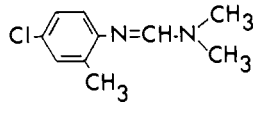
Metabolism of Two Acaricidal Chemicals, *N'*-(4-Chloro-*o*-tolyl)-*N,N*-dimethylformamidine (Chlorphenamidine) and *m*-{[(Dimethylamino)methylene]amino}phenyl Methylcarbamate Hydrochloride (Formetanate)

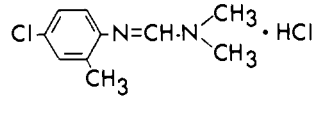
Charles O. Knowles

The metabolism of two acaricides *N'*-(4-chloro-*o*-tolyl)-*N,N*-dimethylformamidine (chlorphenamidine) and *m*-{[(dimethylamino)methylene]amino}phenyl methylcarbamate hydrochloride (formetanate) in mammals is reviewed with emphasis on excretion balance and on the nature and concentration of the urinary metabolites. Their metabolic fate in plants is also discussed, along with data obtained on penetration and translocation in plant tissues. The degradation of chlorphenamidine, formetanate, and related compounds in the presence of soil, microorganisms, and model systems is also reviewed.

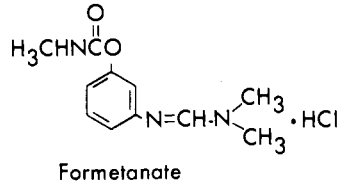
Control of phytophagous mites is essential for successful production of many horticultural, ornamental, and agronomic crops. Since mites readily develop resistance to acaricides, new lethal chemicals, often with modes of action different from existing compounds, have to be continually available. Two of these novel acaricides are *N'*-(4-chloro-*o*-tolyl)-*N,N*-dimethylformamidine or chlorphenamidine and *m*-{[dimethylamino)methylene]amino}phenyl methylcarbamate hydrochloride or formetanate. Chlorphenamidine is formulated as the free base (Galecron EC, CIBA Agrochemical Co.; Fundal EC, NOR-AM Agricultural Products, Inc.) and the hydrochloride salt (Galecron SP; Fundal SP); formetanate is the hydrochloride salt (Carzol, NOR-AM Agricultural Products, Inc.). The structures shown below illustrate the chemical similarity of the acaricides.



Chlorphenamidine (base)



Chlorphenamidine (salt)



Formetanate

In field tests, chlorphenamidine (both base and hydrochloride salt) and formetanate have been used effectively as acaricides on numerous crops (Asquith, 1968; Batiste and Berlowitz, 1969; Cone, 1968; Di Caro, 1967; Dittrich, 1967; Furr and Davis, 1969; Jeppson *et al.*, 1969; Stafford, 1968; Westigard, 1969; Wilson and Oliver, 1969). Moreover, a mixture of chlorphenamidine hydrochloride (60%) and formetanate (30%) plus inert ingredients (10%) gives control of the citrus red mite, *Panonychus citri* (McGregor), on lemons (Jeppson *et al.*, 1969) and the two-spotted spider mite, *Tetranychus urticae* (Koch), on cluster hops (Cone, 1968).

Department of Entomology, University of Missouri, Columbia, Mo. 65201

Chlorphenamidine and formetanate also kill organophosphate resistant mites. In fact, a negative correlation with organophosphate resistance in certain strains of the two-spotted spider mite and the toxicity of chlorphenamidine (Dittrich, 1969) and formetanate (Steinhausen, 1968) has been demonstrated.

Since chlorphenamidine and formetanate are toxic to numerous mite species, some of which have already developed organophosphorus resistance, they would be useful in future mite control programs. Therefore, we have studied certain facets of the toxicology of these compounds to determine any hazards that might be associated with their use. This paper reviews the existing data on the metabolism, movement, and persistence of chlorphenamidine and formetanate in biological systems.

METABOLIC FATE OF CHLORPHENAMIDINE

Metabolism in Mammals. Rats, dogs, and goats treated orally with radioactive chlorphenamidine (tolyl methyl-^{14}C) rapidly metabolized and subsequently eliminated the radioactive components primarily *via* the urine. Cumulative percentages of the dose excreted in the urine 24 hr after treatment were 85% for rats (Figure 1A), 70% and 80% for two dogs (Figure 1B), 65% for a lactating goat, and 80% for a male goat (Knowles and Sen Gupta, 1970b; Sen Gupta and Knowles, 1970b). Rats eliminated 7.5% of the dose in the feces by 72 hr, and only 0.6% and 1.8% of the administered radioactivity was accounted for in dog and goat feces, respectively, during the 72-hr period.

There were differences in the rates of degradation of chlorphenamidine by the three mammalian species. By 24 hr after treatment, 25% of the radioactive material in rat urine partitioned into chloroform, but less than 10% was organosoluble from dog and goat urine. Levels of chlorphenamidine expressed as percentages of organosoluble urinary radioactivity at 24 and 72 hr posttreatment were 9.9 and 2.1 for the rat, 1.3 and 0.2 for the dog, and 0.1 and < 0.1 for the goat. Organosoluble radioactive chlorphenamidine metabolites extracted from rat, dog, and goat urine included *N'*-(4-chloro-*o*-tolyl)-*N*-methylformamidine [demethylchlorphenamidine (II)], 4'-chloro-*o*-formotoluidide (III), 4-chloro-*o*-toluidine (IV), *N*-formyl-5-chloroanthranilic acid (V), and 5-chloroanthranilic acid (VI). (Roman numeral designations following chemical and/or common names in text correlate with

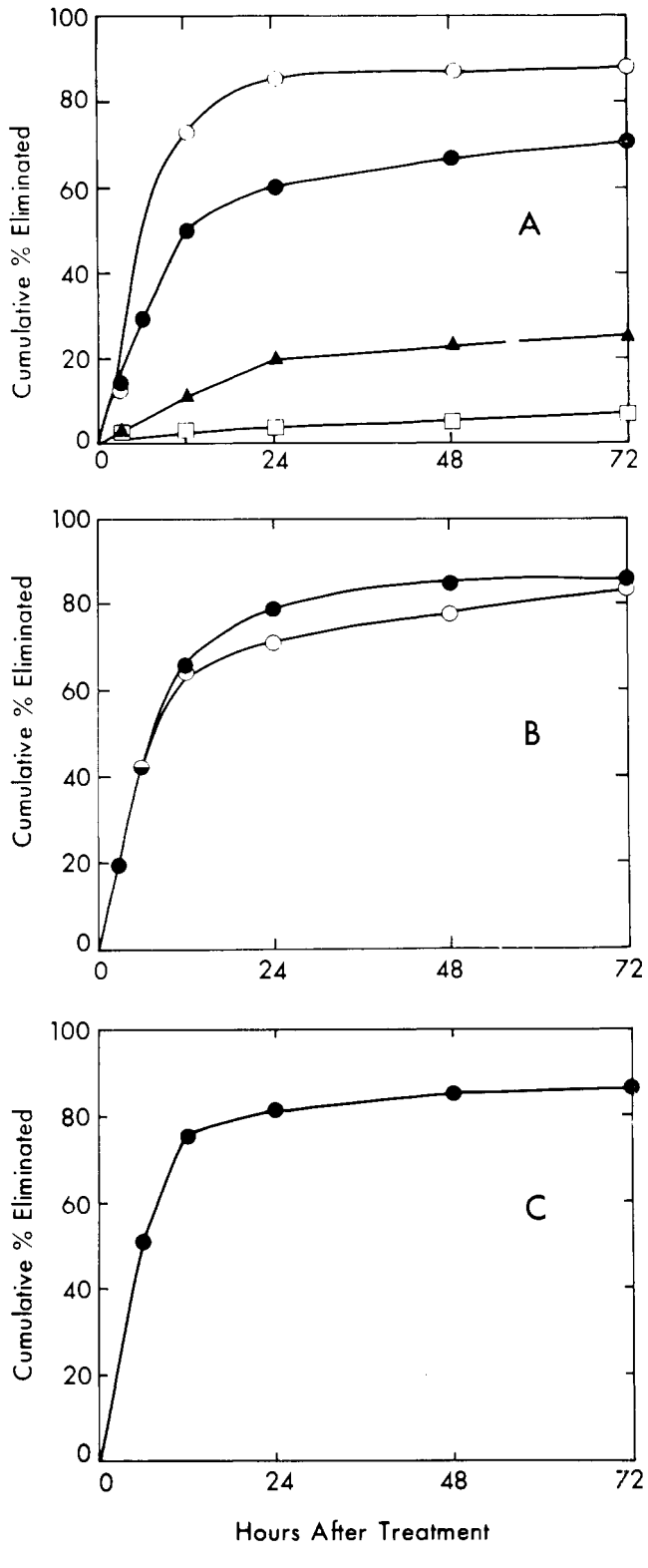


Figure 1. Cumulative percentage of the administered dose eliminated in urine and feces of mammals treated orally with chlorphenamidine-^{14}C, 4-chloro-*o*-toluidine-^{14}C, and formetanate-^{14}C (Knowles and Sen Gupta, 1970b; Sen Gupta and Knowles, 1970a,b). Reprinted with permission: *J. Econ. Entomol.* 63, 10, 856, 951 (1970). A. Chlorphenamidine, rat urine (○); Chlorphenamidine, rat feces (□); 4-chloro-*o*-toluidine, rat urine (●); 4-chloro-*o*-toluidine, rat feces (▲). B. Chlorphenamidine, dog urine (○ and ●). C. Formetanate, rat urine (●)

