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# Effects of dieldrin, Ruelene<sup>®</sup> and DDT on the electrocorticogram in sheep

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EFFECTS OF DIELDRIN, RUELENE<sup>®</sup> AND DDT  
ON THE ELECTROCORTICOGRAM IN SHEEP.

Iowa State University, Ph.D., 1969  
Physiology

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EFFECTS OF DIELDRIN, RUELENE <sup>®</sup> AND DDT  
ON THE ELECTROCORTICOGRAM IN SHEEP

by

Gary Arthur Van Gelder, D.V.M.

A Dissertation Submitted to the  
Graduate Faculty in Partial Fulfillment of  
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Signature was redacted for privacy.

In Charge of Major Work

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1969

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## INTRODUCTION

As the result of the widespread pollution of our environment, the latter part of the 20th Century may become known as the toxicological age. The detrimental aspects of air and water pollution are beginning to come to the surface as health authorities and conservationists are calling these problems to public attention. It would appear that the time has come when industry, and ultimately the consumer, must begin paying the cost of pollution control. Even now the public is paying for the damage done to lakes and streams by industrial and agricultural pollution. The constantly recurring question is how much pollution can the environment and its biological contents endure before a halt must be called.

One group of the offending pollutants are the organic insecticides which have helped the farmer control a wide range of soil, plant, and animal pests and have resulted in increased production and a greater food supply. However, along with the benefits have come environmental pollution and, in some cases, the contamination of food supplies. The significance of these residue levels is unknown. The research reported here is part of a research program designed to provide knowledge of the neurological effects of insecticides so that the cost-benefit equation for insecticides may be more adequately evaluated.

There is no doubt that detectable amounts of chlorinated hydrocarbon insecticides are being found in food, environmental samples and human and animal tissues. These levels range from parts per billion up to parts per million. Further, there is no doubt that significant exposure to organic insecticides can result in serious illness and occasionally death. Furthermore, as will be shown later, it is known that these chemicals produce at least part of their toxic effects by interfering with central nervous system functioning. The crucial question of whether the current residue levels of organic insecticides have any significant detrimental effects is largely unanswered.

In 1964-65, Dr. William Buck, Dr. Richard Talbot, and Dr. George Karas of the Iowa State University faculty were awarded a grant from the National Institutes of Health for the purpose of developing methods and techniques for the assessment of sub-clinical effects of low level exposure to organic insecticides. The premise of the research proposal was that since high exposure to insecticides results in neurotoxicity, there may be neurological effects resulting from lower or subclinical exposure levels. The approach proposed was to study the electrophysiological and behavioral influences of these compounds in the intact animal. A recent statement by Woolley and Barron (1968) exemplified the problem when they remarked that in order to be able to evaluate whether or not DDT and other organic insecticides actually pose a public health problem, more knowledge of its effects at the whole

animal level are required. In particular the effects on the intact nervous system are not well enough understood to explain the clinical symptoms. This is interesting in light of the fact that a greater amount of work has been done on discovering the effects of DDT on animals than with any of the other insecticides. It would also seem that some consider the mechanism of action of organophosphorus insecticides an open and shut case since it is known that they inactivate acetylcholinesterase. However, intuitively from clinical cases, it would appear that other mechanisms are involved since the clinical syndromes differ with the various organophosphorus insecticides.

The work reported here is part of the electrophysiological studies. The author became involved in this project during the summer of 1966 and as research veterinarian was intimately involved in the total research program. During this period 3 men completed advanced degree programs. Previously, Dr. Bruce Sandler (1968) reported on one of the behavioral studies in his Ph.D. dissertation entitled "Dieldrin Exposure and Vigilance Behavior in Sheep", Mr. James Maland (1968) reported on the "Effect of Dieldrin on Visual Discrimination in the Sheep" for a M.S. thesis, and Dr. Donald Sussman's (1967) Ph.D. dissertation was entitled "The Effect of Exposure to Dieldrin on Avoidance Extinction and Conditioned Heart Rate in Sheep". Reports of work on this project have been reported by Sandler et al. (1968a), Sandler et al. (1969b), Sandler et al. (1969c),



Van Gelder et al. (1969a) and Van Gelder et al. (1969b). The major purpose of the research reported here was to provide some understanding of the occurrence of electrocorticographic (ECOG) changes with specific insecticides, paying particular attention to the nature of the changes. It should be noted that this work has already paid one dividend in that the rationale for Dr. Sandler's dissertation was based on work completed with dieldrin as part of this dissertation.

## LITERATURE REVIEW

The literature reviewed here was limited to those publications concerning the levels of insecticides in central nervous system (CNS) tissue and the effects on CNS and peripheral nerve functioning. A large number of references concerning fat, blood, soil and water levels of pesticides have accumulated during the past 20 years as evidenced by reviews of Frazer (1967) and Hodge et al. (1967), and specific reports of Buck and Van Note (1968), Dale and Quinby (1963), Hunter et al. (1963) and Abbott et al. (1968). However, until the biological effects of these residue levels are elucidated, if indeed there are detectable effects, the significance of the published residue data must be held in abeyance.

For purposes of discussion and for understanding the similarities and dissimilarities of clinical toxicity resulting from the various types of insecticides, Radeleff (1964) categorized the organic insecticides into the following 3 major groups: organophosphates, carbamates and chlorinated hydrocarbons. The compounds parathion, diazinon, guthion, malathion, and TEPP represent the organophosphorus group, which all inhibit acetylcholinesterase. A related group consists of the systemic organophosphorus insecticides with Ronnel<sup>®</sup>, coumaphos, Ruelene<sup>®</sup>, and trichlorfon being representative. These compounds also inhibit acetylcholinesterase.

The most widely known carbamate is carbaryl. The carbamates also exert a parasympathomimetic effect by the inhibition of acetylcholinesterase.

The chlorinated hydrocarbon insecticides are further divided into 2 subgroups. The first is comprised of the DDT class, which consists of such compounds as DDT, TDE, and methoxychlor. The second is the cyclodiene class, which consists of compounds such as chlordane, dieldrin, aldrin, endrin, and heptachlor.

Animals exposed to members of the DDT class show an abnormal hypersensitivity to external stimuli. Convulsions are not pronounced and usually occur late in the toxic syndrome. The cyclodiene group, on the other hand, are potent CNS stimulants or depressants. Initially there is hypersensitivity followed by fasciculations of facial and cervical muscles which are usually followed fairly rapidly by clonic spasms of the head, neck and forequarters. The animals then lose coordination, walk aimlessly, assume abnormal postures and may experience clonic-tonic convulsive seizures. Some animals will only be hypersensitive prior to the onset of an explosive convulsive seizure. The convulsive seizures may repeat at regular intervals and may persist until death. Some animals will show severe depression, inappetance, inactivity, emaciation, and death instead of the convulsive syndrome.

## Neurological Aspects of Chlorinated Hydrocarbon Insecticides

### Cyclodiene class

The preponderance of work with this class has centered on aldrin and dieldrin. Hayes (1965) reviewed the absorption, storage, biotransformation and excretion of the chlorinated hydrocarbon insecticides in mammals. Because the ingestion of aldrin results in its epoxidation and resultant storage as dieldrin in the body fat (Ivey et al., 1961), aldrin and dieldrin can be considered together. Quaife et al. (1967) in a review article on the ingestion and storage of aldrin and dieldrin in animals and man concluded that no data have been presented to show that long-term feeding studies on full grown animals result in saturation levels of dieldrin in fat. Hodge et al. (1967) surveyed the literature in an effort to determine the no effect levels of aldrin and dieldrin. Their report showed that the lethal oral dose for aldrin and dieldrin for all species was in the range of 20-70 mg/kg body weight. The lowest exposure level having biological effects was with rhesus monkeys where feeding 5 parts per million (ppm) dieldrin for 1 year resulted in death. This was estimated to be equivalent to a 70 kg man consuming 17.5 mg/day. Other rhesus monkeys tolerated 1 ppm dieldrin in the feed, which was estimated to be equivalent to a 3.5 mg/day exposure in a 70 kg man.

