The Action of Pesticides on Conduction in the Rat Superior Cervical Ganglion

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The toxic action of the organophosphorus insecticide, Parathion, and the carbamate insecticide, Carbaryl, are attributed primarily to their inhibition of cholinesterase resulting in the accumulation of acetylcholine.

However, the mechanism of toxic action of the chlorinated hydrocarbon insecticides--Chlordane, DDT, and Toxaphene-- has not been established. MATSUMURA (1971) and WOOLEY and BARRON (1968) have suggested that DDT may act on the sodium channels of nerve membranes. These investigators have related the instability of the nerve membrane to the toxic symptoms of the intact animal.

Of possible "model" systems, the superior cervical ganglion was selected. As PERRY (1957) has stated, "Here nature provides us with an aggregate of neurons readily accessible to experiments, readily isolated from the influence of other active neurons..."

Therefore, the preganglionic and postganglionic transmission and the O_2 consumption of the superior cervical ganglion of the rat were measured in vitro following the onset of symptoms after oral dosing. From these parameters the effect of insecticides on axonal and synaptic transmission was evaluated in defining its action in the intact animal. The materials tested included the chlorinated hydrocarbon pesticides - Chlordane, DDT, Lindane, and Toxaphene; three organophosphorus compounds - DFP, Parathion, and Paraxon; and the alkaloid - Nicotine.

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METHODS

The compounds reported were administered to Holtzman[1] adult white male rats by oral dosing using peanut oil as a vehicle. An LD_{50} was used for all compounds except Carbaryl, for which 1/10 of an LD_{50} was used.

At the onset of symptoms the animal was killed by a blow to the head. The ganglionic preparation was removed and desheathed. The desheathed preparation was mounted in a volume recording chamber (Figure 1a). The preganglionic branch was stimulated supermaximally with a pulse width of 1 msec using either a Nuclear-Chicago current stimulator or a Tektronix Type 162 waveform generator with a Type 161 pulse generator. The propagated nerve response was displayed on a Tektronix Type RM565 dual beam cathode ray oscilloscope (CRO) using a Type 3A3 differential input and Type 122 low level preamplifier. The CRO tracings were recorded on Polaroid Type 410 film. The potentials displayed on the CRO were typical triphasic responses of a volume recording. A detailed explanation of the properties of volume recording is found in FULTON (1955).

The parameters used were as follows:

1. Conditioning-Test (C-T) Response - The conditioning stimulus rate was approximately 0.5 Hz. The test stimulus followed at 50-800 msec intervals. The test response for the preganglionic and postganglionic amplitudes was expressed as a percentage of the pre- and postganglionic conditioning response, respectively.

2. Frequency Response - The response of the pre- and postganglionic fibers was measured during the second minute of a two-minute stimulation period at a given rate of stimulation. The amplitude of the preganglionic and the area under the curve of the postganglionic response were expressed in percentages of the respective measurements at 1 Hz.

3. O_2 Consumption - The O_2 consumption of the various portions of the nervous system was measured in a Gilson differential respirometer. The values are reported as liters x 10^{-4} of O_2 per gram of wet tissue per hour.

4. Solutions - The media used was that reported earlier (SANTOLUCITO and WHITCOMB, 1971). In addition, some preparations were tested in Ca^{++}-free media.

5. Analyses - Response comparisons were made using the student "t" test with the 95% confidence level considered statistically significant.

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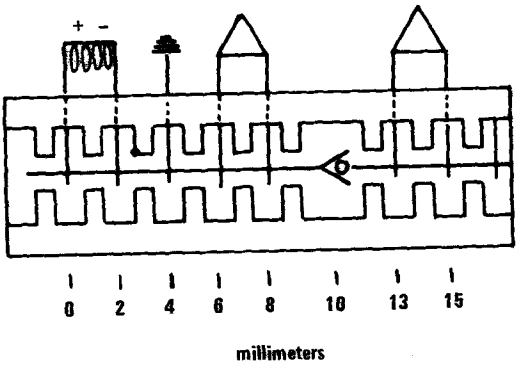
[1]Mention of manufacturer or commercial product name does not constitute endorsement or recommendation for use by the U.S. Environmental Protection Agency.

