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Structure-Activity Correlations in DDT Analogs

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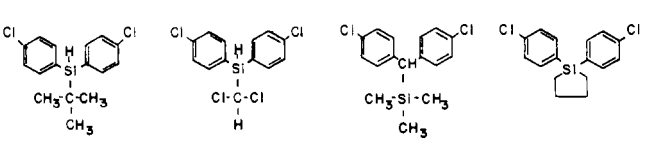
The relationship between the structure of a series of DDT analogs and the insecticidal activity to houseflies and mosquito larvae was examined by means of multiple regression analysis using the substituent constants E_s, π, σ^*, F, and R. The steric substituent constant E_s was the single most important parameter for the correlation of insecticidal activity when substituents are varied on the aromatic ring and on the α-carbon atom.

In connection with our continuing efforts to discover new approaches for the design of selective and biodegradable insecticides, the synthesis and evaluation of the insecticidal activity of a series of silicon-containing analogs of DDT were carried out. The structures of some of these compounds in which silicon occupies a key position in the molecule are given below. The rationale behind the examination of silicon DDT analogs as biodegradable insecticides resides in the greater instability of the C–Si bond compared to the C–C bond to ionic and radical reactions.



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To our disappointment, virtually all of the approximately 20 silicon analogs, including I–IV, were ineffective against houseflies, showing little or no toxicity at 500 μg/g, alone or in combination with the synergist piperonyl

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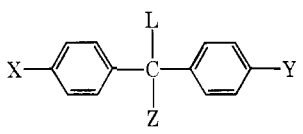
butoxide. The inactivity of these silicon DDT analogs was somewhat surprising, particularly since many of the compounds were direct isosteres of active compounds. Further, it appeared unlikely that these compounds were ineffective because of poor penetrating ability into the housefly, since silicon-containing carbamate esters have been shown to be as active as their carbon analogs (Metcalf and Fukuto, 1965).

There are several possible explanations for the poor insecticidal activity of the silicon DDT analogs. One possibility is that these compounds are too unstable in biological systems and are degraded before they reach the target site, even though they appear to be chemically quite stable and are distillable at elevated temperatures.

Another explanation for the ineffectiveness of the silicon analogs may be that these compounds are inherently inactive owing to poor fit at the site of action. Silicon is approximately 50% larger in size than carbon, and the Si–C bond (1.86 Å) is considerably longer than the C–C bond (1.48 Å). The effect of the increase in bond length may be seen by comparing the average van der Waal's radii of 2.79 and 3.3 Å for the *tert*-butyl and trimethylsilyl moieties, respectively (Charton, 1969). Therefore, the substitution of a silicon atom in a central position in the DDT analog would cause an increase in the overall size of the molecule in all tetrahedral directions and it is possible that this increase in size prevents interaction of the molecule with the DDT receptor site. Although this may be a plausible explanation for the inactivity of silicon DDT analogs, actually there is little theoretical basis for its support. For this reason, it was decided that an analysis of the relationship between the structure of DDT analogs and insecticidal activity was needed for purposes of correlation and prediction of activity.

In spite of the fact that it has been over three decades since the discovery of the insecticidal properties of DDT, relatively little has been published concerning structure–activity relationships among DDT analogs. This is in fact attributable to the absence of a satisfactory account of the mode of action of DDT at the molecular level. The bulk of the published work on DDT structure–activity relations has been based on the use of molecular models and attempts have been made to correlate the size and shape of the model with interaction at a hypothetical DDT receptor site (Holan, 1969; Mullins, 1956; Rogers *et al.*, 1953). This kind of approach has been useful in providing qualitative insight to the DDT structure–activity relationship but has failed to provide quantitative relationships.

Theory. The working model that we have adopted for the mode of action of DDT and related compounds is essentially a modification of the model proposed by Holan (1969). The basic premise is that DDT and its analogs fit in a receptor site of a macromolecule, possibly a protein or lipoprotein in a nerve membrane. The receptor site may be visualized as a cavity or a pouch with a limited amount of flexibility and is by no means a rigid structure. Maximum effect occurs when there is maximum interaction between the DDT molecule and the receptor site, particularly with respect to the four key substituents X, Y, L, and Z (structure below) through van der Waal's



forces (Gunther *et al.*, 1954). For maximum interaction the overall size of the DDT molecule, *i.e.*, summation of the size of X, Y, L, and Z, is critical and any deviation from this size results in reduced interaction, hence reduced activity. The concept of maximum overall size is illustrated in Figure 1. **M** is a substituent approximately the size of Cl, **S** is smaller than the **M**, and **L** is larger than **M.** The point to be made is that good fit with the receptor site is not restricted to symmetrical molecules but also may be obtained with unsymmetrical analogs, as long as the overall size of the molecule remains within the flexible framework of the receptor site. This concept dif-

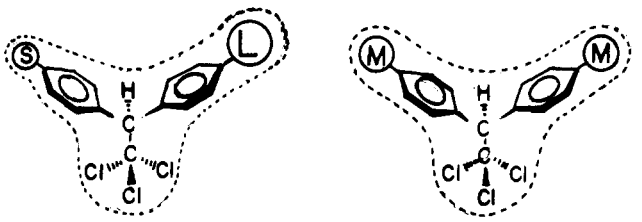


Figure 1. Hypothetical models showing the fit of DDT analogs containing different size ring substituents with the DDT receptor site.

fers from the model of Holan (1969), where the size of each substituent is assumed to be independent of each other and greater reliance is placed on shape symmetry.

