Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2021; 9(5): 46-51

Received: 25-07-2021
Accepted: 27-08-2021

Germain Gil Padonou
(1) Centre de Recherche Entomologique de Cotonou (CREC), Benin
(2) Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Cotonou, Benin
(3) Laboratory of Biology and Molecular Typing in Microbiology, Faculty of Sciences and Technology/University of Abomey-Calavi, Benin

Razaki Ossè
(1) Centre de Recherche Entomologique de Cotonou (CREC), Benin
(2) Université Nationale d'Agriculture, Porto-Novo, Bénin

Arthur Sovi
(1) Centre de Recherche Entomologique de Cotonou (CREC), Benin
(2) Faculty of Agronomy, University of Parakou, BP 123, Parakou, Benin

Albert Sourou Salako
(1) Centre de Recherche Entomologique de Cotonou (CREC), Benin
(2) Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Cotonou, Benin

Rock Aikpon
(1) Centre de Recherche Entomologique de Cotonou (CREC), Benin
(2) Université Nationale des Sciences, Technologies, Ingénierie et Mathématiques, Abomey, Bénin

Boulais Yovogan
(1) Centre de Recherche Entomologique de Cotonou (CREC), Benin
(2) Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Cotonou, Benin

Steve Hougbe
(1) Centre de Recherche Entomologique de Cotonou (CREC), Benin
(2) Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Cotonou, Benin

Esdras Mahoutin Odjo
1. Centre de Recherche Entomologique de Cotonou (CREC), Benin
2. Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Cotonou, Benin

Constantin Adoha
(1) Centre de Recherche Entomologique de Cotonou (CREC), Benin
(2) Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Cotonou, Benin

Lamine Baba-Moussa
(1) Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Cotonou, Benin
(2) Laboratory of Biology and Molecular Typing in Microbiology, Faculty of Sciences and Technology/University of Abomey-Calavi, Benin

Martin C Akogbeto
Centre de Recherche Entomologique de Cotonou (CREC), Benin

Corresponding Author:
Albert Sourou Salako
(1) Centre de Recherche Entomologique de Cotonou (CREC), Benin
(2) Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Cotonou, Benin

Efficacy of brodifacoum 50ppm-based rodenticidal baits on the control of the mice *Mus musculus* in Cotonou

Germain Gil Padonou, Razaki Ossè, Arthur Sovi, Albert Sourou Salako, Rock Aikpon, Boulais Yovogan, Steve Hougbe, Esdras Mahoutin Odjo, Constantin Adoha, Lamine Baba-Moussa and Martin C Akogbeto

DOI: https://doi.org/10.22271/j.ento.2021.v9.i5a.8842

Abstract
In Benin, cities with a high population rate such as Cotonou are colonized by several species of rats such as *Rattus rattus*, *Mastomys sp*, *Mus musculus* and *Crocidura olivieri* which are agents of destruction of stored products especially in the area of the big Dantokpa market. Indeed, anticoagulants are the most frequently used rodenticides worldwide in the fight against these rodents. However, very few studies have evaluated the biological efficacy of high dose rodenticides on the resistant strain of the mouse population (*Mus musculus*) in Cotonou. The present work compared the efficacy of three original brodifacoum-based baits: vertox pellets, vertox block and vertox past on the wild domestic mouse strain (*Mus musculus*) in Cotonou.
The results showed that in the no-choice feeding tests in the laboratory, 100% of the animals died in all experimental groups and zero in the control groups. Time to death did not differ significantly between vertox pellet and vertox block bait in either test/feeding regime. However, with vertox past, a relatively longer time to death was observed. The three baits were therefore tested under field conditions on mouse populations and showed the following efficacy in terms of mouse mortality after three weeks: 83.33% with vertox pellets; 72% with vertox block and 95.33% with vertox past. The results obtained highlighted the good efficacy of the three brodifacoum-based baits in heterogeneous (resistant or not) mouse populations.
However, an additional study is needed with the same anticoagulants only on resistant rodent populations.

