for **25** suggests that the R_{SP} flies are capable of detoxification by other mechanisms. In this regard the LD_{50} of **25** synergized with 5:1 piperonyl butoxide for the S_{NAIDM} strain was 7.5 μg/g. The results indicate that **25** is detoxified to a small extent by the mixed function oxidase enzymes.

Except for the trifluoromethyl derivative (**26**), the other analogues also showed insecticidal activity but none were as potent as **25**. The absence of insecticidal activity of **26** and **27** again points out the deactivating influence of the trifluoromethyl moiety in the Z position. Because of the variety of substitutions which were made relative to the original DDT molecule it was not possible to analyze the data by multiple regression analysis (Fahmy et al., 1973). However, comparison may be made with some of the compounds in Table II with those of the DDT type in which Z is trichloromethyl and L is hydrogen. The direct analogue of compound **25** is 1,1,1-trichloro-2,2-bis(*p*-ethoxyphenyl)ethane. The housefly LD_{50} and *Culex* larvae LC_{50} of this compound are 7.0 μg/g and 0.04 ppm, respectively. While **25** is slightly less toxic to these insects than the corresponding DDT analogue, the toxicities are close enough to support the rationale used for the synthesis of these compounds. A similar comparison may be made with compound **21** and 1,1,1-trichloro-2-*p*-chlorophenyl-2-*p*-anisylethane (housefly LD_{50} 41.5 μg/g and *Culex* larvae LC_{50} 0.058 ppm).

Although the insecticidal activities of the compounds in Table II appear to be slightly less than their related DDT analogues, the results, nevertheless, indicate that derivatives which contain an α-methyl group also may possess high insecticidal activity. To our knowledge, this is the first case where significant insecticidal activity has been demonstrated with compounds of this type. It should be pointed out that the present study was exploratory in nature and only a few compounds were synthesized and evaluated. The approach, however, appears to be worthwhile and a large number of related derivatives remain to be examined. Further work is in progress.

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Toxicity of N-Sulfenylated Derivatives of Insecticidal Methylcarbamate Esters to the Honeybee

Narayana Moorthy Mallipudi and T. Roy Fukuto*

A series of *N*-arylsulfenyl, *N*-alkylsulfenyl, and *N*-aminosulfenyl derivatives of several commercial insecticidal methylcarbamate esters was examined for toxicity to honeybees and houseflies. All but one of the 34 compounds showed high toxicity to the honeybee. 2-Isopropoxyphenyl *N*-methyl-*N*-(2-methyl-4-*tert*-butylphenylsulfenyl)carbamate with a honeybee LD_{50} >800 μg/g was more than 33 times less toxic to honeybees than to houseflies (LD_{50} 24.5 μg/g). In comparison, the parent carbamate, propoxur, was more toxic to the honeybee (LD_{50} 4.5 μg/g) than to the housefly (LD_{50} 24.0 μg/g). The toxicity of 2-isopropoxyphenyl *N*-methyl-*N*-(2-methyl-4-*tert*-butylphenylsulfenyl)carbamate to the honeybee was synergized more than 18-fold by piperonyl butoxide. Compared to propoxur, the *N*-aminosulfenyl derivatives of propoxur were generally of equal toxicity to houseflies, substantially more toxic to mosquito larvae, and much less toxic to mice.

A previous report from this laboratory (Black et al., 1973a) described the favorable toxicological properties of a variety of *N*-arylsulfenyl and *N*-alkylsulfenyl derivatives of some common methylcarbamate insecticides. The insecticidal activity of either parent or derivatized carbamates was approximately the same, but mammalian toxicity was drastically reduced in the case of the derivatives. Based on a comparative metabolism study in the housefly and white mouse, the desired order of selectivity of the sulfenyl derivative *N*-(2-toluenesulfenyl)carbofuran was attributed to different pathways of metabolism between the insect and mammal (Black et al., 1973b). In houseflies, lethal quantities of carbofuran were formed in vivo after topical application of *N*-(2-toluenesulfenyl)-carbofuran, while the mouse preferentially degraded the derivative at the carbamate ester linkage, possibly by carboxylesterase action, to the nontoxic phenol.

