centration of 40 ppm in the soil, five last stage housefly larvae were placed on top of the soil immediately after sample treatment. The mortality after 48 h was recorded. The treatment was then repeated with five additional larvae and freshly treated soil. Similarly, with soil concentrations of 6 and 2 ppm, the last stage larvae of *Diabrotica u. undecimpuntata* were tested.

Residual Spray on Cardboard for Argentine Ant. One-pint cardboard cartons were sprayed with formulations at the rate of 10 mg/ft^2. Sixteen hours after spraying ants were collected and placed in the treated cartons which were capped with fine nylon netting. Mortality counts were taken after 15 min, 1 h, and 3 h of exposure. Each test consisted of three replicates of 30 ants each.

Leaf Feed for Potato Beetle Larvae and Southern Army Worm. Eggplant leaves dipped into test solutions of the respective compounds were offered to the potato beetle larvae for a 24-h feeding period, after which mortality data were recorded. Each test consisted of three replicates of five larvae each. Lima bean leaves dipped into test solutions of the respective compounds were offered to ten larvae of the Southern army worm (late third instar), for a 48-h feeding period. Mortality data were recorded.

Foliage Spray for Alfalfa Weevil. Potted alfalfa plants were sprayed with the test solutions at 2000 ppm. The plants were then infected with alfalfa weevil adults. Mortality counts were taken at 1, 3, and 7 days post spray.

Contact Test for Body Louse. Compounds were screened as body louse (*Pediculus humanus humanus L.*) toxicants by exposing young adult body lice on treated patches of woolen cloth, 3.8 cm in diameter. Duplicate patches were impaled on pinboards, and 0.7 ml of 1% solutions of the compounds in acetone was applied to them by pipet. After the patches were dried, 10 female lice were exposed to them in 50-ml glass beakers for 24 h. Knockdown was recorded at intervals of 15 min, 1 h, and 3 h, and kill at 24 h. Patches on which all lice were dead or knocked down were retested at intervals of 2 to 7 days until one or more lice remained unaffected. After 31 days the tests were terminated, even if the patches were still effective.

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Formamidine Acaricides. Toxicity and Metabolism Studies with Twospotted Spider Mites, *Tetranychus urticae* Koch

Kuo-Mei Chang and Charles O. Knowles*

The toxicity to adult twospotted spider mites of 104 aryl formamidines was studied using a slide-dip technique. For maximum toxicity formamidines must possess an aryl moiety with two substituents, and these two substituents should be located at ring positions two and four. With regard to requirements on the amino nitrogen, it was concluded that at least one substituent should be lower alkyl, preferably methyl, while greater flexibility existed relative to the other amino nitrogen substituent. It could be hydrogen, lower alkyl, methylthiomethyl, or other more complex moieties. Metabolism studies of two aryl formamidines, chlordimeform-^{14}C and *N′*-(4-chloro-*o*-tolyl)-*N*-methylformamidine or demethylchlordimeform-^{14}C, in twospotted spider mites indicated a rapid uptake of each compound accompanied by high internal levels of organosoluble radioactive material. Chlordimeform metabolites included demethylchlordimeform, *N′*-(4-chloro-*o*-tolyl)formamidine, 4′-chloro-*o*-formotoluidide, and 4-chloro-*o*-toluidine. Both chlordimeform and demethylchlordimeform were metabolized slowly by twospotted spider mites as compared with houseflies, a chlordimeform-tolerant insect. Differential metabolism likely plays a role in the selective toxicity of chlordimeform.

There are numerous problems associated with acarine (mites and ticks) control. For example, phytophagous mites reproduce rapidly and in great numbers. They are regarded as genetically plastic organisms, and new races are continually formed. Therefore, strains resistant to conventional acaricides can develop with such rapidity as to threaten the efficacy of an entire class of chemicals. Thus, there is a continuing search for new classes of acaricides.

The formamidines are a class of compounds active as acaricides and insecticides which differ in their mode of action from the chlorinated hydrocarbons, organophosphates, and carbamates (Dittrich, 1966). Moreover, some formamidines are more toxic to organophosphate-

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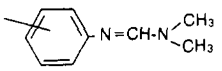
Table I. Toxicity of *N'*-Aryl-*N*,*N*-dimethylformamidines with One Ring Substituent to Twospotted Spider Mites



Compd no.	R	Bp or mp[a]	Mortality, %[b]		LC_{50}, ppm[c]
			48 h	72 h	
1	H	63-66 (0.06)[d]	0.0 (0.0)	1.5 (2.5)	>500
2	F-2	60-62 (0.06)	0.0 (0.0)	18.4 (4.3)	>500
3	F-3	64-70 (0.09-0.15)	4.8 (1.4)	5.4 (2.5)	>500
4	F-4	67-70 (0.06)	5.0 (3.0)	11.3 (4.8)	>500
5	Cl-2	79-90 (0.06-0.07)[e]	5.0 (3.1)	14.8 (0.7)	>500
6	Cl-3	83-89 (0.06)[f]	9.3 (3.1)	16.3 (2.4)	>500
7	Cl-4	82-88 (0.08)[g]	16.8 (4.7)	78.1 (4.9)	<500
8	Br-2	94-95 (0.12)	6.2 (2.4)	31.9 (5.5)	>500
9	Br-4	105-110 (0.25)[h]	5.0 (1.2)	25.0 (7.8)	>500
10	I-2	98-101 (0.05)	4.0 (3.1)	4.2 (4.2)	>500
11	I-3	111-113 (0.11)	0.8 (0.7)	10.0 (4.6)	>500
12	I-4	66-67.5	1.5 (0.9)	18.6 (3.7)	>500
13	CH_3-2		2.0 (2.0)	3.1 (1.8)	>500
14	CH_3-4	69-70 (0.06)	0.8 (0.7)	1.7 (1.7)	>500
15	C_2H_5-2	70-72 (0.15)	5.0 (4.5)	30.6 (9.6)	>500
16	C_2H_5-4	79-88 (0.10-0.12)	1.3 (1.6)	7.5 (2.5)	>500
17	*n*-C_4H_9-4	95-100 (0.06)[i]	7.0 (4.0)	21.1 (4.6)	>500
18	CH_3O-2	85-91 (0.09)	10.5 (4.4)	22.5 (4.3)	>500
19	CH_3O-3	94-98 (0.09-0.11)	6.0 (4.1)	12.5 (2.5)	>500
20	CH_3O-4	92-94 (0.10)[j]	6.8 (1.6)	18.8 (4.7)	>500
21	NO_2-2	123-127 (0.15)[k]	2.8 (1.9)	13.2 (1.2)	>500
22	NO_2-3	51-53[l]	3.0 (0.0)	8.8 (3.7)	>500
23	NO_2-4	79.5-80[m]	1.5 (0.6)	6.3 (2.4)	>500
24	CF_3-2	62-70 (0.06)	5.0 (2.9)	9.5 (4.1)	>500
25	CF_3-3	62-67 (0.06)[n]	5.0 (0.0)	5.4 (3.2)	>500

