Toxic Action of Bisthiosemi, Methylene-bis(1-thiosemicarbazide) and its Acceptability in Rats. Tyuzi KUSANO (Dept. of Applied Entomology, Tottori University, Koyama-Cho 1-1, Tottori), Iwao TOKUMITSU, Koji OGUSHI (Research Department, Kondo Chemical Industry Co., Ltd., Tsumashoji 13-35, Hakata-Ku, Fukuoka) and Hiroshi YAMAMOTO (Pesticide Department, Nippon Kayaku Co., Ltd., Marunouchi 1-2-1, Chiyoda-Ku, Tokyo). Received March 4, 1974. *Botyu-Kagaku*, 39, 70, 1974.

14. ビスチオセミ Methylene-bis(1-thiosemicarbazide) の毒作用ならびに摂取性について

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ビスチオセミおよびその類縁体チオセミカルバジドのネズミに対する毒作用を調べ，比較した．

ビスチオセミの摂取性の良好なことは選択試験，受容閾値の測定，スキナー・ボックスを用いた摂取性試験により明らかとなった．

ビスチオセミはネズミに鼓索神経を介して味覚効果を与えなかった．これはビスチオセミの良好な摂取性の一要因となっているものと考えられる．

In a previous paper (Tokumitsu *et al.*, 1973), we reported that a new thiourea derivative, [bisthiosemi or methylene-bis(1-thiosemicarbazide)] had several superior rodenticidal activities and was able to use as a promising rodenticide. In this paper many findings concerning the toxic action of this poison and its acceptability in rats are described.

Materials and Methods

Male adult rats of the Wistar strain, weighing 180-425 g, and Norway rats, *Rattus norvegicus*, which were caught from residential district of Tottori, weighing 70-210 g were used.

The candidate toxic compounds were bisthiosemi, [methylene-bis(1-thiosemicarbazide)] and TSZ (thiosemicarbazide). Also QU (quinine hydrochloride) was employed as a standard bitter substance.

Toxic action of bisthiosemi and TSZ: Several milliliters of bisthiosemi suspension, being suspended in 5% gum arabic solution, and of 1% TSZ of aqueous solution were offered to tested rats with a stomach catheter. After dose, the poisoning symptoms were observed until the occurrence of death. When 30 g of poisoned wet baits were offered to tested rats kept individually in cages, the poisoning symptoms were observed and the intake amount of poisoned bait was determined. The tested rats fasted on tap water for 6 hours before the feeding test. The composition of poisoned bait (biscuit wet bait) was as follows: 7 g of bisthiosemi or TSZ 10% powder (diluted with corn starch), 43 g of 25% biscuit powder (a mixture of wheat flour and biscuit powder at the rate of 75 : 25), 20m*l* of distilled water. These materials were kneaded uniformly by hands and the poisoned wet baits of both 1-1.5 g per bait and triangular form were made up.

Acceptability of bisthiosemi and TSZ: (1) **Preference test**: The preference of Norway rats to both the plain bait and the poisoned bait containing bisthiosemi or TSZ was investigated. The composition, the form and the preparation of this bait were similar to those of biscuit wet bait mentioned above. In the preliminary test for three days, the coloured plain baits and the noncoloured plain baits were offered to Norway rats and the coloured plain baits were placed at a position prefered by Norway rats. The red pigment, amarance was employed as a coloured material, the concentration of amarance in the plain bait was 0.04% and the addition of amarance to the plain bait did not wholly lower the acceptance of the plain bait. After the preliminary choice test, each 30 g of both the plain baits and the coloured and poisoned baits were offered to the tested rats. The residual amounts of these baits were weighed daily and thus the amounts of the baits eaten were determined.

(2) **Acceptable thresholds**: Three kinds of thres-

holds, the detection taste threshold, the recognition threshold for discrimination and the absolute rejection threshold, of either bisthiosemi or TSZ in solid bait were determined by the bait-choice method being similar to a two bottle choice method described in the paper of Kusano *et al.* (1974). These census baits were prepared by a following method. A mixture of wheat flour and distilled water at the rate of 100 g: 45 m*l* was thoroughly kneaded and many baits of slender rod-form (about 6mm diameter and 25cm length) were made up and these were cut in lengths of 2-2.5 cm each. After keeping at the room temperature for one night, these baits were dried at 60°C for 2 hours. Amarance was employed as a colour material and the concentration of amarance in the solid bait was 0.04%. In the preliminary test for four days 30 g of coloured plain bait and that of non-coloured plain bait were daily offered to albino rats and the coloured plain baits were placed at a position prefered by the rats. Afterwards 30g of the poisoned bait and that of the plain bait were daily offered to the albino rats. The daily concentration of the tested poison was increased stepwise in a geometrical progression at 2 of equal ratio until the intake amount of the poisoned bait lowered less 3g.

