Studies on the Rodenticidal Activity of Methylene-bis(1-thiosemicarbazide) Iwao TOKUMITSU, Koji OGUSHI (Research Department, Kondo Chemical Industry Co., Ltd., Tsumashoji 13-35, Hakata-ku, Fukuoka City), Hiroshi YAMAMOTO (Pesticide Department, Nippon Kayaku Co., Ltd., Marunouchi 1-2-1, Chiyoda-ku, Tokyo) and Tyuzi KUSANO (Faculty of Agriculture, Tottori University, Koyama-cho 1-1, Tottori City). Received June 1, 1973. *Botyu-Kagaku*, 38, 202, 1973.

27. Methylene-bis(1-thiosemicarbazide) の殺そ力について

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Methylene-bis (1-thiosemicarbazide) のドブネズミ，クマネズミおよびハタネズミに対する殺そ力を検討した．本化合物の経口急性 LD_{50} はそれぞれドブネズミ：雄 25 mg/kg，雌 32mg/kg，クマネズミ：雄 76 mg/kg，雌 89 mg/kg，ハタネズミ：雄 42 mg/kg で，その致死作用は速効であった．

本化合物に対する耐性および aversion の発達は認められなかった．また，本化合物に対するドブネズミの感受性には季節および環境温度による影響はなかった．毒餌に対する各ネズミの摂食性は良好で，毒餌摂食によるドブネズミの最少致死量は雄40～50 mg/kg，雌 40～70mg/kg であった．ドブネズミおよびハタネズミに対するフィールドテストではすぐれた防除効果があった．

Introduction

Thiourea rodenticides, i. e. ANTU, thiosemicarbazide and Muritan are not widely used because of their undesirable properties. But, there is the possibility of discovering rodenticides in thiourea derivatives with excellent activities. The rodenticidal property of thiourea derivatives was first discovered in phenylthiourea by Richter in 1942[23]. He found that laboratory animals showed no aversion to eating foods poisoned with phenylthiourea, which suggested the possibility of using this compound as a rodenticide. Preliminary trials of phenylthiourea in the field indicated that its performance as a rodenticide was somewhat erratic, thus a number of other thiourea derivatives were tested. Of these, ANTU bait which possessed a high degree of toxicity was most readily accepted by rats. Although ANTU is highly toxic to Norway rats and to house mice, these animals gain a tolerance to it when fed sub-lethal doses[18]. It is also considerably less toxic to roof rats. Thiosemicarbazide was discovered to be a rodenticide by Dieke *et al.*, but its acceptability was poor, and it was highly toxic to other animals.[3] Instead of producing a superior rodenticide, undesirable features have led to the virtual discontinuance of ANTU as a rodenticide.

We studied the toxicities of scores of thiourea derivatives synthesized by the Ageo Pesticide Laboratory of Nippon Kayaku Co., Ltd.. In the process, methylene-bis(1-thiosemicarbazide) which has a strong rodenticidal activity, was discovered.

In this paper the rodenticidal activities and efficiency of this compound for rat control are described. The killing effect and feeding acceptability are also presented and are compared with those of several single-dose rodenticides.

Meterials and Methods

Rodent : Norway rats, *Rattus norvegicus*, roof rats, *Rattus rattus* and Japanese field voles, *Microtus montebelli*, were used. The former two species came from our laboratory's colonies, and were bred and maintained by the authors. The breeding of these rats is the same as that of Calhoun used for the production of semi-natural colonies of Norway rats[1]. Japanese field voles were captured in a field in Fukuoka.

Chemicals :

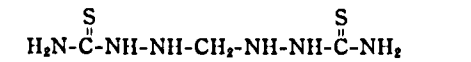
a) NK-15561

chemical name: methylene-bis (1-thiosemicarbazide)

code number: NK-15561

trade name: Kayanex

chemical structure:



appearance : white crystalline powder
melting point : 171-174°C (decomposition)
odor:none
solubility : little solubility in water and the usual organic solvents ; soluble in dimethyl sulfoxide.
stability : decomposes gradually in water. When acids or alkalis are added, the decomposition of this compound is accelerated.

b) ANTU : α-naphthylthiourea
c) norbormide: 5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzilidene)-5-norbornene-2,3-dicarboximide
d) scilliroside
e) thallium sulfate
f) thiosemicarbazide
g) zinc phosphide

Acute oral toxicity : A pre-determined dose of NK-15561, which was suspended in a 3% starch aqueous solution, was administered in the stomach by catheter. Afterward, the rodents were observed for a period of 5 days. The LD_{50}'s were calculated by Finney's Graphic appoximate method[6].

