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Chemistry, Biological Activity, and Uses of Formamidine Pesticides

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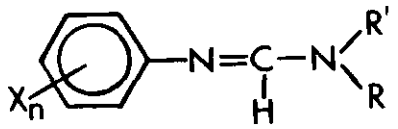
The formamidines, a relatively new group of acaricide-insecticides, are novel both in their range of biological activities and in their mode of action, which is presently unknown. This paper is a review of the historical development, properties, structures, uses, and chemistry of this group of pesticides, with particular emphasis on chlordimeform (Galecron or Fundal), *N'*-4-chloro-*o*-tolyl-*N,N*-dimethylformamidine, and amitraz, 1,3-di-(2,4-dimethylphenylimino)-2-methyl-2-azapropane. Their biological activity and uses are defined by their toxicity to spider mites, ticks, and certain insects, and they are particularly effective against juvenile and resistant forms of these organisms. A significant, but poorly understood feature of their field effectiveness is their breadth of toxic action which includes direct lethality, excitant-repellant behavioral effects, and chemosterilization. They are generally of low hazard for nontarget species with the significant exception of predaceous mites.

Several aspects of the chemistry of these compounds are considered, including structure–activity relations, synthetic pathways, isomerism and configuration, and their chemical and environmental stability. A significant feature of the metabolism and toxicity of these agents is the possible activation of chlordimeform by *N*-demethylation *in vivo*. Strong evidence for this has been presented with the cattle tick, but recent results discussed here *suggest that in other species, i.e., mice, German cockroaches or black cutworm eggs, N*-demethylation is neither a strong activation nor a detoxication reaction.

Introduction

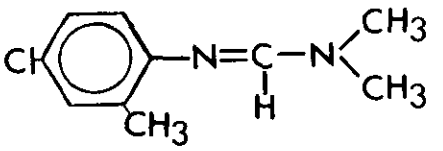
Interest in the trisubstituted formamidines of general structure I, developed strength in the late 1950's and early 1960's and has led to claims for an extremely broad spectrum of useful biological activity for this class of compounds. Such activity



I

ranges from bactericidal and antiprotozoal effects (*1*), through antihelminthic (*1,2*), fungicidal and herbicidal properties (*3–6*) to the insecticidal and acaricidal actions which are the subject of this paper. Additionally the pharmacological activity of several phenyl, naphthyl, and pyridyl dimethylformamidines has been the subject of an extensive series of papers from the Higher Institute of Medicine, Sofia (*7,8*).

Perhaps the most rewarding of these ventures resulted in the discovery of the acaricide-insecticide chlordimeform (II) which was first synthesized in 1963 by Schering A. G. in Germany in a program to develop herbicidal materials.



II

The choice of the critical 2-methyl-4-chloro substituents was based on analogy with the familiar herbicide MCPA (2-methyl-4-chlorophenoxyacetic acid) (V. Dittrich, personal communication). Concurrent investigation of this and

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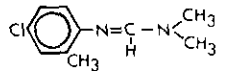
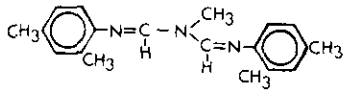
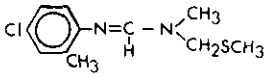
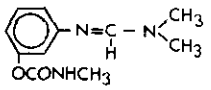
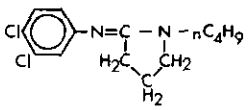
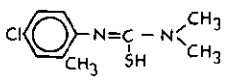
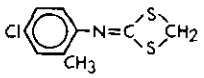
related formamidines at Ciba Limited has since resulted in joint development of this compound. Chlordimeform (previously known as chlorphenamidine) is of novel structure and action, and has proved to be one of the more significant innovations in pesticidal chemistry in the last decade. Inevitably it has opened the way to a number of other promising materials of related structure (Table 1).

Table 1. Formamidine and related pesticides.

Structure	Name and manufacturer
	Chlordimeform (Fundal : Nor-Am/Schering) (Galecron : Ciba-Geigy)
	Amitraz (BTS-27419 : Boots) (U-36059 : Upjohn)
	H-20013 : Hokko
	Formetanate (Carzol : Nor-Am/Schering)
	Clenpyrin (Bayer 6896)
	Chloromethiuron (C-9140 : Ciba-Geigy)
	AC-84633 : Am. Cyanamid

Chlordimeform [*N'*-(4-chloro-*o*-tolyl)-*N,N*-dimethylformamidine] is marketed in the United States as Galecron (Ciba-Geigy) and Fundal (Nor-Am). Among the related compounds, several of which have been introduced overseas, is amitraz (1,3-di-(2,4-dimethylphenylimino)-2-methyl-2-azapropane or, 1,5-di-(2,4-dimethylphenyl)-3-methyl-1,3,5-triazapenta-1,4-diene). This compound was first synthesized by the Boots Co. in England in 1969 (*9,10*), and presently is undergoing field testing in the United States by the Upjohn Co. A close relative of chlordimeform, differing only in the replacement of an *N*-methyl by an *N*-methylthiomethyl group is produced by Hokko Chemical Industry Co. in Japan as Hokupanon (*11*).

Formetanate, as the hydrochloride salt, is a registered insecticide-acaricide (Carzol of Nor-Am) which is both an arylformamidine and an arylcarbamate. However, it appears to exert its toxicity to rats, houseflies, and mites as an anticholinesterase agent rather than a formamidine (*12*), and thus it will not be considered further here. The final three compounds are not formamidines but are included here since they have structural features and biological actions which indicate a close affinity to the formamidines. All are recent compounds and their major current use is for cattle tick control in Australia, South America, and South Africa. The 2-(arylimino)-1-alkyl(or alkenyl)-pyrrolidines of which clenpyrin is a member are produced by Bayer A. G. and their properties have been discussed by Enders et al. (*13*). The structure shown for chloromethiuron is a tautomeric form of the more likely thiourea structure, in which >C=S is replaced by ⩾C-SH to better illustrate its analogy to the formamidines. Its properties have been briefly described by Dittrich and Loncarevic (*14*). It is similar to chlordimeform in effect but more persistent. Finally, AC-84633 is one of a recent series of acaricidal 2-)arylamino)-1,3-diethietanes from American Cyanamid (*15*).

