(4) Arnow, L. E., *J. Biol. Chem.* **118**, 531 (1937).
(5) Balls, A. K., Jansen, E. F., *Advances in Enzymol.* **13**, 321 (1952).
(6) Barthel, W. F., Alexander, B. H., Giang, P. A., Hall, S. A., *J. Am. Chem. Soc.* **77**, 2424 (1955).
(7) Casida, J. E., Chapman, R. K., Stahmann, M. A., Allen, T. C., *J. Econ. Entomol.* **47**, 64 (1954).
(8) Dostrovsky, I., Halmann, M., *J. Chem. Soc.* **1953**, 516.
(9) Epstein, J., Rosenblatt, D. N., Demek, M. M., *J. Am. Chem. Soc.* **78**, 341 (1956).
(10) Epstein, J., Rosenthal, R. W., Ess, R. J., *Anal. Chem.* **27**, 1435 (1955).
(11) Hackley, B. E., Plapinger, R., Stolberg, M. A., Wagner-Jauregg T., *J. Am. Chem. Soc.* **77**, 3651 (1955).
(12) Hartley, B. S., Kilby, B. A., *Nature* **166**, 784 (1950).
(13) Horning, E. C., Horning, M. C., *J. Org. Chem.* **11**, 95 (1946).
(14) Jandorf, B. J., Communications, 3rd International Congress of Biochemistry, Brussels, p. 136, 1955.
(15) Jandorf, B. J., Crowell, E. A., Levin, A. P., *Federation Proc.* **14**, 231 (1955).
(16) Jandorf, B. J., Michel, H. O., Schaffer, N. K., Egan, R., Summerson, W. H., *Discussions Faraday Soc.* **20**, 134 (1956).
(17) Jandorf, B. J., Summerson, W. H., Fleisher, J. H., Johnson, D. V., Abstracts of Papers, 116th Meeting, American Chemical Society, Atlantic City, N. J., p. 42C, 1946.
(18) Jandorf, B. J., Wagner-Jauregg, T., O'Neill, J. J., Stolberg, M. A., *J. Am. Chem. Soc.* **74**, 1521 (1952).
(19) Jansen, E. F., Nutting, M. D. F., Jang, R., Balls, A. K., *J. Biol. Chem.* **179**, 189 (1949).
(20) Kodama, S. K., Morse, M. S., Anderson, H. H., Dunlop, M. K., Hine, C. H., *Arch. Ind. Hyg. and Occupational Med.* **9**, 45 (1954).
(21) Kunitz, M., *J. Gen. Physiol.* **30**, 291 (1947).
(22) Lamb, A. B., Carleton, P. W., Maldrum, W. B., *J. Am. Chem. Soc.* **42**, 251 (1920).
(23) Levin, A. P., Jandorf, B. J., *J. Pharmacol. and Exptl. Therap.* **113**, 206, Footnote 3 (1955).
(24) Mazur, A., *J. Biol. Chem.* **164**, 271 (1946).
(25) Metcalf, R. L., March, R. B., *Ann. Entomol. Soc. Amer.* **46**, 63 (1953).
(26) Meyer, F., Hardebeck, K., Augustiny, U., *Arzneimittel-Forsch.* **5**, 109 (1955).
(27) Morse, M. S., Kodama, S. K., Hine, C. H., *Proc. Soc. Exptl. Biol. Med.* **83**, 765 (1953).
(28) Mounter, L. A., Floyd, C. S., Chanutin, A., *J. Biol. Chem.* **204**, 221 (1953).
(29) Nelson, N., *Ibid.*, **153**, 375 (1944).
(30) Perkow, W., Ullerich, K., Meyer, F., *Naturwissenschaften* **39**, 353 (1952).
(31) Rothenberg, M. A., Nachmansohn, D., *J. Biol. Chem.* **168**, 223 (1947).
(32) Strelitz, F., *Biochem. J.* **38**, 86 (1944).
(33) Wilson, I. B., *J. Biol. Chem.* **190**, 111 (1951).

