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Insecticidal and Neuroexciting Actions of DDT Analogs

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The neuroexciting activity of DDT and its analogs to produce repetitive responses on the nerve cord of *Periplaneta americana* was determined using the extracellular electrode method. The convulsive activity on *P. americana* and the insecticidal effect on *Callosobruchus chinensis* were also examined. It was found that the convulsive and insecticidal activities increase almost proportionally with increase in the neuroexciting activity within a set of *p,p'*-substituted DDT analogs. The intimate connections among these biological effects suggest that symptoms such as convulsion and death caused by DDT analogs are closely related with their neuroexcitory effect and there is a common mode of action in spite of differences in insect species.

DDT-poisoning of insects has been well characterized as producing ataxia, convulsion, and eventual paralysis (1). On the insect axon, DDT exerts two effects; one produces repetitive discharges (2) and the other is an increase in negative after-potential (3). The poisoned axon has been assumed to undergo changes such that the increased negative after-potential can initiate repetitive firings (4, 5). However, neither of these neurotoxic effects has been explicitly proved to be related to the insecticidal activity of DDT and its analogs.

We report that, by using a variety of DDT analogs, the ability to produce repetitive discharges was found to be closely related to insecticidal and convulsive actions.

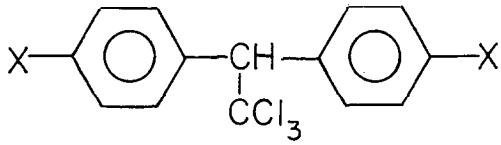
MATERIALS AND METHODS

The DDT analogs in Table 1 were prepared in this laboratory and were highly purified by repeated recrystallizations or distillations. Compounds 1∼12, and 19 were synthesized by variations of Baeyer condensation between chloral and respective substituted benzenes (6–10). Compounds 14, 15, 17, 18, and 20 were derived by the hydrolysis (11), oxidation (12), nitration (13), reduction (13), and oxidative acetylation (11) of compounds 20, 6, 1, 17, and 6, respectively, using reported methods. Compound 13 was prepared by reacting compound 8 with acetic anhydride in the presence of sulfuric acid. The product was a colorless crystal recrystallized from ethanol, mp 141 ∼ 142°C. Anal. Calcd for $C_{18}H_{15}O_4Cl_3$; C, 53.82; H, 3.76. Found C, 53.90; H, 3.99. Compound 16 was obtained by esterification of compound 15 with diazomethane. The colorless product was recrystallized from ethanol, mp 115 ∼ 116°C. Anal. Calcd for $C_{18}H_{15}O_4Cl_3$; C, 53.82; H, 3.76. Found C, 54.02; H, 3.77.

For electrophysiological experiments, the extracellular electrode technique was used with nerve cords of the male adult American cockroach, *Periplaneta americana*, which has all thoracic and abdominal ganglia. The stimulating and recording elec-

TABLE 1

Biological Activities of DDT Analogs



Compound	Substituent X	Repetitive responsiveness $-\log MEC_{RD}$ (mole/liter) *P. americana*	Convulsive activity $-\log MEC_{CA}$ (mole/liter) *P. americana*	Insecticidal activity $-\log LC_{50}$(mole/liter) *C. chinensis*
1	H	4.1 ($\pm$0.1)[a]	0.70 ($\pm$0.10)[a]	1.29 ($\pm$0.05)[b]
2	F	5.1	1.52	2.77
3	Cl	5.7	2.15	3.45
4	Br	5.7	2.30	3.33
5	I	$\leq$4.7	1.52	2.26
6	CH_3	4.7	1.52	2.15
7	C_2H_5	5.0	1.70	2.50
8	OH	4.4	<0.70	—[c]
9	OCH_3	5.7	2.30	3.47
10	OC_2H_5	6.0	2.52	3.28
11	OC_3H_7	5.3	2.00	2.62
12	OC_4H_9	<4.0	1.00	1.42
13	$OCOCH_3$	4.4	1.15	1.70
14	CHO	5.0	1.40	—[c]
15	COOH	<4.1	<0.70	—[c]
16	$COOCH_3$	5.0	1.52	2.30
17	NO_2	5.0	1.70	—[c]
18	NH_2	<3.9	$\leq$1.00	—[c]
19	C_6H_5	<4.7	<1.00	<1.4
20	$CH(OCOCH_3)_2$	4.7	1.30	—[c]

[a] Mean value of at least two runs.
[b] Mean value of three runs.
[c] Not examined.