6. Recording

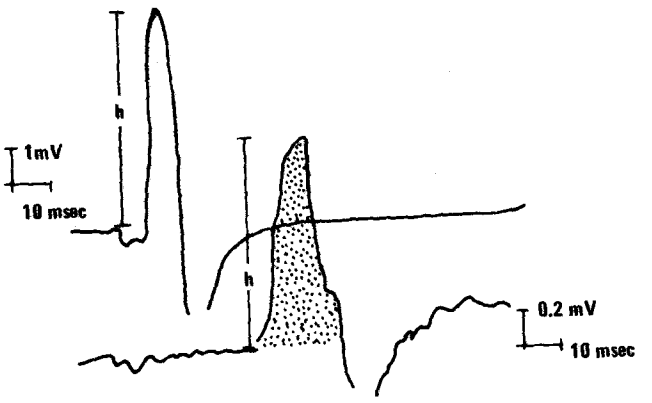
Figure 1a - Superior Cervical Ganglion Recording Chamber.

Figure 1b is a representative tracing of the pre- and postganglionic response recorded on Polaroid film. The positive deflection "h" was used as a measure of amplitude. In addition, the shaded area of the postganglionic response was used as a measure of the number of fibers responding.

Figure 1c represents two superimposed preganglionic responses obtained from pools 6mm-8mm and 6mm-10mm. This demonstrates that the amplitude "h" and duration "d" measured for the preganglionic response occurred in the pool nearest the stimulating electrodes.

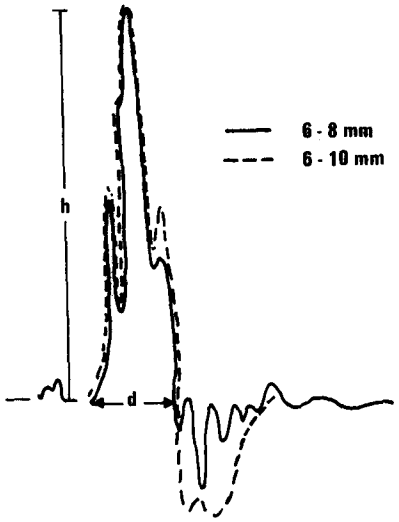


A. SUPERIOR CERVICAL GANGLION RECORDING CHAMBER



B.

Figure 1. Recording chamber and observed waveforms.



C.

RESULTS

Conditioning-Test Response - The amplitude of the test response of the preganglionic and postganglionic fibers of the non-treated group and the treated group, composed of Carbaryl, Chlordane, and DDT, was expressed as percentage of their respective conditioning response. The results are summarized in Table I.

TABLE I

The *in vitro* Test Response Amplitude of Pre- and Postganglionic Fibers from Rats with Toxic Symptoms Following Oral Dosing.

C-T[1] Interval (msec)	Fibers	% of Conditioning Response (mean ± S.D.)[+] Non-treated	Carbaryl	Chlordane	DDT
50	Pregang	88±14*	80±10*	79±22*	75±12*
	Postgang	46±16*	48±17*	55± 9*	46±14*
100	Pregang	97± 6	82±10*	88±10*	79±12*
	Postgang	56±13*	60±17*	67±14*	53±12*
200	Pregang	97± 3	98±14	95± 9	89±10*
	Postgang	66±16*	66±15*	70±18*	57±10*
300	Pregang	98± 3	96±11	103± 9	94± 8
	Postgang	66±10*	78±14*	70±17*	66±16*
400	Pregang	99± 2	102±13	100± 4	96± 6
	Postgang	66± 9*	86±17	70±10*	72±10*
500	Pregang	100± 2	99± 6	103± 7	97± 4
	Postgang	71± 9*	86±16	70±10*	76± 9*

TABLE I (Continued)

C-T[1] Interval (msec)	Fibers	% of Conditioning Response (mean ± S.D.)[+] Non-treated	Carbaryl	Chlordane	DDT
600	Pregang	98± 2	101± 9	99± 5	98± 4
	Postgang	84± 8*	93±10	79± 5*	78±10*
700	Pregang	100± 2	98± 4	95± 5	98± 4
	Postgang	88± 8*	90±13	83± 8*	85±12*
800	Pregang	100± 2	98± 3	99± 8	97± 6
	Postgang	95± 5*	96±11	92± 6*	88±10*

[1]Conditioning-Test.

[+]Each mean represents six trials.

*Significantly different from conditioning response.

The preganglionic 'test' response of the DDT group decremented sooner than the response from the Carbaryl and Chlordane groups. All groups showed a decreased response at the C-T interval of 50 msecs. However, there were no statistically significant differences in preganglionic responses between treated and non-treated groups at any given C-T interval.

