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Widespread exposure to anticoagulant rodenticides among common urban mesopredators in Chicago

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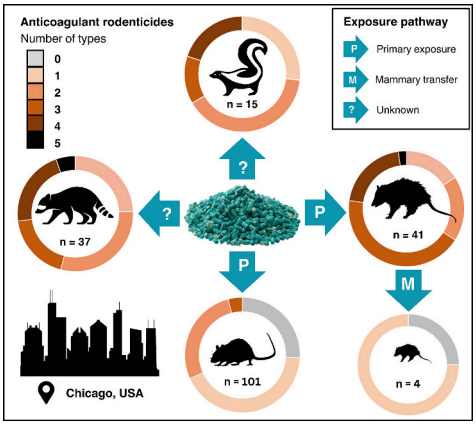
HIGHLIGHTS

- Urban mesopredators can act as an important bridge between target rats and apex predators.
- 100 % of 93 sampled raccoons, skunks, and opossums were exposed to at least one type of AR.
- Mesopredators had mean brodifacoum concentrations at least 6.57 higher than rats, indicating biomagnification.
- We found evidence consistent with mesopredators consuming rat bait and mammary transfer to altricial young.
- Testing wildlife carcasses from pest control operators can provide insights into pesticide exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Anticoagulant rodenticides (ARs) are currently the most common method to control rats in cities, but these compounds also cause morbidity and mortality in non-target wildlife. Little attention has been focused on AR exposure among mesopredators despite their ecological role as scavengers and prey for larger carnivores, thus serving as an important bridge in the biomagnification of rodenticides in food webs. In this study, we sampled liver tissue from raccoons (*Procyon lotor*; $n = 37$), skunks (*Mephitis mephitis*; $n = 15$), and Virginia opossums (*Didelphis virginiana*; $n = 45$) euthanized by pest professionals and brown rats (*Rattus norvegicus*; $n = 101$) trapped in alleys in Chicago, USA to evaluate how often these species are exposed to ARs. We tested whether

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Raccoon
Didelphis virginiana
Virginia opossum
Mephitis mephitis
Striped skunk
Ecotoxicology

mesopredators had a higher prevalence of ARs and to more AR compounds compared to rats and calculated biomagnification factors (mean concentration in mesopredators/rats) as indicators of biomagnification. Of 93 sampled mesopredators, 100 % were exposed to at least one AR compound, mainly brodifacoum (≥80 %), and 79 % were exposed to multiple AR compounds. We also documented teal stomach contents consistent with the consumption of rat bait and altricial young tested positive to the same AR as their mother, suggesting mammary transfer. Of the 101 rats, 74 % tested positive to at least one AR compound and 32 % were exposed to multiple AR compounds. All mesopredator species had biomagnification factors exceeding 1.00 for brodifacoum (6.57–29.07) and bromadiolone (1.08–4.31). Our results suggest widespread exposure to ARs among urban mesopredators and biomagnification of ARs in mesopredators compared to rats. Policies that limit AR availability to non-target species, such as restricting the sale and use of ARs to licensed professionals in indoor settings, education on alternatives, and more emphasis on waste management may reduce health risks for urban wildlife and people in cities around the world.

1. Introduction

Anticoagulant rodenticides (ARs) are a common way to control commensal rat populations in cities around the world (Jacob and Buckle, 2018). ARs kill rats, but by extension harm non-target wildlife, by preventing blood clotting (Rattner et al., 2014). Since the development of warfarin in 1950, ARs have become more potent as rats evolved resistance to first generation ARs (hereafter, FGARs) and therefore, second generation ARs (SGARs) were developed to require fewer feedings to administer a lethal dose (Jacob and Buckle, 2018). As a result, newer products containing SGARs are particularly harmful to non-target wildlife because they are toxic and can be biomagnified in the tissues of non-target wildlife, which occurs when toxicants are accumulated in predator tissues after consuming poisoned prey (Elliott et al., 2014). This biomagnification of ARs also causes morbidity and mortality in many non-target wildlife species that consume poisoned prey (López-Perea and Mateo, 2018), notably predatory birds (Murray, 2020; Thornton et al., 2022; Okoniewski et al., 2021; Elliott et al., 2024), top mammalian carnivores, and endangered species (Sánchez-Barbudo et al., 2012; Riley et al., 2007; Cypher et al., 2014). In an effort to reduce AR exposure to children, pets, and non-target wildlife, the sale of ARs has been under scrutiny (US Environmental Protection Agency, 2008) and has been restricted in several US States and Canadian Provinces (Eisemann et al., 2018; Jacob and Buckle, 2018; Quinn et al., 2019). Despite these recent changes, ARs are still being found in non-target species in alarming concentrations (e.g. Niedringhaus et al., 2021).

Relatively little attention has been focused on AR exposure in mesopredators, particularly in urban areas. Mesopredators may act as an important bridge for the biomagnification of ARs in food webs between the rodents they scavenge upon and larger predators who consume mesopredators such as urban mountain lions (*Puma concolor*; Cashman et al., 1992) and urban coyotes (*Canis latrans*; Shedden et al., 2020). Urban wildlife are particularly at risk for rodenticide exposure (Moriarty et al., 2012) because of the large quantities of ARs distributed in urban areas where rat densities are highest (López-Perea et al., 2019). Alongside rats, mesopredators such as raccoons (*Procyon lotor*), striped skunks (*Mephitis mephitis*; hereafter skunks), and Virginia opossums (*Didelphis virginiana*; hereafter opossums) thrive in North American cities (Magle et al., 2021; Gehrt et al., 2010). These species are successful in urban areas in large part because they are dietary generalists that can forage on anthropogenic foods such as food waste in garbage (Gehrt et al., 2010). As generalists, these urban mesopredators could be exposed to ARs by consuming affected rodents (e.g. opossums, Lotts and Stapp, 2020) or by consuming the poison rat bait itself, which is designed to be palatable. For example, opossums have been observed entering rodenticide bait stations (Burke, 2021). Mammalian mesopredators can also be exposed to ARs through their mother's milk (i.e. mammary or mammillary transfer) before they are weaned, which has been shown experimentally in domestic animals (Horak et al., 2018) and inferred in wild populations (Gabriel et al., 2012). Previous studies have found high rates of AR exposure among small numbers of dead mesopredators (Stone et al., 1999; Sánchez-Barbudo et al., 2012; reviewed in Nakayama et al., 2019). These studies, however, largely tested for ARs in animals with signs of rodenticide poisoning (e.g. bleeding from the mouth), which makes it difficult to generalize these results to wildlife populations. Conversely, Hosea (2000) examined two raccoons and 17 coyotes that were apparently healthy and euthanized for pest control or public safety purposes and still found a high prevalence of exposure (2/2 raccoons and 15/17 coyotes), suggesting underlying exposure in apparently healthy mesopredators. In Europe, Elmeros et al. (2011) found widespread exposure to ARs in stoats (*Mustela erminea*) and 69 weasels (*Mustela nivalis*) with various causes of death and found a negative association between AR concentrations and body condition, suggesting negative health effects from these compounds. Understanding any health threats to urban mesopredators from pesticides is also important for public health because these species are important hosts for zoonotic pathogens such as rabies, leptospirosis, raccoon roundworm (*Baylisascaris procyonis*), and distemper (Lednicky et al., 2004; Oertli et al., 2009; Beltrán-Beck et al., 2012; Glebskiy et al., 2022). Thus, assessing the rates at which urban mesopredators are exposed to rodenticides can help elucidate the impacts of pest management on urban food webs and zoonotic disease dynamics.

To quantify baseline concentrations of AR exposure in urban wildlife, we studied mesopredators in Chicago, IL, USA, the third largest city in the United States (population of 2.6 million; U.S. Census Bureau, 2022) and the city with the most rat complaints nationally for the last nine consecutive years (Orkin, 2023). In Chicago, a combination of government employees, private pest professionals, and the public provide poisoned rat bait for both rats and non-target species in buildings, yards, and alleys. As in many cities, municipal rat management in Chicago mainly relies on city employees baiting rat burrows in alleys with ARs in response to public complaints about rats (Murray et al., 2018). In addition, many residents and businesses privately hire pest professionals to set bait stations and traps in and around buildings. However, in the United States, rodenticide products are available for purchase by the public (EPA, 2023). Comparing the rates of exposure and AR compounds present in mesopredators can help contextualize the sources of AR exposure in non-target species relative to target wildlife but this has not been previously studied.

