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# Brodifacoum as a first choice rodenticide for controlling bromadiolone-resistant *Mus musculus*

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ABSTRACT

Laboratory tests were conducted to estimate the efficacy of brodifacoum in controlling bromadiolone-resistant Tyr139Cys and Leu128Ser/Tyr139Cys house mice in no-choice feeding tests with two short exposure times of 24 and 48 h. The mortality of bromadiolone-resistant house mice in brodifacoum tests was similar 90 and 100% in the respective 24 and 48 h intervals.

There were neither differences in survival rates between the genders nor between the VKOR variants, but consumption was higher in females than in males. Brodifacoum was effective against bromadiolone-resistant house mice in the short exposure test, offering a sound practical alternative for rodent control in situations when bromadiolone is no longer a satisfactory solution. Whether a short-term use of brodifacoum after bromadiolone application reduces environmental risks, compared to using brodifacoum only, needs a further confirmation.



## 1. Introduction

The house mouse (*Mus musculus* L.) is able to cause significant merchandise and financial losses by food consumption, contamination and spillage. Contamination with urine, hair and feces is the leading form of damage that mice cause (Hernandez and Drummond, 1984). A single mouse is believed to be able to produce up to 70 fecal pellets over 24 h which mostly contain pathogenic bacteria and spores of toxinogenic fungi (Frynta et al., 2012). As a host and vector of many ecto- and entoparasites, the house mouse is a species of special epizootiological and epidemiological interest (Battersby et al., 2008; Kataranovski et al., 2010). Damage caused by house mice in storages is mostly very difficult to estimate accurately. Data on economic losses are mostly shown in literature as overall damage resulting from the presence of rodents in storages, i.e. as economic losses caused by mice and rats together. Data from some earlier studies had shown that up to 33 million tons of cereals were being lost each year through rodent damage (Meyer, 1994; Timm, 1994). No recent estimates are available that would show the precise percentage of damage caused in food production and storage facilities globally. In the European Union, an average of 300 million tons of grain is exposed to risks of infestation and contamination, so that even a small percentage of goods damaged by rodents may ultimately result in huge economic losses (Stejskal et al., 2015).

Anticoagulant rodenticides are effectively used for rodent control around the world (Bentley, 1972; Buckle et al., 1994; RRAG, 2012). However, the spreading of resistance to first- and some second-generation anticoagulants, i.e. bromadiolone and difenacoum, has become a threat to efficient rodent control management (Buckle, 2013). Bromadiolone was initially introduced with an objective to enhance the control of warfarin-resistant animals worldwide (Marsh, 1977). Its lower efficacy was first detected in practice and then confirmed in no-choice feeding tests (Rowe et al., 1981). Brodifacoum was introduced worldwide when it was shown to be successful in controlling warfarin-resistant rodents, and its efficacy was higher than the effectiveness of either bromadiolone or difenacoum (Greaves and Cullen-Ayres, 1988). Initial reports have revealed a high degree of efficiency of 0.0005% brodifacoum against populations of warfarin-resistant brown rats (*Rattus norvegicus*) and 100% mortality of roof rats (*Rattus rattus*) and house mice after a single day of feeding on 0.005% brodifacoum (Lund, 1984). Recent field trials have confirmed a high effectiveness of brodifacoum against resistant populations of brown rat (Buckle et al., 2012).

The discovery of the VKORC1 gene, which is assumed to reduce

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the sensitivity of animals to anticoagulants, has opened new paths for developing resistance determination methodologies by using primarily molecular-biological methods. Such methods enable simple monitoring of the development and distribution of resistant populations while preventing the use of non-effective rodenticides (Li et al., 2004; Rost et al., 2004; Pelz et al., 2005, 2007). Tyr139Cys confers strong practical resistance against first-generation anticoagulants and bromadiolone (Endepols et al., 2012). Although difenacoum is generally more effective, acceptable control may be difficult to achieve (Buckle et al., 2013), while brodifacoum 0.005% has been confirmed as effective against Tyr139Cys Norway rats (Buckle and Prescott, 2012; Buckle et al., 2012).

Resistance evolution to any anticoagulant requires its exclusion from further use. In Germany, brodifacoum, flocoumafen and difethialone have been recommended as a solution for control of Tyr139Cys house mouse and Tyr139Cys rat populations (RRAG, 2012; Buckle and Prescott, 2012). These anticoagulants are recommended only in regions in which bromadiolone and difenacoum resistance have been confirmed in order to avoid potential risks to non-target species due to their high toxicity (Esther et al., 2014).

Even though brodifacoum was introduced in Serbia three decades ago, its use has been minimal compared to bromadiolone. Over the past several decades, bromadiolone has been the leading choice in rodent pest management control programs. Our recent research has revealed bromadiolone-resistant populations of house mice in Serbia (Šćepović et al., 2016). All animals were found to carry the Tyr139Cys polymorphism and combination of Leu128Ser and Tyr139Cys (Leu128Ser/Tyr139Cys) polymorphisms. The present study examines the option of using brodifacoum for control of bromadiolone-resistant populations of house mice, especially for controlling the carriers of Leu128Ser/Tyr139Cys, which had earlier been found to have a lower susceptibility to blomadiolone than the carriers of Tyr139Cys (Šćepović et al., 2016).