The postganglionic response for the non-treated, as well as the Chlordane and DDT groups, was reduced at the 800 msec C-T interval. Only the Carbaryl group was able to maintain an equivalent amplitude response at intervals of 300 msec or longer.

Frequency Response - In view of the decremented amplitude of the postganglionic response for the non-treated group, and two of the three treated groups at all C-T intervals, the area under the curve rather than amplitude was subsequently utilized in order to account for desynchronization effects. Furthermore, responses to multiple stimuli at various frequencies appeared to give a better graded response for the non-treated group.

Therefore, the preganglionic amplitude and postganglionic area were expressed as percentage of their respective response at 1 Hz. The results of stimulating at rates of 5 Hz, 10 Hz, 20 Hz, and 1 Hz-terminal are summarized in Table II.

TABLE II

The *in vitro* Rate Responses[1] of the Preganglionic (Amplitude) and Postganglionic (Area) Fibers from Rats with Toxic Symptoms Following Oral Dosing.

Group	Nerve Branch	Rate of Stimulation 5 Hz	10 Hz	20 Hz	Terminal-1 Hz
Non-treated (18 trials)	Pregang	112±20*	102±28	39±38*	95±19
	Postgang	107±38	39±29	NM	93±26
Chlordane (9 trials)	Pregang	111±28	105±28	77±25*	91±28
	Postgang	119±32	30±21*	NM	76±32*
DDT (5 trials)	Pregang	107±31	94±31	59±35*	92±33
	Postgang	130±17*	47±12*	NM	+62±11*
Lindane (6 trials)	Pregang	112±13*	85±25	38±22*	110±28
	Postgang	119±12*	46± 6*	NM	76±11*
Toxaphene (3 trials)	Pregang	116± 6*	108±38	58±14*	113±24
	Postgang	102±16	36± 7*	NM	77±22
Carbaryl (7 trials)	Pregang	108±10	106±10	78± 6*	105± 6
	Postgang	120±25*	51±35*	NM	77±55
Parathion (5 trials)	Pregang	129±13*	116± 8*	75± 9*	100±15
	Postgang	135±26*	54±16*	NM	88±10*
DFP (4 trials)	Pregang	116±12*	106±26	52±25*	92±11
	Postgang	165±66*	69±20*	NM	82±15
Nicotine (4 trials)	Pregang	114± 6*	108± 8	67± 7*	108±23
	Postgang	144±25*	18±21*	NM	+129±17*

[1]All values are means ± S.D. expressed as percentage of the response at 1 Hz.

*Significantly different from its own response at 1 Hz.

+Significantly different from the non-treated response at the same rate of stimulation.

NM - Not measurable.

Preganglionic Response - The preganglionic amplitude response at 5 Hz was increased significantly in six of the nine groups, the exceptions being Chlordane, DDT, and Carbaryl. However, the Carbaryl response was significant only at the 90% confidence level. An analysis of the waveform indicated the source of the increased amplitude at 5 Hz to be the growth of a peak which had been a minor peak at 1 Hz. However, when the stimulating rate was increased to 10 Hz, this increased response was not evident with the exception of the Parathion group. At the stimulation rate of 20 Hz, the preganglionic amplitude was reduced significantly for all groups. Following the high rate of stimulation, the terminal 1 Hz response was determined and was comparable to the initial 1 Hz response.

There was no significant difference in preganglionic amplitude response between non-treated and treated groups at a given rate of stimulation.

Postganglionic Response - When the response at 5 Hz was compared to the initial 1 Hz response, only the DDT, Lindane, and Parathion groups had a significantly greater response. At the 90% confidence level, the Carbaryl, DFP, and Nicotine groups had a significantly greater response. The three remaining groups--non-treated, Chlordane, and Toxaphene--showed no significantly increased response.

When the response at 5 Hz between the non-treated and treated groups was compared, only the DFP group's response was increased significantly. At the 90% confidence level, the response of the Nicotine group was increased significantly.

Of the six groups which had an increased postganglionic response at 5 Hz as compared to 1 Hz, there were four which had an increased preganglionic response. These were DFP, Lindane, Nicotine, and Parathion.

When the stimulation rate was increased to 10 Hz, the postganglionic response, for all groups, was significantly less than their respective 1 Hz response. However, when the treated groups were compared to the non-treated groups, there was no significant difference.

