Penetration, Metabolism, and Synergistic Activity with Carbaryl of Some Simple Derivatives of 1,3-Benzodioxide in the Housefly

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Twenty-three derivatives of 1,3-benzodioxole mainly substituted in the 5 and 6 positions of the ring, and four naphtho-1,3-dioxoles, have been evaluated as synergists for carbaryl (1-naphthyl-*N*-methylcarbamate) at each of four synergist-insecticide ratios against the common housefly (*Musca domestica* L.). Many of these were found to be superior to sesamex, especially at low synergist-insecticide ratios, the most marked in this respect being those incorporating nuclear nitro and methoxy groups. The novel application of electron-capture gas chromatography to the estimation of the compounds containing halogen and nitro groups has enabled data on in vivo penetration and metabolism to be obtained.

In recent years there has been renewed interest in insecticide synergists, particularly with respect to their activity with the carbamate insecticides (*8*, *17*, *19*, *27*, *29*).

Much of this recent work on synergists of the 1,3-benzodioxole (methylenedioxyphenyl) group has been directed towards investigations of the essentiality and chemical specificity of the 1,3-benzodioxole structure (*19*, *29*). The results indicate that in a series of closely related bicyclic systems of this general type, maximum synergistic activity with the carbamate insecticides is dependent on the presence of an intact 1,3-benzodioxole group. This is in general agreement with the results of similar investigations on synergists of this type in combination with the pyrethrin insecticides (*9*, *18*).

Despite these findings, little progress has been made in defining more clearly the minimal molecular requirements for synergistic activity of 1,3-benzodioxole derivatives with the carbamate insecticides. Since a number of rather simple compounds containing the 1,3-benzodioxole nucleus have recently been shown to be extremely effective in synergizing the carbamates (*19*, *29*), it became of interest to examine the activity of a series of these compounds mainly substituted in the 5 and 6 positions of the ring.

Those members of the series which contain halogen (Cl or Br) or nitro substituents could readily be estimated on a quantitative basis by means of gas-liquid chromatography coupled with an electron affinity detector (*28*). This has made possible investigations on such properties as in vivo penetration and metabolism of these synergists in the housefly (*M. domestica* L.) at the levels at which they exert their synergistic activity. Data of this nature have not previously been obtainable because of the general lack of sensitivity of existing chemical and physical methods for determination of the 1,3-benzodioxole ring (*20*).

In the following investigation employing the carbamate insecticide carbaryl (1-naphthyl-*N*-methylcarbamate) the synergistic activity of each of a series of derivatives of 1,3-benzodioxole has been measured at four synergist-insecticide ratios. Where electron affinity properties have allowed, in vivo penetration and metabolism data have also been obtained.

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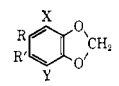
Materials and Methods

Synthesis. Compounds I to XXIII (Table I) were synthesized in the laboratory, using in most cases piperonal (3,4-methylenedioxybenzaldehyde) as the starting material.

1,3-Benzodioxole (I) was prepared in 85% yield as described by Dallacker and Binsack (*7*) by the deformylation of piperonal with palladium on charcoal. It was obtained as a colorless liquid (b.p. 55–59° C. at 10.0 mm., n_D^{23} 1.5385). Careful addition of I to ice-cold nitric acid (sp. gr. 1.42), followed by dilution, gave a yellow solid which on recrystallization from ethanol provided pale yellow needles of 5-nitro-1,3-benzodioxole (VI) [m.p. 147–48° C. (lit. 147° C.)] (*24*). Further treatment of VI with an ice-cold mixture of glacial acetic acid and fuming nitric acid (sp. gr. 1.5) as described by Parijs (*22*) gave 5,6-dinitro-1,3-benzodioxole (XVIII) obtained as yellow needles from ethanol [m.p. 96–98° C. (lit. 100° C.)]. 5-Methylamino-6-nitro-1,3-benzodioxole (XIX) (red needles, m.p. 170–71° C.), and 5-dimethylamino-6-nitro-1,3-benzodioxole (XX) (orange platelets, m.p. 93.5–95° C.) were prepared (*22*) by heating XVIII in a sealed tube for 5 hours at 100° C. with methylamine and dimethylamine (as 33% solutions in alcohol), respectively, followed by recrystallization of the resulting solids from ethanol.

Catalytic reduction of VI using Adam's catalyst in ethanol (*25*) yielded 5-amino-1,3-benzodioxole (VIII), which crystallized as light brown needles [m.p. 39–41° C. (lit. 44.5° C.)] from toluene and ligroin.

