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Exposure pathways of anticoagulant rodenticides to nontarget wildlife

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Abstract Second-generation anticoagulant rodenticides are widely reported to contaminate and poison nontarget wildlife, primarily predatory birds and mammals. Exposure pathways, however, have not been well defined. Here, we examined potential movement of rodenticides from deployment of bait to exposure of small mammals and other biota. At two adjacent working farms, we placed baits containing either brodifacoum or bromadiolone. We monitored movement of those compounds to the surrounding environment by collecting small mammals, birds, and invertebrates. Similar collections were made at a third agricultural setting without active bait deployment, but located among intensive livestock production and regular rodenticide use by farmers. Livers and whole invertebrate samples were analyzed for rodenticides using a sensitive LC-MSMS method. Norway rats (*Rattus norvegicus*) from both baited and non-baited farms had residues of brodifacoum or bromadiolone, implicating rats as an important exposure pathway to wildlife. Among 35 analyzed nontarget small mammals, a single vole had high hepatic residues (18.6 μ/g), providing some indication of a small mammal pathway. One song sparrow (*Melospiza melodia*) sample from a baited farm contained 0.073 μg/g of brodifacoum in liver, while 0.39 μg/g of diphacinone was measured in a pool of carrion beetles (*Dermestes* spp.) from the non-baited farm area, implicating avian and invertebrate components in exposure pathways. Regurgitated pellets of barn owl (*Tyto alba*) selected randomly from baited farms contained no detectable rodenticide residues, while 90 % of owl pellets collected from a variety of farms, and selected for the presence of rat fur, contained detectable anticoagulant residues. We recorded behavior of a captive sample of a representative songbird, the house sparrow (*Passer domesticus*); they readily entered bait stations and fed on (unloaded) bait.

Keywords Anticoagulants · Exposure pathways · Nontargets · Raptor · Rat

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Introduction

Estimates of total damage from invasive rodents, principally Norway rats (*Rattus norvegicus*), are as high as

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$19 billion annually in the USA (Pimentel et al. 2005). In most jurisdictions, the preferred method to control commensal rodent infestations is by use of anticoagulant rodenticides. Their mode of action is to affect vitamin K cycling in the liver preventing synthesis of clotting factors such as prothrombin and leading to fatal hemorrhage (Watt et al. 2005). Widespread use of the first-generation anticoagulant rodenticides (FGARs) including warfarin, chlorophacinone, and diphacinone led to the development of genetic resistance in rodent populations (Thijssen 1995). In the 1970s, second-generation anticoagulants (SGARs) were introduced, being more acutely toxic than first-generation compounds and capable of killing the target rodent after a single feeding (Buckle et al. 1994). Greater effectiveness of the SGARs is due to their higher affinity for hepatic binding sites (Parmar et al. 1987) and longer half-lives in the body (US EPA 2004).

The greater persistence and toxicity of SGAR compounds has resulted in increased incidence of exposure and poisoning of nontarget wildlife, primarily predatory birds and mammals (Hegdal and Colvin 1988;Newton et al. 1990; Shore et al 1996; Tobin et al. 1996; Newton et al. 1999, 2000; Stone et al. 1999, 2003; Howald et al. 1999; Eason et al. 2002; Lambert et al. 2007; Riley et al. 2007; Walker et al. 2008; Albert et al. 2010, Lima and Salmon 2010; Murray 2011; Thomas et al. 2011; Christensen et al. 2012; Gabriel et al. 2012). However, with some exceptions (Merson et al. 1984; Cox and Smith 1990, 1992; Howald 1997; Eason et al. 2002; Brakes and Smith 2005; Lima and Salmon 2010), few studies have examined the exposure pathways from rodenticide bait deployment to nontarget wildlife exposure. The objective of the current work was to examine the process of anticoagulant rodenticide movement into the local environment from their use at livestock farming operations. In cooperation with farmers, we conducted field experiments and monitoring with and without deploying rodenticide bait and measured residues in small mammals, birds, and invertebrates.

Methods

Prey species study design and sample collection

All sampling was done under Animal Care Committee approved methods. Two different study scenarios were employed (scenario 1: Delta Farm A and Delta Farm B and scenario 2: Fraser Valley Farms C and D). In scenario 1, two adjacent livestock operations (Delta Farm A and Delta Farm B) in the municipality of Delta, British Columbia (BC), with and without active Norway rat infestations, were actively treated with SGARs.