[a] Boiling point expressed as °C per millimeter of mercury. Melting point expressed as °C. A Fisher-Johns apparatus was used, and melting points are uncorrected. [b] Toxicity determined by slide-dip technique; concentration of test compounds was 500 ppm. Figures are the mean of at least three tests with the standard deviation given in parentheses. [c] LC_{50} values determined at 72 h. [d] Literature values: 140 °C (18), Counselman (1970) and Duerr et al. (1966); 75-76 °C (0.02), Steiger (1963). [e] Literature values: 120-122 °C (0.02), Counselman (1970) and Duerr et al. (1966); 99-102 °C (0.25), Gerjovich (1965). [f] Literature values: 124 °C (0.05), Duerr et al. (1966); 102-103 °C (0.22), Gerjovich (1965). [g] Literature value: 101-103 °C (0.06), Counselman (1970) and Duerr et al. (1966). [h] Literature value: 173 °C (14), Duerr et al. (1966). [i] Literature value: 106 °C (0.08), Counselman (1970) and Duerr et al. (1966). [j] Literature value: 98 °C (0.03), Duerr et al. (1966). [k] Literature value: 152-154 °C (1.0), Gerjovich (1965). [l] Literature value: 58.5-59.5 °C, Gerjovich (1965). [m] Literature value: 79-80 °C, Steiger (1963). [n] Literature value: 93-95 °C (0.01), Duerr et al. (1966).

resistant mites than to organophosphate-susceptible mites (Dittrich, 1969). Formamidines are extremely useful in mite control programs; therefore, toxicological studies of these compounds are essential.

The purpose of this investigation was to conduct a structure-activity relationship study of formamidines against a common pest mite, the twospotted spider mite, *Tetranychus urticae* Koch. A precise bioassay technique was used to study the toxicity to spider mites of 104 formamidines. Special emphasis was given to the *N'*-aryl-*N*,*N*-dimethylformamidines of which chlordimeform, the first formamidine to be used commercially as an acaricide and insecticide, is a member. A study of the metabolic fate of radiolabeled chlordimeform and of a toxic metabolite *N'*-(4-chloro-*o*-tolyl)-*N*-methylformamidine or demethylchlordimeform in twospotted spider mites also was undertaken.

MATERIALS AND METHODS

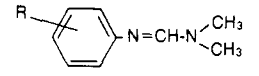
Experimental Organisms. Twospotted spider mites, *Tetranychus urticae* Koch, were reared in the laboratory on lima bean plants (Henderson's Bush variety). Infested seedlings were kept in a plant growth chamber which was operated at 27 °C under continuous light.

Formamidines and Related Compounds. Formamidines used in these toxicological studies are given in Tables I to VI. Compounds 1 to 12, 14 to 51, 53, and 55 to 82 were prepared by reacting the appropriate substituted aniline (Aldrich Chemical Co., Inc., Milwaukee, Wis.) with dimethylformamide (Fisher Scientific Co., St. Louis, Mo.) in the presence of phosphorous oxychloride (Fisher Scientific Company) (Arndt and Steinhausen, 1968; Bredereck et al., 1959). Compounds **83**, **85**, and **86** were similarly prepared using the appropriate substituted aniline and dialkylformamide. All formamidines were purified by short-path distillation or recrystallization. A check of the purity of each compound was made by TLC. When chromatographed on silica gel GF_{254} with a developing solvent mixture of benzene-diethylamine (95:5), many of the compounds gave only one spot indicative of a high degree of purity. Other synthesized compounds contained minor impurities, such as the substituted formanilide and/or the starting substituted aniline.

Compounds **13** (C-14640), **54** (C-10405), **84** (C-8519), **88** (C-22840), **89** (C-22511), **90** (C-8515), **91** (C-8518), **92** (C-8521), **93** (C-9496), **95** (C-9980), **96** (C-22510), **97** (C-22547), **98** (C-22599), and **99** (C-22655) were obtained from CIBA Agrochemical Co., Vero Beach, Fla. Compounds **52** (chlordimeform) and **87** (demethylchlordimeform) were obtained from NOR-AM Agricultural Products, Inc., Woodstock, Ill. Compounds **94** (BTS-27271) and **103** (BTS-23376) were obtained from Boots Pure Drug Co. Ltd., Nottingham, England. Compound **100** (H-20013) was provided by Hokko Industry Chemical Co. Ltd., Tokyo, Japan. Compounds **101** (U-42558), **102** (U-42564), and **104** (amitraz) were obtained from The Upjohn Co., Kalamazoo, Mich.

For metabolism studies, chlordimeform-^{14}C, radiolabeled

Table II. Toxicity of *N*′-Aryl-*N*,*N*-dimethylformamidines with Two Ring Substituents to Twospotted Spider Mites