chemical structures in Figures 11 and 12.) The concentration of the "free" anthranilic acids (V and VI) in the urine increased with time after treatment; the goat was most efficient in converting chlorphenamidine to anthranilic acid derivatives, the dog was intermediate, and the rat was least efficient. Several minor unidentified radioactive metabolites were also present in the urine in some cases.

The nature of the water-soluble radioactive material in dog and goat urine was also investigated (Sen Gupta and Knowles, 1970b). Aliquots of the aqueous fraction from the urine were incubated with β-glucuronidase and β-glucuronidase-aryl sulfatase; the aglycones released were extracted with ether and subjected to tlc for tentative identification. In dog urine at least six unidentified aglycones were present in addition to chlorphenamidine and the "free" identified metabolites mentioned above, whereas aglycones from the goat included chlorphenamidine, the "free" metabolites, and only two unknowns. The cleavage data suggested that the aglycones were present as glucuronides and/or ethereal sulfates. Compounds with amino or carboxyl groups such as 4-chloro-*o*-toluidine (IV), *N*-formyl-5-chloroanthranilic acid (V), and 5-chloroanthranilic acid (VI) are capable of conjugating with glucuronic acid in the body (Williams, 1959). 4-Chloro-*o*-toluidine (IV) and 5-chloroanthranilic acid (VI) could also form sulfamates, since aromatic amines can react with sulfuric acid *in vivo* (Williams, 1959).

There was no appreciable storage of chlorphenamidine-^{14}C equivalents in rat and dog tissues or elimination in goat milk (Knowles and Sen Gupta, 1970b; Sen Gupta and Knowles, 1970b).

The relative importance of the biliary route in the elimination of chlorphenamidine and its radioactive metabolites was investigated by Sen Gupta and Knowles (1970b). A mongrel dog was treated orally with chlorphenamidine at a dosage level of 0.3 mg/kg. Twelve hours prior to treatment a brass cannula was inserted into the gall bladder, and the common bile duct was ligated. Thus, all bile from the dog flowed through the cannula and emptied into a collection balloon. Slightly less than 5% of the administered dose was accounted for in the bile by 72 hr after treatment, with the peak concentration of chlorphenamidine-^{14}C equivalents (2.5 ppm) occurring at 8 hr after treatment. Radioactive compounds extracted from bile and present in the "free" and conjugated form included chlorphenamidine (I), demethylchlorphenamidine (II), 4′-chloro-*o*-formotoluidide (III), 4-chloro-*o*-toluidine (IV), the anthranilic acids (V and VI), and several unidentified compounds that cochromatographed with some of the unknown urinary metabolites. 4′-Chloro-*o*-formotoluidide (III) was the major "free" and conjugated chlorphenamidine metabolite in bile. We concluded that an enterohepatic circulation of chlorphenamidine and metabolites was possible, since total chlorphenamidine-^{14}C equivalents detected in bile were greater than those eliminated in the feces (Sen Gupta and Knowles, 1970b).

Knowles and Sen Gupta (1970b) also studied the metabolism of 4-chloro-*o*-toluidine-^{14}C, a chlorphenamidine metabolite, in the rat. Approximately 71% and 25% of the dose was eliminated in the urine and feces, respectively, by 72 hr after treatment (Figure 1A). However, more than 35% of the radioactive material in the urine still partitioned into ethyl acetate at 72 hr posttreatment; the ethyl acetate fraction was composed of 4-chloro-*o*-toluidine (IV), 5-chloroanthranilic acid (VI), 4′-chloro-2′methylacetanilide, and at least six unidentified metabolites. Therefore, the 4-chloro-*o*-toluidine

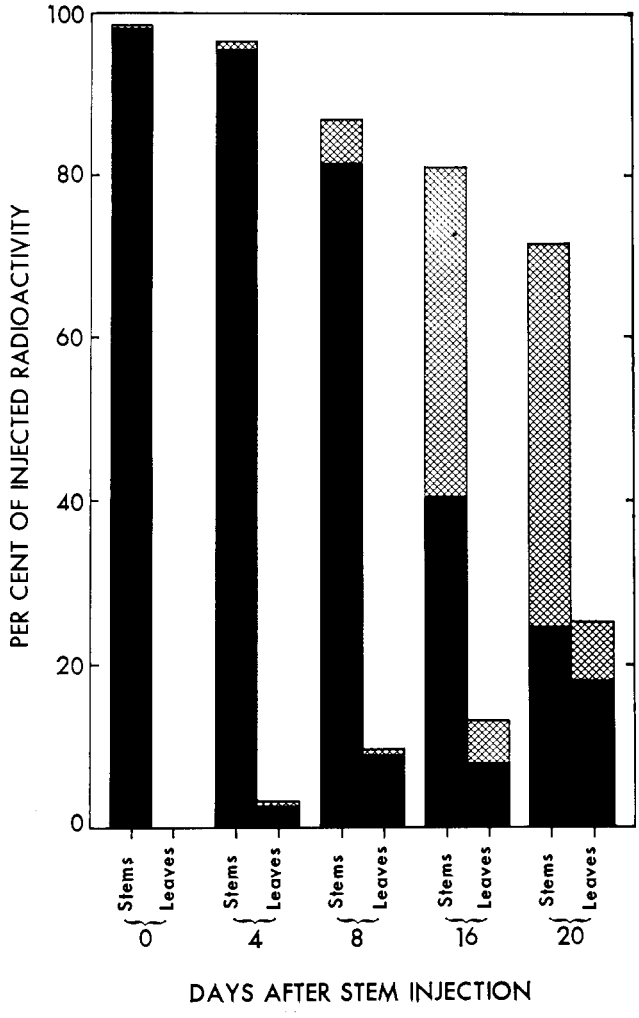


Figure 2. Fate of chlorphenamidine-^{14}C when injected into the stems of apple seedlings (Sen Gupta and Knowles, 1969). Bars = total chlorphenamidine-^{14}C equivalents in stems or leaves; solid black portion denotes concentration of parent compound

was not absorbed as readily and metabolized as rapidly as chlorphenamidine. Total elimination of radiocarbon-containing compounds in the urine and feces was, however, about the same for both compounds by 72 hr after treatment.

Degradation by Enzyme Preparations. When the 600 × G supernatant fraction (nuclear supernatant) isolated from rat liver was fortified with reduced nicotinamide adenine dinucleotide phosphate (NADPH) and incubated with chlorphenamidine-^{14}C for 4 hr, about 34% was converted to other organosoluble products (Knowles and Ahmad, 1970). After correcting this result for decomposition in the control, about 16% of the conversion was enzymatic, since it was blocked by thermal treatment of the nuclear supernatant fraction. Demethylchlorphenamidine (II) was the major *in vitro* organosoluble metabolite and increased from 0.3% in the control to 13.5% in the nuclear supernatant fraction. The demethylation reaction was sensitive to 2-diethylaminoethyl 2,2-diphenylvalerate (SKF-525A), a known inhibitor of mixed function oxidases, but not to diisopropyl phosphorofluoridate (DFP). Other experiments clearly showed that the *N*-demethylation was catalyzed by microsomal enzymes. When NADPH-fortified rat liver microsomes were incubated with chlorphenamidine-^{14}C for 4 hr, about 30% of the organosoluble radioactive material was demethylchlorphenamidine (II); less than 2% was demethylchlorphenamidine (II) in the 100,000 × G supernatant or soluble fraction (Knowles and Ahmad, 1970). Microsomal preparations from the abdomens of houseflies, *Musca domestica* L., also demethylated chlorphenamidine in the presence of NADPH and oxygen (Shrivastava and Knowles, 1970).