Basically, our model informs us that, as the summation of the size of X, Y, L, and Z increases, interaction with the receptor increases, eventually reaching a maximum from which point increase in substituent size results in decreased interaction. Assuming that interaction or fit with the hypothetical receptor site is directly related to insect toxicity, it is possible now to correlate insecticidal activity with free energy parameters on a quantitative basis.

Although several different parameters may be used to estimate the size of a substituent, *e.g.*, van der Waal's radii, etc., we chose to use Taft's steric substituent parameter E_s (Taft, 1956) owing to the belief that fit at the receptor site should be governed primarily by steric effects. Keeping in mind that receptor interaction should approach a maximum and then decrease with increase in the steric parameter E_s, the most reasonable approximation between toxicity and E_s should be parabolic in nature. Therefore, the mathematical model to express toxicity in terms of E_s with change in a single substituent X may be expressed by the following equation where α, β,

$$\log \mathrm{LD}_{50} = \alpha + \beta E_s^{X} + \gamma[E_s^{X}]^2 \qquad (1)$$

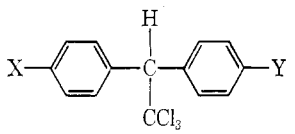
and γ are constants. A generalized equation for changes in more than one substituent would be represented by eq 2, where α', β', and γ' are a new set of constants and i denotes substituent X, Y, L, and Z.

$$\log \mathrm{LD}_{50} = \alpha' + \beta'\Sigma^{i}E_s + \gamma'\Sigma^{i}[E_s]^2 \qquad (2)$$

METHODS

Free Energy Parameters. E_s values used in this study are different from those normally reported (Kutter and Hansch, 1969; Taft, 1956) in that all values were converted to the same scale as those of other free energy parameters, *i.e.*, to a scale relative to H (E_s for H is zero). This was accomplished simply by subtracting the value 1.24 (E_s for H relative to methyl) from all E_s values. E_s for OCH_3 and SCH_3 was calculated from the estimated van der Waal's radii of 1.81 and 1.92, respectively, for these groups (Charton, 1969; Pauling, 1960) using the equation provided by Kutter and Hansch (1969). E_s for ring halogens also was calculated from the same equation using values for maximum van der Waal's radii for F, Cl, Br, and I (Pauling, 1960). E_s for C_2H_5O was estimated by adding to the value calculated for CH_3O (−1.08) the value of −0.09, *i.e.*, the difference between E_s of C_2H_5O and

Table I. Effect of Variation in the X and Y Positions on the Insecticidal Activity of DDT Analogs



Compound	X	Y	E_s^X	E_s^Y	π^X	π^Y	R^X	R^Y	F^X	F^Y	LD_{50}, μg/g Housefly (alone)	LD_{50}, μg/g Housefly (+P.B.)	LC_{50}, ppm, *Culex fatigans*
1	H	H	0	0	0	0	0	0	0	0	2900	575	1.1
2	F	F	−0.46	−0.46	0.15	0.15	−0.34	−0.34	0.71	0.71	30.5	17.0	0.074
3	Cl	Cl	−1.16	−1.16	0.70	0.70	−0.16	−0.16	0.69	0.69	14	5.5	0.07
4	Br	Br	−1.36	−1.36	1.02	1.02	−0.18	−0.18	0.73	0.73	27	10.5	0.074
5	I	I	−1.72	−1.72	1.27	1.27	−0.20	−0.20	0.67	0.67	35	14	1.4
6	CH_3	CH_3	−1.24	−1.24	0.52	0.52	−0.14	−0.14	−0.05	−0.05	100	17.5	0.081
7	C_2H_5	C_2H_5	−1.62	−1.62	1.00	1.00	−0.11	−0.11	−0.06	−0.06	70	29.0	0.18
8	OCH_3	OCH_3	−1.08	−1.08	−0.04	−0.04	−0.50	−0.50	0.41	0.41	45	3.5	0.067
9	OC_2H_5	OC_2H_5	−1.17	−1.17	0.50	0.50	−0.44	−0.44	0.36	0.36	7.0	1.75	0.04
10	CH_3	H	−1.24	0	0.52	0	−0.14	0	−0.05	0	>500	33.0	0.51
11	Cl	CH_3	−1.16	−1.24	0.70	0.52	−0.16	−0.14	0.69	−0.05	62.5	6.5	0.031
12	F	CH_3	−0.46	−1.24	0.15	0.52	−0.34	−0.14	0.71	−0.05	250	25	0.059
13	Br	CH_3	−1.36	−1.34	1.02	0.52	−0.18	−0.14	0.73	−0.05	32.5	1.75	0.04
14	I	CH_3	−1.72	−1.24	1.27	0.52	−0.20	−0.14	0.67	−0.05	120	22.2	0.044
15	F	OCH_3	−0.46	−1.08	0.15	−0.04	−0.34	−0.50	0.71	0.41	47	11.5	0.47
16	Cl	OCH_3	−1.16	−1.08	0.70	−0.04	−0.16	−0.50	0.69	0.41	41.5	7.0	0.058
17	CH_3	OCH_3	−1.24	−1.08	0.52	−0.04	−0.14	−0.50	−0.05	0.41	23.5	4.9	0.085
18	CH_3	OC_2H_5	−1.24	−1.17	0.52	0.50	−0.14	−0.44	−0.05	0.36	9	1.7	0.13
19	OCH_3	OC_2H_5	−1.08	−1.17	−0.04	0.50	−0.50	−0.44	0.41	0.36	16	3.7	0.039
20	SCH_3	SCH_3	−1.29	−1.29	0.62	0.62	−0.19	−0.19	0.33	0.33	225	17.0	0.25
21	SCH_3	OCH_3	−1.29	−1.08	0.62	−0.04	−0.19	−0.50	0.33	0.41	32	4.0	0.11
22	SCH_3	OC_2H_5	−1.29	−1.17	0.62	0.50	−0.19	−0.44	0.33	0.36	32	2.8	0.7
23	CH_3	C_2H_5	−1.24	−1.62	0.52	1.00	−0.14	−0.11	−0.05	−0.06	11	3.0	0.08
24	OCH_3	$CH(CH_3)_2$	−1.08	−2.32	−0.04	1.30	−0.50	−0.14	0.41	−0.10	160	61.5	0.32
25	Cl	$CH(CH_3)_2$	−1.16	−2.32	0.70	1.30	−0.16	−0.14	0.69	−0.10	215	93.5	0.15