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SYNERGISM IN PESTICIDES

Comparative Synergistic Effects of Synthetic 3,4-Methylenedioxyphenoxy Compounds in Pyrethrum and Allethrin Fly Sprays

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The comparative value of 63 synthetic 3,4-methylenedioxyphenoxy derivatives and two related compounds as synergists with either pyrethrins or allethrin was estimated in tests against the housefly, *Musca domestica* L., by the turntable method. Forty-three compounds were demonstrated to be synergistic with each insecticide. Certain ethers, acetals, and esters of aromatic sulfonic acids had strong effect, whereas esters of carboxylic and carbamic acids had little or no effect. The intensity of synergism was so high for 18 compounds—toxicity was raised to at least six times that expected for pyrethrins alone or three times that expected for allethrin alone—that further work was recommended.

The best synergists for pyrethrins and allethrin contain the 3,4-methylenedioxyphenyl group in the molecule, although the presence of this group does not in itself assure synergistic effect (*6*, *8*, *12*). It has recently been shown that sesamolin, obtained from sesame oil and differing structurally from sesamin in containing a 3,4-methylenedioxyphenoxy group in place of one of the 3,4-methylenedioxyphenyl groups (*2*, *3*), is a much more effective synergist with pyrethrins against houseflies, *Musca domestica* L., by the turntable method than is sesamin (*7*). It was, therefore, desirable to prepare other 3,4-methylenedioxyphenoxy derivatives and evaluate them as candidate synergists. The preparation of 66 such compounds has been reported (*1*). The present paper reports the results of tests with 63 of these compounds designed to evaluate them separately in mixtures with pyrethrins or allethrin against the housefly. The purpose of this study is to select the most promising synergists for future, more precise comparison. Similar tests with two compounds that are not 3,4-methylenedioxyphenoxy derivatives are also reported for comparison with those of closely related compounds—1-allyl-3,4-methylenedioxybenzene (known as safrole) and 2-(*p*-methoxyphenoxy)tetrahydropyran.

Purified samples of the synthetic compounds were used. The sample of pyrethrins was the complex contained in the extractives from pyrethrum flowers not further processed for the removal of materials other than the toxicants. It had been proved to be biologically stable when held in the dark at room temperature in a 4% kerosine solution and compared periodically with allethrin. In this sample, 61% of the total pyrethrins consisted of pyrethrin I and cinerin I as determined by the mercury-reduction method. The sample of allethrin was of 94% purity as determined by the hydrogenolysis method.

Sprays of the two insecticides, alone and mixed with each of the adjuncts, and sprays of the adjuncts alone were prepared at concentrations determined by preliminary tests. The sprays containing the insecticides alone were prepared at several concentrations in refined kerosine. One spray of each mixture was prepared. The concentration was usually 100 mg. of pyrethrins plus 1000 mg. of adjunct. or 50 mg. of allethrin plus 500 mg. of adjunct per 100 ml. of kerosine. There were some departures from these concentrations as the result of shortage or low solubility of the adjuncts. For the latter reason acetone was used as an auxiliary solvent in some sprays. One spray was prepared for each adjunct alone at its concentration in the mixture with pyrethrins and in the same solvent.

Method

Knockdown in 25 minutes and mortality in 1 day of laboratory-reared adult houseflies were determined in replicated tests by the Campbell turntable method (*4*). In each test approximately 100 flies averaging 2.5 to 3.5 days of age were used. A number of series of tests were made, each comprising the tests on the same populations of flies and including tests with standard sprays. All sprays in a series were tested simultaneously on each population. Because all the compounds were not available at one time, the number of sprays in a series varied.

Knockdown and Mortality Results

The sprays containing the adjuncts alone caused little or no knockdown and mortality. The standard sprays containing the insecticides alone and the mixed sprays caused complete knockdown. The mean mortality results obtained with them are given in Table I.