4′-Chloro-*o*-formotoluidide-^{14}C, the major hydrolytic decomposition product of chlorphenamidine (Knowles and Sen Gupta, 1969) was converted to 4-chloro-*o*-toluidine (IV) by a rat liver soluble enzyme, and the conversion was inhibited by DFP, but not by SKF-525A (Knowles and Ahmad, 1970).

Metabolism in Plants. Sen Gupta and Knowles (1969) injected chlorphenamidine-^{14}C into the main stem of apple seedlings and then analyzed the stems and leaves at regular intervals (Figure 2). During the first 4 days after injection, greater than 95% of the total radioactivity was localized in the stems, predominately as the parent compound. Twenty days after injection 71.6% of the chlorphenamidine-^{14}C equivalents still remained in the stems. Chlorphenamidine was slowly translocated into the leaves of apple seedlings. Chlorphenamidine-^{14}C equivalents in the leaves at 20 days accounted for 25.4% of the injected radioactivity, and 17.9% was the parent compound. Chlorphenamidine metabolites isolated from leaves and stems included demethylchlorphenamidine (II), 4′-chloro-*o*-formotoluidide (III), 4-chloro-*o*-toluidine (IV), and *N*-(2-methyl-4-chlorophenyl)-D-glucosylamine. However, the major portion of the chlorphenamidine-^{14}C equivalents in the stems at 16 and 20 days was unextractable with chloroform and acetone. The authors stated that this radioactive material possibly represented chlor-

Table I. Persistence of Chlorphenamidine and Chlorphenamidine Hydrochloride when Applied Topically to Leaves of Apple Seedlings and Grapefruit Seedlings[a]

	Percent Applied Radioactivity Recovered at Indicated Days After Treatment					
	0	4	8	12	16	20
Apple Leaves						
Chlorphenamidine-^{3}H-equivalents	99.3[b]	59.4	55.4	55.7	45.1	45.6
Chlorphenamidine	99.0	55.1	51.7	50.8	40.2	40.7
Chlorphenamidine-^{14}C-equivalents	86.3[c]	...	52.6	50.3	45.6	46.2
Chlorphenamidine	86.0	...	50.0	46.3	41.3	41.0
Grapefruit Leaves						
Chlorphenamidine-^{14}C-equivalents	97.8[b]	27.3	35.0	32.4	30.2	23.2
Chlorphenamidine	95.0	9.1	4.8	5.1	2.2	0.9
Chlorphenamidine hydrochloride-^{14}C-equivalents	82.3[c]	16.0	13.2	10.8	9.6	6.7
Chlorphenamidine hydrochloride	64.9	8.6	5.5	1.6	0.6	0.5

[a] Sen Gupta and Knowles (1969); Ehrhardt and Knowles (1970). [b] Analyzed immediately after treatment. [c] Analyzed 1 hr after treatment.

phenamidine degradation products that were complexed with polymeric cell constituents of the plant.

Apple leaves were also treated topically *in situ* with either chlorphenamidine-^{14}C or chlorphenamidine-^{3}H (ring labeled) (Table I). With the exception of the 0 time values, there was close agreement between data obtained in the two experiments. The disparity at 0 time could be explained by the fact that the leaves treated with chlorphenamidine-^{3}H were analyzed immediately after treatment, while those treated with chlorphenamidine-^{14}C were analyzed 1 hr after treatment. The difference, therefore, was a result of volatilization of chlorphenamidine from the leaf surface during he 1-hr time differential. The decrease in radiocarbon between 0 time and 4 days was also due primarily to volatilization of the parent compound. The chlorphenamidine-^{3}H- and chlorphenamidine-^{14}C-equivalents in and on apple leaves after 4 days decreased very slowly with time. Forty-six percent of the applied radioactivity still remained in and on the leaves by 20 days after treatment; 41% was the parent compound. Organosoluble chlorphenamidine metabolites isolated from apple leaves treated topically were similar to those detected in stems and leaves when the compound was injected into the main stem.

Differences were observed in the behavior of chlorphenamidine applied topically to grapefruit leaves (Ehrhardt and Knowles, 1970) as compared to apple leaves (Table I). For example, chlorphenamidine-^{14}C-equivalents in grapefruit leaves were 15 to 30% less than those in apple leaves during the experimental period. Twenty days after treatment of grapefruit leaves the concentration of the parent compound was only 0.9% of the applied radioactivity. An appreciable portion of the radioactive material was unextractable from the grapefruit leaves. Of the 23.2% of the chlorphenamidine-^{14}C equivalents in the grapefruit leaves at 20 days, 16.3% was localized in the leaf residue and was not identified. There was little movement of radioactive material into grapefruit stems, since only 0.8% of the applied radioactivity was detected in the stems 20 days after topical leaf treatment.

Chlorphenamidine hydrochloride-^{14}C was also applied topically to grapefruit leaves (Ehrhardt and Knowles, 1970). Chlorphenamidine hydrochloride-^{14}C equivalents in and on grapefruit leaves were lower than those detected when the base was applied (Table I). However, with the exception of 0 time, the levels of the parent compound in and on grapefruit leaves were comparable to those obtained in the chlorphenamidine experiment. With chlorphenamidine hydrochloride, the concentration of radioactivity in the leaf residue or the stems never exceeded 2% of the applied radioactivity.

The identified organosoluble radioactive metabolites resulting from application of chlorphenamidine and chlorphenamidine hydrochloride to grapefruit leaves were generally similar to those isolated from apple seedlings. However, several minor unidentified metabolites were presented in grapefruit leaves treated with the two compounds, and it was speculated that some were salts of corresponding identified bases.

When evaluating the data in Table I, several variables should be considered. The experiments were conducted at different times and, in some cases, in different greenhouses. Therefore, the rate of volatility of chlorphenamidine and chlorphenamidine hydrochloride from the leaf surface, as well as the transpiration rate of the plants, was probably affected by differences in light, temperature, and relative humidity. The volatility of chlorphenamidine is 4 mg per m^{3} at 30° C (Dittrich, 1966), and the compound evaporates rapidly from glass plates (Geissbühler, 1966; Sen Gupta and Knowles, 1969) and to a lesser degree from bean leaves (Geissbühler, 1966). Evaporation of chlorphenamidine hydrochloride from glass plates and bean leaves occurs much more slowly than chlorphenamidine (Ehrhardt and Knowles, 1970; Geissbühler, 1966). Also, because of the solubility properties of chlorphenamidine hydrochloride, it was necessary to modify the extraction procedure, which resulted in lower recoveries of radiocarbon.

We, therefore, concluded that metabolism and persistence of chlorphenamidine and chlorphenamidine hydrochloride in citrus are similar. Notwithstanding the variables described above, chlorphenamidine is apparently more persistent when applied to apple leaves than to grapefruit leaves. This is due, no doubt, to morphological and physiological differences in apple and citrus leaves which affect partition, volatility, solubility, and adsorbability of the chlorphenamidine molecule.