CH_3O for the alcohol moiety of carboxylic acid esters (Taft, 1956). E_s values determined by Hancock *et al.* (1961) were used for alkyl substituents on the phenyl ring, since they represent true steric effects, separated from hyperconjugation. These values also were used by Kutter and Hansch (1969) in their analysis of monoamine oxidase inhibitors. Except for substituents of the type CHAB, all E_s values for groups in the L and Z positions are those reported by Taft (1956) after normalizing them to *H*. For substituents of the CHAB type (compounds **30, 33, 34, 36,** and **37**), where A and B are different moieties, the E_s values were approximated by taking the mean E_s values of the respective CHAA and CHBB substituents. *A priori*, E_s for CHAB is expected to be intermediate between E_s for CHAA and CHBB. Support for the use of the mean value to represent E_s for CHAB is found in data provided by Taft (1956). For example, the values of E_s reported for $(CH_3)_2CH$, $(C_2H_5)_2CH$, and $(CH_3)(C_2H_5)CH$ are −0.47, −1.98, and −1.13, respectively. The value calculated for $(CH_3)(C_2H_5)CH$ by taking the mean of E_s for $(CH_3)_2CH$ and $(C_2H_5)_2CH$ is −1.23. Similarly, E_s reported for (neopentyl)$_2$CH is −3.18 and that for (CH_3)(neopentyl)CH is −1.85. The value calculated for (CH_3) (neopentyl)CH by taking the mean of E_s for $(CH_3)_2CH$ and (neopentyl)$_2$CH is −1.83. These examples demonstrate that the calculated mean is reasonably close to the experimental value. Since E_s for several of the CHAB-type substituents was not available in the literature (*e.g.*, CHFCl), it was necessary to estimate these values in the manner described.

Toxicity Data. Toxicity data against the housefly (*Musca domestica*), with and without the synergist piperonyl butoxide, and against the mosquito larvae (*Culex fatigans*), were obtained in the laboratories at the University of California, Riverside (Metcalf and Fukuto, 1968) and at the University of Illinois (Metcalf *et al.*, 1971). The data were divided into two categories: the effect of variations in groups X and Y on toxicity, keeping L and Z constant (Table I) and the effect of variations in groups L and Z on toxicity, restricting X and Y to chlorine (Table II).

Multiple regression analyses were performed by using the IBM 360/50 computer, Computing Center, University of California, Riverside, Calif.

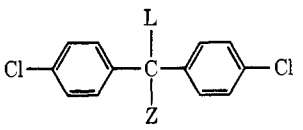
Synthesis of Silicon DDT Analogs. The silicon analogs (I–IV) of DDT were prepared by conventional methods using the appropriate alkyl and aryl Grignard or lithium reagents with a chlorosilane (Petrov *et al.*, 1964). Procedures are indicated below. All pmr spectra were obtained with a Varian T-60 spectrometer in deuterochloroform using TMS as a standard unless otherwise indicated.

