*Proceedings of the Seventh International Conference on Urban Pests*
*William H Robinson and Ana Eugênia de Carvalho Campos (editors) 2011*
*Printed by Instituto Biológico, São Paulo, SP. Brazil.*

# INFLUENCE OF BAIT TYPE AND ACTIVE INGREDIENT ON RODENTICIDE PALATABILITY AND EFFICACY

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**Abstract** In a semi natural test system for efficacy evaluation of rodenticidal products according to the Infectious Diseases Protection Act of the Federal Republic of Germany, 80 choice tests with *Mus musculus* and 62 choice tests with *Rattus norvegicus* from 1984 to 2010 were evaluated. The tests were conducted with groups of wild strain *M. musculus* (15 – 27 mice per group), *R. norvegicus* and *R. rattus* (5–10 rats per group). Bromadiolone, warfarin and zinc phosphide caused a relatively low mortality in *M. musculus* (65% to 85%), although the palatability of baits with theses active ingredients was good with >25% bait consumption related to overall food consumption. All products with second generation anticoagulants as well as coumatetralyl as active ingredients caused high mortality rates in choice trials with *M. musculus* (92% to 98 %). In 72.5% of all choice tests with *M. musculus*, and in 56% of all choice tests with *R. norvegicus* a mortality of ≥ 90% was observed. Bait type (bloc, paste or granular bait) had no significant influence on bait attractivity. In experiments with difenacoum (0.005%), a bait uptake of 35% in relation to overall food consumption (bait + challenge diet) was sufficient to achieve a 100% mortality in *M. musculus*, whereas a bait consumption of 24.5 % resulted in 100% mortality in *R. norvegicus*. Both values are higher than the recommended 20% given in the Technical Notes for Guidance for Rodenticide Efficacy Evaluation for European biocide product authorisation.
**Key Words** *Mus musculus, Rattus norvegicus,* anticoagulants*,* bait consumption

## INTRODUCTION

Rodenticides differ in their toxicity against rodents. For instance anticoagulants, which are used most frequently for rodent control, have a wide range for $LD_{50}$ against their target organisms (Bull, 1976; Meehan, 1978; Lechevin and Poche, 1988). However, the success of rodent control operations with toxic chemicals for oral uptake relies not only on the toxicity of the active substance, but also heavily on palatability of the bait which carries the poison. In our study, we investigate the influence of active substance as well as bait formulation on the overall efficacy of bait products for rodent control. The background of our study is that the Infectious Diseases Protection Act (2001; Infektionsschutzgesetz, IfSG § 18; formerly Bundesseuchengesetz §10c) of the Federal Republic of Germany prescribes the publication of a list of officially tested and approved biocides against health pest organisms. For listing, the respective biocide has to be examined for efficacy and attractiveness. In case of rodenticides, the respective product must be effective enough to cause eradication (100 % mortality) of the target organism population, since the main objective of rodent control in public healthcare is the interruption of the infectious chain from rodents to humans. The Federal Environment Agency of Germany (FEA) is testing the efficacy of rodenticidal products to be listed after the Infectious Diseases Protection Act for more than 25 years with the same experimental setup, allowing a comprehensive comparison of various bait types and formulations.

The FEA rodenticide efficacy test is a semi natural choice laboratory test with groups of wild strain rodents (*Mus musculus*, *Rattus norvegicus* and *Rattus rattus*) and the methods described here are regularly used for practical tests of rodenticide efficacy and attractiveness since that time. Details of the test methods have been described and published as official test guidelines (BBA, 1992; BBA, 1994). The main objective is to test rodenticide bait products under controlled laboratory conditions which simulate their use under practical conditions. Therefore, we use groups of wild strain rodents for our efficacy trials, since they are more likely to exhibit natural rodent behaviour than laboratory strains of single-caged rats or mice.

## MATERIALS AND METHODS

### Study Animals

Wild strains of house mice (*Mus musculus*), brown rats (*Rattus norvegicus*) and roof rats (*Rattus rattus*) were obtained from laboratory colonies kept at the FEA. The wild strain rodents are kept in colonies and are left relatively undisturbed. The rat and mice colonies consist of offspring from animals that had been caught in natural habitats in northern and eastern Germany. New wild-trapped individuals are regularly introduced to the colonies at least every two years. Resistance against rodenticides is regularly tested in the rat and mice strains.