Table I. Synergistic Activity of Some 1,3-Benzodioxoles with Carbaryl



	R	R′	X	Y	LD_{50} (μg./Female Fly) of Carbaryl[a] at Various W./W. Ratios of Insecticide-Synergist 1:1	1:2.5	1:5	1:10
I	H	H	H	H	5.0 (>10.0)[b]	2.6 (>19.2)	1.6 (>31.2)	1.2 (>41.7)
II	H	OH	H	H	6.6 (>7.6)	4.9 (>10.2)	4.6 (>10.9)	2.2 (>22.7)
III	H	CH_3	H	H	1.6 (>31.2)	0.88 (>56.8)	0.59 (>84.7)	0.43 (>116)
IV	H	Cl	H	H	1.12 (>44.6)	0.90 (>55.5)	0.66 (>75.7)	0.50 (>100)
V	H	Br	H	H	0.68 (>73.5)	0.40 (>125)	0.32 (>156)	0.27 (>185)
VI	H	NO_2	H	H	0.33 (>151)	0.20 (>250)	0.17 (>300)	0.11 (>454)
VII	H	CH_3O	H	H	0.36 (>139)	0.25 (>200)	0.18 (>277)	0.16 (>312)
VIII	H	NH_2	H	H	3.1 (>16.1)	1.9 (>263)	1.0 (>50.0)	0.61 (>82.0)
IX	Cl	Cl	H	H	0.56 (>89.3)	0.35 (>143)	0.26 (>192)	0.20 (>250)
X	Br	Br	H	H	0.36 (>139)	0.24 (>208)	0.18 (>277)	0.13 (>385)
XI	Cl	Cl	Cl	Cl	0.48 (>104)	0.33 (>151)	0.22 (>220)	0.19 (>263)
XII	Br	Br	Br	Br	1.75 (>28.6)	1.2 (>41.7)	1.3 (>38.5)	0.80 (>62.5)
XIII	Cl	CH_3O	H	H	0.26 (>192)	0.15 (>330)	0.12 (>417)	0.084 (>600)
XIV	Br	CH_3O	H	H	0.22 (>220)	0.15 (>330)	0.11 (>454)	0.085 (>600)
XV	NO_2	CH_3O	H	H	0.15 (>330)	0.11 (>454)	0.094 (>532)	0.088 (>568)
XVI	NO_2	Cl	H	H	0.90 (>55.5)	0.56 (>89.3)	0.39 (>128)	0.26 (>192)
XVII	NO_2	Br	H	H	1.85 (>27.0)	1.03 (>48.5)	0.60 (>83.3)	0.44 (>114)
XVIII	NO_2	NO_2	H	H	2.9 (>17.2)	1.95 (>25.6)	2.1 (>24.0)	1.6 (>31.2)
XIX	NO_2	$NHCH_3$	H	H	0.19 (>263)	0.125 (>400)	0.108 (>463)	0.086 (>581)
XX	NO_2	$N(CH_3)_2$	H	H	0.19 (>263)	0.117 (>427)	0.084 (>595)	0.060 (>833)
XXI	CHO	NO_2	H	H	[c]	[c]	[c]	[c]
XXII	CHO	Cl	H	H	3.3 (>15.1)	2.0 (>25.0)	1.3 (>38.5)	1.0 (>50.0)
XXIII	CHO	Br	H	H	2.15 (>23.2)	1.48 (>33.8)	0.94 (>53.2)	0.74 (>67.6)
	Sesamex				0.44 (>114)	0.24 (>208)	0.165 (>303)	0.105 (>476)

[a] LD_{50} for carbaryl alone = >50.0 μg./fly.

[b] Figures in parentheses indicate synergistic ratio = $\frac{LD_{50} \text{ carbaryl alone}}{LD_{50} \text{ carbaryl + synergist}}$

[c] Satisfactory log dosage-mortality lines could not be obtained.

An excellent yield of 5-hydroxy-1,3-benzodioxole (sesamol, II) [m.p. 62–64° C. (lit. 64–65° C.)] was obtained from piperonal by the method of Markus (*16*), and its methyl ether (VII), readily prepared by treatment of the sodium salt with dimethyl sulfate (*12*), distilled as a colorless volatile oil [b.p. 108–10° C. at 15.0 mm., n_D^{22} 1.5415]. Chlorination of VII in glacial acetic acid gave 5-chloro-6-methoxy-1,3-benzodioxole (XIII) which was recrystallized from ethanol (m.p. 95–96.5° C.). Elemental analysis: Calculated for $C_8H_7ClO_3$, C 51.47, H 3.75; found C 51.60, H 3.95. Bromination in a similar manner yielded 5-bromo-6-methoxy-1,3-benzodioxole (XIV) (m.p. 78–80° C.). Elemental analysis: Calculated for $C_8H_7BrO_3$, C 41.56, H 3.03; found C 41.93, H 2.99. Careful addition of VII to cold 50% nitric acid gave a solid from which yellow needles of 5-methoxy-6-nitro-1,3-benzodioxole (XV) (m.p. 138.5–39.5° C.) were obtained after crystallization from ethanol. Elemental analysis: Calculated for $C_8H_7NO_5$, C 48.73, H 3.55; found C 49.39, H 3.84.

Chlorination of piperonal in glacial acetic acid (*21*, *22*) afforded a mixture of 5-chloro-6-formyl-1,3-benzodioxole (XXII) [m.p. 114.5–15.5° C. (lit. 115° C.)] and 5,6-dichloro-1,3-benzodioxole (IX) [m.p. 76–77° C. (lit. 80–81° C.)]. The corresponding bromo derivatives, XXIII (m.p. 126–27° C.) and X (m.p. 84–85° C.), were prepared in a similar manner by bromination of piperonal in glacial acetic acid (*21*). A more effective method of obtaining the dibromo compound (X) consisted of treating piperonylic acid with bromine in aqueous sodium carbonate (*13*). 5-Formyl-6-nitro-1,3-benzodioxole (XXI) [m.p. 96–98° C. (lit. 98° C.)] was prepared by the nitration of piperonal with cold concentrated nitric acid (sp. gr. 1.42) (*24*).