Our study of urban mesopredator exposure to ARs included four specific goals. First, we evaluated how often urban raccoons, skunks, and opossums are exposed to different ARs in terms of prevalence, concentrations, and number of AR compounds. Second, we documented evidence consistent with potential AR exposure routes during mesopredator necropsies. Third, we examined where mesopredators are more likely to be exposed to ARs in relation to human activity. We hypothesized that human population density largely determines the distribution of ARs throughout a city. As such, we predicted that mesopredators sampled in neighborhoods with higher population density would have higher AR prevalence and be exposed to more AR compounds. Fourth, we compared AR exposure in mesopredators to rats. We hypothesized that mesopredators would exhibit more evidence of biomagnification compared to target rodents because they forage at a higher trophic level and are physically larger; therefore, they likely are better able to survive

exposures to multiple doses of ARs over time. We therefore predicted that raccoons, opossums, and skunks, and particularly older individuals, would exhibit higher AR prevalence, higher concentrations, and exposure to more AR compounds relative to rats. This study provides important information about the impacts of rat management on urban wildlife health, which can inform future policy efforts to mitigate non-target exposure to ARs in urban environments.

2. Methods

2.1. Sample collection

Mesopredators were collected in July–October 2022 throughout the North Side of Chicago, IL, USA and into surrounding suburbs at 56 unique locations (Fig. 1). Wildlife carcasses for this study were obtained by ABC Wildlife Humane Control and Prevention in accordance with administrative code, part 525 nuisance wildlife control permits section 525.45 disposition of animals due to homeowners requesting the removal of wildlife from their homes (Illinois General Assembly, 2017a; Illinois General Assembly, 2017b). Raccoons were trapped with roof-mounted traps near an entry hole in the roof or eaves. Skunks and opossums were trapped with cage traps set near their burrows under stoops and decks. After euthanasia by CO2 following regulatory guidelines (AVMA, 2020.S7.6.3.2), carcasses were frozen at −20 °C to ensure sample quality and condition of the specimen. Following review by the Lincoln Park Zoo IACUC, this study was deemed exempt because it involved animal carcasses that had already been euthanized for pest control purposes. Location, species, and date of euthanasia was recorded for each specimen. This sampling design targets mesopredators using habitats near buildings, potentially making them more likely to come into contact with ARs if ARs are preferentially used in residential areas or if poisoned animals are more likely to seek out anthropogenic resources (i.e. garbage cans, litter, compost bins, etc.). We do not believe this is a concern because previous work has shown AR exposure in many types of urban habitats such as parks, golf courses, and industrial areas (Cypher et al., 2014) and that habitat selection does not change following AR exposure (Walther et al., 2021).

In addition to mesopredator sampling, rats were trapped using snap traps within tamper-resistant housings (Fast Catch Station with Rodent Alert, B & G Equipment Company, Inc., USA and Big Snap-E Rat Trap, Kness Mfg. Co., Inc., USA) in 16 alleys in four wards (i.e. neighborhoods) during the same time period (August–November 2022) as part of a concurrent study (Murray et al., 2024). Rat traps were checked daily and specimens were frozen at −20 °C. Location and date of trapping was recorded for each specimen.

Carcasses were thawed for 24 h before being processed between August 2022–January 2023. During processing, each specimen was sexed, weighed, and external morphometric measurements including total body length, tail length, length of hind foot, and ear length were recorded. Age class was assigned using a combination of criteria. Individuals with clear reproductive activity (i.e. lactating females or with fetuses or joeys for opossums) were identified as adults. Then once specimen skulls were cleaned, they were further aged based on dental wear and tooth eruption based on modifications to other mammalian studies (see Hernandez et al., 2017; Gardner, 1973; Van Valkenburgh and White, 2021). Individuals with any deciduous teeth or any teeth which were not fully erupted, were assigned sub adult or juvenile categories, whereas any individual with fully erupted and permanent teeth were considered adults.

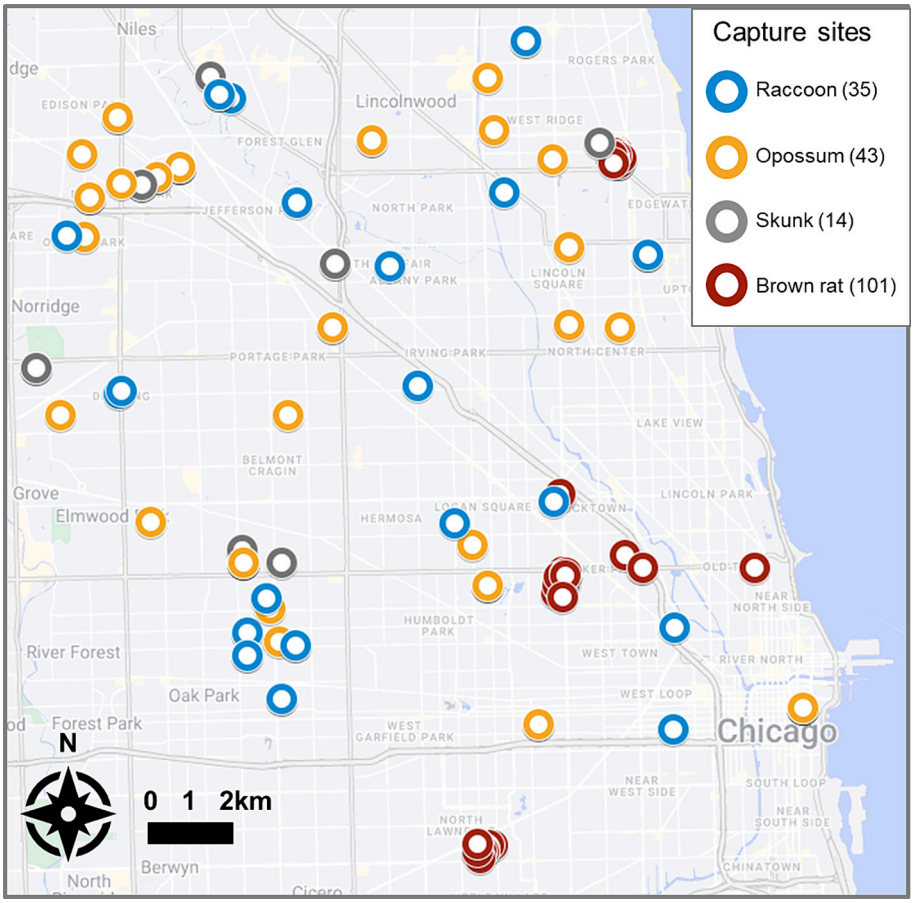

Fig. 1. Spatial distribution of mesopredators and rat samples in the North Side of Chicago, IL, USA.

