EEG, ECG, and Respiratory Response to Acute Insecticide Exposure

K. M. Hyde, J. C. Crandall, K. E. Kortman, W. K. McCoy

Biology Department
Point Loma College
3900 Lomaland Drive
San Diego, Calif. 92106

Despite intensive efforts over the last 30 years, the mode of insecticide action remains largely undetermined. Cholinergic enzyme systems have been identified as targets of organophosphates (HAYES, 1975) whereas respiratory, cardiovascular, reproductive, and most prominently, neurological dysfunctions are implicated vectors in organochlorine toxicity (SHANK-LAND, 1964; EMERSON et al, 1964; HYDE et al, 1973). Additional evidence supporting organochlorine neurotoxicity has recently been provided through electrographic studies in this laboratory (HYDE and FALKENBERG, 1976) and elsewhere (WOOLLEY and BARRON, 1968; JOY, 1973, 1976). Many of these investigations have, however, concentrated on *in vitro* responses or on individual physiological indices; rarely considering more than one responding system. We feel that a more definitive knowledge of intoxicative pathways might be obtained by monitoring simultaneous reactions in the intact animal.

Our objective was therefore to compare acute electrophysiological effects of representative organochlorine (OCl) and organophosphate (OP) insecticides on the laboratory rat. Concurrent neurological, respiratory, and cardiac involvement were primary concerns.

METHODS

Each of 14 male Sprague-Dawley rats weighing between 240-280 g was equipped with chronic dural electrodes to record spontaneous cortical EEGs. The calvarium was exposed by reflecting overlying muscle and periosteum and electrode implantations performed as described previously (HYDE and FALKENBERG, 1976). Predetermined coordinates were stereotactically located slightly anterior and lateral to the bregma-midsagittal and lambda-midsagittal junctions. A dental drill, affixed to the stereotaxic unit, was then used to prepare four burr holes at these specified points. Self-tapping stainless steel screws contacting the dura served as recording macroelectrodes. Bipolar EEGs were subsequently obtained from contralateral

Bulletin of Environmental Contamination & Toxicology,
0007-4861/78/0019-0047 $1.80

frontal and occipital montages thereby permitting analysis of simultaneous records from general motor and sensory regions.

Individually housed rats were given a minimum of 7 days to recover from electrode implantation and then randomly assigned to one of 7 treatment groups; 2 rats per group. Treatments consisted of lethal ip injections of each of 3 OCls (chlordane, 300 mg/kg; dieldrin, 60 mg/kg; and toxaphene, 70 mg/kg) and 3 OPs (azodrin, 20 mg/kg; phosdrin, 3 mg/kg; and vapona, 40 mg/kg). Technical insecticides[1] were emulsified in a Tween 80-isotonic saline solution and adjusted so that injections of not more than 0.7 ml delivered the desired dosage. Two control rats received 0.5 ml of the vehicle only.

Spontaneous EEGs, ECGs, and respiratory rates were monitored concurrently on a 4-channel Physiograph^{R} employing rectilinear pens. Respiration and ECGs were recorded via bilateral needle electrodes inserted subcutaneously at the 6-7 rib level along the midaxillary line. In all cases, mild restraining anesthesia (~10 mg/kg sodium pentobarbital) was used to facilitate data retrieval by reducing consequences of external stimulation and somatic activity on electrographic events.

Bioelectrical recordings were secured from all subjects for at least 60 sec prior to insecticide exposure. Because of prolonged toxicity, 60-sec tracings were collected every 5 min, or as events dictated, from rats given OCls, whereas, animals administered OPs were monitored continuously until expiration. Tracings were obtained intermittently throughout a one-hour period from control rats.

Quantitative evaluations were conducted on individual 12-sec tracings representing 5 progressive stages of intoxication from each subject. Data analyzed were: cardiac rates, voltages, and morphological changes in ECGs; respiratory rates and wave structure; and EEG frequency, amplitude, and waveform. Electrocortical rates were calculated as mean spontaneous activity (MSA) from frontal and occipital leads. A compensating polar planimeter was employed to determine mean EEG voltage in accordance with the methodology of BRUCK (1960).

RESULTS

Observed toxic symptoms resembled published accounts and therefore will not be considered in detail except for a brief comparison of treatments.

[1] Insecticide suppliers: Hercules Incorporated, Shell Chemical Company, and Velsicol Chemical Corporation.