Gradual addition of 5-chloro-6-formyl-1,3-benzodioxole (XXII) to ice-cold nitric acid (sp. gr. 1.42) gave a yellow solution which yielded a solid on dilution (*21*). Recrystallization of the latter from ethanol gave yellow needles of 5-chloro-6-nitro-1,3-benzodioxole (XVI) [m.p. 65–66° C. (lit. 70° C.)]. A similar procedure starting with XXIII gave the corresponding 5-bromo-6-nitro-1,3-benzodioxole (XVII) [m.p. 84–85° C. (lit. 87–89° C.)] (*21*).

4,5,6,7-Tetrabromo-1,3-benzodioxole (XII) was obtained according to Raiford and Oberst (*23*) by refluxing 5-bromo-6-formyl-1,3-benzodioxole (XXIII) with an excess of bromine (containing 1% aluminum bromide) at 100° C. for 3 hours. It was recrystallized

from ethanol as white needles [m.p. 208–10° C. (lit. 208–09° C.)]. The corresponding tetrachloro compound (XI) was obtained as white needles [m.p. 169–71° C. (lit. 171° C.)] by ring closure of tetrachlorocatechol (m.p. 192–93° C.), with dibromomethane in a similar manner to that described by Campbell *et al.* (*5*).

5-Methyl-1,3-benzodioxole (III) and 5-chloro-1,3-benzodioxole (IV) were synthesized by ring closure with dibromomethane of the dipotassium salts of the corresponding catechols as described earlier (*28*).

The monobromo derivative (V) was prepared according to Jones and Robinson (*13*) by passing 1.2 molar equivalents of bromine (in a stream of air) through a solution of 1,3-benzodioxole (I) in glacial acetic acid. It was obtained as a volatile colorless oil (b.p. 112–16° C. at 28.0 mm., n_D^{22} 1.5836).

Bioassay. The required solutions of carbaryl and the various synergists were prepared in acetone. They were applied topically to the thoraxes of 4-day-old, female susceptible houseflies (*M. domestica* L., P.I.L. strain, maintained unselected in the laboratory for many years), in a volume of 1 μl., delivered from a microdrop applicator developed in this laboratory (*10*). Twenty flies were used at each concentration on each of three different days, the insects being treated under carbon dioxide anesthesia and subsequently held on filter paper disks under inverted glass dishes at 25–26° C. Sugar solution was provided. Mortality counts were made at intervals following treatment, that taken at 24 hours being employed in the final assessment of toxicity. The mean 24-hour mortality at each concentration was plotted on log × probability paper and the log dosage-mortality line was fitted by eye. The LD_{50} values were obtained directly from these lines. None of the synergists themselves were toxic at the concentrations at which they were employed in combination with carbaryl.

Gas-Liquid Chromatography and Penetration Data. Groups of 10 4-day-old female susceptible houseflies (*M. domestica* L.), of the same strain employed in the bioassay, were topically treated with 5 μg. of synergist under carbon dioxide anesthesia. They were subsequently held at 25–26° C. for varying lengths of time (½ to 48 hours) either under inverted glass dishes on filter paper disks, or in loosely stoppered 250-ml. Erlenmeyer flasks. Sugar solution was provided. Following the lapse of the required time period, the flies were briefly knocked down with carbon dioxide, and were rinsed three times with 4-ml. aliquots of an acetone-ligroin (40° to 60° C. boiling fraction) (1 to 1) mixture. The rinses were combined and diluted to a volume dictated by the response of the particular compound to the electron capture detector. The rinsed flies were then transferred to a Potter Elvehjem homogenization tube with a loosely fitting glass plunger, and 2.5 ml. of extraction solvent, acetone-ligroin (1 to 4) plus solid sodium sulfate, was added. The flies were homogenized, the tube being cooled in an ice bath during the process, and the homogenate was centrifuged. The supernatant was decanted, and the process repeated twice further, the supernatants being combined in a 10-ml. glass-stoppered graduated tube. The combined extracts were washed with 4 ml. of water and the organic phase was diluted to 5 ml. with ligroin. Aliquots of this solution (usually 1 to 2 μl.) were used for gas chromatographic estimation.

Estimations were carried out on either a Pye Panchromatograph, employing a ^{90}Sr macroargon ionization detector (used at low voltage for electron capture), or a Shandon gas chromatograph provided with a 100-mc. tritium foil electron capture detector of the type first described by Lovelock (*15*). In each case nitrogen was used as the carrier gas. Columns of various lengths (4, 2, and ½ feet) were employed (*28*) using a packing consisting of a stationary phase of 2.5% Apiezon + 0.5% Epikote on Celite (100- to 120-mesh). Column conditions (temperature and gas flow) were adjusted for each compound so as to give retention times suitable for rapid quantitative measurement (Table III). Peak heights were compared with those on a standard curve obtained on the same day under identical column conditions.