trodes were placed between first and second and between fifth and sixth abdominal ganglia, respectively. Nerve cords were placed in Ringer's solution containing 210 m*M* NaCl, 2.9 m*M* KCl, 1.8 m*M* $CaCl_2$, and 2.0 m*M* phosphate buffer (pH 7.2) with various concentrations of DDT analogs at 20($\pm$1)°C for 30 min. A small amount of methanol used as the dispersing agent (0.1 $\sim$0.5 (v/v) %) had no effect on nervous functions. Without toxicants, nerve conduction occurred only when stimuli over a certain threshold (250 mV, 0.01 msec) were applied. This value was almost unchanged after treatment with DDT analogs. Within 30 min after starting immersion with more than a given concentration of DDT analogs, nerve cords reached a state where a single stimulus over the threshold could induce a repetitive response. The minimum molar concentration of DDT analogs producing this repetitive response to an electric stimulus twice as strong as the threshold, MEC_{RD}[1], was determined. Values of $-\log MEC_{RD}$ are shown in Table 1. Action potentials induced by the stimulus after treat-

[1] Abbreviations: MEC_{RD}; Minimum effective concentration for repetitive action, MEC_{CA}; Minimum effective concentration for convulsive action, LD_{50}; half lethal dose.

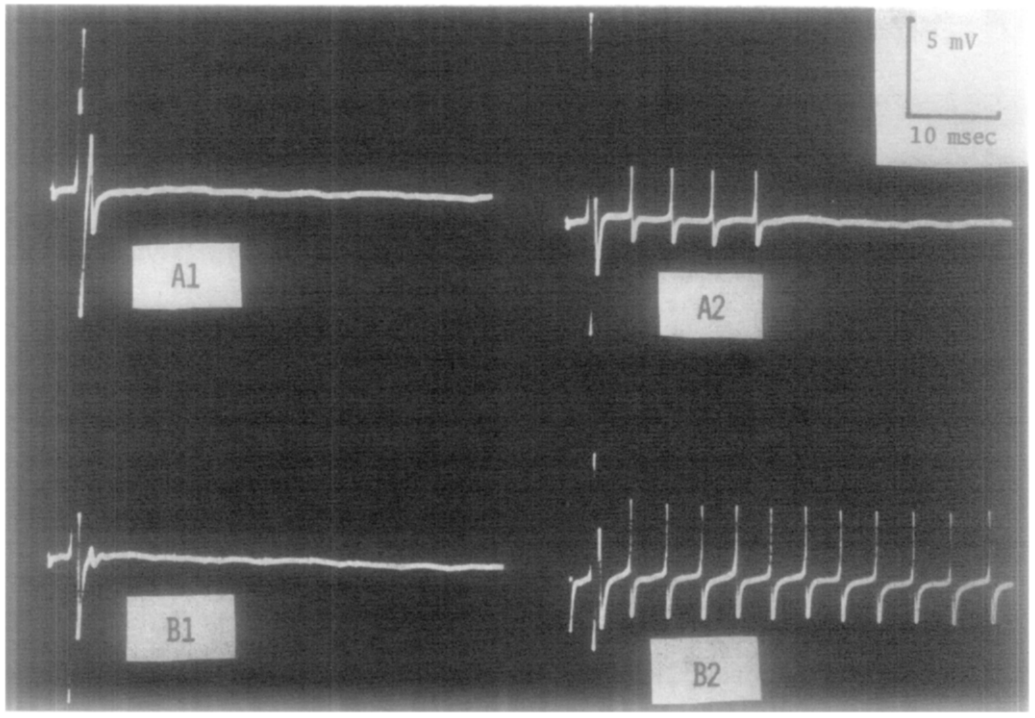


FIG. 1. *Action potentials induced by a single stimulus, recorded after treatment for 30 min with DDT and ethoxychlor. With concentrations less than the threshold (A_1 and B_1), only the normally conducted impulses were observed. With more than the threshold concentrations (A_2 and B_2), a train of firings was induced immediately after the normal response. A_1: DDT $1 \times 10^{-6}M$; A_2: DDT $2 \times 10^{-6}M$; B_1: ethoxychlor $5 \times 10^{-7}M$; B_2: ethoxychlor $1 \times 10^{-6}M$.*

ment with DDT(3) and ethoxychlor(10) are recorded in Fig. 1.

The convulsive action of DDT analogs was examined using male adult American cockroaches. One microliter of various concentrations of DDT analogs (methanol solution) was injected into the abdomen (between 3rd and 4th segments) with a microsyringe. Injected cockroaches were placed in a container at 20(±1)°C. This quantity of methanol alone had no effect on the cockroaches. From observations of symptoms, the minimum effective concentration which produced convulsive action within 3 hr after injection, MEC_{CA}, was determined. Values of $-\log MEC_{CA}$ are shown in Table 1. The convulsive action of DDT analogs was characterized by violent reflex quivering of legs. With the minimum effective dose, DDT analogs caused only convulsions; not paralysis or death.