Evaluation of Joint Action

Table I also shows the insecticide equivalents necessary to assess the relative toxicity of the mixtures. To obtain these equivalents and to estimate their experimental error and so obtain the requirements to demonstrate synergism, the following procedure was used: In each series a weighted regression equation of probit mortality on log concentration was computed for the standard insecticide by the method described by Finney (*5*). From this equation the logarithms of the insecticide equivalents were calculated for the individual mortalities obtained with all the sprays in the series. From an analysis of variance of these log concentrations the insecticide equivalent required for a significant difference from the actual insecticide content was calculated. This requirement, together with the regression equation and the relative standard error, is also reported in Table I. The equivalents reported are the means of the individual equivalents; the equivalents of the mean mortalities may be calculated directly with the use of the equations. As a precise comparison of estimations from mortalities approaching 100% should not be relied upon, the calculated equivalents for mortality levels above 95% are not given.

For mixtures containing acetone its toxicity has been allowed for in the calculation of the equivalents. This may be done with accuracy because of a study of similar tests on acetone-kerosine sprays containing pyrethrins or allethrin (unpublished), and the fact that ratios of toxicity by this method have been shown to be reproducible. The study shows that, between 0 and 75%, for an increment of 25% in acetone content, the ratio of toxicity is increased 55%. The effect of the smaller amounts may therefore be interpolated; the ratio of toxicity (insecticide equivalent of an acetone-kerosine mixture divided by the actual insecticide content) is 1.04 for a mixture containing 2.5% of acetone, 1.09 for 5%, 1.19 for 10%, 1.30 for 15%, and 1.42 for 20%. As may be seen, the values for the higher amounts approach significant differences in themselves. Therefore, to permit comparison of the mixtures containing acetone, the equivalents reported are adjusted to equivalents in kerosine alone by means of these ratios. The required equivalents to demonstrate synergism are also those in kerosine alone.

For mixtures containing precisely 100 mg. of pyrethrins or 50 mg. of allethrin per 100 ml., the insecticide equivalents, when those for the allethrin mixtures are multiplied by 2, are expressions of relative per cent toxicity. For the other mixtures the equivalents are to be adjusted according to the insecticide concentration used.

Because the adjuncts were nontoxic at the concentrations used, any significant increase in toxicity over that of the insecticide in each mixture may be ascribed to synergistic action. There were 22 compounds that either were not synergistic or caused such slight synergism (toxicity increased in their mixtures but 70% or less) that the increase was not significant in these tests. All but two, safrole and 2-(*p*-methoxyphenoxy)tetrahydropyran, are examples of compounds that lack pronounced synergistic effect despite the presence of the 3,4-methylenedioxyphenoxy group in the molecule; they are given below. Exceptions to the insecticide-synergist proportion of 1 to 10 are noted in parentheses. In the names appearing in this and subsequent groups R refers to 3,4-methylenedioxyphenyl and RO refers to 3,4-methylenedioxyphenoxy.

The 22 following compounds were not synergistic.

	Pyrethrins	Allethrin
Acetic acid		
Chloro-, R ester		
(RO)-, butyl ester		
Benzoic acid		
R ester		
o-Chloro-, R ester		
p-Chloro-, R ester		
o-Ethoxy-, R ester		
n-Butyric acid, R ester		
Caproic acid, R ester		
Carbamic acid		
1-Naphthyl-, R ester	(1:3)	(1:4)
Phenyl-, R ester	(1:3)	(1:3)
Carbonic acid		
n-Butyl R diester		
Ethyl R diester		
Isobutyl R diester		
Cyclohexanecarboxylic acid, R ester		
Ether, R trimethylsilyl		
2-Furoic acid, R ester		
Methane, bis(RO)-	(1:5)	
Palmitic acid, R ester		
Propionic acid, R ester		
Pyran, tetrahydro-, 2-(*p*-methoxyphenoxy)-		
Safrole		

In 43 compounds synergism was demonstrated and its approximate intensity with each insecticide—insecticide equivalent divided by actual content—is given below.