Results and Discussion

The synergistic activity of the series of compounds (I to XXIII) is shown in Table I, from which it is immediately obvious that large variations in activity result from relatively minor changes in chemical structure. Carbaryl was virtually nontoxic towards the strain of flies employed, which withstood a dose of 50 μg. per fly with impunity and consequently the synergistic ratios obtained are spectacular. In Table I, the various ratios of synergist to carbaryl are given on a weight basis, but because of considerable variation in the molecular weights of these compounds a better method of presenting the data is afforded by describing these ratios in terms of moles of synergist per mole of carbaryl. This enables direct comparisons to be made on the relative efficiency of these compounds in terms of the molar concentration of the 1,3-benzodioxole ring, the moiety responsible for synergistic activity. Plots of log LD_{50} of carbaryl against the molar ratio (synergist-carbaryl) are shown in Figure 1, *A*, *B*, and *C*, for the methoxy-, nitro-, and halogen-containing compounds, respectively. Figure 1*A* also includes the data for compounds I, II, and VIII and sesamex is incorporated into each of these plots for comparative purposes.

As has been reported for both the pyrethrins (*18*) and the carbamates (*19*, *29*), the parent compound 1,3-benzodioxole (I) shows only slight synergistic activity, substantial recovery occurring after a rapid initial knockdown effect. Other compounds of low activity were the three aldehydes (XXI to XXIII), 5-amino-1,3-benzodioxole (VIII), and sesamol (II); sesamol and the nitroaldehyde (XXI) are the least effective members of the series. It seems probable that all these compounds are rapidly metabolized by the insect, each having either a group which can be conjugated directly, or one which can be conjugated after prior oxidation.

Figure 1*A* shows most of the methoxy-containing members of the series to be efficient synergists for carbaryl. Although 5-methoxy-1,3-benzodioxole (VII)

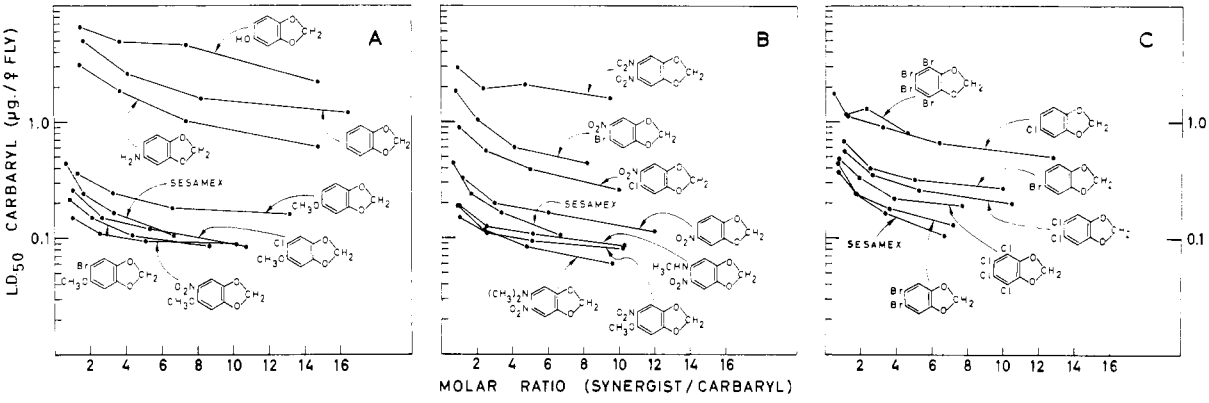


Figure 1. Relation of log LD_{50} carbaryl to molar ratio (synergist-carbaryl) of substituted 1,3-benzodioxoles in the housefly

is not itself as effective as sesamex, additional substitution in the ring with chloro and bromo groups further enhances the activity of the molecule to give compounds having synergistic ratios ranging from >200 at the 1 to 1 (w./w.) ratio to >600 at the 1 to 10 ratio. The combination of a nitro and a methoxy group as in compound XV results in one of the most active compounds evaluated, having a synergistic ratio of >330 even at the 1 to 1 (w./w.) ratio, making it approximately three times more active than sesamex. These results are in general agreement with those of Kerr (*14*), who discussed the increased synergistic activity towards the pyrethrins resulting from the incorporation of nuclear methoxy groups in the 1,3-benzodioxole structure. Other reports in the literature point out the possible activating effects of nitro substitution with respect to pyrethrin synergism (*18*).

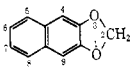
Figure 1*B* shows that compounds containing nitro substituents varied considerably in synergistic activity. Whereas 5-nitro-1,3-benzodioxole (VI) shows considerable activity, and is protected along with other similar compounds in a German patent (*1*), the inclusion of a second nitro group to give compound XVIII results in a compound of very low activity. This compound penetrates insect tissues extremely slowly and there is a possibility that it has a direct effect on the penetration of carbaryl, as especially at the higher synergist-carbaryl ratios the log dosage-mortality lines show a trend away from linearity. The nitroaldehyde (XXI) was even more marked in this respect and it was not found possible to obtain satisfactory LD_{50} values for carbaryl in combination with this compound. Although compounds XVI and XVII, which contain a halogen in combination with the nitro group, show a fairly rapid knockdown, subsequent recovery results in their intermediate activity as synergists. This can satisfactorily be explained from penetration data. The most effective members of the nitro-containing series are those incorporating methoxy (XV), methylamino (XIX), and dimethylamino (XX) substituents, the latter being the most active of all the compounds evaluated in this investigation, having a synergistic ratio of >830 at the 1 to 10 (w./w.) ratio.