Clinical reactions to OCl insecticides were detected as early as 18 min but generally delayed by 30-45 min. Sequential symptomatology included hypersensitivity, myogenic tremors, a strong tendency to forward progression, exaggerated standing, respiratory difficulty, and tonic-clonic convulsions. Extremely animated convulsions were characteristic of OCl intoxication followed by muscular paralysis and death.

Organophosphate poisoning revealed a different toxic syndrome. Symptoms appeared much earlier (within 0.5-3 min) and were distinguished by muscle fasciculation, salivation, urination, diarrhea, slight cyanosis of extremities, lacrimation, and severe dyspnea preceding mortality. Clinical responses to the OPs were thus consistent with established effects of cholinesterase inhibition (O'BRIEN, 1967) although toxic signs associated with azodrin were slower to develop and less intense than those produced by either phosdrin or vapona. Massive convulsions were not evoked by any of the OPs tested; death seemed to result from respiratory failure in every case.

Neurotoxicity

Preinjection and control EEGs consisted of a mixture of surface-negative high frequency beta discharges superimposed on less numerous slower delta and theta waves. The MSA from the frontal cortices of 14 rats was 17.67 ±0.53 Hz and was slightly elevated from the occipital leads (18.78 ±0.69 Hz). Moderate average amplitudes of 77.1 ±8.5 and 83.2 ±8.9 μV were measured from the frontal and occipital brain areas, respectively. These pretreatment values changed only nominally during exposure to the vehicle solution.

Neuroelectrical effects of OCls. Except for variation in latency, the 3 OCls tested produced comparable alterations in electrocortical recordings. The earliest disturbance was recorded within 15 min of chlordane injection, 21 min post-dieldrin, and delayed 42 min with toxaphene; seizures required a much longer time to evolve. All changes were propagated in the form of dispersed spikes or as isolated paroxysmal volleys issued synchronously from the two brain areas (Fig. 1).

Although at times seizures began and ended abruptly, preconvulsive features generally became progressively more severe until tonic-clonic episodes dominated the EEG. These epileptogenic type discharges in turn became successively more pronounced, particularly from the occipital cortex during exposure to chlordane and dieldrin. Seizures exhibited fast, extremely high-voltage alpha and beta waves (MSA of 36.4 ±4.3 Hz); spike amplitudes reached