Dittrich (1966) applied a chlorphenamidine emulsion to the lower surface of bean leaves and found that spider mites feeding from the upper surface were killed. He later showed that mites were also killed when allowed to feed on the leaves of bean seedlings which were placed in a nutrient solution containing chlorphenamidine under conditions that precluded any vapor toxicity (Dittrich, 1967). He concluded that in bean seedlings at least chlorphenamidine was translocated in toxic amounts from the roots to the leaves and from the lower leaf surface to the upper portion of the leaf. Radiochemical analyses indicated there was apparently little movement of chlorphenamidine into stems when applied topically to grapefruit leaves (Ehrhardt and Knowles, 1970), and only slow movement of chlorphenamidine into leaves following the stem injection of apple seedlings (Sen Gupta and Knowles, 1969). Therefore, Ehrhardt and Knowles (1970) carried out additional translocation studies with chlorphenamidine, using grapefruit seedlings. When chlorphenamidine-^{14}C was applied topically in acetone solution to growing leaves, radioautographs prepared 8 days after treatment showed extensive diffusion of radioactivity throughout the treated leaf tissue (Figure 3). At the time of radioautography, however, no ^{14}C was detected in the veins or in the petioles (Figure 3). A similar pattern was seen in identical experiments using chlorphenamidine hydrochloride-^{14}C in methanol solution. Therefore, either both base and salt penetrate at a similar rate or there exists on the leaf surface a certain equilibrium between free base and salt regardless of which form is applied. In this latter case the lipophilic base would probably penetrate the cuticle more readily than the salt. Geissbühler (1966) suggested that either the base form of chlorphenamidine is rapidly dissolved or adsorbed in the waxy coating of the cuticular layer, or plant exudates, *e.g.*, calcium oxalate, present on the leaf surface buffer the material at a pH at which a considerable portion of the free base is converted to a salt form. The observation that chlorphenamidine hydrochloride as well as chlorphenamidine volatilize from bean leaves (Geissbühler, 1966) also suggests that an equilibrium exists between free base and salt on the leaf surface. A knowledge of the pK_a of the chlorphenamidine tertiary nitrogen and of the pH at the leaf surface would help clarify this point.

There was no detectable movement of radioactivity into adjacent stems and leaves 8 days after chlorphenamidine-^{14}C was applied to two lower leaves or two upper leaves (Figure 4). Slight movement of radioactivity into stems and leaves did occur when chlorphenamidine hydrochloride-^{14}C was applied topically to two lower leaves (Figure 5). Appreciable acropetal movement of radioactivity into stems and leaves was

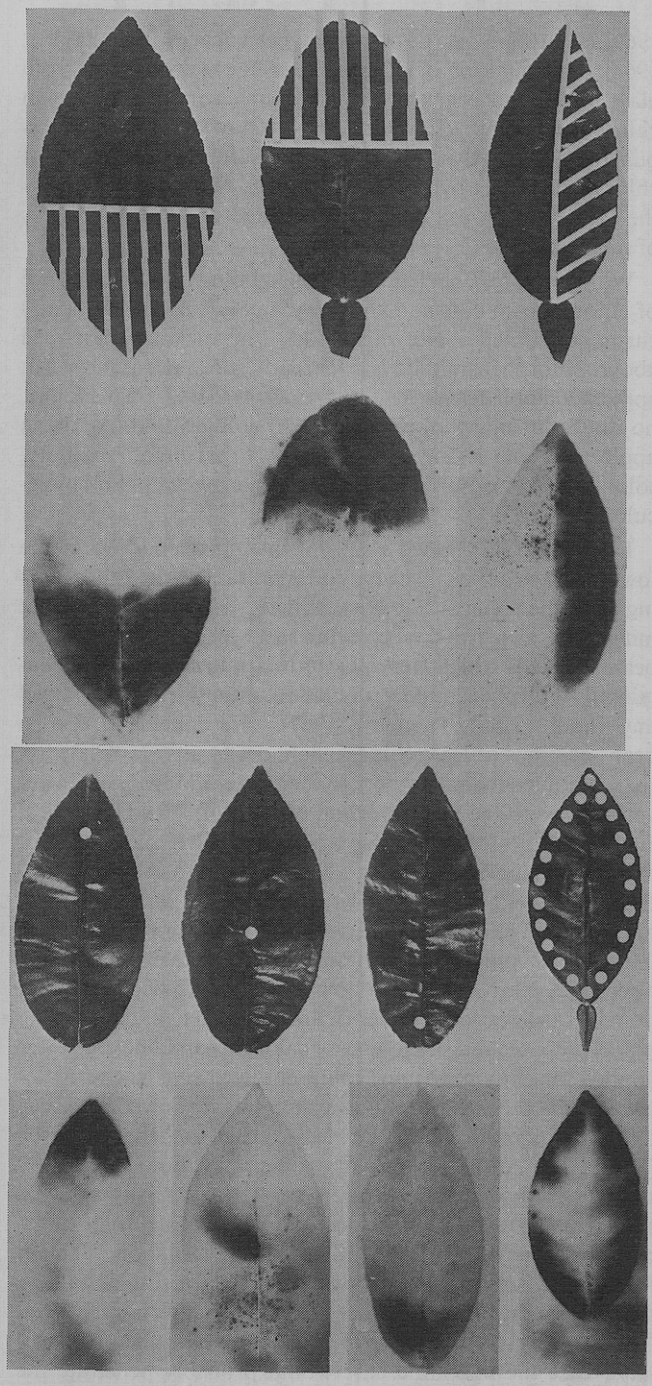


Figure 3. Translocation following partial topical application of chlorphenamidine-^{14}C to leaves of grapefruit seedlings *in situ* (Ehrhardt and Knowles, 1970). Reprinted with permission: *J. Econ. Entomol.* 63, 1306 (1970). Each leaf received 5×10^4 cpm of the acaricide; white lines and dots indicate treated areas. Plants were kept in greenhouse for 8 days prior to radioautography

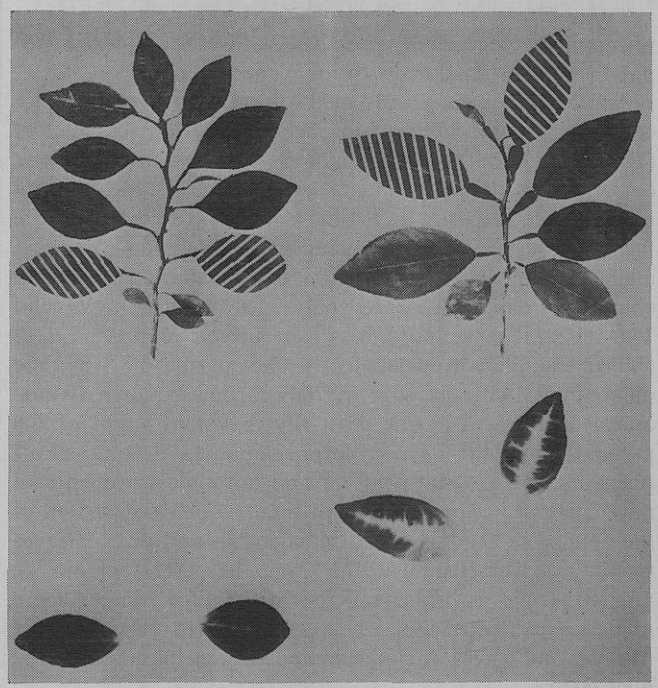


Figure 4. Radioautographs prepared 8 days after topical application of chlorphenamidine-^{14}C to two lower and two upper leaves of grapefruit seedlings at a radioactivity level of 10^5 cpm per leaf (Ehrhardt and Knowles, 1970). Reprinted with permission: *J. Econ. Entomol.* 63, 1306 (1970)

apparent when either chlorphenamidine-^{14}C (Figure 6) or chlorphenamidine hydrochloride-^{14}C was injected into the main stem of grapefruit seedlings. Thus, in those cases where movement of chlorphenamidine and its hydrochloride was detected in leaves and stems of citrus plants, it occurred mainly in the direction of the xylematic transpiration stream.

METABOLIC FATE OF FORMETANATE

Metabolism in Rats. Rats were treated orally with radioactive formetanate (ring-^{14}C), and the urine, feces, and selected tissues were analyzed to determine its metabolic fate (Sen Gupta and Knowles, 1970a). Absorption, metabolism, and elimination of the acaricide were rapid; 75% of the administered dose was excreted in the urine during the initial 12 hr after treatment (Figure 1C), with the peak concentration of formetanate-^{14}C equivalents detected at the 6-hr sampling interval. An additional 8% of the dose was eliminated via the feces during the 72-hr experimental period, and the peak level of fecal radioactivity occurred between 12 and 24 hr posttreatment.