Keywords: efficacy, rodenticidal baits, brodifacoum, *Mus musculus*, Cotonou

Introduction
Urbanization is constantly increasing, especially in Africa. Nowadays, nearly 50% of the global population lives in urban areas and the phenomenon is not expected to slow down [1]. Projections estimate that 60% of the global population will be living urban areas by 2030 [2]. This growing urbanization observed in Africa has generated increasing environmental modifications such as such as deforestation around cities and transportation routes, occupation of cultivable land that destroyed several natural habitats suitable for different wildlife species [3]. At the opposite, it has also favoured the creation of new ecological niches in urban areas for several synurbic species such as birds, bats, squirrels and mice that takes advantage of this opportunity to adapt, proliferate and live. Due to their voracious feeding habits and their ability to carry pathogens, they become harmful in cities. In Benin, cities with a high population density such as Cotonou are not spared and rats are agents of destruction of stored products, particularly in the area of the large Dantokpa market. In this city, species like *Rattus rattus*, *Mastomys sp*, *Mus musculus* and *Crocidura olivieri* are mainly found [4]. According to Corrigan [5], the high reproductive and adaptative capacity, as well as the opportunistic feeding mode of these small mammals allow them to adapt to the urban environment. The problems caused by these rodents can be seen on crops as well as in the nature and human environment. Population growth in urban areas, climate change, and aging urban infrastructures in some Benin cities such as Cotonou, Porto-Novo, Ouidah, Abomey, and Lokossa may contribute to the problem in the upcoming years if nothing is done.

It appears necessary for these cities to put in place effective and sustainable control programs to mitigate the negative effects resulting from the presence of rats. Although the bioecology of rats as well as the technology to exterminate them are known, many large urban centers still fail to adequately control their population [6]. Anticoagulants have gradually become essential tools to control these rodents since their rodenticide potential was discovered when they were formulated as bait in the 1950s. The main advantage of anticoagulants based rodenticides is undoubtedly their progressive mode of action that consists of stopping blood clotting, leading to fatal hemorrhage, which occurs between 4-12 days. When rodents are invasive, they become devastating. That's why, rodenticides are used in both culture and wildlife protection, as well as in urban areas. Brodifacoum is one of the most potent second-generation anticoagulants, which were developed to overcome the problems of resistance to first-generation anticoagulants in the 1970s. The present study evaluated the efficacy of three different formulations of brodifacoum-based vertox granules in the control of mice sampled in Cotonou, Benin.

Materials and Methods

Experimental animals

To minimize genetic variation issues, wild house mouse (*Mus musculus*) that were used as part of the present study, were collected from a single small population found in abandoned buildings in Cotonou. Water and food (corn paste and smoked fish) were provided to the animals housed in pairs in standard plastic cages (30×15×15 cm), under standard laboratory conditions (temperature 19–22 °C, 12:12 light cycle). For the experiments, they have been individually housed in experimental wire cages (26 × 17 × 17 cm) (Fig. 1).

The three tested formulations of brodifacoum-based baits includes: vertox pellets, vblock and, vertox pasta bait.

Fig 1: Experimental cages each containing a specimen of *Mus musculus*

Characteristics of rodents

The rodents used are in the form of granules or paste impregnated with a solution of 0.005% Brodifacoum (50 mg/kg), ready to use. Their mode of action results in blood coagulation disorders (anticoagulant) and the appearance of hemorrhagic syndromes. The delayed effect of these molecules allows to bypass the distrust of rodents. Rodent corpses dry out and rarely smell. Of the three rodents used, two are red pellet baits based on cereals with enhanced palatability and a bittering agent (denatonium benzoate 0.001%): these are vertox pellets (A) and vertox blocks (B) (Fig. 2). Vertox Pasta Bait (C) is a pasty rodenticide formulated from a mixture of culinary grade wheat flour, packaged in 10 g biodegradable paper bags (Fig. 2). It is a bait that provides a complete meal for rodents and can be used successfully when rodents have refused other baits. The use of synthetic peanut flavors allows the product to be used in sensitive situations where the use of nut products would not be allowed.

A. B. C.

Fig 2: Photos of vertox pellets (A), vertox pasta bait (B) and vertox blok (C) based on brodifacoum at 50ppm used in the tests.

Multiple day feeding test

In total, 29 males (average body weight = 143.19 g) and 31 females (average body weight = 133.69 g) wild house mouse (*Mus musculus*) were used (Table 1). These mice were assigned at random to either the experimental group (24 males and, 26 females) or the control one (5 males and, 5 females).