At the stimulation rate of 20 Hz, the postganglionic response for all groups was too reduced to be measurable.

When the stimulation rate was returned to 1 Hz, the response of the non-treated group returned to its initial 1 Hz response. Four groups--Chlordane, DDT, Lindane, and Parathion--had a significantly reduced terminal 1 Hz response. In contrast, the Nicotine group had a significantly increased response.

When the treated and non-treated groups were compared, the DDT group's postganglionic response was significantly decreased.

O_2 Consumption of Nerve Tissue - *In vitro* O_2 consumption measurements were determined for the superior cervical ganglion, cerebral cortex, cerebellum, brain stem, spinal cord, vagus nerve, and sciatic nerve. These tissues were from non-treated and treated groups. The treated group consisted of either Parathion, Chlordane, DFP, Carbaryl, or DDT treated animals. Measurements were also made on tissues from non-treated animals which were exposed *in vitro* to Paraoxon. The results, expressed as liters x 10^{-4}/grams wet tissue/hour are summarized in Table III.

It will be observed that the compounds given orally did not produce a consistent inhibition pattern of O_2 consumption. Only Paraoxon, which was added *in vitro* produced a consistent inhibition of O_2 consumption.

TABLE III

In vitro O_2 Uptake of Nerve Tissue from Rats Pretreated with Pesticides.

Group	Superior Cervical Ganglion	Vagus Nerve	Sciatic Nerve	Cerebral Cortex	Cere-bellum	Brain Stem	Spinal Cord
Non-treated (9)[1]	11.5 ±5.0[2]	2.2 ±1.5	1.8 ±1.2	1.9 ±0.7	1.8 ±0.7	1.5 ±0.2	1.5 ±0.3
Parathion (15)	7.5 ±1.5	3.8 ±1.4	1.2 ±0.2	1.4 ±0.2	1.5 ±0.3	1.2* ±0.1	NM[3]
Chlordane (5)	6.6 ±2.3	4.8 ±2.2	1.2 ±0.3	1.3 ±0.1	1.3 ±0.1	1.4 ±0.4	1.7 ±0.4
DFP (5)	10.2 ±5.6	3.6 ±1.7	1.0 ±0.1	1.5 ±0.2	1.4 ±0.1	1.1* ±0.2	1.5 ±0.4
Carbaryl (5)	12.6 ±3.8	7.9* ±3.8	2.0 ±1.4	1.6 ±0.2	1.7 ±0.2	1.4 ±0.2	1.5 ±0.3
DDT (15)	6.9 ±6.3	5.2* ±2.1	.87 ±.37	1.6 ±0.1	1.7 ±0.2	1.5 ±0.5	NM
Paraoxon[4] (3)	4.0* ±1.9	NM	1.2 ±1.3	0.8* ±0.5	0.7 ±0.2	0.8* ±0.2	NM

[1]Value in parenthesis indicates number of animals.

[2] x 10^{-4} liters/gram/hour. Mean ± standard deviation.

[3]No measurements.

[4]Paraoxon was added to media at time of *in vitro* measurement.

*Significantly different from the non-treated tissue.

DISCUSSION

The effects of DDT and the other chlorinated hydrocarbons were evaluated on the mode of action of DDT suggested by MATSUMURA (1971) and WOOLEY and BARRON (1968). None of the parameters of this experiment suggested that the sodium channels in the nerve membrane were altered. There was no evidence of spontaneous firing of the preganglionic and postganglionic fibers in Ca^{++}-free or Ca^{++}-containing media from animals treated with the chlorinated hydrocarbons.

The only significant difference between the chlorinated hydrocarbon group and the non-treated group was the decreased postganglionic area for the terminal 1 Hz stimulation with DDT-treated animals. Since the preganglionic response was not altered, it would appear that this was a synaptic transmission effect rather than an axonal transmission effect.

O_2 Consumption - Just as BROOKS et al. (1949) were able to demonstrate an inhibition of O_2 uptake by DFP in the isolated frog brain, the addition of Paraoxon *in vitro* significantly reduced the O_2 uptake in the superior cervical ganglion and portions of the central nervous system. The failure to observe a comparable effect with the compounds administered by oral dosing is consistent with the *in vivo* findings of JOVIC et al. (1971) for Soman and DFP. Thus, the present results support JOVIC's conclusion that "no significant inhibition of oxygen uptake occurs in cerebral preparations from animals killed during the first exitatory period of poisoning."

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