Insecticidal activities of DDT analogs were determined using male adult azuki bean weevils, *Callosobruchus chinensis*. Twenty weevils were dipped for 3 min into an emulsion containing various concentrations of DDT analogs, then were placed in a glass container at 20(±1)°C. As emulsifying agents, xylene [2 (v/v) %] and a surfactant, Toxanon 500 [0.1 (v/v) %, Sanyo Kasei Co., Japan] were used, neither of which had any effect on azuki bean weevils at these concentrations. LC_{50}s of DDT analogs were determined by plotting the mean percentage of mortalities (of three runs) 3 days after dipping treatment converted to probit vs log dose. Values of $-\log LC_{50}$ are shown in Table 1.

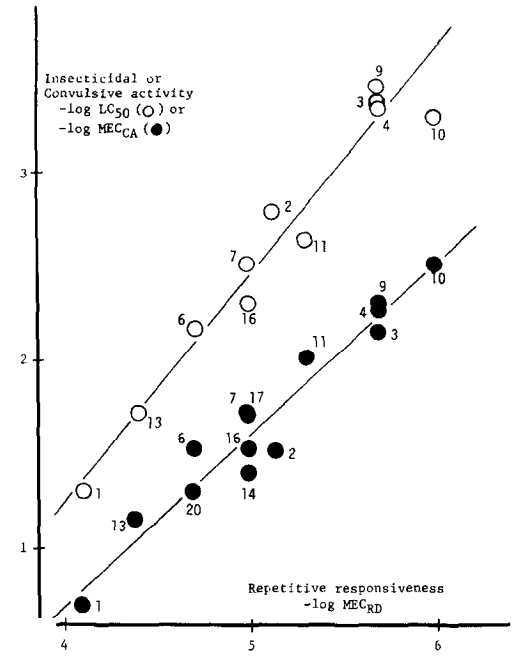


FIG. 2. *Relationships among insecticidal, convulsive, and neuroexciting activities of DDT analogs.*

RESULTS AND DISCUSSION

The minimum concentration of DDT which produced repetitive discharges on nerve cords of the American cockroach at 20°C was 2×10^{-6} *M*. This value is very close to that obtained by Matsumura and Patil for the isolated giant axon of the German cockroach at 24°C ($1 \sim 3 \times 10^{-6}$ *M*) (14). For this effect, ethoxychlor is the most active of the compounds including DDT and methoxychlor (9).

The covulsive activity of DDT analogs decreases in the order of ethoxychlor > DBrDT (4) ~ methoxychlor ~ DDT > propoxychlor (11). As shown in Fig. 2, the convulsive activity of DDT analogs is closely related to the neuroexciting ability to produce repetitive discharges. Equation (1) was obtained for 14 analogs using the method of least squares; omitting values where distinct activity values could not be determined. In this and following equations, *n* is the number

$$-\log MEC_{CA} = 0.91(\pm 0.14) \times (-\log MEC_{RD}) - 2.95(\pm 0.72)$$
$$n = 14,\ s = 0.126,\ r = 0.971 \quad [1]$$

of compounds studied, *s* is the standard deviation, *r* is the correlation coefficient, and figures in parentheses are the 95% confidence intervals. The value of the slope of Eq. [1], which is close to one (0.91), indicates the intimate connection between the neuroexciting and convulsive actions of DDT analogs. The value of the intercept suggests the extent of dilution of the DDT analogs injected into cockroach bodies. The weight of a male adult American cockroach is about 1 g, i.e., equivalent to 1 ml in volume as a first approximation .If distribution of DDT analogs in the cockroach body is uniform, then the extent of dilution corresponds approximately to 1 μl/1 ml or -3 on a log scale. In fact, the value of the intercept of Eq. [1], -2.95, is very close to -3. Thus, the convulsive action of DDT analogs is closely correlated with their neuroexciting activity.

For some compounds (5, 8, 15, 18, and 19), definite values of MEC_{RD} or MEC_{CA} were unable to be determined. While compounds 5 and 19 are too low in solubility to obtain an adequate concentration to exhibit sufficient response, compounds, 8, 15, and 18 would be partly ionized and have difficulty in reaching the nerve membrane. The sheath of the cockroach nerve probably serves as a barrier to diffusion.