None of the several halogen-containing compounds studied were as effective as sesamex in synergizing carbaryl (Figure 1*C*). In the case of the chlorinated compounds (IV, IX, and XI) a steady increase in activity is associated with increasing chloro substitution, the tetrachloro derivative (XI) being only slightly less active than sesamex. The monobromo (V) and dibromo (X) compounds similarly show this trend and are in fact more efficient synergists than the corresponding chloro compounds (IV and IX). The trend is reversed, however, by 3,4,5,6-tetrabromo-1,3-benzodioxole (XII), which has only slight activity. The possible commercial significance of compounds IX and X has recently been indicated by their inclusion in a Dutch Shell patent (*25*).

In addition to the variations in synergistic activity discussed above, variations between different synergists have also been observed with respect to the rate of knockdown of treated flies. In general the presence of halogen groups within the molecule was associated with rapid knockdown, although subsequent recovery makes them usually less effective synergists than the slower-acting nitro compounds, where less recovery occurs. Some recovery invariably occurs even when there remains a significant amount of synergist within the insect, and it is likely that under these conditions the carbaryl is slowly degraded at the cholinesterase site (*30*). Alternatively it is possible that even in the presence of high internal concentrations of synergist, the microsomal detoxication pathways are never completely inhibited, and a slow breakdown of carbaryl is still possible. Such a situation could easily exist if the synergist was competing with carbaryl at the enzymic level. The steady increase in toxicity associated with increasing synergist-carbaryl ratio (Figure 1) indicates that even at the 10 to 1 (w./w.) ratio the maximum insecticidal potential of carbaryl is not realized and is evidence that a competitive situation at the detoxication site results in incomplete blockage of the system. A mathematical treatment of the data presented here will be published shortly, and should clarify this point (*11*).

The synergistic activities of four naphtho-1,3-dioxoles are shown in Table II. The parent compound, naphtho-[2,3-*d*]-1,3-dioxole (XXIV), is the most active

Table II. Synergistic Activity of Some Naphtho-1,3-dioxoles with Carbaryl



		LD_{50} (μg./Female Fly) of Carbaryl[a] at Various W./W. Ratios of Insecticide-Synergist			
		1:1	1:2.5	1:5	1:10
XXIV	Unsubstituted	0.24 (>208)[b]	0.173 (>289)	0.114 (>438)	0.103 (>485)
XXV	4-Nitro	0.46 (>187)	0.28 (>178)	0.22 (>220)	0.175 (>286)
XXVI	4,9-Dibromo	1.0 (>50.0)	0.55 (>90.9)	0.37 (>135)	0.32 (>156)
XXVII	4,5,6,7,8,9-Hexachloro[c]	>5.0	>5.0	[d]	[d]

[a] LD_{50} for carbaryl alone = >50.0 μg./fly.

[b] Figures in parentheses indicate synergistic ratio = $\frac{LD_{50} \text{ carbaryl alone}}{LD_{50} \text{ carbaryl + synergist}}$.

[c] Derivative of 1,2-methylenedioxynaphthalene.

[d] Data not obtained because of extreme insolubility in acetone.

member of the group, in marked contrast to the inactivity of 1,3-benzodioxole (I). At a 1 to 1 (w./w.) ratio the former compound is approximately twice as effective as sesamex and it appears that 5,6-fusion of an additional benzene ring (as in naphthalene) is equivalent to simpler forms of 5,6-disubstitution in 1,3-benzodioxole. 4-Nitronaphtho-[2,3-*d*]-1,3-dioxole (XXV) is also an outstanding synergist, although substitution is ortho to the dioxole ring where some steric effects might be expected to occur. It is slightly less active than XXIV, however, and as the 4,9-dibromonaphtho-[2,3-*d*]-1,3-dioxole (XXVI) in which both the ortho positions are occupied, shows a further decrease in synergistic activity, steric hindrance may be of some importance as discussed with similar compounds in the benzene series. The hexachloro derivative (XXVII) shows only slight activity, but in this case the very low solubility in acetone did not permit testing at high synergist-insecticide ratios.

The structural requirements for synergistic activity with carbaryl appear to be remarkably nonspecific. Although 1,3-benzodioxole (I) itself shows only slight synergism, substitution in the benzene ring with almost any group or atom, with the exception of hydroxyl, results in a marked increase in activity. Most efficient in this respect are the nitro and methoxy groups, although a number of the halogenated derivatives are also outstanding. The extreme effectiveness of compounds XIX and XX, which contain a methylamino and dimethylamino group, respectively, may indicate that these groups have an effect in addition to the 1,3-benzodioxole ring. This would not be surprising in view of their structural similarity with the *N*-methylcarbamates. Electronic interactions of the several substituents are complicated and any correlations between these and their possible activating effects on the dioxole ring are not immediately obvious.