2.2. Rodenticide screening

To measure AR concentrations, liver samples were sent to the Pennsylvania Animal Diagnostic Laboratory System (PADLS) Toxicology Laboratory, which screened for eleven ARs. To do so, livers were subsampled (≥20 g for mesopredators; ≥2 g for rats) into sterile polyethylene storage bags (Whirl-pak, Nasco, Fort Atkinson, WI) and frozen at −20 °C before shipment. PADLS quantified AR concentrations using the QuEChERS (quick, easy, cheap, effective, rugged, and safe) and high-performance liquid chromatography (HPLC) methods and instruments described in Vudathala et al. (2010) and Facka et al. (2023). Briefly, detection limits were established by spiking 0.2 g or 1 g of liver tissue. Liver samples were then homogenized, extracted, and purified before the supernatant was dried for analysis. Anticoagulant rodenticide detection and quantification was then performed using an AB Sciex API 4000 or Agilent 6470 Liquid chromatography-tandem mass spectrometry (LC-MS/MS) system. The API 4000 system utilized a Shimadzu LC-20 HPLC system with an Agilent Poroshell C18 column (3 × 50 mm, 2.7 μm), while the 6470 system used an Agilent 1290 Infinity LC system with a Poroshell C18 column (2.1 × 50 mm, 2.7 μm). The mobile phases consisted of 5 mM ammonium acetate in water and methanol. Each compound had an established limit of quantification (LOQ), which varied by compound as follows for mesopredator liver samples (all in μg/g): brodifacoum (0.025), bromadiolone (0.100), chlorophacinone (0.100), coumachlor (0.025), coumafuryl (0.025), dicoumarol (0.100), difenacoum (0.025), difethialone (0.025), diphacinone (0.050), pindone (0.200), warfarin (0.025). Rat samples were analyzed earlier in 2023 and had different detection limits for brodifacoum (0.010 μg/g), bromadiolone (0.025), chlorophacinone (0.050), coumachlor (0.100), coumafuryl (0.100), dicoumarol (0.100), difenacoum (0.010), difethialone (0.050), pindone (0.100), and warfarin (0.100). For any comparisons between rats and mesopredators, we used the higher detection limit. Positive results below the LOQ were recorded as trace amounts. For samples at or above the LOQ, AR concentrations were recorded as parts per million (ppm), which is equivalent to micrograms per gram (μg/g), on a wet weight basis.

2.3. Routes of exposure

During necropsy, we visually examined mesopredator stomach contents for teal-colored seeds consistent with the coloration of many types of bait blocks and pellets containing ARs. We also collected liver samples from a subset of altricial opossum young (i.e. joeys) in the pouch to determine if ARs may be transferred through milk (i.e. mammary transfer). This analysis was limited to joeys large enough to provide adequate liver tissue for testing (≥2 g of liver tissue).

2.4. Statistical analysis

2.4.1. AR exposure and human population density

We tested whether mesopredators had higher AR concentrations or were exposed to more AR compounds if they were located in a neighborhood with higher population density. We used population density as a proxy for urban development and an indicator that more people would be available to use ARs. We accessed human population density data at the neighborhood level through the Chicago Metropolitan Agency for Planning's (CMAP) Community Data Snapshots (CMAP, 2023).

To compare population density with mesopredator concentrations of multiple ARs simultaneously, we used a Principal Components Analysis (PCA). The PCA was used to re-ordinate and identify latent variables that best describe the variation of the most common AR compounds in our analysis (brodifacoum, bromadiolone, difenacoum, difethialone, diphacinone). The PCA was conducted on a correlation matrix of the rodenticide values for all species. Using Correlation matrix PCs is the appropriate choice for datasets where different changes of scale are conceivable for each variable (Jolliffe and Cadima, 2016). Subsequently, most ecological studies use a correlation matrix based PCA on abiotic and habitat variables (Peres-Neto et al., 2003). Given that the chemical and molecular formulas per pesticide are different, we opted for a correlation matrix rather than a covariance matrix for our PCA. PC loading importance was assessed as loadings >0.30 and <−0.30 were classified as important and loadings with >0.50 and <−0.50 as highly important following Hair et al. (1987). We used a correlation matrix, rather than a covariance matrix, because the scale of each rodenticide was not identical and because concentration values of below detection limits are recorded as zero μg/g and precluded a log transformation. Biplots of PC scores visualized the species best associated with the pattern and subsequent ARs PCs (Fig. 2).

We then used Linear Mixed Models (LMM) with PC Dimension 1 or Dimension 2 as a metric of AR concentrations as the response variable with individual loadings values tied to each individual mesopredator. As explanatory variables in our model we included neighborhood population density as a spatial covariate and the sex, age, and species of a sample as individual covariates. To test whether mesopredators in denser neighborhoods were more likely to be exposed to more AR compounds, we used Generalized Linear Mixed Models (GLMM) with a Poisson distribution with the number of AR compounds as the response variable. In these models, we again included sex, age class, population density, and species. In both models (i.e. concentrations and number of AR compounds), we included location ID as a random effect to account for individuals trapped at the same building.

2.4.2. AR exposure in mesopredators compared to rats

To test the hypothesis that mesopredators would exhibit more biomagnification of ARs relative to target rats, we estimated the prevalence and concentrations of specific ARs in raccoons, opossums, skunks, and brown rats using a Hurdle model, which is a two-part regression analysis (Zuur et al., 2009). The first part of our Hurdle model estimated the likelihood of observing non-zero versus zero observations using logistic regression, which in our case represented individuals with and without a detectable concentration of a given AR. The second part of our Hurdle model estimated the expected concentration of a given AR of each species using the data from individuals with a detectable concentration of AR (i.e., it conditionally models all non-zero observations). We fitted a Hurdle model to AR concentration data for brodifacoum, bromadiolone, difethialone, and diphacinone because these were the most common AR compounds in our dataset (i.e. were present in ≥17 % of all 198 sampled individuals) and thus we had sufficient sample size. In this model, the first component compares the proportion of individuals who have concentrations at or above the detection limit for a given AR (i.e. prevalence). To do so, we converted AR concentrations to a binary response variable (1 = AR concentration at or above the detection limit, 0 = AR concentration below the detection limit) and used logistic regression. Our model included species as a categorical covariate, treating brown rat as the reference category, and so this portion of the model quantified the proportion of individuals within a species that had detectable concentrations of the aforementioned ARs and whether this quantity differed between mesopredators and rats. In this analysis, we used rats as the reference category because we were interested in comparing non-target to target species. The second component of our Hurdle model is conditional on the first component, and compares the AR concentrations among individuals with a concentration above the detection limit for a given AR. For this conditional model, we subset the data down to individuals who had detectable concentrations of a given AR and used gamma regression, again using species as a categorical variable. This second model therefore quantifies the average concentration of each AR in individuals of a species, conditional on it being detected. With these two components of the model, we can therefore compare the prevalence and concentrations for specific ARs among mesopredators and rats. Finally, to estimate overall AR concentrations across each species with uncertainty we bootstrapped our data and refitted our hurdle model 2000 times to generate a distribution of

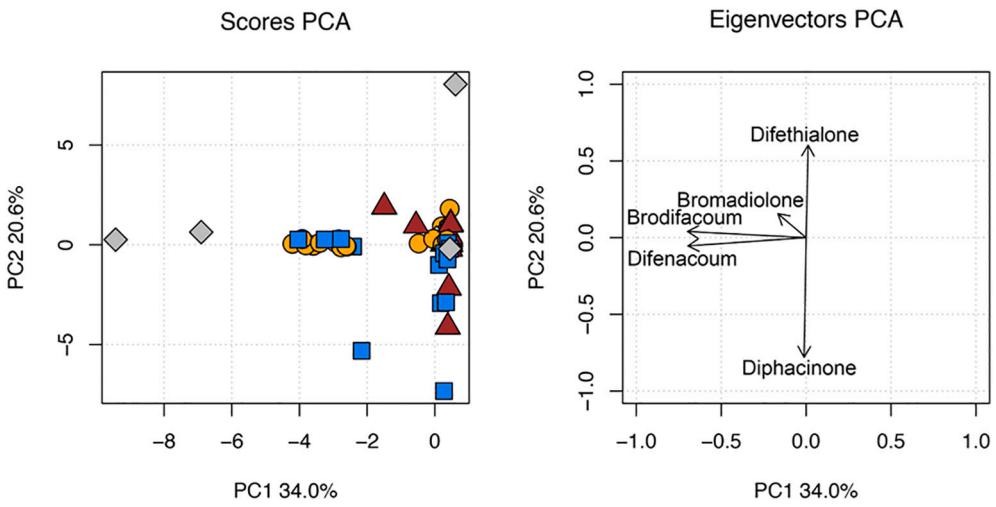

Fig. 2. Distribution of individual mesopredator eigenvalues identified as opossums as orange circles, raccoon as blue squares, skunks as gray diamonds, and rats as brown triangles (a) and clustering of anticoagulant rodenticide compounds (b) along the two main axes of a Principal Components Analysis.

prevalence and conditional AR concentration values for each species. The product of these two quantities (i.e., the proportion of the population with detectable concentrations of an AR compound multiplied by the average conditional AR concentration) represents the average AR concentration for each species, from which we calculated median estimates and 95 % confidence intervals. To test whether mesopredators were more likely to be exposed to more AR compounds relative to rats, we used Generalized Linear Mixed Models (GLMM) with a Poisson distribution with the number of AR compounds as the response variable. In this model, we included sex, age class, and species as explanatory variables.