(3) **Acceptability of bisthiosemi and TSZ:** The acceptability of bisthiosemi and TSZ in albino rats was investigated with the Skinner Box of Osaka University type (Kawamura and Kasahara, 1964). The plain pellet and the poisoned pellet for this apparatus were made up by hands with wheat flour as a base material. Suitable weight of one pellet was about 100mg, the test time was one hour and the fundamental data on this experimental conditions will be published in another paper. Tested albino rats were offered only tap water for 8 hours from 10 a.m. to 6 p.m. and after the experiment of one hour with the Skinner Box the albino rats had fed *ad libitum* commercially available solid baits (MF) until 10 a.m. of next day. After the rate of lever-pressing and the intake amount in the preliminary test from 4 to 5 days with the plain pellet reached approximately a definite level, the experiment with the poisoned pellet was carried out. Bisthiosemi and TSZ were employed as the test poison. The acceptability of the poisoned pellet was evaluated by the lever-pressing reaction, the intake amount of the poisoned pellet and the residual amount of the poisoned one.

(4) **Gustatory nerve response:** The integrated responses of the whole chorda tympani nerve to bisthiosemi, TSZ and QU applied to the tongue surface of albino rats were recorded by a platinum wire electrode (100 μ in diameter). Supernatant layer of 1% hypersaturated solution of bisthiosemi was used as a test solution, and TSZ and QU were used as 0.2% and 1% aqueous solutions, respectively. The exposure of the whole chorda tympani nerve and the recording method of the integrated response were similar to those described in the previous report of Kusano *et al.* (1971).

Results

Comparison of poisoning symptoms between bisthiosemi and TSZ: In the case of bisthiosemi, the initial phase of the poisoning symptoms in albino rats occurred about 85-136 min. after the oral administration of a lethal dose and this was an abnormal posture such as being strained fore legs and gazing on an upward point for a while, or the sudden occurrence of running convulsions without this abnormal behavior. Then the poisoned rats showed repeatedly running convulsive seizure, and followed by raising a creaky voice and secreting saliva. Some rats bit strongly cage bar, so that their lips and tongues bled slightly. Afterwards violent convulsions of several times accompanied by jumping and rolling forth and back in the cage. After the last systemic and tonic convulsions, the death occurred. Such very characteristic poisoning symptoms were quite similar to those of TSZ. The latent time from the dose to the initial symptoms and the survival time in bisthiosemi were slightly longer than those in TSZ. When 1% bisthiosemi bait or 1% TSZ bait was offered to albino rats, the latent time (94 ± 19 min.) and the survival time (206 ± 122 min.) in bisthiosemi bait with five rats were longer than those (the former 81 ± 18 min., the latter 124 ± 32 min.) in TSZ bait with six rats. The intake

amount (2.8±0.7 g) of 1% bisthiosemi bait was slightly larger than that (2.0 ± 0.7 g) of 1% TSZ bait. Since the hydrolysis of bisthiosemi in the presence of acid yielded TSZ[5] and the poisoning symptoms of both bisthiosemi and TSZ were very similar except the latent time and the survival time in the present experiment, the primary action of bisthiosemi may possibly be caused by TSZ, that is produced by the activation in the stomach.

Acceptability of bisthiosemi and TSZ:

(1) **Preference test:** The results are given in Table 1. Norway rats preferred distinctly 1% bisthiosemi bait to plain bait and the acceptance of 1% TSZ bait showed a similar tendency but the intake amount of the former was considerably higher than that of the latter. All the rats in this test were dead.

(2) **Acceptable thresholds in poisoned bait:** In many albino rats the recognition threshold for discrimination in TSZ and bisthiosemi coincided with the detection taste threshold of these poisons, respectively. As shown in Table 2, the mean value of the recognition threshold for discrimination in bisthiosemi was about 3 times higher than that of TSZ. In the bisthiosemi group one albino rat died without discrimination for both the poisoned bait and the plain bait but in the TSZ group such a phenomenon was not observed. The mean value of the absolute rejection threshold in the bisthiosemi group also was about 4 times higher than that of the TSZ group. From these results, bisthiosemi bait is more difficult to be discriminated by a gutatory sense of rats than TSZ bait.

(3) **Acceptance test with the Skinner Box:** In

Table 1. Preference test with 1% TSZ bait and 1% bisthiosemi bait in Norway rats.