Compd no.	R	Bp or mp[a]	Mortality, %[b] 48 h	Mortality, %[b] 72 h	LC$_{50}$, ppm[c]
26	F-2,4	62-63 (0.14)	15.4 (5.7)	82.1 (7.9)	235 (.85)
27	Cl-2,3	115-119 (0.15)	4.3 (2.7)	20.0 (4.6)	>500
28	Cl-2,4	120-125 (0.2)	95.8 (2.3)	100.0 (0.0)	14 (0.23)
29	Cl-2,5	104-112 (0.23-0.25)	8.5 (3.1)	10.8 (7.2)	>500
30	Cl-2,6	89-91 (0.1)	8.0 (3.5)	19.1 (8.5)	>500
31	Cl-3,4	108-128 (0.15)	15.5 (5.8)	27.5 (1.4)	>500
32	Cl-3,5	97-100 (0.05)[d]	6.8 (2.7)	23.5 (5.0)	>500
33	Br-2,4	117-125 (0.06)	5.5 (4.6)	54.3 (12.9)	<500
34	Br-2,5	134-137 (0.25)	10.5 (5.6)	25.0 (2.1)	>500
35	Br-2,6	105-107 (0.1)	1.5 (0.9)	16.2 (7.7)	>500
36	CH_3-2,3	77-78 (0.11)	18.5 (4.5)	21.6 (9.6)	>500
37	CH_3-2,4	73-74 (0.14)	70.5 (6.1)	96.0 (1.5)	110 (0.77)
38	CH_3-2,5	76 (0.15)	0.8 (0.7)	28.6 (3.2)	>500
39	CH_3-2,6	67-70 (0.06-0.1)	21.8 (4.6)	62.5 (8.5)	<500
40	CH_3-3,4	84-85 (0.15)[e]	5.3 (2.2)	17.5 (6.3)	>500
41	CH_3-3,5	84-86 (0.15)	3.5 (1.7)	7.4 (3.7)	>500
42	CH_3O-2,4	110-117 (0.06)	0.1 (0.6)	15.0 (3.2)	>500
43	CH_3O-2,5	117-120 (0.06)	7.3 (3.4)	16.7 (7.4)	>500
44	CH_3O-3,4	127-144 (0.06)	2.3 (0.7)	8.8 (4.6)	>500
45	CH_3O-3,5	122-129 (0.06)	5.5 (2.5)	9.7 (4.7)	>500
46	NO_2-2,4	106-107[f]	3.0 (0.0)	6.6 (1.2)	>500
47	F-2, CH_3-4	75 (0.11)	80.0 (2.3)	96.0 (1.5)	155 (0.74)
48	Cl-2, CH_3-4	92-93 (0.11)	82.5 (0.7)	100.0 (0.0)	48 (0.67)
49	Cl-2, CH_3-6	78 (0.15)	21.6 (9.7)	42.9 (10.1)	>500
50	Cl-3, CH_3-2	94-96 (0.15)[g]	71.8 (5.1)	96.1 (2.9)	100 (0.69)
51	Cl-3, CH_3-4	102-104 (0.15-0.2)[h]	11.8 (4.3)	15.8 (5.4)	>500
52	Cl-4, CH_3-2	[i]	100.0 (0.0)	100.0 (0.0)	19 (.83)
53	Cl-5, CH_3-2	100-101 (0.2)	6.8 (2.4)	17.1 (2.8)	>500
54	Br-4, CH_3-2		92.5 (3.2)	100.0 (0.0)	42 (0.47)
55	CH_3-2, NO_2-3	133-140 (0.22-0.25)	1.5 (0.9)	11.8 (3.5)	>500
56	CH_3-2, NO_2-5	86-86.5	4.8 (2.8)	14.3 (4.7)	>500
57	CH_3-2, NO_2-6	106-110 (0.16)	4.3 (2.7)	16.3 (5.1)	>500
58	CH_3-4, NO_2-2	67-67.5	0.0 (0.0)	0.0 (0.0)	>500
59	CH_3O-2, CH_3-5	93-96 (0.09)[j]	4.3 (1.2)	11.3 (2.4)	>500
60	CH_3O-4, CH_3-2	104-106 (0.2)[k]	1.5 (0.9)	30.9 (5.2)	>500
61	CH_3O-2, NO_2-4	78.5-80.5	18.0 (4.3)	22.5 (2.5)	>500
62	CH_3O-4, NO_2-2	141-160 (0.15)	1.3 (1.2)	13.2 (5.2)	>500
63	Cl-2, NO_2-4	112-113	1.5 (0.6)	5.5 (0.0)	>500
64	Cl-2, NO_2-5	97-98.5	3.0 (2.1)	15.0 (4.6)	>500
65	Cl-4, NO_2-2	73.5-74	3.0 (0.0)	5.3 (4.8)	>500
66	Cl-4, NO_2-3	83	6.0 (2.0)	7.9 (2.4)	>500
67	Cl-2, CF_3-5	80-87 (0.06)	20.0 (3.5)	22.5 (3.2)	>500
68	(-OCH_2O-)-3,4	117-122 (0.11)	12.8 (3.6)	27.0 (3.7)	>500

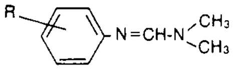
[a] Boiling point expressed as °C per millimeter of mercury. Melting point expressed as °C. A Fisher-Johns apparatus was used, and melting points are uncorrected. [b] Toxicity determined by slide-dip technique; concentration of test compounds was 500 ppm. Figures are the mean of at least three tests with the standard deviation given in parentheses. [c] LC$_{50}$ values determined at 72 h; slope given in parentheses. [d] Literature values: 120–122 °C (0.15), Counselman (1970) and Duerr et al. (1966); 133–135 °C (0.25), Gerjovich (1965); 136–140 °C (0.35), Winberg (1964). [e] Literature value: 93–94 °C (0.05), Counselman (1970) and Duerr et al. (1966). [f] Literature value: 107.5–108.5 °C, Winberg (1964). [g] Literature value: 94 °C (0.05), Counselman (1970) and Duerr et al. (1966). [h] Literature value: 123–125 °C (0.01), Counselman (1970) and Duerr et al. (1966). [i] Literature values: 163–165 °C (14), mp 35 °C, Counselman (1970); 156–157 °C (0.4), Arndt and Steinhausen (1968). [j] Literature value: 92–95 °C (0.05), Duerr et al. (1966). [k] Literature value: 160–161 °C (9), Counselman (1970) and Duerr et al. (1966).

at the tolyl carbon atom (specific activity 4.1 mCi/mmol), was provided by CIBA-GEIGY Corp., Greensboro, N.C. Demethylchlordimeform-^{14}C was synthesized from 4-chloro-*o*-toluidine-*tolyl*-^{14}C (specific activity 10 mCi/mmol), *N*-methylformamide, and phosphorus oxychloride. This synthesis was reported by Benezet and Knowles (1976a). The radiochemical purity of the chlordimeform-^{14}C and demethylchlordimeform-^{14}C was 96 and 93%, respectively.

The following nonradioactive compounds were examined as potential chlordimeform and demethylchlordimeform metabolites; 4-chloro-*o*-toluidine, 4′-chloro-*o*-formotoluidide, 4′-chloro-2′-methylacetanilide, 5-chloroanthranilic acid, *N*-formyl-5-chloroanthranilic acid, 2,2′-dimethyl-4,4′-dichloroazobenzene, *N*′-(4-chloro-*o*-tolyl)formamidine (didemethylchlordimeform), and 3-(4-chloro-*o*-tolyl)urea. The sources and properties of these compounds with exception of the urea derivative have been reported (Benezet and Knowles, 1976a; Knowles and Sen Gupta, 1970; Sen Gupta and Knowles, 1970). The urea derivative was recently synthesized and characterized (Benezet and Knowles, 1976b).

Toxicity Studies. The contact slide-dip method was used to study the toxicity to twospotted spider mites of the formamidines and related compounds (Dittrich, 1962; Voss, 1961). Twenty adult spider mites (males and females) were adfixed dorsally to a strip of double coated masking tape attached to a microscope slide by using a

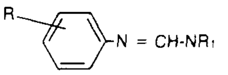
Table III. Toxicity of *N*′-Aryl-*N*,*N*-dimethylformamidines with Three, Four, and Five Ring Substitutents to Twospotted Spider Mites



