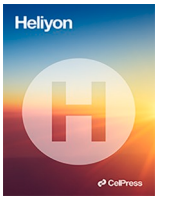
Heliyon 10 (2024) e29471

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

A strategy to improve rodent control while reducing rodenticide release into the environment

Tanja Blažić [a,*], Bojan Stojnić [b], Svetlana Milanović [c], Goran Jokić [a]

[a] Institute of Pesticides and Environmental Protection, Zemun, 11080, Belgrade, Serbia
[b] Faculty of Agriculture, University of Belgrade, Serbia
[c] Faculty of Veterinary Medicine, University of Belgrade, Serbia

ARTICLE INFO

Keywords:
Anticoagulant combination
Rattus norvegicus
Rodenticide
Non-target
Reduced dose
Environment-friendly

ABSTRACT

In addition to having a negative impact on the health of people and domestic animals, rodents often cause enormous damage to the environment by disrupting natural biodiversity. The negative impacts of rodents in urban and rural areas have required intensive use of rodentcides in spite of the proven risk of secondary poisoning of non-target predators and scavengers. Continuous and intensive use of rodenticides has led to environmental pollution through their retention in the environment. Commensal rodents are predominantly managed with anticoagulant rodenticides, which are very persistent in the environment and move up the food chain and accumulate in the bodies of predators and scavengers. Generally, the use of anticoagulant rodenticides continues, and there is a need to take appropriate measures to reduce their harmful impact. The efficacy of second generation anticoagulants (bromadiolone, difenacoum and brodifacoum), combined either mutually or with chlorophacinone at reduced doses (0.001 % and 0.0008 %), in controlling brown rats (*Rattus norvegicus*) was tested in a four-day no-choice feeding test. Combinations of second generation anticoagulants were more effective than the combination of chlorophacinone and second generation anticoagulants. The results indicate that combinations of different anticoagulants at multifold lower doses than the standard may provide a successful tool for brown rat control and a more environment-friendly method of rodent control and protection of non-target animals.

1. Introduction

Despite the long and intensive efforts to eradicate the Norway rat, its explosive demography, adaptable ecology and opportunistic behaviour have ensured its survival as one of the high-ranking species on the list of pests that cause grave economic losses and endanger the health of humans, as well as domestic animals [1–6]. The fact that rats are known to be hosts of a long list of resistant bacterial strains, which is why they are considered an important reservoir of antibiotic resistance, is an additional threat to human and animal health [2,7]. Many studies have documented direct and indirect impacts of rodents on ecosystem properties. Rodents remain one of the most widespread and damaging invasive mammal species, especially on islands [8–10]. They cause serious detriment through predation and competition. There have been several reported cases of native species being driven to extinction by invasive rodents [10–12].

* Corresponding author.
E-mail address: tanja.scepovic@pesting.org.rs (T. Blažić).

https://doi.org/10.1016/j.heliyon.2024.e29471
Received 14 December 2023; Received in revised form 1 March 2024; Accepted 8 April 2024

Rodents are also the cause of some more recent bird extinctions, and have been implicated in the extirpation of numerous small mammals, invertebrates and plant species [8–11]. Global conservation actions aimed to prevent or slow down extinctions and protect biodiversity are costly. At least US $21.5 billion has been spent annually worldwide on biodiversity conservation [13].

Anticoagulants, which are the most widespread type of rodenticides intended for rodent control, are highly toxic compounds [14–16]. Research is therefore increasingly focusing on incorporating environmentally-friendly programmes in rodent control practice [17–20]. The goal is to discover the most effective method of control of rodent numbers while causing the least possible environmental pollution and threat to non-target animals. As rodenticide application has proved to be the most effective way of controlling rodents, research is focusing on modifications of rodenticide treatments [15,21,22].

The European Commission has recently accepted a recommmendation of the European Chemical Agency (ECHA) that all anticoagulants currently available for use at rates exceeding 30 ppm (0.003 %) be banned for amateur uses [15,22]. On the one hand, rodenticide application at reduced doses alleviates ecological impact and lowers environmental residues of anticoagulants, while on the other hand it raises the question of efficacy of such rodenticides. Dose reduction would also expectedly require longer feeding periods before lethal dose is achieved, and consequently an extended period of animal mortality, which would result in their being exposed as prey to predators for longer periods of time, and in increasing the risk of secondary and tertiary poisoning [16,23–26]. Research has already shown that some rodenticides, such as brodifacoum or cholecalciferol, are effective at lower doses [21,22]. However, there is insuficient evidence of the efficacy of anticoagulants at additionally reduced doses and their potential synergistic activity.