The nature and concentration of the radioactive material in the urine were also investigated. Between 60% (at 6 hr) and 80% (at 72 hr) of the radiocarbon-containing components in the urine were water-soluble. Incubation of the 12-hr water-soluble components from rat urine with β-glucuronidase and β-glucuronidase-aryl sulfatase followed by extraction of the mixture with ethyl ether revealed that almost 90% of the radioactivity in the original aqueous fraction was in the form of enzymatically hydrolyzable conjugates. The major aglycone was 3′-hydroxyacetanilide (XIV), comprising 49 and 69% of

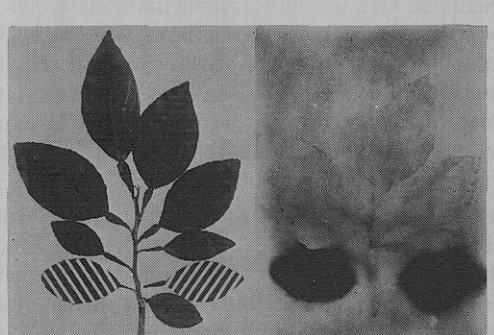


Figure 5. Radioautograph prepared 8 days after topical application of chlorphenamidine hydrochloride-^{14}C to two lower leaves of a grapefruit seedling at a radioactivity level of 10^5 cpm per leaf (Ehrhardt and Knowles, 1970). Reprinted with permission: *J. Econ. Entomol.* 63, 1306 (1970)

Table II. Fate of Formetanate When Applied Topically to Leaves of Orange Seedlings[a]

	Percent of Applied Radioactivity Recovered at Indicated Days After Treatment					
	0	4	8	12	16	20
Leaves						
Organic Solvent Fraction						
Formetanate[b]	93.3	34.8	30.2	23.8	22.7	20.5
Metabolites[b]	5.0	35.9	36.7	34.6	31.0	25.9
Aqueous Fraction	<0.1	16.1	20.0	22.6	22.6	27.0
Leaf Residue	<0.1	6.9	8.4	10.3	15.8	18.3
Stems	<0.1	0.4	0.9	1.3	3.2	3.6
Total Recovery of Radiocarbon, %	98.3	94.1	96.2	92.6	95.3	95.3

[a] Knowles and Sen Gupta (1970a). [b] Summation of percentage radioactivity in leaf rinse and leaf extract.

the ether-soluble radioactivity resulting from treatment with β-glucuronidase and β-glucuronidase-aryl sulfatase, respectively. Other aglycones included 3′-hydroxyformanilide (XII), 3-formamidophenyl methylcarbamate (X), *m*-aminophenol (XIII), formetanate (VII), and *m*-{[(methylamino)methylene]amino}phenyl methylcarbamate [demethylformetanate (VIII)]. We concluded that 3′-hydroxyacetanilide (XIV), 3′-hydroxyformanilide (XII), and *m*-aminophenol (XIII) were present *in vivo*, probably as *O*-conjugates with glucuronic acid and sulfuric acid and that formetanate (VII), demethylformetanate (VIII), and 3-formamidophenyl methylcarbamate (X) were probably *N*-glucuronides formed through the *N*-methylcarbamyl moiety and/or *O*-glucuronides formed through the enol form.

Ethyl acetate-soluble radioactive compounds extracted from rat urine included formetanate (VII), demethylformetanate (VIII), *m*-aminophenol (XIII), 3-formamidophenyl methylcarbamate (X), 3′-hydroxyformanilide (XII), and 3′-hydroxyacetanilide (XIV). The major "free" formetanate metabolite in the urine was 3-formamidophenyl methylcarbamate (X), accounting for 15% and 8.5% of the total radioactive material in the urine at 6 and 72 hr, respectively. The parent compound comprised only 0.2% of the urinary radioactivity 72 hr after treatment.

Levels of radioactivity in tissues 72 hr after treatment of rats with formetanate were low. The liver contained the maximum concentration of 0.178 ppm with formetanate-^{14}C equivalents in the other tissues analyzed, ranging from 0.033 to 0.091 ppm (Sen Gupta and Knowles, 1970a).

Degradation by Rat Liver Homogenates. Ahmad and Knowles (1970) studied the degradation of formetanate-^{14}C in various centrifugal fractions prepared from rat liver homogenates. Although the formetanate was highly unstable under the assay conditions employed, quantitative differences in the degradation products were evident. The 600 × G supernatant (nuclear supernatant) and the 100,000 × G supernatant (soluble) fractions were most active in the degradation of the acaricide. In contrast to the *N*-demethylation of chlorphenamidine, formetanate was converted to demethylformetanate (VIII) by a soluble DFP-sensitive enzyme. The degradation of demethylformetanate (VIII) to an unidentified metabolite was apparently catalyzed by a soluble SKF-525A-sensitive enzyme(s). Other soluble enzymes were involved in cleaving formetanate to *m*-{[(dimethylamino)methylene]amino}phenol (IX), hydrolyzing 3-formamidophenyl methylcarbamate (X) to 3′-hydroxyformanilide (XII), and deformylating 3′-hydroxyformanilide (XII) to *m*-aminophenol (XIII). The low activity of the microsomal fraction even in the presence of NADPH suggested that mixed function oxidases were not very active in formetanate degradation under the conditions employed.

Metabolism in Orange Seedlings. Since formetanate is an effective acaricide on citrus, Knowles and Sen Gupta (1970a) studied the fate of the compound when applied topically to leaves of growing orange seedlings (Table II). The concentration of formetanate (VII) in the leaf rapidly decreased from 93.3% of the applied radioactivity at 0 time to 34.8% by 4 days. This was followed by a lower rate of loss, since 20.5% of the applied radioactivity was still formetanate 20 days posttreatment. At 0 time, all of the formetanate as well as the other radioactive components could be removed from the orange leaves with two brief methanol rinses. However, at 20 days only 0.5% of the formetanate was in the methanol rinse fraction; the remaining 20% of the formetanate was in the leaf extract. The formetanate metabolites in the leaf rinse and leaf extract included demethylformetanate (VIII), 3-aminophenyl methylcarbamate (XI), *m*-aminophenol (XIII), 3-formamidophenyl methylcarbamate (X), and 3′-hydroxyformanilide (XII); *m*-{[(dimethylamino)methylene]amino}-

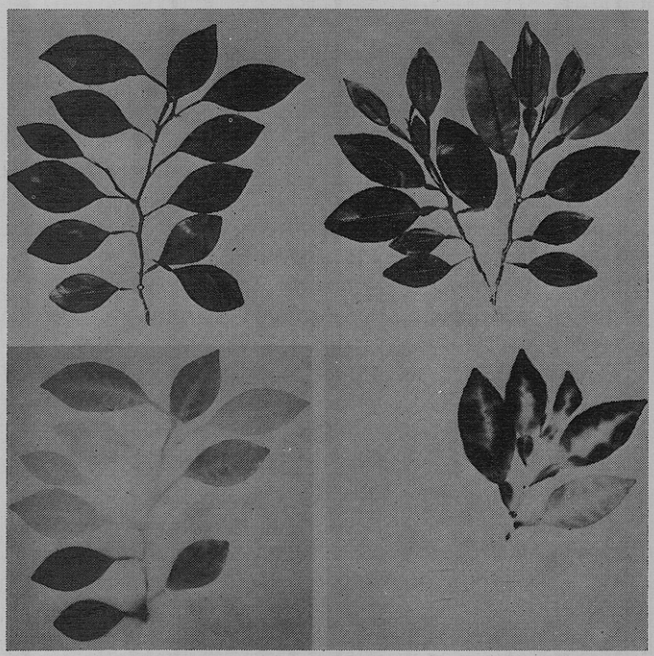


Figure 6. Translocation of chlorphenamidine-^{14}C 8 days after lower and upper stem injection of grapefruit seedlings with 10^5 cpm of the acaricide (Ehrhardt and Knowles, 1970). Reprinted with permission: *J. Econ. Entomol.* 63, 1306 (1970). Injection site indicated by black and white dot on stem