Killing effect by baiting : Poisoned bait was prepared from maize powder, wheat flour, rice bran, corn oil, sugar, water and the thiourea derivative. After mixing and kneading these materials, the poisoned bait was dried[20]. Ten grams of this bait was given to individual rats kept in separate cages (25 cm wide, 35 cm long, 20cm high), each of whom fed at liberty for one night (17 hours). The weight of the residual bait was recorded the next morning, after which, the survival or death of the rodents was observed over a 5 day period.

Killing effect by dusting : Tests were carried out on two run-way linked compartments (one with a nest box and one with a bait-box) in the rearing cage (70cm wide, 150cm long, 60cm high). The poisoned dust prepared with talc as the diluent was dusted on the run-way at the rate of 300g per square meter daily for 5 days. These tests were designed to produce contact with the dust, whenever the rats went and returned on the run-way between the nest-box and the bait-box. The survival or death of the rats was observed for over a 5 day period.

The above experiments were carried out in the laboratory at room temperature (15-20°C).

Field tests :

a) Norway rats : A control operation was carried out at a farm in Fukuoka in September, 1971. The farm contained a cattle-shed, lumber room and dwelling, which were surrounded by rice-fields. The distance from neighboring houses was about 100 meters. The cattle were fed with the leavings of cook shops from the town. Thus, the test area was heavily infested with many Norway rats because of abundant food and shelter.

These poisoned baits were used for the control test; 1) 1.0% NK-15561 dry bait. This bait was formed from the test chemical, maize powder, wheat flour, rice bran, corn oil and sugar into cylinders (5 mm in diameter and 5 mm long, 0.2g in weight). 2) 1.0% NK-15561 wet bait. This bait was prepared from cut raw sweet potato and the test chemical into cubes (3 mm cubes, 1.0 g in weight).

Control effects were evaluated in comparison with the intake amounts of plain bait (wheat grain) before and after the operation. Bait was placed on dishes, 50 g per dish, on the runways. Wheat grain was placed in dishes on the 1st-3rd day and on the 8th-10th day, and poisoned baits were placed there on the 4th-7th day.

The control effects with poisoned dry or wet bait are shown in Tables 10 and 11. From these data, the degree of control was calculated using the following equation:

$$\text{control effect A}(\%) = \left(1 - \frac{\text{maximum intake of wheat grain per day after the control operation}}{\text{maximum intake of wheat grain per day before the control operation}}\right) \times 100$$

b) Japanese field voles:

i) Administering poisoned bait in vole holes; A control operation was carried out at a rice-field (1.6 are) in Fukuoka in February, 1972. In this

rice-field, many holes of the Japanese field voles, *Microtus montebelli*, were observed.

2% NK-15561 baits were formulated into a type of dry pellet at the rate of 0.2 g per pellet using maize powder, wheat flour, rice bran, corn oil and the test chemical. Plain bait was formulated by the same method. This was used before and after the control operation. Unpoisoned and poisoned baits were placed in holes at the rate of three pellets per hole. Unpoisoned bait was used on the first, second, fifth and sixth day. Poisoned bait was used on the third and fourth day. The number of residual pellets was checked daily.

The control effect was evaluated using the following equation:

$$\text{control effect B}(\%) = \left(1 - \frac{\text{number of "living" holes after control operation}}{\text{number of "living" holes before control operation}}\right) \times 100$$

ii) Test by the bait-box method; Recently, bait-boxes have been used in the control program, to curtail labor and to prevent danger. Thus, the control operation for Japanese field voles was carried out using bait-boxes in the fields (18 are) of Fukuoka in June, 1972.

