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Insecticidal and Neuroexcitant Actions of DDT Analogs on the Cockroach, *Periplaneta americana*

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Abstract

The American cockroach (Periplaneta americana) was used to test 14 DDT analogs for their ability to cause a) death and b) repetitive firing in the abdominal nerve cord. P,p'-DDT, p,p'-DDD, p,p'-methoxychlor, and o,p'-DDT were all found to induce repetitive firing in the nerve cord after a twice threshold stimulus, but only p,p'-DDT, p,p'-DDD, and p,p'-methoxychlor produced mortality within 4 days from injection of the pesticides. Also, cockroaches exposed to sublethal doses of p,p'-DDT and p,p'-methoxychlor were found to exhibit repetitive firing in the abdominal nerve cord three weeks after injection of the pesticide. The 'cause and effect' relationship between neuroexcitation and lethality is therefore questioned, at least in the case of cockroaches.

Introduction

Studies on the structure and insect toxicity of DDT analogs have produced a series of hypotheses (ROGERS, et al., 1953; GUNTHER, et al., 1954; MULLINS, 1956; HOLAN, 1969) which relate their disruptive action to steric fit with receptor molecules. FAHMY, et al. (1973) have shown that even the symmetry of the DDT molecule can be dispensed with, without loss of insecticidal activity, as long as the overall dimensions of the molecule remain within certain limits. They have therefore suggested that the receptor site is sufficiently flexible to allow changes in molecular geometry within a certain overall size. WU, et al. (1975) have argued, however, that a better estimate of structure-activity relationships for DDT analogs would be obtained if one were to use the effects at the primary site of action as the experimental monitor. NARAHASHI and HAAS (1968) and PICHON (1976) have shown, in lobster and cockroach axons respectively, that DDT is responsible for causing prolonged sodium conductance

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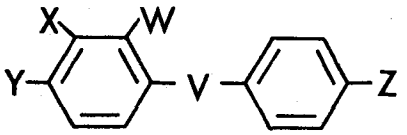
during the action potential (by delaying sodium inactivation) together with a reduced outward potassium conductance. These changes could explain the repetitive firing in arthropod axons after DDT treatment (ROEDER and WEIANT, 1948; LALONDE and BROWN, 1954; NARAHASHI and YAMASAKI, 1960). WU, et al. therefore determined the structure-activity relationships of 14 DDT analogs by their ability to cause repetitive firing in crayfish axons. While the existence of other 'primary' sites of action (e.g., the nerve mitochondrial Mg^{2+}-ATPase (DESAIAH, et al., 1974) is still a matter of debate (JACKSON and GARDNER, 1977)), at present the best substantiated site of action for DDT compounds seems to be a direct interaction with nerve membranes (BARNOLA, et al., 1971; GARDNER and BAILEY, 1975). It is still necessary however to show that the appearance of repetitive firing in insect axons is linked to insect mortality. UCHIDA, et al. (1974) have attempted to show a 'cause and effect' relationship by comparing the concentration of a range of symmetrical DDT analogs needed to cause repetitive firing in cockroach axons with that necessary to produce insecticidal effects. Unfortunately they only compared the neurotoxicity in cockroaches with the mortality of bean weevils. In the present study we have used 14 DDT analogs, both symmetrical and unsymmetrical, and compared the concentrations at which they produced repetitive firing in the cockroach abdominal nerve cord with the concentrations required to produce mortality in the same species.