The most recent development (*16*) is a further series of promising variants on the chlordimeform theme which have been patented by the Upjohn Co. In these structures the key feature is the presence of an *N*-sulfenyl group, e.g., *N*-thiophenyl (U-42558) or *N*-thiotrichloromethyl (U-42662) in place of one of the two *N*-methyl groups of chlordimeform. Related structures are based on N'-2,4-dimethylphenylformamidine. These compounds have notable residual and systemic activity.

Biological Activity and Uses

The formamidines have a unique and fascinating spectrum of biological activity, which is reviewed in Table 2. Naturally such sweeping categorizations of what is sensitive or insensitive are not absolute, but they do apply quite widely based on current knowledge. Sensitive organisms include a broad range of acarines. In agricultural practice important target groups would be phytophagous and parasitic mites, and ticks, particularly those of cattle. Additional and economically very significant activity is shown against a narrower group of insects, in particular

Table 2. Generalized selectivity pattern of formamidine pesticides.

Sensitive organisms	Insensitive organisms
Acarines	Invertebrates
Phytophagous mites	Most insects, including parasites, predators and pollinators
Predaceous mites	Spiders
Ticks	Vertebrates
Insects	Fish
Lepidoptera	Birds
Hemiptera	Mammals

Lepidoptera, although sensitivity varies considerably (*17*), and some Homoptera (e.g., aphids, scales, leafhoppers, and psyllids). In this regard, amitraz has not been described as having a strong action on most lepidopterous species, but it is active against Homoptera (*10*).

Toxicity of these materials to most other insects is quite low and, in general, beneficial insects such as parasites, predators, and pollinators are not severly reduced under normal conditions of field usage (*10,18–20*). Predatory spiders seem to be relatively insensitive (*20,21*), but predatory mites are clearly susceptible in many instances and several authors have concluded that chlordimeform is not compatible with pest management practices where such predators are a key factor (*22–24*).

Vertebrates in general are not severely threatened by the acute toxicity of chlordimeform or amitraz. For both compounds, LC_{50} (96 hr) values for fish range from 1 to 10 ppm, and birds (quail and ducks) have 8-day LD_{50} values of over 1000 ppm in the diet (*10,20*). Acute toxicity values for mammals are presented later, but the formamidines are not highly hazardous in this respect either. Subacute and chronic toxicity tests are reported to be satisfactory, e.g., chlordimeform in 2-yr feeding studies with rats and dogs had a maximum no-effect level of 250 ppm. This level was also without effect in three-generation reproduction studies in the rat (*20*) and translates to an acceptable daily intake (ADI) of 0.06 mg/kg-day for man, assuming a 100-fold safety factor. Residue tolerances in the United States generally range from 1 to 25 ppm in edible plant material and 0.05 to 0.25 ppm in animal products (*20*).

The mechanisms by which the formamidines reduce insect populations in the field are unusually complex and still not fully understood.

An important component of their action is direct lethality. This is particularly marked with the immature stages of the target species. With Lepidoptera, chlordimeform acts largely as an ovicide with sensitivity rarely extending beyond the earliest larval instars (*25,26*). In fact the term, "ovicide" may be a misnomer, since death occurs at about the time the young larvae are emerging from the egg (*17*; R. M. Hollingworth, unpublished data). With mites, the eggs and young larvae are again most sensitive, but here significant mortality is obtained even with the nymphs and adults (*20,27*). Again, considerable embryonic development occurs in the mite egg before death (*28*).

However, even in those cases where no direct lethal action occurs, chlordimeform may express a useful toxic effect less directly by at least two other general mechanisms. First, it has the property of exciting certain behavioral patterns, e.g., it shows a strong repellant–antifeedant action on both lepidopterous larvae and mites in laboratory (*20,29*) and field tests (*29,30*). In what may be a related effect, chlordimeform has the useful and unusual property of causing hyperexcitation and detachment of feeding ticks (*31,32*). In one of these studies (*31*), amitraz, H-20013, and AC-84633 had a similar effect, but chloromethiuron was inactive. Furthermore Phillips (*33*) has shown that after feeding chlordimeform to adult moths, their patterns of flight, mating, and oviposition are severely disturbed, e.g., flight was continual even during the day which is normally a time of rest, and difficulties were seen in the separation of the sexes after mating.

A second effect of chlordimeform on fecundity arises through its direct sterilizing action, causing both decreased egg production and diminished hatchability in both lepidopterans (*17,33*) and ticks (*34*). In the case of the bollworm, reduced fecundity may carry over into subsequent generations (*33*). Clenpyrin has been shown to have a similar series of actions i.e. causing irritation and paralysis, and reduced egg production in cattle ticks, probably by different mechanisms (*13*), and amitraz, chloromethiuron and H-20013 all show sterilizing activity against ticks (*9,34*).

Overall, control of insects, mites, and ticks by the formamidines is relatively slow, often taking several days for full effect. Significant vapor phase and systemic actions are also shown by chlordimeform, as discussed later, and thus according to circumstances it may act as a contact or stomach poison or as a fumigant.

From these biological properties the uses and limitations of the formamidines will be evident. Registered uses of chlordimeform in the United

States currently are against mites and lepidopterous species on cotton (particularly the *Heliothis* group and leaf perforator), deciduous fruits (e.g., codling moth, peach twig borer, pear psylla, and mites), and walnuts (e.g., codling moth, walnut aphid, mites), and against several lepidopterous pests of cole crops. Major uses elsewhere for the formamidines include control of rice stem borers, citrus pests, and cattle ticks.