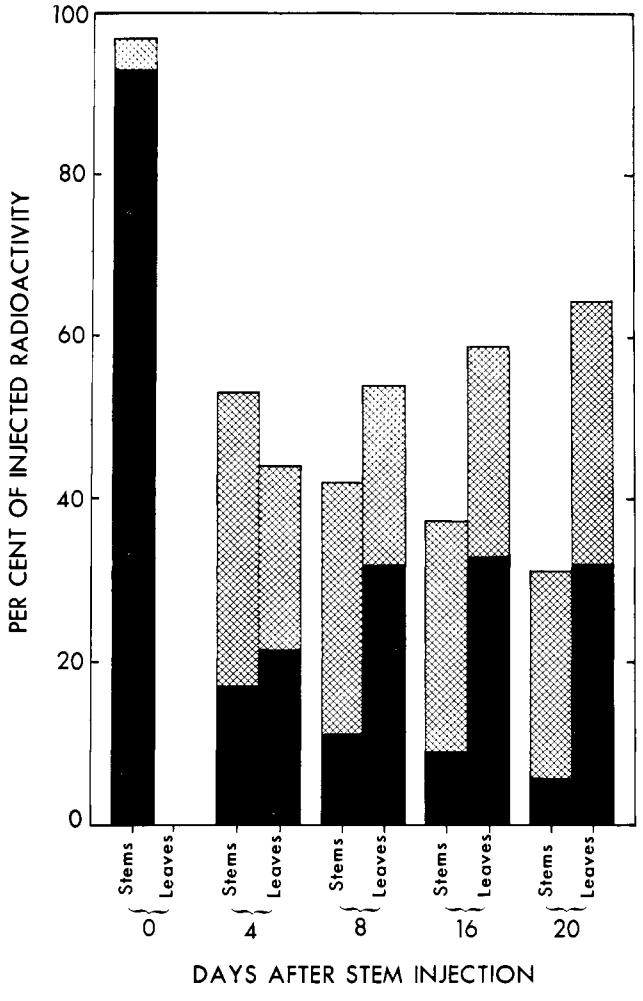


Figure 7. Fate of formetanate-^{14}C when injected into the stems of orange seedlings (Knowles and Sen Gupta, 1970a). Bars = total formetanate-^{14}C equivalents in stems or leaves; solid black portion denotes concentration of parent compound

phenol (IX) was detected in low concentrations only in the leaf extract.

Formetanate was metabolized in orange leaves to water soluble components (Table II). The greatest increase of radioactivity in the aqueous phase occurred during the initial 4 days after treatment (<0.1 to 16.1%), and even after 20 days only a further 10.9% of applied radioactivity was present in this fraction. The radioactivity in the leaf residue increased with time after treatment with a maximum of 18.3% of the applied radioactivity unextractable from the leaf tissue after 20 days (Table II).

Combustion analysis of the "stems," which included all of the plant except the leaves, revealed that only low levels of radioactivity were present with the maximum concentration of formetanate-^{14}C equivalents detected in the stems (3.6%) occurring after 20 days (Table II).

The high total recovery of radiocarbon, which averaged 95% during the 20-day posttreatment period, indicated that little, if any, formetanate volatilized from the plant surface (Table II).

When orange seedlings were injected in the main stem approximately 10 cm above the soil level with formetanate-^{14}C (Knowles and Sen Gupta, 1970a), there was a rapid increase in radioactivity in the leaves (Figure 7). Twenty days after injection, about 65% of the applied radioactivity was in the leaf tissue. The formetanate metabolites found in the stems and leaves were similar to those found in and on the leaves when the compound was applied topically to leaves. *m*-{[(Dimethylamino)methylene]amino}phenol (IX) was an exception, since it was not detected following stem injection.

A study was made of the nature of the water-soluble radioactive metabolites detected in the stems and leaves of orange seedlings at 20 days post injection. These components made up 15% (stems) and 10% (leaves) of the total injected radioactivity (Knowles and Sen Gupta, 1970a). Approximately 60% of these radiocarbon-containing compounds partitioned into ethyl acetate when the aqueous fractions isolated from the stems and leaves were incubated with β-glucosidase. The aglycones were identified as formetanate (VII), *m*-aminophenol (XIII), 3-formamidophenyl methylcarbamate (X), and 3′-hydroxyformanilide (XII); the last compound (XII) was the major component.

In these studies of formetanate metabolism in orange seedlings (Knowles and Sen Gupta, 1970a) all leaves from the plant were combined for analysis, making it impossible to determine if translocation occurred following foliar application. Experiments indicated, however, that formetanate was translocated from the stems into the leaves when the compound was injected directly into the main stem. Thus, further studies were conducted to investigate the movement of formetanate after topical application to leaves or injection into stems of orange seedlings (Johannsen and Knowles, 1970). Formetanate-^{14}C in methanol was streaked on the upper surface of orange leaves *in situ*, or applied as discrete droplets. Radioautographs were then prepared after the plants were held in the greenhouse for 8 days. Figure 8 shows that the direction of movement of the radioactivity was toward the distal end of the leaf and occurred primarily via the veins with little diffusion of the radioactivity into surrounding tissue. There was no detectable movement of radioactivity into the petiole, even when application of formetanate was made at the proximal end of the leaf blades. Also, there was no detectable movement of radioactivity into adjacent leaves or stems when formetanate was streaked on two basal leaves or two upper leaves of orange seedlings (Figure 9). On the other hand, injection of formetanate into the stems resulted in migration of radioactivity in the stems and leaves above, but not below, the injection site (Figure 10). In this case the radioactivity was most apparent in the midrib and veins of the leaf blades, and followed the xylematic transpiration stream.

These experiments suggest that formetanate penetrated the leaf slowly when topically applied. This is not surprising, since formetanate is a polar salt and the citrus leaf has an apolar waxy cuticle. Once penetration occurred, the radioactivity was localized in the veins rather than in the peripheral margins of the leaf. This slow movement from the vascular tissue probably results from the binding or adsorption of formetanate and/or its metabolites to components of the vascular system. The walls of the xylem are composed of lignified cellulose and contain many fixed acidic radicals capable of ionically bonding various positive ions that move in the transpiration stream (Canny, 1963).

OTHER CONSIDERATIONS

Since foliar spray is the usual method of applying chlorphenamidine and formetanate to plants, small amounts of the compounds could then enter the soil. The observation that small quantities of 4-chloro-*o*-toluidine and *m*-aminophenol were formed in plants treated with chlorphenamidine (Ehrhardt and Knowles, 1970; Sen Gupta and Knowles, 1969)

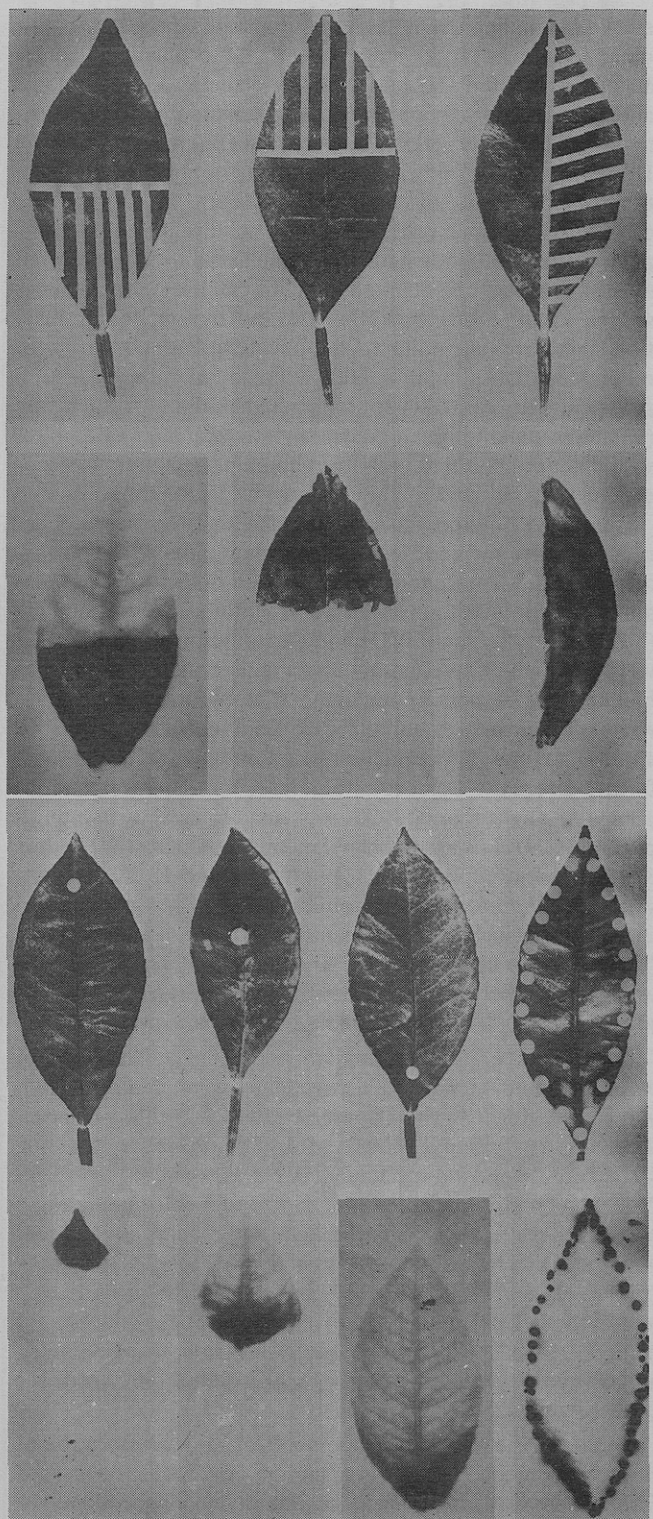


Figure 8. Translocation following partial topical application of formetanate-^{14}C to leaves of orange seedlings *in situ* (Johannsen and Knowles, 1970). See Figure 3 for legend