Materials and Methods

Adult cockroaches (Periplaneta americana) were obtained from Carolina Biological Co., Burlington, North Carolina, U.S.A. Aldrich analytical grades (99+%) were used for p,p'-DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane); o,p'-DDT (1,1,1-trichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethane); p,p'-DDD (1,1-dichloro-2,2-bis(p-chlorophenyl)ethane) and p,p'-DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene). Chromatographically pure samples of p,p'-methoxychlor (p,p'-MeO-DDT) (1,1,1-trichloro-2,2-bis(p-methoxyphenyl)ethane); o,p'-DDD (1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl) ethane); m,p'-DDD (1,1-dichloro-2-(m-chlorophenyl)-2-(p-chlorophenyl)ethane); o,p'-DDE (1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethylene); p,p'-Cl-DDT (1,1,1,2-tetrachloro-2,2-bis(p-chlorophenyl)ethane); p,p'-F_3-DDT (1,1,1-trifluoro-2,2-bis(p-chlorophenyl)ethane); p,p'-DDA (bis(p-chlorophenyl)acetic acid) and p,p'-dichlorobenzophenone were kindly provided by Dr. H.V. Morley, Agriculture Canada, Central Experimental Farm, Ottawa, Ontario, Canada. P,p'-Perthane (1,1-dichloro-2,2-bis (p-ethylphenyl)ethane) and p,p'-OH-DDT (dicofol) (1,1,1-trichloro-2-hydroxy-2,2-bis(p-chlorophenyl)ethane) were obtained from the Rohm and Haas Company, Philadelphia, Pa. U.S.A. Structural formulae are represented in Table 1.

TABLE 1

DDT Analogs Tested on Cockroach Nerve Cords



DDT Analogs	V	W	X	Y	Z
p,p'-DDT	$HCCCl_3$	H	H	Cl	Cl
p,p'-OCH_3-DDT (methoxychlor)	$HCCCl_3$	H	H	OCH_3	OCH_3
p,p'-DDD	$HCCHCl_2$	H	H	Cl	Cl
o,p'-DDT	$HCCCl_3$	Cl	H	H	Cl
o,p'-DDD	$HCCHCl_2$	Cl	H	H	Cl
m,p'-DDD	$HCCHCl_2$	H	Cl	H	Cl
Cl-DDT	$ClCCCl_3$	H	H	Cl	Cl
F_3-DDT	$HCCF_3$	H	H	Cl	Cl
OH-DDT (dicofol, Kelthane)	$HOCCCl_3$	H	H	Cl	Cl
p,p'-C_2H_5-DDD (Perthane)	$HCCHCl_2$	H	H	C_2H_5	C_2H_5
DDA	HCCOOH	H	H	Cl	Cl
p,p'-DDE	$CCCl_2$	H	H	Cl	Cl
o,p'-DDE	$CCCl_2$	Cl	H	H	Cl
Dichlorobenzophenone	C=O	H	H	Cl	Cl

Electrophysiological studies: The abdominal nerve cord of Periplaneta americana was exposed from the ventral surface. All the lateral nerves were cut and adhering tissues removed as completely as possible. The abdominal viscera were removed leaving the nerve cord suspended above the empty shell of the abdominal exoskeleton. Pairs of chlorided silver wires were used for recording and stimulating. To avoid contamination they were soaked overnight in 5% Decon 75 (BDH) to remove all traces of organochlorine compounds after each experiment. A pair of electrodes was placed between the first and second ganglia for stimulation. A similair pair was placed between the fourth and fifth ganglia to record nerve activity. The nerve cord was crushed with forceps proximal to the stimulating electrodes and distal to the recording electrodes giving, in effect, an isolated nerve cord. This procedure was adopted because preliminary experiments had shown that sensory input obscured the data from the nerve cord.

The nerve cords were kept moist with saline containing 210 mM NaCl, 2.9 mM KCl, 1.8 mM $CaCl_2$, and 2.0 mM phosphate buffer (pH 7.2) (UCHIDA, et al., 1974). They were exposed to various concentrations of DDT analogs at room temperature (approx. 21°C) for up to 2 hours. Acetone was used as a dispersing agent at a maximum concentration of 1% (v/v). This amount of acetone alone in the saline had no observable effect on the nervous activity. The minimum effective concentrations of the DDT analogs necessary to produce repetitive discharges (MEC_{RD}) after a single stimulus twice as strong as threshold (approx. 0.2V for monophasic pulses of 0.4msec duration) were determined. The stimulus strength was maintained unaltered throughout an experiment as stimuli considerably larger (e.g., x5) than threshold were able to produce a prolonged nerve cord discharge even in control preparations.