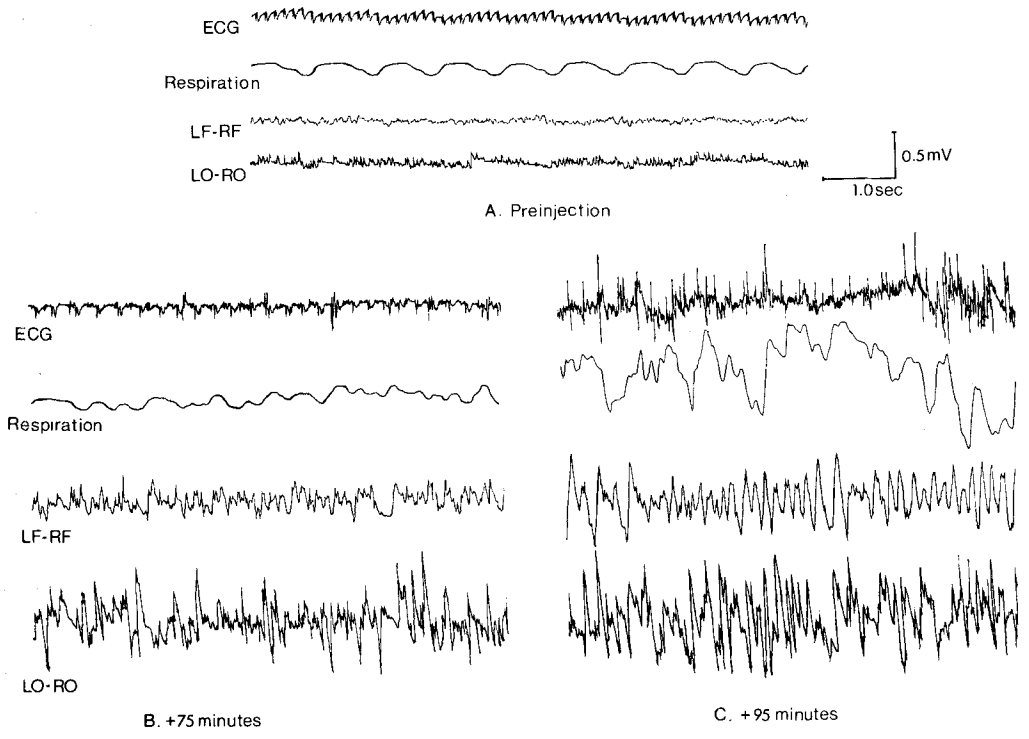

Figure 1. Simultaneous preconvulsive and convulsive records issued during ip chlordane injections (300 mg/kg). EEG amplitude and frequency increased markedly as seizures developed, especially from the occipital cortex. ECGs became disorganized coincident with convulsive episodes showing arrythmic tachycardia. Dyspnea was reflected by large arching waves (C).

730 μV from the frontal cortex and exceeded 960 μV in occipital tracings (Fig. 1).

Each convulsive episode alternated with relatively inactive periods of variable duration indicative of a refractory phenomenon. Mean frequency of frontal EEGs diminished to as low as 11.9 ±0.7 Hz during these interseizures and culminated in either another convulsion or ultimatley in isoelectric tracings.

Neuroelectrical effects of OPs. In contrast to OCl toxicity, OP exposure produced non-episodal EEG patterns (Fig. 2). Although somatic tremors were characteristic features of OP poisoning, they were not visibly reflected in electrocortical records. Nonsignificant ($p > 0.05$) increases in the MSA from predosage rates to 18.40 ±0.36 Hz for frontal tracings and 19.60 ±0.82 Hz from the occipital neocortex were recorded by late phases of intoxication. Concomitant with these frequency alterations was a moderate decrease in amplitude over the course of exposure suggestive of cerebral anoxia (KOOI, 1971).

Cardio-Respiratory Response

Control animals exhibited no significant change in ECG rate, ECG voltage, or in respiratory activity during the one-hour exposure to the vehicle solution. Mean cardiac rate prior to injection was 471.6 ±7.3 BPM and 470.3 ±9.6 BPM after one hour. Spontaneous respiration from the 14 pretreatment rats was expressed as rhythmic sinusoidal tracings of 107.5 ±4.4 breaths/min and, likewise, demonstrated no significant change in rhythmicity throughout the control period.

OCl influence on ECGs and pneumographs. The 3 OCls were essentially equipotent with respect to cardiac and respiratory rates (Table 1). Bradycardia was a consistent reaction to each OCl; mean heart rate subsided from 417.5 ±26.7 BPM predosage to 388.5 ±11.2 and 335.0 ±20.3 BPM at 30 and 45 min postinjection, respectively. However, by ~65 min cardiac rhythm had returned to or exceeded preinjection rates and continued to increase so that tachycardia distinguished late stages of intoxication (Fig. 1C).

ECG structure remained unaltered during bradycardia, except for a moderate voltage decrease (toxaphene) or a slight increase (chlordane and dieldrin). However, during tachycardia a gradual deterioration of waveform occurred. Elevation of surface-positive deflections, arrythmias, and dissociation of wave complexities were unifromly manifested. Nevertheless, waves became somewhat obscured as toxicity progressed