An extremely significant general feature of the formamidine group in most of these uses is that the many acarines which have developed severe resistance to chlorinated hydrocarbon, organophosphate, and carbamate insecticides are generally as sensitive, or more sensitive, to the formamidines than are susceptible strains (*9,10,13,35,36*). Evidence of negatively correlated cross resistance has also been observed with chlordimeform in BHC-resistant rice stem borers (*30*).

The fact that, particularly with insects, only the early immature stages are susceptible presents a problem for control in infestations where older larvae or adults are present. For this reason, and in situations where insensitive species (e.g., boll weevils) are also damaging, combinations of chlordimeform with other insecticides such as toxaphene, methyl parathion, azinphosmethyl, and formetanate have often been used. A further favorable possibility in using such mixtures is that significant synergism may occur. This has been shown clearly in a number of studies with organophosphates, nicotine, and pyrethrins in mites and insects (*27,37,38*). In a recent publication, Plapp (*39*) found evidence of synergism in larvae of the tobacco budworm using mixtures of chlordimeform with numerous organophosphates, two pyrethroids, carbaryl, and the new anticuticular compound TH-6040. The greatest synergism was seen in those compounds with lowest innate toxicity. These observations raise the important question of the possibility of synergism with similar mixtures in vertebrates and beneficial insects.

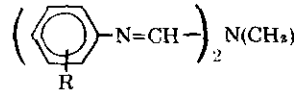
Because of its limited toxicity over the total life cycle and lack of prolonged persistence, chlordimeform in many situations is used in multiple application programs as long as egg-laying persists (*20*). In cotton this may mean application as often as every 3 to 5 days, and 7 to 10 days on cole crops.

Structure–Activity Relations

Even from the limited number of compounds shown in Table 1, a reasonably clear pattern for a successful insecticide-acaricide emerges. Ignoring the aberrant compound, formetanate, each material is a disubstituted-phenyl derivative with the combination of substituents limited to chloro and methyl. In particular, successful structures tend to be 2-methyl-4-chloro or 2,4-dimethyl. Even with clenpyrin, although the 3,4-dichloro analog was chosen for commercial development, the 2-methyl-4-chloro and 2,4-dihalo analogs are appreciably more toxic than clenpyrin to the cattle tick in laboratory tests (*13*). Obviously there is an intriguing specificity for substitution in the phenyl ring which is further illustrated by the data in Table 3, taken from structure–activity studies in the amitraz series (*9,40* Monosubstituted compounds were uniformly inactive. Although mitraz, the 2,4-dimethyl analog (III), is highly toxic to both ticks and mites, the presence of a 3-methyl group, e.g., 2,3-dimethyl (IV) or 2,3,4-trimethyl (V), selectively destroys activity against mites. On the other hand, the 2,4,5-trimethyl derivative (VI) is effective on mites but not on ticks, although the 2,5-dimethyl compound (VII) is inactive against mites. Perhaps the most surprising compound of all is the 2-methyl-4-chloro analog (IX), which has high activity against the tick but none against the mite, even though these mites are highly sensitive to the same substituent pattern in chlordimeform! Halogen substitution in the *ortho* position (XI–XIII) gives activity against ticks but not mites.

Abo-Khatwa and Hollingworth (unpublished data) studied the variation in toxicity of a number

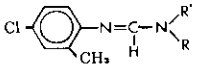
Table 3. Effect of different phenyl substituents on the toxicity of symmetrical triazapentadienes of the amitraz family.



Compound	R	LC50, mg/l[a] *Boophilus microplus* (larvae)	LC50, mg/l[a] *Tetranychus urticae* (eggs/larvae)
III	2,4-diCH3[b]	3	2
IV	2,3-diCH3	40	>1000
V	2,3,4-triCH3	30	>1000
VI	2,4,5-triCH3	>500	13
VII	2,5-diCH3	–	>1000
VIII	2,4,6-triCH3	85	280
IX	2-CH3,4-Cl	18	>1000
X	2-CH3,4-F	40	>1000
XI	2-F,4-CH3	40	500
XII	2-Br,4-CH3	85	>1000
XIII	2,4-diCH3,6-Cl	85	>1000

[a]Data from Harrison et al. (*9,40*).
[b]Amitraz.

Table 4. Variation of biological activity with structure in the *N'*-4-chloro-*o*-tolyl formamidines.



Compound	R	R'	*Tetranychus urticae* (ova)[a] % soln.	*Tetranychus urticae* (ova)[a] Mortality, %	*Boophilus microplus* (adult) DD_{50}, μg/tick[b]
II[c]	CH_3	CH_3	0.01	100	0.65
XIV	C_2H_5	C_2H_5	0.05	80	—
XV	*n*-C_3H_7	*n*-C_3H_7	0.05	85	0.90
XVI[d]	CH_3	CH_2SCH_3	—	—	0.012
XVII	H	CH_3	0.01	100	0.0009
XVIII	H	C_2H_5	—	—	0.03
XIX	H	*n*-C_3H_7	0.05	99	—
XX	H	*i*-C_3H_7	—	—	0.40
XXI	H	*n*-C_4H_9	—	—	0.07
XXII	H	*i*-C_4H_9	0.05	85	0.12
XXIII	H	*s*-C_4H_9	0.05	80	—

[a]Data from Arndt and Steinhausen (*41*).
[b]Dose which causes 50% of ticks to detach from host. Data from Stone et al. (*31*).
[c]Chlordimeform.
[d]H-200B.

of *N'*-substituted phenyl-*N,N*-dimethylformamidines to the eggs of the black cutworm and concluded that toxicity was favored by high lipid solubility, a relatively small group in the *para* position of the ring, and a bulky one in the *ortho* position. Interestingly, rather similar molecular requirements were needed for successful inhibition of monoamine oxidase by these same agents, while mitochondrial uncoupling depended solely on the lipophilicity of the molecule. The most toxic members of this series again were the 2-methyl-4-chloro, 2-methyl-4-bromo, and 2,4-dimethyl analogs.

