BIOLOGIJA. 2005. Nr. 4. P. 77–81

Toxicity and effectiveness of indirect anticoagulants bramodiolone and brodifacoum

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We established the toxicity and effectiveness of the preparation R1 containing 0.05% of bromodiolone and R2 containing 0.05% of brodifacoum. We calculated the parameters of acute toxicity of these preparations by using Litchfield–Wilcoxon and Kölber methodologies. We observed mice and rats 10 days after giving them the anticoagulants, we recorded their time of death and dissected them. The parameters of bromadiolone LD_{50} were determined: 1.7 mg/kg (1.41–2.04 mg/kg) for white mice and 1.1 mg/kg (1.0–1.2 mg/kg) for white rats. The parameters of brodifakoum LD_{50} were 0.4 mg/kg (0.34–0.46 mg/kg) for white mice and 0.30 mg/kg (0.21–0.42 mg/kg) for white rats.

Granules of R2 and wax blocks of R1 were equally effective against mice and rats. All rats died within 4 to 8 days and mice 6 to 9 days after having eaten the bromadiolone bait. All rats died within 3 to 8 days and mice 5 to 9 days after having eaten the brodifacoum bait. These anticoagulants act when given orally, they are characterised by a latent period and gradual increase in effect, they inhibit blood coagulation in affected tissues, and the animal dies because of haemorrhage.

Key words: rats, mice, rodenticide, bromadiolone, brodifacoum

INTRODUCTION

About 22 species of rodents live in Lithuania. The most harmful rodents are rats and mice. There are two species of rats in Lithuania. These include brown rat (*Rattus norvegicus*), also called Norway rat, Irish rat, common or sewer rat, and black rat (*Rattus rattus*), also called house, ship or Alexandrian rat. House mice (*Mus musculus, Mus domesticus*) are the most spread rodents in Lithuania. The problems caused by these pests are important and arise practically in all fields of economy and industry: food, agriculture, human services, etc.

Different methods (chemical, mechanical, biological, etc.) are used for extermination of rodent species in one or several foci. One of them is application of rodenticides. The breakthrough in the field of rodent extermination came in the beginning of 1950s when rodenticides of anticoagulant group were developed. They provided with a possibility to achieve a 100% extermination of a rodent population. Long-term anticoagulants are more effective. Indirect anticoagulants act only *in vivo* in the liver. They interfere with transformation of the vitamin K_1 oxide into the active form of vitamin K_1 and with transportation of vitamin K to liver cells. After decline in the amount of vitamin K, the synthesis of prothrombin, proconvertin and blood coagulation factors IX and X as well as coagulation phases gets disturbed [3, 5, 7, 9, 11]. Indirect anticoagulants diminish blood coagulation (the effect depends on the level of vitamin K in the body) only after a latent period (after 12 to 72 hours) and their effect is long-lasting (2 to10 days). After passing to the blood these anticoagulants are bound by proteins, they accumulate in the liver, lungs, spleen, kidneys and penetrate through the placenta into the foetus [4, 11, 12]. These anticoagulants act when given orally, they have a latent period and gradually increase in effect, inhibit blood coagulation in affected tissues, and the animal dies because of haemorrhage. Though it seems dramatic, it causes much lower stress for the dying animal in comparison with almost all rodenticides of acute and subacute action. Besides, the action of anticoagulants is slower, the shortest period until the death is 2 to3 days, 6 to 7 days on average.

Usually the concentrations of these anticoagulants are 0.05% to 0.0025% [2, 7, 9, 11].

It is possible to select and mix a rodenticide with a bait basis or to acquire prepared baits with rodenticides (loose grains, pellets, wax pieces). It is im-

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possible to determine a general-purpose base of baits, though rodents select preferably whole and chopped grains. According to observations, the bait becomes more alluring for rodents after addition of sugar (5%) and oil (5%). Colours and substances of unpleasant taste (detergents) are added in order to avoid occasional eating of the bait by humans.

The aim of the present study was to establish the toxicity and effectiveness of the preparations R1 containing 0.05% of bromodiolone and R2 containing 0.05% of brodifacoum.