The insecticidal activity of DDT analogs against azuki bean weevils is also closely related to their repetitive activities on cockroach nerve cords as shown in Eq. [2] and Fig. 2. Since such relationships as Eqs. [1] and [2] hold regardless of the difference in

$$-\log LC_{50\text{weevil}} = 1.19(\pm 0.22) \times (-\log MEC_{RD}) - 3.50(\pm 1.12),$$
$$n = 11,\ s = 0.180,\ r = 0.972 \quad [2]$$

test objects and insect species, the differences in the route of administration and in

the body construction of different insect species play only minor roles on the variation in biological activities. Neither penetration through the integument of insects nor translocation to the nervous system seem to be crucial, except in ionizable analogs.

The same situation is also observed with toxicity against houseflies. Since the metabolic inactivation of DDT analogs is significant in houseflies, toxicity data using inhibitors of metabolizing enzymes such as piperonyl butoxide should be compared (10, 15). A good linear relationship as shown in Eq. [3] is derived for LD_{50} values (mole/kg) recalculated from the original data by Metcalf and

$$-\log LD_{50_{housefly}} = 1.23(\pm 0.53) \times (-\log MEC_{RD}) - 2.05(\pm 2.87)$$
$$n = 6,\ s = 0.315,\ r = 0.953 \quad [3]$$

his co-workers (15). The resemblance between the slopes of Eqs. [2] and [3] seems to indicate that the mechanism for the insecticidal action of DDT analogs is at least common between azuki bean weevils and houseflies.

The present work clearly indicates that repetitive activity is closely correlated to the insect toxicity of DDT analogs. Further studies showing how the increase in the negative afterpotential caused by DDT is related to insect toxicity are required.

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REFERENCES

1. T. Yamazaki and T. Ishii, Studies on the mechanism of action of insecticides (VI): DDT symptoms in the cockroach, with special reference to the effect of temperature, *Oyo-Kontyu* **9**, 87 (1953).
2. T. Yamazaki and T. Ishii, Studies on the mechanism of action of insecticides (VII): Activity of neuron soma as a factor of development of DDT symptoms in the cockroach, *Botyu-Kagaku* **19**, 1 (1954).
3. T. Yamazaki and T. Narahashi, Studies on the mechanism of action of insecticides (VIII): Increase in the negative after-potential of insect nerve by DDT, *Botyu-Kagaku* **22**, 296 (1957).
4. T. Narahashi and T. Yamazaki, Mechanism of increase in negative after-potential by dicophanum (DDT) in giant axons of the cockroach, *J. Physiol.* **152**, 122 (1960).
5. T. Narahashi, Effects of insecticides on excitable tissues, *Advan. Insect Physiol.* **8**, 1 (1971).
6. M. Hamada, T. Sasakawa, and M. Ohno, Studies on the correlation between chemical constitution and insecticidal activity of halogenated aromatic componds I: DDT and its related compounds (I), *Botyu-Kagaku* **10**, 9 (1948).
7. M. Hamada and M. Ohno, Studies on the correlation between chemical constitution and insecticidal activity of halogenated aromatic compounds IV: Studies on DDT related compounds (II), *Botyu-Kagaku* **13**, 19 (1949).
8. P. Müller, Über Zusammenhange zwischen Konstitution und insektizider Wirkung I, *Helv. Chim. Acta* **29**, 1560 (1946).
9. L. Haskelberg and D. Lavie, Derivatives of 1,1,1-trichloro-2,2-diphenylethane, *J. Amer. Chem. Soc.* **69**, 2267 (1947).
10. R. L. Metcalf, I. P. Kapoor, and A. S. Hirwe, Biodegradable analogues of DDT, *Bull. WHO* **44**, 363 (1971).
11. Y. Perron and R. Barre, Synthesis of 1,1,1-trichloro-2,2-bis-(*p*-cyanophenyl)-ethane, *Can. J. Chem.* **30**, 203 (1952).
12. Y. Perron, On the oxidation of 1,1,1-trichloro-2,2-bis-(*p*-tolyl)-ethane, *Can. J. Chem.* **31**, 4 (1953).
13. S. Kirkwood and P. H. Phillips, The antitubercular action of 1,1,1-trichloro-2,2-bis-(*p*-aminophenyl)-ethane, *J. Amer. Chem. Soc.* **69**, 934 (1947).
14. F. Matsumura and K. C. Patil, Adenosine triphosphatase sensitive to DDT in synapses of rat brain, *Science* **166**, 121 (1969).
15. R. L. Metcalf and T. R. Fukuto, The comparative toxicity of DDT and analogues to susceptible and resistant houseflies and mosquitos, *Bull. WHO* **38**, 633 (1968).