### Experimental Setup

For testing the efficacy and attractiveness of the rodent baits in no-choice and choice tests, groups of mice (15–27 individuals per group; male : female ratio 1:1 up to 1:2) and rats (5–10 individuals per group; male : female ratio 2:1 up to 1:2) were removed from their colonies and placed in test chambers. Prior to the start of the tests, animals were given three to five days of familiarization with the test chamber; during this period, they were fed with dry bread *ad libitum*. After this period, the rodenticide bait and in choice tests also challenge diet (placebo bait) was offered for a defined and species-specific baiting period (see below). The floor space in the test chambers was 6 m² for rats and 5 m² for mice. Each chamber had two windows and was not artificially illuminated. To faciliate the cleaning of the chambers at the end of the experiments as well as to prevent structural damage by the rodents, its walls, floor and ceiling were tiled. Chambers were empty except for an animal shelter (wooden nest boxes), a water supply and feeding dishes. During experiments, animals had *ad libitum* access to water, the rodenticide product and, in choice experiments, challenge diet.

### Efficacy and Attractiveness Testing

In no-choice tests, a feeding tray with the rodenticide product was offered *ad libitum* to groups of house mice and roof rats for 21 days and to brown rats for 10 days (baiting period). In the choice tests, two feeding dishes were offered to the animals, one with the rodenticide product and one with a challenge diet (i.e. non-poisoned food/placebo bait), both in the same amounts. Both food dishes were placed at the opposite end of the nest boxes. Bait and challenge diet was offered *ad libitum* for 14 days for *Rattus norvegicus* and 28 days for *Mus musculus* and *Rattus rattus*. The challenge diet for *Mus musculus* was oat flakes, and wheat grain for *Rattus norvegicus* and *Rattus rattus.* Distance between the feeding dishes (poison bait and challenge diet) was 80 cm to 120 cm in all experiments. Each day, the places of both dishes were interchanged to avoid spatial conditioning. After the baiting period, surviving rats were fed with wheat and surviving mice with oat flakes *ad libitum* and were observed for 14 days in both types of tests (choice and no-choice). The main parameter tested in the no-choice experiments was mortality, and in choice tests bait attractiveness. Bait attractiveness was calculated as percentage of rodent bait taken up in total over the whole baiting period in relation to the overall food consumption (bait and challenge diet). In all trials, rodenticide bait and food were replaced once a day, and water was replenished. The number of dead animals, the condition of surviving animals, and behaviour of the rodents were recorded daily. The amount of bait and/or food consumed was calculated daily using an electronic balance (PG5002-S Delta Range, Mettler Toledo GmbH, Giessen, Germany, exactness = 0.1 g).

### Products Tested

Since the application of producers or distributors for their bait products to be included in the list of specifically efficacious rodenticides according to the Infectious Diseases Protection Act (IfSG-list) is voluntary and the details of the test results are confidential, all products are anonymized and their exact composition cannot be revealed. The IfSG-list defines three major bait types, *bloc bait* which is form stable and consists of grain and other digestible material embedded in a paraffin block, *paste bait* which has the consistency of dough and contains usually flour, small grain particles and fat and *granular bait*, which can be either pellets (small or powdered grain with a binder) or cereals and grain.

Until 2008 for efficacy tests of rodenticide products a no-choice as well as a choice test was mandatory. From 2008 on, choice tests were made only in cases when not enough data were available for the principal efficacy of an active substance in its given concentration in the product. Instead, a pair of at least 2 choice trials was conducted for each product. Thus, we divided our results in a pre-2008 and a post-2008 period and evaluated the results accordingly. Some trials with an experimental setup which deviated from the one described here were omitted in this study. Moreover, cellulose and plaster based baits against rats and mice have also been tested at the FEA, but

since the test results have been described in detail elsewhere and it is questionable from these results that cellulose and plaster have a rodenticidal potential (Schmolz 2010), we did not include them here.