Bis-(*p*-chlorophenyl)-*tert*-butylsilane (I). Bis-(*p*-chlorophenyl)chlorosilane was prepared according to Barry (1949). This was reacted with *tert*-butyllithium in pentane to give I: bp 132–135° (0.3 mm); $n^{25}D$ 1.5737; pmr 9 H singlet at δ 1.0 for *tert*-butyl protons, 1 H singlet at 4.6 for SiH, 8 H multiplet at 7.2–7.6 for aromatic protons. *Anal.* Calcd for $C_{16}H_{18}SiCl_2$: C, 62.13; H, 5.83. Found: C, 62.27; H, 6.42.

Bis-(*p*-chlorophenyl)dichloromethylsilane (II). This compound was prepared by reacting bis-(*p*-chlorophenyl)-chlorosilane with dichloromethyllithium (Köbrich *et al.*, 1964) in tetrahydrofuran at −70°. The product, bp 152–154 (0.2 mm) after several redistillations, was judged to be about 80% II from analysis of its pmr spectrum and based on the relative integrals of SiH and $CHCl_2$ protons. Pmr spectrum gave a doublet at δ 5.3 (J = 1 Hz) for SiH proton, a doublet at 5.8 (J = 1 Hz) for $CHCl_2$ proton, and a multiplet at 7.3–7.8 for aromatic protons.

Bis-(*p*-chlorophenyl)trimethylsilylmethane (III). This

Table II. Effect of Variation in the L and Z Positions on the Insecticidal Activity of DDT Analogs



Compound	L	Z	E_s^L	E_s^Z	π^L	π^Z	σ^{*L}	σ^{*Z}	LD_{50}, μg/g, houseflies	LC_{50}, ppm, *Culex fatigans*
3	H	CCl_3	0	−3.30	0	1.70	0	1.00	2.0	0.070
26	H	CBr_3	0	−3.67	0	2.32	0	1.00	>500	0.550
27	H	CF_3	0	−2.40	0	0	0	0.92	>500	0.250
28	H	$CHCl_2$	0	−2.78	0	1.32	0	0.70	20	0.038
29	H	CHF_2	0	−1.91	0	0.18	0	0.70	>500	0.950
30	H	CHClF	0	−2.35	0	0.85	0	0.70	155	0.120
31	F	CCl_3	−0.46	−3.30	−0.17	1.70	1.10	1.00	125	0.092
32	F	$CHCl_2$	−0.46	−2.78	−0.17	1.32	1.10	0.70	4.1	0.024
33	F	CHClBr	−0.46	−2.94	−0.17	1.52	1.10	0.70	2.8	0.021
34	F	CHClF	−0.46	−2.34	−0.17	0.85	1.10	0.70	220	0.110
35	F	$CHBr_2$	−0.46	−3.00	−0.17	1.72	1.10	0.70	7.0	0.022
36	F	CHBrF	−0.46	−2.51	−0.17	0.93	1.10	0.70	70	0.085
37	F	$CHBrCH_3$	−0.46	−2.41	−0.17	1.64	1.10	0.30	9.0	0.070
38	F	CHF_2	−0.46	−1.91	−0.17	0.18	1.10	0.70	>500	0.810

compound was prepared by adding 1 equiv of *p,p'*-dichlorobenzhydryl chloride to a mixture of equivalent amounts of trimethylchlorosilane and magnesium in ether at room temperature. The product (III), after distillation, was crystallized from ethanol, mp 69–72°. Pmr spectrum showed a 1 H singlet at δ 3.4 for benzylic proton, an 8 H multiplet at 7.1–7.35 for aromatic protons, and a 9 H singlet at 0 for trimethylsilyl protons (TMS was not used as the standard and all signals are related to the internal trimethylsilyl absorption). *Anal.* Calcd for $C_{16}H_{18}SiCl_2$: C, 62.13; H, 5.83. Found: C, 62.86; H, 6.40.

Bis-(*p*-chlorophenyl)tetramethylenesilane (IV). Dichlorotetramethylenesilane was prepared according to West (1954). This was treated with 2 equiv of *p*-chlorophenyllithium to give IV: bp 142–146 (0.025 mm); $n^{25}D$ 1.5897. Pmr spectrum showed a 4 H multiplet at δ 1.05–1.2 for $SiCH_2$ protons, a 4 H multiplet at 1.6–1.85 for methylene protons, and an 8 H multiplet at 7.2–7.5 for aromatic protons. *Anal.* Calcd for $C_{16}H_{16}SiCl_2$: C, 62.54; H, 5.21. Found: C, 62.66; H, 6.16.

RESULTS

The data presented in Tables I and II were subjected to multiple regression analysis. In accordance with our model, the variables ΣE_s and ΣE_s^2 were forced into the regression equation and other variables (*cf.* Tables I and II) which might contribute significantly to the regression sums of squares at the 0.05-level of probability were entered into the analysis. The data also were analyzed by a program which allowed the stepwise addition of significant variables, but in no case did any variable or appropriate combination contribute more significantly to the regression equation than $\Sigma E_s + \Sigma E_s^2$, as anticipated by the model.