Twenty bait-boxes were placed 10 meters apart. Twenty grams of unpoisoned bait was used per bait-box on the first, second, third and seventh day. The same amount of poisoned bait was used in the boxes on the fourth, fifth and sixth day. The amount of residual bait was weighed daily.

The control effect was evaluated using the following equation:

$$\text{control effect C}(\%) = \left(1 - \frac{\text{intake of unpoisoned bait per day after the control operation}}{\text{maximum intake of unpoisoned bait per day before the control operation}}\right) \times 100$$

Results and Discussion

Acute oral toxicity

The killing effects of NK-15561 due to oral administration to Norway rats, roof rats and Japanese field voles are shown in Table 1. Oral LD_{50}'s to rodents were: Norway rats, male 25mg/kg and female 32mg/kg; roof rats, male 76mg/kg and female 89mg/kg; Japanese field voles, male 42mg/kg.

NK-15561 was effective for the three kinds of rodents and the selective toxicity was very low. The main poisoning symptom induced by NK-15561 was that of violent convulsions. This symptom was quite similar to that evoked by thiosemicarbazide as observed by Dieke[4]. It has been confirmed that the hydrolysis of NK-15561 in the presence of acids gives thiosemicarbazide[19]. Therefore, it is reasonable to assume that the poisoning symptom of NK-15561 is the same as that of thiosemicarbazide.

The latent period before poisoning symptoms occurred was 1-2 hours after oral administration. Poisoned rodents died within 1.5-3 hours. Thus, we concluded that NK-15561 is an acute-action rodenticide, as is thiosemicarbazide.

Seasonal susceptibility of Norway rats to NK-15561 was tested at 5°C in January and at 25°C in July. As shown in Table 1, the toxic effect of NK-15561 does not seem to vary in either season.

Acceptability of NK-15561 bait and its killing effect

As shown in Table 2, the intake amount of 0.5-2.0% NK-15561 bait for Norway rats was 1.4 g to 2.5 g. The influence of the concentration of NK-15561 was not remarkable. The killing effect of NK-15561 increased with the increase in concentration of NK-15561 in poisoned bait. This suggests that the suitable concentration of NK-15561 in bait is about 2.0%. The minimum lethal dose of NK-15561 was 40-50 mg/kg on males and 40-70 mg/kg on females.

The acceptability of NK-15561 bait and its killing effect on roof rats and Japanese field voles are shown in Table 3. With 1.0% NK-15561 bait, seven roof rats died after taking doses above 94.1 mg/kg and one roof rat survived with a dose of 69.6 mg/kg. Three Japanese field voles died after taking doses above 125.0 mg/kg.

The killing effects of 1.0% thiosemicarbazide bait varied, and mortalities did not increase with an increase in the dose. Only 9 rats of 20 treated died. (Table 4) The toxicity or thiosemicarbazide by oral administration was quite high, but the

Table 1. Acute oral toxicity of NK-15561 for Norway rats, roof rats and Japanese field voles

Species	Sex	Environmental temperature (°C)	Dose given (mg/kg B. W.)	Average B. W.	Mortality	Latent period	Survived period	LD_{50} (mg/kg B. W.)
R. n.	♂	5	20	200	1/5	60~150 min.	150~180min.	25
			30	188	4/5			
			40	202	5/5			
			50	192	5/5			
		25	30	145	2/3	65~135 min.	150~170min.	
			40	142	3/3			
			50	164	3/3			
			60	160	3/3			
	♀	5	20	220	0/5	70~150 min.	130~170min.	32
			30	224	2/5			
			40	194	4/5			
			50	190	4/5			
		25	30	160	1/3	65~125 min.	140~250min.	
			40	165	3/3			
			50	155	3/3			
			60	172	3/3			
R. r.	♂	23	63	150	1/5	>6 hrs.	6~17 hrs.	76
			79	162	3/5			
			100	146	5/5			
			200	133	3/3		150~180min.	
			400	137	3/3		155~170min.	
	♀	23	79	142	2/5	>6 hrs.	6~17 hrs.	89
			100	166	3/5			
			126	156	4/5			
			200	137	3/3		150~180min.	
			400	143	3/3		145~170min.	
M. m.	♂	23	40	27	2/5	55~80 min.	150~300min.	42
			60	27	4/5			
			80	29	5/5			

R. n.: *Rattus norvegicus*, *R. r.*: *Rattus rattus*, *M. m.*: *Microtus montebelli* B. W.: body weight

killing effect by baiting was poor. Thus, we concluded that NK-15561 bait is more effective on rats and voles than is thiosemicarbazide bait.