Synergistic action is especially attractive from the ecotoxicological aspect [18,27–31]. Combinations of second and first generation anticoagulants might be more applicable in practice from the environmental perspective than combinations of second generation anticoagulants mutually, regardless of the reduced contents of their active ingredients. First generation anticoagulants have more acceptable DT_{50} and Log Pow values, which indicates their persistence in soil and ability to bioaccumulate in living organisms [32–36]. Compared to second generation, first generation anticoagulants do not tend to bioaccumulate and have been found less toxic [37]. As second generation anticoagulants are classified into the PBT (persistent, bioaccumulative and toxic) group of substances and do not meet environmental and public health safety criteria, it is necessary to test their toxicity to brown rats at lower doses and in combinations of two anticoagulants, relaying on a potential synergistic action. Reduced efficacy of any rodenticide due to insufficient consumption of bait may misguide into a conclusion that bait is not toxic enough.

A lack of reliable and effective methods of rodent control justifies the use of anticoagulant rodenticides in practice. Their detrimental effect on the environment and wildlife, yet unavoidable status so far, have made it necessary to improve the existing rodent pest control program. From an economic point of view, reduced contents of active ingredients would significantly lower the production cost of products for brown rat control. Environmentally-safe rodenticides could also minimize environmental pollution and threat to non-target animals. The present study therefore focused on testing the efficacy of combinations of first and second generations of anticoagulants in concentrations lower than standard application rates for commercial products in order to avoid or reduce negative impact on non-target animals and the environment.

2. Materials and methods

2.1. Animals

Wild-born rats were captured in Belgrade suburbs. Norway rats were captured in facilities of an abandoned cattle farm where no eradication of rodents had been conducted in previous years. Sixty mature and healthy animals, their body weight ranging from 124 g to 407 g, were used in the trials. On arrival, all animals were sexed and individually housed. The rats were acclimatized for three weeks. Water and laboratory standard diet were provided ad libitum during acclimatization. The sixty animals were divided in 10 groups intended for 10 separate experiments (3 males and 3 females per group).

2.2. Baits

Placebo bait was prepared by mixing crushed wheat grain and corn oil. Test baits were prepared by mixing placebo bait with appropriate amounts of liquid anticoagulant concentrates. All liquid concentrates (0.25 % of active ingredients) were commercially available and supplied by Ekosan D.O.O. (Serbia). The following anticoagulants were used in the trial: chlorophacinone, bromadiolone, difenacoum and brodifacoum.

All test baits contained combinations of two anticoagulants, both of which were applied at the same concentration. Both anticoagulants in each combination were applied at 0.001 % or 0.0008 %. Variations in active ingredient content in the prepared baits were within limits of ±8 %. All baits were prepared in the Laboratory of Applied Zoology, while active ingredient contents in the baits were checked in the Laboratory of Chemistry of the Institute of Pesticide and Environmental Protection.

2.3. Experimental design

Experiments were conducted using no-choice feeding tests [38]. Each animal was kept in an individual cage. Standard laboratory feed was replaced with test baits. All animals were fed on laboratory-made baits, which consisted of different anticoagulant combinations. The animals were given test bait without any other choice of diet. Water was available ad libitum throughout the test period.

Baits were offered and replenished daily. Rats were fed for four consecutive days and bait consumption was recorded. Spilled food

was collected daily under each cage. After testing was completed, the animals were given standard laboratory diet, the same they had been offered during acclimation. Besides the acclimation period of 21 days, and four-day test period (period of exposure to test ingredients), animal survivors of test feeding were also monitored for further survival over a subsequent observation period of up to two weeks. Symptoms of poisoning, and day of death were also recorded. All dead animals were autopsied to determine the cause of death.

The following combinations of anticoagulants were offered to 6 groups of animals and all combinations were made in 0.001 % concentration for both anticoagulants: chlorophacinone + bromadiolone (0.001 % + 0.001 %), chlorophacinone + difenacoum (0.001 % + 0.001 %), chlorophacinone + brodifacoum (0.001 % + 0.001 %), bromadiolone + difenacoum (0.001 % + 0.001 %), bromadiolone + brodifacoum (0.001 % + 0.001 %) and difenacoum + brodifacoum (0.001 % + 0.001 %). An additional concentration of 0.0008 % (0.0008 % + 0.0008 %) was made for four anticoagulant combination that achieved complete mortality at the higher concentration.