Several reports of accidental or suicidal exposures to aldrin or dieldrin have been published. Schwar (1965) reported

a case where dieldrin was injected intravenously by a 31 year old woman in which convulsive seizures and death occurred within hours. The terminal blood dieldrin level was 50 ppm. Three cases of occupational exposure to aldrin or dieldrin were reported by Bell (1960). The patients complained of nausea, vomiting and headaches which could not be controlled by analgesics. In one case a fat sample taken 8 months after recovery from clinical toxicity contained 1 ppm dieldrin. In another clinical case, the electroencephalogram (EEG) was recorded one week after an acute toxic exposure. Random bilateral 4-7 hertz (formerly CPS, now abbreviated Hz) activity was found. The alpha wave activity (8-12 Hz) was normal and blocked on visual attention. No significant changes were noted with photic stimulation. Electroencephalograms recorded two and one-half months later were normal. The fat dieldrin level was 40 ppm 44 days after exposure. Conley (1960) reported that some people poisoned by dieldrin report a loss of memory.

Hoogendam et al. (1962) reported that industrial employees exposed to toxic levels of dieldrin and/or endrin showed some bilateral synchronous spike and wave complexes of a 3 Hz frequency in the EEG. Bilateral synchronous theta (4-7 Hz) activity was frequently found with a general slowing of the EEG and an increased amplitude. Most EEGs returned to normal in 3-6 months. Over a 9 year period the industrially exposed group had a 20% rate of EEG abnormalities compared to 9% for an office

control group (Hoogendam et al., 1965). During these 9 years of observation there were 17 poisonings resulting in convulsive seizures.

Endrin has also been reported to cause irregular slow wave EEG activity with some irregular spiking in rats. Electroencephalographic changes were not found in rats receiving 3.5 mg endrin/kg body weight although convulsive seizures were induced (Speck and Maaske, 1958).

Hunter and Robinson (1967) fed professional human volunteers 10, 50, or 211  $\mu\text{g}$  dieldrin for 18 months. There were no clinical signs of illness and the following parameters remained within normal ranges: plasma alkaline phosphatase, red cell and plasma cholinesterase, hemoglobin, packed cell volume, white cell count, differential white count, plasma protein, blood urea nitrogen, serum glutamic pyruvic transaminase, serum glutamic oxalacetic transaminase, electrocardiogram, electroencephalogram and electromyogram. At the end of 18 months the 50  $\mu\text{g}$  group had a fat level (1.12 ppm) 4 times the general population (0.23 ppm) and the 211  $\mu\text{g}$  group's fat level (3.64 ppm) was 15 times the general population residue level. They estimated an exposure level of 25  $\mu\text{g}/\text{day}$  for the general population.

Casarett et al. (1968) reported brain residue data for heptachlor epoxide, DDE, DDD, DDT and dieldrin from 32 autopsies of general population. The average brain dieldrin level was 0.0031 ppm and for DDE 0.0831 ppm. The total brain chlorinated

hydrocarbon level was 0.0989 ppm and the total fat residue was 5.89 ppm.

The biological half-life of dieldrin in man was calculated to be 97 days by Brown et al. (1964). The blood dieldrin residue toxicity threshold for man and dog was estimated to be in the 0.15-0.20 ppm range.

Jolly (1954) reported that sheep poisoned on dieldrin do not show brain lesions which agrees with observations by Duncan<sup>1</sup>. Kitselman (1953) cited degeneration of cerebral cortical cells in dogs fed 0.2 mg dieldrin/kg body weight for 2 months.

A limited amount of information is available on the neurological mechanisms of action of dieldrin. Hathway (1965) reported significant increases in rat cerebral lactate and pyruvate during dieldrin and telodrin induced convulsive seizures. The lactate levels rose during convulsions and pyruvate only after a convulsion. Alanine levels were elevated prior to convulsive seizures. Telodrin increased glutamine and decreased glutamate levels prior to convulsions. The free ammonia levels in the brain were elevated with both insecticides but were higher with telodrin.

Preincubating rat brain slices with dieldrin dissolved in olive oil inhibited oxygen consumption due to interference with

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<sup>1</sup>Duncan, R. Veterinary Pathologist, Department of Veterinary Pathology, Iowa State University, Ames, Iowa. Observations on histological sections of brain from sheep exposed to dieldrin. Private communication. 1969.

dehydrogenases and cytochrome b. Dieldrin also inhibited anaerobic glycolysis and the hydrolysis of acetylcholine (Hosein and Proulx, 1960). In addition, they stated that the results indicated mitochondrial damage in brain cells following dieldrin, metrazol, camphor, or ammonium chloride induced convulsions.

Lethal doses of dieldrin in rats resulted in elevated levels of alpha-alanine and gamma-aminobutyrate in the brain. DDT did not have this effect (Witter and Farrior, 1964).

Gowdey et al. (1952) reported on the pharmacological properties of aldrin. Injecting aldrin intravenously in spinal cats resulted in muscle twitches of the rear limbs. Blocking the blood supply to the pelvic limbs did not block the onset of the twitches. The extensor reflex could be elicited with weaker currents and reflex excitability of the spinal cord was initially increased and then depressed.

#### DDT class

The lowest level of dietary DDT that was shown to have a biological effect was 2.5 ppm, which increased the microsomal epoxidation of aldrin to dieldrin (Gillett, 1968).

Laws et al. (1967), based on experience with 35 men with 11-19 years occupational exposure to DDT, estimated a daily intake of 17-18 mg/man/day. Fat residue levels were 38-647 ppm compared to 8 ppm for the general population.



Dale et al. (1963) reported that the concentration of DDT in the brain of rats was directly proportional to the severity of signs of poisoning. Woolley and Runnells (1967) showed that gray matter of the CNS accumulated DDT to a greater extent than white matter. The DDT concentrations were twice as high in whole brain after 6 and 12 hours of exposure than in the spinal cord, but after 24 hours the concentrations were nearly identical.

Crescitelli and Gilman (1946) administered a DDT emulsion intravenously to pentobarbital anesthetized cats and monkeys and reported increased amplitude of the cerebellar rhythm with peak effects occurring within two hours post administration. In unanesthetized, curarized monkeys given 50-75 mg DDT/kg body weight spike-like waves occurred in the electrical activity of both the cerebellum and cerebrum. Fast waves recorded from the motor cortex were in temporal synchrony with fast waves from the cerebellum. Transient periods of fast waves were usually noted 2-3 hours after DDT administration.

In 1953, Pollock and Wang stated that DDT caused an increased amplitude of the cerebral and cerebellar electrical activity in cats. Desi et al. (1966) fed rats 40 mg DDT/kg body weight for 6 days and recorded increased frequency and amplitude in the EEG. In rats fed 20 mg DDT/kg body weight the EEG amplitude increased after the first week and the frequency increased after 4 weeks of exposure. There was no difference

in the ability of the rats to perform a food-maze behavioral problem. Rats fed 5, 10, or 20 mg DDT/kg body weight were reported to have an increased frequency synchronization with a stroboscope as evidenced by photic activation of the EEG (Farkas et al., 1968).

The most extensive work on the CNS effects of DDT was recently reported by Woolley and Barron (1968). Giving 100 mg DDT/kg body weight to rats resulted in increased amplitude and occurrence of sinusoidal fast waves in the olfactory bulb and the prepyriform cortex. The electrical activity of the frontal cortex tended toward a continuous arousal pattern with an increased amplitude. Spikes appeared in the cerebellar electrical activity that matched the frequency of the whole body tremors. Slow waves disappeared from the midbrain reticular formation and medial geniculate body. They concluded that the EEG changes were similar to behavioral arousal and suggested that DDT stimulated the reticular activating system.

The activity of spinal dorsal roots in spinal rats was increased following DDT administration. Excised tibial nerves from such rats lost the positive afterpotential. The absolute refractory period was increased by a factor of one-third and the relative refractory period by a factor of two (Shankland, 1964).

The effect of DDT on the invertebrate nervous system has been studied more extensively than on mammals. Yeager and

Munson (1945) showed that DDT acted directly on the sensory nerves of the cockroach causing repetitive discharges. Roeder and Weiant (1945) and Tobias et al. (1946) corroborated this finding and described a similar action on motor nerves at higher concentrations of DDT. Later Roeder and Weiant (1948) showed that the initial symptoms of DDT poisoning in cockroaches were due to induced repetitive discharges originating in sense cells associated with the campaniform sensillae located on the legs.

