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Original Article

Systemic Acaricidal Activity of Chloromethanesulfonamide against Three Species of Phytophagous Mites and Its Toxicity to Rats, Mice and Carp*

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Chloromethanesulfonamide (CMSA) is a systemic acaricidal compound which has stronger activity than dimethoate and disulfoton against the citrus red mite, *Panonychus citri*, the two-spotted spider mite, *Tetranychus urticae* and the carmine mite, *Tetranychus cinnabarinus*. LC_{50} values of chloromethanesulfonamide against the larvae, protonymphs, deutonymphs and female adults of *T. cinnabarinus* were 74.4, 148.6, 183.1 and 286.6 ppm respectively. CMSA showed a systemic acaricidal activity against *P. citri* when painted on the trunk and leaf surface of a mandarin orange tree or when applied to the soil surface near the tree. Its toxicity to mammals and carp was low.

INTRODUCTION

Seki[1] reported that the mite, *Panonychus citri* was resistant to organophosphorus compounds in Japan in 1958. Since the introduction of dicofol in 1962, the acaricide dicofol had been frequently applied in orchards and greenhouses in Japan and as a consequence spider mites had developed a resistance by 1968.[2] Their resistance to acaricides presented a serious problem, which researchers have been trying to solve ever since. Matsui *et al.*[3] reported that some chloromethanesulfonamide derivatives had a mothproofing action and that *N*-2, 4, 5-trichlorocyclohexyl-chloromethanesulfonamide was most effective among them. Our screening tests with sulfonamide derivates showed that chloromethanesulfonamide (CMSA) was most effective against phytophagous mites, such as the citrus red mite, *P. citri*, the carmine mite, *T. cinnabarinus* and the two-spotted spider mite, *T. urticae*. We report here on the acaricidal activity and systemic action of CMSA against the mites.

MATERIALS AND METHODS

1. *Animals*

Mites: Citrus red mites, *Panonychus citri* McGREGOR, were collected from mandarin orange trees at Mikkabi-cho, Shizuoka Prefecture and reared on Satsuma orange trees in the orchard at the Kumiai Chemical Life Science Institute, Kikugawa-cho, Shizuoka Prefecture. Carmine mites, *Tetranychus cinnabarinus* BOISDUVAL and two-spotted spider mites, *Tetranychus urticae* KOCH, were collected from bean plants in Shimizu City, Shizuoka Prefecture and from a rose field in Kikugawa-cho respectively, and successively reared on kidney bean seedlings in the greenhouse of our Institute.

Mice and Rats: Male and female mice of the CRJ-JCR strain (Japan Charles River Co., Ltd.) and male and female rats of the CRJ-SD

* Acaricidal Activity of Chloromethanesulfonamide against Phytophagous Mites (Part 1).

strain (Japan Charles River Co., Ltd.) were purchased at 6 weeks of age. After acclimatization to the test conditions for 1 week, they were divided into groups of 10 mice/rats each. At the start of the study, male mice weighed 31–42 g and female mice 23–30 g, and male rats weighed 257–306 g and female rats 162–200 g. Ten mice per cage and two rats per cage were housed in plastic cages (Econ B-type, Japan Claire Co., Ltd.) spread with wooden flakes. The animals were reared in an air-conditioned room (temperature: 22±2°C, humidity: 55±5%) and given a solid diet (Oriental Yeast Industry Co., Ltd.) and water *ad libitum*. Food was withheld from the animals for 16 hr before the application of CMSA.

Carp: Carp 5 cm in average body length were acclimatized for a week and bred in a water-filled container (water temperature: 25±0.5°C, pH: 7.2±0.2, oxygen level in water: 4.2±2.8 mg/*l*).

2. Acaricides and Insecticides

Chloromethanesulfonamide (CMSA) of 96% technical grade was synthesized and formulated as 80% W.P. at the Chemical Research Institute, Kumiai Chemical Industry Co., Ltd. Dicofol (40% E.C.) was purchased from Nihon Nohyaku Co., Ltd. Dimethoate (43% E.C.) and disulfoton (5% granule) were formulated at our company. Aldicarb (10% granule) was supplied by Union Carbide Co., Ltd.