Room temperature was kept within 20–22 °C range and relative humidity varied from 40 to 60 %, while the light/dark cycle was 12:12 h. Ventilation was done at 15 min intervals for complete refreshment of air. Water was provided ad libitum.

2.4. Data analysis

Consumption of anticoagulants was calculated for each animal using the formula: consumption over exposure period (g)/initial body weight (g).

Statistical analysis was conducted using ANOVA and Tukey's HSD test to examine the influence of different anticoagulant combinations on rat consumption.

The influence of anticoagulant combinations on survival time was checked by non-parametric ANOVA and multiple comparisons of mean ranks for all tested groups.

The influence of anticoagulant combinations on rat appetite and rate of action was compared by *t*-test. In all analyses *P*-values below 0.05 were considered statistically significant.

Combinations which contained anticoagulants from different groups (second and first generation anticoagulants) will be presented in the following text as "II + I". All combinations containing second generation anticoagulants will be presented in the following text as "II + II". Abbreviations are also used for all combinations of anticoagulants: Brom + Chlor (bromadiolone + chlorophacinone), Dif + Chlor (difenacoum + chlorophacinone), Bdf + Chlor (brodifacoum + chlorophacinone), Brom + Dif (bromadiolone + difenacoum), Brom + Bdf (bromadiolone + brodifacoum) and Dif + Bdf (difenacoum + brodifacoum).

3. Results

3.1. Mortality

The combinations Dif + Chlor, Dif + Brom, Dif + Bdf and Brom + Bdf caused 100 % mortality at 0.001 % concentration. Based on these results we decided to carry out an additional experiment with a further reduced dose (0.0008 %) of all four combinations. The combinations Brom + Chlor and Bdf + Chlor were not tested at the additionally reduced concentration because mortality was below 100 % when the higher (0.001 %) concentration was applied.

All II + II combinations successfully achieved 100 % mortality with 0.001 % concentration. The same results were observed for two combinations when 0.0008 % concentration was applied (Table 1). Regarding II + I combinations, only Dif + Chlor reached 100 % mortality at the higher concentration.

3.2. Survival time

Using multiple comparisons of mean ranks for all groups we confirmed that there were no statistical differences in survival time between the tested combinations (H = 15.18; P = 0.09; df = 9; N = 60). Nominally, the highest median value was confirmed for

Table 1
Efficacy of anticoagulant combinations at two different concentrations against brown rat.

Anticoagulant generation	Anticoagulant combination	ABW (g)	SP min-max (day)	Mortality
	0.001 %			
II + I	Dif + Chlor	215.2	5–10	6[a]/6
	Brom + Chlor	289.7	2–5	2/6
	Bdf + Chlor	298.0	6–7	3/6
II + II	Dif + Brom	187.8	5–7	6/6
	Dif + Bdf	223.8	4–8	6/6
	Brom + Bdf	196.0	5–8	6/6
	0.0008 %			
II + I	Dif + Chlor	214.7	5–6	4/6
II + II	Dif + Brom	174.7	3–11	5/6
	Dif + Bdf	195.2	6–8	6/6
	Brom + Bdf	198.3	4–6	6/6

[a] number of not survived animals; ABW-average body weight; SP-survival period.

animals treated with the Dif + Brom (0.001 %) combination (Md = 18 days) and the lowest value (Md = 5 days) for Brom + Chlor (0.001 %) combination (Fig. 1).

3.3. Anticoagulant consumption

T-test showed statistical differences in bait consumption between the first and last test day for Dif + Chlor (0.001 %), Bdf + Chlor (0.001 %), Brom + Bdf (0.001 %) and Brom + Bdf (0.0008 %) combinations (Table 2). Nominally higher bait consumption was confirmed on the first test day for all anticoagulant combinations.

There was no confirmed influence of active ingredient concentration on bait consumption. No significant differences were confirmed in bait consumption for the four anticoagulant combinations used in two different concentrations (0.0008 % and 0.001 %). Bait consumption was compared only for the first test day between two groups of animals that consumed baits with different concentrations of the same anticoagulant combination (Table 3).