Consideration of the structures in Table 1 reveals that there is scope for considerable variation in the structure of the rest of the molecule, at least as far as toxicity to cattle ticks is concerned. However, there is also significant specificity. For example, consider the data in Table 4 which shows the variation in sensitivity of a mite and a tick species to various *N'*-4-chloro-*o*-tolyl formamidines (*31,41*). In the dialkyl series (compounds II, XIV–XVI) all compounds show considerable activity but the dimethyl compound is preferable against the mites. Neither the dimethyl (II) nor dipropyl (XV) compound is highly effective in causing tick detachment, but the *N*-methyl *N*-methythiomethyl analog (H-20013, XVI) is potent. Among the *N*-monoalkyl substituents (compounds (XVII–XXIII) the methyl analog XVII is clearly superior in both tests though other alkyl substituents are also effective. The difference in potency to *Boophilus* between compounds II (chlordimeform) and XVII (its *N*-demethyl analog) is extraordinary high, i.e., about 700-fold. The complexity which can arise is typified by the fact that this clear superiority of the *N*-demethyl derivative (XVII) in causing detachment of ticks is not seen in considering either direct toxicity or sterilizing action (*34*) where chlorodimeform (II) is just as potent.

Structure–activity data are also available for the clenpyrin (*13*) and amitraz (*9,40*) series. With amitraz the highest activity against cattle ticks and mites is found with the *N*-methyl compound and longer alkyl chains again reduce or eliminate activity.

However in considering structure–activity relations of many of these analogs the possibility should be kept in mind that metabolic activation e.g., by conversion of an *N,N*-dialkyl to an *N*-monoalkyl form may have a significant influence on overall toxicity, especially with ticks, as discussed later.

Chemistry of the Pesticidal Formamidines

Synthesis

A number of routes to the *N*-arylformamidine structure are available, of which three stand out in terms of facility and generality. These are shown in Figure 1. The reaction of a substituted formamide with an aniline in the presence of an acid halide (e.g., $POCl_3$, $SOCl_2$, $COCl_2$, or an arylsulfonylhalide) has been widely used for the synthesis of formamidines (*1,41,42*) and provides one commercial route to chlordimeform (*43*). Alternatively, an arylformamide can be used in place of the aniline (*44*). Replacement of the usual dialkylformamide

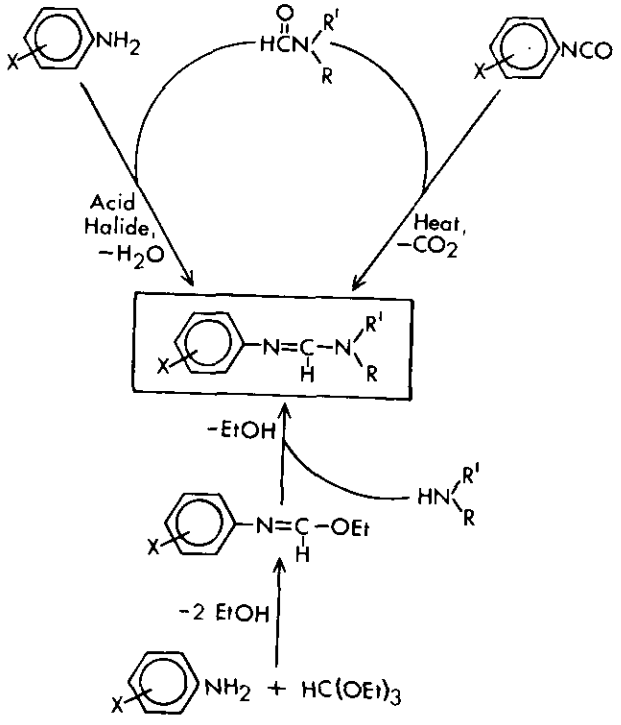


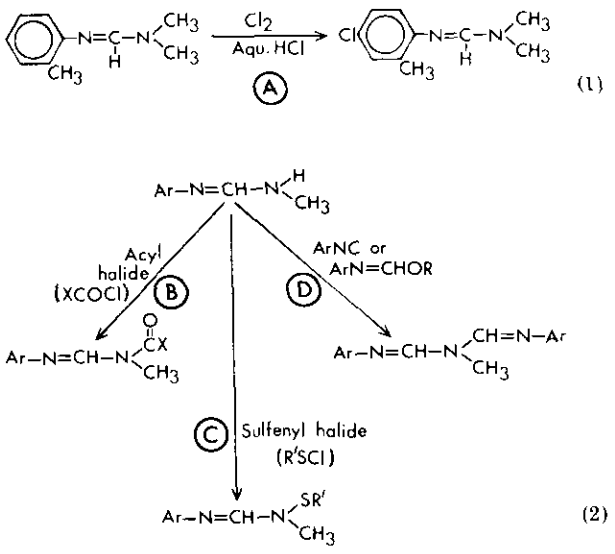
FIGURE 1. Synthetic routes to the *N*-arylformamidines.

by an *N*-alkylpyrrolidone yields products of the clenpyrin group (*13*).

A second general method involves heating a mixture of an arylisocyanate with a formamide, the reaction being marked by the evolution of CO_2 (*4,5*). Finally, the reaction of an aniline with triethyl orthoformate yields an intermediate formimidate ester which further combines with an amine to yield the desired formamidine (*45,46*). This is the logical method for the synthesis of the triazapentadienes such as amitraz since the reaction of 2,4-dimethylaniline and methylamine in the presence of triethyl orthoformate, yields *N'*-2,4-dimethyl-*N*-methylformamidine which will react at the free -NH group with a further equivalent of the same formimidate intermediate to yield amitraz, as shown in eq. (2), pathway D (*9*).

Other routes to the arylformamidines include the reaction of an aniline and an amine in the presence of HCN (*47*), the reaction of an aniline with *s*-triazine in the presence of a substituted amine (*48*), the oxidation of the corresponding aryl dialkyl thiourea with H_2O_2 (*49*), and the reaction of an aniline with dimethylformamide dimethylacetal (*50*).