The two Delta farms, labeled A and B, were 500 m apart with the farmer at A reporting a heavy Norway rat and domestic mouse (*Mus musculus*) infestation of his barn during fall/winter, while B was relatively free of rats. In January of 2006 when contacted, both farms were actively using rodenticides. Farm A was using brodifacoum pellets in homemade unsecured bait stations. Inspection of the stations revealed the bait to be in poor condition; most had been consumed, and the remains were putrefied/moldy. Farm B was using bromadiolone blocks in tamper resistant bait stations. Over the winter at both sites, we observed spilled cereal cattle feed available to rodents in the barn buildings. Both farms also had active barn owl (*Tyto alba*) winter roosts. Beginning in late January, and for a 3-week period prior to the experiment at each location, we searched the main cattle barn and immediate surrounding grounds for evidence of rodent activity and collected any carcasses. Twenty kill traps (Victor©) were randomly placed within barn buildings on both sites to target mice and rat species. Peanut butter and rolled oats were used as bait. Traps were checked twice daily.

Following the 3-week surveillance period, the farmers' respective rodent control bait stations were removed from each location. In late February, we placed new Protecta© side kick tamper resistant rodent bait stations at each farm. Six stations were placed in the same locations inside the barns as previous farmer-placed bait stations. In an effort to detect the movement of chemicals into the local food chain, we switched the products from those used by the respective farmer at each site. Thus, farm A bait stations were supplied with block form bromadiolone, Bell Laboratories Inc. CONTRAC Blox Rodent Bait (Bromadiolone 0.005 %). Farm B bait stations were supplied with block form brodifacoum, Bell Laboratories Inc. FINAL Blox Rodent Bait (Brodifacoum 0.005 %). Over the following 3 weeks, barns and bait stations were visited twice daily to monitor bait usage and collect dead/alive rodents/birds/insects/mollusc species. In late March, 3 weeks after placement of the new bait stations, small

mammal trapping transects were established. Trapping was done with Sherman live traps. Ten traps were placed at 10 m intervals in transects at distances of 50 and 100 m in parallel from each of the respective main barns for a total of 20 traps at each of farms A and B. Fields were fallow forage fields mainly covered in fescue.

For scenario 2, we focused on an area of the neighboring Fraser Valley near the municipality of Abbotsford B.C., the site of intensive poultry production and rodenticide use, and a focal area of owl poisonings by SGARs over the period 1999 to 2006 (Albert et al. 2010). A site was selected with two adjacent cooperating farms, farms C and D, for proximity (500–800 m) to commercial poultry operations. The focal farms were not actively using rodenticide and did we not undertake an active bait switching and placement experiment. However, many of the surrounding operations were actively baiting with anticoagulants. Using a simple monitoring design, we sampled resident rodents/birds/invertebrates around these two farms to test for movement of rodenticides from areas of intensive use to surrounding farms. During the winter months (January–April 2006), neighboring land use around barn buildings was mainly forage grass (orchard grass and fescue). We placed ten Sherman traps at random distances from farm buildings at intervals of approximately 20 m.

Under both scenarios, small mammal traps were pre-baited with rolled oats and apple slices and left open for a period of 7 days. Traps were visited twice daily to check for specimens once activated. At both the Delta and Fraser Valley locations, we also sampled barn buildings and adjacent areas for house sparrows (*Passer domesticus*), song sparrows (*Melospiza melodia*), and European starlings (*Sturnus vulgaris*). Both air rifles (0.177 cal) and 12 gauge shotguns were used. Attempts were made to collect any insect and mollusc samples found within/around barns or in and around bait stations. Insect activity was minimal during winter months. In April, carcasses of rats found dead at farm A, and presumed to have died of bromadiolone poisoning, were placed outside in fields at farm C in order to collect known insect visitors to carcasses such as flies (*Musca* spp.) or carrion beetle (*Dermestes* spp.). Carrion baits were covered by a wire enclosure to prevent scavenging by vertebrates. Carrion was checked every 2 days until beetles were found and fly larvae (maggots) were collected. Collections continued until the carrion was completely consumed or decayed.

Collection and examination of owl pellets

At farms A and B, barn owl (*T. alba*) pellets were collected opportunistically beneath roost areas. Pellets were also collected later at nine other farms in Delta and the adjacent municipality of Surrey from active nests during breeding season. At the latter sites, pellets were screened for the presence of rat fur and selected for analysis. For each pellet, prey items were identified using remnant bone pieces. The number of individuals per prey type was determined by pairing each skull with the correct number of ischia, left and right mandibles, tibiae/fibulae, or in the case of birds, each skull with sternum, gizzard sac and feet. The remaining bones contained within the pellet were assembled to determine the minimum number of additional individuals whose skull may have been crushed.