External symptoms were also recorded, such as bleeding nose, anus and feces, lethargy, laboured breathing and piloerection. These symptoms became obvious one or two days before death. Animal weight was reduced during the experiment because their appetite was quenched by eating anticoagulants. Autopsy showed that all dead animals had signs of internal bleeding provoked by eating anticoagulants.

4. Discussion

Rodent trapping and application of repellents are more environmentally-friendly control methods but certain limitations are involved in practice. Mechanical removal of rodents is more labour- and time-consuming, and requires more traps, while repellents have limited effectiveness in outdoor applications [39]. To date, effective biological rodent control measures, such as fertility control of rodents, have not been demonstrated at landscape scales, and very few such products have achieved registration [40]. Current practice has shown that application of anticoagulants is the most effective method of controlling rodents.

Relying on the synergistic activity of reduced doses of two combined anticoagulants would help identify environmentally more acceptable but equally effective means for rodent control. The results of the present study indicate that certain combinations of anticoagulants at 0.001 % and 0.0008 % concentrations could be successfully used to control brown rats. The tested concentrations of anticoagulants were three times lower than the current limit in the EU for anticoagulants allowed for amateur use (0.003 %). The anticoagulant concentration of 0.005 % is still allowed for professional use [15,22]. The efficacy of difenacoum combinations with chlorophacinone, bromadiolone and brodifacoum at 0.001 % concentration, and brodifacoum with bromadiolone and difenacoum at 0.0008 % was 100 % in the laboratory. These are the first results of examination of the susceptibility of wild-born brown rats to combinations of anticoagulants at multiple-reduced doses. The results of a research by Endepols et al. [29] on brown rat indicated a great potential of the combination coumatetralyl + cholecalciferol but neither component was applied at reduced dosage (0.0375 % + 0.0250 %). No-choice feeding tests with possums (*Trichosurus vulpecula*) and ship rats (*Rattus rattus*) achieved mortality of 87 % and 86 %, respectively. The results were reported by Eason et al. [31] and the full dose of diphacinone was applied in combination (0.005 %) with vitamin D_3 dose reduced from 0.08 % to 0.06 %. Data from a research by Singla et al. [28] showed a great potential of the combination bromadiolone + cholecalciferol at decreased contents of both active ingredients (0.001 % + 0.005 %) for controlling bandicoot rat (*Bandicota bengalensis*). Most tested combinations were found to fail in causing symptoms fast, i.e. bait consumption was not found to change during the test. Initial symptoms mostly resulted in changed animal behavior and reduced bait consumption [28,29,41,42]. In the present study, combinations that included brodifacoum concentration of 0.001 % (Brom + Bdf and Bdf + Chlor), as well as the combination Brom + Bdf at 0.0008 % concentration, provided faster action as bait consumption on the last test day was two or more times lower. The combination of brodifacoum and difenacoum at both concentrations showed no significant difference in bait

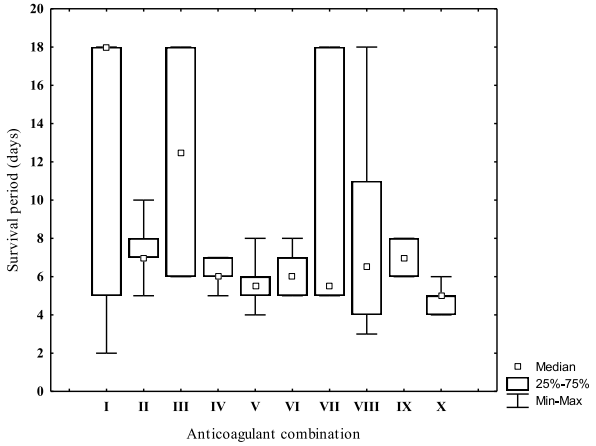


Fig. 1. Survival period in ten groups of animals fed on ten different anticoagulant combinations.

Table 2
T-test results after comparison of bait consumption (g/g bw) between test day 1 and test day 4.