Compd no.	R	Bp or mp[a]	Mortality, %[b] 48 h	72 h	LC_{50}, ppm[c]
69	Cl-2,4,5	91.5-92[d]	2.3 (0.7)	4.0 (1.2)	>500
70	Cl-2,4,6	70-71[e]	7.8 (3.7)	30.9 (8.5)	>500
71	CH_3-2,4,6	74-76 (0.09)	19.1 (7.5)	64.6 (2.4)	<500
72	CH_3O-3,4,5	143-145 (0.15)	9.3 (2.4)	17.5 (3.2)	>500
73	CH_3O-2,4, Cl-5	187-188	6.5 (2.8)	11.4 (3.2)	>500
74	CH_3-2,5, NO_2-4	103	12.0 (4.3)	26.4 (3.7)	>500
75	CH_3-4,5, NO_2-2	93-94	8.0 (3.5)	17.5 (4.3)	>500
76	Cl-2,4, NO_2-6	92-93	11.8 (3.1)	15.3 (4.3)	>500
77	Cl-2,5, NO_2-4	138-138.5	5.5 (1.4)	8.6 (4.6)	>500
78	Cl-2,6, NO_2-4	159.5-160[f]	1.5 (0.6)	9.5 (2.4)	>500
79	Cl-4,5, NO_2-2	122-122.5[g]	5.3 (3.2)	31.9 (8.3)	>500
80	I-2,6, NO_2-4	164-164.5	19.3 (9.3)	36.1 (7.5)	>500
81	F-2,3,5,6	68-70 (0.16)	4.8 (2.0)	24.3 (3.1)	>500
82	F-2,3,4,5,6	64 (0.15)	25.5 (0.9)	55.7 (3.1)	<500

[a] Boiling point expressed as °C per millimeter of mercury. Melting point expressed as °C. A Fisher-Johns apparatus was used, and melting points are uncorrected. [b] Toxicity determined by slide-dip technique; concentration of test compounds was 500 ppm. Figures are the mean of at least three tests with the standard deviation given in parentheses. [c] LC_{50} values determined at 72 h; slope given in parentheses. [d] Literature value: 85 °C, Steiger (1963). [e] Literature value: 73-73.5 °C, Gerjovich (1965). [f] Literature value: 160-162 °C, Steiger (1963). [g] Literature value: 126.5-127.5 °C, Gerjovich (1965).

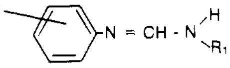
Table IV. Toxicity of *N*′-Aryl-*N*,*N*-dialkylformamidines to Twospotted Spider Mites



Compd no.	R	R_1	Bp or mp[a]	Mortality, %[b] 48 h	72 h	LC_{50}, ppm[c]
83	CH_3-2, Cl-4	$(C_2H_5)_2$	108-109 (0.14)	72.0 (1.5)	98.6 (1.1)	50 (.63)
84	CH_3-2, Cl-4	$(n\text{-}C_3H_7)_2$	109-111 (0.15)	60.0 (7.1)	96.1 (2.4)	51 (1.0)
85	CH_3-2, Cl-4	$(i\text{-}C_3H_7)_2$	100 (0.2)	18.0 (8.7)	47.1 (5.2)	>500
86	CH_3-2,4	$(C_2H_5)_2$		18.0 (2.0)	25.7 (3.1)	>500

[a] Boiling point expressed as °C per millimeter of mercury. Melting point expressed as °C. A Fisher-Johns apparatus was used, and melting points are uncorrected. [b] Toxicity determined by slide-dip technique; concentration of test compounds was 500 ppm. Figures are the mean of at least three tests with the standard deviation given in parentheses. [c] LC_{50} values determined at 72 h; slope given in parentheses.

Table V. Toxicity of *N*′-Aryl-*N*-monoalkylformamidines to Twospotted Spider Mites

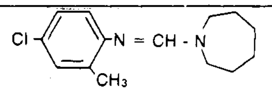
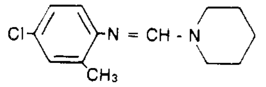
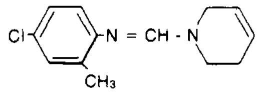
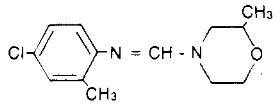
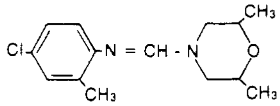
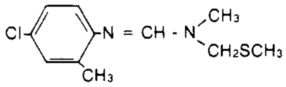
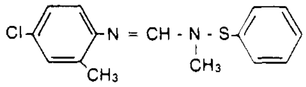
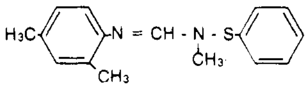
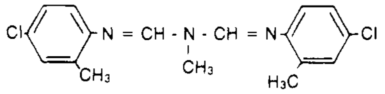
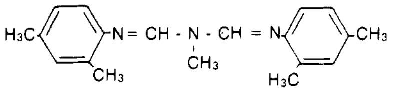


Compd no.	R	R_1	Mortality, %[a] 48 h	72 h	LC_{50}, ppm[b]
87[c]	CH_3-2, Cl-4	CH_3	98.4 (1.6)	100.0 (0.0)	12 (.70)
88	CH_3-2, Cl-4	C_2H_5	36.2 (11.1)	93.6 (3.1)	150 (.50)
89	CH_3-2, Cl-4	$i\text{-}C_3H_7$	30.8 (2.2)	96.9 (2.6)	50 (.81)
90	CH_3-2, Cl-4	$n\text{-}C_4H_9$	16.3 (3.2)	55.4 (3.7)	<500
91	CH_3-2, Cl-4	$i\text{-}C_4H_9$	12.5 (3.2)	47.3 (6.0)	>500
92	CH_3-2, Cl-4	$s\text{-}C_4H_9$	10.0 (3.5)	39.2 (5.4)	>500
93	CH_3-2, Cl-4	$t\text{-}C_4H_9$	4.0 (1.3)	29.7 (3.7)	>500
94[d]	CH_3-2,4	CH_3	87.0 (6.7)	100.0 (0.0)	72 (.44)

[a] Toxicity determined by slide-dip technique; concentration of test compounds was 500 ppm. Figures are the mean of at least three tests with the standard deviation given in parentheses. [b] LC_{50} values determined at 72 h; slope in parentheses. [c] Demethylchlordimeform. [d] BTS-27271.

small brush which had been dipped in distilled water. The slide was immersed and shaken for 5 s in an emulsion of the test compound. Water-soluble compounds were dissolved in water containing Triton X-100 (Rohm and Haas, Philadelphia, Pa.). Those compounds which were not water soluble were first dissolved in a small amount of acetone; Triton X-100 was added followed by the distilled water. The final concentration of Triton X-100 was 0.1%. To minimize any decomposition of the formamidines, formulations of test compounds were used immediately after preparation. Mites were treated at approximately the same time each day to minimize any differences in sensitivity associated with daily rhythms. At least three tests were conducted at each dosage level. The treated slides were placed on the top of a moist sponge in petri dishes which contained water to maintain the