Observations of bait station use by songbirds

In early September, house sparrows were lured by playing house sparrow song recordings and mist netted. The birds were then held in an outdoor aviary. Food (mixed millet and finch seed) in a ground feeder, water, and shelter were provided for a 1-week acclimatization period. During the second week, a Protecta© bait station was placed on the floor of the aviary in a position that replicated normal use of a rodent bait station for rodent control (up against the wall of the aviary). The same bird seed mix was placed inside the bait station, where bait would normally be placed. The usual ground feeder with the same seed mix remained in the aviary. A web camera (Swann bulletcam SW-P-BCC, Port Melbourne, Australia) was affixed to the side of the aviary and a monitor as well as a hard drive DVD recorder (Pioneer DVR-550H, Tokyo, Japan) allowed the observer to view and record sparrow activity in the aviary. The camera was set up to record from 0730–2030 hours. Sparrow activity was recorded for 2 days. The bait station was then removed from the aviary.

During the third week, a bait station was placed in the same location in the aviary. Inert grain-based bait blocks were placed inside the station. The ground feeder with seed remained in the aviary. Sparrow activity was observed and recorded for 2 days. The bait station was then removed from the aviary, and the bait blocks were examined to determine the degree of pecking or chewing, possibly by rodents entering the station.

Chemical analysis

Chemical analysis was conducted at the National Wildlife Research Center in Ottawa, Ontario, Canada, by methods described in Albert et al. (2010). Briefly, 0.50 g of liver sample was ground in a mortar with about 5 g of anhydrous sodium sulfate (Fisher no. S420-3). The ground mixture was extracted with acetonitrile (EMD Omnisolv, AX0142-1, HPLC Grade) shaken vigorously for 15 min then centrifuged. The total supernatant was evaporated under a stream of nitrogen in a water bath. A portion was transferred into a test tube and evaporated to dryness. The sample was reconstituted in acetonitrile and cleaned up by solid phase extraction using one or two Sep-Pak plus tC18 conditioned cartridges (cat WAT036810) rinsed with acetonitrile. The eluate was evaporated to dryness, reconstituted in MeOH, and filtered through an Acrodisc® syringe filter with a 0.45 μm PVDF membrane. A dilution was made, and internal standards were added before analysis. Samples were analyzed by LC-MSMS, liquid chromatography mass spectrometry, (Waters Alliance 2695 HPLC). The method detection limit was 0.005 μg/g for diphacinone and difethialone and 0.002 μg/g for warfarin, brodifacoum, chlorophacinone, and bromadiolone. The standards were all analytical grade (>98 % purity). Recoveries at low and high level were >70 % for all compounds. The addition of a known amount of coumatetralyl (5 pg/μL—transition 291.00>140.90) and flocoumafen (1 pg/μL—transition 541.40>382.00) to each sample prior to the injection allowed monitoring for ion suppression. A blank containing 100 % methanol was injected between each sample to monitor for any possible contamination due to carry over.

Commercial use of rodenticides in British Columbia

We compiled data from the files of the British Columbia Ministry of Environment, Pesticides Branch, Surrey, BC over the period from 1995 to 2009 for the Lower Mainland region of the province. Data were confined to use by registered commercial applicators who are compelled by the law to provide summaries of their product usage.

Statistics

Differences among the numbers of small mammals tested at each site were tested using nonparametric *t* statistics. Trends in pesticide usage were examined by linear regression.

Results

Sample collection

A summary of the sample collections is provided in Table 1. Given the variation in sample sizes and scatter in the data, statistical testing options were limited. The number of rats trapped or found dead at farm A increased significantly after baiting with bromadiolone (Mann–Whitney *U* test (one-tailed); U=269, p=0.039, r=0.39). There were no differences in the number of voles trapped between farms A and B (Mann–Whitney *U* test; U=212.5, p=0.72, r=−0.08).

Rodenticide residues in prey and invertebrate samples

At farm A, two rats were collected in February prior to deployment of bromadiolone baits. The kill-trapped rat was analyzed for rodenticides, and no residues were detected. Over the period March 6 to March 15 following deployment of bromadiolone, 21 rats were collected in and around the main barn at farm A. Due to resource limitations, samples were prioritized for residue analysis. Of two rats live-trapped on March 7, one was analyzed for rodenticides and contained no detectable residues (Table 2). Of the 19 rats found dead, three were selected randomly for chemical analysis; all of which contained hepatic residues of bromadiolone ranging from 2.87 to 4.26 μg/g. Two also contained residues of brodifacoum at concentrations <1 μg/g.