Anticoagulant combination	T value	P value	Ms±SD[a] (day 1)	Ms±SD (day 4)
0.001 %				
Dif + Chlor	3.50	0.0057	0.100 ± 0.006	0.041 ± 0.015
Dif + Brom	1.78	0.1058	0.061 ± 0.009	0.040 ± 0.007
Dif + Bdf	1.69	0.1211	0.082 ± 0.009	0.053 ± 0.014
Brom + Chlor	1.45	0.1778	0.053 ± 0.010	0.030 ± 0.013
Brom + Bdf	3.13	0.0106	0.091 ± 0.007	0.039 ± 0.014
Bdf + Chlor	3.37	0.0071	0.081 ± 0.008	0.046 ± 0.006
0.0008 %				
Dif + Chlor	0.41	0.6915	0.119 ± 0.011	0.112 ± 0.015
Dif + Brom	1.33	0.2523	0.058 ± 0.013	0.033 ± 0.013
Dif + Bdf	0.94	0.3642	0.086 ± 0.010	0.072 ± 0.011
Brom + Bdf	3.15	0.0102	0.120 ± 0.018	0.045 ± 0.015

[a] Ms±SD−mean value ± standard deviation.

Table 3
Influence of active ingredient concentration on bait consumption (g/g bw).

Anticoagulant combination	Content (%)	Ms±SD[a]	T value	P value
Dif + Chlor	0.0008	0.12 ± 0.03	1.37	0.2014
	0.001	0.10 ± 0.02		
Dif + Brom	0.0008	0.06 ± 0.03	0.17	0.8689
	0.001	0.06 ± 0.02		
Dif + Bdf	0.0008	0.09 ± 0.02	0.30	0.7677
	0.001	0.08 ± 0.02		
Brom + Bdf	0.0008	0.12 ± 0.04	1.44	0.1800
	0.001	0.09 ± 0.02		

[a] Ms±SD - mean value ± standard deviation.

consumption on the first and last test day.

Comparing the II + I and II + II combinations, II + II proved to be the more effective. All tested II + II combinations achieved 100 % mortality at the concentration of 0.001 %. The same result was achieved by the lower test concentration (0.0008 %) of both combinations that included brodifacoum as a component, while a single animal died in a group of animals fed on the combination Dif + Brom.

The results of this research show that the reduced doses of II + II combinations were more effective than II + I combinations. The combination of difenacoum and chlorophacinone at 0.001 % concentration promised potentially good results in the field. Mortality was 100 %, and bait consumption analysis revealed a fast evolution of symptoms as bait consumption decreased 60 % on the last test day. First-generation anticoagulants are generally less toxic and persistent than second-generation anticoagulants and therefore less of a hazard to nontarget species through secondary poisoning and bioaccumulation [36,37]. Chlorophacinone is known to have lower values of degradation in soil and bioaccumulation in living organisms (DT_{50} = 128 days, log Pow = 2.42), in contrast to bromadiolone, difenacoum and brodifacoum (DT_{50} = 52–252 days, log Pow = 4.07; DT_{50} = 439 days, log Pow = 7.6; DT_{50} = 300 days, log Pow = 8.5), which have shown higher values [32–35]. Lower DT_{50} of chlorophacinone indicated its faster degradation in soil. Also, its lower log Pow indicated that it is a water-soluble substance that is more readily eliminated and generally with a lower bioaccumuation potential, which makes it a suitable component for combinations.

The presented results indicate the validity of use of anticoagulant combinations at reduced contents of active ingredients (0.001 % and 0.0008 %) in rodent control procedures. High mortality (100 %) resulting from the application of all combinations containing 0.001 % active ingredients, and some combinations with lower concentration (0.0008 %), indicate satisfactory toxicity and effectiveness in laboratory no-choice tests. However, additional choice and field tests are required in order to determine the acceptability and efficacy of rodenticide combinations under practical conditions before final validation of their application in practice.

5. Conclusion

The results infer that anticoagulant combinations can successfully control brown rats in doses that are lower than the standard application rate (0.001 %), and even at the additionally reduced dose of 0.0008 %. As the combination difenacoum + chlorophacinone (0.001 %) achieved complete mortality and fast-evolving symptoms, while having environmentally safer components, that combination may be considered a better choice in rodent control than II + II combinations at the same concentration. The application of anticoagulant combinations at multiple-reduced doses would be highly significant not only from the aspect of effective rodent control, it would be equally important from an ecotoxicological aspect due to the reduced release of rodenticides into the environment. The present results are the first step towards a more comprehensive research aimed at examining the acceptability and efficacy of rodenticide combinations under practical conditions in choice and field tests. Data concerning the parameters such as blood clotting

time, vitamin K levels, hepatic residue concentrations of deceased rats or expression of resistance genes also need to be provided in further research.