Killing effect of the dusting test

10% NK-15561 dust exposed on the run-way was enough to kill Norway rats (Table 5). But the toxic effect of 2% NK-15561 dust was poor; 60% of the rats died within 4 days with successive exposure. The consumption of the dust was high on the first day, but afterwards decreased markedly.

Aversion to NK-15561 bait

Rats which survive the feeding of a dose of sub-lethal poisonous bait show an aversion to the same bait given again[2,8,26]. This poison bait aversion has been demonstrated for ANTU, arsenious oxide, barium carbonate and red squill powder. This phenomenon may depend on the

Table 2. Intake amounts of NK-15561 bait and the killing effect on Norway rats

Concentration %	No. of rats	Av. B. W.	Av. amount of bait eaten per rat (g)	Av. dose taken (mg/kg B. W.)	Mortality	Minimum dose taken by the dead (mg/kg B. W.)	Maximum dose taken by the survivors (mg/kg B. W.)
0.5	4 ♂	133	1.6	60.2	75	55.2	42.3
	4 ♀	153	1.2	39.2	25	91.2	40.0
1.0	14 ♂	178	2.8	157.3	93	52.7	21.3
	18 ♀	164	2.4	146.3	72	45.2	66.7
1.5	7 ♂	163	1.9	174.8	70	125.0	20.0
	7 ♀	136	1.6	176.5	100	80.0	20.0
2.0	11 ♂	165	2.5	303.0	100	102.0	
	13 ♀	173	1.3	150.3	92	40.0	28.6

B. W.: body weight, Av.: average

Table 3. Intake amounts of NK-15561 bait and the killing effect on roof rats and Japanese field voles

Rodents			Poisoned Bait			Dose Taken (mg/kg B. W.)	Results
Species	B. W. (g)	Sex	Type	Concentration (%)	Amount eaten (g)		
R. rattus	115	♂	dry	1.0	0.8	69.6	survival
	160	♂	〃	2.0	0.8	100.0	death
	150	♀	〃	〃	1.2	160.0	〃
	170	♀	〃	〃	0.8	94.1	〃
	150	♀	〃	〃	1.0	133.3	〃
	120	♀	〃	〃	1.0	166.7	〃
	140	♀	〃	〃	1.2	171.4	〃
	110	♂	wet	〃	0.6	109.1	〃
M. montebelli	32	♂	dry	2.0	0.2	125.0	death
	30	♂	〃	〃	0.3	200.0	〃
	24	♂	wet	〃	0.4	333.3	〃

B. W.: body weight

conditioning of rats to pain or to an unpalatable taste or odor from the bait materials or poison.

From the above results, we inferred that all test rats will survive when 0.1% NK-15561 bait is offered. Thus, 0.1% NK-15561 bait was given to rats on the first day and all survived after eating doses of 29.1-51.3 mg/kg. These intake doses were at higher levels than those for 0.5-2.0% poisoned bait. Plain bait was given on the following three days and 1.0% NK-15561 bait was given on the fifth day. The second intake amount of NK-15561 bait was less than the first. But, all the test rats were fed lethal doses and died (Table 6). Accordingly, the ability of Norway rats to develop an aversion to NK-15561 is low.

Tolerance

Some rodents develop a tolerance to rodeticides after taking sublethal doses. For example, the tolerance to ANTU is built up rapidly within a few days[3,24,27], and a thiosemicarbazide tolerance is well enough developed for the rat to survive with a two-fold dose of LD_{50} given by repetitious administration of a sub-lethal dose[4].