Effect of Variation in X and Y Positions. For the series of 25 symmetrical and unsymmetrical DDT analogs in which X and Y are varied (Table I), the following equations relating toxicity (LD_{50}) against houseflies, with and without the synergist piperonyl butoxide, with E_s were formulated. Without synergist

$$\log LD_{50} = 3.24\ (\pm 0.35) + 1.52\ (\pm 0.31)\ \Sigma E_s + 0.65\ (\pm 0.14)\ \Sigma E_s^2 \quad (3)$$
$$n = 24;\ r = 0.736;\ s = 0.41$$

with synergist

$$\log LD_{50} = 2.69\ (\pm 0.25) + 1.85\ (\pm 0.22)\Sigma E_s + 0.85\ (\pm 0.11)\Sigma E_s^2 \quad (4)$$
$$n = 25;\ r = 0.874;\ s = 0.31$$

n is the number of compounds in the analysis, r is the correlation coefficient, and s is the standard error.

Comparison of eq 3 and 4 shows that the use of piperonyl butoxide significantly improves the correlation between housefly toxicity and the steric substituent constant E_s. Since piperonyl butoxide is regarded as an inhibitor of microsomal mixed function oxidase (Metcalf *et al.*, 1971), the improved correlation between synergized toxicity and E_s serves to point out the importance of the effect of oxidative metabolism on the insecticidal activity of DDT analogs. The relationship between synergized housefly toxicity and E_s is presented graphically (Figure 2) by plotting log LD_{50} observed against log LD_{50} calculated according to eq 4. Several of the compounds, notably **6, 7, 12, 14,** and **20,** appear to be considerably less toxic than expected from the equation, while a few (**9, 13,** and **18**) are more toxic. Since enzymatic dehydrochlorination of DDT and related compounds is not blocked by piperonyl butoxide, it is possible that these compounds deviate from the calculated fit because of differences in their susceptibility to dehydrochlorination by DDT dehydrochlorinase. However, it should be pointed out that the deviation of these points from the line is not as serious as may appear from casual observation of the figure, since most of the deviations are in the region where high toxicity is expected. For example, the largest deviation occurs with compounds with a calculated LD_{50} in the range of 4–6.5 μg/g (log LD_{50} 0.6–0.8), whereas the actual observed LD_{50} is in the range of 2–16 μg/g (log LD_{50} 0.3–1.2). Considering the inherent variability usually found in toxicity data and the number of compounds in the analysis, the fit of the points to the regression line is quite good.

A composite of data developed in two different laboratories over a period of about 15 years (Metcalf and Fukuto, 1968; Metcalf *et al.*, 1971) was used in the analysis of mosquito toxicity. For the 25 compounds in Table I, stepwise selection of variables by significance did not provide any significant correlation between toxicity and any of the

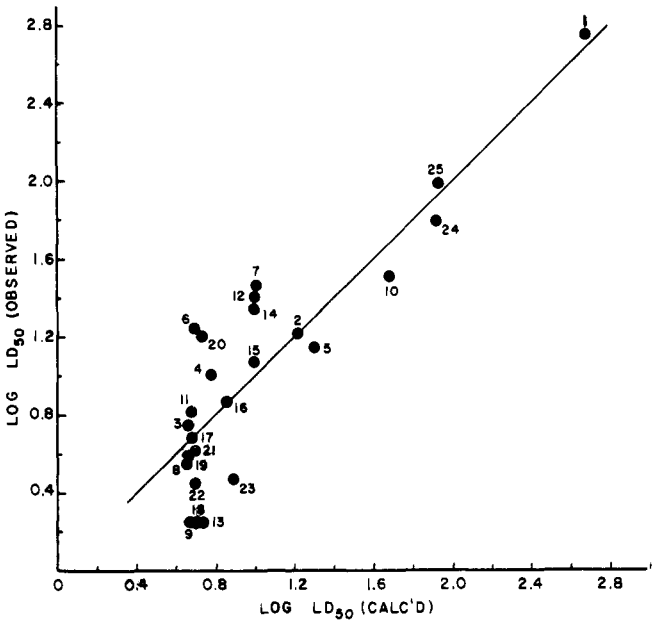


Figure 2. Correlation between observed synergized housefly toxicity and toxicity calculated according to eq 4 for variation in X and Y positions of DDT analogs.

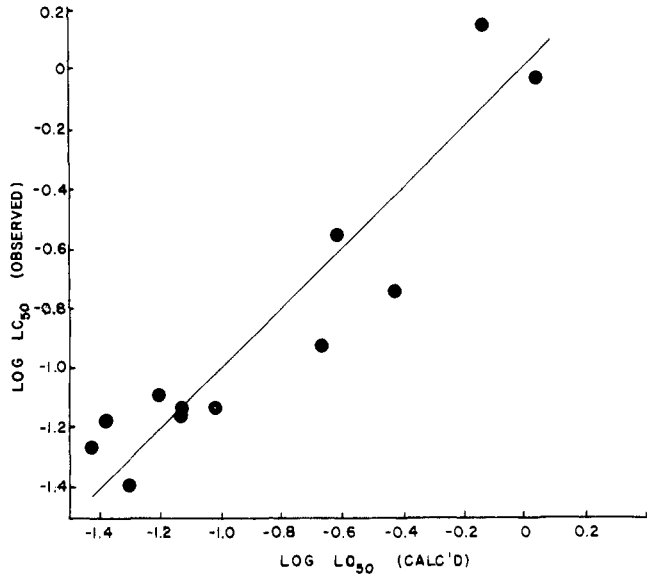


Figure 3. Correlation between observed mosquito larvae toxicity and toxicity calculated according to eq 6 for variation in X and Y positions in DDT analogs.