MATERIALS AND METHODS

White mice of both sexes weighing 20 to 33 g and white rats weighing 300 to 400 g were used in finding the indicators of acute toxicity of the anticoagulants. Mice and rats were divided into groups, 6 mice and 6 rats in each. The laboratory animals were kept in similar conditions; they were fed twice a day and always had drinking water. Different amounts of the anticoagulants were fed orally to white mice and white rats of each group. The mice and rats were observed 10 days after administration of the anticoagulants, the time of death was recorded and the dead animals were dissected. We calculated the parameters of acute toxicity of the preparations by using the Litchfield–Wilcoxon and Kölber methodologies [17]. Based on the findings, we determined the lethal doses LD_{50} of the preparations according to the classification of substance toxicity [7, 15, 17].

The effectiveness of the anticoagulants was elucidated for white mice and white rats. Mice and rats were divided into groups, each including 10 mice and 10 rats. Different amounts of the anticoagulants were fed orally to white mice and white rats of each group. The mice and rats were observed for 10 days after administration of the anticoagulants, the time of death was recorded and the dead animals and then dissected. The research work met the regulations and ethical principles of the use of animals in research.

The findings were analysed and statistical data were calculated with the help of Epi Info software (1996; Centers for Disease Control & Prevention (CDC), U. S. A., Version 6.04). Arithmetical means of findings (M) and standard deviation (SD) were calculated. The Student multiple comparison method was applied to determine the significance criterion for difference between the groups (p). The difference was considered statistically significant at $p < 0.05$.

RESULTS

White mice and white rats which had received bromadiolone showed the main clinical symptoms of intoxication after a latent period (24 to 36 hours). The mice and rats were sleepy, they did not eat, were weakened, breathed with difficulty, had bleeding mucosa, their urine and faeces became red, some of animals vomited with bloody secretions. The animals died after a period of 3 to 9 days. Autopsy of the animals demonstrated the following: haemorrhages in internal organs, mucosa and muscles, blood in the abdominal cavity, and increased permeability of blood vessels. Bromadiolone LD_{50} parameters were established during the test (Tables 1 and 2) and reached 1.7 mg/kg (1.41–2.04 mg/kg) for white mice and 1.1 mg/kg (1.0–1.2 mg/kg) for white rats. According to the regulations of pharmacopoeia and chemotherapy, medical substances with LD_{50} 1 to 100 mg/kg belong to highly toxic substances [15].

Table 1. **Calculation of brodiolone acute toxicity parameters for mice according to the Litchfield–Wilcoxon method**

Indicators		Dose of bromiodolone, mg per 1 kg of weight						
Dose, mg/kg		1.0	1.2	1.4	1.6	1.8	2.0	
Effect		0/6	1/6	2/6	3/6	5/6	6/6	
Visible effect, % (S)		1.6	16.6	33.3	50.0	83.3	96.8	
Expected effect, % (L)		5.0	22.0	42.0	63.0	85.0	90.0	
Difference between S and L		3.4	5.4	8.7	13.0	1.7	6.8	
Component, X^2		0.024	0.018	0.03	0.07	0.0028	0.04	0.1848
Value of tested x^2		1.1088						
X^2 value when p = 0.05		7.82						
Confidence limits, LD_{50}	S	1.25						
	N	12						
	f ED_{50}	1.2						
Toxicity parameters, mg/kg	LD_0	0.65 mg/kg						
	LD_{16}	1.4 mg/kg						
	LD_{50}	**1.7 mg/kg (1.41–2.04) when P = 0,05**						
	LD_{84}	1.8 mg/kg						
	LD_{100}	3.5 mg/kg						

Table 2. **Calculation of brodiolone acute toxicity parameters for white rats according to the Litchfield–Wilcoxon method**

Indicators		Dose of bromiodolone, mg per 1 kg of weight						
Dose, mg/kg		0.8	0.9	1.0	1.1	1.2	1.3	
Effect		0/6	1/6	2/6	3/6	5/6	6/6	
Visible effect, % (S)		1.3	16.6	33.3	50.0	83.3	97.4	
Expected effect, % (L)		4.0	17.2	40.0	60.0	82.0	92.0	
Difference between S and L		2.7	0.6	13.3	10.0	1.3	5.4	
Component, X^2		0.024	0.018	0.03	0.07	0.0028	0.04	0.1848
Value of tested x^2		1.1088						
X^2 value when p = 0.05		7.82						
Confidence limits, LD_{50}	S	1.16						
	N	18						
	f ED_{50}	1.09						
Toxicity parameters, mg/kg	LD_0	0.6 mg/kg						
	LD_{16}	0.9 mg/kg						
	LD_{50}	**1.1 mg/kg (1.0–1.2) when P = 0.05**						
	LD_{84}	1.2 mg/kg						
	LD_{100}	1.8 mg/kg						