## RESULTS

***Mus musculus***

From 1986 to 2010, 57 no-choice-tests were conducted with groups of *Mus musculus* with brodifacoum 0.005% (8 trials), bromadiolone 0.005% (5), chlorophacinone 0.0075% (3), coumatetralyl 0.0375% (1), difenacoum 0.005% (16), difethialone 0.0025% (3), flocoumafen 0.005% (8) and warfarin 0.04 to 0.08% (8). Of these trials 1 trial with bromadiolone (79% mortality), 1 trial with chlorophacinone (87% mortality), 1 trial with coumatetralyl (87% mortality) and 4 of 8 trials with warfarin (40 to 80% mortality) were below the required mortality of 100%.

From 1984 to 2008, 54 choice tests with bait products against *Mus musculus* were evaluated. Table 1 gives the number of products for all active ingredients; the only non-anticoagulant was zinc phospide. Bromadiolone, warfarin and zinc phosphide caused a relatively low mortality in mice (65% to 85%), although the palatability of baits with theses active substances was good with >25% bait consumption related to overall food consumption. All products with second generation anticoagulants as well as coumatetralyl caused high mortality rates in choice trials (92% to 98 %) with no significant differences between the substances (Kruskal Wallis H-Test, $p>0.05$).

13 Products were tested after 2008 with 2 choice trials each (total of 26 trials). Active substances were brodifacoum 0.005% (2 products), chlorophacinone 0.0075% (2 products), difenacoum 0.005% (8 products) and zinc phosphide 0.8% (1 product). Bait types were paste, granular and block baits. In 23 trials, the mortality was 100%, in 2 trials the mortality was higher than 90%, and in 5 trials the mortality was below 90% (15% to 53.3 %). Again, difenacoum was most frequently used as active substance (8 products, 16 trials).

**Table 1.** Choice trials with *Mus musculus*. The number of trials (n) is equal to number of products tested until 2008, whereas from 2008 on the number of products tested is half the number of trials (explanation see text). SD = standard deviation.

| **Trials before 2008**<br>Active substance and concentration | n | Mortality (± SD) [%] | Mortality range (min – max) [%] | Number of trials with mortality ≥ 90% |
|---|---|---|---|---|
| Brodifacoum 0.005% | 5 | 95 (± 12) | 73 - 100 | 4 |
| Bromadiolone 0.005% | 4 | 68 (± 39) | 20 - 100 | 2 |
| Coumatetralyl 0.0375% | 4 | 94 (± 7) | 87 – 100 | 2 |
| Difenacoum 0.005% | 17 | 92 (± 19) | 27 – 100 | 14 |
| Difethialone 0.0025% | 9 | 95 (± 5) | 87 – 100 | 7 |
| Flocoumafen 0.005 | 6 | 97 (± 3) | 95 – 100 | 3 |
| Flocoumafen 0.0025% | 3 | 98 (± 3) | 95 – 100 | 3 |
| Warfarin 0.08% | 3 | 85 (± 16) | 67 – 95 | 2 |
| Zinc phosphide 0.4% | 3 | 65 (± 13) | 50 - 73 | 0 |
| **Trials after 2008** | | | | |
| Brodifacoum 0.005% | 4 | 100 | all 100 | 4 |
| Chlorophacinone 0.0075% | 4 | 58 (± 46) | 15 – 100 | 2 |
| Difenacoum 0.005% | 16 | 97 (± 12) | 53 – 100 | 15 |
| Zinc phosphide 0.8% | 2 | 44 (± 9) | 38 – 50 | 0 |

***Rattus norvegicus***

From 1986 to 2010, 51 no-choice trials were made with *Rattus norvegicus* with brodifacoum 0.005% (3 trials), bromadiolone 0.005% (7), chlorophacinone 0.0075% (5), coumatetralyl 0.0375% (7), difenacoum 0.005% (10), difethialone 0.0025% (1), flocoumafen 0.005% (3) and warfarin 0.04 to 0.08% (15). All trials resulted in 100%

mortality after a baiting period of 10 d. The same results were received in 14 no-choice trials with *Rattus rattus* with brodifacoum 0.005% (4 trials), bromadiolone 0.005% (1), difenacoum 0.005% (7), difethialone 0.0025% (1), and flocoumafen 0.005% (1), with an eradication of the test population within the baiting period of 21 d in all trials.