Test compound	Sex	Body weight (g)	Preference test with plain bait (g/rat)			Preference test with poisoned bait and plain bait					Mortality
							Intake amount		Dose eaten		
			1st day	2nd day	3rd day		g/rat	g/100g B. W.	mg/rat	mg/100g B. W.	
TSZ	♂	176	W 8.6	9.0	7.9	PB	10.0	5.7	9.0	5.0	Death
			R 6.7	7.2	10.0	TSZ	0.9	0.5			(<24 hrs)
	♀	105	W 3.3	6.7	3.8	PB	0.0	0.0	11.0	11.0	〃
			R 3.5	3.9	7.7	TSZ	1.1	1.1			
	♀	115	W 1.1	5.6	5.0	PB	0.0	0.0	8.0	7.0	〃
			R 0.0	6.1	6.0	TSZ	0.8	0.7			
		Mean	W 4.3	7.1	5.6	PB	3.3	1.9	9.3	7.7	
			R 3.4	5.7	7.9	TSZ	0.9	0.7			3/3
Bisthiosemi	♂	145	W 0.0	5.8	5.1	PB	0.4	0.3	22.0	15.0	Death
			R 0.0	4.0	5.3	Bis	2.2	1.5			(<24 hrs)
	♂	187	W 0.0	8.0	6.3	PB	0.6	0.3	29.0	16.0	〃
			R 0.0	7.8	10.0	Bis	2.9	1.6			
	♀	96	W 0.0	0.0	2.9	PB	0.2	0.2	23.0	24.0	〃
			R 0.0	10.0	8.4	Bis	2.3	2.4			
		Mean	W 0.0	4.6	4.8	PB	0.4	0.3	24.7	18.3	
			R 0.0	7.3	7.9	Bis	2.5	1.8			3/3

R:Coloured bait, W and PB: Non-coloured bait, Bis: Bisthiosemi.

Table 2. Acceptable thresholds of TSZ and bisthiosemi in albino rats.

Test compound	No. of rats	Detection taste threshold (%, Mean±S. D.)	Recognition threshold for discrimination (%, Mean±S. D.)	Absolute rejection threshold (%, Mean±S. D.)
TSZ	5	0.0053±0.0075	0.014±0.017	0.038±0.026
Bisthiosemi	4	0.031±0.037	0.040±0.036	0.141±0.094

Body Weight of Rats: 180-250g.

the TSZ group, the rate of bar-pressing for bait at 0.5 and 1% decreased slightly and at 2% considerably. The left-over pellets increased with the increase of the concentration of TSZ. The intake amount of TSZ pellet at 0.5% was much lower than that of plain pellet and decreased with the increase of the concentration of TSZ. The rate of bar-pressing for water did not show a definite tendency in the TSZ group but the drunk volume of water tended to decrease slightly. In the bisthiosemi groups of 1 and 2%, the rate of bar-pressing for bait reduced to about 80 percent of a normal level and was about as large as that with the TSZ groups. The left-over amount of bisthiosemi pellet was more than that of plain pellet but markedly less than that of the TSZ pellet. The intake amount of bisthiosemi pellet was larger than that of the TSZ pellet. The rate of bar-pressing for water and the intake of water in the case of bisthiosemi pellet increased slightly. These results indicate that the acceptability of bisthiosemi in albino rats is more superior than that of TSZ (Table 3).

(4) **Gustatory nerve response**: When 2m*l* of supernatant layer of hypersaturated solution of bisthiosemi was applied on the tongue surface of the albino rat, no integrated response was recorded from the chorda tympani nerve as shown in Fig. 1. Whereas, in the cases of TSZ and QU definite integrated responses were recorded. The result indicates that bisthiosemi does not exert an unpleasant gustatory effectiveness to the rats. Difference of gustatory effectiveness on gustatory nerve between bisthiosemi and TSZ might be related to that of water solubility. Also the response of off-type of the chorda tympani nerve in TSZ and bisthiosemi was observed, although its physiological significance has not been analyzed as yet.

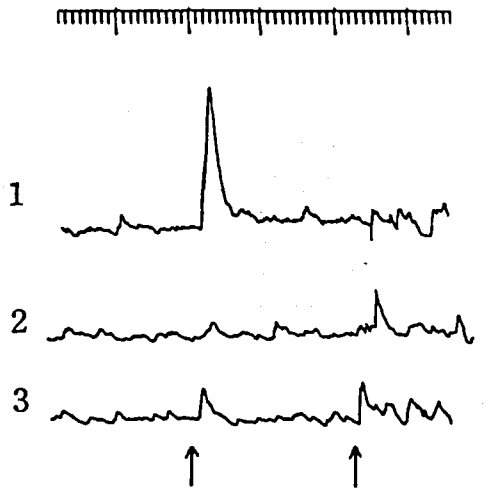

Fig. 1. Integrated responses of the whole chorda tympani nerve to QU, TSZ and bisthiosemi.
1 ; 0.2% QU, 2 ; 1% bisthiosemi, 3 ; 1% TSZ. The first arrow ; application of test solution. The second arrow ; application of running water. Time is in seconds.