Having obtained the basic substituted formamidine, further reactions may yield derivatives



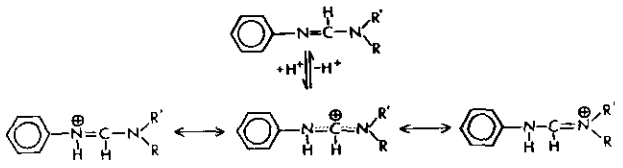
with interesting properties. Thus, an alternative commercial route to chlordimeform [eq. (1)] consists of preparing *N'*-*o*-tolyl-*N*,*N*-dimethylformamidine as above followed by chlorination in aqueous HCl (*43,51*). Presumably the 6-chloro derivative would also be formed to some extent.

Where the initial product is an *N*-monomethylformamidine, additional reactions at the free N-H group are possible. The formation of amitraz in this way [eq. (2D)] was mentioned above. The same product is formed by using an arylisocyanide in the presence of a metal or metal oxide catalyst, or even by condensation of two molecules of the intermediate *N'*-aryl-*N*-methylformamidine with the release of methylamine (*9*). Other possibilities include acylation with a carboxylic or carbonic acid halide [eq. (2B)] (*46*), or sulfenylation with an aryl-, alicyclicyl-, alkenyl-, or alkylsulfenyl chloride [eq. (2C)] (*16*).

Formamidines as Bases

A most significant property of the formamidines is their basicity. Thus chlordimeform is a base of medium strength [pK_a 6.8 in 50% aqueous methanol (*43*)] and forms stable salts with acids. Amitraz is described as a weak base and attempts to produce salts have not been successful, leading to decomposition (*9,40*).

It is generally accepted that in formamidines, protonation occurs at the imino nitrogen (*52,53*). As shown in eq. (3), two resonance forms may be drawn with the distribution of charge in the formamidinium cation, depending on the nature of the groups on the N atoms.



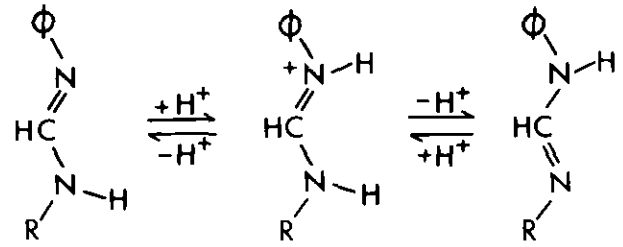
(3)

Since the pK_a value of chlordimeform is near 7, at physiological pH significant amounts of both the free base and ion will be present. This fact must be kept in mind in considering the possible interactions of these compounds with a potential target system; e.g., Abo-Khatwa and Hollingworth (*54*) found that the uncoupling action of chlordimeform on rat liver mitochondria increased with pH over the range 6.2–8.7, indicating that the free base was the active species in this reaction.

Isomerism in the Formamidines

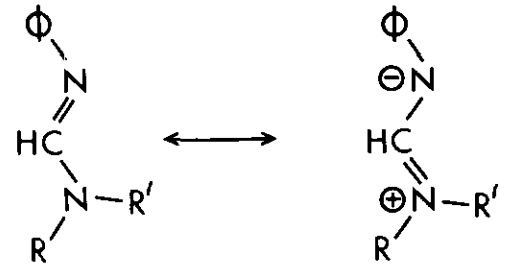
The configurational and electronic structure of amidines presents complexities and remains a subject of investigation. Prevorsek (*55*) outlined three types of isomerism of substituted formamidines which are applicable to the compounds under discussion here.

Tautomerism is possible only with *N,N'*-di- and *N*-mono-substituted formamidines, e.g., the *N*-demethyl analog of chlordimeform. The tautomeric forms are shown in eq. (4). The proportion of the two forms XXIVa and XXIVb present in solution clearly depends on the electronic nature of the substituents on the nitrogen atoms which govern their comparative basicities. In the *N'*-aryl-*N*-alkylformamidines, the nitrogen with the alkyl substituent will be the more basic, and thus tautomer XXIVa is favored.



XXIVa XXIVb (4)

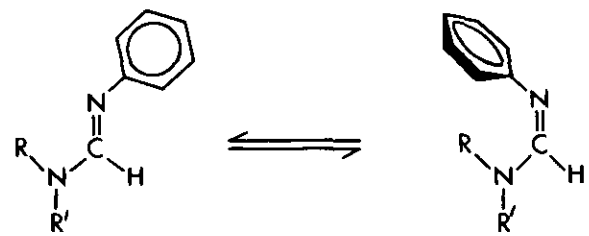
Hindered rotation around the C–N$(CH_3)_2$ bond arises due to resonance effects; e.g., ignoring resonance involving the aryl moiety, structures such as those shown in eq. (5) may be drawn.



XXVa XXVb (5)

The existence of forms such as XXVb gives this bond a partial double bond character, and, incidentally, ensures that the formamidine group is approximately planar (*55*). Several proton NMR studies have shown a magnetic nonequivalence for the protons of R and R' in these and closely related structures (*50,56–58*) which is attributed to the restricted rotation about the C—N bond. Obviously, if R ≠R', and a significant enough rotational barrier exists, two stereoisomers may be present. However, for the free base form, enthalpies of activation ($\Delta G\ddagger$) for this rotation lie in the range of 13–16 kcal/mole, considerably below the 23 kcal/mole needed to allow separation of the isomers at room temperature (*59*). The rotational barrier for the conjugate acid would be higher (*57*), in the region of 20 kcal/mole for the *N*-phenyl-*N,N*-dimethylbenzamidium ion (*58*).