To more precisely evaluate the potential for specific ARs to bioaccumulate in urban mesopredators, we calculated biomagnification factors (BMF; van den Brink et al., 2016). BMFs are particularly relevant when predator and prey species are sampled using the same tissue type and in the same area and season, as we have in our study. We calculated BMFs as the ratio of the mean AR concentration in a mesocarnivore species, as estimated by the Hurdle model, divided by the mean concentration in rats. We calculated BMFs for each mesopredator-rat pair and for each of the four ARs with enough data for us to estimate mean concentrations using the Hurdle model (diphacinone, brodifacoum, bromadiolone, difethialone). The BMF is a ratio and a value of >1.00 indicates biomagnification of ARs in predator tissue relative to prey tissue.

To visualize any associations between AR compounds with species and age classes, we generated a heat map matrix using the visweb function with the R package bipartite (v. 2.19). This matrix displays the prevalence of specific ARs along a gradient for each combination of species and age class. All analyses were performed using R Statistical Software (v4.2.2; R Core Team, 2022).

3. Results

3.1. Mesopredator exposure to ARs

We sampled liver tissue from 41 opossums, 37 raccoons, and 15 skunks (Tables 1, S1). Of these, 100 % (93/93) tested positive (value > detection limit) for at least one AR compound. Specifically, 100 % tested positive for at least one SGAR (brodifacoum, difethialone, bromadiolone, or difenacoum), 25.8 % (24/93) tested positive for an intermediate generation anticoagulant rodenticide (IGAR; diphacinone), and 2.2 % (2/93) tested positive for a first-generation anticoagulant rodenticide (FGAR; warfarin) (Fig. 3). Brodifacoum was the most commonly detected AR compound in mesopredator samples and was detected in 89 % of raccoons (concentration range: 0.025–0.71 μg/g), 80 % of skunks (0.025–2.9 μg/g), and 87 % of opossums (0.025–0.87 μg/g). Bromadiolone was the second most commonly detected AR compound in mesopredator samples and was detected in 41 % of raccoons (concentration range: 0.10–2.20 μg/g), 33 % of skunks (0.10–5.2 μg/g), and 58 % of opossums (0.10–3.0 μg/g). Further, 79 % of mesopredators tested positive for more than one AR compound (Fig. 4). The largest proportion of mesopredators tested positive for three AR compounds (30.1 %), followed by two AR compounds (26.9 %) but two raccoons (5.4 %) and one opossum (2.4 %) tested positive for five AR compounds (Fig. 4). None of the sampled mesopredators tested positive for chlorophacinone, coumachlor, coumafuryl, dicoumarol, or pindone.

3.2. Routes of exposure

We observed one opossum with visible signs consistent with consuming teal bait blocks or pellets containing ARs (Fig. 5a, b). Individual 1240 was a female juvenile opossum who weighed 0.95 kg. There were no external lesions upon observation. Upon gross necropsy of the intestinal tract, seeds with a teal (blue/green) hue was noted throughout the intestine and the contents of the stomach, consistent with the texture and coloration of bait blocks and pellets containing ARs (Fig. 5a, b). Upon toxicological analysis of the liver, female 1240 was positive for two ARs, bromadiolone (0.41 μg/g) and difethialone (1.5 μg/g) respectively.

We also documented the first known mammary transfer of ARs in urban wildlife, specifically to opossum joeys. We tested liver tissue from four opossum joeys from the mother's pouch (Fig. 5c). Individual 1199 was a female adult opossum who weighed 2.8 kg. Gross necropsy revealed no external lesions and organs were unremarkable (i.e. expected color and size). Upon assessment of the gastrointestinal tract, roundworms were found throughout including the mouth (Fig. 5d). The female had eight joeys present in her pouch. The joeys were lightly furred but did not yet have their eyes open, aging them at <64 days old and therefore dependent on maternal milk as weaning typically starts at 70 days (Pollock and Arbona, 2018). The female (1199) tested positive for two different ARs, brodifacoum (0.051 μg/g) and difethialone (0.4 μg/g). We sampled the livers of four of the eight joeys and all but one (three of four) were positive for trace amounts of difethialone (0.025 μg/g).

3.3. AR exposure and human population density

When we combined the most common AR compounds using a PCA,

Table 1
Summary of anticoagulant rodenticide prevalence and hepatic concentrations (in μg/g) for raccoons ($n = 37$), skunks ($n = 15$), opossums ($n = 41$) and brown rats ($n = 101$) collected in Chicago, IL. Median and maximum observed concentrations are shown for all compounds that were detected above the limit of quantification (LOQ). Estimated mean concentrations and 95 % confidence intervals (CI) for diphacinone, brodifacoum, bromadiolone, and difethialone were calculated using a Hurdle model and contingent on an animal testing positive for that particular anticoagulant rodenticide compound. Positive results below the LOQ were recorded as trace amounts.

Anticoagulant compound	Metric	Raccoon	Skunk	Opossum	Brown rat
First generation					
Warfarin	Positives (Prevalence)	2 (5 %)	0 (0 %)	0 (0 %)	0 (0 %)
	Median concentration (μg/g)	<LOQ	–	–	–
	Maximum concentration (μg/g)	<LOQ	–	–	–
	Estimated mean concentration (95 % CI)	–	–	–	–
Coumachlor	Positives (Prevalence)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
	Median concentration (μg/g)	–	–	–	–
	Maximum concentration (μg/g)	–	–	–	–
	Estimated mean concentration (95 % CI)	–	–	–	–
Dicoumarol	Positives (Prevalence)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
	Median concentration (μg/g)	–	–	–	–
	Maximum concentration (μg/g)	–	–	–	–
	Estimated mean concentration (95 % CI)	–	–	–	–
Intermediate generation					
Chlorophacinone	Positives (Prevalence)	0 (0 %)	0 (0 %)	0 (0 %)	2 (2 %)
	Median concentration (μg/g)	–	–	–	<LOQ
	Maximum concentration (μg/g)	–	–	–	<LOQ
	Estimated mean concentration (95 % CI)	–	–	–	–
Diphacinone	Positives (Prevalence)	14 (38 %)	4 (27 %)	5 (13 %)	9 (9 %)
	Median concentration (μg/g)	0.14	0.05	0.05	0.05
	Maximum concentration (μg/g)	1.7	0.05	0.13	0.95
	Estimated mean concentration (95 % CI)	0.38 (0.20, 0.73)	0.05 (0.02, 0.17)	0.07 (0.03, 0.18)	0.20 (0.09, 0.45)
Second generation					
Brodifacoum	Positives (Prevalence)	33 (89 %)	12 (80 %)	36 (88 %)	16 (16 %)
	Median concentration (μg/g)	0.03	0.04	0.03	0.01
	Maximum concentration (μg/g)	0.71	2.90	0.87	0.07
	Estimated mean concentration (95 % CI)	0.09 (0.05, 0.16)	0.41 (0.16, 1.04)	0.16 (0.10, 0.27)	0.01 (0.01, 0.03)
Bromadiolone	Positives (Prevalence)	15 (41 %)	5 (33 %)	24 (58 %)	66 (67 %)
	Median concentration (μg/g)	0.03	0.03	0.32	0.03
	Maximum concentration (μg/g)	2.20	5.20	3.00	18.10
	Estimated mean concentration (95 % CI)	0.76 (0.13, 4.63)	2.36 (0.10, 5.36)	0.59 (0.15, 2.33)	0.55 (0.23, 1.28)
Difenacoum	Positives (Prevalence)	6 (16 %)	2 (13 %)	9 (22 %)	0 (0 %)
	Median concentration (μg/g)	<LOQ	<LOQ	<LOQ	–
	Maximum concentration (μg/g)	<LOQ	<LOQ	<LOQ	–
	Estimated mean concentration (95 % CI)	–	–	–	–
Difethialone	Positives (Prevalence)	24 (65 %)	11 (73 %)	37 (90 %)	17 (17 %)
	Median concentration (μg/g)	0.03	0.03	0.03	0.07
	Maximum concentration (μg/g)	0.80	6.9	1.50	0.90
	Estimated mean concentration (95 % CI)	0.13 (0.06, 0.26)	0.66 (0.23, 1.93)	0.18 (0.10, 0.32)	0.22 (0.09, 0.52)

