



Exposure of nontarget white-footed mice (*Peromyscus leucopus*) to second-generation anticoagulant rodenticides in an urban context

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Received: 24 April 2026 / Accepted: 8 October 2026

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Abstract The pathways of nontarget exposure to anticoagulant rodenticides (ARs) are poorly understood and have yet to be examined in Ontario, Canada. We sought to determine if second-generation anticoagulant rodenticide (SGAR) baiting practices used by pest management professionals targeting commensal rodents may be causing rodenticide spillover into nontarget rodents in urban wildland areas, which could act as a vector of ARs to predators. We used snap traps to capture white-footed mice (*Peromyscus leucopus*) in urban wildland areas of Toronto and Vaughan, Ontario, near ongoing rodenticide baiting programs utilizing bromadiolone and difethialone products. We detected bromadiolone in only nine individuals and difethialone in two individuals trapped out of a total of 111. These exposed white-footed mice were caught at only five out of seven study sites. AR concentrations were between 0.008 and 0.03 µg/g of liver tissue for bromadiolone and 0.008 and 0.023 µg/g for difethialone, which may be sublethal for raptors. SGAR residues were detected in

individuals caught at 0 m, 5 m, 20 m, 40 m, 70 m, and 100 m from active rodenticide stations. SGAR-positive mice did not differ significantly from SGAR-negative mice in terms of sex, age, body condition, distance to the AR source, capture date, or capture site. Four exposed individuals were in “poor” body condition. This indicates that the pest management industry’s use of rodenticides in these urban and suburban settings is likely causing some degree of nontarget spillover in Toronto and the Greater Toronto Area. Additionally, nearby residential SGAR usage may have accounted for some of these exposures.

Keywords Rodenticide · Exposure · Nontarget · Difethialone · Bromadiolone · *Peromyscus*

Introduction

Anticoagulant rodenticides (ARs) are the gold standard for low-maintenance rodent control programs worldwide (Endepols, 2002). ARs rose to popularity because of their ability to prevent rodent bait shyness, through delayed effect lethality by means of vitamin K deprivation and subsequent hemorrhage (Rost et al., 2004). Numerous taxa including mammals (Hindmarch et al., 2025), birds (Regnery et al., 2024), invertebrates (Williams et al., 2023), reptiles (Lettoof et al., 2020), amphibians (Rowley et al., 2024), and even fish (Kotthoff et al., 2019) are exposed to these products. Predators that consume rodents exposed to

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10661-026-16038-x>.

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ARs can thus be secondarily exposed (López-Perea et al., 2019; Sainsbury et al., 2018). Second-generation anticoagulants (SGARs) are currently the most popular class of ARs and are known for their propensity to bioaccumulate and magnify in higher trophic levels (Jaramillo-Q. et al., 2024). The exceedingly toxic SGARs were designed specifically to control “warfarin-resistant” commensal rodents, namely, rats *Rattus* spp. and house mice *Mus musculus* (Boyle, 1960), which evolved insusceptibility to the first generation of these poisons (Rost et al., 2004). However, the exact pathways of nontarget exposure are not well understood (Elmeros et al., 2019), making the creation of responsible rodenticide usage programs particularly difficult. This is a particular issue of concern in Canada, where research on rodenticide resistance, predator exposure, and prevention is sparse (Elliott et al., 2014; Thomas et al., 2011; Thornton et al., 2022).

Peridomestic rodent species use human habitations and structures intermittently (Douglass et al., 2006), unlike commensals, which are characteristically synanthropic and often reliant on human activity (Weissbrod et al., 2017). Peridomestics may be the main focus of rodent control programs or end up as bycatch, and they may also occupy different spatial niches where they are sympatric with commensals (Frye et al., 2021). Given that the majority of pest management professionals (PMPs) in Canada have few commercial first-generation anticoagulant (FGAR) alternatives for rodent infestations, it is likely that peridomestic, native nontarget rodents (NTRs), like *Peromyscus* spp. (deer and white-footed mice), are heavily exposed to SGARs, as is the case with nontargets in other regions (Elmeros et al., 2019). *Peromyscus* mice can move great distances from infested buildings quickly (Calisher et al., 1999) and may have a preference for natural landscapes (Hummell et al., 2023) where predators may be more abundant, making them a main pathway of secondary exposure (Geduhn et al., 2016). They may also be the preferred prey of Ontario’s predators (Baker & Brooks, 1981). Commensals are the sole target of SGARs in Canada and are often less available to predators after poisoning because of their stricter synanthropy; they are often subterranean and more abundant inside and around human structures where predators may be less prevalent (Geduhn et al., 2014; Kaufman & Kaufman, 1990; Walther et al., 2021). In addition, although not