Acetylcholine was observed to accumulate in nervous tissue of cockroaches, flies, and crayfish poisoned on DDT (Tobias et al., 1946). In frogs the peripheral nervous system was found to be involved in DDT poisoning (Isaacson, 1968).

The voltage clamp technique was applied to the study of DDT poisoned giant axons of lobsters by Narahashi and Haas (1967). Following 20-60 minutes of incubation in a DDT solution there were repetitive afterdischarges. It was shown that DDT delayed the turning off of the peak sodium conductance and inhibited potassium conductance, which resulted in a prolongation of the falling portion of the axon potential.

#### Neurological Aspects of Organophosphorus Insecticides

There are few reports on the neurophysiological effects of the organophosphorus insecticides. Crowley and Johns (1966) using electromyographic techniques demonstrated depolarization

blockade of the neuromuscular junction in a man with malathion toxicosis.

Recalling that the organophosphorus insecticides inhibit cholinesterase, it is possible to gain some insight into their possible neurological effects by reviewing the literature of the known anticholinesterase agents represented by physostigmine or eserine, neostigmine and the highly reactive cholinesterase inhibitor di-isopropyl fluorophosphate (DFP).

The administration of acetylcholine via carotid injection resulted in an increased amplitude and frequency of the EEG (Bonnet and Bremer, 1937).

In 1947 Grob et al. administered DFP to men and observed EEG changes similar to those seen in patients with grand mal epilepsy, but no convulsions were precipitated. Freedman et al. (1949) followed this with a study showing that intracarotid administration of DFP initially caused increased frequency and decreased amplitude of the EEG. As the dosage was increased the initial EEG changes progressed to an increase in amplitude followed by the appearance of large amplitude slow waves. This in turn was followed by the appearance of grand mal activity, which regressed to a pattern of high frequency spikes interspersed with irregular slow waves. The earliest EEG changes were noted when brain cholinesterase activity was reduced to 8.7% of normal. Grand mal seizure activity occurred when

cholinesterase activity was 0.6% of normal. Atropine blocked the occurrence of the grand mal pattern.

Bouzarth and Himwich (1951) followed this with a study in which rabbits were given DFP intravenously. Grand mal-like convulsive patterns appeared in the following order: thalamus, limbic cortex, or caudate nucleus, and the motor cortex.

Wescoe et al. (1948) gave DFP intravenously to 3 cats and a monkey and found increased frequency and decreased voltage in the EEG within a few minutes after administration.

The direct application of eserine to the cortex of anesthetized rabbits and cats resulted in blockage of large slow waves and caused the appearance of an arousal type EEG activity. Acetylcholine applied to the cortex reduced the amplitude of the slow waves while acetylcholine and eserine together produced large rapid rhythmical waves which could be blocked by atropine (Miller et al., 1940).

Bradley and Elkes (1953) studied the effect of physostigmine and neostigmine in conscious cats. Physostigmine produced a fast activity EEG similar to the normal arousal pattern. Neostigmine failed to show an EEG effect at doses which produced marked peripheral effects.

Funderburk and Case (1951) reported that cats receiving eserine showed an arousal type EEG and the animals did not sleep during this time.

Physostigmine given intravenously to cats reduced the surface negative potential from the medial suprasylvian gyrus but only slightly reduced this response in the anterior sigmoid gyrus. Reduction in the surface negative components of the anterior sigmoid responses to repetitive stimulation of the nucleus centralis lateralis (recruiting response) and the ventralis posteriolateralis (augmenting response) was marked. These effects were reversed by the administration of atropine (Hance et al., 1963). Villablanca (1966) demonstrated that atropine and eserine were pharmacologically active when applied to the chronically isolated cortex.

Physostigmine but not neostigmine was shown by Mrsulja et al. (1968) to decrease the glycogen concentration in mesencephalon, cerebral cortex, cerebellar cortex and medulla.

Since atropine is used as the treatment for organophosphorus insecticide toxicosis, the literature was reviewed for the EEG effects of atropine. Funderburk and Case (1951) produced a sleep-like EEG pattern with 0.5-1.0 mg atropine/kg body weight in curarized cats. Wescoe et al. (1948) showed that atropine and scopolamine caused high voltage slow waves of 6-10 Hz. Higher doses of atropine reduced the frequency to 3-8 Hz.

Bradley and Elkes (1953) gave 2-3 mg atropine/kg body weight intraperitoneally to conscious cats and recorded high amplitude slow waves, interspersed by bursts of fast activity.

These animals did not show a cortical alerting response to sensory stimuli although the animal could be behaviorally aroused. The effects of hyoscyamine were similar to atropine.

Got and Polyva (1963) isolated cholinesterases with high specific activities from sheep brain. Eleven bands of hydrolytic enzymes with organophosphate degrading activity were found in the mouse brain by Sakai and Matsumura (1968).

#### Electrocorticogram of Ruminants

There are two specific aspects of the ruminant electrocorticogram (ECoG) which have been brought to light. The first is the possible influence of rumination and the second is whether or not ruminants sleep.

Concerning the influence of rumen constituents on the ECoG, White and Samson (1956) demonstrated in rabbits that the intravenous injection of 4 millimole/kg body weight of buffered fatty acid anions of propionate, butyrate, valerate and caproate evoked high amplitude slow wave ECoG activity. The longer chain fatty acids were more effective. Comparable solutions of glucose, NaCl, lactate, acetone, and betahydroxybutyrate had no detectable effect on the ECoG activity. Bell in 1958 reported that the ECoG pattern of goats during rumination showed high amplitude waves with spindles similar to those recorded during somnolence. The intravenous infusion of butyric or valeric acid caused the development of an ECoG similar to that of the

drowsy animal and produced signs of drowsiness. Bell (1960) later reported that when a goat ruminated during a somnolent state the ECoG settled down to a low frequency pattern and the animals were more difficult to arouse during these periods.

In a study on sleep in goats Balch (1955) reported that they never entered the state of deep sleep shown by other species but had periods of rest and somnolence scattered somewhat irregularly over the 24 hours. During somnolence the ECoG changed to a pattern normally associated with deep sleep or anesthesia. At this time goats show attitudinal changes but did not exhibit the normal signs of sleeping. Klemm (1966) disputed these findings by recording periods of apparent sleep and paradoxical sleep EEG activity from goats. He further reported that goats while either standing or lying had an arousal type EEG. He also reported that the EEG activity during rumination was one of alertness although his published EEG tracings, due to chewing artifacts, are not conclusive for this point of view.



## MATERIALS AND METHODS

## Animals

Adult female sheep 2 years of age were used in this study. The animals were of a Columbia-Rambouliet cross obtained from the Western sheep regions of the United States. The animals were maintained in unheated outside sheds and fed a diet of chopped hay, soybean oil meal, cracked corn, trace mineral salt and dicalcium phosphate.

## Fabrication of Electrodes

Nylon screws<sup>1</sup> (0.25" or 0.375" X 8-32) with binder heads served as the basis for the ECoG recording electrodes. A 0.0625" diameter hole was bored through the long axis of each screw. A 4.5 cm piece of 30 gauge stainless steel suture wire was placed through the hole in the shaft of the screw. The end of the wire on the head end of the screw was bent into a loop approximately 1.5 mm in diameter and filled with solder<sup>2</sup>. On the other end of the wire at a point 1.3 cm from the end the wire was bent 180° back onto itself. The new end which then consisted of a double strand was bent into an "L" shape so that

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<sup>1</sup>Small Parts, Inc. Box 792, Biscayne Annex, Miami, Fla. 33152.

<sup>2</sup>Ersin, L. M. P., 62% tin, 36% lead, 2% silver, melting point 354° F. Allied Electronics, 100 N. Western Avenue, Chicago, Illinois, 60680.

a hook 1.5 mm long or slightly less was formed. The doubled wire was then pulled back into the shaft of the screw until the hooked end rested tightly against the end of the screw, (Fig. 1). The electrodes were washed and steam autoclaved.

An electrode plug and wire connector was assembled prior to surgery with a miniature 7 pin socket connector<sup>1</sup> with a locking clip serving as the basic unit. A 9 cm length of 22 gauge wire with color coded vinyl insulation was soldered to each of the 7 pins. A different colored wire was used for each pin and the color-pin combination was standardized to facilitate the identification of leads during the implantation procedure. A small amount of acrylic plastic<sup>2</sup> was placed around the wire-connector junctions and the finished unit (Fig. 1) washed and steam autoclaved.