Mixtures of 1,3-benzodioxole (I) with carbaryl produced an initial knockdown followed by recovery, so perhaps the synergistic potential of the former compound is not fully realized because of instability in vivo. The effect of 5- and/or 6-substitution in the benzene series, or 5,6-fusion as in the naphthalene compounds, may be to give added stability to the dioxole nucleus at the site of action. This may be either physical (decreased volatility, etc.) or chemical stability to the oxidative and conjugative enzyme systems within the insect.

Penetration of Synergists into Flies. The fact that many of the compounds considered contain either halogen or nitro substituents in the benzene ring makes possible their rapid and sensitive estimation by means of electron-capture gas chromatography (*28*). Previous methods for detecting synergists of the 1,3-benzodioxole class have relied chiefly on chemical methods involving the colorimetric determination of the products of ring cleavage (*2*). Although these are useful for qualitative work and quantitative estimation at the microgram level, they have not been sufficiently sensitive for application to investigations of such factors as penetration and metabolism. The gas chromatographic technique used in this investigation makes possible detection at the nanogram level and below (Table III), and may prove a useful addition to radiotracer techniques in biochemical studies on the mode of action and metabolic fate of these compounds in biological systems.

The approximate detection limits are indicated in Table III, the order of electron affinity of the three major substituents being NO_2 > Br > Cl. Although mononitro compounds could readily be determined at the required level, monobromo derivatives were found more difficult, and in the chlorinated compounds at least two chlorine atoms were found to be required for satisfactory detection characteristics.

The penetration data are shown in Table IV, and typical examples of the curves obtained are illustrated in Figure 2. Marked differences exist within the series of compounds investigated.

Two groups of compounds are notable for their rapid elimination from insect tissues: the three aldehydes (XXI, XXII, and XXIII) and the halogenated nitro derivatives (XVI and XVII).

Although disappearance from the outside of the insect is fairly rapid in the case of the three aldehydes (Figure 2*A*), only trace amounts can be detected within the insect, and total recovery (rinse plus extract) falls rapidly, until after 8 hours only traces are recovered. This may partially explain the low synergistic activity

Table III. Gas Chromatographic Conditions for Estimation of Some Insecticide Synergists

Compound	Column Length, Ft.	Temp., ° C.	Gas Flow, Cc./Min.	Retention Time, Min.	Approx. Detection Limit, G.
VI	2	160	290	1.8	10^{-10}
IX	4	160	150	1.9	10^{-9}
X	2	160	290	1.7	10^{-10}
XI	2	172	290	2.4	10^{-10}
XII	1/2	187	150	2.7	$<10^{-10}$
XIV	1/2	125	100	2.0	10^{-9}
XV	2	193	290	1.6	10^{-10}
XVI	2	185	175	1.7	10^{-10}
XVII	2	190	290	1.3	10^{-10}
XVIII	1/2	160	100	3.0	$<10^{-10}$
XIX	1/2	187	150	1.1	10^{-10}
XX	4	187	150	4.0	10^{-10}
XXI	1/2	162	100	1.3	10^{-10}
XXII	2	190	290	0.8	10^{-10}
XXIII	1/2	150	100	1.4	10^{-10}
XXV	1/2	187	150	1.1	$<10^{-10}$
XXVI	1/2	187	150	2.6	$<10^{-10}$
XXVII	1/2	187	150	12.6	10^{-10}

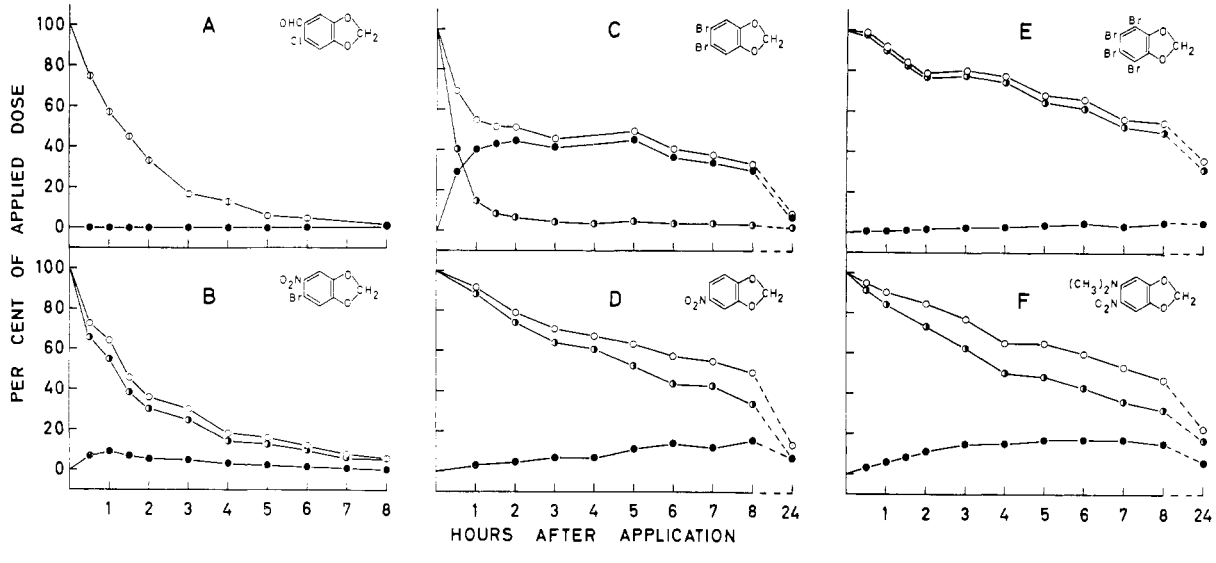


Figure 2. Penetration curves for substituted 1,3-benzodioxoles in the housefly

● Internal extract
◑ External rinse
○ Total recovery (rinse + extract)
◌ Coincidence of external rinse and total recovery

of these aldehydes with carbaryl and suggests a very rapid and efficient metabolic removal, probably through oxidation [or reduction (*6*)] and conjugation of the aldehyde group. The fact that these compounds exhibit any synergistic activity at all is interesting in view of the small amount of material accumulating in insect tissues.