3. The Synthetic Process of CMSA

3.1 The reaction formula

$$(CH_2S)_3 + 9\,Cl_2 + 6\,H_2O \longrightarrow 3\,ClCH_2SO_2Cl + 12\,HCl$$

$$ClCH_2SO_2Cl + 2\,NH_3 \longrightarrow ClCH_2SO_2NH_2 + NH_4Cl$$

3.2 The procedure

Into a mixture of 110 g (0.8 mol) of trithian and 275 g (0.33 mol) of concentrated hydrochloric acid, 568 g (8.1 mol) of chlorine was passed at 15–20°C. After the reaction mixture was allowed to stand for 30 min, the organic layer was concentrated under reduced pressure at 70°C to give 222 g (62.2%) of chloromethanesulfonyl chloride (CMSC). Into a solution of 25 g (0.17 mol) of CMSC in 100 ml of acetonitrile ammonia was passed with stirring until the reaction was completed while monitored by a gas liquid chromatograph. Then the precipitate was filtered off and washed with hexane to give 24.1 g (97.0%) of CMSA in white needles.

4. Measurement of Chemical and Physical Properties of CMSA

Melting point: Followed the EPA guideline[4]

Boiling point: Followed the OECD guideline[5]

Solubility: Followed the EPA guideline[6]

Stability to light: Conformed to Residure Reviews[7]

pH: Followed the method of T. Hata[8]

5. Acute Toxicity to Mammals

Oral administration: CMSA was given by means of gavage of 25% suspension in 0.25% carboxymethyl cellulose (CMC) aqueous solution to rats at 4 ml/100 g body weight and to mice at 0.4 ml/100 g body weight.

Percutaneous administration: CMSA prepared in the form of 33% suspension in 0.25% CMC aqueous solution was applied to the dorsal region of a mouse from which fur had been clipped (1.5×2 cm²). The applied dose was 0.15 ml/10 g body weight. After the application the site was kept covered with an adhesive plaster for 24 hr to prevent the test compound from being removed.

Subcutaneous administration: CMSA prepared in the form 25% suspension in sterilized 0.25% CMC aqueous solution was subcutaneously injected to mice at 0.1–0.2 ml/100 g body weight.

Intraperitoneal administration: CMSA prepared in the form of 25% suspension in sterilized 0.25% CMS aqueous solution was intraperitoneally injected to mice at 0.1–0.2 ml/10 g body weight. The mortality was investigated 5 days after treatment and the LD_{50} was calculated on the basis of the mortalities.

6. Fish Toxicity

A fixed dose of 1, 5, 10, 20 or 50 g of CMSA was added to 10 *l* of water in a container. In each container five carp weighing 2 g were reared and their survival rates were examined after 48 hr, from which the TLm values were calculated.

7. Toxicity against Adult Females of Three Species of Phytophagous Mites

In separate experiments, (a) adult female citrus red mites were transferred onto the upper surface of a mandarin orange leaf, (b) adult female carmine mites were transferred onto the upper surface of a kidney bean leaf which had been cut into a disk 1.5 cm in diameter, and (c) adult female two-spotted spider mites were transferred onto the upper surface of a kidney bean leaf disk 1.5 cm in diameter. These leaves or leaf disks were placed on a 0.4% agar in a petri dish 9 cm in diameter.[9]

Each test solution (3 ml) was sprayed on the mites with a rotary-type spray apparatus (Mizuho Scientific Co., Ltd.). The treated mites were kept at 25°C and their mortality was recorded 48 hr after treatment. About 100 mites were tested at each dose level and their mortalities were corrected by Abott's formula.[10] The LD_{50} values were calculated according to Bliss's method.[11] Tests were replicated three times.

8. Toxicity against Various Stages of Carmine Mites

Eggs and larvae were obtained by confining 12–13 adult females to a kidney bean leaf placed on a 0.4% agar in a 9-cm-diameter petri dish for about 12 hr. Then the adults were removed and the eggs were kept at 25°C for several days until larvae, protonymphs or deutonymphs emerged. All unhatched eggs and overstage mites were removed just prior to spraying,[12] and mites of those stages were treated by the method previously described. Assays were repeated three times. Ten mites were placed on a leaf disk on average and their mortality was recorded 48 hr after treatment. Abott's formula was used to correct the mortality percentages. The LC_{50} values were calculated following Bliss's method.