The effects of lethal doses on rats surviving sublethal doses are shown in Table 7. The killing effects of 2.0% NK-15561 bait and its acceptability to the surviving rats after exposure to 2.0 % dust are shown in Table 8. These results show that Norway rats may not develop a tolerance for NK-15561.

Table 4. Intake amounts of 1.0% thiosemicarbazide bait and the killing effect on Norway rats

Rats B.W. (g)	Rats Sex	Amount eaten (g)	Dose taken (mg/kg B.W.)	Days survived (over a 5 day period)
275	♂	0.1	3.6	survival
280	♂	0.2	7.1	< 1
215	♂	0.3	14.0	< 1
165	♂	〃	18.4	< 1
160	♂	〃	18.7	survival
275	♂	0.6	21.8	survival
150	♀	0.4	26.7	< 1
300	♂	1.5	50.0	< 1
140	♂	0.8	57.1	survival
155	♂	1.0	64.5	survival
145	♂	1.1	75.9	survival
155	♂	1.2	77.4	survival
160	♂	1.3	81.3	< 1
140	♂	1.6	114.3	survival
165	♂	2.0	121.2	survival
165	♂	〃	121.2	< 1
180	♂	2.3	127.8	survival
125	♂	1.6	128.0	survival
160	♀	2.5	156.3	< 1
105	♀	2.3	219.0	< 1

Table 5. Effects of NK-15561 dust on Norway rats

Concentration %	Amount of dust	Rats No.	Rats Av. B.W. (g)		Results (day) 1st	2nd	3rd 4th
2	15 g/400 cm²	10 (1♂,9♀)	161	Intake of food-stuff (g)	20	60	115
				Consumption of dust (g)	14	5	5
				No. of dead	5/10	5/10	5/10
10	15 g/500 cm²	5 (5♂)	157	Intake of food-stuff (g)	19		
				Consumption of dust (g)	14		
				No. of dead	5/5		

Table 6. The learning test of Norway rats given NK-15561 bait

Rats B.W. (g)	Rats Sex	1st day Concentration (%)	1st day Intake (g)	1st day Dose (mg/kg)	2nd-4th day	5th day Concentration (%)	5th day Intake (g)	5th day Dose (mg/kg)	Results
150	♂	0.1	7.7	51.3	plain bait	1.0	2.5	166.7	death
160	♂	〃	7.8	48.1	〃	〃	2.7	168.8	〃
150	♂	〃	7.5	50.0	〃	〃	2.6	173.3	〃
165	♂	〃	4.8	29.1	〃	〃	3.6	218.2	〃
140	♀	〃	6.0	42.9	〃	〃	1.3	92.9	〃
120	♀	〃	5.0	41.7	〃	〃	2.0	166.7	〃
160	♀	〃	7.9	49.4	〃	〃	2.7	168.8	〃
150	♀	〃	6.1	40.7	〃	〃	3.4	226.7	〃

Table 7. Tolerance test of Norway rats to NK-15561

Rats		1st day	4th day		
B. W. (g)	Sex	Dose (mg/kg)	Dose (mg/kg)	Latent time (min.)	Survival time (min.)
200	♂	10	20	75	120
210	♂	15	30	120	survival
165	♀	15	30	80	115
210	♂	20	40	120	survival
160	♀	20	40	100	115
130	♂	7.5	50	110	survival
150	♀	10	〃	85	240
120	♂	2.5	60	80	240
140	♀	〃	〃	90	240
120	♂	5.0	70	80	115
135	♀	7.5	〃	55	240
130	♀	5.0	80	75	155
130	♀	30	90	65	130
130	♀	40	〃	130	240

Table 8. Toxic effects of 2.0% NK-15561 bait on Norway rats surviving exposure to 2.0% NK-15561 dust

Rats		Amount eaten (g)	Dose (mg/kg)	Results
B. W. (g)	Sex			
85	♀	0.6	141.2	death
95	♀	0.6	126.3	〃
115	♂	2.7	469.6	〃
230	♂	2.7	234.8	〃

Comparison of toxicity and acceptability with other rodenticides

The LD_{50}'s of several single-dose rodenticides to rodents are shown in Table 9. An acute oral lethal dose of NK-15561 is equal to that of zinc phosphide and is two or three-fold that of thiosemicarbazide.