Experimental animals were fed continuously with 5g of each rodenticide bait per day per individual until all animals died, while control animals were fed with a standard meal (Corn paste and smoked Fish).
The mice were examined on a daily basis. The weight of the food which has not been eaten by the animals was determined. The same procedures were followed daily, and the time at which each animal died was recorded. The experiment was conducted until the last experimental individual died. Water was provided at will.

Single day feeding test
We tested 32 males (mean body weight =142.89 g) and 32 females (mean body weight = 132.28 g) (Table 1). These mice were randomly assigned to either the experimental group (n = 27 males, 27 females) or the control one (5 males, 5 females). The experimental animals were fed with a 5g rodenticide bait for 24 hours; the bait was then replaced with a standard diet. The control animals were given a standard diet. Water was provided at will. Treatments and experimental procedures followed here were the same as in the multi-day feeding test.

Table 1: Number and average body weight of mice in the multi-day feeding test

Species of mice	*Mus musculus*	
Sex	Males	Females
Feeding over several days		
Number	29	31
Mean body weight (g)	143.19	133.69
Feeding over one day		
Number	32	32
Mean body weight (g)	142.89	132.28

Field experiment
Rodent activity was measured one week prior to the brodifacoum-based bait testing. For this purpose, the consumption of a non-toxic monitoring bait was evaluated one week before and three weeks after the treatment. The monitoring baits (54 g) were placed at the study site in a facility (approximately 10 × 10 m) similar to that dwells both human populations and mice. Thereafter, 15g of vertox pellets or vertox block or vertox pasta bait were placed at baiting points at 8 m intervals on the paths used by the mice and near the burrows. Shelters of plastic pipe sections (Fig. 3) were used to prevent rodents from being disturbed while eating. Monitoring of baits allowed their weighing, replenishment, and replacement with fresh samples on a weekly basis. Bait effectiveness was assessed through the percentage reduction in rodent activity which served as an estimate of mortality.

Fig 3: Baiting mice with brodifacoum-based rodenticides in the community

Measured indicators and data analysis
The indicators measured includes:
- Food consumption on day 1 (g/g)
- Dose of brodifacoum on day 1 (g/g)
- Time to death (days)
- Average food consumption per day (g/g)
- Mortality rate (%).

To assess the time to death of mice in the laboratory feeding experiments, a survival analysis comparing multiple samples was performed separately for the single and multi-day (s) feeding tests. To visualize the survival data, Kaplan-Meier survival curves showing the likelihood of survival in a given time interval were used. Over several days, individual food consumption per experiment was expressed as being the amount of food consumed per day per gram of individual body weight. The food (bait or standard diet) consumed on the first day and the corresponding dose of ingested brodifacoum, both expressed per gram of individual body weight, were analyzed separately for the feeding test performed over one or several day(s). The mortality rate was estimated in terms of reduction of rodent activity post-treatment compared to pre-treatment based on the consumption of monitoring baits for both experiments.

Results
Laboratory experiments.
Food intake and brodifacoum dosage
For multi-day feeding, the average bait consumed per experiment differed significantly between experimental groups (Table 2) ($p<0.05$). Multiple comparison of average food consumption per day revealed that mice consumed significantly less of the rodenticide vertox pasta (1.5±0.1 g) than vertox pellets (3.8±0.2 g), vertox block (4.4±0.4 g), and standard bait (control) (average amount of bait consumed per day: 15.5±1.5 g) ($p<0.05$) (Table 2).
A similar trend was observed during the single day feeding test. Indeed, vertox pasta was consumed less than the other two ($p<0.05$). Mice in the control group ingested significantly more standard bait than those of experimental groups that consumed rodenticides baits ($p<0.05$) (Table 2).
The mean values for each rodenticide bait and the corresponding dose of brodifacoum ingested are presented in Table 2.
All experimental mice ended up to die while no mortality was observed in the control mice.