The focus of this study was to determine exposure of nontarget small mammals; thus, all seven voles and two shrews trapped along the field transects at farm A were analyzed. None contained detectable residues of the rodenticide compounds included in the analytical method. A sample of slugs collected from bait stations at farm A had detectable residues of brodifacoum.

At farm B, no rats were collected. From a total of 16 nontarget small mammals trapped at this site following deployment of brodifacoum bait, only one sample, an adult male vole trapped 50 m from the barn in late March, had detectable albeit very high (18.6 μg/g) residues of brodifacoum and traces of bromadiolone (Table 2). A sample of 6 starlings (*S. vulgaris*) analyzed contained no residues; however, a song sparrow

Table 1 Details of sample collections for rodenticide residue analyses at four farms in the Fraser Valley region of British Columbia, Canada. Farms A and B were baited with rodenticide. Farms C and D were within an area of intensive poultry farming. See "Methods" section for more details

Location	Date	Organism	Number found dead	Trapped (in barn)	Trapped (50 m)	Trapped (100 m)	Collected (bait station)	Collected (on carrion)
Farm A (bromadiolone baited)	Feb. (pre-baiting)	Rat (*Rattus norvegicus*)	1	1				
	March (baiting)		19	2				
	April	Vole (*Microtus* spp.)			4	3		
	April	Shrew (*Sorex* spp.)				2		
	April	Slug (*Arion* spp.)					8	
	April	Snail (*Monadenia* spp.)					8	
	May	Beetle (*Carabid* spp.)					9	
Farm B (brodifacoum baited)	March–April (baiting)	Vole (*Microtus* spp.)			2	5		
		Shrew (*Sorex* spp.)			3	3		
		Deer mouse (*Peramyscus* spp.)			2	1		
	February	Song sparrow		1				
	February	Starling (*S. vulgaris*)		7[a]				
	March–April	Worm (*Eisenia* spp.)					8	
		Wasp (*Paravespula* spp.)					8	
Farms C and D (not baited)	March–April	Rat (*Rattus norvegicus*)			2			
	March	Vole (*Microtus* spp.)			3	5		
	March	Shrew (*Sorex* spp.)			1	1		
	May	Starling (*S. vulgaris*)		9[a]				
	March	Slug (*Arion* spp.)					8	
	May	Maggot (*Musca* spp.)						8
	May	Carrion beetle (*Dermestes* spp.)						8

[a] Shot in barn, see "Methods" for details

was also found in a small mammal trap at this location with detectable levels of brodifacoum.

At the farm C site, one of the two rats trapped at this site in the 50-m transects had residues of diphacinone and brodifacoum. Vole and starling samples had no detectable residues. A sample of carrion beetle also contained residues of diphacinone.

Rodenticide residues in barn owl pellets

No rodenticide residues were detected in the pooled samples of randomly selected barn owl pellets from either farm A or B (Table 3). Of the ten pellets from various locations in Delta and Surrey that were selected based on the presence of rat fur, nine contained detectable residues of at least one compound, difethialone. Seven of those pellets contained detectable residues of both difethialone and another compound tentatively identified as hydroxyl-chlorophacinone.

Use of bait stations by house sparrows

House sparrows readily entered the bait station to eat seed when there was choice between seed in the station and seed in the open. Sparrows also entered the bait stations containing only bait blocks. Evidence of bait block consumption was present with pecking marks and crumbs littering the bait station. There was no evidence from video recordings that small mammals had entered the aviary or gone into the bait stations. The marks on the bait blocks were consistent with pecking by birds and not gnawing by rodents.

Table 2 Mean rodenticide residues in sampled organisms from four farms in the Fraser Valley, British Columbia, Canada. Farms A and B were baited with rodenticide. Farms C and D were within an area of intensive poultry farming. See Methods section for more details. Values are expressed as ug/g wet weight (number with detectable residues in brackets)