parameters. Forcing ΣE_s and ΣE_s^2 in the regression analysis resulted in eq 5. However, when the analysis was re-

$$\log LC_{50} = 0.99\ (\pm 0.29)\ \Sigma E_s + 0.46\ (\pm 0.14)\ \Sigma E_s^2$$
$$n = 25;\ r = 0.595;\ s = 0.4 \qquad (5)$$

stricted to the compounds evaluated in the early study (Metcalf and Fukuto, 1968), *i.e.*, compounds **1–11** and the compound where X is Cl and Y is H, a satisfactory correlation was obtained (eq 6). In both eq 5 and 6 the con-

$$\log LC_{50} = 1.63\ (\pm 0.21)\ \Sigma E_s + 0.93\ (\pm 0.12)\ \Sigma E_s^2$$
$$n = 12;\ r = 0.933;\ s = 0.21 \qquad (6)$$

stant for the intercept was nonsignificant and was effectively zero. The relationship between observed LC_{50} and LC_{50} calculated according to eq 6 is shown graphically in Figure 3.

On the other hand, analysis of the mosquito toxicity data provided in the more recent study of DDT analogs (Metcalf *et al.*, 1971), *i.e.*, compounds **12–25**, gave poor correlation between toxicity and E_s ($r = 0.433$). The basis for the poor correlation, in light of the highly satisfactory correlation obtained for the other compounds, is not known. It is noteworthy that **1–9** are symmetrical compounds while the remaining, except for **20**, are unsymmetrical DDT analogs. Whether asymmetry in the molecule is responsible for the poor correlation is conjectural at this time. It should be pointed out, however, that asymmetry did not affect the correlation on houseflies.

In general, correlation of toxicity with the steric substituent constant E_s was reasonably satisfactory with variation in the X and Y positions when oxidative metabolism was minimized by piperonyl butoxide. The successful use of the sum of the steric effects in the X and Y position in correlating insecticidal activity suggests that the DDT receptor site is quite flexible and is not as rigid as depicted by Holan (1969). This model adequately explains why DDT analogs which contain a substituent smaller than chlorine in the X position and a substituent larger than

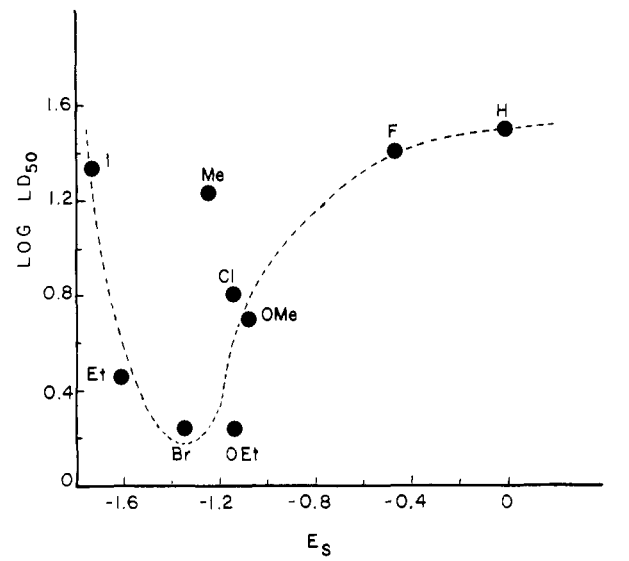


Figure 4. Relationship between observed synergized housefly toxicity and E_s for substituent X for 1,1,1-trichloro-*p*-methyl-*p'*-X-diphenylethanes.

chlorine in the Y position are toxic. Evidently, the degree of interaction between the substituents X and Y with the receptor site largely depends upon the sum of the steric effects produced by both X and Y, as suggested in Figure 1. A graphic illustration of this concept is presented in Figure 4, where synergized log LD_{50} against houseflies is plotted against E_s for different substituents in one position (X) and the rest of the molecule is held constant (Y = methyl). With the exception of the point representing the symmetrical dimethyl analog (X = Y = methyl), the plot is strikingly similar to the well known potential energy diagram for diatomic molecules. The figure shows that there is a region where maximum interaction occurs between the compound and the receptor site and interaction decreases as the sum of E_s for X and Y is above or below this region.