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Less emphasis has been placed in past years on studies concerned with differences in toxicity of insecticidal materials toward different insect species, particularly with regard to differences in response between beneficial and harmful insects. Although organophosphorus esters containing the diisopropoxy moiety, e.g., diisopropyl parathion, are known to be peculiarly safe to the honeybee while still retaining high activity toward other insects (Metcalf and Frederickson, 1965; Camp et al., 1969; Dauterman and O'Brien, 1964), carbamate insecticides generally are considered to be highly toxic to the honebee (Georghiou and Atkins, 1964). Because of the favorable order of selectivity achieved between insects and mammals by derivatization of carbamate insecticides, an assessment of the toxicity of derivatized methylcarbamates to the honeybee also was made. This report is concerned with the toxicological properties of a series of N-sulfenylated derivatives of commercial methylcarbamate insecticides with special focus on the toxicological response observed in honeybees.

MATERIALS AND METHODS

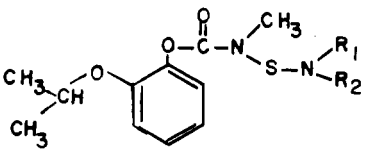
The methylcarbamate insecticides carbofuran, propoxur, and carbaryl were obtained from their respective manufacturers as technical materials and were purified by recrystallization from appropriate solvents. Several of the *N*-arylsulfenyl and *N*-alkylsulfenyl derivatives of the carbamates were obtained from a previous study (Black et al., 1973a) and the rest were synthesized by the procedure described by Black et al. (1973a). The purity of products were checked by thin-layer chromatography (solvent system, ether/hexane 1:3). Only a single TLC spot was seen under UV light. *N,N*-Dialkylaminosulfenyl chlorides were prepared immediately before use by condensing sulfur dichloride or sulfur monochloride with the appropriate amine in anhydrous diethyl ether (Weiss and Schulze, 1962).

Synthesis of *N*-Aminosulfenyl Derivatives of Carbamates. These derivatives of insecticidal methylcarbamates were synthesized by the reaction between the methylcarbamate and *N,N*-dialkylaminosulfenyl chloride in pyridine (Fukuto et al., 1975). The following procedure for the synthesis of 2-isopropoxyphenyl *N*-dipropylaminosulfenyl-*N*-methylcarbamate is typical. Freshly distilled dipropylaminosulfenyl chloride (5.03 g, 0.03 mol) was slowly added to a solution of 6.27 g (0.03 mol) of propoxur in 30 mL of dry pyridine under nitrogen. The reaction mixture was stirred for 18 h and poured into water, and the aqueous mixture was extracted with ether/hexane (1:1). The extract was washed in turn with 5% cold hydrochloric acid, water, saturated sodium bicarbonate, and finally with saturated sodium chloride and dried over sodium sulfate. Evaporation of the solvent gave a dark-brown oil. Addition of hexane to the oil resulted in the precipitation of most of the unreacted propoxur. The hexane solution was concentrated and final purification was carried out by silica gel column chromatography using hexane and ether/hexane (1:3) as the eluting solvents. The final product was a viscous oil. Elemental analysis for the *N*-aminosulfenyl derivatives of propoxur are presented in Table I. Structures also were confirmed by NMR using a Varian T-60 spectrometer.