The feeding amounts of 1.0%, 1.5% and 2.0% NK-15561 bait, 1.0% thiosemicarbazide bait, 1.0% norbormide bait, 0.03% scilliroside bait, 1.0% zinc phosphide bait and 0.3% thallium sulfate bait per Norway rat are shown in Figure 1 and Table 10.

The feeding amounts of each poisoned bait and the mortality of the majority of test rats were: 0.1-0.9 g with 1.0% norbormide, 40%; 0.1-0.9 g with 0.03% scilliroside, 70%; 1.0-1.9 g with 1.0% zinc phosphide, 45%; 3.0-3.9 g with 0.3% thallium sulfate, 89%; 1.0-1.9 g with 2.0%

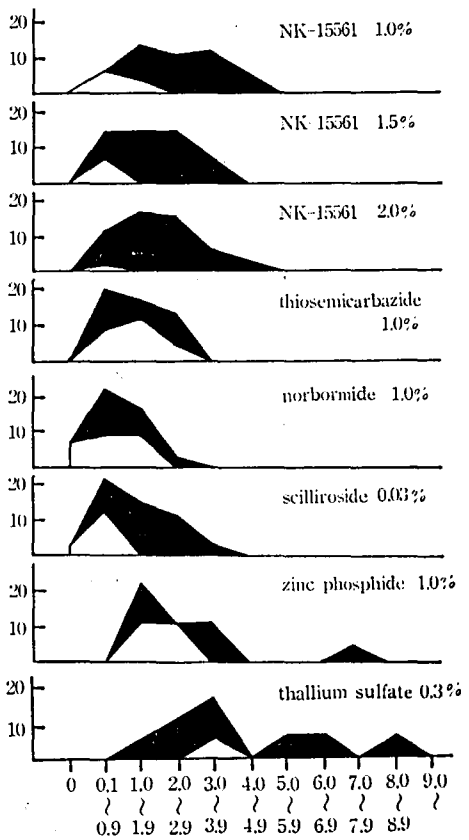


Fig. 1.

Table 9. Acute oral toxicities of single-dose rodenticides to rodents

Rodenticides	Species	LD_{50} (mg/kg)	References
Thiosemicarbazide	*R. norvegicus* (breeding)	15	Richter, 1950[25]
	R. norvegicus (wild)	10	
	R. norvegicus (Adult)	13	Dieke, 1949[4]
	R. norvegicus (Young)	19	
	R. rattus	23	
ANTU	*R. norvegicus* (breeding)	5	Richter, 1950[25]
	R. norvegicus (wild)	7	
	R. rattus	250	Dieke & Richter, 1946[3]
Zinc phosphide	*R. norvegicus*	40±2.9	Dieke & Richter, 1946[3]
	R. norvegicus	40	Holden, 1947[27]
	R. rattus	50	Heinz, 1951[27]
	C. r. bedfordiae	30	Higuchi, 1965[9]
Norbormide	*R. norvegicus* ♂	13.0	Roszkowski, 1965[26]
	R. norvegicus ♀	10.0	
	R. n. albino ♂	15	
	R. n. albino ♀	5.3	
	R. rattus ♂+♀	52	
Red squill conc. powder	*R. norvegicus* ♂	279±29	Dieke & Richter, 1946[3]
Red squill conc. powder	*R. norvegicus* ♀	133±10	
scilliroside	*C. r. bedfordiae*	1.62	Kinoshita, 1963[13]
Thallium sulfate	*R. norvegicus*	15.8±0.9	Dieke & Richter, 1946[3]
	C. r. bedfordiae	23	Higuchi, 1965[9]
	M. m. m. albino	29	Kusano, 1969[16]

Table 10. Toxic effects of rodenticide baits on Norway rats

Rodenticides	Concentration (%)	No. of rats	Av. B. W. (g)	Mortality (%)	Intake dose (mg/kg)	
					Minimum dose in the dead	Maximum dose in the survivors
Thiosemicarbazide	1.0	16 ♂	190	45	7.1	128.0
		4 ♀	143		26.1	64.5
Norbormide	1.0	20 ♂	175	40	8.0	147.0
Scilliroside	0.03	19 ♂	139	70	1.45	1.03
		9 ♀	206		0.53	0.18
Zinc phosphide	1.0	9 ♂	187	45	65.0	330.0
Thallium sulfate	0.3	7 ♂	131	89	41.0	110.0
		2 ♀	78		53.0	no survivors

NK-15561, 96%.