registered or designed for the control of native NTR, PMPs can still perform general rodent control outside and around structures and fence lines using bromadiolone in Canada (but not with any other SGARs, Health Canada, 2012), possibly exposing *Peromyscus* spp. or other nontargets to this chemical (Hindmarch et al., 2018). PMPs and farmers are the only parties authorized to apply SGARs in structural settings in Canada (Health Canada, 2012). Most studies of primary and secondary nontarget exposure focus on agricultural use patterns (Tosh et al., 2012), but which of these two parties may be more responsible for AR spillover is unknown.

Nontarget SGAR exposure research often involves testing rodents captured near rodenticide usage sites for SGAR exposure rather than predator carcasses. This allows scientists to approximate the secondary poisoning risk to nontargets in different contexts (Hughes et al., 2013). This may provide a less biased gauge of risk associated with AR usage practices (Tosh et al., 2011). Variation in exposure risk to rodent predators or scavengers can be affected by the rodent species being exposed, which may be related to landscape factors, as well as the distance to the AR source (Geduhn et al., 2014, 2016). Males and sexually mature adult rodents may be exposed more often or to greater concentrations of ARs as a result of their larger home range and spatial foraging proclivity (Gaitan & Millien, 2016; Metzgar, 1973; Wolff, 1985), which may change seasonally (Getz, 1961). A greater quantity of rodenticide bait at an extermination site may correspond with higher exposure risk, especially among rodents found closer to bait stations (Tosh et al., 2012). Nontarget *Peromyscus* spp. mice may enter structures (Douglass et al., 2006; Frye et al., 2021) and may be mistaken for house mice, or SGARs may be deployed before visual species identification occurs (Hindmarch et al., 2018). *Peromyscus leucopus* may have large home ranges of over 7000 m² (Gaitan & Millien, 2016) and may leave their urban forest habitats to enter structural rodenticide stations alongside commensals (Baldwin et al., 2014).

Canadian PMPs use locked, tamper-proof bait stations to house rodenticides used for rodent control (Richardson, 2026). These stations typically utilize paraffinized bock baits containing the SGAR bromadiolone, formulated at 50 ppm (0.005%), for exterior rodent control in Canada, given bromadiolone’s status as the only SGAR permitted for exterior usage (Health

Canada, 2012). Such bromadiolone baits may be used for interior control, along with difethialone (0.0025%) and brodifacoum (0.005%), which are registered for indoor usage only. According to typical labels for these SGAR products, 1–2 baits are to be set every 8–11 feet for house mice and 3–16 baits every 15–30 feet for rats (Health Canada, 2026). Permanent rodenticide baiting, such as that which occurs year-round in Canada on commercial properties or in agricultural areas (Hindmarch et al., 2018), is cited as a principal cause for nontarget rodent and predator exposure to ARs in Europe. Permanent baiting may be most dangerous in agricultural settings, likely as a result of the exposure of nontargets like *Apodemus* spp. (field and wood mice, Elmeros et al., 2019; Endepols et al., 2015; Geduhn et al., 2016). These NTRs are known to subsequently retreat to open fields or wildland areas after consumption of SGAR baits, thereby acting as a “vector” through which rodenticides enter predator-friendly landscapes (Tosh et al., 2012). Various commercial and food handling establishments often do or are required to employ professional pest management services in Ontario under regulation 493/17 (Government of Ontario, 2017), and these programs commonly utilize some form of rodenticide treatment for the duration of the contract. Canadian SGAR labels allow for many baits to be used “until activity ceases” (Health Canada, 2026). The percentages of sampled NTR exposed to rodenticides vary in studies of secondary poisoning pathways but are typically between roughly 12 and 48% (Brakes & Smith, 2005; Endepols et al., 2003; Walther et al., 2021). No exact rate of exposure appears to be associated with hazard levels that put predator species’ populations at risk, but patterns related to increased predator exposure rates can be used to correct SGAR usage programs through ongoing rodent species assemblage monitoring, intermittent baiting, or pulse baiting (CRRU 2018).