Insect and Mammalian Bioassay. The toxicities of the derivatized methylcarbamates to a susceptible strain (NAIDM) of female houseflies (*Musca domestica*) were obtained by the usual procedures (March and Metcalf, 1949). The test compound in 1 μL of acetone was applied directly to the notum of the fly and mortality was estimated 24 h after treatment. Toxicity to the honeybee was determined in a similar manner after application of the test compound on the dorsal surface of the thorax following immobilization in groups of 20 to 40 per wire mesh cage by keeping the cage for 5–10 min in a cold room maintained at 40 °F. By using this method, mortality in control replicates was always zero. Following treatment, the bees were placed in 5³-in. wire cages, and the lid of the cage was fitted with a small feeding vial inserted into a rubber stopper. Feeding vials were filled to two-thirds capacity with a 50% (v/v) solution of honey in water and plugged with cotton. The cage lid was held in position with rubber bands, and the feeding vial was placed in a vertical position so that the cotton plug was in contact with the honey–water mixture. The treated bees were held for 24 h in a room maintained at 80 °F with 12 h of light, 12 h of dark, and 65% relative humidity. Mortality was observed after 24 h. Generally, three to five replicates of 20 bees per replicate were treated at each dosage level on three different days. The average weight of a worker honeybee was 125 ± 5 mg. The toxicity of the compound in combination with piperonyl butoxide to honeybees was determined by applying a constant dose of 200 μg/g of piperonyl butoxide prior to application of the test material. Fourth instar mosquito larvae (*Culex pipiens quinquefasciatus* Say) were treated according to the method of Georghiou et al. (1966). Toxicity tests to mosquito larvae were carried out in glass beakers instead of waxed paper cups.

Mouse toxicity was determined orally on 4-month-old female Swiss white mice (25–30 g) obtained from Simonsen Inc., Gilroy, CA, using corn oil as the carrier according to usual procedure (Hollingworth et al., 1967). The test compounds were dissolved in corn oil, and 100 μL was introduced orally by means of a syringe equipped with a small animal feeding probe. Mortality was evaluated 48 h after treatment. The average percentage mortality of the replicates within each dose was plotted on logarithm–probit paper and the LD_{50} (dosage required to kill 50% of the test population) was obtained from an eye-fitted line.

Table I. Elemental Analyses of N-Aminosulfenylated Derivatives of Propoxur[a]



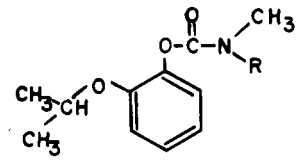
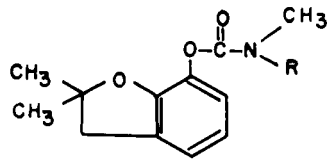
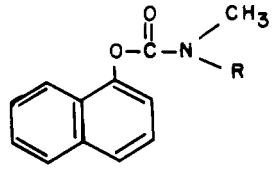
compound no.	R_1	R_2	analysis	
			calcd	found
XXV	C_2H_5	C_2H_5	C, 57.66	57.30
			H, 7.74	7.57
XXVI	n-C_3H_7	n-C_3H_7	C, 59.97	60.01
			H, 8.28	8.28
XXVII	n-C_4H_9	n-C_4H_9	C, 61.92	61.97
			H, 8.75	8.54
XXVIII	$-(CH_2)_5-$		C, 59.23	59.16
			H, 7.45	7.41

[a] Purified by silica gel column chromatography.

RESULTS

Insecticidal Activity. Data for the toxicity of the various N-arylsulfenylated derivatives of insecticidal methylcarbamates to honeybees and houseflies are given in Table II. Toxicity ratios, defined as LD_{50} of the compound against the honeybee divided by the LD_{50} of the compound against the housefly, also are given. It is apparent from the data in Table II that most of the various substituted phenylsulfenylated derivatives of propoxur and

Table II. Toxicity of *N*-Arylsulfenyl and *N*-Alkylsulfenyl Derivatives of Insecticidal Methylcarbamates to Houseflies and Honeybees

compd	R	housefly LD_{50}, μg/g[a]	honeybee LD_{50}, μg/g	toxicity ratio[b]
				
I	H(propoxur)	24.0	4.5	0.19
II	phenylsulfenyl	36.0	6.4	0.18
III	4-tolylsulfenyl	36.0	8.0	0.22
IV	3-tolylsulfenyl	23.5	6.9	0.29
V	2-tolylsulfenyl	24.0	10.0	0.42
VI	2,4-xylylsulfenyl	27.5	16.8	0.61
VII	2-isopropylphenylsulfenyl	28.5	5.4	0.19
VIII	4-*tert*-butylphenylsulfenyl	9.0	36.0	4.00
IX	2-methyl-4-*tert*-butylphenylsulfenyl	24.5	>800.0	>32.70
X	4-bromophenylsulfenyl	26.5	14.8	0.52
XI	4-bromo-2-methylphenylsulfenyl	37.0	15.0	0.41
XII	CH_3	65.0	3.0	0.05
				