The nitrochloro (XVI) and nitrobromo (XVII) compounds (Figure 2*B*) are also rapidly eliminated by the insect, the internal concentration rapidly reaching a maximum of about 10% of the applied 5-μg. dose 1 to 1½ hours after treatment, and thereafter falling until at 8 hours only trace amounts can be detected. It seems possible that these compounds are readily conjugated by the insect and rapidly excreted as mercapturic acids in a manner similar to that described in mammals for similar halogenated nitrobenzenes (*4*). This involves the replacement of the halogen atom [or possibly the nitro group (*3*)] by an *N*-acetylcysteine moiety, the cysteine originating from glutathione. Within ½ hour of treatment with these compounds the flies begin to excrete yellow material which is not the intact material and which cannot be detected on the gas chromatographic columns employed. Attempts have

not yet been made to identify these metabolites.

The remaining compounds show much greater persistence than those discussed above but show marked differences with regard to their rate of penetration and the extent to which they accumulate within the insect.

Three compounds (IX, X, and XIV), all halogen-containing, are remarkable for their extremely rapid disappearance from the outside of the insect and their accumulation within. This is most marked with the dichloro compound (IX) and when flies treated with 5 μg. of this material were held in Erlenmeyer flasks, 60% of this dose was found to have penetrated the fly within 1/2 hour. The corresponding dibromo derivative (X) was found to penetrate only slightly less rapidly. In both cases a maximum internal concentration approximating 80% of the applied dose was reached after about 2 hours, and this fell slowly over 8 hours. Twenty-four hours after treatment approximately 20% of the amount applied still remained within the flies. When the treated flies were held after treatment on filter paper under inverted glass dishes, a maximum of only 40% of the applied dose was found within the flies (Figure 2*C*), although the rate of penetration as measured by external disappearance had apparently increased. This suggests that under the dishes considerable loss occurs through volatilization, a factor which is not observed to the same extent in the enclosed environment of the flasks. The apparently rapid penetration of these compounds may in fact involve uptake in the vapor phase through the respiratory system rather than direct penetration through the insect cuticle. In a number of other compounds evaluated, especially some of the more volatile oils such as 1,3-benzodioxole (I), and the monohalogenated derivatives (IV and V) this volatilization factor could account for considerable losses, and may be partially responsible for their low synergistic activity. Unfortunately, penetration data on these compounds are not available. The differences in penetration characteristics between flask-held flies and those held under glass dishes were not obvious with the other compounds studied.

In spite of the losses discussed above, substantial concentrations of the dihalogenated compounds are found within the insect and the fact that these are not such active synergists as many of the others suggests that they are innately less efficient at the site of action. On the other hand, it may prove that uptake through the respiratory system in some manner results in a lower accumulation at the site of action than that resulting from direct cuticular penetration, and may therefore indicate the importance of the cuticle as a major site of detoxication.

At the other extreme to compounds IX, X, and XIV, a number of compounds penetrate rather slowly and in these cases internal concentrations never attain a high level. Thus the poor in vivo synergistic activity of 5,6-dinitro-1,3-benzodioxole (XVIII) and the tetrabromo derivative (XII) (Figure 2*E*), may be partially attributed to the very low internal levels reached by these compounds, which is never more than 2% of the 5 μg. applied in the case of XVIII. These two compounds must possess considerably less synergistic potential at the site of action than compounds XV and XIX, since although the latter attain internal concentrations of only 8 and 3%, respectively, of the applied dose they are among the most active compounds evaluated in the benzene series. In addition, 4-nitronaphtho-[2,3-*d*]-1,3-dioxole (XXV) and 4,9-dibromonaphtho-[2,3-*d*]-1,3-dioxole (XXVI), also outstanding synergists, attain only very low internal levels.

The extremely slow penetration of the tetrabromo derivative (XII) is interesting in view of the rapid internal accumulation found with the corresponding dibromo compound (X) and may further be evidence of the fact that the major mode of ingress of the latter is accomplished in the vapor phase.

The remaining compounds on which penetration data have been obtained (VI, XI, and XX) (Figures 2, *D* and *F*, and 3*A*) fall into an intermediate group. Maximum internal concentrations rise to approximately 20% of the 5 μg. applied. Each is an effective in vivo synergist for carbaryl.