PC1 explained 34.0 % of the variance, with more positive values indicating higher concentrations of brodifacoum, difenacoum, and bromadiolone (Fig. 2). PC2 explained 20.6 % of the variance. PC1 was negatively associated with brodifacoum and difenacoum, whereas PC2 was positively and significantly positively associated with difethialone and significantly negatively associated with diphacinone (Table S2). Visualization of the species most associated with the variation in AR concentrations can be seen in Fig. 2.

Contrary to our predictions, we found no difference in AR concentrations or number of AR compounds present in mesopredators based on the human population density in the neighborhood where the animal was euthanized (all $p \geq 0.22$).

3.4. AR exposure in mesopredators compared to rats

We sampled liver tissue from 101 rats (Table 1). Of these, 73.2 % (74/101) tested positive for at least one AR compound. Specifically, 73 % tested positive for at least one SGAR (brodifacoum, difethialone, bromadiolone, or difenacoum), 9 % tested positive for an IGAR

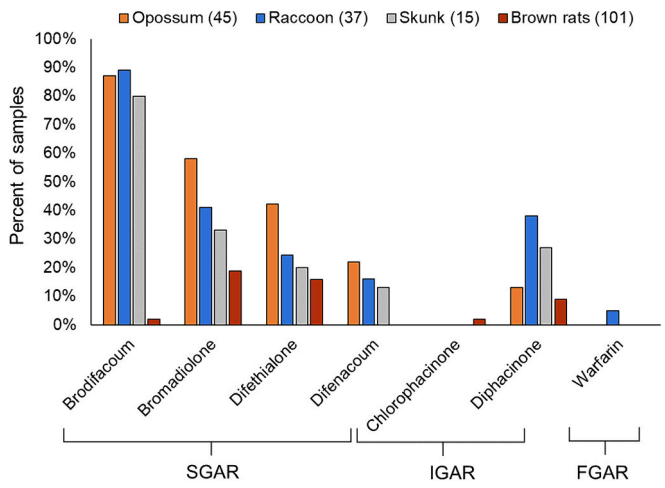

Fig. 3. Prevalence of anticoagulant rodenticide residues in mesopredator and rat liver samples from Chicago, IL, USA. Specific anticoagulant rodenticide compounds are labeled as second generation (SGAR), intermediate generation (IGAR), and first generation (FGAR). The limit of quantification (LOQ) thresholds for the AR compounds are as follows (all in μg/g): brodifacoum (0.025), bromadiolone (0.100), difethialone (0.050), difenacoum (0.025), chlorophacinone (0.050), diphacinone (0.050), warfarin (0.025).

(diphacinone, chlorophacinone), and none tested positive for an FGAR (warfarin) (Fig. 3). Further, 32 % of rats tested positive for more than one AR compound (Fig. 4). None of the sampled rats tested positive for difenacoum, coumachlor, coumafuryl, dicoumarol, or pindone.

Overall, mesopredators had a higher prevalence of exposure to any AR compound relative to rats (100 % of mesopredators and 74 % of rats; Goodness of fit $\chi^2 = 25.15$, $p < 0.001$). This pattern was consistent for SGARs (100 % of mesopredators, 73 % of rats; $\chi^2 = 26.31$, $p < 0.001$), IGARs (25 % of mesopredators, 9 % of rats; $\chi^2 = 8.80$, $p = 0.003$), and FGARs (2 % of mesopredators, 0 % of rats; Fig. 3). The prevalence of ARs also varied by species and age class. Based on our heat map of age classes by species, brodifacoum was the most prevalent of all AR compounds and mesopredators had higher cumulative AR prevalence relative to rats regardless of age class (Fig. 6; Table 1). Further, juveniles tended to have the lowest AR prevalence relative to other age classes (Fig. 6; Table 1). Mesopredators were also significantly more likely to be exposed to multiple AR compounds relative to rats (mespredators: 78 % ≥ 2 compounds; rats: 32 % ≥ 2 compounds; Table 2). Relative to rats, mesopredators had significantly higher prevalence of exposure to brodifacoum, bromadiolone, difethialone, and diphacinone ($p \leq 0.0003$; Table 2).

Using the Hurdle model, we estimated expected AR concentrations in mesopredators and rats which were conditional on the animal being exposed to that particular AR. These conditional concentrations ranged from 0.014 μg/g (rats) to 0.407 μg/g (skunks) for brodifacoum, from 0.547 μg/g (rats) to 2.356 μg/g (skunks) for bromadiolone, from 0.127 μg/g (raccoons) to 0.662 μg/g (skunks) for difethialone, and 0.050 μg/g (skunks, rats) to 0.382 μg/g (raccoons) for diphacinone (Table 1). We also calculated overall expected concentrations that were estimated via bootstrapping, which included all individuals including those which tested negative. For these unconditional estimates of AR concentrations, the estimated concentrations of bromadiolone were highest of any AR compound across all species and skunks tended to have higher concentrations of brodifacoum, bromadiolone, and difethialone relative to raccoons, opossums, and rats (Fig. 7).

Based on the estimated mean concentrations of brodifacoum, bromadiolone, difethialone, and diphacinone from the Hurdle model, we found that all mesopredator species had BMF values exceeding 1.00 for brodifacoum and bromadiolone, indicating biomagnification of these compounds in predator tissues (Table 3). The BMF values were highest for brodifacoum (6.57–29.07), the most common SGAR in our dataset, and lowest for diphacinone (0.25–1.91), an IGAR (Table 3). Further, skunks tended to have higher BMF values (0.25–29.07) relative to opossums (0.33–11.57) and raccoons (0.57–6.57; Table 3).

4. Discussion

We found ubiquitous exposure to ARs in urban mesopredators. Of the mesopredators in our study, 100 % were exposed to at least one anticoagulant rodenticide and 79 % had been exposed to multiple AR compounds. Mesopredators (non-target species for ARs) also had a higher prevalence of AR exposure and were exposed to more AR compounds than brown rats (target species for ARs). Importantly, the majority (53 %) of mesopredators in our sample were exposed to three or more ARs relative to only 4 % of rats. This exposure among non-target wildlife demonstrates how the widespread use of ARs in cities can have far reaching and unintended impacts with implications for wildlife health.

Although we found universal exposure to ARs in urban mesopredators, it can be difficult to interpret how hepatic AR concentrations

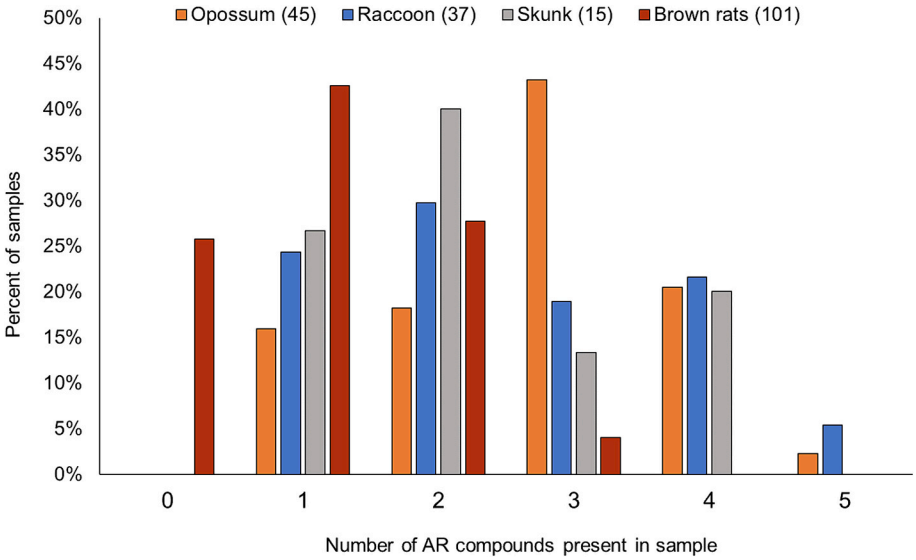

Fig. 4. Distribution of the number of anticoagulant rodenticide compounds detected in liver samples of individual animals, colored by species.