Syn-anti isomerism is a clear possibility, based on the >C=N-bond as shown in eq. (6). The presence of these isomers is a second possible source of the R,R' magnetic nonequivalence mentioned above. However, there is considerable doubt whether the *syn*-isomer exists in any significant amounts with *N'*-aryl-*N,N*-dimethylformamidines. Space-filling molecular models show the *syn* isomer to be sterically crowded and several studies have claimed on the basis of proton NMR data that



Trans, Anti, E Cis, Syn, Z (6)

only the *anti* isomer is present (*50,56,57*). However, Filleux et al. (*60*) have cited evidence for the existence of the *syn* isomers of related formamidines using ^{13}C-NMR.

With *N'*-aryl-*N*-monoalkylformamidines there is a greater possibility that the *syn* isomer may exist, since they are less crowded in this configuration. Thus Wellman and Harris (*61*) concluded with several *N'*-aryl-*N'*-tert-butylformamidinium salts that not only does the *syn* isomer exist, but it actually may be the favored form in the equilibrium, depending on the nature of the aryl substituent. The group most favoring the *syn* configuration was *o*-methyl, which is also the most constant feature of the successful formamidine pesticides as discussed above.

Clearly the conformational status of the pesticidal formamidines is in need of definition as an aid to structure-activity and mode of action studies.

Physical and Chemical Characteristics

The ability to form salts with chlordimeform and some other formamidines has important implications for variations in the volatility, solubility, stability, and toxicity of the formamidines in the environment, or in different types of formulation. Formulations commonly used for chlordimeform employ the free base in emulsifiable concentrates or the hydrochloride salt in soluble powder form.

The data in Table 5, derived from the technical data sheets of the manufacturers (*20,62*) and Weighton et al. (*10*), compares some physical properties and acute toxicities for chlordimeform base, its hydrochloride salt, and amitraz. The relatively high volatility of chlordimeform base is easier to grasp when quoted as a saturation content for air of 4 mg/m^3 at 20°C (20 mg/m^3 at 35°C) (*20*). Thus, as already mentioned, this compound is capable of efficient fumigant action which may be of considerable importance under field conditions (*28,63*), e.g., exposure of the eggs of *Tetranychus telarius* to the saturated vapor for as little as 25 sec gave over 80% mortality (*27*). The salt of chlordimeform naturally has low volatility, as does amitraz.

Even as the free base, chlordimeform has appreciable water solubility. This is probably a major reason for its translocatability in plants; e.g., it is taken up by the roots of bean (*63*) and rice (*14,30*) plants and transported acropetally with systemic toxicity to plant-feeding pests. Translaminar movement and toxicity is seen when chlordimeform is applied to bean leaves (*28*), and extensive movement to the periphery of grapefruit leaves is seen when it is applied centrally (*64*). Storage and movement in the plant are considered to enhance its residual effectiveness. Despite the solubility of chlordimeform as both base and salt, it does not appear to leach readily from its site of application in the soil (*62*). Amitraz by contrast is rather insoluble in water. It shows translaminar but no strong systemic action (*10*).

Table 5 also presents data for the acute toxicity of these compounds to rats by oral or dermal exposure. The free base and salt forms of chlordimeform differ little in their oral toxicity particularly if considered on a molar basis. However the dermal toxicity, probably a more realistic indicator of hazard in the field, is much lower with the salt than the base, presumably due to differences in liposolubility and penetration through the skin. In one case (*62*) a dermal LD_{50} of 255 mg/kg was reported for a 50% emulsifiable concentrate of chlordimeform to mice, and some problems with toxicity to cattle have been observed in cattle dips at higher doses (*36*). Thus it appears that some caution might be called for in exposure by the dermal route. Amitraz is of generally low toxicity to rodents, although significant species variation occurs, with the oral LD_{50} for dogs and baboons lying in the 100–250 mg/kg range (*10*).

Chemical and Biological Stability

Hydrolysis: Values for the hydrolytic stability of these formamidines are shown in Table 5 and the

Table 5. Physical properties and acute toxicity of formamidines.

	Mp, °C	Vapor pressure (20°C), torr	Solubility (H_2O, 20°C)	Toxicity in rat as acute LD_{50}, mg/kg Oral	Dermal	Half life (H_2O, pH 7)
Chlordimeform base	32	3.5×10^{-4}	250 ppm	162-220	640	42 hr (30°C)
Chlordimeform HCl	225-227 (decomp.)	2.2×10^{-7}	>50%	280-325	~4000	—
Amitraz	87–88	3.8×10^{-7}	<1 ppm	600	>1600	>12 weeks (20°C)

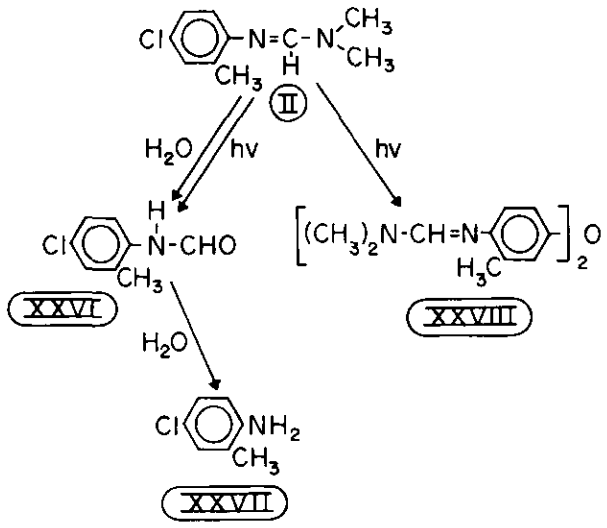


FIGURE 2. Pathways of aqueous hydrolysis and photodecomposition of chlordimeform.

pathway for hydrolysis of chlordimeform is presented in Figure 2. The primary product is the *N*-arylformamide, *N*-formyl-4-chloro-*o*-toluidine (XXVI), which is slowly converted to 4-chloro-*o*-toluidine (XXVII) by further hydrolysis. Amitraz on hydrolysis first yields *N'*-2,4-xylenyl-*N*-methylformamidine as a major product which may then follow the analogous pathway to chlordimeform, first yielding the *N*-formylxylidine, and then the unsubstituted xylidine (*9*). At pH 7.0, chlordimeform is hydrolyzed rather rapidly, while amitraz is relatively stable (Table 5). However, in each case, the rate of hydrolysis is strongly pH-dependent; e.g., Roulston et al. (*36*) report a half-life for chlordimeform at 10°C of about 38 days at pH 7 and 8 days at pH 8. At 30°C these values are reduced to about 3 and 0.5 days, respectively. A solution of the hydrochloride salt (pH 3–4) shows no appreciable hydrolysis over several days (*20,65*), and acid buffering drastically enhanced stability of chlordimeform in cattle dips (*36*). By contrast, amitraz is relatively stable as a finely divided suspension at alkaline pH (20% decomposition in 12 weeks at pH 12, 20°C) but is unstable in acid (86% decomposition in 1 day at pH 2, 20°C) (*9*).