Stainless steel screws<sup>3</sup> (0.375" or 0.750" X 6-32) (Fig. 1) were washed and autoclaved prior to surgical implantation. These screws served as anchors in the cranium for the implant mass.

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<sup>1</sup>Amphenol 126-198, Allied Electronics, 100 N. Western Avenue, Chicago, Illinois, 60680.

<sup>2</sup>Cranioplastic, Plastic Products Co., P.O. Box 1204, Roanoke, Va.

<sup>3</sup>Small Parts, Inc., Box 792, Biscayne Annex, Miami, Florida, 33152.

Fig. 1. Nylon screw-stainless steel wire  
dural electrodes, stainless steel  
anchor screws and electrode plug  
connector assembly

Fig. 2. Exposed skull with electrodes and  
anchor screws in place. The bottom  
of the picture is the anterior part  
of the skull



## Surgical Procedure

The sheep were held off feed for at least 12 hours prior to surgery. One hour before induction of anesthesia each sheep was given 32 mg of atropine sulfate subcutaneously. The head and neck were clipped and a short acting anesthetic<sup>1</sup> was given via the jugular vein. The anesthetized sheep was carried from the preparation room to the surgery suite and strapped to the surgery table. The trachea was intubated and the endotracheal tube connected to a closed system anesthesia machine<sup>2</sup>. Methoxyflurane<sup>3</sup> was used as the surgical anesthetic agent. The head was positioned and fastened in a stereotaxic frame<sup>4</sup> to provide rigid support and fixation.

The dorsal portion of the head was clipped, scrubbed and 3 applications of 70% ethyl alcohol applied. The animal was draped and the surgical procedure started.

A 10 cm midline incision was made from the poll to the caudal edge of the cranium. A cold cautery probe<sup>5</sup> was used to control hemorrhage. The subcutaneous tissue was bluntly

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<sup>1</sup>Pento-short ®, Haver-Lockhart, Kansas City, Mo.

<sup>2</sup>Veterinary Anesthesia Machine, Heidbrink Model 960, Ohio Chemical and Surgical Equipment Co., Madison, Wisconsin.

<sup>3</sup>Metofane ®, Pitman-Moore, Indianapolis, Indiana.

<sup>4</sup>David Kopf Instruments, 7324 Elmo St., Tujunga, California, 91042.

<sup>5</sup>Wappler Cold Cautery Unit, American Cystoscope Makers Inc., Electro Medical Division, Pelham Manor, New York.

dissected to expose the skull. All soft tissue was removed from the skull and special care was taken to provide a clean, dry bone surface. Holes were drilled for the 6 active and 1 reference electrodes. Each hole for the 6 active electrodes penetrated the full thickness of the bone, but care was taken not to disturb the dura mater. The electrodes were placed as follows:

- 1) Frontal electrodes- 3 cm anterior and 1 cm lateral to bregma over the right and left frontal cortex.
- 2) Parietal electrodes- 1 cm anterior and 2 cm lateral to bregma over the right and left parietal cortex.
- 3) Occipital electrodes- 2 cm posterior and 1.5 cm lateral to bregma over the right and left occipital cortex.
- 4) Reference electrode- on the midline at bregma.

After the holes were bored, each was threaded with a machine tap and an electrode screwed into place. It was possible to determine when the electrode contacted the dura mater by sliding the wire up and down in the nylon screw as the screw was turned into place. The reference electrode penetrated approximately half the thickness of the skull.

Generally, the short electrodes were long enough for the frontal and parietal regions while the long electrodes were used for the occipital region.

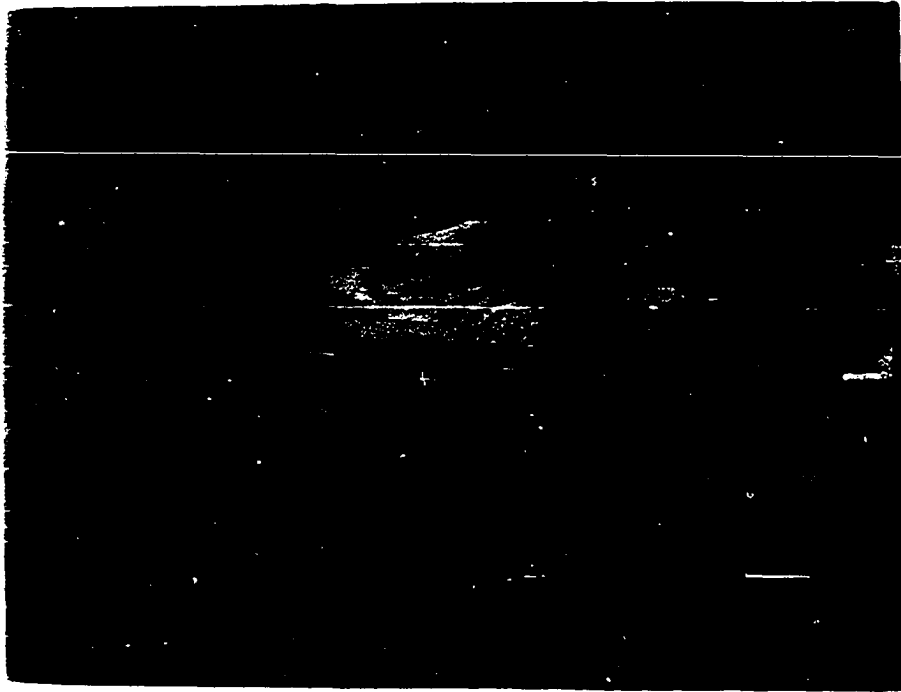
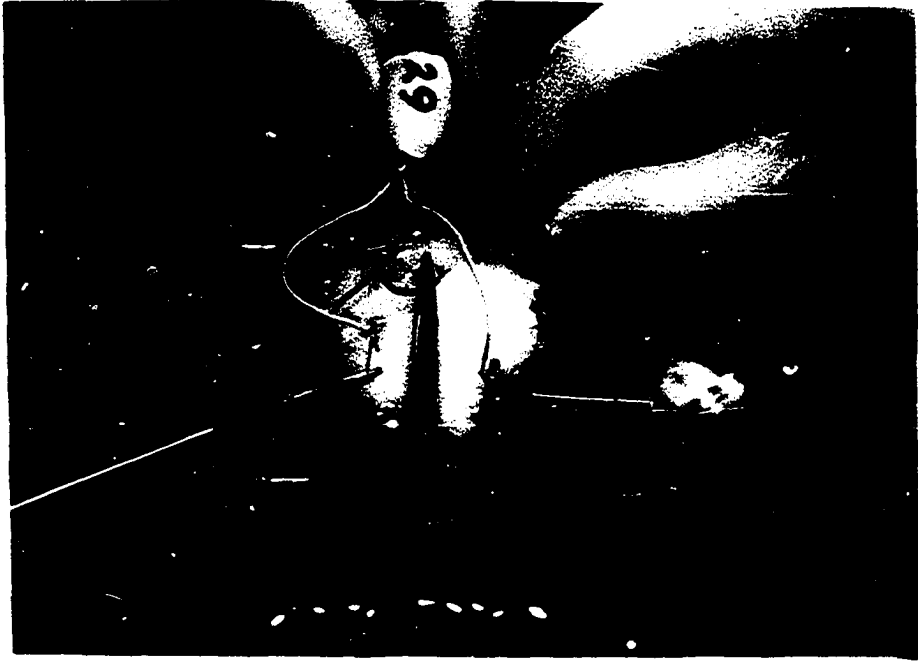
Holes for 10-15 anchor screws were bored into but not through the skull. These holes were located anterior, posterior, and lateral to the electrodes. Care was taken to insure that at least 4 anchor holes were placed sufficiently lateral so that the anchor screws projected horizontally to provide maximum anchorage. After tapping the holes, the stainless steel anchor screws were put in place (Fig. 2). The exposed skull was then dried, cleaned with gauze and covered with a 1 cm thick layer of acrylic plastic. The acrylic was pressed firmly into place around the anchor screws and electrodes and tightly against the skull. Care was taken to assure that the acrylic did not cover soft tissue around the margins and that each electrode wire protruded above the acrylic. The acrylic was allowed to harden for 10-15 minutes during which time the wires of the electrode pin connector assembly were trimmed to the proper length.