The acceptability of 2.0% NK-15561 bait was better than that of 1.0% norbormide or 0.03% scilliroside baits. The killing effect was higher than that of 1.0% zinc phosphide or 0.3% thallium sulfate baits. Therefore, NK-15561 is clearly superior to some single-dose rodenticides.

Control effect for Norway rats

The control effect with 1.0% NK-15561 dry bait was 51.4% and that with 1.0% NK-15561 wet bait was 97.5%. The poisoned wet bait was more effective with Norway rats than was the poisoned dry bait. It seems that the great difference between the control effect of both the dry and wet baits reflects a disparity in food preference to bait materials and in the amount of intake.

The intake amount of wheat grain per rat per day, the rat population, the number of killed rats, the death rate, the average amount of intake of poisoned bait and the average intake dose can be inferred from the results given in Tables 11 and 12. An assumption about the population of Norway rat was made, from the intake of wheat grain, according to Thompson's method[29].

Although there is no reliable value for the daily intake amount of wheat grain per rat, the amount per rat has generally been regarded as about 30g[28]. We estimated about 15 g for one Norway rat with a body weight of 200g in the laboratory. Granting that the Norway rat did not move, the intake amount was constant and the wheat grain given was not stored by the rat. Feeding amounts of 30g, 15g and 10g were not suitable based on the discovery of the number of dead, the average intake dose (that in each case becomes 100% lethal dose) and bait acceptability. Also, 4 g is not suitable as the average intake dose (that becomes a sub-lethal dose). Thus, if the intake amounts of wheat grain lie in a range of from 5.0 g to 7.5 g, the following calculation can be made :

For 1.0% NK-15561 dry bait,	
rat population before control:	184-276
number of killed rats:	94-141
discovery rate of the dead:	31-47%
average amount of intake of poisoned bait per rat:	1.3-1.7g
average intake dose:	65-85 mg/kg
For 1.0% NK-15561 wet bait,	
rat population before control:	99-149
number of killed rats:	97-145
discovery rate of the dead:	10-14%

Table 11. Field test of 1.0% NK-15561 dry bait given to Norway rats

Bait	Wheat grain			1.0% NK-15561 dry bait				Wheat grain		
Day	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Amount of bait (g)	2500	2500	2500	2500	2500	2500	2500	2500	2500	2500
Intake of bait (g)	862	1355	1380	241	49	17	12	581	677	647
No. of stations	50	50	50	50	50	50	50	50	50	50
No. of stations with taken bait	28	44	39	27	13	4	5	23	22	22
No. of dead discovered				37	4	2	1			

Table 12. Field test of 1.0% NK-15561 wet bait given to Norway rats

Bait	Wheat grain			1.0% NK-15561 wet bait				Wheat grain		
Day	11th	12th	13th	14th	15th	16th	17th	18th	19th	20th
Amount of bait (g)	1250	1250	1250	1250	1250	1250	1250	1250	1250	1250
Intake of bait (g)	745	605	681	256	72	35	20	25	35	20
No. of stations	25	25	25	25	25	25	25	25	25	25
No. of stations with taken bait	21	22	23	20	13	3	1	2	2	2
No. of dead discovered				9	4	1				

average amount of intake of poisoned bait per rat: 2.6-4.0 g
average intake dose: 130-200 mg/kg

The amount of intake of poisoned bait per rat in the control operation with 1.0% NK-15561 wet bait was two-fold that with 1.0% NK-15561 dry bait. Thus, the control effect with poisoned wet bait is clearly higher than that with poisoned dry bait. This effect was also evaluated from lethal doses (male: above 55mg/kg, female: above 93mg/kg) and from sub-lethal doses (male: under 42 mg/kg, female: under 67 mg/kg). Therefore, the suitable concentration of NK-15561 in dry bait should be about 2.0%. The discovery rate of the dead depends considerably on the ecological and sociological behavior of Norway rats. Also, a control operation with poisoned wet bait, carried out after the control operation with poisoned dry bait, showed excellent results. This suggests that NK-15561 produces no tolerance in Norway rats.