Collection Site	Organism	N^a	N^b	Warfarin concentration	Diphacinone concentration	Chlorophacinone concentration	Brodifacoum concentration	Bromadiolone concentration
Farm A (bromadiolone baited)	Rat (*Rattus norvegicus*)	22	5	ND	ND	ND	0.01–0.15 (2)	2.87–4.26 (3)
	Vole (*microtus* spp.)	7	7	ND	ND	ND	ND	ND
	Shrew (*Sorex* species)	2	2	ND	ND	ND	ND	ND
	Slug (*Arion* spp.)	8	1 pool	ND	ND	ND	0.073 (1)	ND
	Carabid beetle (*Carabidae* spp.)	9	1 pool	ND	ND	ND	ND	ND
	Snail (*Monadenia* spp.)	48 s	1 pool	ND	ND	ND	ND	ND
Farm B (brodifacoum baited)	Vole (*Microtus* spp.)	7	7	ND	ND	ND	18.6 (1)	0.01 (1)
	Shrew (*Sorex* spp.)	6	4	ND	ND	ND	ND	ND
	Deer mouse (*Peramyscus* spp.)	3	3	ND	ND	ND	ND	ND
	Sparrow (*Melospiza melodia*)	1	1	ND	ND	ND	0.073 (1)	ND
	Starling (*S. vulgaris*)	7	4	ND	ND	ND	ND	ND
	Worm (*Eisenia* spp.)	8	1 pool	ND	ND	ND	ND	ND
	Wasp (*Paravespula* spp.)	8	1 pool	ND	ND	ND	ND	ND
Farms C and D (not baited)	*R. norvegicus*	2	2		0.64 (1)	ND	0.41 (1)	ND
	Vole (*Microtus* spp.)	9	9	ND	ND	ND	ND	ND
	Starling (*S. vulgaris*)	9	3	ND	ND	ND	ND	ND
	Carrion beetle (*Dermestes* spp.)	8	1 pool	ND	0.39 (1)	ND	ND	ND
	Maggots (*Musca* spp.)	8	1 pool	ND	ND	ND	ND	ND

[a] Number sampled, see "Results" section for details

[b] Numbered analyzed, see "Results" section for details

Table 3 Rodenticide residues in barn owl pellets collected from agricultural barn sites in the lower Fraser Valley region of British Columbia (microgram per gram wet weight)

Collection site	Disposition	Prey contents	N^a	N^b	Hydroxyl-Chlorophacinone	Brodifacoum	Bromadiolone	Difethialone
Delta (7..363)	Rat fur present	Mainly rat[c]	1	1	0.164	ND	ND	0.009
Delta (88ST35)	Rat fur present	Mainly rat	1	1	0.124	ND	ND	0.098
Delta (CWS33)	Rat fur present	Mainly rat	1	1	0.037	ND	ND	0.225
Surrey (7..764)	Rat fur present	Mainly rat	1	1	0.006	ND	ND	ND
Surrey (MP2)	Rat fur present	Mainly rat	1	1	0.177	ND	ND	0.096
Surrey (18..204)	Rat fur present	Mainly rat	1	1	0.024	ND	ND	0.310
Surrey (16..661)	Rat fur present	Mainly rat	1	1	ND	ND	ND	0.141
Surrey (70..764)	Rat fur present	Mainly rat	1	1	0.022	ND	ND	0.050
Surrey (MP5)	Rat fur present	Mainly rat	1	1	ND	ND	ND	0.121
Delta farm A (baited)	Random selection	Mixed, mainly vole[d]	10	2 pools	NR	ND	ND	NR
Delta farm B (baited)	Random selection	Mixed, mainly vole	10	2 pools	NR	ND	ND	NR

Rodenticides analyzed warfarin, diphacinone, and chlorophacinone (all non-detected), a compound tentatively identified as hydroxyl-chlorophacinone, brodifacoum, bromadiolone, and difethialone

[a] N number sampled

[b] N number analyzed

[c] *Rattus norvegicus*

[d] *Microtus* species

Commercial use of rodenticides in British Columbia

Use of eight rodenticides by licensed commercial applicators and farmers in the most heavily populated area of BC, the Lower Mainland, is shown in Fig. 1. There was a significant trend of overall increasing use of rodenticides over the time period 1995 to 2009 (r^2=0.44, df=12, p<0.05). Bromadiolone was the dominant compound used in every year, and there was a significant increase over the period 2005 to 2009 (r^2=0.59, p<0.05). Over the same time period, sales of brodifacoum decreased but the trend was not significant (r^2=0.66, df=3, p>0.05)

Discussion

The evidence presented here points towards targeted Norway rats as one and possibly the most important source of rodenticides to secondary consumers at least to species such as barn owls, barred owls (*Strix varia*), and/or great horned owls (*Bubo virginianus*), which will frequent or visit farmyards and nearby lands (Hindmarch et al 2012; Hindmarch et al. submitted). Because the original focus was on nontarget small mammals, we only analyzed five of the rats found dead or trapped at farm A. But given the large number of rats collected soon after baiting began and the consistently elevated residues of bromadiolone in three rats and lower concentrations of brodifacoum (probably from the farmer's earlier baiting) in two animals, it seems likely that all or most of the rats that died in that period would have had a rodenticide body burden. The finding of residues of a SGAR compound and possibly of a FGAR only in barn owl pellets containing rat fur further implicates targeted rats as a pathway to raptors. Pellets were shown in a previous study to be a means of eliminating part of a FGAR and a SGAR dose (Newton et al. 1994).