We sampled white-footed mice *Peromyscus leucopus* from fragmented urban forests adjacent to commercial buildings employing permanent SGAR baiting to determine if this nontarget species is being exposed to SGARs. We hypothesized that nontarget white-footed mice would be differentially exposed to SGARs as a function of their sex, age class, and distance from rodenticide bait stations in urban landscapes. We hypothesized that males relative to females, and mature individuals relative to immature individuals, would be exposed more often and

to higher concentrations of AR chemicals, especially when closer to exterior bait stations, as a result of their larger home ranges and foraging proclivities (Gaitan & Millien, 2016; Metzgar, 1973; Wolff, 1985). We also hypothesized that bromadiolone would be the most commonly detected chemical in white-footed mouse liver tissue, due to its popularity (Elliott et al., 2014) and being registered for outdoor usage (Health Canada, 2012).

Methods

Field methods

All trapping was conducted under an Ontario Ministry of Natural Resources and Forestry (OMNRF) Wildlife scientific collector’s permit (WSCA 1106092) and the approval of Laurentian University’s Animal Care Committee. White-footed mice were captured by snap trapping (lethal sampling) using Victor™ M7 snap traps inside secured bait stations (Bell Laboratories Protecta EVO® EXPRESS™). Stations were modified with sheet metal to prevent the entry of larger animals through the ingress point (similar to Bell et al., 2019). Similar trapping methods have been used previously for detecting rodenticide residues in small mammals available to predators (Brakes & Smith, 2005; Sage et al., 2008; Lima and Salmon 2010; Winters et al., 2010; Tosh et al., 2011; Elliott et al., 2014; Geduhn et al., 2016; Desvars-Larrive et al., 2017; Elmeros et al., 2019; Murray & Sánchez, 2021; Walther et al., 2021; Mahamat et al., 2024).

Seven “wildland” sites (municipal parks composed of mixed deciduous forests) across Toronto and Vaughan, Ontario, adjacent to commercial buildings with ongoing, permanent exterior rodenticide programs, were selected as sites for trapping. Rodenticide program details were confirmed through phone call discussions or emails with property managers. The general days or weeks when bait stations were visited by PMPs (e.g., every first and third week of the month) were provided and were used to approximate the elapsed time from rodenticide station checks and refills relative to sampling days. Sites 1, 2, 3 and 4 were in Vaughan, site 5 was in Scarborough, site 6 was in North York, and site 7 was in Etobicoke (see Supplementary Material 1). Five sampling

sites were owned by the Toronto and Region Conservation Authority and two were owned by the City of Vaughan. Written permission for trapping was obtained from both parties before the sampling period began on June 28th, 2024. Rodent trapping at each site occurred once every 3 weeks until the end of August, to obtain rodent carcasses at different times in relation to scheduled PMP site visits, when rodenticide refills may occur. However, we could not confirm with property managers or pest control companies the exact amount of bait consumed or replenished. A total of three trapping events, when traps were set and checked the following day, occurred per site, and overall, there were 334 trap nights.

At each site, a transect was created as the basis for a trapline using ArcGIS® Online. The transect was drawn perpendicular to the foundation wall of the building where AR was in use, and trapping stations were placed at specific, uniform distances (see Supplementary Material 2). Coordinates were stored on a Garmin™ GPS map 64s and used for setting stations in the field. Distances chosen were 20 m, 40 m, 70 m, and 100 m, approximating the distance intervals used in previous studies (Elliott et al., 2014; Elmeros et al., 2019). The width of chosen wildland areas restricted our ability to have each of these exact distance points at each site. The 20-m distance was used for sites 2, 3, 6 and 7; 40 m and 70 m for all sites; and 100 m for sites 1, 2, 4, 5, and 6. Trap stations were also placed at the edge of the wildland area (either 5, 15, 20, or 40 m from the base of the building's foundation) at all sites. In one case, the edge of a wildland was 31 m from the rodenticide use building but was still incorporated into the 40 m category for analytical purposes. Finally, to quantify urban lands within each trapping location on the transect line, the percentage of developed space (buildings, hardscapes, and manicured lawns) was assessed at each distance location within the estimated home range size of *P. leucopus* (7600 m² circular area, Gaitan & Millien, 2016).