The failure of the resonance substituent constant R to appear in the regression equations indicates that van der

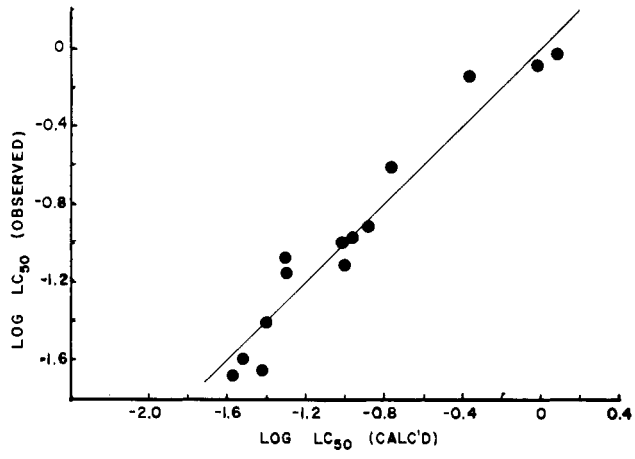


Figure 5. Correlation between observed mosquito larvae toxicity and toxicity calculated according to eq 9 for variation in L and Z positions of DDT analogs.

Waal's interaction forces are primarily responsible for DDT-receptor interaction (Gunther *et al.*, 1954).

Effect of Variation in L and Z Positions. The importance of the size of the downward group Z on the insecticidal activity of DDT analogs is well known. Holan (1969) has indicated that the downward projection of DDT and the corresponding dichlorocyclopropane analogs are similar and of optimum size (6.0–6.3 Å). Although downward projection into a membrane cavity to account for the effect of Z on toxicity has been useful in correlating structure with insecticidal activity, this type of projection analysis has ignored the effect of the simultaneous presence of the group in the L position. To illustrate this point, the replacement of the benzylic hydrogen in DDT by fluorine (**31**) does not effectively change the downward projection of the trichloromethyl moiety; however, housefly toxicity drops by a factor of about 63. On the other hand, replacement of the benzylic hydrogen in DDD (**28**) with fluorine resulted in a compound (**32**) which was only twofold less toxic than DDT. From these examples, it is evident that the effect of substituents on the α-carbon should be examined together and not separately.

Toxicological properties and the various parameters for DDT analogs where L and Z are varied are given in Table II. Analysis of the data provided eq 7 for unsynergized housefly toxicity.

$$\log LD_{50} = 14.14\ (\pm 4.0) + 9.36\ (\pm 3.0)\ E_s + 1.12\ (\pm 0.47)\Sigma E_s^2 + 4.31\ (\pm 1.37)\Sigma\sigma^* \quad (7)$$

$$n = 9;\ r = 0.856;\ s = 0.31$$

For mosquito larvae, eq 8 and 9 were obtained.

$$\log LC_{50} = 5.29\ (\pm 1.06) + 4.90\ (\pm 0.92)\Sigma E_s + 0.76\ (\pm 0.16)\Sigma E_s^2 + 1.76\ (\pm 0.43)\Sigma\sigma^* \quad (8)$$

$$n = 14;\ r = 0.889;\ s = 0.29$$

$$\log LC_{50} = 7.27\ (\pm 0.72) + 6.03\ (\pm 0.57)\Sigma E_s + 0.76\ (\pm 0.09)\Sigma E_s^2 + 2.27\ (\pm 0.26)\Sigma\sigma^* + 0.37\ (\pm 0.08)\Sigma\pi^2 \quad (9)$$

$$n = 14;\ r = 0.970;\ s = 0.16$$

The relationship represented by eq 9 is presented graphically in Figure 5.

While significant correlation was not obtained when each of the parameters was used alone, the combination of E_s and σ^* was highly significant and indicated that steric and polar effects contributed to the interaction of L and Z with the receptor site in both houseflies and mosquito larvae. Against mosquito larvae, significant improvement in correlation was obtained by the introduction of a π^2 term (eq 9). Although the coefficient for this term (0.37) is small, it does point out the importance of the lipophilic character of L and Z on toxicity to mosquito larvae.

For groups on the α-carbon atom, eq 7–9 predict that higher log LD_{50} or log LC_{50} (lower toxicity) will be obtained for substituents with approximately equal E_s values but larger σ^* values. This is quite surprising since virtually all of the more toxic DDT analogs contain polar groups in the Z (or L) position; *i.e.*, groups with reasonably large positive σ^* constants. However, of the compounds examined, σ^* for the Z substituent resides in a rather narrow range compared to the corresponding E_s values and, therefore, any conclusions concerning the effect of σ^* on toxicity must be tempered with caution. It is possible that there is also a value of σ^* where maximum toxicity effects would be observed, similar to that for E_s. Unfortunately, however, data were not available for DDT analogs with a diverse range of σ^* values for the Z substituent, particularly those with negative values.

The general absence of π, except for π^2 in eq 9, in the different regression equations deserves comment. For the various substituents X, Y, L, and Z examined in this analysis, the values of E_s were more or less in parallel to the corresponding π values. By forcing π and π^2 into the regression analysis, equations were obtained which gave good fit to the data, but this fit was not as satisfactory as that obtained with E_s and E_s^2. For example, for the 12 compounds represented by Figure 4, $\Sigma\pi$ and $\Sigma\pi^2$ produced an equation with correlation coefficient r of 0.738, while ΣE_s and ΣE_s^2 gave an r value of 0.933. This indicates that the effect of π is related to E_s but the latter is a more useful parameter for correlation studies with DDT analogs.