However, the presence of residues in a putative prey item does not necessarily mean exposure to raptors or other predators/scavengers. In a related study at similar agricultural settings in the Fraser Valley, BC, barred owls and great horned owls tended to consume primarily voles, mainly *Microtus townsendi*, throughout the year. The proportion of rats in the diet increased with degree of urbanization of habitat, presumably as the availability of voles decreased (Hindmarch and Elliott,

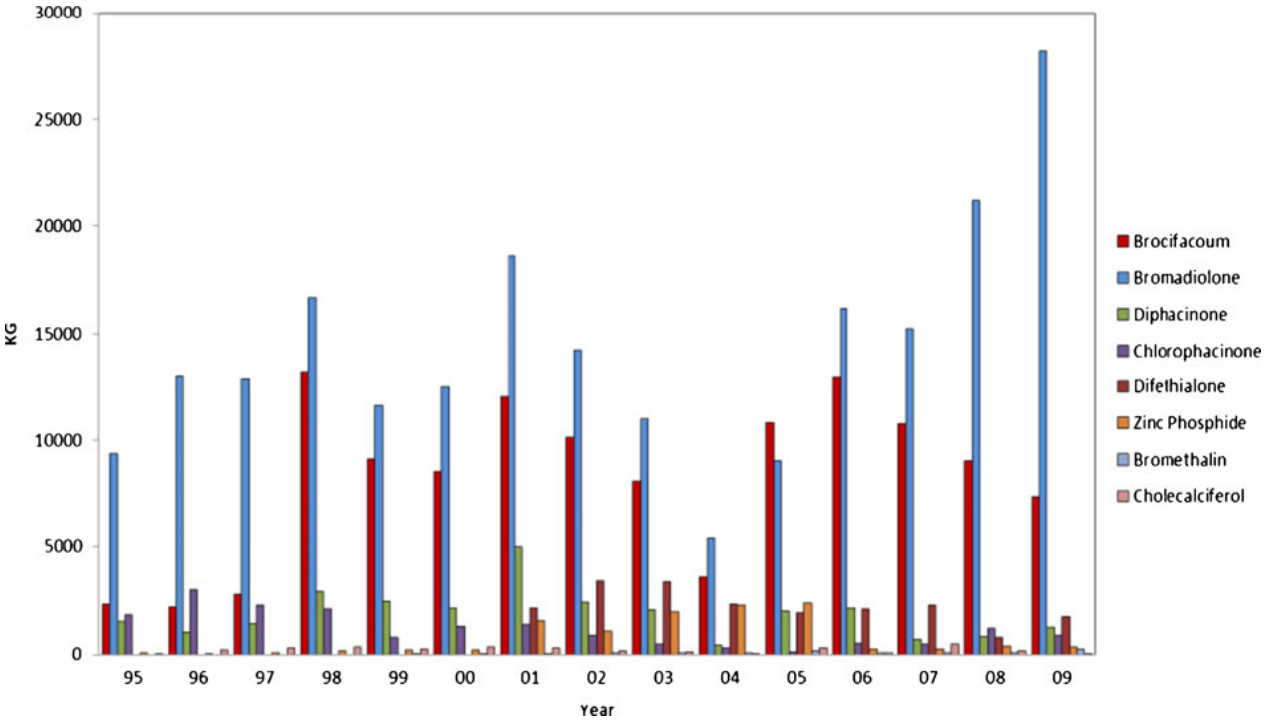

Fig. 1 Pesticide sales to commercial applicators, Lower Mainland Region of British Columbia, Canada, 1995 to 2009, as obtained from the provincial regulator's files

submitted). Similarly, during the breeding season, when voles are particularly abundant and available, there was little evidence of exposure of barn owl nestlings to rodenticides based on measurement of blood coagulation times (Webster et al., in prep). A study of polecats (*Mustela putorius*) in Britain reported a higher incidence of AR residues resulting from their seasonal pattern of feeding on farmyard rats during the autumn (Shore et al. 1996).