	Pyrethrins	Allethrin
Carbamic acid		
o-Tolyl-, R ester	2	2
m-Tolyl-, R ester	2	2
p-Tolyl-, R ester	2	1
Ether		
Allyl R	2	2
p-Bromobenzyl R	2 (1:9)	3 (1:9)
2-Chloroallyl R	2	2
3-Chloroallyl R	2	2
2,4-Dichlorobenzyl R	2	2
3,4-Dichlorobenzyl R	2	2
Acetaldehyde, 2-ethylhexyl R acetal	3	3
Ether		
n-Amyl R	3	2
n-Butyl R	3	3
o-Chlorobenzyl R	3	2
p-Chlorobenzyl R	3	2
Isoamyl R	3	2
p-Nitrobenzyl R	3 (1:7)	2 (1:7)
Propyl R	3	2

Table I. Joint Toxic Action against Houseflies of Certain 3,4-Methylenedioxyphenoxy

	With Pyrethrins			With Allethrin		
Adjunct[a] and Standard	Concentration, mg./dl.	Arithmetic mean mortality in 1 day, %	Geometric mean pyrethrins equivalent[b], mg./dl.	Concentration, mg./dl.	Arithmetic mean mortality in 1 day, %	Geometric mean allethrin equivalent[b], mg./dl.
Series 1. Four Replicates						
Pyran, tetrahydro-, 2-(RO)	100 + 500	72.9	471	50 + 250	69.1	139
Standard	506	79.4	544	200	84.9	194
	338	57.3	306	133	71.6	144
	225	45.0	229	88.9	43.4	87.8
	150	28.4	153	59.3	21.6	56.4
Required to demonstrate synergism			131	..	..	65.4
Equation for standard		$Y = 2.525X - 1.090$			$Y = 3.516X - 1.998$	
Relative standard error of insecticide equivalent, 10.6%						
Series 2. Two Replicates						
Ether						
Benzyl R	100 + 1000	63.4	458	50 + 500	74.2	112
o-Chlorobenzyl R	100 + 1000	49.1	321	50 + 500	69.5	103
p-Chlorobenzyl R	100 + 1000	46.1	300	50 + 500	61.9	90.3
2,4-Dichlorobenzyl R	100 + 1000	33.8	219	50 + 500	71.8	107
Standard	338	51.2	338	133	82.6	133
	150	20.4	150	50.0	26.3	48.5
Required to demonstrate synergism			136	..	..	67.9
Equation for standard		$Y = 2.433X - 1.122$			$Y = 3.657X - 1.847$	
Relative standard error of insecticide equivalent, 11.4%						
Series 3. Two Replicates						
Chrysanthemumic acid, R ester	100 + 1000	56.4	410	50 + 500	59.5	93.4
Ether						
p-Bromobenzyl R	100 + 920[c]	37.7	236	50 + 460[d]	81.9	141
2-Chloroallyl R	100 + 1000	24.8	172	50 + 500	55.6	88.0
3-Chloroallyl R	100 + 1000	36.7	250	50 + 500	57.1	89.3
3,4-Dichlorobenzyl R	100 + 1000	31.6	217	50 + 500	67.8	109
Standard	338	48.5	337	133	77.9	135
	150	19.6	148	50.0	25.0	49.8