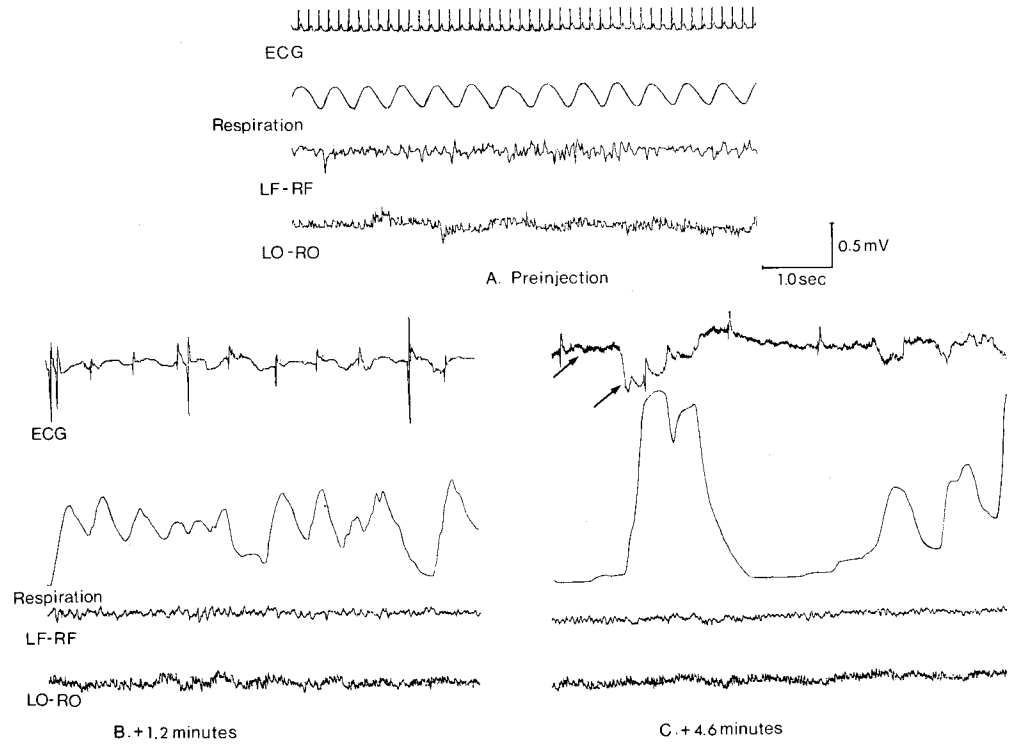

Figure 2. Selected tracings collected before and during lethal Vapona intoxication (40 mg/kg). EEGs display progressive decrease in energy indicative of cerebral anoxia. Respiratory waves reflect extreme dyspnea. ECGs show increasing bradycardia and elevation of amplitude as well as the influence of respiratory difficulty and somatic tremors (arrows).

due to EMG and respiratory interference (Fig. 1C). During latter stages of poisoning recurrent periods of relative ECG quiescence alternated with active episodes paralleling EEG behavior.

Pulmonary rhythm remained essentially unaltered through early exposure, however, as intoxication advanced, dyspnea and ancillary signs of respiratory distress generated slightly faster, medium-amplitude waves (Fig. 1B). By late stages of poisoning pneumographic records exhibited accelerated shallow respiration superimposed on deep arching waves coincident with cardiac disturbances and neuroelectric seizures. During these severe convulsions, respiratory tracings became erratic, typified by a meandering baseline and a preponderance of rapid, abnormal patterns (Fig. 1C). Respiratory response to OCl exposure seems to follow both EEG and ECG activity in that normalized periods were evoked during interseizures. Breathing rates increased significantly over the exposure period (62%; $p < 0.01$) but tended to slow to near normal rhythms during interseizures.

TABLE 1

Changes in cardiac and respiratory rates during exposure to organochlorine and organophsophate insecticides.

Insecticide	Total Percentage Change[a] ECG Rates	Respiration	Time to Death[b]
OCls:			
Chlordane	+26.0	+65.5	94.8 ±4.8
Dieldrin	+24.5	+64.0	97.6 ±0.6
Toxaphene	+17.5	+56.5	112.0 ±10.0
OPs:			
Azodrin	-25.0	-51.0	11.5 ±3.5
Phosdrin	-66.5	-62.0	1.7 ±0.5
Vapona	-77.0	-63.5	4.2 ±1.9

[a] Values are expressed as the percentage change from pretreatment to late stage of toxicity.

[b] Time given in min from injection ±SE; irreversible respiratory and EEG silence, when occurring together, were criteria for determining time of death.

Organophosphate effect on ECGs and respiration. Progressive post-organophosphate bradycardia and reduced pulmonary rates were demonstrated from all animals (Table 1). A mean preinjection ECG rate of 410.7 ±23.6 BPM was reduced to 266.7 ±59.6 BPM by mid-intoxication and severely depressed to 173.9 ±44.9 BPM a short time prior to death. No reversal in bradycardia was recorded and chaotic ECGs showing effects of somatic tremors inevitably preceded mortality (Fig. 2). Gross wave deformities originated within 45 sec (phosdrin), 60 sec (vapona), and within 3 min of azodrin exposure.