A hemostat, which served as a heat sink, was clamped to each electrode wire and a color coded wire from the connector pin assembly was soldered to each electrode (Fig. 3). The use of low melting point solder reduced the amount of heat required to form a good connection and the use of heat sinks prevented thermal energy from reaching the dura mater. After assuring good solder connections, the wires and electrodes were pressed down onto the first layer of acrylic making sure that no two wires touched. A second layer of acrylic was then placed over

Fig. 3. Soldering electrode plug connector assembly to electrodes

Fig. 4. Head positioned in stereotaxic frame with finished electrode implant





the first, incorporating the acrylic that was previously applied to the connector assembly. Thus, all the wires and solder joints were embedded in the second layer of acrylic. The acrylic mass was then shaped and smoothed to yield a finished implant and the flesh wound closed with a purse string suture, drawing the skin up around the base of the implant (Fig. 4).

The animal was removed to the recovery room and placed on rubber mats to prevent damage to the implant.

Each animal was allowed at least two weeks for post-surgical recovery before recordings were made.

#### Recording Procedure

All electrocorticographic (ECOG) recordings were taken via radiotelemetry<sup>1</sup> and animal activity was observed with closed circuit television<sup>2</sup> (CCTV). Each radiotelemetric transmitter had 2 channels and, typically, each animal was equipped to carry 2 transmitters so that 4 leads of ECOG could be recorded simultaneously (Fig. 5).

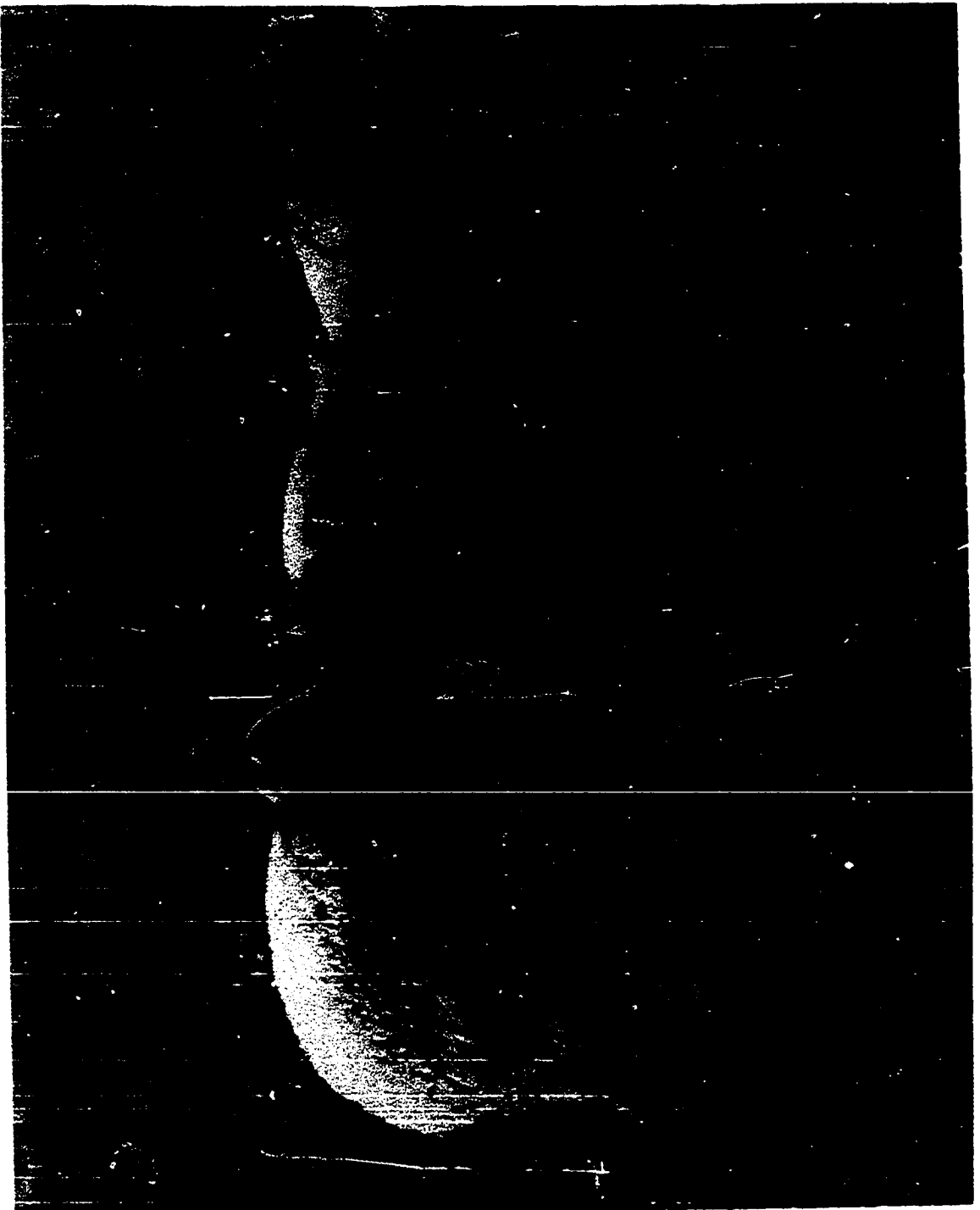
The transmitters were fastened in a harness which were strapped to the back. The harness was constructed from army surplus nylon webbing 3.9 cm wide and a piece of leather 27 by 34 cm. The leather was riveted to the nylon straps and

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<sup>1</sup>Bio Com, Inc., Culver City, California.

<sup>2</sup>General Electric Co., C. H. Schuller Co., Des Moines Iowa.

**Fig. 5. Sheep wearing telemetry harness**



the leather pouches for the transmitters and battery power supplies were riveted to the top of the leather. The piece of leather underneath the transmitters served to keep wool from touching the transmitters and interfering with cable connections.

All ECoGs were recorded from the right and left frontal and occipital electrodes utilizing a common indifferent reference electrode.

All ECoG recordings were taken from animals housed in pens inside the laboratory building. The CCTV camera was equipped with a wide angle lens and positioned so that the experimental animal was continually in view.

The telemetric receivers, CCTV monitor and polygraph recorder<sup>1</sup> were located in an instrumentation room remote from the animal observation area.

Recordings were obtained during the day and evening hours while the animals were eating, standing, alert and resting.

The polygraph was equipped with 2 event marker pens, each of which could be deflected either up or down or simultaneously up and down. By combining the displacement of the two pens, 16 unique codes could be generated, one of which was redundant, namely, when neither pen was activated. Eighteen lock down pushbuttons were installed on the polygraph so that depressing

---

<sup>1</sup>Model 7 Polygraph, Grass Instruments Co., Quincy, Mass.

one of 15 buttons resulted in a unique code being entered on the EEG record. The remaining 3 were used as stop buttons to terminate the code. The codes and their meaning are given in Fig. 6. Thus, it was possible to efficiently and rapidly enter on the ECoG record, while it was being taken, the behavioral or physiological state of the animal which was important for the later interpretation of the recordings.

The amplitude-frequency response curves for the recording system used in this work is given in Fig. 7.

The calibration of the recording system was checked daily by applying a 100  $\mu$ v peak to peak 7 Hz sine wave to the transmitter inputs and adjusting the gain of the polygraph amplifiers to a 1 cm pen deflection. All records were made at a chart speed of 25 mm per second.

Control ECoG records were taken from each animal for at least one week prior to exposure to the specific insecticide.

#### Analytical Procedure

The ECoG records were analyzed by visual observation and studied taking into account the physical or physiological state of the animal. Representative control records were selected for each animal and used as standards to compare with the post-exposure records.

In the Ruelene<sup>®</sup> study, whole blood cholinesterase activity was determined by the method of Radeleff and Woodard (1956).