Survival analysis
In the multi-day feeding test, all experimental mice that consumed brodifacoum rodenticide died and all control mice survived. Combined data from males and females shows no difference between sexes ($p>0.05$). Mean survival times differed significantly between experimental groups (chi-square=44.11; $p<0.001$). Multiple comparisons of mean times revealed that mice fed with vertox pellet (3.3 days) or vertox block (3.6 days) bait died earlier than those fed with vertox pasta (7.3 days) ($p<0.05$) (Table 2).
In the single day feeding test, the mean survival times did not differ significantly between the experimental groups (chi-square=8.6, p=0.075) (Table 2); this time was 4.8 days with vertox pellet bait, 5.2 days with vertox block and 6.55 days with vertox past bait respectively ($p>0.05$) (Table 2).
However, survival time at which 50% of the mice died (TL50) varied between experimental groups and between

different types of food. The survival time of 50% (TL50) of the mice after one-day feeding or multi-day feeding with vertox pellets or vertox block are shorter compared to those observed from feeding with vertox past ($p<0.05$). This time (TL50) was relatively shorter after multi-day feeding with vertox pellets (3 days) and vertox block (3.85 days) but with no significant difference compared to that observed after one-day feeding with the same rodenticides: vertox pellets & vertox block (5 days). However, the time (TL50) is similar after multi-day feeding (8 days) and one-day feeding (7 days) with past vertox (Fig. 4 & Fig. 5).

All experimental mice ended up to die while no mortality was observed in the control mice.

Table 2: Bait consumption, brodifacoum dosage and time to death in laboratory feeding experiments.

	Vertox pellets	Vertox block	Vertox pasta	Control
Multiple day feeding test				
1st day food consumption (g/g)	5 ± 0^{c} (136.05)	4.8 ± 0.1^{c} (137.91)	3.6 ± 0.2^{b} (139.47)	13.6 ± 0.8^{a} (139.8)
1st day brodifacoum dosage (g/g)	5 (136.05)	5 (137.91)	5 (139.47)	-
Time to death (days)	3.3^{a}	3.6^{a}	7.3^{b}	-
Average food consumption per day (g/g)	3.8 ± 0.2^{c}	4.4 ± 0.4^{c}	1.5 ± 0.1^{b}	15.5 ± 1.5^{a}
Mortality rate (%)	100	100	100	0
Single day feeding test				
1st day food consumption (g/g)	5 ± 0^{a} (141.76)	4.6 ± 0.2^{a} (133.32)	3.5 ± 0.2^{b} (137.4)	16.5 ± 0.8^{c} (136.41)
1st day brodifacoum dosage (g/g)	5 (141.76)	5 (133.32)	5 (137.4)	-
Time to death (days)	4.84^{a}	5.2^{a}	6.55^{a}	-
Mortality rate (%)	100	100	100	0

a,b,c :The values of the same variable with different letters are statistically different ;