XIII	H(carbofuran)	6.7	0.42	0.06
XIV	phenylsulfenyl	9.3	0.52	0.05
XV	4-tolylsulfenyl	9.0	0.72	0.08
XVI	3-tolylsulfenyl	6.5	0.66	0.10
XVII	2-tolylsulfenyl	3.7	0.52	0.14
XVIII	4-*tert*-butylphenylsulfenyl	2.7	1.42	0.53
XIX	2-methyl-4-*tert*-butylphenylsulfenyl	7.5	2.34	0.31
XX	4-bromophenylsulfenyl	9.0	0.80	0.09
XXI	4-bromo-2-methylphenylsulfenyl	11.3	0.8-4.0	
XXII	2-isopropylphenylsulfenyl	8.3	0.8-4.0	
				
XXIII	H(carbaryl)	>900.0	18.4	<0.02
XXIV	2-methyl-4-*tert*-butylphenylsulfenyl	>500.0	>800.0	>1.6

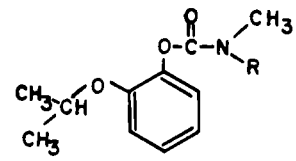
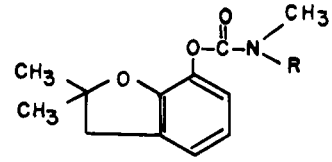
[a] Values from Black et al. (1973a). [b] LD_{50} of compound against honeybees/LD_{50} of compound against houseflies.

carbofuran were highly toxic to the honeybee. In the propoxur series, very little difference was observed between the toxicity ratios of propoxur (I) and sulfenylated derivatives II, III, IV, and VII with toxicity ratios ranging from 0.19 to 0.29. Slight improvement in bee toxicity was observed with the bromine containing derivatives (X, XI) and for the 2-tolyl-(V) and 2,4-xylylsulfenyl derivatives, but all of these compounds were substantially more toxic to the honeybee than to the housefly. Introduction of a *tert*-butyl moiety in the phenylsulfenyl ring resulted in a derivative (VIII) which was fourfold less toxic to the honeybee than to the housefly and was eightfold less toxic to the honeybee than propoxur. Further insertion of a methyl moiety in the 2 position of the phenyl group, giving the 2-methyl-4-*tert*-butylphenylsulfenyl derivative (IX), resulted in a compound which was virtually nontoxic to the honeybee while remaining as effective as propoxur to the housefly. Thus, the introduction of a single methyl group in VIII increased bee safety more than eightfold.

In contrast to the propoxur derivative, the corresponding substituted phenylsulfenyl derivatives of carbofuran were all more toxic to the honeybee than to the housefly, including the 4-*tert*-butyl- and 2-methyl-4-*tert*-butylphenylsulfenyl analogues. However, conversion of carbaryl to the 2-methyl-4-*tert*-butylphenylsulfenyl derivative resulted in a compound which was more than 44-fold less toxic to the honeybee than carbaryl (compare XXIII with XXIV).

In the presence of piperonyl butoxide, an inhibitor of mixed-function oxidase enzyme activity (Wilkinson, 1968; Casida, 1970), the toxicity of IX to honeybees was significantly increased (LD_{50} 44.0 μg/g). Black et al. (1973a) and Fahmy et al. (1978) also reported high synergism with piperonyl butoxide for N-sulfenylated derivatives of methylcarbamates and for *N,N'*-thiodicarbamates in houseflies. The high degree of synergism obtained with piperonyl butoxide probably resides in the ability of the synergist to protect the methylcarbamate from detoxication by mixed-function oxidase enzymes after its formation from the derivative. The high toxicity of IX in combination with piperonyl butoxide to bees clearly suggests a significant role for mixed-function oxidase enzymes in bees. Metcalf et al. (1966) suggested that the unusually high susceptibility of bees to insecticides might

Table III. Toxicity of *N*-Aminosulfenyl Derivatives of Insecticidal Methylcarbamates

compd	R	LD_{50}, μg/g housefly	LD_{50}, μg/g honeybee	toxicity ratio[a]	*Culex pipiens* LC_{50}, ppm	mouse (oral) LD_{50}, mg/kg
						