From 1986 to 2008, 41 choice tests with bait products and from 2008 to 2010 21 trials with 10 products against *Rattus norvegicus* were evaluated. Table 2 gives an overview of the experiments. Compared to house mice, the results are more heterogenous for brown rats. Only products with coumatetralyl caused 100 % mortality in all trials, whereas all other active substances had the potential to eradicate the test populations, since at least one trial per active substance product group yielded in a complete eradication of the rat groups. Table 2 shows the range for mortalities, and in contrast to tests with house mice, several product groups produced trials with zero mortality (difenacoum, difethialone, warfarin), whereas in all trials with house mice at least some of the test mice were killed by the rodenticide.

**Table 2.** Choice trials with *Rattus norvegicus*. The number of trials (n) is equal to number of products tested until 2008, whereas from 2008 on the number of products tested is half the number of trials, with the exception of one product with difenacoum, which was tested in 3 trials. (explanation see text). SD = standard deviation.

| **Trials before 2008**<br>Active substance and concentration | n | Mortality (± SD) [%] | Mortality range (min – max) [%] | Number of trials with mortality ≥ 90% |
|---|---|---|---|---|
| Brodifacoum 0.005% | 2 | 70 (± 42) | 40 - 100 | 1 |
| Bromadiolone 0.005% | 4 | 96 (± 8) | 83 - 100 | 3 |
| Chlorophacinone 0.005% | 2 | 100 | all 100 | 2 |
| Coumatetralyl 0.0375% | 6 | 100 | all 100 | 6 |
| Difenacoum 0.005% | 6 | 45 (± 44) | 0 – 100 | 1 |
| Difethialone 0.0025% | 4 | 80 (± 40) | 0 – 100 | 3 |
| Flocoumafen 0.005 | 2 | 90 (± 14) | 80 – 100 | 1 |
| Warfarin 0.04 – 0.08% | 15 | 80 (± 29) | 0 – 100 | 8 |
| **Trials after 2008** | | | | |
| Brodifacoum 0.005% | 2 | 100 | all 100 | 2 |
| Chlorophacinone 0.0075% | 4 | 71 (± 34) | 33 – 100 | 2 |
| Difenacoum 0.005% | 15 | 65 (± 42) | 0 – 100 | 6 |

***Rattus rattus***

Until 2008, 3 products with brodifacoum (0.005%), 1 product with bromadiolone (0.005%), 2 products with difethialone (0.0025%) and 1 product with flocoumafen (0.005%) as active ingredients were tested with *R. rattus*. All of these trials resulted in a mortality of 100%. 7 products with difenacoum (0.005%) were tested, with an average mortality of 89% (SD 19%, range 57% - 100%, 2 products below 90% mortality). From 2008 to 2010, 4 products with difenacoum (0.005%) were tested in 8 choice trials, with an average mortality of 81 % (SD 30%, range 12.5% - 100%, 4 trials below 90% mortality).

**Bait consumption**

In choice trials with *Mus musculus*, the bait consumption as fraction of the overall food uptake (bait + challenge diet) ranged from 1% up to 83%. Interestingly, both values (max and min) were obtained in trials with bloc baits, whereas mean consumption of granular baits and paste baits was 38% (SD 21%, n = 14) and 27% (SD 12%, n = 8). In choice trials with *Rattus norvegicus*, the range for bait consumption was even more pronounced and ranged from 0% to 99.6%. Figure 1 shows an evaluation of bait consumption of all trials from 1984 to 2010 with products with difenacoum (0.005%) as active ingredient in relation to mortality. For *Mus musculus*, the lowest bait consumption to achieve 100% mortality was 14.2%; however, one product with a bait consumption of 34.9% resulted in a mortality of only 85%. A bait consumption of more than 35% always resulted in 100% mortality. In *Rattus norvegicus*, the

lowest value for bait consumption leading to 100% mortality was 11.6%, but as it is the case with mice, two products with a bait consumption of 13% and 14% resulted in a mortality of 83% and 86%, respectively. Thus, the critical value for bait consumption to receive an eradication of the test population was 24.5%. All trials with a bait consumption ≥ 24.5% had a mortality of 100%. This value is lower for *Rattus rattus* with 15.8% bait consumption. The lowest value for a bait consumption of roof rats leading to 100% mortality was 4.6%.

In a comparison of all choice trials with rats and mice, consumption is not clearly dependent on bait type and shows a considerable variation (Figure 2).