and formetanate (Knowles and Sen Gupta, 1970a), and that the chloroaniline produced by degradation of certain chloroacylanilide herbicides formed azo derivatives in soil (Bartha, 1968; Bartha and Pramer, 1967) and under peroxidase conditions (Bartha *et al.*, 1968) prompted additional studies of the fate of chlorphenamidine, formetanate, and certain of their metabolites.

Consequently, the degradation of chlorphenamidine-^{14}C by bacteria (*Aerobacter aerogenes* and *Serratia marcesens*), fungi

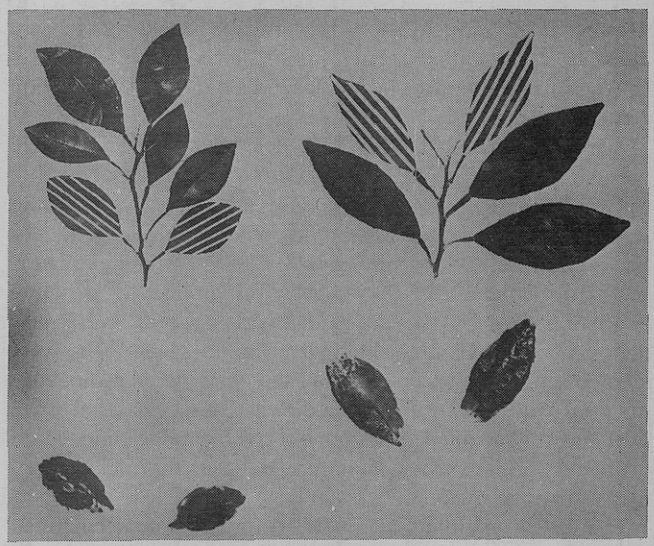


Figure 9. Radioautographs prepared 8 days after topical application of formetanate-^{14}C to two lower and two upper leaves of orange seedlings at radioactivity level of 10^5 cpm per leaf (Johannsen and Knowles, 1970)

(*Fusarium moniliforme* and *Rhizopus nigricans*), and an actinomycete (*Streptomyces griseus*) was studied by Johnson and Knowles (1970). The compound was metabolized extensively by all five microbial species. 4′-Chloro-*o*-formotoluidide (III) was the major organosoluble degradation product in the case of the two bacterial and fungal species. With *Streptomyces griseus*, the single actinomycete used, 4-chloro-*o*-toluidine (IV) was by far the predominant metabolite, accounting for 65% of the recovered radioactivity 48 hr after inoculation with chlorphenamidine. 4-Chloro-*o*-toluidine was also formed by the other microorganisms, but the symmetrical azo derivative of 4-chloro-*o*-toluidine was not detected in any of the microorganisms tested.

Ercegovich (1970) incubated soil with exaggerated concentrations (500 ppm) of nonradioactive chlorphenamidine and 4-chloro-*o*-toluidine. After 150 days, 4-chloro-*o*-toluidine was present in chlorphenamidine-treated soil, but its azo derivative was not detected (lower limit of detectability 0.1 ppm).

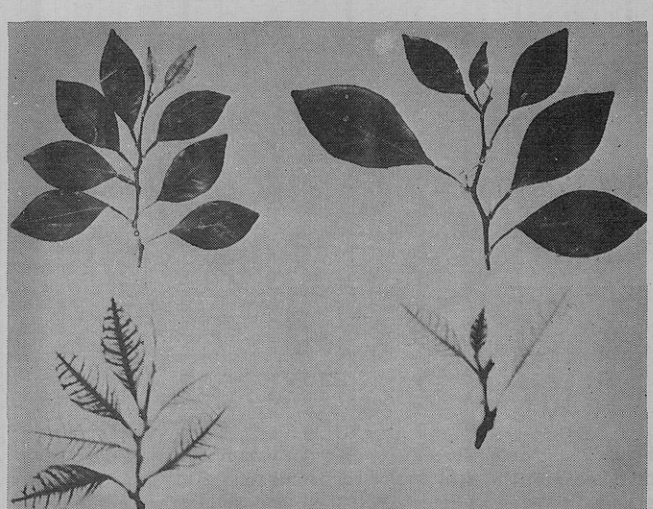


Figure 10. Translocation of formetanate-^{14}C 8 days after lower and upper stem injection of orange seedlings with 10^5 cpm of the acaricide (Johannsen and Knowles, 1970). Injection site indicated by black and white dot on stem

However, 2,2′-dimethyl-4,4′-dichloroazobenzene was present in 4-chloro-*o*-toluidine-treated soil at 20 days, and it increased in concentration up to 150 days, as judged by visual inspection of tlc plates.

Formetanate-^{14}C was rapidly degraded when incubated with an alkaline soil (pH 8.0) in laboratory tests, since 50% of the formetanate decomposed in less than 2 days (Arurkar and Knowles, 1970a). The major degradation products identified during the 16-day test period were 3-formamidophenyl methylcarbamate (X), 3′-hydroxyformanilide (XII), and *m*-aminophenol (XIII), but demethylformetanate (VIII) and and *m*-{[(dimethylamino) methylene]amino}phenol (IX) were also present. We speculated that the unidentified radioactive material (30% of the applied radioactivity recovered after 16 days) was either formetanate or a formetanate decomposition product(s) complexed with naturally occurring soil components (Arurkar and Knowles, 1970a).

The fate of *m*-aminophenol-^{14}C, a formetanate decomposition product, in an alkaline soil was studied by Sonawane and Knowles (1970). Although several unidentified radiocarbon-containing compounds were extracted from the soil, none cochromatographed with authentic 3,3′-dihydroxyazobenzene.

Knowles *et al.* (1969a,b) incubated high concentrations of nonradioactive 4-chloro-*o*-toluidine with horseradish peroxidase-hydrogen peroxide and with divalent iron-hydrogen peroxide. In both cases a product identical to 2,2′-dimethyl-4,4′-dichloroazobenzene as determined by ultraviolet, visible, and infrared spectrographic methods, melting point, and mixed melting point was isolated in low yields (<1%). Many other colored derivatives were also formed in both systems. The symmetrical azo derivative of *m*-aminophenol was not detected when formetanate or *m*-aminophenol was allowed to react under similar conditions (Arurkar and Knowles, 1970b).

This area warrants further study, since the toxicological significance of azo formation from pesticide degradation products in soil is not fully understood.

SUMMARY AND CONCLUSIONS

Chlorphenamidine and formetanate contain the dimethylaminomethyleneamino moiety linked to a substituted benzene ring. Thus, from a biochemical point of view, both acaricides undergo certain similar reactions *in vivo*. This conclusion is based on metabolites identified in plant leaves and/or stems and in mammalian urine during our studies of chlorphenamidine and formetanate metabolism. Chlorphenamidine (I) and formetanate (VII) underwent *N*-demethylation in plants and animals forming demethylchlorphenamidine (II) and demethylformetanate (VIII), respectively (Figures 11 and 12). However, the *in vitro* metabolic data suggest that *N*-demethylation of chlorphenamidine and formetanate is catalyzed by different enzymes at least in rats. Conversion of chlorphenamidine (I) to demethylchlorphenamidine (II) occurred chiefly in the microsomal fraction of rat liver homogenates supplied with exogenous NADPH and oxygen. This reaction, therefore, is similar to the oxidative *N*-dealkylation of other xenobiotics catalyzed by microsomal mixed function oxidases. In contrast to chlorphenamidine, formetanate (VII) was metabolized to demethylformetanate (VIII) by a soluble enzyme(s) from rat liver homogenates, and the conversion was inhibited by DFP but not by SKF-525A.