I	H(propoxur)	24.0	4.5	0.19	0.33	62
XXV	*N,N*-diethylaminosulfenyl	37.0	5.9	0.16	0.014	500
XXVI	*N,N*-dipropylaminosulfenyl	39.0	2.8	0.07	0.008	>1000
XXVII	*N,N*-dibutylaminosulfenyl	27.0	4.0	0.15	0.0064	>1000
XXVIII	*N*-piperidinosulfenyl	34.5	5.3	0.15	0.02	>1000
						
XIII	H(carbofuran)	6.7	0.42	0.06		2
XXIX	*N,N*-dimethylaminosulfenyl	15.0[b]	0.61	0.04		10-20[b]
XXX	*N,N*-diethylaminosulfenyl		0.64			
XXXI	*N,N*-dipropylaminosulfenyl		0.74			
XXXII	*N,N*-dibutylaminosulfenyl		0.64			
XXXIII	*N*-piperidinosulfenyl		0.4			
XXXIV	morpholinosulfenyl	10.5[b]	0.53	0.05		10-12[b]

[a] LD_{50} of compound against honeybees/LD_{50} of compound against houseflies. [b] Values from Fukuto et al. (1975).

be due to an inefficient microsomal detoxication system. This suggestion was compatible with the observation that the combination of piperonyl butoxide and carbaryl was only 2.9-fold more toxic to the honeybee than carbaryl alone (Georghiou and Atkins, 1964). However, Gilbert and Wilkinson (1974) reported the presence of a relatively efficient microsomal oxidase system in several developmental stages of the honeybee. The specific activity of the epoxidase in preparations from 7-day-old drone larvae was found to be comparable to that of the hepatic microsomal oxidases of several mammals (Wistar white rat, rabbit) and to preparations from the tissues of other insect species (housefly and *Acheta domesticus*).

The data in Table III show that the toxicity of the *N*-aminosulfenyl derivatives of propoxur and carbofuran to the honeybee and housefly were generally equal to that of the parent methylcarbamates. The series, as a whole, did not reveal any favorable selectivity differences between houseflies and honeybees. The aminosulfenyl derivatives of propoxur were more toxic to mosquito larvae than propoxur. The data suggested that mosquito larvae toxicity increases as the number of carbon atoms in the aliphatic amino moiety is increased. Lipophilicity of the carbamate derivative plays an important role in determining effectiveness against mosquito larvae (Fahmy et al., 1978). In accordance with these results, of the various aminosulfenyl derivatives of propoxur (XXV to XXVIII), the *N*-dibutylaminosulfenyl moiety (XXVII) showed the highest larvicidal activity.

Toxicity to White Mice. Data for the toxicity of the *N*-aminosulfenyl derivatives of propoxur to mice also are given in Table III. The derivatives (XXV–XXVIII) were extremely safe to mice, showing LD_{50} values greater than 1000 mg/kg. The safety of these derivatives to mice probably is attributable to preferential metabolic detoxication of the derivative to nontoxic phenols. A previous metabolism study with *N*-(2-toluenesulfenyl)carbofuran (Black et al., 1973b) in the white mouse indicated that the arylsulfenyl group substitution on the carbamate moiety allows the mouse to carry out metabolic reactions leading to less toxic products which are rapidly conjugated.

DISCUSSION

While replacement of the carbamate hydrogen atom of a toxic methylcarbamate insecticide by a sulfenyl moiety generally results in products which are substantially safer to mammals than the parent methylcarbamate (Black et al., 1973a; Fahmy et al., 1974, 1978), most of the sulfenylated derivatives which were examined for toxicity to the honeybee were highly toxic to this insect. In fact, of the 34 different sulfenylated derivatives of propoxur, carbofuran, and carbaryl evaluated, only three showed a toxicity ratio greater than one although many were safer to bees than the parent methylcarbamate. The most notable exception was the 2-methyl-4-*tert*-butylphenylsulfenyl derivative of propoxur (IX) which was more than 178-fold safer to the honeybee than propoxur. Although the 4-*tert*-butyl moiety in the ring appeared to introduce the greatest amount of honeybee safety in these derivatives, no obvious relationship between the structure of the carbamate derivative and honeybee toxicity was apparent.