Table VI. Toxicity of Miscellaneous Formamidines to Twospotted Spider Mites

Compd no.	Structure	Mortality, %[a] 48 h	72 h	LC_{50}, ppm[b]
95		6.6 (2.5)	27.0 (3.5)	>500
96		4.0 (1.3)	40.5 (5.8)	>500
97		3.5 (2.1)	17.1 (9.0)	>500
98		13.2 (7.0)	26.1 (3.6)	>500
99		0.0 (0.0)	29.7 (3.1)	>500
100[c]		70.3 (2.3)	100.0 (0.0)	39 (.49)
101[d]		100.0 (0.0)	100.0 (0.0)	6 (.92)
102[e]		54.5 (4.0)	100.0 (0.0)	62 (.54)
103[f]		68.8 (0.0)	100.0 (0.0)	36 (1.1)
104[g]		82.1 (3.3)	98.3 (1.7)	36 (1.4)

[a] Toxicity determined by slide-dip technique; concentration of test compounds was 500 ppm. Figures are the mean of at least three tests with the standard deviation given in parentheses. [b] LC_{50} values determined at 72 h; slope given in parentheses. [c] Hokko-20013. [d] Upjohn-42558. [e] Upjohn-42564. [f] BTS-23376. [g] Amitraz, Upjohn-36059, BAAM.

humidity. Mortality was recorded at 48 and 72 h following treatment. Spider mites were considered moribund if no twitching of the appendages occurred following gentle probing with a brush. Data were corrected for control mortality by Abbott's formula. The control mortality never exceeded 4% at 72 h but did increase appreciably in preliminary experiments conducted at longer time intervals.

The initial screening of the formamidines was conducted at a concentration of 500 ppm (0.05%), and the mean percentage mortality figure was used for ranking compounds. Those compounds giving less than 25% mortality at 72 h were considered inactive. Compounds giving 25 to 49% mortality at 72 h were regarded as having low activity, and those yielding between 50 and 80% were regarded as having moderate activity. Compounds giving greater than 80% mortality at 72 h were regarded as having high activity, and LC_{50} values were subsequently determined from dosage–mortality curves derived from 12 different concentrations of the test compound.

Metabolism Studies. Approximately 100 mg of twospotted spider mites, were brushed from infested bean leaves into a metabolism chamber which was precoated with about 100 000 dpm of purified chlordimeform-^{14}C or demethylchlordimeform-^{14}C. For initial studies the metabolism chamber consisted of a standard glass scintillation vial and cap. A quarter inch diameter hole was drilled into the scintillation vial cap, and a piece of nylon gauze was glued to the interior side to prevent escape of the mites. In experiments with this metabolism chamber, appreciable variation in the recovery of total radioactivity was observed. To minimize this variation a modified metabolism chamber was devised (Figure 1). This modified or closed chamber also consisted of a standard 22-mm glass scintillation vial; however, a 2.5 mL plastic sample cup containing calcium sulfate was glued to the top of the modified cap.

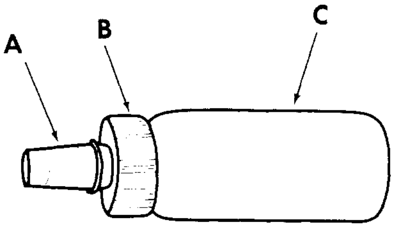


Figure 1. Metabolic chamber used in formamidine-spider mite studies. Legend: A, 2.5 mL plastic cup; B, scintillation vial cap; and C, glass scintillation vial.

Table VII. Chromatographic Behavior of Chlordimeform and Related Compounds[a]

Compound	R_f value in	
	Solvent system A	Solvent system B
Chlordimeform	0.66	0.70
Demethylchlordimeform	0.36	0.48
Didemethylchlordimeform	0.08	0.18
4′-Chloro-*o*-formotoluidide	0.16	0.31
4-Chloro-*o*-toluidine	0.43	0.55
4-Chloro-2-methylacetanilide	0.17	0.36
5-Chloroanthranilic acid	0.00	0.00
N-Formyl-5-chloroanthranilic acid	0.00	0.00
2,2′-Dimethyl-4,4′-dichloroazobenzene	0.73	0.79
3-(4-Chloro-*o*-tolyl)urea	0.00	0.07

[a] TLC was carried out on glass plates coated with a 500-μm layer of silica gel FG_{254}. Solvent system A = benzene-diethylamine (95:5). Solvent system B = benzene-acetone-diethylamine (75:20:5).

Spider mites in the metabolism chamber were rotated for 2 min initially on a Fisher minimill to facilitate contact of test organism and acaricide. The metabolism chambers containing the mites were held for exposure periods of 0, 1, 3, 6, 12, and 24 h at room temperature. At least three replicates were included at each time interval.

Following the desired exposure period, the mites were removed from the metabolism chamber and rinsed three times with 10 mL of acetone. The acetone rinses were combined, the volume was reduced to 5 mL, and two 0.1-mL aliquots were radioassayed (mite rinse).

The radioactivity in all samples was measured on a Picker Liquimat 220 liquid scintillation spectometer. The scintillation counting solution consisted of a mixture of 2,5-diphenyloxazole (12 g) and 1,4-*bis*-2-(4-methyl-5-phenyloxazolyl)benzene (120 mg) dissolved in toluene (2 L) and methyl Cellosolve (1 L) (Knowles and Sen Gupta, 1969). Ten milliliters of the cocktail was used per sample for radioanalysis. Data were corrected for counting efficieny, background, and dilution.

Following the acetone rinse, the spider mites were homogenized in 3 mL of acetone using a ground glass homogenizer and pestle. The homogenate was transferred to a centrifuge tube and centrifuged at low speed. The supernatant was decanted, and the precipitate was homogenized again in 3 mL of acetone. This acetone extraction was repeated. The three acetone extracts were combined and concentrated to a volume of 1 mL, and two 0.1-mL aliquots were radioassayed (organic extract). The remaining organic extract was subjected to TLC, autoradiography, and radioassay for separation, identification, and quantification of the radioactive components.

TLC of the organic extract was accomplished on 20 × 20 cm glass plates coated with a 500-μm layer of silica gel GF_{254} (Analtech, Inc., Newark, Del.). The solvent system for TLC was benzene–diethylamine (95:5) or benzene–acetone–diethylamine (75:20:5); R_f values for chlordimeform and related compounds are given in Table VII. A saturated chamber was used. Autoradiographs were prepared with Kodak no-screen x-ray film; the exposure period was usually 14 days.

The mite residue remaining after acetone extraction was extracted three times with 3-mL aliquots of water. The aqueous extracts were combined, and two 0.1-mL aliquots were radioassayed (aqueous extract).

The mite residue remaining after extraction with acetone and water was dried at 28 °C, and the total radioactivity in the residue was determined by oxygen flask combustion and subsequent radioassay of the trapped $^{14}CO_2$ (mite residue) (Sen Gupta and Knowles, 1969).

To the metabolism chamber was added 3 mL of acetone, and the chamber was swirled. Following evaporation of the acetone, 10 mL of scintillation fluid was added, and the radioactivity was determined (container rinse).