Table 3. **Calculation of brodifacoum acute toxicity parameters for mice according to the Litchfield–Wilcoxon method**

Indicators		Dose of bromiodolone, mg per 1 kg of weight						
Dose, mg/kg		0.08	0.1	0.2	0.3	0.4	0.5	
Effect		0/6	1/6	2/6	3/6	5/6	6/6	
Visible effect, % (S)		3.2	14.6	48.0	72.0	85.3	92.7	
Expected effect, % (L)		10.0	22.0	42.0	63.0	85.0	82.7	
Difference between S and L		6.8	7.4	6.0	9.0	0.3	10.0	
Component, X^2		0.04	0.03	0.015	0.03	0.0015	0.06	0.1765
Value of the tested x^2		1.059						
X^2 value when p = .0.05		7.82						
Confidence limits, LD_{50}	S	1.3						
	N	18						
	f ED_{50}	1.16						
Toxicity parameters, mg/kg	LD_0	0.14 mg/kg						
	LD_{16}	0.28 mg/kg						
	LD_{50}	**0.4 mg/kg (0.34–0.46) when P = 0.05**						
	LD_{84}	0.54 mg/kg						
	LD_{100}	1.0 mg/kg						

The white mice and white rats that received brodifacoum showed clinical symptoms of intoxication after a latent period (24 to 36 hours). The mice and rats were sleepy, they did not eat, were weakened, breathed with difficulty, had bleeding mucosa, their urine and faeces became red, some of the animals vomited with bloody secretions. The animals died after a period of 3 to 8 days. Autopsy of the animals demonstrated the following: haemorrhages in internal organs, mucosa and muscles, blood in the abdominal cavity, and increased permeability of blood vessels.

The brodifacoum LD_{50} parameters established during the test (Tables 3 and 4) were 0.4 mg/kg (0.34 to 0.46 mg/kg) for white mice and 0.30 mg/kg (0.21 to 0.42 mg/kg) for white rats. According to the regulations of pharmacopoeia and chemotherapy, medical substances with LD_{50} 1 to 100 mg/kg belong to high toxicity substances [15].

Data of Table 5 show that the bait with 0.005% of bromadiolone was effective in extermination of mice and rats. All rats died during a period of 4 to 8 days and mice during a period of 6 to 9 days after administration of wax blocks.

Data of Table 6 show that the bait with 0.005% of brodifacoum was effective in extermination of mice and rats. All rats died during a period of 3 to 8 days and mice during a period of 5 to 9 days after administration of granules.

Table 4. **Calculation of brodifacoum acute toxicity parameters for white rats according to the Litchfield–Wilcoxon method**

Indicators		Dose of bromiodolone, mg per 1 kg of weight						
Dose, mg/kg		0.09	0.1	0.2	0.3	0.4	0.5	
Effect		0/6	1/6	2/6	3/6	5/6	6/6	
Visible effect, % (S)		2.9	16.6	33.3	50.0	83.3	96.8	
Expected effect, % (L)		9.0	13.0	30.0	54.0	76.0	90.0	
Difference between S and L		6.1	3.3	3.3	4.0	7.3	6.8	
Component, X^2		0.05	0.01	0.005	0.005	0.03	0.05	0.145
Value of the tested x^2		0.87						
X^2 value when p = 0.05		7.82						
Confidence limits, LD_{50}	S	1.8						
	N	18						
	f ED_{50}	1.4						
Toxicity parameters, mg/kg	LD_0	0.08 mg/kg						
	LD_{16}	0.1 mg/kg						
	LD_{50}	**0.30 mg/kg (0.21–0.42) when P = 0.05**						
	LD_{84}	0.45 mg/kg						
	LD_{100}	0.8 mg/kg						

Table 5. **Effectiveness of wax blocks containing bromadiolone**

Rodents	Level of bromadiolone in the bait, %	Number of animals	Died, %	Day of death		Percentage of toxic bait in the ration
				average	interval	
White rats	0.005 %	10	100	5	4–8	70.5
White mice	0.005 %	10	100	7	6–9	58.5

Table 6. **Effectiveness of granules containing brodifacoum**

Rodents	Level of bromadiolone in the bait, %	Number of animals	Died, %	Day of death		Percentage of toxic bait in the ration
				average	interval	
White rats	0.005 %	10	100	5.3	3–8	51.3
White mice	0.005 %	10	100	7.6	5–9	33

DISCUSSION

Rodents may appear everywhere where food is stored, processed or saled. They are especially spread in rural areas, farms and fields where they cause much harm to harvest and products. Food damage by rodents makes about 10% of the general world food production. Fedding on foodstuffs and cereals, rodents contaminate them and disseminate different infectious diseases. The most dangerous infections disseminated by rodents include plague, cholera, leptospirosis, yersiniosis, trichinosis, salmonellosis, scabies, rabies, epizootic aphthae and anthrax.