Required to demonstrate synergism			151	..	..	75.5
Equation for standard		$Y = 2.323X - 0.910$			$Y = 3.388X - 1.430$	
Relative standard error of insecticide equivalent, 16.3%						
Series 4. Two Replicates						
Ether						
n-Amyl R	100 + 1000	49.8	326	50 + 500	67.9	84.5
Isoamyl R	100 + 1000	44.5	281	50 + 500	71.5	90.7
p-Nitrobenzyl R	90 + 670[e]	56.2	275	45 + 340[f]	69.4	69.3
Pyran, tetrahydro-, 2-(*p*-methoxy phenoxy)-[g]	100 + 1000	23.6	146	50 + 500	33.1	47.8
Standard	338	50.9	337	133	88.3	135
	150	24.0	148	50.0	35.5	49.9
Required to demonstrate synergism						
For mixtures containing usual amount of insecticide			147	..	..	73.7
For mixtures containing 0.9 that amount			133			66.3
Equation for standard		$Y = 2.044X - 0.144$			$Y = 3.668X - 1.604$	
Relative standard error of insecticide equivalent, 15.1%						
	Series 5. Four Replicates			**Series 6. Four Replicates**		
Acetic acid						
R ester	100 + 1000	22.1	150	50 + 500	40.3	66.6
Chloro-, R ester	100 + 1000[e]	32.4	175	50 + 500[c]	49.8	70.9
(RO)-, butyl ester	100 + 1000	18.0	129	50 + 500	37.1	63.4
Benzoic acid						
R ester	100 + 1000	20.5	145	50 + 500	32.8	57.8
p-Chloro-, R ester	100 + 1000[c]	21.0	134	50 + 500[c]	43.4	63.9
o-Ethoxy-, R ester	100 + 1000[c]	14.2	106	50 + 500[c]	49.6	70.7
n-Butyric acid, R ester	100 + 1000	12.1	110	50 + 500	20.7	43.7
Caproic acid, R ester	100 + 1000	16.3	126	50 + 500	41.0	67.1
Carbonic acid						
n-Butyl R diester	100 + 1000	17.2	128	50 + 500	31.4	55.4
Ethyl R diester	100 + 1000	11.2	102	50 + 500	30.8	56.8
Isobutyl R diester	100 + 1000	15.3	123	50 + 500	40.4	66.0
Cyclohexanecarboxylic acid, R ester	100 + 1000	18.9	138	50 + 500	36.7	61.6
Ether						
2-(2-*n*-butoxyethoxy)ethyl R	100 + 1000	99.1	>1000	50 + 500	99.5	>200
n-Butyl R	100 + 1000	49.4	271	50 + 500	82.2	144
2-(2-Chloroethoxy)ethyl R	100 + 1000	93.4	926	50 + 500	91.8	189
Cyclohexyl R	100 + 1000	91.5	1170	50 + 500	88.0	171
2-Cyclohexylethyl R	100 + 1000	93.0	871	50 + 500	74.0	125
Cyclopentyl R	100 + 1000	82.2	574	50 + 500	84.2	149
2-(2-Ethoxyethoxy)ethyl R	100 + 1000	99.3	>1000	50 + 500	98.3	>200