Two trapping stations were placed within 2 m of each coordinate location, with entry holes facing north–south, and labeled “A” or “B.” By extension, traps inside were labeled N or S (north/south). Stations were left in place with unset traps baited with peanut butter and hamster feed for between 1 and 6 weeks. Traps were set on weekends only (Fridays

or Saturdays) and checked the following day within 24 h (Saturday/Sunday) and left unset until the next trapping period. All sites had four trapping locations (i.e., four distance intervals), eight trapping stations (two per distance interval), and 16 individual mouse traps (two per trapping station), with the exception of site 1, which had three locations, six stations, and twelve traps. Trapped rodents were removed, and sex, age, body weight, and length were recorded. Carcasses were placed in labeled storage bags detailing exact date and location and frozen on the day of capture.

Laboratory methods

Only adult (mature and immature) white-footed mice, with testes present or perforated vaginas, were used for liver analysis. Liver samples were sent to the University of Guelph Animal Health Lab for liquid chromatography tandem mass spectrometry testing (methods similar to Thornton et al., 2022). A Shimadzu LC30AD chromatography system combined with a Sciex 4000 ESI-MS/MS detector was used, with a XDB-C18 5 µm 2.1 × 150 mm column and Synchronis C18 5 µm 10 × 2.1 mm guard column (see Smith et al., 2017). A method blank with six nonzero calibration levels was analyzed at the beginning and end of all analytical runs for calibration, and the average of these was taken for quantification. A correlation coefficient of at least 0.95 was used as a baseline for quadratic fitting, and a blank spiked with 100 ppb AI was analyzed for at least 52–157% recovery.

Anticoagulant rodenticide analytes included the following, with limits of detection and quantification (ppb), respectively, in brackets: brodifacoum (19, 67), bromadiolone (6, 21), difethialone (1, 4), flocoumafen (2, 5), difenacoum (2, 7), warfarin (2, 5), chlorophacinone (2, 5), diphacinone (6, 20), pindone (3, 11), dicoumarol (1, 3), coumatetralyl (1, 3), coumachlor (3, 8), coumafuryl (14, 50), and valone (3, 8). Only liver residues were analyzed and concentrations given in µg/g wet weight. Metabolites were not analyzed, meaning that recent exposures may not have been detected due to the time lapse needed after consumption for AR residues to accumulate in liver tissue.

Statistical methods

Contingency tables were used to conduct Fisher's tests to compare frequencies of exposed and unexposed white-footed mice based on sex, age, or capture site. Length (millimeters) and weight (grams) were graphed and correlated using linear regression to calculate body weight residual as a metric of body condition, as a function of the individual's distance from the least squares line of best fit (i.e., negative or positive residual). This value was then used as a predictor of exposure. Nonparametric Mann–Whitney *U* tests were used to compare body morphometry measurements and environmental factors that may relate to exposure at the capture site. Factors used for predicting rodenticide exposure and concentration included rodent sex, rodent age, the number of rodenticide stations at the adjacent building, percentage of developed land within the estimated home range, distance from AR source, and Julian date of capture. We compared these metrics between exposed (AR positive) and unexposed (AR negative) individuals, exposed simply meaning that liver residues were not detected. Other bodily AR metabolites were not analyzed. Effect sizes were also calculated. Linear regression was also used to compare these variables to concentration of AR chemical detected and checked for heteroskedasticity and normality of residuals with Breusch–Pagan and Shapiro–Wilk tests, respectively. Principal component analysis was then used to capture covariation between these variables, and regression was performed with PC1 against the AR concentrations of exposed individuals. Logistic regression was used to determine if the number of bait stations at a site had any relationship with exposure status

(exposed or unexposed). Statistical analyses were performed in R Studio (R Core Team 2024, R version 4.4.2). Packages used included “lme4” and “RStatix.”

Results

Trapping success for all seven sites was 47% (158 captures/334 trap nights). A total of 11/111 adult white-footed mice tested were positive for anti-coagulant rodenticides (9.9%, see Table 1), all of which were second-generation compounds (SGARs), either difethialone (two mice) or bromadiolone (nine mice). Exposed mice were caught at sites 1, 3, and 4 (Vaughan), as well as 6 and 7 (Toronto) (see Supplementary Material 1).

Concentrations of bromadiolone ranged between 0.008 and 0.03 µg/g of liver tissue (av. 0.015 µg/g) and were 0.008 and 0.023 µg/g for difethialone. Difethialone was only found at sites 3 and 4 (both in Vaughan, borders separated by Rutherford Road by < 100 m). Never was more than one SGAR detected in a single individual mouse. All sites were mixed deciduous-coniferous forests, and there was no difference in the proportion of exposed individuals caught at these five sites (Fisher's exact $p=0.39$). Two or more exposed individuals were never caught at the same distance at a single site, meaning that a single trap box or trap never caught more than one exposed mouse throughout the duration of the study period. One exposed *P. leucopus* (exposed to difethialone) was caught 100 m from a rodenticide use building, and one (exposed to bromadiolone) was caught directly beside a bait station on one occasion (0 m).