**Fig. 6. Codes entered on electrocorticalgraphic records to characterize physical state of the sheep**

DATA CHANNEL 4



- ALERT
- STANDING
- LAYING DOWN
- GOT UP
- LAI D DOWN
- RESTING
- WALKING
- EATING
- CHEWING
- DRINKING
- SLEEPING
- ROOM LIGHT ON
- ROOM LIGHT OFF
- CLINICAL SIGN
- CONVULSION

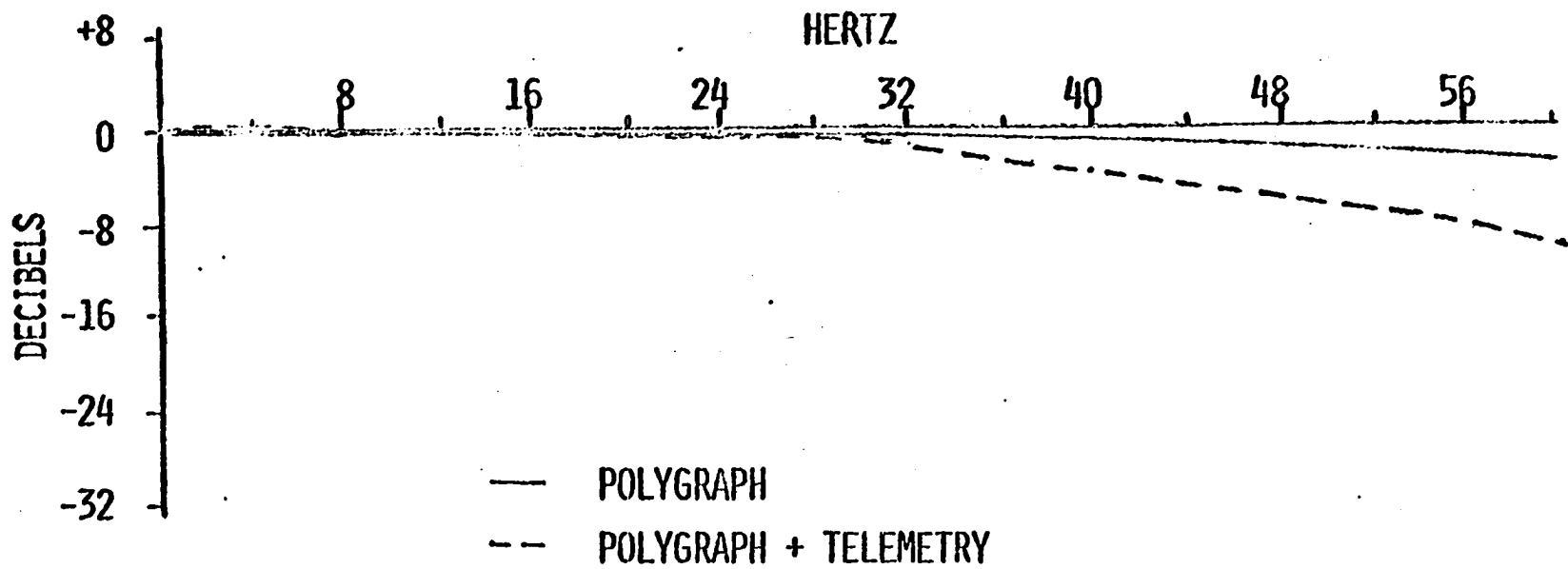
DATA CHANNEL 1



EVENT MARKER CODES



**Fig. 7. Amplitude-frequency response curve of  
the recording system**



## Exposure of Animals

The insecticides used in this study included the cyclodiene chlorinated hydrocarbon dieldrin<sup>1</sup> (hexachloroepoxyoctahydro-endo, exo, dimethanonaphthalene), DDT<sup>2</sup> (dichlorodiphenyl-trichloroethane) and the systemic organophosphate Ruelene<sup>®</sup><sup>3</sup> (4-tert-butyl-2-chlorophenyl methyl methylphosphoramidate).

All insecticides were used in a powder form given orally in a gelatin capsule. Each sheep was exposed daily until clinical toxicity developed or definitive ECoG changes occurred. The exposure record for each animal is included in the results section of this manuscript.

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<sup>1</sup>Technical Dieldrin (100%) supplied courtesy of Shell Chemical Co., New York.

<sup>2</sup>Technical DDT (100%) supplied courtesy of Giegy Chemical Corporation, Ardsley, New York.

<sup>3</sup>Technical Ruelene<sup>®</sup> (100%) supplied courtesy of Dow Chemical Co., Midland, Michigan.

## RESULTS AND DISCUSSION

## Control Electroencephalograms of Sheep

In all the ECoG records reported herein, the same 4 areas of the cortex were recorded, namely, the right and left frontal cortex and the right and left occipital cortex.

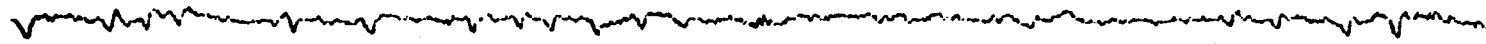
The pre-exposure ECoGs for each animal were analyzed and representative tracings selected for comparison with the post-exposure ECoG for each sheep. Representative tracings were selected for presentation herein.

The ECoGs of sheep which were standing and behaviorally alert are shown in Figs. 8 and 9. This same type of ECoG pattern was also recorded while sheep were lying down and behaviorally alert (Fig. 10). The term "arousal ECoG" will be used herein to mean this type of activity characterized by a low amplitude and fast frequency wave form.

When the sheep were resting or inattentive the ECoG was of higher amplitude and slower frequency (Fig. 11). This record was recorded while the sheep was lying down with its head extended and resting on the floor. Behaviorally this animal appeared to be resting. The term "resting ECoG" will be used to mean this type of activity. This type of activity was also recorded from an animal that appeared to be resting in a standing position (Fig. 12). Often the ears would be drooping and the head lowered when this type of ECoG pattern occurred.

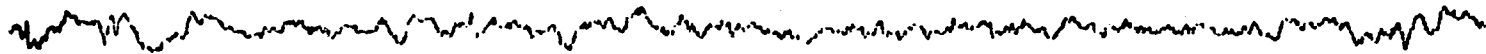
**Fig. 8. Control ECoG of a behaviorally alert  
standing sheep (Sheep 337)**

ECOG OF AN ALERT NORMAL SHEEP



LEFT FRONTAL

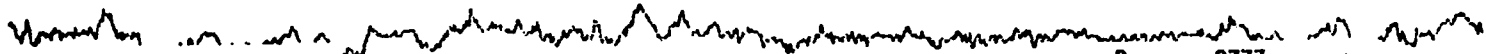
100  
1 SECOND



LEFT OCCIPITAL



RIGHT FRONTAL



RIGHT OCCIPITAL

RUELENE-S337-CONTROL

**Fig. 9. Control ECoG of a behaviorally alert  
standing sheep (Sheep 311)**

ECOG OF AN ALERT NORMAL SHEEP

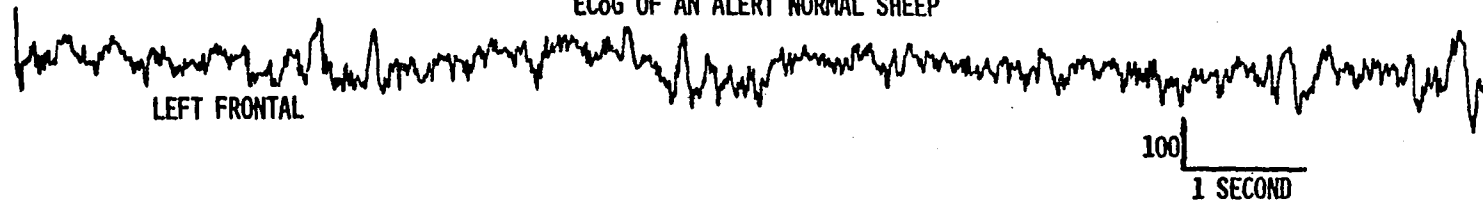
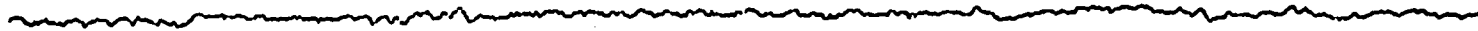




Fig. 10. Control ECoG of a behaviorally alert  
sheep in sternal recumbency (Sheep 337)

ALERT ECoG OF A RECUMBENT SHEEP



LEFT FRONTAL



LEFT OCCIPITAL



RIGHT FRONTAL



RIGHT OCCIPITAL

RUELENE-S337-CONTROL

Fig. 11. Control ECoG of a sheep in sternal  
recumbency while behaviorally resting  
(Sheep 311)