Data availability

Data will be made available on request.

Ethics statement

The experiment was conducted in accordance with ethical principles and was approved by the Ministry of Agriculture, Forestry and Water Management (Republic of Serbia) - Veterinary Directorate (No. 323-07-04943/2020-05/2, 29.05.2020).

CRediT authorship contribution statement

Tanja Blažić: Data curation, Investigation, Writing – original draft. **Bojan Stojnić:** Formal analysis. **Svetlana Milanović:** Investigation. **Goran Jokić:** Writing – review & editing, Investigation, Supervision.

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.

Acknowledgements

This research was funded by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia (grants 451-03-66/2024-03/200214 and 451-03-66/2024-03/200143).

References

[1] S. Battersby, R.B. Hirschhorn, B.R. Amman, Commensal rodents, in: Public Health Significance of Urban Pests, WHO Regional Office for Europe, Copenhagen, Denmark, 2008, pp. 387–419.
[2] A. Desvars-Larrive, W. Ruppitsch, S. Lepuschitz, M.P. Szostak, J. Spergser, A.T. Febler, S. Schwarz, S. Monecke, R. Ehricht, C. Walzer, I. Lončarić, Urban Brown Rats (*Rattus norvegicus*) as Possible Source of Multidrug-Resistant Enterobacteriaceae and Meticillin-Resistant *Staphylociccus* Spp, 2019. Vienna, Austria, 2016 and 2017, Eurosurvelliance, https://doi:10.2807/1560-7917.ES.2019.24.32.1900149.
[3] K. Modlinska, W. Pisula, The Norway rat, from an obnoxious pest to laboratory pet, Elife (2020), https://doi.org/10.7554/eLife.50651.
[4] C.C. Udechukwu, C.A. Kudi, P.A. Abdu, E.A. Abiayi, O. Orakpoghenor, Prevalence of Leptospira interrogans in wild rats (Rattus norvegicus and Cricetomys gambianus) in Zaria, Nigeria, Heliyon 7 (2021) e05950.
[5] E.G. Anaduaka, N.O. Uchendu, R.O. Asomadu, A.L. Ezugwu, E.S. Okeke, T.P.C. Ezeorba, Widespread use of toxic agrochemicals and pesticides for agricultural products storage in Africa and developing countries: possible panacea for ecotoxicology and health implications, Heliyon 9 (4) (2023) e15173.
[6] Z. Zhuang, L. Qian, J. Lu, X. Zhang, A. Mahmood, L. Cui, H. Wang, X. Wang, S. Yang, L. Ji, T. Shan, Q. Shen, W. Zhang, Comparison of viral communities in the blood, feces and various tissues of wild brown rats (Rattus norvegicus), Heliyon (2023) e17222, https://doi.org/10.1016/j.heliyon.2023.e17222.
[7] F.M. Gakuya, M.N. Kyule, P.B. Gathura, S. Kariuki, Antimicrobial resistance of bacterial organisms isolated from rats, East, Afr. Med. J. 78 (2001) 646–649.
[8] D.R. Towns, I.A.E. Atkinson, C.H. Daugherty, Have the harmful effects of introduced rats on islands been exaggerated? Biol. Invasions 8 (2006) 863–891.
[9] J.S. Athens, Rattus exulans and the catastrophic disappearance of Hawai'i's native lowland forest, Biol. Invasions 11 (2009) 1489–1501.
[10] J.J.H. St Clair, S. Poncet, D.K. Sheehan, T. Szekely, G.M. Hilton, Responses of an island endemic invertebrate to rodent invasion and eradication, Anim. Conserv. 14 (2011) 66–73.