DISCUSSION

Overall, the steric substituent constant E_s appears to be the single most important parameter for the correlation of housefly and mosquito larvae toxicity when substituents are varied in the X, Y, L, and Z positions in DDT analogs. The successful use of ΣE_s and ΣE_s^2 in correlating insecticidal activity with structure strongly indicates that there is a reasonable amount of flexibility in the DDT receptor site. The receptor site may be visualized as a flexible cavity which can accommodate DDT analogs of varying dimensions as long as the overall size of the molecule does not deviate substantially from that of DDT. Compounds possessing the same geometry as that of DDT but which fall outside the range of maximum interaction because of their overall size, *i.e.*, smaller or larger, therefore are not expected to be toxic. The organosilicon analogs (**1–4**) of DDT probably fall into this category.

It was not possible to examine the effect of simultaneous variation in all four positions (X, Y, L, and Z) on insecticidal activity, owing to an insufficient number of compounds for analysis. However, there is evidence to indicate that the effect of ring substituents is not independent of that of substituents on the α-carbon atom. For example, the toxicity of Perthane [1,1-dichloro-2,2-bis-(*p*-ethylphenyl)ethane] to houseflies synergized with piperonyl butoxide is approximately twice that of synergized DDT. In comparison, the toxicity of synergized 1,1,1-trichloro-2,2-bis-(*p*-ethylphenyl)ethane, the *p*-ethyl analog of DDT, is only one-sixth that of synergized DDT. This suggests that when group X or Y or both are altered, insecticidal activity may be maintained by appropriate

alteration of L or Z. The E_s values for the chloro and ethyl moieties are −1.16 and −1.62, respectively. Changing the ring substituent from chloro to ethyl, therefore, can be compensated for by substituting dichloromethyl (E_s = −2.78) for trichloromethyl (E_s = −3.30) in the α position.

From the preceding discussion, it is obvious that a large number of new DDT analogs still remain to be synthesized and examined for insecticidal activity. The model that we have developed in predicting the insecticidal activity of DDT analogs is by no means infallible but it provides a more systematic approach to the design of new compounds. The synthesis of new DDT analogs with predictable activity from our analysis is currently in progress.

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The *p*-Value Approach to Quantitative Liquid–Liquid Extraction of Pesticides and Herbicides from Water. 3. Liquid–Liquid Extraction of Phenoxy Acid Herbicides from Water

Irwin H. Suffet

The goal of aqueous herbicide analysis is the recovery of 100% of an herbicide for qualitative and quantitative analysis. Liquid–liquid extraction is the method of choice for quantitative recovery from water. The *p*-value concept is useful in developing an understanding of the liquid–liquid extraction process in order to select optimum experimental conditions to approach 100% herbicide recovery. The best solvents for extraction of phenoxy acid herbicides are ethyl acetate and ether. The best solvents for simultaneous extraction of 2,4-D or 2,4,5-T and their *n*-butyl and isopropyl esters are ether and ethyl acetate (2,4-D and esters) and benzene (2,4,5-T and esters). Possible variation of the *p*-value caused by alteration of natural water characteristics was tested. The apparent *p*-values for 2,4-D obtained with waters from different sources and the *p*-value found in distilled water were found to be consistent. Adsorption characteristics of herbicide esters were changed upon adjustment of turbid water to the aqueous characteristics of the *p*-value method. Therefore the aqueous sample should be filtered before adjustment of aqueous conditions for liquid–liquid extraction. The *p*-value gives a theoretical guide (an F_n value) for development of an aqueous residue procedure. Recovery data from the literature and a recovery study at high concentration confirmed the calculated F_n value. A two-step serial extraction with 200 and 50 ml of ethyl acetate under *p*-value conditions is the choice for extracting 99% of 2,4-D from 1 l. of aqueous solution.

The first step of aqueous residue analysis consists of liquid–liquid extraction (LLE). It is critical to know the efficiency of the LLE step if the data are to be valid as "actual" aqueous concentration data for interpreting ecological problems such as persistence and transport of herbicides in aquatic environments (Faust and Suffet, 1966, 1969, 1972; Suffet and Faust, 1972a). In fact, at low herbicide levels (ng/l.), the efficiency of the LLE step can set the lower limit of detectability of the analytical method. Similar emphasis on the initial extraction step of residues from plant and soil material has been stated by Wheeler and Frear (1966) and Chiba and Morley (1968), respectively.

At the pH of natural waters (pH 5 to 9), chlorinated phenoxy acid herbicides can exist as ionized salts, amine salts, or esters. Since phenoxy acid herbicides are primarily applied as ester derivatives, it appears important to know the concentration of both ester and anion of the free acid to assess herbicide fate. Therefore analytical procedures should individually determine both the free acid herbicide and its ester forms.

A "standard method" of aqueous herbicidal analysis has not been developed which meets the criterion of analytical acceptability of less than 50% total error (McFarren *et al.*,

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