RESTING ECoG IN A RECUMBENT SHEEP

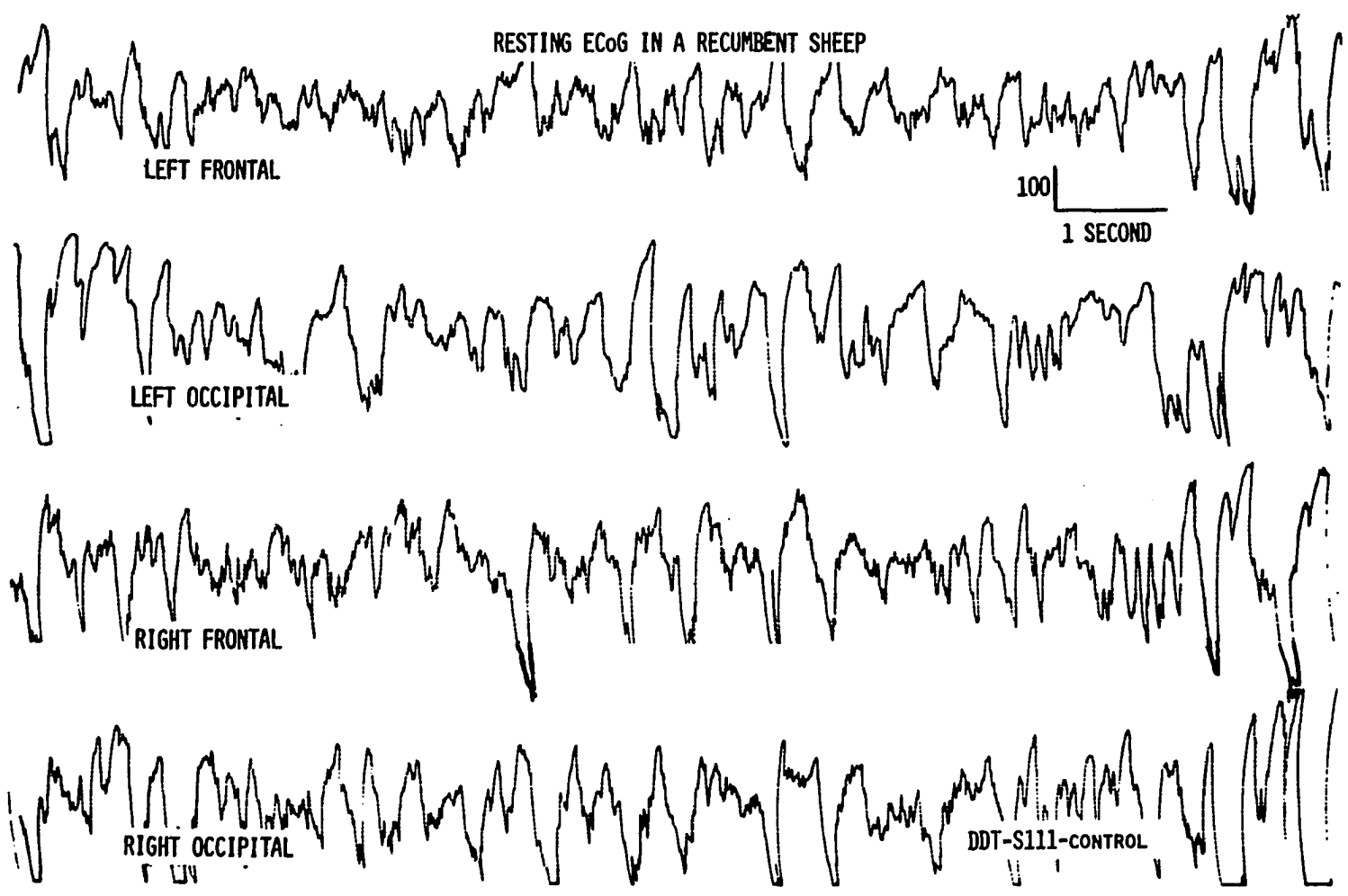
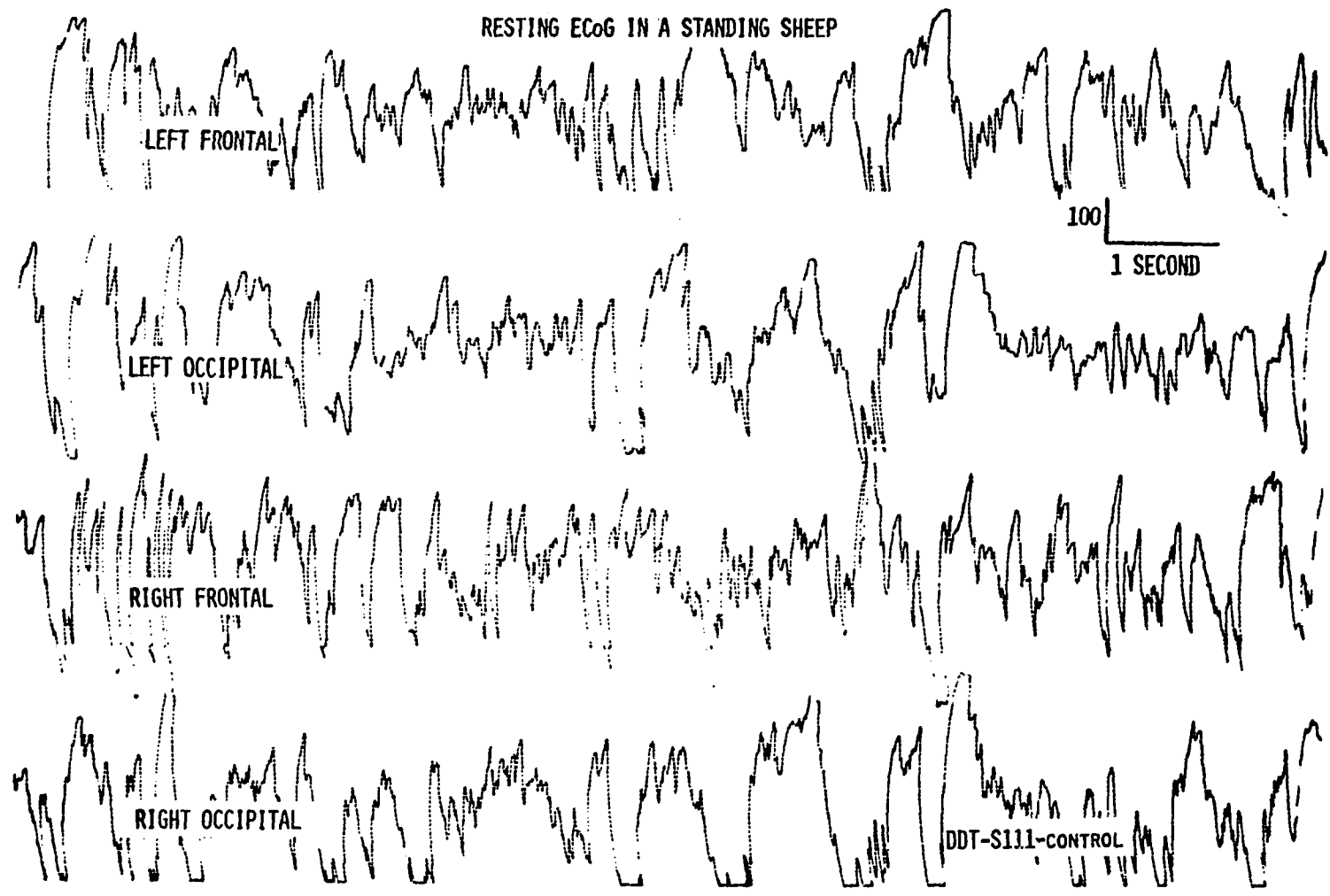


Fig. 12. Control ECoG of a standing sheep while behaviorally resting (Sheep 111)

RESTING ECoG IN A STANDING SHEEP



During arousal ECoG activity spindles of 6-8 Hz often occurred. This was more frequently seen in the occipital leads, but also occurred in the frontal leads (Fig. 13). The duration of each spindle was from less than a second to 10 or more seconds in duration (Fig. 14). These were easily recognized because of the well defined wave form.