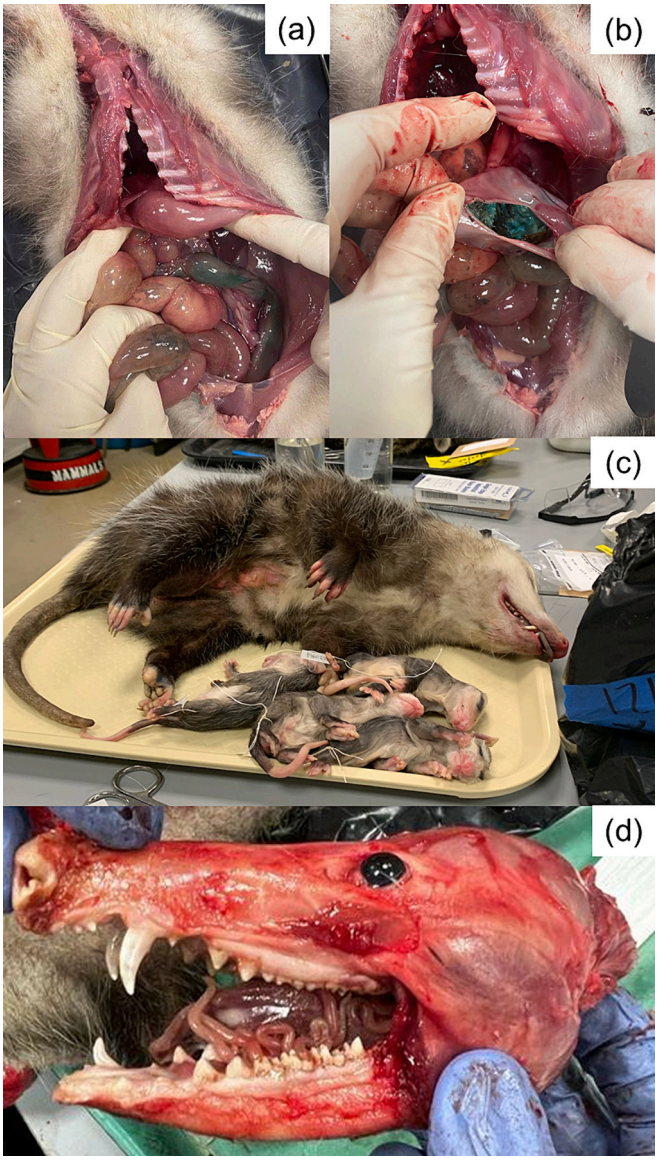

Fig. 5. Stomach contents of Virginia opossum (ND1240) during necropsy, with an external (a) and internal view (b). The teal color is consistent with the coloration of bait blocks and pellets containing ARs. (c) Virginia opossum 1199, an adult female opossum with eight altricial joeys, aged at 6–7 weeks based on appearance. Both the mother and three of the four sampled joeys tested positive for difethialone, suggesting mammary transfer of anticoagulant rodenticides. (d) Individual 1199 also had worms (species unknown) throughout the gastrointestinal tract, including the mouth. Photo credit: Jacqueline Y. Buckley (a, b, d) and Maureen H. Murray, (c).

affect the health of non-target wildlife. For example, the reported hepatic concentration threshold for SGARs associated with toxicosis varies between studies, different species can be more sensitive to AR exposure than others, and allometric scaling values for estimating toxicity based on body size are AR-specific (Rattner and Harvey, 2020). However, we detected bromadiolone concentrations that exceeded those in skunks (0.28 μg/g) and opossums (0.8 μg/g) who had died with signs of rodenticide poisoning (Stone et al., 1999) in four of the skunks (0.85–5.2 μg/g) and six of the opossums (1.0–3.0 μg/g) in our study. Similarly, we found brodifacoum concentrations that exceeded those in raccoons (0.32 μg/g) and opossums (0.18 μg/g) that had died with signs of rodenticide poisoning (Stone et al., 1999) in three of the raccoons (0.42–0.71 μg/g) and 11 of the opossums (0.23–0.87 μg/g) in our study. These comparisons suggest that the concentrations we detected likely

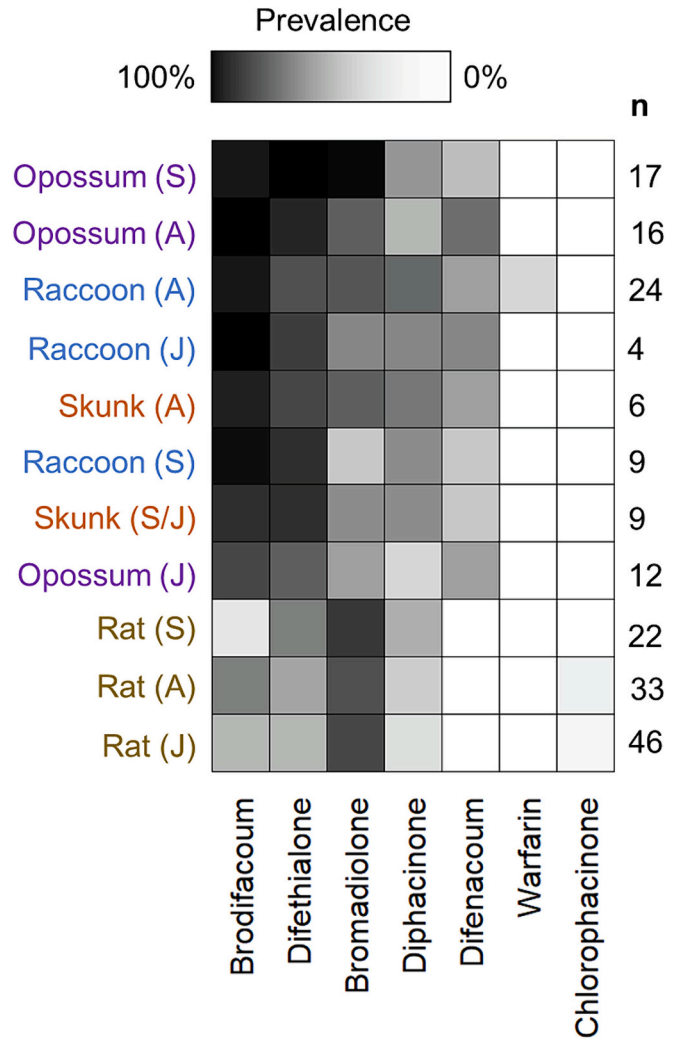

Fig. 6. Heat map showing the prevalence of each anticoagulant rodenticide compound by species and age group (adult = A, subadult = s, juvenile = J). Darker squares indicate a higher proportion of individuals that tested positive (i.e. prevalence).

Table 2
Comparison of anticoagulant rodenticide (AR) exposure in mesopredators (opossum, n = 41; raccoon, n = 37; skunk, n = 15) relative to brown rats (n = 101) trapped in the same sampling period. A Generalized Linear Model with Poisson distribution was used for the number of AR compounds present in a sample and a Hurdle model was used to compare the prevalence of individuals above detection limits for the four most common ARs in our dataset. Rat was the reference category for this among-species comparison.