Seasonal variation in behavior of rats will also affect their availability as prey and likely the degree to which they are contaminated with rodenticide residues. A recent study of rat behavior in urban parts of Vancouver found that Norway rats tend to move from outside to indoor environments with the onset of cooler weather in the fall, thus causing evident infestations and efforts to control them, primarily using rodenticides (Himsworth et al. 2013). We did not monitor throughout an annual cycle; however, the rat infestation at farm A reportedly dated from the previous fall. At the farm C setting, we trapped two rats in April along the 50 m (from barn) transects, one contained residues of brodifacoum and diphacinone. It would appear that during the spring, at least some rats were using the surrounding areas to forage or to move between sites. Brodifacoum exposure presumably came from feeding in or near a barn or other building. Exposure to diphacinone in an agricultural setting could come from many registered uses, including field use for vole control (PMRA 2010). Use of or movement through fields by rats may also increase the potential for capture by a variety of raptors and possibly partially explains the relatively high incidence of rodenticide exposure in many other species, including great horned owls and red-tailed hawks (*Buteo jamaicensis*).

How rats or other small mammals behave when they are suffering from symptoms of rodenticide toxicosis is relevant to their availability to predators. In a study of the ecotoxicological consequences of use of brodifacoum to eliminate rats on a seabird colony, a proportion of the targeted rat population were shown to die above ground and be available to avian scavengers (Howald et al. 1999). Having no data on the population of the rat infestation at barn A, we do not know the proportion of the total that was retrieved. However, again, we found 19 dead animals indicating that some portion of the rodenticide-poisoned population died above ground and were available to be predated or scavenged.

The potential toxicity of the range of bromadiolone concentrations in liver of rats from farm A, approximately 3 to 4 μg/g, is not clear. Owls, for example, will

consume most of the carcass, and the whole body rodenticide content is difficult to determine as rats actively feeding at bait stations may have large quantities of undigested bait in their intestines (Howald et al. 1999). Bromadiolone is less toxic to birds than brodifacoum, although the data base is limited. The reported LD_{50} for bobwhite quail (*Colinus virginianus*) ranges from 138 to 170 mg/kg, as opposed to, for example, 3.3 mg/kg brodifacoum in the California quail (*Callipepla californica*) (US EPA, 2004). There is limited data on toxicity of bromadiolone to birds of prey. Mendenhall and Pank (1980) reported that it took 10 days of repeated feeding of bromadiolone poisoned rodents to barn owls to cause mortality, as opposed to 3 days for brodifacoum. However, recent reports by Rattner et al. (2011, 2012) on diphacinone toxicity to birds of prey show that raptors are more sensitive to coagulopathies caused by that compound than common avian test species.

At all farms, 32 small mammals were collected and analyzed for rodenticide residues and only one had detectable residues. Voles and other small wild rodents can inhabit vegetated areas adjacent to buildings and potentially encounter and consume rodenticide baits. However, in the present study, nontarget small mammals were all trapped in field transects and there was no evidence from other trapping that they regularly entered barns. Results from vole trapping indicate that populations were comparable among fields at the three farm sites, and thus, our sampling was consistent. One vole did contain high concentrations of brodifacoum, indicating that some individuals will enter barns and feed at bait stations. Thus, nontarget small mammals may also provide an exposure pathway. Other published studies of target and nontarget exposure to rodenticide bait have produced varying results. Lima and Salmon (2010) trapped a variety of native California rodents in habitats adjacent to urban and agricultural areas heavily baited with rodenticides. Only trace concentrations, mainly of SGARs, were found in those rodent samples. Cox and Smith (1990) placed bromadiolone baits at six farms in England and monitored rat and other small mammal populations for 6 weeks. There was no evidence of vole species feeding at bait stations or exposed to rodenticide. At indoor only baited farms, exposure of wood mice *Apodemus sylvaticus* was limited. At all outdoor baited farms, there was evidence of feeding and poisoning of wood mice and local populations of *A. sylvaticus* decreased. In another study, an FGAR, coumatetralyl, deployed outdoors in a variety of agricultural and game-feeding bait scenarios, resulted in extensive exposure of nontarget small mammals (Brakes and Smith 2005).

We found detectable concentrations of brodifacoum in a songbird sample, providing some evidence of a possible pathway to bird-eating species. Raptors such as *Accipiter* hawks, considered to be primarily bird-eaters, have been found with rodenticide residues (Stone et al. 1999; Thomas et al. 2011; Elliott unpubl. data). Further support for the potential for songbird exposure is provided by our video evidence of willingness at least of house sparrows to enter bait stations and to feed on pellets and even peck at paraffin bait blocks. There is a published account of songbirds (chaffinches, *Fringilla coelebs*) poisoned by an experimental application of the rodenticide, calciferol, in southern England, presumably by entering the bait stations to feed (Quy et al. 1995). A recent report documented poisoning of songbirds from feeding on diphacinone treated grain-based baits deployed for prairie dog control (Nimish et al. 2013). The case of songbirds is further problematic as there is some potential of secondary exposure via feeding on invertebrates that fed directly on bait or even tertiary exposure via necrophagus insects which fed on primarily poisoned rodents, but those pathways have not been well established (Howald 1997). Detectable residues of brodifacoum in a sample of slugs, and diphacinone in a sample of carrion beetles provide some evidence for invertebrates as a source by which these chemicals can contaminate and potentially biomagnify in food chains, possibly as suggested above by invertebrate-feeding birds. Exposure and even poisoning of invertebrates has been studied quite extensively in New Zealand (Eason et al. 2002; Hoare and Kelly 2006).