RESULTS

Toxicity of Formamidines. The toxicity to adult twospotted spider mites of *N′*-aryl-*N*,*N*-dimethylformamidines with one ring substituent is given in Table I. Of the 25 compounds examined, only four showed activity. Compound **7**, the 4-Cl derivative, showed moderate activity while the 2-Br (**8**), 4-Br (**9**), and 2-C_2H_5 (**15**) derivatives demonstrated low activity.

Table II gives the toxicity to adult twospotted spider mites of *N′-aryl-N,N-dimethylformamidine with two ring* substituents. Forty-three compounds in this category were examined, and 16 were active. Eight compounds yielded greater than 80% mortality at 72 h and were considered highly active. Of these eight compounds, seven were substituted in the 2 and 4 positions of the benzene ring. These compounds and their 72-h LC_{50} values were: **26** (F-2,4), 235 ppm; **28** (Cl-2,4), 14 ppm; **37** (CH_3-2,4), 110 ppm; **47** (F-2, CH_3-4), 155 ppm; **48** (Cl-2, CH_3-4), 48 ppm; **52** (Cl-4, CH_3-2), 19 ppm; and **54** (Br-4, CH_3-2), 42 ppm. The other highly active formamidine with two ring substituents was **50** (Cl-3, CH_3-2), LC_{50} 100 ppm. Formamidines with two ring substituents showing moderate activity included **39** (CH_3-2,6) and **33** (Br-2,4). Formamidines with low activity included **60** (CH_3O-4, CH_3-2), **38** (CH_3-2,5), **31** (Cl-3,4), **49** (Cl-2, CH_3-6), **34** (Br-2,5), and **68** [(-OCH_2O-)-3,4]. Twenty-seven of the 43 formamidines with two ring substituents were inactive (Table II).

The toxicity to adult twospotted spider mites of 12 *N′*-aryl-*N*,*N*-dimethylformamidines with three ring substituents is given in Table III. Five compounds were active. Compound **71** (CH_3-2,4,6) was moderately active; **80** (I-2,6, NO_2-4), **79** (Cl-4,5, NO_2-2), **70** (Cl-2,4,6), and **74** (CH_3-2,5, NO_2-4) showed low activity.

The toxicity to adult twospotted spider mites of one *N′*-aryl-*N*,*N*-dimethylformamidine with four ring substituents and one *N′*-aryl-*N*,*N*-dimethylformamidine with five ring substituents also is given in Table III. Compound **82**, a pentafluoro substituted derivative, was moderately active; **81**, the tetrafluoro derivative, was inactive.

Table IV presents the toxicity to adult twospotted spider mites of *N′*-aryl-*N*,*N*-dialkylformamidines. All four compounds showed activity. When R was 4-chloro-*o*-tolyl, high activity was shown with **83** (R_1 = diethyl), LC_{50} 50 ppm, and with **84** (R_1 = di-*n*-propyl), LC_{50} 51 ppm. Compounds **85** (R_1 = diisopropyl) showed low activity. Compound **86** (R = 2,4-xylyl; R_1 = diethyl) also showed low activity.

Table V gives the toxicity to adult twospotted spider mites of *N′*-aryl-*N*-monoalkylformamidines. In the 4-chloro-*o*-tolyl series (**87** to **93**), three compounds possessed high activity. They were **87** (R = methyl), LC_{50} 12 ppm; **89** (R_1 = isopropyl), LC_{50} 50 ppm; and **88** (R_1 = ethyl), LC_{50} 150 ppm. Compound **90** (R_1 = *n*-butyl) was moderately active, while **91** (R_1 = isobutyl), **92** (R_1 = *sec*-butyl), and **93** (R_1 = *tert*-butyl) possessed low activity. Compound **94**, the 2,4-xylyl derivative (R_1 = methyl) was highly active with a LC_{50} of 72 ppm.

Table VI gives the toxicity to adult twospotted spider mites of several miscellaneous formamidines. Compounds **95** to **99** include the 4-chloro-*o*-tolyl derivatives with cyclic nitrogen-containing substituents; **95**, **96**, **98**, and **99** showed

Table VIII. Metabolism of Chlordimeform-^{14}C by Twospotted Spider Mites[a]

Distribution and nature of radioactive material	% recovered radioactive material after indicated h of exposure					
	0	1	3	6	12	24
External radioactive material						
Container rinse	93.9	36.0	15.5	12.5	9.1	12.6
Mite rinse	2.7	16.6	12.6	20.8	17.5	7.3
Internal radioactive material						
Chlordimeform	0.6	43.3	57.9	45.9	36.9	35.3
Demethylchlordimeform	0.4	1.4	4.6	6.9	10.5	11.1
Didemethylchlordimeform	0.1	<0.1	0.1	0.3	0.8	1.6
4′-Chloro-*o*-formotoluidide	0.4	0.4	1.4	1.4	2.6	4.6
4-Chloro-*o*-toluidine	0.7	0.8	2.4	1.7	1.9	2.0
Unknown I	<0.1	<0.1	0.2	0.3	0.9	1.0
TLC origin	<0.1	0.4	1.8	2.7	7.1	6.9
Aqueous extract	1.2	1.0	3.3	7.1	12.1	16.9
Mite residue	<0.1	0.1	0.2	0.4	0.6	0.7
Applied radioactivity recovered, %	93.5	90.3	90.7	91.7	96.5	100.7

[a] Figures are the mean of at least three experiments. Solvent system for TLC was benzene–acetone–diethylamine (75:20:5). Unknown I chromatographed at R_f 0.09.

Table IX. Metabolism of Demethylchlordimeform-^{14}C by Twospotted Spider Mites[a]

Distribution and nature of radioactive material	% recovered radioactive material after indicated h of exposure					
	0	1	3	6	12	24
External radioactive material						
Container rinse	97.1	41.6	31.3	54.2	27.8	33.2
Mite rinse	1.2	18.3	8.9	11.6	15.6	13.9
Internal radioactive material						
Demethylchlordimeform	0.3	19.8	39.4	20.8	22.3	12.4
Didemethylchlordimeform	0.2	1.4	2.1	1.0	3.0	2.0
4′-Chloro-*o*-formotoluidide	0.2	9.8	6.6	3.3	6.9	8.2
4-Chloro-*o*-toluidine	0.3	2.0	3.2	1.2	3.0	5.5
TLC origin	0.2	5.1	6.3	5.4	14.7	9.0
Aqueous extract	0.3	1.7	1.9	2.1	5.8	13.3
Mite residue	0.2	0.3	0.3	0.4	0.9	2.5
Applied radioactivity recovered, %	97.5	91.7	94.8	99.6	87.9	93.7

[a] Figures are the mean of at least three experiments. Solvent system for TLC was benzene–acetone–diethylamine (75:20:5).

only low activity. Compound **97** was inactive.

Compound **100**, the only *N*′-aryl-*N*-methyl-*N*-alkylthioalkylformamidine examined was highly active with a LC_{50} of 39 ppm (Table VI).

Both *N*′-aryl-*N*-methyl-*N*-thiophenylformamidines were highly active; **101** had a LC_{50} of 6 ppm, and **102** had a LC_{50} of 62 ppm (Table VI).