Photodecomposition: Photodecomposition of chlordimeform is also pH-dependent. Su and Zabik (*65*) have found that an aqueous solution of chlordimeform hydrochloride (pH 3.1) was unaffected by mercury lamp irradiation for up to 12 days at 25°C , while a solution of the free base at pH 7–8 was decomposed to the mixture shown in Figure 2, consisting of the *N*-formylchlorotoluidine (XXVI, 95%) and a bisformamidine (XXVIII, 5%) in which the two phenyl groups are connected by an ether bridge. Free-radical mechanisms were suggested for each route.

Photodecomposition of chlordimeform has also been studied on silica gel chromatographic plates (*66*) with irradiation by long- and short-wave ultraviolet light, fluorescent light, and sunlight (under glass) over periods of 10 to 20 hr. Again the major product was XXVI (Fig. 2) with ultraviolet light or sunlight. Compound XXVIII was not reported, but a number of other degradation products were found, some unidentified, which were relatively minor with the exception of short-wave ultraviolet irradiation where 15% of the material was converted to largely unknown polar compounds. Fluorescent light caused little decomposition, but the other treatments resulted in 12% decomposition in 10 hr (sunlight) or 25% in 20 hr (either ultraviolet light). Considerable degradation occurred, even on the plates kept in the dark as controls.

Data are lacking on the photochemistry of amitraz, but both it and chlordimeform, alone or in formulation, are relatively stable to heat (*10,20*).

Biodegradation: Extensive studies of the metabolism and fate of chlordimeform have been published, particularly by Knowles and his coworkers, (*67*), but data for amitraz still await publication. Since this topic is reviewed elsewhere in an accompanying paper from this symposium (*68*), only the briefest outline is given here. Figure 3 shows the general metabolic pathways for chlordimeform in most organisms studied including vertebrates, invertebrates, plants, and microorganisms. In addition to the familiar *N*-formylchlorotoluidine and chlorotoluidine which are major metabolites, additional oxidative reactions are seen, i.e., ring methyl oxidation yielding

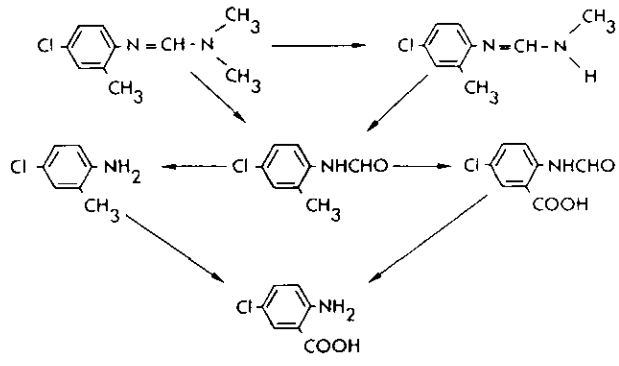


FIGURE 3. Generalized pathways of biodegradation of chlordimeform [Reprinted with permission from J. Econ. Entomol. 63: 856 (1970).]

anthranilic acids and *N*-demethylation to yield *N*-demethyl chlordimeform. Several unidentified and bound metabolites are generally found, and all of the metabolites may be present as conjugates which yield to arylsulfatase/β-glucuronidase cleavage.

Breakdown by soil microorganisms is quite rapid (*68–70*), yielding, besides the metabolites shown in Figure 3, dehalogenation products (*69*) and a malonic acid conjugate of the released chlorotoluidine (*70*). In mammals (dog, goat, and rat) uptake of an oral dose and its excretion are both rapid and complete, 65–85% of the dose appearing as urinary metabolites within 24 hr. Little storage occurs in the tissues (*67*).

The overall picture of the physical and biological stability of chlordimeform therefore clearly suggests that it is quite unlikely to accumulate in the environment or within organisms. Although detailed studies have not been published, general values for the stability in soil indicate a half-life in the range of 2–5 weeks (*62,70,71*), and its instability in neutral aqueous solution has been a barrier to its use in cattle dips (*36*). Measurements of the disappearance of chlordimeform from several fruits after normal field application (*71,72*) indicated that residues dissipated rather rapidly. Although residue levels varied widely initially, depending on the type of fruit, generally within 3 to 6 weeks after spraying they were below 1 ppm. Peaches, however, which had the highest initial residue (6.6 ppm at 14 days) still had 1.6 ppm after 70 days. Residues were mainly confined to the peel of the fruits and it was concluded that losses were largely due to weathering and growth dilution.

Several studies have been published on the fate and residues of chlordimeform applied to plants. Generally a very rapid and extensive initial loss, presumably by volatilization, is followed by a much slower loss and degradation of the remaining residue in and on the leaves over several weeks. However, the details of this process vary with the plant species and growing conditions. Ehrhardt and Knowles (*64*) found 70% loss of a chlordimeform deposit from grapefruit leaves after 4 days. Internal levels of the parent material or its *N*-demethyl analog in the leaves were always minor (2% or less of the dose). The initial loss from apple leaves was less extensive (40% in 4 days), and a much larger fraction of the applied chlordimeform was present in the leaf and was still increasing (to 11.6% of the dose) 21 days after application (*67*). Again, only minor amounts of the *N*-demethyl metabolite were present. The situation was rather different with cotton leaves (*73*) where, after an initial extensive loss (60% in 4 days, most of this in the first 4 hr), up to 17% of the dose was present as the *N*-demethyl analog after 2–3 weeks, with little of the parent chlordimeform present. Analytical methods for chlordimeform and its residues have been reviewed by Voss et al. (*43*).