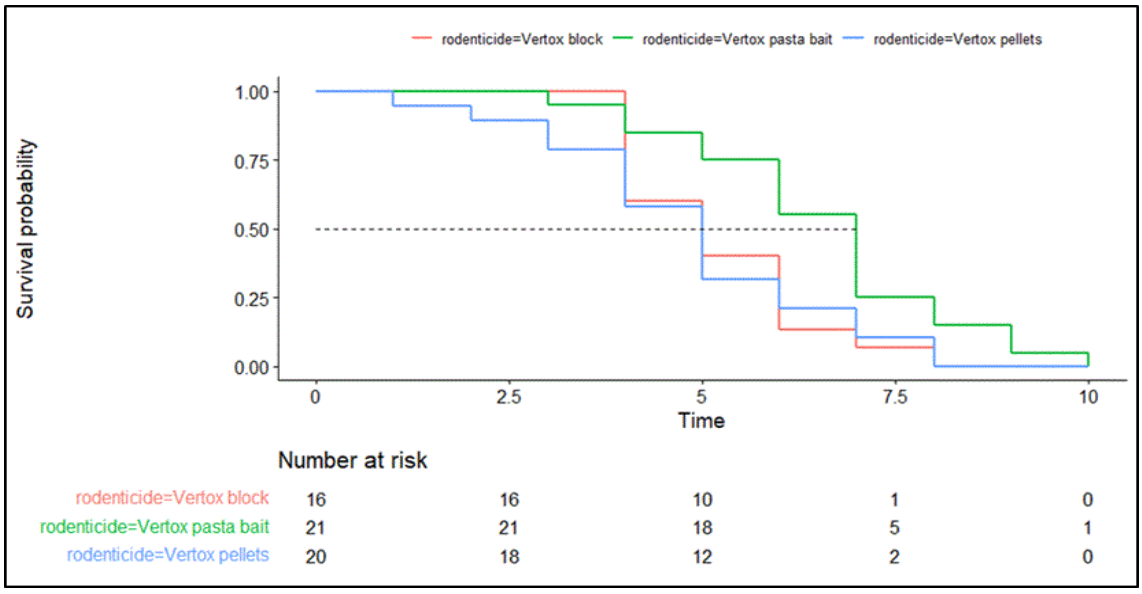

Fig 4: Survival curve of mice after one day of rodenticide feeding

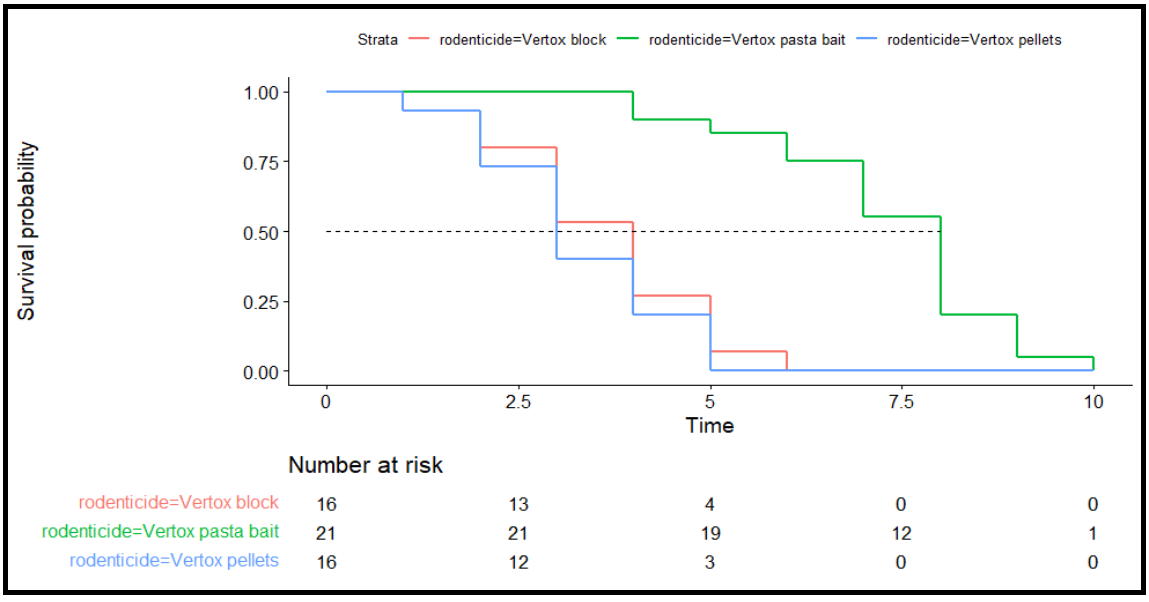

Fig 5: Survival curve of mice after multi-day rodenticide feeding

Field experiment.

The total consumption of standard baits by house mice in the three selected field houses reveals the strong presence of mice in the domestic environment. The reduction in rodent activity was estimated to be 82.66% and 72% for the vertox pellets and vertox block, respectively, over three weeks of baiting. However, the consumption of vertox past by community rodents was not 100% during the first week as was the case with vertox pellets and vertox block. The consumption of this rodenticide decreased significantly already in the second week. Evaluation of the treatment periods revealed differences in bait consumption over the course of the experiment; consumption decreased significantly at three weeks of baiting (vertox pellets: chi-square = 18.13, p = 0.00205; vertox block: chi-square = 13.89; p = 0.0001; vertox past: chi-square = 10.00; p = 0.001) (Table 3). The data recorded in Table 3 show a decrease in rodenticide consumption over time.

Table 3: Bait consumption by house mice before and after treatment in the field.