Apart from those compounds such as the aldehydes (XXI to XXIII) and the halogenated nitro derivatives (XVI and XVII), which fall into distinct groups for reasons already discussed, and possibly the dihalogenated compounds (IX and X) where volatility leads to complications, a remarkable similarity becomes obvious within most of the remaining members of the group. This can be seen in Table IV, in the column

Table IV. Penetration into Housefly of Some Nitro- and Halogen-Containing 1,3-Benzodioxoles and Naphtho-1,3-dioxoles

Compound	Method of Holding	Maximum Internal Concn.[a]	Duration of Max. Internal Concn.[b]	Total % Recovery at 8 Hr.[c]
VI	D[d]	18–20	8–18	49.6
IX	D	35–42	1–8	35.4
	F[e]	70–75	1–8+	74.5
X	D	40–45	1–5	33.2
	F	80–85	2–8+	76.3
XI	D	15–25	4–24+	49.2
XII	D	4–6	6–24+	55.0
XIV	F	55–60	3–6	48.0
XV	D	4–8	7–24+	74.3
XVI	D	9–12	1/2–1 1/2	1.0
XVII	D	7–10	1/2–1 1/2	6.0
XVIII	D	1 1/2–2	4–8+	53.0
XIX	D	2–3	3–24+	50.9
XX	D	11–17	2–8+	46.9
XXI	D	Trace	1–8+	20.0
XXII	D	Trace	...	1.0
XXIII	D	1 1/2–2	3–8+	9.1
XXV	D	1 1/2–2 1/2	2–24+	53.2
XXVI	D	5–8	4–24+	56.2
XXVII	D	Trace	1–24+	66.8

[a] % of applied dose of 5 μg./fly.
[b] Hours after application.
[c] Sum of external rinse and internal extract expressed as per cent of applied dose.
[d] Dishes.
[e] Flasks (see text).

representing the total per cent recovery at 8 hours. This figure, indicating the total amount of unchanged material (rinse plus extract, as a percentage of the applied dose) remaining 8 hours after treatment, should indicate by difference the total amount of metabolism. This figure remains remarkably constant at about 45 to 55%, which might indicate the existence of a common metabolic process. The only common factor within this group of compounds is the dioxymethylene ring and it seems possible that the difference between the total recovery curve and the dose applied represents the in vivo metabolic cleavage of this ring. A direct result of this relatively constant rate of metabolism is that the internal concentration of any particular compound is directly proportional to its rate of penetration (Figure 2). The decrease in total recovery is not a measure of physical loss, as prolonged washing of the holding vessels yields only traces of unchanged material. Compounds XV and XXVII with total recoveries at 8 hours of 74.3 and 66.8%, respectively, appear to be exceptions, and no explanation is immediately obvious to explain their greater in vivo stability.

To substantiate evidence of metabolism, tests have been conducted under completely anoxic conditions, the flies being held after treatment in glass-stoppered Erlenmeyer flasks in an atmosphere of pure nitrogen. Under these conditions the total recovery at 8 hours was found to be 96.4% in the case of 4,5,6,7-tetrachloro-1,3-benzodioxole (XI) (Figure 3*B*), compared with 49.2% (Figure 3*A*) obtained for the same compound in air. This is indicative of the existence of an oxidative mechanism capable of degrading this compound, although the possibility of secondary complications resulting from anoxia cannot be entirely ruled out.

Initial tests have been conducted in vitro with compound XI employing the NADPH-dependent (reduced nicotinamide adenine dinucleotide phosphate) microsomal system obtained from pig liver. Only a very small amount of metabolism of XI could be demonstrated (about 2%) in terms of recovery of unchanged material. Treatment of the deproteinized incubation mixture with alkali and dimethyl sulfate, followed by extraction into ligroin and subsequent gas chromatographic analysis, showed a small peak having the same retention time as the dimethyl ether of tetrachlorocatechol and suggested a small amount of metabolic cleavage of the 1,3-benzodioxole ring to the corresponding catechol. Further metabolic studies are at present in progress.

It was initially hoped that correlations would be found between the in vivo synergistic activity and the rate of internal accumulation of the several compounds evaluated. Unfortunately, this has not been entirely satisfactory, although the results indicate that large variations in penetration rate exist and that the speed of onset of symptoms and subsequent knockdown are probably related to this factor.

The marked synergistic activity of a number of the compounds evaluated in this study, combined with the fact that most are synthesized by relatively simple procedures from piperonal, one of the cheapest commercial sources of the 1,3-benzodioxole ring, suggests

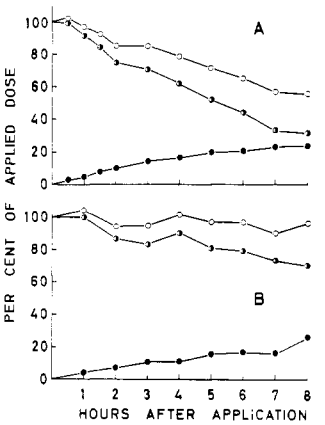


Figure 3. Penetration of 4,5,6,-7-tetrachloro-1,3-benzodioxole in the housefly

A. In air
B. In nitrogen
● Internal extract
◑ External rinse
○ Total recovery (rinse + extract)

that compounds of this type may have commercial significance. The introduction of a cheap effective synergist for the carbamates could result in the widespread agricultural application of the phenomenon of carbamate synergism, which has hitherto been mainly confined to laboratory investigations.

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