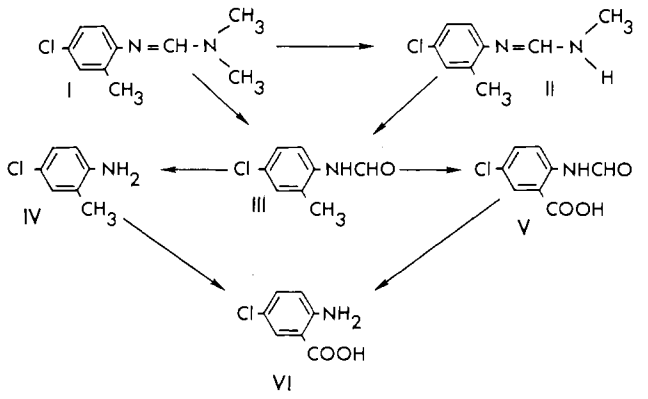


Figure 11. Metabolic path for chlorphenamidine. Roman numerals correlate with chemical and/or common names given in text. Compounds I through VI detected in "free" and conjugated form in mammals (Knowles and Sen Gupta, 1970b; Sen Gupta and Knowles, 1970b). Compounds I through IV isolated from apple seedlings (Sen Gupta and Knowles, 1969), grapefruit seedlings (Ehrhardt and Knowles, 1970), and microorganisms (Johnson and Knowles, 1970). Reprinted with permission: *J. Econ. Entomol.* 63, 856 (1970)

Chlorphenamidine, formetanate, and their *N*-demethyl derivatives were metabolized in plants and animals to *N*-formyl compounds. Chlorphenamidine (I) and demethylchlorphenamidine (II) were converted to 4′-chloro-*o*-formotoluidide (III), and formetanate (VII) and demethylformetanate (VIII) were similarly converted to 3-formamidophenyl methylcarbamate (X) (Figures 11 and 12). The *N*-formyl derivatives were subsequently metabolized to their corresponding substituted aniline. 4′-Chloro-*o*-formotoluidide (III) was transformed directly to 4-chloro-*o*-toluidine (IV), and 3-formamidophenyl methylcarbamate (X) was metabolized to *m*-aminophenol (XIII) *via* 3-aminophenyl methylcarbamate (XI) and/or 3′-hydroxyformanilide (XII) (Figures 11 and 12). The conversion of 4′-chloro-*o*-formotoluidide (III) to 4-chloro-*o*-toluidine (IV) and 3′-hydroxyformanilide (XII) to *m*-aminophenol (XIII) may be effected by the known soluble rat liver formamidase (Mehler and Knox, 1950) that catalyzes the hydrolysis of *N*-formyl-L-kynurenine to L-kynurenine and formate.

Differences in the mammalian and plant metabolism of chlorphenamidine and of formetanate were demonstrated. When rats, dogs, and goats were treated with chlorphenamidine, the tolyl methyl moiety of 4′-chloro-*o*-formotoluidide (III) and 4-chloro-*o*-toluidine (IV) was oxidized to yield *N*-formyl-5-chloroanthranilic acid (V) and 5-chloroanthranilic acid (VI), respectively. These anthranilic acids (V and VI) were not detected as chlorphenamidine metabolites in plants (Figure 11). In the case of formetanate, *m*-{[(dimethylamino)methylene]amino}phenol (IX) and 3-aminophenyl methylcarbamate (XI) were detected only in orange seedlings, and 3′-hydroxyacetanilide (XIV) was isolated only from rat urine (Figure 12).

Studies of the penetration, metabolism, and translocation of chlorphenamidine, chlorphenamidine hydrochloride, and formetanate in plants indicate once again the important role of bases and their corresponding salts in biological systems. Chlorphenamidine and chlorphenamidine hydrochloride are relatively stable molecules, and an equilibrium between free base and salt probably exists regardless of which form is applied to the plant foliage. Therefore, penetration with subsequent access to the transpiration stream resulting in long-range plant systemic action can occur. Because of the instability of its free base (Jenny, 1969), formetanate must be used in the salt form. The very slow movement of the salt into the leaf enables degradation to keep pace with penetration, resulting in negligible long-range systemic activity.

This review indicates that we have amassed considerable data on the fate of chlorphenamidine and formetanate in higher plants, mammals, microorganisms and/or soil. To

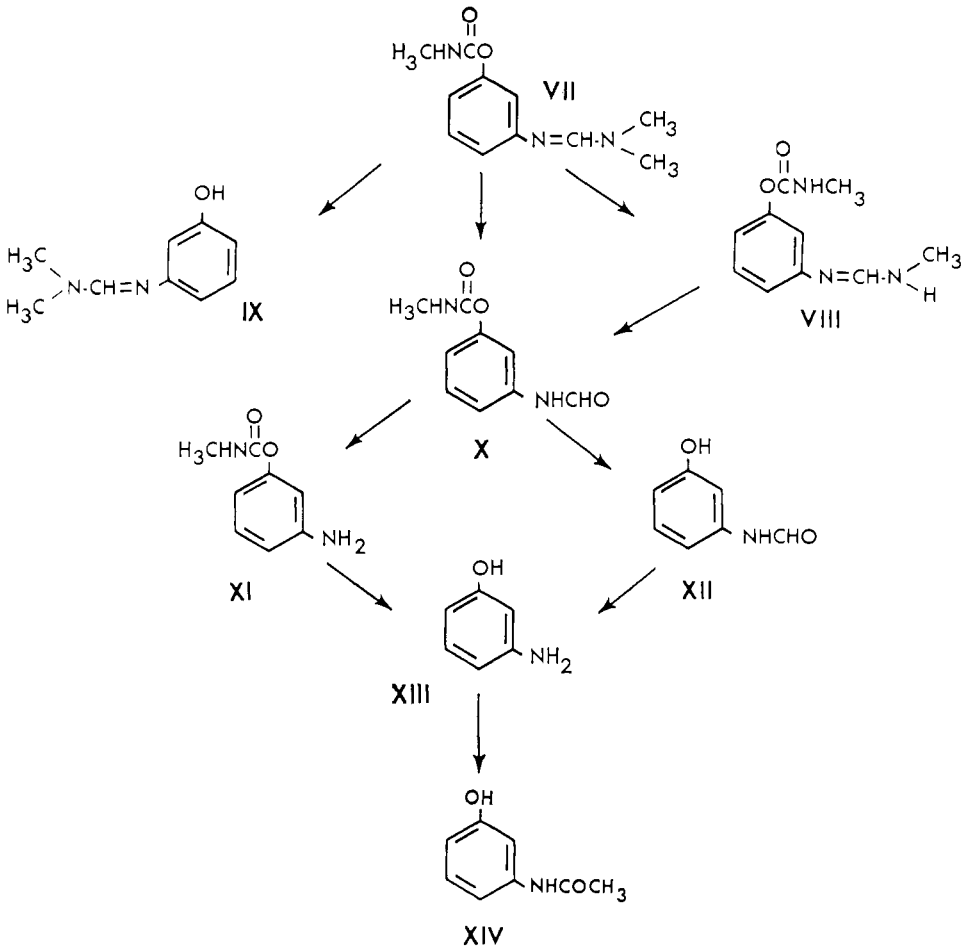


Figure 12. Metabolic path for formetanate. Roman numerals correlate with chemical and/or common names given in text. Compounds VII, VIII, X, XII, XIII, and XIV detected in "free" and conjugated form in rat urine (Sen Gupta and Knowles, 1970a). Compounds VII through XIII detected in orange seedlings (Knowles and Sen Gupta, 1970a). Compounds VII, VIII, IX, X, XII, and XIII isolated from formetanate-treated soil (Arurkar and Knowles, 1970a). Reprinted with permission: *J. Econ. Entomol.* 63, 615 (1970)

provide an even more complete understanding of the metabolism of chlorphenamidine and formetanate in biological systems, future studies should examine the metabolic fate of these compounds in insects and especially in mites—the target organisms.

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