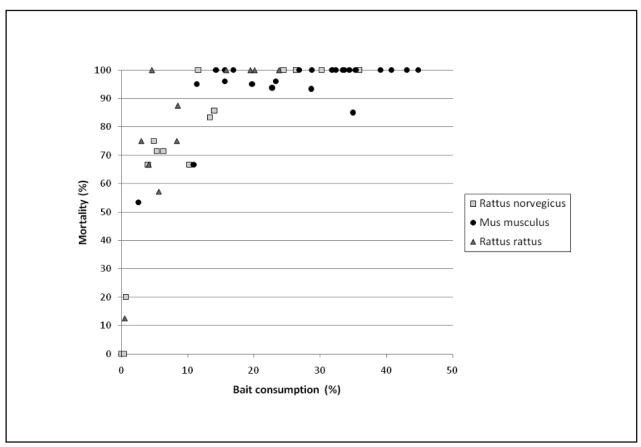


**Figure 1.** House mouse, brown rat and roof rat mortality in relation to bait consumption for products with difenacoum 0.005%. Number of trials is for *Mus musculus* n = 24, *Rattus norvegicus* n = 19, *Rattus rattus* n = 11. Two trials with *R. norvegicus,* 4 trials with *R.rattus* and 5 trials with *M. musculus* with a bait consumption > 50% which had a mortality of 100% are not shown in this graph. Data from all experiments 1984 to 2010.

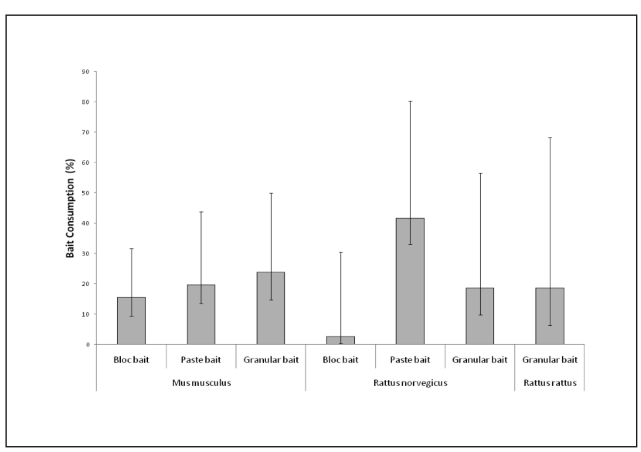


**Figure 2.** Influence of bait type on bait consumption in all trials from 1984 to 2008. Bars indicate median, small bars 1. and 3. quartile. Number of trials: *M. musculus* bloc bait n = 8, paste bait n = 18, granular bait n = 28, *R. norvegicus* bloc bait n = 10, paste bait n = 2, granular bait n = 29, *R. rattus* granular bait n = 12.

## DISCUSSION

The principal efficacy of rodenticides was tested in a large number of no-choice tests. The only cases were the mortality was below 100% were trials with bromadiolone, chlorophacinone, coumatetralyl and warfarin, all in trials with house mice, which are less susceptible against these active ingredients than rats are (Meehan, 1984). In 72.5% of all choice tests with mice from 1984 to 2010, a mortality of ≥ 90% was achieved. This value is lower in choice tests with brown rats, since in only in 56% of all trials from 1986 to 2010, a mortality of ≥ 90% was

observed, although all no-choice tests resulted in 100% mortality. This clearly highlights the importance of bait attractiveness in rodent control operations. The Technical Notes for Guidance for Rodenticide Efficacy Evaluation for the European biocide product authorisation set a threshold value of 20% bait consumption as sufficient for bait attractiveness in choice trials (European Community 2009).