Until recently, consumers in Canada could purchase commercial anticoagulant rodenticide baits in the form of pellets, loose meal, paraffin blocks, or packet baits available from various companies and in varying concentrations (PMRA 2006). However, as of December 2012, new Canadian conditions of use came into effect. Products intended for the domestic retail market can only contain FGARs, warfarin, chlorophacinone, and diphacinone, and must be sold in or with a tamper resistant bait station. Agricultural and commercial control of commensal rodents must be by government licensed or sanctioned applicators only. Brodifacoum and difethialone continue to be available for use by licensed applicators, but can only be used inside

buildings and placed in tamper resistant bait stations or in locations not accessible to children, pets, livestock or non-target wildlife. Bromadiolone is available for outdoor used by licensed applicators, but must be deployed in tamper proof stations within 15 m of buildings and along fence lines within 100 m of buildings in securely fastened tamper resistant bait stations (PMRA 2010). Similar new restrictions have also been brought into effect by the US EPA (US EPA, 2011; http://www.epa.gov/pesticides/mice-and-rats/cancellation-process.html), but at the time of writing were subject to litigation.

These risk mitigation measures are intended to reduce contamination and poisoning of nontarget wildlife (as well as domestic pets and children) to the more toxic products containing brodifacoum and difethialone. Recent data on commercial use of rodenticides in southwestern BC shows a significant trend away from brodifacoum and towards bromadiolone usage. Bromadiolone is a less toxic alternative to the other SGAR products registered in Canada, although as discussed, the avian toxicity data base is limited. Areas of uncertainty in these new risk mitigation measures include the degree to which rats may feed indoors on bait formulations containing the more toxic SGARs, brodifacoum, and difethialone and move outside from unsealed buildings, particularly barns or sheds. We saw evidence of that happening in data from the present study. Other sources of uncertainty include the potential for technically noncompliant uses. Owners of small rural properties, such as hobby farms, may obtain brodifacoum or difethialone from agricultural supply stores and deploy them around their properties either outside or in unsealed buildings.

Outreach and education (stewardship) programs, such as those in effects in other countries (e.g., in the UK http://www.farminguk.com/news/Stewardship-the-way-forward-for-anticoagulant-rodenticides-in-the-UK_26037.html), backed up by enforcement programs are likely the best way to address such issues. Recently, we conducted a survey of rodenticide usage among farmers in the Fraser Delta. The main objectives were to evaluate the role farmers as part of the process by which anticoagulants enter the ecosystem and assess farmers' knowledge level and attitudes surrounding rodenticide usage (Hindmarch, unpubl. data). Overall, farmers were well aware of the precautionary measures needed to reduce rodent populations on their property. However, the survey documented a lack of knowledge regarding correct use of rodent control products containing SGARs. In particular, among berry farmer, 28 % (n=33) of the respondents reported noncompliant use in berry fields of products containing brodifacoum or difethialone.

In California, noncompliant use of rodenticides by outdoor (and illegal) marijuana grow operations has emerged as a source of exposure to nontarget wildlife on public lands even in remote locations (Gabriel et al. 2012). Similar marijuana grow operations are widespread in British Columbia (Clare et al. 2010), but possible illegal pesticide usage has not been investigated.

Conclusions

Under the conditions of the present study of late winter and spring in agricultural settings on the south coast of BC, Canada, targeted rats provided the greatest potential pathway of second-generation rodenticides to wildlife predators. However, there was evidence of small mammals, songbirds, and invertebrates as possible exposure pathways to secondary consumers.

Recommendations of further research and surveillance include: (1) more investigation of exposure of both target and nontarget small mammals (and possibly birds) which are potential prey of vertebrate predators and scavengers. Studies should be conducted throughout at least one annual cycle in conjunction with active bait deployment; studies should consider the potential transport of rodenticide residues from indoor use in unsealed buildings, but should probably focus on outdoor use of bromadiolone, which in Canada is permitted along fence lines; (2) ongoing surveillance of nontarget wildlife mortality and strategic monitoring of residues; and (3) generation of an improved data base for comparative avian toxicity of bromadiolone.

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