Because of the unusual selectivity of *N*-(2-methyl-4-*tert*-butylphenylsulfenyl)propoxur, this compound is being investigated further for comparative metabolism in the honeybee and housefly.

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Residues of Croneton and Its Sulfoxide and Sulfone Metabolites in Citrus (*Clementine* Trees) following a Soil Treatment for the Control of *Aphis spiraecola*

Nadav Aharonson,* Ilan Neubauer, Isaac Ishaaya, and Benjamin Raccah

Residues of the insecticide croneton, [2-(ethylthio)methyl]phenyl *N*-methylcarbamate, and its sulfoxide and sulfone metabolites were determined in *Clementine* (*C. reticulata* Blanco) leaves and fruits following soil treatment with 2, 6, and 18 g of active ingredient (AI) per tree. The analytical procedure was improved to separate croneton from its two oxygen analogues. Croneton was extracted with petroleum ether and its sulfoxide and sulfone metabolites with chloroform; all were silylated with bis(trimethylsilyl)trifluoroacetamide and determined by gas–liquid chromatography using the flame photometric detector. The carbamate was found to accumulate in the leaves and mainly in its oxidized forms. Soil treatment with 18 g of AI/tree resulted in a slow accumulation in the leaves during the first 12 days (up to 2 ppm), followed by a much faster rate. After 42 days the total residue level was 12 ppm (0.4 ppm croneton, 5.6 ppm sulfoxide, and 6.2 ppm sulfone). Residues in the leaves were proportional to the dosage applied to the soil. An average level of 4–6 ppm of the carbamate in the leaves provided satisfactory control of the spirea aphid. Similar results were found when the spirea aphids were fed on a synthetic diet containing similar levels of the insecticide.

Croneton (Bay HOX 1901), [2-(ethylthio)methyl]phenyl *N*-methylcarbamate, is a systemic insecticide exhibiting specific action against aphids (Bayer AG, 1974). It was chosen as one of several potential systemic candidates for *Aphis spiraecola* Patch. control in citrus trees by soil application. [*Aphis spiraecola* has recently been named *Aphis citricola* Van der Goot by Dr. D. Hille Lambers (*Entomol. Ber.* **35**, 59 (1975))]. While many insecticides exhibit aphicidal properties, only those providing foliar protection for at least 2–3 weeks were of interest (Bullock, 1972). Investigations of the efficacy of soil treatment with systemic insecticides for citrus insect and mite control have grown in number in recent years (Brooks, 1968; Tashiro et al., 1969; Shaw, 1970; Bullock, 1972; Milne and de Villiers, 1975). However, there are no data available on the systemic action and residues of croneton in citrus trees.

Residues of croneton were determined in recent studies as total carbamate by oxidizing croneton and croneton sulfoxide to the sulfone form (Dräger, 1974) or by thin-layer chromatography of ^{14}C-carbonyl- and ^{14}C-ring-labeled croneton (Nye et al., 1976).

The present work reports an improved analytical method for determining separately the residues of croneton and its sulfoxide or its sulfone metabolites (Figure 1) in *Clementine* leaves and fruit. This report is also concerned with the uptake, accumulation, and persistence of croneton or its biologically active metabolites in the trees and their relation to aphid control.

EXPERIMENTAL SECTION

Field Treatment. Croneton 10% granular formulation was applied to the surface of the soil around the base (100 cm diameter) of 4-year-old *Clementine* trees, variety Mikhal, at three rates: 2, 6, and 18 g of AI/tree (160 trees/acre). Overhead irrigation at the rate of 140 L/tree and natural rainfall (0.4 in) provided the equivalent of 4 in. of precipitation following application. Weekly irrigation was continued during the summer.

The insecticide was applied in April to plots of three trees, replicated four times for each of the three dosages. Aphid populations were determined by examining ten young growth terminals per tree (120 terminals per treatment). Heavy infestation was recorded as percent of

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