[11] H.P. Jones, B.R. Tershy, E.S. Zavaleta, D.A. Croll, B.S. Keitt, M.E. Finkelstein, G.R. Howald, Severity of the effects of invasive rats on seabirds: a global review, Conserv. Biol. 22 (2008) 16–26.
[12] M. Saunier, M. Amy, M. Baumann, F. Bignon, A. Cartraud, Q. d'Orchymont, J. Gazal, A. Goguelat, M. Lemenager, S. Marinesque, S. Orlowski, M. Pierre Etienne, L.C. Matthieu, Long-term Monitoring Highlights the Positive Responses of the Seabird Community to Rat Eradication at Tromelin Island, Western Indian Ocean, Conservation Sciences and Practice, 2024, https://doi.org/10.1111/csp2.13083.
[13] A. Waldron, A.O. Mooers, D.C. Miller, N. Nibbelink, D. Redding, T.S. Kuhn, J.T. Roberts, J.L. Gittleman, Targeting global conservation funding to limit immediate biodiversity declines, Proc. Natl. Acad. Sci. USA 110 (29) (2013) 12144–12148.
[14] W. Erickson, D. Urban, Potential Risks of Nine Rodenticides to Birds and Nontarget Mammals: a Comparative Approach, United States Environmental Protection Agency, Washington, 2004. https://www.fluoridealert.org/wp-content/pesticides/EPA-HQ-OPP-2006-0955-0005.pdf.
[15] EC, Commision Regulation (EU) 2016/1179. EC (European Commission), Off. J. Eur. Union (L195/11-L195/25) (2016).
[16] H. Alomar, A. Chabert, M. Coeurdassier, D. Vey, B. Philippe, Accumulation of anticoagulant rodenticides (chlorofacinone, bromadiolone and brodifacoum) in a non-target invertebrate, the slug, *Deroceras reticulatum*, Sci. Total Environ. (2018), https://doi.org/10.1016/j.scitotenv.2017.08.117.
[17] S.A. Shumake, R.T. Sterner, S.E. Gaddis, Repelents to reduce cable gnawing by wild Norway rats, J. Wildl. Manag. 64 (2000) 1009–1013.
[18] D.R. Morgan, J. Arrow, M.P. Smith, Combining aspirin with Cholecalciferol (Vitamin D3)- a potential new tool for controlling possum populations, PLoS One (2013), https://doi.org/10.1371/journal.pone.0070683.
[19] T.T. Tran, L.A. Hinds, Fertility control of rodent pests: a review of the inhibitory effects of plant extracts on ovarian function, Pest Manag. Sci. 69 (2013) 342–354.
[20] M. Frankova, B.K. Eliasova, P. Rodl, R. Aulicky, D. Frynta, V. Stejskal, Monitoring of *Rattus norvegicus* based on non-toxic bait containing encapsulated fluorescent dye: laboratory and semi-field validation, J. Stored Prod. Res. 64 (2015) 103–108.
[21] S. Hix, P. Aylett, D. MacMorran, C.T. Eason, S. Sam, J.G. Ross, A. Miller, S.C. Ogilvie, Low-dose cholecalciferol bait for possum and rodent control, N. Z. J. Agric. Res. 5 (2012) 207–2015.
[22] M. Frankova, V. Stejskal, R. Aulicky, Efficacy of rodenticide baits with decreased concentrations of brodifacoum: validation of the impact of the new EU anticoagulant regulation, Sci. Rep. 9 (1) (2019) 16779. https://doi:10.1038/s41598-019-53299-8.
[23] B. Walther, A. Geduhn, D. Schenke, J. Jacob, Exposure of passerine birds to brodifacoum during management of Norway rats on farms, Sci. Total Environ. 762 (2021) 144160. https://org.10.1016/j.scitotenv.2020.144160.