Insecticidal activity: Male and female adult cockroaches were temporarily immobilized with CO_2, and injected between the 3rd and 4th abdominal segments with 1 µl of acetone containing various concentrations of DDT analogs. This amount of acetone alone was found to be nontoxic to Periplaneta americana. After the injection, the insects were kept in 2 litre flasks with food (1:1 rolled oats and brewer's yeast) and water provided. Mortality counts were made daily for four days. Failure to exhibit locomotion was taken as the criterion for death (COCHRAN, 1955).

Results

Of the 14 DDT analogs examined for their effects upon the abdominal nerve cord, only four were found to produce repetitive firing under the experimental conditions used. P,p'-DDT, p,p'-MeO-DDT, p,p'-DDD and o,p'-DDT all produced trains of impulses in response to a twice threshold strength stimulus within 30 minutes of exposure. Control nerve cords only gave a single action potential in response to such a stimulus. P,p'-DDT was the most effective of the compounds studied: the minimum

effective concentration which produced repetitive firing was 8×10^{-7}M (Fig. 1A). Methoxychlor was almost as effective: MEC_{RD} was 5×10^{-6}M (Fig. 1B). With p,p'-DDD, a concentration of 2.5×10^{-5}M (Fig. 1C) was needed, while for o,p'-DDT, MEC_{RD} was 5×10^{-4}M (Fig. 1D). All the other analogs were tested at 10^{-3}M but failed to induce repetitive discharges in the cockroach central nerve cord even after 2 hours of exposure.

The data for the insecticidal studies are presented in the form of dosage-mortality curves fitted according to the statistical procedure of Finney as outlined by BUSVINE (1971). Each point on the curves (Figs. 2-4) was obtained by exposing 10 cockroaches to a given concentration of pesticide. Only p,p-DDT, p,p'-MeO-DDT and p,p'-DDD proved toxic to the cockroaches. The LD_{50} values, expressed as µmoles/roach, are shown in Table 2.

TABLE 2

Male and female LD_{50} data

Analog	Male LD_{50} (± S.D.) (µmoles/roach)	Female LD_{50} (± S.D.) (µmoles/roach)
p,p'-DDT	0.017 ± 0.004	0.033 ± 0.007
methoxychlor	0.029 ± 0.006	0.044 ± 0.01
p,p'-DDD	0.19 ± 0.04	0.27 ± 0.08

None of the other analogs tested exhibited insecticidal activity even when the cockroaches were injected with 0.5mg analog/roach (equivalent to 1.43 µmoles DDT or 1.39 µmoles MeO-DDT). Since o,p'-DDT produced repetitive firing in the central nerve cord, a higher dose of this analog (3.5mg per roach: equivalent to 10 µmoles DDT or 9.75 µmoles MeO-DDT) was injected, but no insecticidal effect was found.

Cockroaches which had survived for 3 weeks after LD_{50} injections of p,p'-DDT (n = 5) or methoxychlor (n = 5), and which had recovered from initial hyperexcitability, were found to exhibit repetitive firing in their nerve cords (with the standard conditions described in Materials and Methods) without further administration of pesticide.