Derivatives and Two Related Compounds with Pyrethrins or Allethrin

	With Pyrethrins			With Allethrin		
Adjunct[a] and Standard	Concentration, mg./dl.	Arithmetic mean mortality in 1 day, %	Geometric mean pyrethrins equivalent[b], mg./dl.	Concentration, mg./dl.	Arithmetic mean mortality in 1 day, %	Geometric mean allethrin equivalent[b], mg./dl.
2-Ethylhexyl R	100 + 1000	74.0	479	50 + 500	80.7	134
R 2-*n*-octyl	100 + 1000	63.4	361	50 + 500	71.6	115
R Trimethylsilyl	100 + 1000	18.8	137	50 + 500	44.9	71.3
Propyl R	100 + 1000	51.5	284	50 + 500	62.1	94.1
2-Furoic acid, R ester	100 + 1000[h]	21.1	120	50 + 500[c]	41.9	61.6
Palmitic acid, R ester	100 + 1000	6.4	80.8	50 + 500	32.5	57.7
Propionic acid, R ester	100 + 1000	17.3	129	50 + 500	32.8	56.8
Standard	759	89.4	752	200	89.7	171
	506	79.3	540	133	82.4	150
	338	61.5	347	88.9	66.3	108
	225	40.3	226	59.3	37.9	61.1
	150	20.9	146	39.5	7.2	30.8
Required to demonstrate synergism			144	..	..	68.7
Equation for standard		$Y = 2.962X - 2.224$			$Y = 3.783X - 2.146$	
Relative standard error of insecticide equivalent, 15.8%					13.3%	
	Series 7.	Four Replicates		Series 8.	Four Replicates	
Acetaldehyde						
2-(2-*n*-butoxyethoxy)ethyl R acetal	100 + 1000[c]	99.6	>1000	50 + 500[d]	100	>170
2-*n*-butoxyethyl R acetal	100 + 1000	99.4	>1000	50 + 500	98.6	>170
n-Butyl R acetal	100 + 1000	74.4	371	50 + 500	83.3	107
2-Chloroethyl R acetal	100 + 1000	88.3	666	50 + 500	93.3	160
2-(2-Ethoxyethoxy)-ethyl R acetal	100 + 1000[i]	100	>1000	50 + 500[i]	100	>170
Ethyl R acetal	100 + 1000	84.4	552	50 + 500	91.5	191
2-Ethylhexyl R acetal	100 + 1000	68.7	318	50 + 500	90.7	141
Isobutyl R acetal	100 + 1000	76.0	391	50 + 500	87.2	115
p-Dioxane, 2-(RO)-	100 + 1000[h]	99.5	>1000	50 + 500[c]	96.9	>170
Ether						
Allyl R	100 + 1000	49.1	185	50 + 500	84.7	109
2-*n*-Butoxyethyl R	100 + 1000	99.0	>1000	50 + 500	96.0	182
Furan, tetrahydro-, 2-ethoxy-5-(RO)-	100 + 1000	99.5	>1000	50 + 500	98.3	>170
Safrole[g]	100 + 1000[c]	35.6	119	50 + 500[d]	67.8	71.3
Standard	759[i]	95.8	1080	200	95.3	>170
	506	84.7	595	133	93.8	158
	338	66.1	292	88.9	81.9	98.1
	225	56.4	224	59.3	51.6	54.1
	150	44.0	163	39.5	34.6	40.1
Required to demonstrate synergism			176	..	..	79.3
Equation for standard		$Y = 2.278X - 0.189$			$Y = 3.379X - 0.817$	
Relative standard error of insecticide equivalent, 26.8%					20.6%	
	Series 9.	Four Replicates		Series 10.	Four Replicates	
Acetaldehyde, 2-methoxyethyl R acetal	100 + 1000	99.5	> 900	50 + 500	98.6	>220
Benzenesulfonic acid, R ester	100 + 1000[f]	99.8	> 900	50 + 500[h]	99.1	>220
p-chloro-, R ester	100 + 1000[f]	95.5	823	50 + 500[h]	92.6	181
Benzoic acid, *o*-chloro-, R ester	100 + 1000[h]	33.8	138	50 + 500[c]	49.0	65.8
Carbamic acid						
1-Naphthyl-, R ester	100 + 300[e]	33.9	114	50 + 200[e]	37.8	41.7
Phenyl-, R ester	100 + 300[h]	30.1	125	50 + 150[h]	45.1	56.2
o-Tolyl-, R ester	100 + 500[f]	60.7	228	50 + 250[h]	78.3	107
m-Tolyl-, R ester	100 + 1000[h]	51.7	204	50 + 500[e]	71.8	99.1
p-Tolyl-, R ester	100 + 1000[e]	58.3	197	47.5 + 475[h]	44.8	56.1
Methane, bis(RO)-	100 + 500[e]	45.4	145	50 + 500[e]	67.4	70.4
2-Naphthalenesulfonic acid, R ester	100 + 1000[f]	74.9	316	50 + 500[h]	79.0	114
p-Toluenesulfonic acid, R ester	100 + 1000[f]	91.6	575	50 + 500[h]	93.9	185
Standard	759	93.1	898	200[i]	91.8	189
	506	80.1	481	133	82.9	151
	338	67.4	342	88.9	63.8	93.4
	225	48.4	227	59.3	37.1	58.4
				39.5	17.0	38.0
Required to demonstrate synergism			148	..	..	87.1
Equation for standard		$Y = 2.772X - 1.571$			$Y = 3.443X - 1.416$	
Relative standard error of insecticide equivalent, 16.8%					26.1%	