[24] R.K. Broughton, K.R. Searle, L.A. Walker, E.D. Potter, M.G. Pereira, H. Carter, D. Sleep, D.G. Noble, A. Butler, A.C. Johnson, Long-term trends of second generation anticoagulant rodenticides (SGARs) show widespread contamination of a bird-eating predator, the Eurasian Sparrowhawk (Accipiter nisus) in Britain, Sci. Total Environ. (2022), https://doi.org/10.1016/j.envpol.2022.120269.
[25] A. Carrera, I. Navas, P. María-Mojica, A.J. García-Fernandez, Greater predisposition to second generation anticoagulant rodenticide exposure in red foxes (Vulpes vulpes) weakened by suspected infectious disease, Sci. Total Environ. (2024), https://doi.org/10.1016/j.scitotenv.2023.167780.
[26] J. Regnery, S. Rohner, J. Bachtin, C. Mohlenkamp, O. Zinke, S. Jacob, P. Wohlsein, U. Siebert, G. Reifferscheid, A. Friesen, First evidence of widespread anticoagulant rodenticide exposure of the Eurasian otter (Lutra lutra) in Germany, Sci. Total Environ. (2024), https://doi.org/10.1016/j.scitotenv.2023.167938.
[27] D.K. Kocher, K. Navjot, Synergistic effect of bromadiolone and cholecalciferol (vitamin D3) against house rat, *Rattus rattus*, Int. J. Curr. Res. Biosci. 2 (2013) 73–82.
[28] N. Singla, S. Kaur, M. Javed, Rodenticidal potential of bromadiolone and cholecalciferol in synergism against *Bandicota bengalensis*, Crop Protect. 72 (2015) 163–168.
[29] S.E. Endepols, N. Klemann, D. Richer, F.R. Matuschka, The potential of coumatetralyl enhanced by cholecalciferol in the control of anticoagulant-resistant Norway rats (*Rattus norvegicus*), Pest. Manag. Sci. 73 (2017) 280–286.
[30] N. Singla, S. Kaur, Rodenticides baits of cholecalciferol, bromadiolone and their combinations against lesser bandicoot rat, *Bandicota bengalensis*, Pestic. Res. J. 30 (1) (2018) 85–91.
[31] C. Eason, L. Shapiro, C. Eason, D. MacMorran, J. Ross, Diphacinone with cholecalciferol for controlling possums and ship rats, N. Z. J. Zool 47 (2019) 106–120. https://doi:10.1080/03014223.2019.1657473.
[32] EFSA, Peer review of the pesticide risk assessment of the active substance difenacoum, EFSA Scientific Report 218 (2008) 1–58.
[33] EFSA, Conclusion on the peer review of the pesticide risk assessment of the active substance bromadiolone, EFSA J. 8 (2010) 1783, https://doi.org/10.2903/j.efsa.2010.1783.
[34] Brodifacoum- ECHA, Evaluation of active substances. Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products, Assess. Rep. (2016) 1–37. https://echa.europa.eu/documents/10162/fa3f5493-6089-bbf3-ec81-84b79b56f259.
[35] ECHA, Chlorophacinone- Evaluation of active substances, Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products, Assessment report. https://www.echa.europa.eu/documents/10162/e623897b-b36e-7527-7ed4-12c9aa311639, 2016.
[36] S.M. Weir, J.F. Thomas, D.N. Blauch, Investigating spatial patterns of mercury and rodenticide residues in raptors collected near the Charlotte, NC, USA, metropolitan area, Environ. Sci. Pollut. Res. 25 (2018) 33153–33161, https://doi.org/10.1007/s11356-018-3229-y.
[37] J. Fischer, A. Friesen, A. Geduhn, S. Hein, S. Jacob, B. Jahn, A. Kalle, A. Kehrer, I. Nöh, E. Petersohn, C. Riedhammer, R. Rissel, A. Schlötelburg, E. Schmolz, B. Schwarz-Schulz, C. Stahr, U. Trauer-Kizilelma, K. Wege, S. Wiec, Authorisation of anticoagulant rodenticides in Germany FAQ on environmental risks, risk Mitigation measures and best practice. https://www.umweltbundesamt.de/en/publications, 2019.
[38] EPPO, Efficacy evaluation of rodenticides: laboratory tests for evaluation of the toxicity and acceptability of rodenticides and rodenticide preparations – PP1/113(2), in EPPO (2004): efficacy Evaluation of Plant Protection Products Miscellaneous, Rodenticides (2004) 23–35.
[39] S. Hansen, C. Stolter, J. Jacob, Effect of plant secondary metabolites on feeding behavior of microtine and arvicoline rodent species, J. Pest. Sci. 89 (2016) 955–963.
[40] K. Jacoblinnert, J. Jacob, Z. Zhang, L.A. Hinds, The status of fertility control for rodents-recent achievements and future directions, Integr. Zool. 17 (6) (2022) 964–980.
[41] T. Šćepović, G. Jokić, A. Esther, D. Kataranovski, P. Vukša, S. Đedović, M. Vukša, VKOR variant and sex are the main influencing factors on bromadiolone tolerance of the house mouse (*Mus musculus* L.), Pest Manag. Sci. 72 (2016) 574–579.
[42] T. Blažić, G. Jokić, A. Esther, S. Đedović, Susceptibility of house mouse carriers of Tyr139Cys and Leu128Ser/Tyr139Cys VKOR variants to difenacoum, Int. J. Pest Manag. 69 (2023) 103–108, https://doi.org/10.1080/09670874.2020.1853277.