Control effects on Japanese field voles

i) **The effect of placement in holes** (Table 13): The greater part of the poisoned bait was taken mostly on the first day. Unpoisoned bait was taken only slightly on the ridge of the rice-field after the control operation. The consumption of poisoned bait was equal to about 40% of the consumption of unpoisoned bait. The control effect of 2.0% NK-15561 bait on Japanese field voles was excellent, at 96.6%.

ii) **The effect with the bait-box** (Table 14): Poisoned bait was taken mostly on the first and second day. The total amount of intake of poisoned bait reached 34% of the maximum intake for unpoisoned bait per day before the control operation. The control effect of NK-15561 on Japanese field voles was excellent, at 86.9%. The disappearance of poisoned bait placed in the holes decreased rapidly on the second day, but still showed a decrease on the third day with the bait-box method. This may be due to the low exposure of the voles with bait-boxes placed at a distance 10 meters.

Summary

The rodenticidal activity of NK-15561, methylene-bis(1-thiosemicarbazide), for Norway rats, roof rats and Japanese field voles was assessed in the laboratory and in the field.

The main toxic symptoms produced by NK-15561 were violent convulsions, similar to the symptoms of thiosemicarbazide. Convulsions in Norway rats and Japanese field voles occurred within 1 to 1.5 hours after administration. Most

Table 13. Control test of 2.0% NK-15561 bait given to Japanese field voles by placement in their holes

Bait	Unpoisoned bait		2.0% NK-15561 bait		Unpoisoned bait
Day	1st	2nd	3rd	4th	5th
No. of bait	264	264	264	264	264
No. of bait taken	245	263	101	6	7
No. of holes used	88	88	88	88	88
No. of holes from which bait was taken	82	88	36	2	3

Table 14. Control test of 2.0% NK-15561 bait on Japanese field voles using bait-boxes

Bait	Unpoisoned bait			2.0% NK-15561 bait			Unpoisoned bait
Day	1st	2nd	3rd	4th	5th	6th	7th
No. of boxes	20	20	20	20	20	20	20
Amount of bait (g)	400	400	400	400	400	400	400
No. of boxes from which bait was taken	15	20	16	12	8	2	7
Amount taken of bait (g)	183	199	195	36	29	3	26

of the poisoned rodents died within 1.5 to 3 hours. The speed of the toxic effect of NK-15561 on roof rats was less than that on Norway rats. These results indicate that NK-15561 is an acute-acting rodenticide.

The acute oral LD_{50}'s of NK-15561 for rodents were: Norway rats, male 25 mg/kg and female 32mg/kg; roof rats, male 76 mg/kg and female 89 mg/kg ; Japanese field vole, male 42 mg/kg. The minimum lethal dosages of NK-15561 bait were 40-50 mg/kg for males and 40-70 mg/kg for female Norway rats.

Furthermore, the NK-15561 susceptibility of Norway rats did not vary with the season nor with environmental temperatures. It was also difficult for surviving rats to develop an aversion or tolerance for NK-15561 after taking sub-lethal doses.

The acceptability of NK-15561 to rats and voles was generally superior to ANTU, thiosemicarbazide, norbormide and scilliroside. The killing effects of NK-15561 were superior to zinc phosphide and thallium sulfate. NK-15561 was also effective as a tracking powder. The concentration in bait of NK-15561 required to kill rodents was estimated about 2%.

Control effects of poisoned bait containing NK-15561 were : 97.5% with 1.0% wet bait against Norway rats; 96.6% with 2.0% dry bait against Japanese field voles by placement in holes; 86.9% with 2.0% dry bait against Japanese field voles using bait-boxes.

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