[a] R = 3,4-methylenedioxyphenyl; RO = 3,4-methylenedioxyphenoxy.
[b] Equivalents in acetone-kerosine sprays adjusted to equivalents in kerosine only.
[c] 5% acetone.
[d] 2.5% acetone.
[e] 20% acetone.
[f] 15% acetone.
[g] Not an RO compound.
[h] 10% acetone.
[i] 2 replicates.

2-Naphthalenesulfonic acid, R ester	3	2
Acetaldehyde		
n-Butyl R acetal	4	2
Isobutyl R acetal	4	2
Chrysanthemumic acid, R ester	4	2
Ether		
2-*n*-Octyl R	4	2
Benzyl R	5	2
2-Ethylhexyl R	5	3
Pyran, tetrahydro-, 2-(RO)-	5 (1:5)	3 (1:5)
Acetaldehyde, ethyl R acetal	6	3.5 or greater
Ether, cyclopentyl R	6	3
p-Toluenesulfonic acid, R ester	6	3.5 or greater
Acetaldehyde, 2-chloroethyl R acetal	7	3
Benzenesulfonic acid, *p*-chloro-, R ester	8	3.5 or greater
Acetaldehyde		
2-(2-*n*-butoxyethoxy)ethyl R acetal	9 or greater	3.5 or greater
2-*n*-Butoxyethyl R acetal	9 or greater	3.5 or greater
2-(2-Ethoxyethoxy)ethyl R acetal	9 or greater	3.5 or greater
2-Methoxyethyl R acetal	9 or greater	3.5 or greater
Benzenesulfonic acid, R ester	9 or greater	3.5 or greater
p-Dioxane, 2-(RO)-	9 or greater	3.5 or greater
Ether		
2-(2-*n*-Butoxyethoxy)ethyl R	9 or greater	3.5 or greater
2-*n*-Butoxyethyl R	9 or greater	3.5 or greater
2-(2-Chloroethoxy)ethyl R	9 or greater	3.5 or greater
Cyclohexyl R	9 or greater	3.5 or greater
2-Cyclohexylethyl R	9 or greater	3
2-(2-Ethoxyethoxy)ethyl R	9 or greater	3.5 or greater
Furan, tetrahydro-, 2-ethoxy-5-(RO)-	9 or greater	3.5 or greater

The last 18 compounds listed approach the effectiveness of the best commercial synergists—sulfoxide (*11*), sulfone (*9*), piperonyl butoxide (*10*), propyl isomer (*10*), and piperonyl cyclonene (*6*)—as judged with the same method of application and against the housefly. Further work with the new compounds designed to make more precise comparison of such strong effects is therefore warranted.

Discussion

Safrole was included for comparison with allyl 3,4-methylenedioxyphenyl ether. As the compounds differ structurally only in the ether linkage and the ether was established as synergistic whereas safrole was not, the comparison furnishes another example of the importance of this linkage. However, the possession of this linkage alone does not give assurance of synergistic effect. With a compound related to the highly synergistic tetrahydropyran derivative but not containing the 3,4-methylenedioxy group—2-(*p*-methoxyphenoxy)-tetrahydropyran—synergism was not found with either insecticide. In this case the replacement of the methylenedioxy group with the *p*-methoxy group destroyed synergistic effect.

In so far as comparisons could be made, the more synergistic a compound was with pyrethrins the more synergistic it was in general with allethrin. However, the intensity of synergism had a pronounced trend to be greater with pyrethrins.