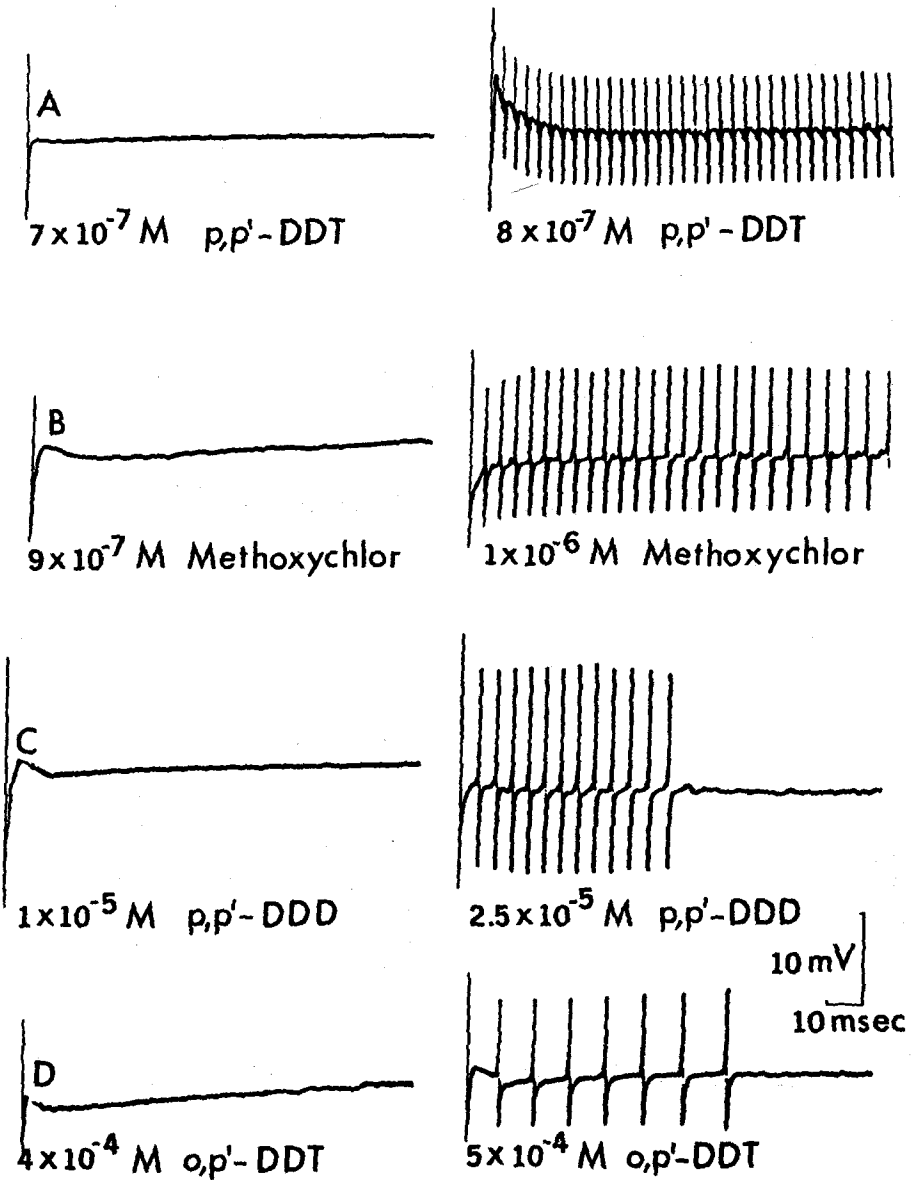
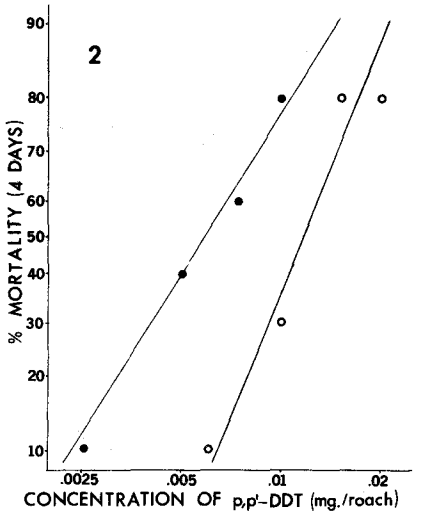
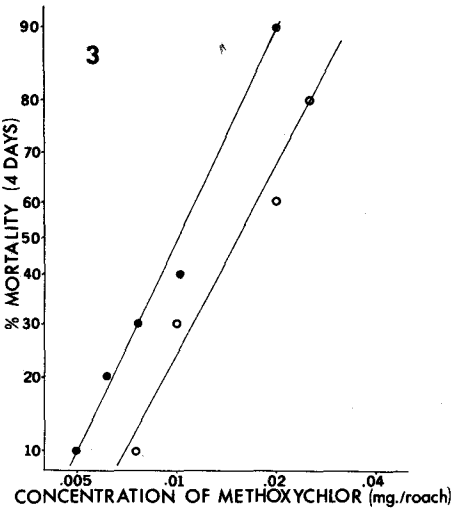
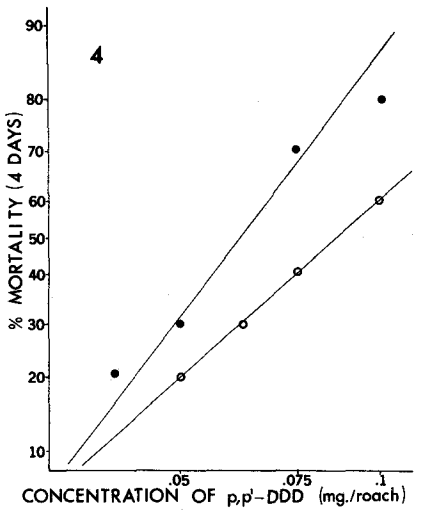