House 1 : Vertox pellets			**P-value**
Pre-test food consumption (g)	Standard bait (g)	Uningested bait (g) (%)	
1 week	54	0 (0)	NA
Rodenticide consumption during the test (g)	Initial dose	Uningested bait (g) (%)	
1 week	15[a]	0 (0) [a]	-
2 week	15[a]	4 (26.66) [a]	0.107
3 week	15[a]	12.5 (83.33) [b]	0.00205
House 2 : Vertox block			
Pre-test food consumption (g)	Standard bait (g)	Uningested bait (g) (%)	
1 week	54	0 (0)	NA
Rodenticide consumption during the test (g)	Initial dose	Uningested bait (g) (%)	
1 week	15	0 (0) [a]	-
2 week	15	3 (20) [a]	0.223
3 week	15	10.8 (72) [b]	0.0001
House 3 : Vertox past			
Pre-test food consumption (g)	Standard bait (g)	Uningested bait (g) (%)	
1 week	54	0 (0)	NA
Rodenticide consumption during the test (g)	Vertox pasta	Uningested bait (g) (%)	
1 week	15	5 (33.33) [a]	-
2 week	15	10.2 (68) [a]	0.125
3 week	15	14.3 (95.33) [b]	0.001

a,b,c :The values of the same variable with different letters are statistically different;%: Percentage reduction in rodent activity or mortality rate.

Discussion

This work evaluated the efficacy of three brodifacoum-based anticoagulants in the laboratory and in the field in *Mus musculus* house mice. Overall, the results of the two laboratory feeding tests showed comparable efficacy for the three baits: vertox pellets, vertox block and vertox pasta. However, the time to death of mice in the multi-day and single-day feeding tests did not differ in all comparisons except with one of the three baits tested (vertox pasta). With the multi-day feeding test, a much longer time to death of the mice (7.3 days) was observed compared to the single feeding test (6.5 days) with the vertox pasta bait. Continued feeding of the vertox pasta bait could lead to the ingestion of a much larger dose than necessary of the active ingredient [7, 8]. These overdosed individuals may create a higher risk of secondary poisoning in non-target species such as their predators and others [9, 10, 11]. Since all mice tested were dead 10 days after having consumed each of the three baits, *Mus musculus* strain was considered sensitive to the various products tested. However, one of the key factors in successful rodent control is the attractiveness and palatability of the baits [12]. In the single day feeding test performed on mice, higher consumption of vertox pellets, and vertox block was observed compared to vertox pasta, but without any significant difference. Thus the vertox block feeding was repeated and extended over several days. The poor palatability of vertox pasta bait did not affect its effectiveness. Similar results were previously published on the decrease in palatability and bait efficacy at low (20 ppm) and high (50 ppm) doses of brodifacoum in house mice [13]. However, this low palatability of vertox pasta does not affect its efficacy.

To validate our preliminary laboratory results, we further tested in the field the effectiveness of the baits in a population of house mice. A low consumption of bait was recorded after the first week of application. Suppression of bait consumption indicated a reduction in the mouse population, which was further supported by the near-zero post-treatment consumption of bait.

Such suppression of consumption indicated a reduction in the mouse population, which was further supported by the almost zero post-treatment consumption of the bait. These results confirm the work of Frankova *et al.* [14] who recently demonstrated that anticoagulant baits have the potential to substantially decrease food intake shortly after initial bait consumption, thereby shortening the activity period of overdosed individuals.

Based on the 100% mortality rate obtained in the laboratory and the relatively short time of 4 days on average with vertox pellets and vertox block, and one week with vertox pasta to cause death, we conclude these three products are effective on the populations of the mice tested. Indeed, according to the criteria of the European and Mediterranean Organization for the Protection of Plants (2009), a rodenticide is considered effective when it displays a mortality rate greater than 90% within 20 days. In the present study, efficacy of rodenticides at lab was followed by low bait consumption after the first week of field application. The results of the current study suggest that the different tested products have a positive impact in the control of rodents which are pests for crops and foodstuffs in Benin. It also contributes to complement the existing documentation on the susceptibility of mice (*Mus musculus*) to brodifacoum in Benin as the need for relevant

data is crucial for future decisions regarding anticoagulant rodenticides at 50 ppm in general. To generalize the use of the tested rodenticides, future research challenges should include regular testing in wild populations of mice to ensure effective control of these animals.