Development of new anticoagulants (brodifacoum, flocoumafen) allowed introducing a new method, the so-called “feeding the bait with intervals”. The toxicity of these new anticoagulants to rodents is higher if compared to that of other anticoagulants, and rodents swallow easier a lethal dose when eating once. The bait may be more rarely changed, and the labour and material resources may be saved because of a higher toxicity and other characteristics of these anticoagulants.

Brodifacoum belongs to the group of highly toxic substances (LD_{50} 0.3 to 0.4 mg/kg of weight); it cumulates in the body of rodents. Other researchers [1, 2, 4, 14] have found that LD_{50} of this anticoagulant is 0.26 to 0.68 mg/kg of weight for rodents, 0.5 to 2.0 mg/kg for pigs, 3.0 to 3.56 mg/kg for dogs, 23 to 26 mg/kg for cats. After accumulation of the anticoagulant in the body of rodents (after 24 to 36 hours), blood coagulation gets disturbed, the permeability of blood vessels increases, haemorrhages appear and the animals die. The bait containing 0.005% of brodifacoum was effective in extermination of mice and rats. All rodents died within a period of 3 to 9 days after administration of the bait granules. Other researchers obtained similar data [3, 7, 9, 16]. They found that all rats and 90% of mice died 4 to 9 days later after administration of the anticoagulant.

Bromadiolone belongs to high toxicity substances according to the toxicity data for rodents (LD_{50} 1.1 to 1.7 mg/kg). Other researchers [4, 6, 10] found that oral LD_{50} of this anticoagulant is 1.12 to 1.25 mg/kg of weight for rats, 1.70 to 1.80 mg/kg for mice, 15 to 20 mg/kg for dogs, 25 to 30 mg/kg for cats.

The bait containing 0.005% of bromadiolone was effective in extermination of mice and rats. All rodents died within 4 to 9 days after administration of wax blocks. Also other researchers tested wax blocks containing bromadiolone [3, 7, 10, 13]. They, similarly to us, have found that wax bocks are better eaten by rats and therefore the anticoagulant is more effective in them.

Received 6 September 2005
Accepted 11 November 2005

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NETIESIOGINIØ ANTIKOAGULIANTØ BRAMODIOLONO IR BRODIFAKOUMO TOKSIÐKUMO BEI EFEKTYVUMO TYRIMAI

S a n t r a u k a

Nustatytas preparato R1, turinèio 0,05% bramodiolono, ir R2, turinèio 0,05% brodifakoumo, toksiðkumas ir efektyvumas. Bandomøjø preparatø ûmaus toksiðkumo parametrus apskaièiavome naudodamiesi Litèfildo-Vilkoksono ir Kiolberio metodikomis. Bramodiolono LD_{50} parametrai: baltosioms pelytëms – 1,7 mg/kg (1,41–2,04 mg/kg); baltosioms þiurkëms – 1,1 mg/kg (1,0–1,2 mg/kg). Brodifakumo LD_{50} parametrai: baltosioms pelytëms – 0,4 mg/kg (0,34–0,46 mg/kg); baltosioms þiurkëms – 0,30 mg/kg (0,21–0,42 mg/kg).

R2 granulës ir R1 vaðkiniai blokeliai vienodai efektyvûs prieð peles ir þiurkes. Suëdusios bramodiolono jaukà visos þiurkës nugaiðo per 4–8 dienas, pelës – per 6–9 dienas. Suëdusios brodifakoumo jaukà visos þiurkës nugaiðo per 3–8 dienas, pelës – per 5–9 dienas. Ðie antikoaguliantai, suvartojus juos oraliai, pasiþymi latentiniu periodu ir palaipsniu efekto didëjimu, stabdo kraujo kreðumà paþeistuose audiniuose ir gyvûnas þûva nuo hemoragijos.

Raktaþodþiai: þiurkës, pelës, rodenticidai, bramodiolonas, brodifakoumas