Table 1 Comparison of parameters for rodenticide-exposed (Pos.) and unexposed (Neg.) individual white-footed mice, based only on liver residue analysis ($n = 111$). Bromadiolone and difethialone-positive mice combined ($n = 11$). The follow-

ing parameters were compared between exposed and unexposed individuals: (a) body length (mm), (b) weight (g), (c) body weight residual (g), and (d) %Urban land within estimated home range at the capture site

	AR positive ($n = 11$)		AR negative ($n = 100$)		MW p value	Effect size (r)
	Median	(IQR)	Median	(IQR)		
Body length (mm)	94	(12.5)	90	(8)	0.82	0.02
Weight (g)	25	(7)	21	(5)	0.22	0.12
Body weight residual (g)	0.48	(3.37)	0.07	(2.89)	0.11	0.15
%Urban land	15.79%	(38.55%)	13.71%	(39.62%)	0.29	0.08

Fisher's exact test for exposure rate of mature and immature males and females = 0.45

Of the exposed individuals, two were immature adult females (18%), two were mature adult females (18%), and seven were mature adult males (64%). There was no significant difference in the proportion of exposed vs unexposed males and females (Fisher's exact $p = 1$) or matures and immatures (Fisher's exact $p = 0.68$). Mann–Whitney U tests were used for unifactorial comparisons of exposed ($n = 11$) and unexposed ($n = 100$) individuals, and there was no difference between these groups in terms of body length, weight, body condition, or the percentage of urban land at the capture location (all $p > 0.05$, Table 1). Four exposed mice were in “poor” condition; that is, they weighed less than expected given their body length. Two individuals (18%) were caught within 1 week of a scheduled PMP site visit (when station checks and refills occur), five (45%) were caught 2 weeks after, two (18%) were caught 3 weeks after, and two (18%) were caught 4 weeks after (these were the only four categories).

Predictors were incorporated for PCA (urban land %, sex, age class, Julian date of capture, distance from AR building), and there was no significant correlation between exposure concentration of bromadiolone and principal component one ($p = 0.15$). Difethialone-exposed mice ($n = 2$) were not incorporated in this analysis, because this SGAR is formulated at 0.0025%, as opposed to 0.005%, in rodenticide baits (Health Canada 2019). PC1 explained 66.04% of the variance across %UrbanLand (0.91), sex (0.55), age (−0.95), Julian date (−0.85), and distance (−0.72). There was also no correlation between exposure concentration or any of the predictors singly (all $p > 0.05$). Finally, the number of bait stations at each site was not related to AR exposure status of mice (logistic regression $p = 0.16$, see Supplementary Material 3).

Discussion

We attempted to determine factors associated with SGAR exposure in a nontarget urban rodent species (*Peromyscus leucopus*) resulting from ongoing commercial SGAR usage, in Toronto and Vaughan, Ontario. This is one of the few observational, in situ examinations of real-world AR spillover from urban commercial operations, as opposed to agricultural studies (Geduhn et al., 2016) and controlled studies

where researchers simulate AR usage by placing their own bait stations (Elmeros et al., 2019), or through modeling (Topping & Elmeros, 2016). Although *Peromyscus* spp. are known to use bait stations set for the control of commensals (Burke et al., 2021), wild-caught *Peromyscus* spp. appear to have only been previously tested for rodenticide residues twice: Elliott et al. (2014) found no residues in three *P. maniculatus* total on farms in British Columbia, and Lima and Salmon (2010) found trace levels (sample size and details not given). Our data show no significant correlates or predictors for exposure, or differences between exposed and unexposed mice, possibly due to low sample size which diminished statistical power (see Table 1). We could not determine when or exactly where these mice were exposed to the chemicals detected. Other tissues, as well as metabolites, were not tested, meaning there may have also been some individuals exposed recently before capture that had not accumulated residues in liver tissue, thus leaving them out of our overall sample.