9. Systemic Acaricidal Action by Different Methods

9.1 Systemic action by soil treatment

Effect on citrus red mite: Fixed doses of CMSA (80% W.P.) were soaked into the soil surface around a 2-year-old natsukan orange tree in a 10-cm-diameter pot which was infested with citrus red mites. The test was repeated on two other such trees in the greenhouse. The number of adult female citrus red mites was counted 3 hr before treatment and 7, 14, 22 and 33 days after treatment.

Effect on carmine mites: The soil surface of a 15-cm-diameter pot which had an eggplant infested with carmine mites was soaked with fixed doses of CMSA. The test was repeated twice in the greenhouse. The number of adult female carmine mites was counted 3 hr before treatment and 7, 12, 23 and 32 days after treatment.

Effect on two-spotted spider mites: One kilogram a.i. of CMSA (80% W.P.) per 10 a was soaked into the soil surface of a nursery bed (1 m × 1 m) where chrysanthemum grew in the greenhouse. Four plants were sampled in each plot and the number of adult female mites infesting the plants were counted 3 hr before treatment and 9, 17, 24 and 39 days after treatment.

9.2 Systemic action by trunk treatment

Seventy-one milligrams a.i. of CMSA diluted with 10 ml water was painted on the main trunk, half the perimeter of the main trunk or around a part of a subsidiary trunk of 3-year-old mandarin orange trees planted in a 30-cm-diameter pot and infested with citrus red mites. The test was repeated twice in the greenhouse. The number of adult females was counted 3 hr before treatment and 7, 10, 20 and 30 days after treatment. The control efficacy of the compounds was calculated by Okudai's method[13] using the following formula:

$$\text{Control effect} = \left(1 - C_b \sum_{i=1}^{n} T_{ai} \Big/ T_b \sum_{i=1}^{n} C_{ai}\right) \times 100$$

n = Frequency of inquiry after treatment. C_b = Number of nontreated mites before treatment. C_{ai} = Number of nontreated mites of at the i-th inquiry after treatment. T_b = Number of mites from a treated plot before treatment. T_{ai} = Number of mites from a treated plot at the i-th inquiry after treatment.

9.3 Systemic action by leaf treatment

0.5, 1.0, 2.5 or 5.0 mg a.i. of CMSA diluted with 0.5 ml water was painted with a small brush on the leaves (upper or under surface) of satsuma orange seedlings planted in a 10-cm-diameter pot. To prevent mite invasion,

Tanglefoot was painted around the leaves. Twenty female adults per leaf were transferred onto the surface of the opposite side of the painted leaves and their mortality was examined 48 hr after treatment. Mites were transferred to the treated leaves 1, 6, 12 and 24 days after the application of chemicals to the leaves in the greenhouse. The experiment was repeated twice. The average treated area on a leaf was 22.7 cm^2 and the average temperature during the test period was 26°C. Control efficacy was calculated by Okudai's method.

RESULTS

The properties of CMSA are shown in Table 1. CMSA is stable to light, heat (80°C), acids and neutral conditions, but unstable to alkalis. The toxicity of CMSA to mammals and fish is shown in Table 2. CMSA is low in toxicity to mice, rats and carp.

Table 3 presents the LC_{50} values of CMSA and dicofol against adult female citrus red mites, carmine mites and two-spotted spider mites. The LC_{50} values of CMSA were 222.9, 286.6 and 258.9 ppm respectively, and those of dicofol 46.8, 37.1 and 984.9 ppm, respectively. Table 4 shows the LC_{50} values of CMSA against each developmental stage of carmine mites. The LC_{50} values to larvae, protonymphs, deutonymphs and female adults were 74.4, 148.6, 183.1 and 286.6 ppm respectively.

Table 5 shows the systemic action of CMSA by soil treatment against citrus red mites on a mandarin orange tree, Table 6 against carmine mites and Table 7 against two-spotted spider mites.

CMSA 250 g a.i./10 a was more effective on citrus red mites than dimethoate 2000 g a.i./10 a and CMSA 100 g a.i./10 a was more effective on carmine mites than disulfoton 1500 g a.i./

Table 1 Chemical and physical properties of chloromethanesulfonamide.