FIG. 1. Action potentials from the cockroach central nerve cord induced by a single stimulus, recorded after 30 min. treatment with the indicated DDT analog. With concentrations less than the threshold (left) only a single impulse was observed. With more than the threshold concentration (right) trains of impulses followed a single stimulation.







FIGS. 2 - 4.

The toxicity of injected p,p'-DDT (Fig. 2), methoxychlor (Fig. 3) and p,p'-DDD (Fig. 4) to adult male (closed circles) and female (open circles) American cockroaches.

Discussion

The minimum effective concentrations of p,p'-DDT and p,p'-MeO-DDT found to induce repetitive discharges in the central nerve cord of Periplaneta americana at 21°C were 8×10^{-7} M and 10^{-6}M respectively (average from 5 unsexed cockroaches each). This is in good agreement with the value previously obtained (UCHIDA, et al., 1974) for male cockroaches of 2×10^{-6}M at 20°C for both compounds. The LD_{50} values for p,p'-DDT and p,p'-MeO-DDT

are close to those reported by COCHRAN (1955) for American cockroaches. Also, in agreement with COCHRAN, we found that females were more resistant than males to the compounds studied.

Following the procedure of UCHIDA et al. (1974), we plotted the -log MEC_{RD} against -log LD_{50}. However, our data show that while a correlation can be obtained if one only considers the three compounds that produced both repetitive firing and mortality (correlation coefficient 0.98), the relationship clearly does not hold in the case of o,p'-DDT which produced repetitive firing but not mortality. One possibility is that the apparent correlation is fortuitous, though it is more probable that o,p'-DDT is preferentially metabolized, or is prevented from reaching its site of action in vivo in some other way (BITMAN, et al., 1971; BROOKS, 1974). In houseflies (BROOKS, 1974) the action of o,p'-DDT can by synergized by mixed function oxidase inhibitors, suggesting that, at least in this case, it does not normally realize its intrinsic toxicity, though generalizations from one insect species to another are not always applicable (METCALF and FUKUTO, 1968).

However, these arguments do not account for our long-term neurophysiological data. Some cockroaches survived injections of LD_{50} doses of p,p'-DDT or p,p'-MeO-DDT, recovered from early symptoms of hyperexcitability, and yet still exhibited repetitive firing in the abdominal nerve cord three weeks later. A similar observation (for DDT) has been made in cockroaches by GAMMON (personal communication). At best this evidence suggests that the insect was able to adapt to the effects of the pesticides on its nervous system, but does not corroborate any 'cause and effect' argument linking repetitive firing activity in the nervous system to mortality.

It therefore still remains a possibility that DDT and its toxic analogs can have several different effects, the sum total or most crucial of which conspire to kill the animal.

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