Potential Toxicity of Metabolites

With the exception of the *N*-demethyl derivative, the known major metabolites of chlordimeform are of limited acute toxicity, although the toxicity of the minor photoproduct XXVIII (Fig. 2) does not seem to have been examined. However, some concern has been expressed over the possibility of conversion of the common metabolite 4-chloro-*o*-toluidine to a potentially reactive and carcinogenic azo derivative, a common route for chloroanilines in soil (*74*). The situation to 1970 in this respect is reviewed by Knowles (*67*) but definitive data were lacking then, and little has been published since. In the studies with soil microorganisms reviewed above, however, no evidence for the formation of azo derivatives from chlordimeform was presented.

The fact that the *N*-demethyl metabolite of chlordimeform has considerable toxicity both to pest species (Table 4, XVII) and vertebrates raises an important question of its contribution to overall toxicity. The importance of this question is intensified by the studies of the toxicity of chlordimeform and its *N*-demethyl analog to cattle ticks; e.g., Knowles and Roulston (*34*) showed that cotreatment of ticks with chlordimeform and methylenedioxyphenyl synergists such as piperonyl butoxide eliminated the toxicity of chlordimeform, but slightly enhanced toxicity of the *N*-demethyl compound. The reasonable interpretation that synergists eliminated the oxidative *N*-demethylation of chlordimeform and that *N*-demethyl chlordimeform is the major toxicant is supported by later studies of the effect of piperonyl butoxide on the penetration and metabolism of chlordimeform in cattle ticks (*75*), and the fact that in tick detatchment studies the *N*-demethyl compound is about 700-fold more effective than chlordimeform (Table 4). Other evidence supporting the idea that *N*-monomethylformamidines (e.g., formed from amitraz) are the actual toxicants for ticks is discussed by Knowles and Roulston (*34*) and Stone et al. (*31*).

Recently we have examined this idea further with other invertebrates and mice (R. M. Hollingworth, unpublished data). The effects of pretreatment with synergists is shown in Table 6. With black cutworm eggs and the German cockroach the toxicity of chlordimeform is slightly enhanced by piperonyl butoxide. With the mouse, piperonyl butoxide (400 mg/kg, intraperitoneally 2–3 hr

Table 6. Effect of several pretreatments affecting microsomal oxidation on toxicity of chlordimeform.

Pretreatment	Black cutworm ova, LC_{50}, hr[a]	German cockroach, topical LD_{50}, mg/kg	Mouse oral LD_{50}, mg/kg
Control	6.2	650	245 (195–310)
Piperonyl butoxide	4.1	555	340 (255–435)
SKF-525A	—	—	256 (215–305)
Control	—	—	238 (206–275)
Phenobarbital	—	—	298 (264–337)

[a]*Time of exposure to vapor causing 50% mortality.*

before the chlordimeform) does reduce toxicity as with cattle ticks, but the effect is slight, and SKF-525A (50 mg/kg, intraperitoneally 2–3 hr before the chlordimeform) has no significant effect on toxicity to the mouse, although it has been shown to be an effective *in vitro* inhibitor of the *N*-demethylation of chlordimeform by rat liver microsomes (*76*). The corollary of the decrease of microsomal *N*-demethylation by the use of inhibitors is to increase the level of microsomal oxidations by pretreatment with phenobarbital. If *N*-demethylation is a crucial activation process *in vivo*, this pretreatment should then increase toxicity. In fact, it decreases it slightly. Thus none of the pretreatments used here had any very marked effect on toxicity. Similarly, piperonyl butoxide did not antagonize toxicity of chlordimeform to houseflies (*77*).

Thus it seems that the concept of activation of chlordimeform by *N*-demethylation applies only to ticks among the organisms so far examined. However, it remains to be shown by metabolic studies that the treatments described above do in fact alter the level of the *N*-demethyl analog *in vivo* in the fashion predicted.

Table 7. Comparative toxicity of chlordimeform and its *N*-demethyl analog.

	Chlordimeform	*N*-Demethyl analog
German cockroach LD_{50}, mg/kg	650	555
Black cutworm ova LC_{50}, % soln.[a]	0.04	0.12
Mouse LD_{50}, mg/kg		
Oral	285	230
IP	104	93
Monoamine oxidase, rat liver I_{50}, μmole/l.	29	24
Uncoupling, rat liver UR_{50}, μmole/l[b].	35	65

[a]Determined by dipping method.

[b]UR_{50} is the concentration needed to stimulate state 4 respiration by 50% above control rate.

The comparative toxicity of chlordimeform and its *N*-demethyl metabolite to the same organisms is presented in Table 7. In each case, the *N*-demethyl analog shows toxicity broadly comparable to the parent material, and is marginally but clearly the more toxic of the two compounds to mice. Data are also presented for their comparative potency *in vitro* as inhibitors of monoamine oxidase and as mitochondrial uncoupling agents. Although neither of these biochemical lesions has yet been plausibly related to acute toxicity, it is clear that again the two compounds are of generally comparable potency in these biochemical actions.

A reasonable preliminary conclusion from these results, contingent on further studies in progress, is that the *N*-demethylation of chlordimeform in most organisms is close to a neutral process toxicologically, since the *N*-demethyl derivative itself differs only marginally in potency from the parent compound.

Clearly, in conjunction with the continuing research into mode of action and therapeutics, which are the most pressing unanswered questions concerning the pesticidal formamidines, there is a very real need for further study of the pharmacological and biochemical effects, and toxicological significance of the major degradation products of these materials.

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