Melting point	72°C
Boiling point	140–145°C/1–3 mmHg
Solubility	Soluble in water (400 g per 1000 ml at 20°C)
	Soluble in acetone (1010 g per 1000 ml at 25°C)
	Soluble in methanol (1450 g per 1000 ml at 25°C)
	Scarcely soluble in xylene (5 g per 1000 ml at 25°C)
Stability	Stable to light (4.5% decomposition rate after 1 day after under UV-light)
	Stable to heat (0.2% decomposition rate after 60 days at 40°C)
	Stable in acid and neutral medium and unstable in alkali medium
	Decomposition rate; 0.1% in 0.1 N HCl after 7 days at a room temperature
	0% at pH 7.0 after 7 days at a room temperature
	100% in 0.1 N NaOH after 7 days at a room temperature
pH	purified (10% solution) 4.0

Table 2 Toxicity of chloromethanesulfonamide to mammals and fish.

Test method	Animal		LD_{50} & TLm_{48}
Acute oral: LD_{50}	Mouse	(male)	6300 mg/kg
	Mouse	(female)	6700 mg/kg
	Rat	(male)	6050 mg/kg
	Rat	(female)	6600 mg/kg
Dermal: LD_{50}	Mouse	(male)	5000 mg/kg
	Mouse	(female)	5000 mg/kg
Subcutaneous: LD_{50}	Mouse	(male)	2660 mg/kg
	Mouse	(female)	2960 mg/kg
Intraperitoneal: LD_{50}	Mouse	(male)	2710 mg/kg
	Mouse	(female)	2690 mg/kg
Fish toxicity: TLm_{48}	Carp		1260 ppm

Table 3 LC_{50} values of chloromethanesulfonamide against female adults of citrus red mites, carmine mites and two-spotted spider mites.

Chemical	Species of mites	Regression line	LC_{50} (ppm)	(95% confidence limit)	n	χ^2	Pr
Chloromethanesulfonamide	Citrus red mite	$Y=5+2.083\ (X-2.348)$	222.9	(177.6–279.8)	3	0.921	$0.8<p<0.9$
	Carmine mite	$Y=5+2.274\ (X-2.458)$	286.6	(226.7–356.7)	1	0.370	$0.5<p<0.6$
	Two-spotted spider mite	$Y=5+1.288\ (X-2.413)$	258.9	(186.2–356.6)	1	0.374	$0.5<p<0.6$
Dicofol	Citrus red mite	$Y=5+3.278\ (X-1.669)$	46.8	(38.8–58.6)	2	0.045	$0.97<p<0.99$
	Carmine mite	$Y=5+5.395\ (X-1.590)$	37.1	(33.9–41.1)	2	0.973	$0.6<p<0.7$
	Two-spotted spider mite	$Y=5+2.127\ (X-2.993)$	984.9	(815.0–1198.9)	2	5.783	$0.05<p<0.1$

Table 4 LC_{50} values of chloromethanesulfonamide against different developmental stages of carmine mites.

Developmental stage	Regression line	LC_{50} (ppm)	(95% confidence limit)	n	χ^2	Pr
Larvae	$Y=5+1.903\ (X-1.878)$	74.4	(60.1–93.5)	3	0.017	$0.99<p$
Protonymphs	$Y=5+1.610\ (X-2.212)$	148.6	(111.4–220.5)	2	3.453	$0.1<p<0.2$
Deutonymphs	$Y=5+2.014\ (X-2.702)$	183.1	(146.6–226.7)	2	6.793	$0.02<p<0.05$
Female adults	$Y=5+2.274\ (X-4.789)$	286.6	(226.7–356.1)	1	0.374	$0.5<p<0.6$

Table 5 Control efficacy of chloromethanesulfonamide by soil treatment against citrus red mites infesting on natsukan orange trees.

Chemical	Dosage (a.i. g/10a)	Number of mites per pot					Control efficacy
		3 hr before treatment	Days after treatment				
			7	14	22	33	
Chloromethanesulfonamide	1000	77	0	1	4	0	99
	500	48	6	2	3	2	97
	250	105	16	9	14	3	95
Dimethoate	2000	27	8	28	16	35	58
No treatment	—	35	68	23	80	90	

10 a and dimethoate 1000 g a.i./10 a, but less effective than aldicarb 500 g a.i./10 a. In the case of two-spotted spider mites, CMSA 100 g a.i./10 a was more effective than aldicarb 1000 g a.i./10 a and dimethoate 2000 g a.i./10 a.