Conclusion

The present study compared the biological efficacy of three new generation anticoagulant baits under laboratory and field conditions on mice (*Mus musculus*). In the laboratory no-choice feeding tests, 100% of the animals died in all treated groups and 0% in the control groups. The time to death did not differ in the two types of tests/feeding regimes except with one of the three baits tested (vertox pasta). The field-tested baits showed good efficacy of all three brodifacoum-based baits in a mouse population. However, further study is needed with the same baits on anticoagulant-resistant rodent populations.

Ethical approval.

Guidelines for animal welfare applicable at international, national, and/or institutional levels were followed. The protocol of the present study was reviewed and approved by the Institutional Ethical Committee of the Center for Research in Entomology of Cotonou (Grant No. IORG005698).

Acknowledgements

We are grateful to the Society for the Development of Cotton (SODECO) which supported financially this study. We thank all those who assisted in the collection of mouse (*Mus musculus*) specimens in the Dontokpa market and in the analysis of the data, including Aziz Affolabi, Kola Azoua and Bruno Akinro.

References

1. Ritchie H, Roser M. "Urbanization". Published online at OurWorldInData.org; 2018. Retrieved from: 'https://ourworldindata.org/urbanization'.
2. Meyer A. Urban Commensal Rodent Control: Fact or Fiction? in Rats, Mice and People: Rodent Biology and Management, Singleton, G.R., Hinds, L.A., Krebs, C.J., and Spratt, D.M., Eds., Canberra: Australian Centre for Int. Agr. Res. 2003, 446-450.
3. Abernethy K, Maisels F, White LJT. Environmental issues in Central Africa Annu. Rev. Environ. Resour 2016;41:1-33.
4. Houemenou G, Kassa B, Libois R. Ecologie, diversité spécifique et abondance des petits mammifères de la ville de Cotonou au Bénin (Afrique de l'Ouest). Int. J Biol. Chem. Sci 2014;8(3):1202-1213.
5. Corrigan RM. Rodent Control: A Practical Guide for Pest Management Professionals. GIE Publishers, Cleveland, OH 2001, 355.
6. Battersby S, Hirschhorn RB, Amman BR. Commensal rodents. In X. Bonnefoy, H. Kampen, & K. Sweeney (Eds.), Copenhagen, Denmark: World Health Organization, Regional Office for Europe. Public Health Significance of Urban Pests 2008, 387-419.
7. Siddiqi Z, Blaine WD. Anticoagulant resistance in house mice in Toronto, Canada. Environ. Health Rev 1982;32:49-51.
8. Wheeler R, Priddel D, O'Dwyer T, Carlile N, Portelli D. Evaluating the susceptibility of invasive black rats (*Rattus rattus*) and house mice (*Mus musculus*) to brodifacoum as a prelude to rodent eradication on Lord Howe Island. Biol. Invasions 2019;21:833-845.
9. Fisher P, O'Connor C, Wright G, Eason CT. Anticoagulant residues in rats and secondary non-target risk. DOC Science Internal Series 2004.
10. Thomas PJ, Mineau P, Shore RF, Champoux L, Martin PA. Second generation anticoagulant rodenticides in predatory birds: Probabilistic characterisation of toxic liver concentrations and implications for predatory bird populations in Canada. Environ. Int 2011;37:914-920.
11. Koivisto E, Santangeli A, Koivisto P, Korkolainen T, Vuorisalo T. The prevalence and correlates of anticoagulant rodenticide exposure in non-target predators and scavengers in Finland. Sci. Total Environ 2018;642:701-707.
12. Morriss GA, O'Connor CE, Airey AT, Fisher P. Factors influencing palatability and efficacy of toxic baits in ship rats, Norway rats and house mice. Science for Conservation 2008;282:1-26.
13. Redfern R, Gill J, Hadler M. Laboratory evaluation of WBA 8119 as a rodenticide for use against warfarin-resistant and nonresistant rats and mice. J Hyg. (Lond.) 1976;77:419-426.
14. Frankova M, Stejskal V, Aulicky R. Suppression of food intake by house mouse (*Mus musculus*) following ingestion of brodifacoum-based rodenticide bait. Crop Prot 2017;100:134-137.