Our overall exposure prevalence was relatively low (9.9%, 11/111), and the most commonly detected chemical was bromadiolone (9/11). Comparable studies using rodent liver tissue as an AR detection matrix have typically found exposure rates between 12 and 48% in peridomestic and nontarget rodents, namely, *Apodemus* spp., *Clethrionomys* spp., *Microtus* spp., and *Arvicola* spp. (Brakes & Smith, 2005; Elmeros et al., 2019; Geduhn et al., 2014; Sage et al., 2008; Tosh et al., 2011). However, these studies were all conducted in agricultural settings, which have less contrast with surrounding natural landscapes than urban areas which may deter rodent travel and foraging (Ortiz-Jimenez et al., 2025). Human activity in urban areas may increase *P. leucopus* giving-up density (Persons & Eason, 2017), and thus decrease foraging. Dense vegetation in urban forests may even decrease *P. leucopus*' usage of nearby yards and human structures (Hummell et al., 2023). NTR may therefore be more receptive to rodenticide bait stations in agricultural regions (Patergnani et al., 2010). Additionally, despite liver tissue typically holding the majority of AR residues (Rached et al., 2022), using homogenized whole-body tissue may have yielded much higher rates of exposure (Sage et al., 2008), since this method accounts for residues that may be on the body or in the digestive tract alone. Our effect sizes for unifactorial comparisons were all well below

the threshold even for small effects (Table 1), indicating that exceedingly large sample sizes would be needed to show significant differences between body morphometrics and urban land within estimated home ranges of exposed and unexposed mice.

A previous study on raptors collected throughout the province of Ontario also showed bromadiolone to be the most prevalent chemical detected, followed by brodifacoum (which we did not detect) and difethialone, but at much greater concentrations and prevalence rates than we present here (Thornton et al., 2022). Considering the biomagnifying nature of SGARs (Buckley et al., 2024; López-Perea et al., 2019) and the trophic level of raptors relative to *Peromyscus* spp., this is axiomatic. However, in contrast with our results, it may also indicate that raptors could be a vacuum for AR poisoned rodents, selectively choosing them (Cox & Smith, 1990), or at least, consuming more proportionally than a trapping study could ever detect. A previous study does suggest that raptors may even prefer to hunt in regions where small rodents are being poisoned (Vyas et al., 2017).

Bromadiolone was detected from *P. leucopus* caught in close proximity (0–20 m) and further away (40, 70, and 100 m) from active bait stations, with an average concentration of 0.015 µg/g. Apparently, no bromadiolone LD50 values are available for *Peromyscus leucopus*, but considering previous studies have shown 100% susceptibility of *P. maniculatus* to 0.02% (200 ppm) chlorophacinone (a far less persistent FGAR) after 3 days of feeding (Marsh et al., 1977), the concentrations we detected may have been lethal and are comparable to those found for NTR in previous studies (Geduhn et al., 2014). These doses could affect a great number of wildlife that may be secondarily exposed, including raptors. Different taxa are variable in their susceptibility to ARs (Eckel et al., 2020; Lemus et al., 2011), and residues may persist for some time after the exposure in an otherwise healthy animal. However, thresholds for sublethal and lethal effects in raptors are reportedly between 0.1 and 0.2 µg/g (Carromeu-Santos et al., 2026). We also found no significant correlation between distance from the source and concentration of AR chemical in liver tissue. However, regarding brodifacoum (formulated at either 50 or 25 ppm in Canada), liver residues rarely exceed 1 µg/g, and the highest concentrations are typically found during the

AR baiting period (Geduhn et al., 2014). Concentrations are also typically higher in target species than nontargets (Geduhn et al., 2014; Walther et al., 2021) and at closer distances to the source (Elmeros et al., 2019) but not always (Tosh et al., 2012).

The fact that our trapping success, a proxy for rodent density (Kaboodvandpour et al., 2010), was relatively high at all sites (47% catch rate/334 trap nights) suggests that rodenticide programs at the adjacent usage buildings may not be decreasing native rodent density. Thus, the SGARs used may not be spilling over enough to threaten predators. However, we do acknowledge that our trapping was not designed to estimate population density or size of these rodents. In contrast, prior studies showed that prairie dog *Cynomys ludovicianus* control with chlorophacinone did not affect *Peromyscus* density (Deisch et al., 1990; McCaffrey et al., 2009), and others have shown that similar species' populations (*Apodemus* spp.) can recover quickly from declines resulting from mass poisoning (Bell et al., 2019), meaning that white-footed mice may persist despite anticoagulant pressure. Seasonal feeding and movement patterns may change this, however, and given that our trapping only took place in the summer, it may be possible that exposure rates, rodent density, and associated secondary exposure risk could change as a function of seasonal factors (Colvin, 1984; Tosh et al., 2011; Geduhn et al., 2016). Commercial facilities have previously noted that exterior AR stations may go untouched by target and nontarget species alike for long periods of time, possibly due to the absence of rodents, spatial or temporal rodent feeding preferences, and feeding competition (Hindmarch et al., 2018), and this, along with rodent species assemblages (Geduhn et al., 2016), may even vary with the seasons.