Table 8 shows the systemic action of CMSA on citrus red mites by trunk treatment. When painted around the main trunk, CMSA's control efficacy was almost equal to that by soil treatment at the same dose level. When painted around half the perimeter of the main trunk, its control efficacy was equal to that by soil treatment at 1000 g a.i./10 a. When painted around a part of the subsidiary trunk, its control efficacy was limited only in an upper part of the branches above the treated region.

Figures 1 and 2 show the systemic action of CMSA against citrus red mites on the leaf surface of a mandarin orange tree. CMSA's acaricidal activity was higher when painted on the under surface of a leaf than when painted on the upper surface.

Table 6 Control efficacy of chloromethanesulfonamide by soil treatment against carmine mites infesting on eggplant plants.

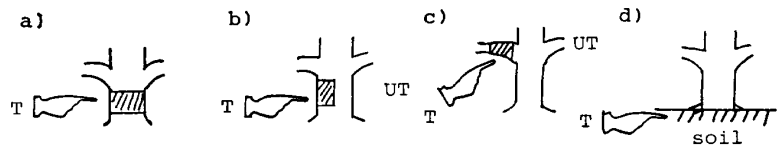
Chemical	Dosage (a.i. g/10 a)	Number of mites per pot					Control efficacy
		3 hr before treatment	Days after treatment				
			7	12	23	32	
Chloromethane-sulfonamide	4000	40	6	1	2	2	94
	2000	42	15	3	2	5	87
	1000	45	23	8	3	3	81
Disulfoton	1500	34	26	5	23	20	50
	1000	64	44	14	38	44	50
Aldicarb	1000	38	1	0	0	0	99
	500	34	6	2	0	0	95
Dimethoate	1000	42	15	15	18	18	64
	500	45	33	13	13	17	61
No treatment	—	54	71	54	67	44	

Table 7 Control efficacy of chloromethanesulfonamide by soil treatment against two-spotted spider mites infesting on chrysanthemum plants.

Chemical	Dosage (a.i. g/10 a)	Number of mites per pot					Control efficacy
		3 hr before treatment	Days after treatment				
			9	17	24	39	
Chloromethane-sulfonamide	1000	49	8	6	1	0	96
Aldicarb	1000	55	5	16	8	3	93
Dimethoate	2000	58	68	31	75	57	53
No treatment	—	42	65	47	76	169	

Table 8 Control efficacy of chloromethanesulfonamide against citrus red mites by trunk treatment.

Painting position	Dosage (a.i. mg/tree)	Number of mites per pot					Control efficacy
		3 hr before treatment	Days after treatment				
			7	10	20	30	
Whole part of main trunk[a)]	71	152	1	4	5	5	98
Half part of main trunk[b)]	71 T[e)]	68	1	3	0	29	90
	UT[f)]	84	2	5	4	30	90
Part of subsidiary trunk[c)]	71 T	111	2	4	3	19	95
	UT	110	35	50	8	25	78
Soil treatment[d)]	71	118	8	1	1	5	97
No treatment	—	103	181	228	17	74	



e) Treated site
f) Untreated site

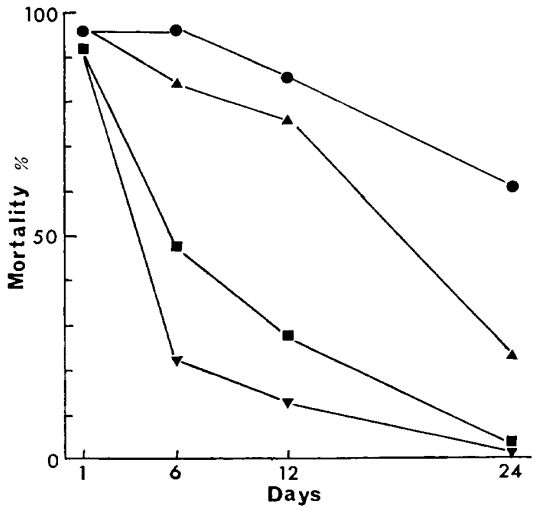


Fig. 1 Systemic acaricidal activity of chloromethanesulfonamide when painted on the upper surface of a mandarin orange leaf.