The two diaryltriazapentadienes (**103** and **104**) were highly active with a LC_{50} of 36 ppm (Table VI).

Didemethylchlordimeform was highly active with a 72-h LC_{50} value of 245 ppm. 3-(4-Chloro-*o*-tolyl)urea gave low activity (38% mortality at 72 h). 4-Chloro-*o*-toluidine, 4′-chloro-*o*-formotoluidide, and 2,2′-dimethyl-4,4′-dichloroazobenzene were inactive.

Metabolism of Chlordimeform. Table VIII gives the distribution of radioactivity following treatment of twospotted spider mites with chlordimeform-^{14}C. Generally, the external radioactive material (container and mite rinse) decreased with increasing exposure time; the total external radioactive material at zero time was 96.6%, and this had decreased to 19.9% by 24 h. Initially the majority of the external radioactive material was in the container rinse; however, at the latter time intervals the majority was in the mite rinse.

The majority of the internal radioactive material was organosoluble (Table VIII). This material increased from 2.2% of the recovered radioactive material at zero time to a maximum of 68.4% at 3 h and by 24 h still comprised 62.5%. The radioactivity in the aqueous extract also increased with exposure time; the maximum level of radioactive material in aqueous extract was 16.9% at 24 h. Levels of radioactivity remaining in the mite residue after extraction were low and did not exceed 0.7% of the recovered radioactive material. The total applied radioactivity recovered averaged 93.9% with a range of 90.3 to 100.7% (Table VIII).

Table VIII also gives the nature and concentration of organosoluble radioactive materials isolated from twospotted spider mites treated with chlordimeform-^{14}C. Chlordimeform (I) increased from 0.6% of the recovered radioactive material at zero time to a peak level of 57.9% at 3 h and decreased to 35.3% by 24 h. Demethylchlordimeform (II) increased from 0.4% at zero time to a maximum level of 11.1% at 24 h. Levels of didemethylchlordimeform (III) were low and did not exceed 1.6% of the recovered radioactive material. 4′-Chloro-*o*-formotoluidide (IV) increased from 0.4% at zero time to a maximum of 4.6% at 24 h. Levels of 4-chloro-*o*-toluidine (V) also were low and did not exceed 2.4%. Unidentified radioactive material at the TLC origin increased from 0.1% at zero time to a maximum of 7.1% at 12 h. The unknown at R_f 0.09 never exceeded 1.0%; the chromatographic behavior of this material was similar to that 3-(4-chloro-*o*-tolyl)urea.

Metabolism of Demethylchlordimeform. The distribution of radioactivity following treatment of twospotted spider mites with demethylchlordimeform-^{14}C is given in Table IX. The total external radioactive material at zero time was 98.3% and decreased to 47.1% by 24 h. In this case the majority of the external radioactive material was in the container rinse throughout the entire 24-h period.

Most of the internal radioactive material was organosoluble (Table IX). This material increased from 1.2% of the recovered radioactive material at zero time to a maximum of 57.6% at 3 h. The radioactivity in the aqueous extract also increased with exposure time; the maximum level of radioactive material in aqueous extract was 13.3% at 24 h. Levels of radioactivity remaining in the mite residue after extraction were low and did not exceed 2.5% of the recovered radioactive material. The total applied radioactivity recovered averaged 94.2% with a range of 87.9 to 99.6% (Table IX).

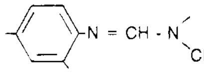
Table IX also presents the nature and concentration of organosoluble radioactive materials isolated from twospotted spider mites treated with demethylchlordimeform-^{14}C. Demethylchlordimeform (II) increased from 0.3% at zero time to a maximum level of 39.4% at 3 h, and by 24 h still comprised 12.4% of the recovered radioactive material. Levels of didemethylchlordimeform (III) were low and did not exceed 3.0% of the recovered radioactive material. 4′-Chloro-*o*-formotoluidide (IV) increased from 0.2% at zero time to a maximum of 9.8% at 1 h. 4-Chloro-*o*-toluidine (V) increased from 0.3% at zero time to a peak of 5.5% at 24 h. Unidentified radioactive material at the TLC origin increased from 0.2% at zero time to a maximum of 14.7% at 12 h.

DISCUSSION

The toxicity of 104 formamidines to the twospotted spider mite was studied using a slide-dip technique. Fifty-seven compounds gave less than 25% mortality at 72 h at a concentration of 500 ppm (0.05%) and were considered inactive. Twenty-two compounds gave mortality in the range of 25 to 49% and were regarded as having low activity, and six compounds gave mortality in the range of 50 to 80% and were regarded as having moderate activity. Nineteen compounds gave greater than 80% mortality and were considered highly active. From the results reported herein it was possible to draw several conclusions relative to the structural requirement of formamidines for maximum toxicity to adult twospotted spider mites. It was obvious that the aryl moiety should contain two substituents and that these substituents should be located at ring positions 2 and 4. The following evidence supported this conclusion. None of the 25 monosubstituted aryl derivatives possessed high activity, although four compounds exhibited moderate or low activity. They were substituted at ring position 2 with bromine (**8**) or ethyl (**15**) or at ring position 4 with chlorine (**7**) or bromine (**9**). None of the compounds substituted at ring position 3 were active. Five of the 12 *N′*-aryl-*N*,*N*-dimethylformamidines with three ring substituents were active. However, only the trimethyl derivative (**71**) was moderately active. Only one *N′*-aryl-*N*,*N*-dimethylformamidine with four ring substituents and one with five ring substituents were examined; neither was highly active. Of the 43 *N′*-aryl-*N*,*N*-dimethylformamidines with two ring substituents, 16 were active, and each active compound had one of the two substituents at ring positions 2 or 4. Further, the most active compounds were substituted at ring positions 2 and 4. Sixteen 2,4-substituted aryl formamidines were tested, and seven were highly active (**26**, **28**, **37**, **47**, **48**, **52**, **54**). Compound **50** (Cl-3, CH_3-2) was the only non-2,4-disubstituted formamidine with high activity. Compound **33** (Br-2,4) possessed moderate activity, but the other seven 2,4-disubstituted compounds (**42**, **46**, **58**, **61**, **62**, **63**, **65**) contained methoxy and/or nitro substituents and were inactive. Thus, in the *N′*-aryl-*N*,*N*-dimethylformamidines, high activity was associated with halogen (chlorine, bromine, fluorine) and methyl.