Labored breathing was rapidly and equably produced by injection of each OP insecticide. Completely disordered tracings were projected within 0.5-3 min in response to phosdrin or vapona but were prolonged 6 min with azodrin. Nevertheless, progressive dyspnea was reflected in all OP tracings. Predosage respiratory rates were significantly reduced (59%; $p < 0.01$) by late stages of intoxication.

DISCUSSION

The remarkable distortion of spontaneous cortical potentials recorded during OCl exposure reveals an inordinate cerebral sensitivity to these compounds. Of the two brain areas examined, the occipital cortex displayed greater excitability, especially in response to chlordane and dieldrin. Epileptiform seizures from this region yielded faster discharges, higher spike amplitudes, and more vertexlike waves than those elicited from the frontal cortex. Similar accentuation of sensory modalities have been observed in laboratory rats administered DDT (WOOLLEY and BARRON, 1968). Likewise, cats exposed to dieldrin experienced sensitization of the somatosensory cortex (JOY, 1976), whereas endrin produced irritability of the avian visual telencephalon (REVZIN, 1966). Furthermore, rats given chronic chlordane at levels incapable of producing overt behavioral changes nevertheless revealed significant alterations of cerebrocortical potentials; changes which were precipitously aggravated by food deprivation (HYDE and FALKENBERG, 1976). Because of the importance of visual, proprioceptive, and motor input to the survival of nontarget mammals, any functional deficit attributable to direct or accumulative OCl exposure is likely to be profound and therefore requires careful assessment.

We did not confirm previous evidence of an anterior-posterior (motor-sensory) evolution of OCl-induced seizures (JOY, 1976); instead, all neuroelectrical disturbances originated synchronously from the frontal and occipital cortices. Indeed, cardiogenic and respiratory anomalies also tended to observe a synchronization with EEG seizures. Our data on

postseizure synchrony extends the findings of JOY (1973) wherein hypersynchrony was a distinctive attribute of preconvulsive EEGs. In addition, relatively inactive interseizure EEGs, ECGs, and pneumographs were consistently recorded during middle and late stages of poisoning. Periodic refractoriness appears to be a common feature of OCl neurotoxicity since oscillations of this nature have also been obtained from rats exposed to DDT (SHANKLAND, 1964) and from anesthesized cats subjected to dieldrin (JOY, 1976).

The absence of electrocortical disturbances following lethal OP injections is evidence of substantial differences in the mechanisms of OP and OCl intoxication. Failure to alter cortical potentials may reflect an inability of these compounds to effectively reach higher brain centers due to rapid peripherally-induced lethality and/or to inadequate penetration into cortical neurons (SHARMA et al, 1973; JOY, 1976). Alternatively, impulse transmission within the central and peripheral nervous systems may be sufficiently dissimilar to produce or contribute to differential neurotoxicity (SHARMA et al, 1973). This aspect of toxicity warrants further investigation.

REFERENCES

BRUCK, M.A., Clin. Neurophysiol. 12, 528 (1960).

EMERSON, T.E., Jr., BRAKE, C.M., and HINSHAW, L.B., Can. J. Physiol. Pharmacol. 42, 41-51 (1964).

HAYES, W.J., Jr., Toxicology of Pesticides. Williams and Wilkins, Baltimore. 580 pp (1975).

HYDE, K.M., GRAVES, J.B., WATTS, A.B., and BONNER, F.L. J. Wildl. Manage. 37(4), 479-484 (1973).

HYDE, K.M., and FALKENBERG, R.L., Toxicol. Appl. Pharmacol. 37, 499-515 (1976).

JOY, R.M., Neuropharmacology. 12, 63-76 (1973).

JOY, R.M., Toxicol. Appl. Pharmacol. 35, 95-106 (1976).

KOOI, K.A. Fundamentals of Electroencephalography. Harper and Row, New York. 260 pp (1971).

O'BRIEN, R.D., Insecticides: Action and Metabolism. Academic Press, New York. 332 pp (1967).

SHANKLAND, D.L., Toxicol. Appl. Pharmacol. 6, 197-213 (1964).

REVZIN, A.M., Toxicol. Appl. Pharmacol. 9, 75-83 (1966).

SHARMA, R.P., SHUPE, J.L., and POTTER, J.R., Toxicol. Appl. Pharmacol. 24, 645-652 (1973).

WOOLLEY, D.E., and BARRON, B.A., Toxicol. Appl. Pharmacol. 12, 440-454 (1968).