wet weight for barn owls (Thomas et al. 2011). Our study found no correlation between liver residues and oral brodifacoum dose, similar to Gray et al. (1994), and no correlation between liver residues and the proportion of quail with hemorrhage or pallor (Fig. 8). However, a correlation was uncovered between increasing PT and the proportion of each treatment group experiencing AR signs ($p < 0.0001$; Fig. 4). Taken together, our study (1) supports the recommendation that liver residues be used to conÞrm SGAR exposure but not be relied upon as an indicator of toxicosis (Murray 2011) and (2) shows that PT is a useful tool to aid in monitoring and assessing risk of SGARs to a speciÞc population of concern.

Evaluation of prothrombin times in barn owls

Barn Owls (Tyto alba) were used to evaluate PT as a biomonitoring assay due to a concurrent study that enabled access to both urban and agricultural chicks and adults during the breeding season. As barn owls are designated as Threatened by COSEWIC (Committee on the Status of Endangered Wildlife in Canada; Environment Canada 2011), and hunt for rodents in environments where AR use

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