## 2. Materials and methods

### 2.1. Animals

House mice (*Mus musculus* L.) used in the test procedure were 3-months old offsprings of wild bromadiolone-resistant mice originally trapped indoors in storages in several Belgrade suburbs. The wild bromadiolone-resistant animals were survivors of a 21-day bromadiolone no-choice feeding test in a former research (Šćepović et al., 2016).

The animals were housed individually in standard plastic cages, dimension 320 × 200 × 135 mm. The floor of the boxes, under the feeding bowls was formed by wire mesh, which allowed the decay of the spilled baits. Under boxes, the filter paper was laid to ease the collection of the spilled baits.

The test animals were reared under laboratory conditions: 20 ± 2 °C, relative humidity varying from 40 to 70%, and a 12/12 h light/darkness photoperiod. Water was always provided *ad libitum*. During adaptation and recovery, the animals were offered standard diet for laboratory mouse “LM2 19% proteins”, manufactured at the Subotica Institute of Veterinary Medicine, Serbia. The animals were fed on standard laboratory diet for lab mice, except during the no-choice feeding tests with bromadiolone and brodifacoum, respectively.

### 2.2. Bait preparation

Unpoisoned bait was prepared by mixing coarsely cut wheat, maize oil and wholemeal flour at 90:5:5 ratio. The poisoned baits containing 0.005% bromadiolone or 0.005% brodifacoum were made by mixing unpoisoned baits and liquid concentrate products suplied by EkoSan d.o.o., Serbia. After application to 22 ml of liquid concentrate of bromadiolone and brodifacoum (products content 0.25% of active ingredients) per one kg of placebo bait, the plastic bag was immediately shaken, with small brake, for no less than 10–15 min. Generally, by visually color observation, equal distribution commonly achieved after 7–10 min. Percent of active ingredient content in the baits of 0.0048–0.0051% for bromadiolone and 0.0047–0.0051% for brodifacoum was confirmed by the Chemical Laboratory of the Institute of Pesticides and Environmental Protection.

### 2.3. Feeding tests

The bromadiolone no-choice feeding test was conducted according to OEPP/EPPO (2004). One hundred and nine animals (47 males and 62 females) were used in the feeding test. Poisoned baits containing 0.005% bromadiolone were offered in feeding bowls during 21 days of the no-choice feeding test. Leftover bait was weighed daily and replaced with fresh bait in clean bowls. Water was provided *ad libitum*. Days survived before death were recorded and all dead animals were autopsied. The mice that survived 21 days feeding with bromadiolone bait and the following 21 days of observation period were classified as bromadiolone-resistant animals. From a series of consecutive bromadiolone no-choice feeding trials we had obtained 20 bromadiolone-resistant animals, which were then reared in the laboratory for at least seven months before proceeding to a brodifacoum trial.

The brodifacoum no-choice feeding test was conducted according to procedures described by Redfern et al. (1976). Brodifacoum bait was offered in separate no-choice trials over 24 or 48 h feeding periods. Each test included 10 animals (5 males and 5 females) that survived the bromadiolone test. Bait consumption was measured daily. Leftover bait was weighed every day and replaced with fresh bait in clean bowls. Water was received *ad libitum*.

All survivors of the brodifacoum trials were under observation for an additional period of 21 days. The animals were fed on standard laboratory diet for laboratory mice during observation before and after the tests. Days to death were recorded and all dead animals autopsied.

### 2.4. VKORC1 sequencing

Tissue samples of the animals that survived bromadiolone trial were investigated for nucleotide sequence changes in the VKORC1 gene (GenBank number NM 178 600 was considered as the ‘wild-type’ sequence). DNA was extracted from tissue using Puregene core kit A (Qiagen, Hilden, Germany). The three VKORC1 exons were sequenced by the company GATC (Konstanz, Germany) using the Sanger sequencing method (Sanger et al., 1977) and an ABI 3730 XL DNA sequencer.

### 2.5. Data analysis

Sum consumption of bromadiolone and brodifacoum baits was calculated for each animal using a formula: consumption over exposure period (g)/initial body weight (g). All consumption values are presented as the mean ± standard deviation. The dose survived is the total amount of active ingredient (mg) eaten by an animal in a feeding test per body weight (kg) that has not caused mortality, in contrast to lethal dose which resulted in the animal's death. T-test was used to analyse differences between genders in bromadiolone and brodifacoum tolerance, i.e. the sum consumption.

Non-parametric ANOVA and Mann-Whitney *U* test were used to analyse the influence of gender and VKOR variant on survival time. ANOVA was also used to analyse the influence of exposure time to survival time. P-values of less than 0.05 were considered as