Also, there were requirements for the substituents on the amino nitrogen. It was concluded that at least one substituent should be lower alkyl, perferably methyl, while greater flexibility existed relative to the other amino nitrogen substituent. It could be hydrogen, lower alkyl, methylthiomethyl or other more complex moieties. The following evidence supported these contentions. In the *N′*-(4-chloro-*o*-tolyl)-*N*,*N*-dialkylformamidines, the dimethyl derivative (**52** or chlordimeform) was most active, followed by the diethyl derivative (**83**) and the di-*n*-propyl derivative (**84**); the diisopropyl derivative (**85**) was appreciably less active. In the *N′*-(2,4-xylyl)-*N*,*N*-dialkylformamidines, a similar relationship was observed in that the dimethyl derivative (**37**) was more active than its diethyl homologue (**86**). With the *N′*-(4-chloro-*o*-tolyl) *N*-monoalkylformamidines activity to adult spider mites decreased in the following order: methyl (**87** or demethylchlordimeform) > isopropyl (**89**) > ethyl (**88**) > *n*-butyl (**90**) > isobutyl (**91**) > *sec*-butyl (**92**) > *tert*-butyl (**93**). Formamidines with cyclic substituents at the amino nitrogen showed only low activity (**95**, **96**, **98**, **99**) or were inactive (**97**). Compounds **100** to **104** with amino nitrogen substituents of methyl and methylthiomethyl (**100**), methyl and phenylthio (**101**, **102**), and methyl and substituted aryl–N=CH– (**103**, **104**) were highly active.

Finally, it was observed that maximum toxicity to adult twospotted mites was associated with the following moiety:



The toxicity by different methods and to different strains of twospotted spider mites of some of these aforementioned compounds has been reported previously. For example, Dittrich (1966) reported that the 24-h LC_{50} value for chlordimeform (**52**) against the twospotted spider mite was 430 ppm with a slide-dip technique and 340 ppm with a leaf-dip technique. In a subsequent paper (Dittrich, 1969), it was reported that the 24-h LC_{50} value for chlordimeform was 62.2 ppm for an organophosphate-susceptible strain and 5.8 ppm for an organophosphate-resistant strain. Harrison et al. (1973) used a leaf spray technique and investigated the toxicity to twospotted spider mites of 38 formamidines. In this test, compounds **1**, **6**, **7**, **9**, **17**, **20**, **25**, **31**, **40**, **51**, **70**, **71**, **83**, **88**, and **92** each had 72-h LC_{50} values of 1000 ppm or greater. Chlordimeform (**52**) yielded an LC_{50} of 230 ppm; demethylchlordimeform (**87**) and BTS-27271 (**94**) yielded LC_{50} values of 120 ppm. Compound **91** gave an LC_{50} of 620 ppm. Thus, there were several apparent disparities between our study and that of Harrison et al. (1973). These included **7**, **71**, **83**, and **88** which were appreciably more toxic to twospotted spider mites by the slide-dip method.

Arndt and Steinhausen (1968) studied the toxicity of several formamidines including **1**, **6**, **13**, **14**, **51**, **52**, **83**, **84**, **87**, **91**, and **92** to postembryonic stages of twospotted spider mites following the spraying of infested bean plants. Structure–activity relationships were difficult to discern because of the experimental design. However, chlordimeform (**52**) and demethylchlordimeform (**87**) were most active, followed by **91**, **83**, **84**, and **92**. Compounds **1**, **6**, **13**, **14**, and **51** were inactive (Arndt and Steinhausen, 1968). These data generally compared favorably with those of the present study.

Gemrich et al. (1976) studied the toxicity to adult twospotted spider mites of **101** and **102** four days after spraying infested bean plants. LC_{50} values were 42 ppm for **101** and 13 ppm for **102**.

There are three practical laboratory bioassay methods commonly used to evaluate the efficacy of acaricides against twospotted spider mites, the slide-dip, leaf-dip, and plant spray. For our toxicity studies, the slide-dip was the method of choice, although we recognize that one of the techniques utilizing a natural substrate might give a better indication of control potential of the compounds under field conditions. Our major aim was to elucidate the intrinsic toxicity of each formamidine to the adult twospotted spider mite. Thus it was important to minimize those variables which could interfere with this measurement. With the slide-dip method the dosage is known more accurately than with a plant spray. Further, formamidines can undergo base–salt conversions on leaf surfaces, and there is some evidence that this can influence toxicity. Finally, with the mites adfixed to the glass slide, the end point can be determined more precisely than with other methods.

Metabolism studies of chlordimeform-^{14}C in twospotted spider mites indicated a rapid uptake of the compound accompanied by high internal levels of organosoluble radioactive material. Chlordimeform metabolites identified by cochromatography included demethylchlordimeform, didemethylchlordimeform, 4′-chloro-*o*-formotoluidide, and 4-chloro-*o*-toluidine. The unknown at R_f 0.09 may be 3-(4-chloro-*o*-tolyl)urea. Benezet and Knowles (1976b) have recently elucidated a urea pathway for chlordimeform metabolism in rats and mice.

Uptake of demethylchlordimeform-^{14}C by twospotted spider mites also was rapid, and levels of internal organosoluble radioactive materials were quite high. Demethylchlordimeform metabolites identified included didemethylchlordimeform, 4′-chloro-*o*-formotoluidide, and 4-chloro-*o*-toluidine.

Chlordimeform and demethylchlordimeform were not rapidly metabolized by twospotted spider mites. This slow metabolism of chlordimeform also has been observed in other chlordimeform-susceptible insects and acarines, such as the cabbage looper, *Trichoplusia ni* (Hübner) (Crecelius and Knowles, 1976), the cattle tick *Boophilus microplus* (Canestrini) (Knowles and Schuntner, 1974), and the rice steam borer, *Chilo suppressalis* Walker (Morikawa et al., 1975) and is in contrast to the rapid metabolism observed with the chlordimeform-tolerant house fly, *Musca domestica* L. (Knowles and Shrivastava, 1973). It appears that differential metabolism plays a role in the selective toxicity of chlordimeform to insects and acarines.

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Metabolism of *O*-Ethyl *S*-4-Chlorophenyl Ethanephosphonodithioate (N-2596) in the Rat

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The experimental insecticide, *O*-ethyl *S*-4-chloro[U-^{14}C]phenyl ethanephosphonodithioate ([^{14}C]N-2596), is rapidly metabolized to detoxification products in rats receiving a single oral dose of [^{14}C]N-2596. Within 4 days of administration, the radiocarbon had been excreted mostly in the urine as conjugates of 4-chloro-3-hydroxyphenyl methyl sulfone. The primary metabolic biotransformations arise from scission of either N-2596 or its oxygen analogue to yield 4-chlorothiophenol which is then S-methylated, sulfoxidized, and ring hydroxylated. Neither N-2596 nor its oxygen analogue were detected in the urine of treated rats. Tissue residues based on total ^{14}C analysis decreased rapidly to negligible levels during an 8-day holding period.

Several phosphonate esters (organophosphorus esters with a P–C bond) have gained acceptance in recent years as highly effective pesticides. The metabolism of these compounds was recently reviewed by Menn and McBain (1974).

Included in this group of compounds is the experimental soil insecticide, N-2596 (*O*-ethyl *S*-4-chlorophenyl ethanephosphonodithioate). It has shown particular efficacy

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