Rodents are known to remove and cache rodenticide baits (Brakes & Smith, 2005), which could also explain our lower exposure prevalence rates, as they may continue to feed on them at a steady rate after their removal from the bait station or abandon them. This could give the impression of lower exposure initially but may result in exposure even after baits have been removed (Brakes & Smith, 2005). Rodents may not consume this cached rodenticide for some time, and hence, depending on changes in food availability, exposure rates may rise when cached food provides the most attainable form of food (Brakes

& Smith, 2005). However, given that the risk of secondary exposure is said to be high in heterogeneous urban-wildland landscapes (Burke et al., 2021; Lohr, 2018; Silveira et al., 2024), our low exposure rate may reflect that snap trapping is not a representative sampling method. Considering that lethal snap traps have previously been used to sample rodents for AR assays and yielded higher rates of exposure (Geduhn et al., 2014) and the usefulness of snap traps in studies of rodent density and disease (Herawati & Purnawan, 2021; Karell et al., 2010; Marquez et al., 2019; Peitz et al., 2001), it would seem that snap traps are a reliable and representative sampling tool. Our low exposure rate could also indicate that patterns of urban exposure are different in Ontario than in other regions. This could possibly be due to SGAR restrictions or use practices (Eckel et al., 2020).

Brodifacoum may not be favored by industry professionals and may be used less often than bromadiolone in Canada (Elliott et al., 2014), which may explain its absence from our samples. However, brodifacoum is typically the most frequently detected chemical in nontarget rodents, corresponding with its half-life being recorded as greater than any other AR available in many regions (Eckel et al., 2020), including Ontario (Thomas et al., 2017). This chemical may also be due more to its greater popularity and more frequent usage in other regions. Few baits available in Canada contain brodifacoum, none of which are in soft bait or liquid formulation (which may increase palatability to rodents), and these can only be applied indoors (Health Canada, 2012). This may explain why brodifacoum was not detected in our samples and why difethialone was only detected twice, at two adjacent sites from mice caught within 1 km of each other. However, the frequent detection of brodifacoum in Ontario raptors (35%, Thornton et al., 2022) suggests that this exposure may be occurring by means of a different landscape or species exposure pathway.

Despite being an “indoor-only” chemical (Health Canada, 2012), difethialone was detected 100 m from a rodenticide use building at site 4, but could also have originated from residential usage at a nearby townhome complex less than 100 m from the study building (Bartos et al., 2011), or illegal outdoor usage (Thompson et al., 2014). Although the half-lives of all SGARs may be similar (in liver or plasma, Fisher et al., 2003), difethialone may actually be less persistent than bromadiolone (~118 days vs. 318 days

liver half-life, Seljetun et al., 2020), despite having lower LD50 for target commensal rodents (Baxter et al., 2022). This could explain its lower detection prevalence. In contrast, bromadiolone, which has likely been the most popular SGAR among Canadian commercial operators since the late 1990s (Elliott et al., 2014), is available in numerous block, pellet, and soft bait products for indoor and outdoor usage. It was detected much more frequently than difethialone in our samples (9/11 positives), though still at a low rate. Our detection of difethialone at two sites (3 and 4) indicates that white-footed mice may move indoors, consume SGAR chemicals which they should not be exposed to, and then travel some distance from the source before death, posing a risk to predators, assuming that difethialone was applied legally at these sites. The fact that no FGAR chemicals were detected, and that no exposed individuals tested positive for multiple chemicals, indicates that homeowners and commercial operators are not using such products, *P. leucopus* is not receptive to them or exposed to them, or that they are perhaps able to metabolize them (Poche & Mach, 2000) below the detection levels of the equipment used. It may also be the case that a larger sample size would have allowed us to detect FGARs.