Response variable	Species	β (SE)	z	p
Number of AR compounds present	Opossum	0.93 (0.14)	6.77	<0.0001
	Raccoon	0.79 (0.15)	5.33	<0.0001
	Skunk	0.72 (0.20)	3.63	0.0003
Brodifacoum prevalence	Opossum	4.64 (0.77)	5.99	<0.0001
	Raccoon	3.78 (0.60)	6.35	<0.0001
	Skunk	3.06 (0.70)	4.36	<0.0001
Bromadiolone prevalence	Opossum	2.01 (0.41)	4.88	<0.0001
	Raccoon	1.08 (0.42)	2.57	0.01
	Skunk	0.77 (0.60)	1.27	0.20
Difethialone prevalence	Opossum	3.82 (0.59)	6.48	<0.0001
	Raccoon	2.21 (0.44)	5.08	<0.0001
	Skunk	2.61 (0.64)	4.07	<0.0001
Diphacinone prevalence	Opossum	0.56 (0.56)	1.00	0.32
	Raccoon	1.83 (0.49)	3.76	0.0002
	Skunk	1.31 (0.68)	1.93	0.05

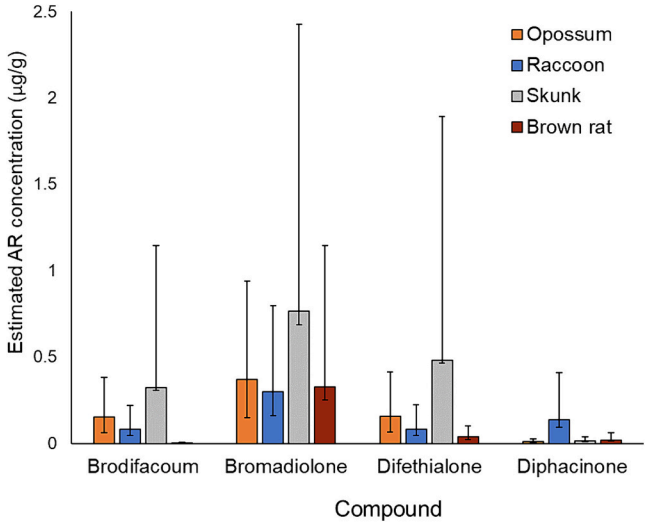

Fig. 7. Predicted hepatic concentrations of four common anticoagulant rodenticides in 93 mesopredators and 101 brown rats in Chicago, IL, USA. These unconditional estimates were calculated using a Hurdle model that accounted for individuals that tested negative for these compounds.

Table 3
Biomagnification factor (BMF) values for raccoons (n = 37), striped skunks (n = 15) and Virginia opossums (n = 41) in relation to brown rats (n = 101). The BMF is a ratio of estimated mean anticoagulant rodenticide (AR) concentrations in a predator relative to prey, and therefore a value >1.00 indicates biomagnification of a particular AR in predator tissue relative to prey tissue.

	Raccoon	Skunk	Opossum
Diphacinone	1.91	0.25	0.33
Brodifacoum	6.57	29.07	11.57
Bromadiolone	1.39	4.31	1.08
Difethialone	0.57	3.00	0.81

cause health harms and signs of toxicity in our study animals. Future research on physiological changes associated with chronic sublethal exposure to ARs, and in particular any additive or synergistic effects of exposure to multiple AR compounds, would help predict health risks for urban wildlife.

In addition to acute toxicity, exposure to ARs can also cause downstream effects on health. For example, bobcats (*Lynx rufus*) exposed to FGARs and SGARs were more likely to die of notoedric mange (Riley et al., 2007; Serieys et al., 2018a). These disease outcomes may be due to immune dysfunction and associated susceptibility to infection, because AR exposure was associated with elevated lymphocytes and suppressed neutrophils in bobcats (Serieys et al., 2018b). Immune dysfunction following AR exposure may also explain why brown rats exposed to ARs were significantly more likely to carry *Leptospira interrogans*, the bacteria that causes leptospirosis, in Chicago (Murray and Sánchez, 2021). Given that raccoons and skunks can carry zoonotic pathogens such as rabies (Oertli et al., 2009), raccoon roundworm (Beltrán-Beck et al., 2012), canine distemper virus (Lednicky et al., 2004), and live in close proximity to people, it is important to prevent human actions that create health risks for mesopredators and, in turn, public health. More holistically, preventing health risks for urban mesopredators can help maintain healthy urban ecosystems because these species play important roles as scavengers, predators for invertebrates, and prey for larger carnivores.

Unlike obligate carnivores, it is difficult to determine whether omnivorous mesopredators had primary or secondary exposure to ARs. We detected AR exposure in altricial opossum joeys, suggesting that ARs can be transferred in the mother's milk (i.e. mammary or mammillary transfer) before young are able to forage independently. Mammary transfer has been demonstrated experimentally in cows (Horak et al., 2018), incidentally in sheep (Moriceau et al., 2020), and observed in the wild in fishers (*Pekania pennanti*; Gabriel et al., 2012). However, this is the first report of mammary transfer of ARs to altricial young still in the mother's pouch and therefore with no access to other foods. Juvenile exposure to ARs in other species and any resulting biological impacts for the health and survival of young, and thus species fitness, is underresearched for wildlife conservation. Experimental work has demonstrated that different AR compounds have varying potential to transfer from mother to fetus, either during gestation or lactation (Chetot et al., 2020), further underscoring the need for more research. We also observed visual evidence of primary AR exposure in one juvenile opossum, suggesting that non-target mesopredators are accessing bait directly, be it accidental or deliberate. While our research was conducted in common urban mesopredators, these species serve as an environmental toxicity sentinel for protected and endangered species. More research documenting routes of exposure and other components of the exposure pathway for ARs in common and rare species is needed to identify vulnerable demographics and strengthen regulatory policies.

Perhaps because we detected at least one AR compound in all sampled mesopredators, we failed to find a relationship between the degree of urban density (i.e. population density) and the prevalence of AR exposure, their concentrations, or the number of AR compounds detected in mesopredators. Other studies have found a positive relationship between urban development and AR exposure (e.g. López-Perea et al., 2019; Cypher et al., 2014; Silveira et al., 2024); however, Chicago may be so large and densely developed that all of our samples may have exceeded a threshold in human activity that resulted in widespread AR exposure. It is also important to consider that the mesopredators sampled in our study were euthanized by pest control professionals because they lived in or adjacent to homes or businesses. As such, these individuals may have been more likely to access rodenticide products deployed in or adjacent to structures. Future studies could collect samples from animals with different causes of death (e.g. road-killed, euthanized by pest control, rehabilitated, found dead) to explore any sources of bias. Given the paucity of data on AR exposure in urban wildlife, our results still provide insights into health risks for animals living near humans, which will likely increase as urbanization expands.

Our results support our hypothesis that mesopredators experience biomagnification of ARs relative to target rats. As with prior studies, we found that brodifacoum was the most common AR detected in mesopredators and was associated with the highest BMF values (Table 3), which is particularly concerning as it has high toxicity (i.e. low LD50) relative to other SGARs (Stone et al., 1999; Hosea, 2000). We also found that brodifacoum was always present in individuals exposed to multiple AR compounds and that bromadiolone was only detected in individuals exposed to multiple AR compounds, aligning with previously documented patterns in AR exposure (Stone et al., 1999; Hosea, 2000). Importantly, we extend these findings to individuals that were sampled regardless of any signs of rodenticide poisoning. Skunks in particular had relatively high estimated SGAR concentrations (Fig. 7), and relatively high BMF values (Table 3) compared to raccoons and opossums. In addition to primary exposure from unsecured poison rat bait, skunks may also experience a greater degree of secondary exposure to ARs because they are more carnivorous compared to raccoons and opossums. Skunks consume small mammals but a large proportion of their diet is composed of invertebrates (Greenwood et al., 1999), which can also contain ARs from environmental contamination (Alomar et al., 2018). While raccoons and opossums are known to consume human-associated foods in urban areas (Nicholson and Cove, 2022), no such dietary shift has been documented in skunks. As such, skunks may experience more trophic transfer of ARs from poisoned prey relative to raccoons and opossums. Relative to rats, mesopredators had greater exposure to ARs, both in terms of prevalence and exposure to multiple AR compounds. This is likely because mesopredators have larger body sizes relative to

rats and therefore they are less likely to die following exposure to small doses of ARs. These doses to multiple compounds can then accumulate in body tissues over time. This accumulation of ARs in tissues likely explains why older mesopredators had higher AR prevalence (Fig. 6).