●: 5 mg, ▲: 2.5 mg, ■: 1.0 mg, ▼: 0.5 mg.

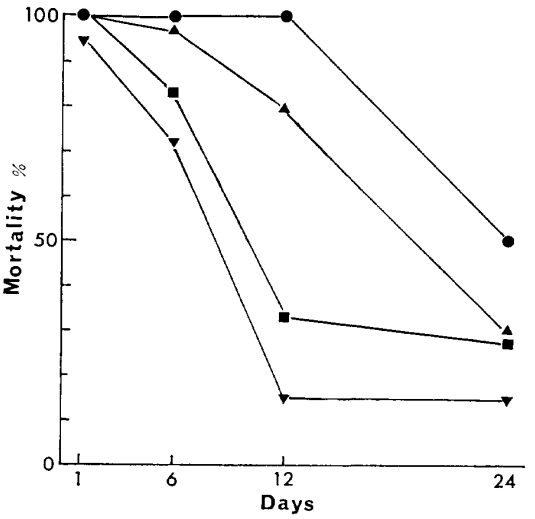


Fig. 2 Systemic acaricidal activity of chloromethanesulfonamide when painted on the under surface of a mandarin orange leaf.

Refer symbols in Fig. 1.

DISCUSSION

Ishii[14] mentioned that most of systemic insecticides, such as systox, disulfoton, phosdrin and dimethoate, have an acaricidal activity against adults, nymphs and larvae, except for eggs, of phytophagous mites belonging to Tetranychidae.

The present experiments have proved CMSA has an acaricidal activity against citrus red mites, carmine mites and two-spotted spider mites. CMSA was acaricidally active against the female adults, protonymphs, deutonymphs and larvae but not against the eggs of carmine mites.

The solubility of CMSA in water was, therefore, assumed to be a factor to determine a systemic acaricidal action as was in the case with organophosphorus insecticides, such as systox, disulfoton and dimethoate. Fukuda *et al.*[15] confirmed the systox's systemic acaricidal effect against citrus red mites when applied to the soil surface of a mandarin orange tree pot. Ripper[16] confirmed that the treatment with dimefox on the bark of coffee trees against *Planococcus kenyae* (Le Pelly) needs only half the volume necessary for soil treatment to obtain the same effect. Fukuda *et al.* confirmed fluoroethyl fluoroacetate's systemic insecticidal effect against arrowhead scales, *Unaspis yanonensis* KUWANA, and its systemic insecticidal effect against arrowhead scales only above the treated spot when applied to the trunk. Similarly, CMSA showed a systemic acaricidal activity against citrus red mites on a mandarin orange tree when applied to the soil surface, or painted around the main trunk, branches or on the leaf surface. CMSA thus appear to show a systemic acaricidal activity against mites when applied to any part of plant.

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要　　約

クロロメタンスルホンアミドの3種のハダニに対する浸透殺ダニ活性ならびにラット，マウス，コイに対する毒性*

小川温樹，嶋津朋徳，西村立己

クロロメタンスルホンアミドはミカンハダニ，ニセナミハダニおよびナミハダニに対し殺ダニ活性を示した．ニセナミハダニの卵を除く，幼虫，第一若虫，第二若虫，雌成虫に殺虫力を示し，LC_{50} 値はそれぞれ，74.4，148.6，183.1，286.6 ppm であった．またミカンハダニ，ニセナミハダニ，ナミハダニに対し，土壌灌注処理において，ジメトエートやダイスルフォトンよりすぐれた浸透殺ダニ力を示した．ミカンハダニに対する柑橘樹の樹冠下への土壌処理，樹幹塗布，葉面塗布処理において，浸透殺ダニ作用を示した．これらの結果よりクロロメタンスルホンアミドはジメトエートやダイスルフォトンのような浸透殺虫剤より高い浸透殺ダニ力をもつと考えられた．

* クロロメタンスルホンアミドのハダニ類に対する活性（第1報）