Table 3. Acceptance test with the Skinner Box in albino rats.

Test compound	No. of rats	Concentration (%)	No. of bar-pressing for bait**	No. of pellet eaten	Weight of pellet eaten	No. of residual pellet		No. of bar-pressing for water**	Volume of water drunk (m*l*)	
						Plain	Poison		Plain bait	Poisoned bait
TSZ	3	0.5	0.93±0.30*	0.48±0.13*	0.51±0.17*	1.3±0.5	18.7±9.9	0.8±1.6*	2.9±0.2	1.5±0.8
	3	1	0.92±0.38	0.23±0.08	0.25±0.09	3.8±5.1	27.7±18.4	2.7±1.4	3.0±2.0	1.8±0.9
	3	2	0.74±0.16	0.28±0.03	0.30±0.05	0.0	14.3±8.7	0.6±0.3	3.2±1.4	3.0±1.5
Bisthiosemi	3	1	0.85±0.28	0.62±0.18	0.58±0.14	0.8±0.6	3.7±3.1	1.5±1.2	2.3±2.2	4.2±2.0
	2	2	0.78±0.13	0.82±0.10	0.75±0.07	2.3±1.8	3.0±1.0	1.8±0.3	0.3±0.2	1.5±1.3

*: An index to normal value 1. **: Total number; it was not necessarily accompanied by the release of bait or water. All values are expressed as mean±S.D. Body weight: 180-280 g.

Summary

Toxic action, acceptability and qustatory effectiveness of the new rodenticide, bisthiosemi [methylene-bis (1-thiosemicarbazide)] with albino rats and Norway rats were investigated.

(1) The primary action of bisthiosemi may possibly be caused by TSZ being produced by the activation in the stomach.

(2) It became clear through the preference test, the determination of acceptable thresholds and the acceptance test with the Skinner Box that the acceptability of bisthiosemi in rats was better and larger than that of TSZ.

(3) By the application of bisthiosemi hypersaturated solution applied on the tongue surface of the rat, no electrical change except the response of off-type was caused in the chorda tympani nerve. Its cause might be related to the extreme insolubility in water.

(4) The good acceptability of bisthiosemi might be related to both the lack of gustatory effectiveness for the chorda tympani nerve of the rat and slightly slower toxic action than TSZ.

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哺乳動物のフェロモン：雄えだづのかもしかの耳下腺に含まれる活性物質の同定

Mammalian Pheromone: Identification of Active Component in the Subauricular Scent of the Male Pronghorn. Dietland Müller-Schwarze, Christine Müller-Schwarze, Alan G. Singer, Robert M. Silverstein, *Science*, 183, 860～862 (1974).

えだづのかもしかの雄は耳下腺の分泌物をこすり付けて，人間の鼻でもそれと判る匂いを付け，なわ張りのマークとしている．他の雄が付けた匂いを見つけると，あらたにその場所に匂付けを行なう．耳下腺をペンタンで抽出し，揮発性成分をGC分析した結果，8成分が同定された．エステル化後GC-Mass分析により2-methyl butyric acid (1) と isovaleric acid (2) (各々10μg/分泌腺) の存在が，13-methyl-1-tetradecanol (3) と12-methyl-1-tetradecanol (4) (各々30μg/分泌腺) が Mass, IR, NMR 等の結果同定され，残り4成分 (各々70～100μg/分泌腺) はメタノリシス後 (1) (2) のメチルエステルと (3) (4) を与えること，および機器分析の結果から 13-methyl tetradecyl 3-methyl butyrate, 12-methyl tetradecyl 3-methyl butyrate, 13-methyl tetradecyl 2-methyl butyrate, 12-methyl tetradecyl 2-methyl butyrate と同定した．これら8種の化合物およびその混合物の生物試験を行なった結果，isovaleric acid が最も強い活性を示し，その他のエステルやアルコール類は先駆物質，希釈剤あるいは揮散制御剤として働いていると思われる．その他の成分や当該成分の組成化の相異が各個体のマークの識別に役立っていると思われる．

(桑原保正)