The results of this study show that a bait consumption of 20% must not necessarily result in 100% mortality, at least not in products with difenacoum (0.005%) against *Mus musculus* and *Rattus norvegicus* (Figure 1). Bait attractiveness may be dependent on bait type (bloc, paste or granular), but our results do not show significant trends for attractiveness. Rather, it is more likely that the bait attractiveness relies on the bait composition, and even bloc bait, which are sometimes regarded as the least attractive bait type (Smythe, 1976; Clapperton, 2006) can be very attractive for rats with a bait consumption as high as 99% as in two of the trials evaluated in this study. Second generation anticoagulants did not differ much in their efficacy, neither in coice nor no-choice tests. The predominant active ingredient in products tested at the FEA was difenacoum (0.005%), and choice trials with house mice had a range for mortality from 27 – 100 %, and in brown rats from 0 -100%. The amount of bait which is taken up by an individual rat or mouse must not be very high to lead to death, but the efficacy of a rodenticide relies rather on a sufficient bait uptake by *all* group members, as it was the case in a trial with roof rats (Figure 2), were a bait consumption as low as 4.6% lead to 100% mortality of the rat group.

Foraging behaviour of rats and mice is complex, and includes social learning (Berdoy and Drickamer, 2007; Galef, 2007) as well as different degrees of neophobia and bait shyness which are major obstacles in rodent control. Choice tests for rodenticide efficacy testing should therefore take the natural social behaviour of rodents in account, since the attractiveness of a rodenticidal product is of key importance. Most active ingredients like second generation anticoagulants do not differ much in their principal efficacy, whereas the amount of bait consumption, which is crucial for a successful rodent control operation, may vary considerably.

## ACKNOWLEDGEMENTS

The author would like to thank Agnes Kalle for help in compiling data.

## REFERENCES CITED

**BBA (Biologische Bundesanstalt) 1992**. Richtlinie für die Prüfung von Nagetierbekämpfungsmitteln gegen Wanderratten, Reihe 9 - 3.2, Saphir Verlag, Ribbesbüttel, Germany. (In German.)

**BBA (Biologische Bundesanstalt) 1994**. Richtlinie für die Prüfung von Nagetierbekämpfungsmitteln gegen Hausmäuse, Reihe 9 - 3.1 Saphir Verlag, Ribbesbüttel, Germany. (In German.)

**Berdoy, M. and Drickamer L.C. 2007.** Comparative Social Organization and Life History of *Rattus* and *Mus*. *In*: Sherman P.W. and Wolff J., eds. Rodent Societies, Chicago: University of Chicago Press

**Bull, J.O. 1976.** Laboratory and field investigations with difenacoum, a promising new rodenticide. Proceedings of the 7th Vertebrate Pest Conference; 9-11 Mar 1976, Monterey, CA, USA. University of California, Davis, USA. http://digitalcommons.unl.edu/vpc7/5 (March 17 2011)

**Clapperton, B.K. 2006.** A review of the current knowledge of rodent behaviour in relation to control devices. Science for Conservation 263. Scientific Monograph Series of the New Zealand Department of Conservation, Wellington, New Zealand.

**European Community 2009.** Technical Notes for Guidance on Product Evaluation Appendices to Chapter 7 Product Type 14 Efficacy Evaluation of Rodenticidal Biocidal Products. http://ecb.jrc.ec.europa.eu/documents/Biocides/TECHNICAL_NOTES_FOR_GUIDANCE/TNsG_PRODUCT_EVALUATION/Revised_ Appendix_Chapter_7_PT14_2009.pdf (March 17 2011)

**Galef, B.G., Jr. 2007.** Social learning by rodents. *In*: Sherman P.W. and Wolff J., eds. Rodent Societies, Chicago: University of Chicago Press

**Lechevin, J.C. and Poche R.M. 1988.** Activity of LM 2219 (difethialone), a new anticoagulant rodenticide, in commensal rodents. Proceedings of the Thirteenth Vertebrate Pest Conference; 1-3 Mar 1988, Monterey, CA, USA. University of California, Davis, USA. http://digitalcommons.unl.edu/vpcthirteen/13 (March 17 2011)

**Meehan, A.P. 1978.** Rodenticide activity of bromadiolone – a new anticoagulant. Proceedings of the 8th Vertebrate Pest Conference; 7-9 Mar 1978, Sacramento, CA, USA. University of California, Davis, USA. http://digitalcommons.unl.edu/vpc8/31 (March 17 2011)

**Meehan, A.P. 1984.** Rats and Mice: Their Biology and Control. East Grinstead: Rentokil Library.

**Schmolz, E. 2010.** Efficacy of anticoagulant-free alternative bait products against house mice (*Mus musculus*) and brown rats (*Rattus norvegicus*). Integr. Zool. 5: 44-52.