A recent study conducted in California determined that commensal rodents may be a main vehicle of coyote *Canis latrans* AR exposure detected in the state, in spite of heavy AR restrictions (Stapp et al., 2025). However, given warmer year-round climates in the American west, the preponderance of black rats *Rattus rattus* in California, and the fact that we detected only one commensal at any of our trapping sites (1/254 total captures) suggest commensals may be able to uniquely capitalize on the California climate. They may become abundant in areas frequented by predators and act as a vector of rodenticides (Stapp et al., 2025). The only commensal rodent we captured (house mouse) was trapped near a fenceline in a park, adjacent to a building where rodenticide usage could not be confirmed, for which reason this sample was not tested. Alternatively, the parking lots separating wildland areas from bait stations at our sites may have acted as a deterrent (Krijger et al., 2017), confining most *P. leucopus* to the wildland areas, which may be a common occurrence in urban regions (Harris and Munshi-South 2017). Given that no exposure was detected at site 5, where there was a unique rise

in elevation and a drop of over 100 m down a prominent slope, elevation may have some influence on the movement of rodents between wildland and urban areas, perhaps acting as a barrier against exposure. However, some degree of elevation drop was present at all other sites.

The fact that exposed rodents were caught at every distance location from identified rodenticide stations suggests that the likelihood of exposure may be more influenced by season, sex/age demographic, or body morphology than proximity to the chemical source. Given that only 4/11 individuals were in “poor” body condition, with exposure concentrations in the mid-range for our sample, larger and more dominant individuals, especially matures, may have better access to all local food sources than smaller subordinates. Three of the four SGAR-positive mice in poor condition were adult males, and the fourth was an immature female, caught directly beside a rodenticide bait station. They were exposed only to bromadiolone, with concentrations ranging between 0.008 (immature female) and 0.014 µg/g. Larger, healthier prey may also be preferred by raptors (Karell et al., 2010) and more likely to consume rodenticides (Caut et al., 2007). Most individuals which tested positive for SGAR residue (7/11) were also caught within 1 or 2 weeks of scheduled PMP visits. This is consistent with previous research that suggests exposure rates climb shortly after baiting begins and decrease as the baiting period goes on (Walther et al., 2021). However, residues in dead rodents may be exceedingly high (Winters et al., 2010), meaning that any individuals which we may have missed using our trapping methods as a result of their being moribund or dead could represent the greatest, unknown risk. No dead rodents were ever found while setting up, checking, or removing trapping stations in and around the transects. However, a systematic search for dead rodents, which are notoriously difficult to find, was not conducted.

Conclusion

Based on these findings, we conclude that professional usage of second-generation anticoagulant rodenticides in urban regions of Toronto and Vaughan is likely leading to the exposure of a non-target rodent (*Peromyscus leucopus*). These may be

a pathway of secondary exposure of rodent predators. However, in some cases, these exposures may have been due to nearby residential usage of SGARs at a townhome complex. Given the minority of individuals in the sample which were exposed, we were unable to identify any patterns of exposure which may be useful for risk mitigation. However, we can say that SGAR usage near wildland areas, where nontarget rodents and their predators may be more abundant and where commensals may be lacking, should be avoided. Pest management professionals should also ensure that they are identifying the rodent species present at extermination sites and continue to monitor species assemblage over time to ensure they are not exposing nontargets.

Acknowledgements The authors would like to thank Dr. Claire Jardine, Dr. Catherine Cullingham, and Dr. Jason Munshi-South for their initial reviews of the thesis from which this manuscript was derived and also the City of Toronto and City of Vaughan for allowing for the collection of rodents on municipal lands.

Author contribution Both authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by L. Richardson. The first draft of the manuscript was written by L. Richardson and A. Schulte-Hostedde edited previous versions of the manuscript. Both authors read and approved the final manuscript.

Funding This research was funded by an NSERC Discovery grant to AISH.

Data availability Research data will be made available upon request.

Declarations

Declarations All authors have read, understood, and complied as applicable with the statement on “ethical responsibilities of authors” as found in the instructions for authors.

Ethics approval All trapping collections were conducted under an Ontario Ministry of Natural Resources and Forestry (OMNRF) Wildlife scientific collector’s permit (WSCA 1106092) and the approval of Laurentian University’s Animal Care Committee. OpenAI’s ChatGPT was used to assist in writing R Studio scripts for data analysis, which were subsequently checked for correctness and modified for customization.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests Luigi Richardson is and has been, since 2020, a pest management technician operating in Toronto and the greater Toronto area, with different companies.

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