The only esters found to have an appreciable synergistic effect were those of certain aromatic sulfonic acids. With each insecticide the intensity was only moderate for the ester of 2-naphthalenesulfonic acid, but relatively high for the others.

With but one exception, synergism was demonstrated in mixtures containing the ethers. The exception was the trimethylsilyl compound, which is, in fact, not a true ether, containing the linkage C—O—Si instead of C—O—C. In general, the greater the number of carbon atoms in the substituting group the greater was the intensity, especially when the group was cyclic or contained an alkoxy group. Introduction of a bromine or chlorine atom into a chain did not increase the intensity; in fact, for the benzyl analog and its halogenated derivatives (and a nitro derivative as well) there was a decrease.

The acetals were highly synergistic, those containing an alkoxy group in the substituted chain resulting in the highest intensity with either insecticide.

The urethan derivatives—that is, the esters of carbamic acids—resulted in little or no synergism.

Conclusions

Forty-three compounds were demonstrated to be synergistic with either pyrethrins or allethrin. Certain ethers, acetals, and esters of aromatic sulfonic acids had strong effect, whereas esters of carboxylic and carbamic acids had slight or no effect. The intensity of synergism was so high for 18 compounds—the toxicity of the mixtures was at least six times that expected for pyrethrins alone or three times that expected for allethrin alone—that further work is recommended with them.

At the concentrations tested knockdown of flies in 25 minutes was complete with all sprays containing the insecticides. Sprays containing the compounds alone caused no or negligible knockdown and mortality.

Literature Cited

(1) Beroza, Morton, J. Agr. Food Chem. **4**, 49 (1956).
(2) Beroza, Morton, *J. Am. Chem. Soc.* **77**, 3332–4 (1955).
(3) Beroza, Morton, *J. Am. Oil Chemists' Soc.* **31**, 302–5 (1954).
(4) Campbell, F. L., Sullivan, W. N., *Soap* **14** (6), 119 (1938).
(5) Finney, D. J., "Probit Analysis," University Press, Cambridge, 1952.
(6) Gersdorff, W. A., Mitlin, Norman, *J. Econ. Entomol.* **45**, 519–23 (1952).
(7) Gersdorff, W. A., Mitlin, Norman, Beroza, Morton, *Ibid.*, **47**, 839–42 (1954).
(8) Gersdorff, W. A., Mitlin, Norman, Gertler, S. I., U. S. Dept. Agr., Bur. Entomol. and Plant Quarantine **E-848** (1952).
(9) Gersdorff, W. A., Mitlin, Norman, Nelson, R. H., *J. Econ. Entomol.* **48**, 9–11 (1955).
(10) Gersdorff, W. A., Nelson, R. H., Mitlin, Norman, *Ibid.*, **44**, 921–7 (1951).
(11) *Ibid.*, **45**, 905–8 (1952).
(12) Schroeder, H. O., Jones, H. A., Lindquist, A. W., *Ibid.*, **41**, 890–4 (1948).

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Microbiological and Chick Assay of Vitamin B_{12} Activity in Feed Supplements and Other Natural Products—Correction

In the article on "Microbiological and Chick Assay of Vitamin B_{12} Activity in Feed Supplements and Other Natural Products" [J. Agr. Food Chem. **4**, 364 (1956)] the following corrections should be made in Table II. In Diet I 30 grams of soybean meal were used, and 2.3 grams of Mico mix instead of calcium carbonate. In Diet II 2.0 grams of calcium carbonate were used.

Footnote *b* of Table II should read, beginning with the second line: phosphate, 225; magnesium sulfate, 125; manganese sulfate monohydrate, 20; potassium iodide, 0.3; zinc acetate, 0.7; aluminum sulfate (alunogenite), 0.8; ferric citrate, 25; copper sulfate pentahydrate, 1.0; cobalt acetate, 0.2; and nickel chloride, 0.1 gram.

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