If rats are more likely to die following AR exposure, it is surprising that so many rats (73 %) tested positive for SGARs, some at quite high concentrations (18.1 and 9.35 μg/g for bromadiolone). These rats, which tested positive but survived until they were trapped, could have been predated upon by other species leading to secondary exposure in non-target wildlife. In addition, the high proportion of rats who had consumed SGARs but still survived until the time of trapping suggests suboptimal efficacy of these products for efficiently killing rats. For example, a high proportion of rats may have survived AR exposure if they were genetically resistant, which has been demonstrated for FGARs in many rat populations (McGee et al., 2020). SGARs were created in part because of the resistance target species had acquired to FGARs and have a longer half-life usually making single feedings lethal for target species (Pelz et al., 2005). These lethal doses (LD50) were established in laboratory rodent populations and may not reflect the lethal dose required for current wild rodent populations (WHO, 1995; McGee et al., 2020). These results suggest that the overuse of AR throughout Chicago causes biomagnification in urban mesopredators and allows rats to survive for at least short periods following ingestion.

Based on the high prevalence of non-target exposure we detected in our sample, current regulations to prevent AR exposure in non-target species are insufficient and require further review and enforcement. Currently, federal regulation in the US dictates that ARs may be placed within 50 ft of a human-made structure (US Environmental Protection Agency, 2023a, 2023b). Several US states and Canadian provinces have recently restricted the availability of SGARs altogether or only for use by licensed professionals with the goal of preventing AR mis-use (e.g. Government of British Columbia, 2023). California, a US state with relatively strict environmental regulation, has an additional regulatory system (California Department of Pesticide Regulation, CDPR), which requires an additional review of pesticides by the US Environmental Protection Agency (Quinn et al., 2019). In 2014, after evidence of the health effects of SGARs on wildlife, the state of California implemented CDPR 2013, which further restricts the sale of SGARs to only licensed applicators and restricts the placement of ARs within the proximity of manmade structures (California Department of Pesticide Regulation, 2013). Banning the use of ARs will prevent exposure in non-target wildlife and will require more emphasis on sanitation and physically excluding rodents from infrastructure. However, in most places, including Chicago, homeowners can access many products containing SGARs in limited quantities without a license. Specifically, the high prevalence of brodifacoum and bromadiolone we observed in our sample may be due to their availability to homeowners. Based on a review of publicly available rodenticides listed at major retailers (e.g. Lowes, Walmart) we found 19 products containing brodifacoum and 30 products containing bromadiolone as the main active ingredient (see Supplemental Material for details on web searches and products). EPA regulations state that pelleted baits are no longer permitted for consumer markets (US Environmental Protection Agency, 2023a, 2023b). However, we found pellet products containing diphacinone and brodifacoum at Home Depot and Walmart (Supplemental Material). Based on observations in Chicago over the past 20 years, one author who is a pest professional has seen rodenticide-treated products outside of housings hundreds of times, but never associated with a licensed professional. These observations include a building engineer putting rodenticide blocks on paper plates in the docks of their warehouses, rodenticide pellets sprinkled on the ground around restaurant dumpsters, and homeowners sprinkling rodenticide pellets to form a barrier around their house (R. Fyffe, pers. obs.). In conversation, residents can be unaware that these products must be in a tamper-resistant station if outdoors and that using rodenticides to control species such as squirrels is not permitted. These experiences, in addition to the high prevalence in AR exposure we observed in mesopredators, underscore the need for restrictions on AR use and availability to the public such as recently proposed AR mitigation guidelines from the EPA (US Environmental Protection Agency, 2023a, 2023b).

In light of our data and these observations, we would recommend restricting the availability of products containing ARs to only licensed professionals. Lower-income residents may not be able to afford licensed pest professionals and so free municipal abatement programs and educational workshops are imperative to provide equitable rat abatement. For example, the 3-1-1 rat reporting and baiting program in Chicago (City of Chicago, 2023) is a free service to residents. Any municipal program would also need public engagement efforts in communities with less trust in government. Additional policies to protect vulnerable communities from rat exposure are also needed, such as stronger tenants' rights with respect to landlord accountability for controlling and excluding rodents similar to the laws for other pests (i.e. bed bugs; City of Chicago, 2024). Further, the context of the AR application should be strongly considered, for example restricting use to indoor settings or areas with a higher risk of human-rat contact.

More research is needed to understand whether ARs actually cause rat population declines to properly weigh the risks and benefits of ARs to manage rats (Quinn, 2019). Currently, there lacks clear evidence that AR use causes rat population declines, but there is evidence that ARs causes acute mortality (Murray, 2011; Niedringhaus et al., 2021) and immune dysfunction (Serieys et al., 2018a, 2018b) in wildlife and is associated with a higher prevalence of zoonotic infection in poisoned rats (Murray and Sánchez, 2021). Until public access to products containing ARs is restricted, understanding the motivations of homeowners to use ARs, their awareness of risks (e.g. Morzillo and Mertig, 2011), and whether they use products properly (Bartos et al., 2012) are important to design messaging campaigns to reduce AR mis-use by homeowners. More broadly, more emphasis on other human behaviors such as food waste and garbage containment may reduce the demand for rodenticides in cities (Parsons and Munshi-South, 2020). Balancing the need for pest control in cities with preventing health risks for urban wildlife is a core aspect of coexisting with wildlife in urban ecosystems (Hunold and Mazuchowski, 2020).

5. Conclusions

We found universal exposure of urban mesopredators to second generation anticoagulant rodenticides (SGARs) with evidence of primary exposure (stomach contents consistent with the coloration of bait blocks and pellets containing ARs) and mammary transfer (through milk to young who are not yet weaned). We also found that mesopredators had been exposed to more AR compounds relative to rats and found evidence of biomagnification (i.e. biomagnification factors > 1.00) for non-target mesopredators relative to rats in most (75 %) comparisons. Other urban predators are likely also at risk for AR exposure due to the large proportion of live rats with detectable and sometimes quite high concentrations of SGARs. Although it is resource-intensive, surveillance in animals not suspected of AR poisoning can help reveal the extent of AR exposure in non-target species. The high prevalence of exposure to ARs we observed in non-target mesopredators, in addition to stomach contents visually consistent with the coloration of rat bait blocks and pellets, suggests that products containing ARs are not being used in accordance with their guidelines to prevent access by non-target species. Greater restrictions and enforcement on the contexts in which ARs can be used (e.g. only by licensed professionals) and education and access to alternative methods with municipal support for residents, are needed to protect the health of wildlife, humans, and the ecosystems we share.

CRediT authorship contribution statement

Jacqueline Y. Buckley: Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization.

Maureen H. Murray: Writing – review & editing, Writing – original draft, Visualization, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Noé U. de la Sancha:** Writing – review & editing, Visualization, Resources, Formal analysis, Data curation. **Mason Fidino:** Writing – review & editing, Formal analysis. **Kaylee A. Byers:** Writing – review & editing, Conceptualization. **Rebecca Fyffe:** Writing – review & editing, Resources, Project administration, Data curation. **Seth Magle:** Writing – review & editing, Resources, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

We have included our dataset as Supplemental Material.

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Ethics statement

Wildlife was euthanized in accordance with AVMA-approved techniques for wildlife. This study made opportunistic use of carcasses from wildlife samples legally harvested from a pest management company in Chicago, IL, and was therefore exempt from Lincoln Park Zoo Animal Care and Use Committees (IACUC) protocol approval.,

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.175883.

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