#### Effect of Dieldrin on the Electroencephalogram

A total of 16 sheep were exposed to varying levels of dieldrin (Table 1) and ECoGs recorded. Generally ECoGs were recorded at 30 minute intervals with each record 2-3 minutes in length.

The highest exposure studies were conducted first to determine the type of changes in ECoG activity which could be expected with lower exposure levels. Generally, as soon as definitive ECoG changes occurred the experiment was terminated. Some of the first animals studied were exposed until convulsive seizures occurred.

In all animals exposed to dieldrin abnormal bilateral hypersynchronous slow waves occurred in the ECoG arousal pattern. These hypersynchronous slow waves, referred to as slow wave spindles because of their shape, were usually in the frequency range of 3-6 Hz and 150-400  $\mu$ v amplitude (Fig. 15). Initially each spindle was 1-2 seconds in duration and occurred

Fig. 13. Eight Hz spindle activity of short duration in frontal and occipital leads in a control ECoG (Sheep 111)



SPINDLE ACTIVITY IN CONTROL ECoG



**Fig. 14. Prolonged eight Hz spindle activity in frontal and occipital leads in a control ECoG (Sheep 334)**

PROLONGED SPINDLE ACTIVITY IN CONTROL ECoG

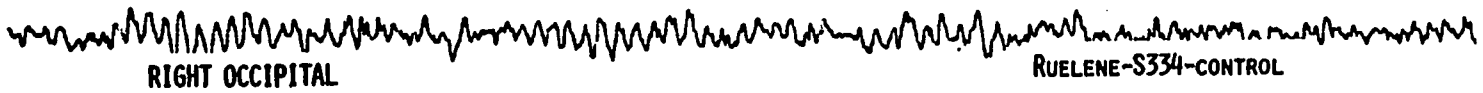
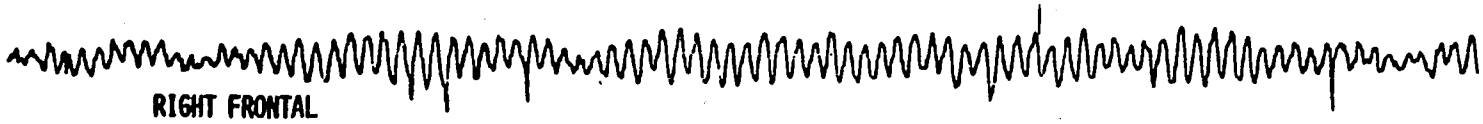
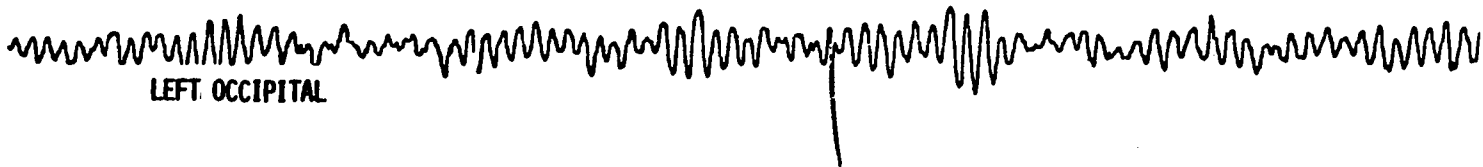
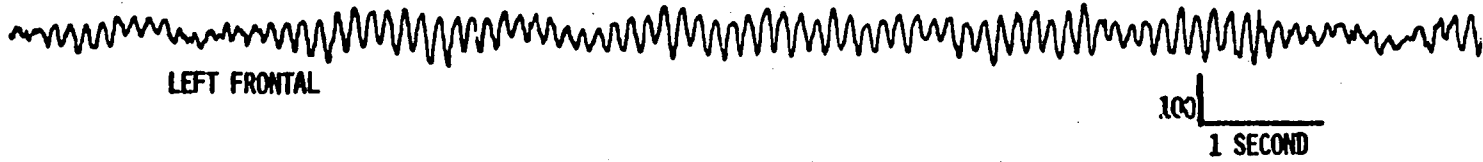
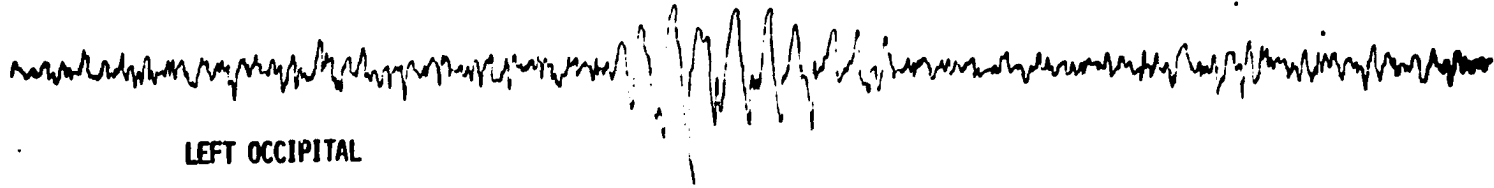
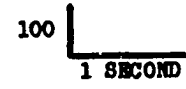


Fig. 15. Simultaneous slow wave spindles in  
ECoG following 1 exposure to dieldrin.  
Sheep 443, dosage: 25 mg/kg body  
weight

SLOW WAVE SPINDLE



LEFT FRONTAL



LEFT OCCIPITAL



RIGHT FRONTAL



RIGHT OCCIPITAL

DIELDRIN-S443-25-DAY 1

a periodically. A prominent feature was the usual simultaneous occurrence in all 4 leads. As exposure continued the slow wave spindles occurred more frequently (Fig. 16).

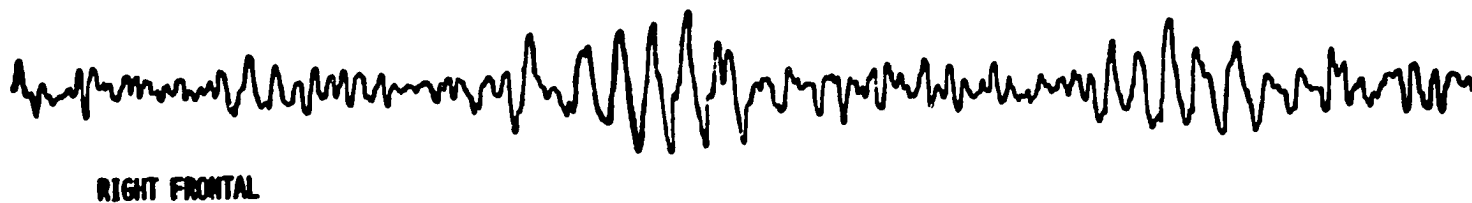
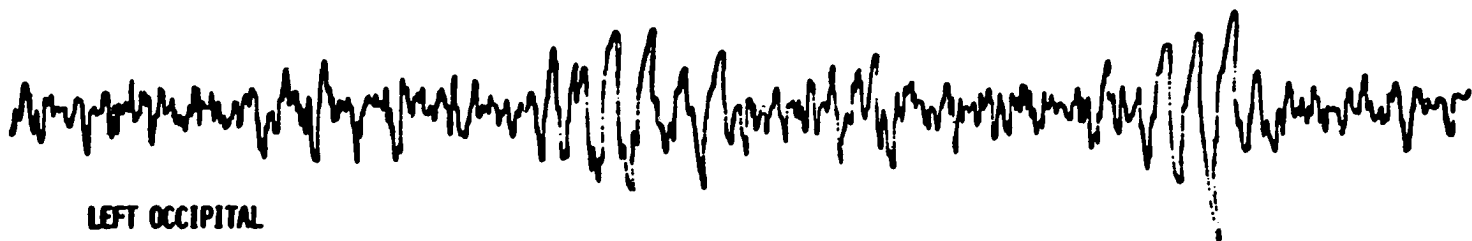
Table 1. Sheep exposed to dieldrin

Sheep	Dosage (mg/kg body weight)	Number of daily exposures	Comment
443	25	7	Convulsion day 4, 6
488	25	5	Convulsion day 4
339	25	4	Convulsion day 4
455	25	3	Convulsion day 3
498	25	5	
104	20	3	
122	20	3	
124	20	4	
130	20	4	
446	15	25	Convulsion day 26
474	10	45	Convulsion day 45
499	5	40	
494	5	43	
485	1	30	
463	1	20	
121	0.5	42	

Following exposure to 25 and 20 mg dieldrin/kg body weight the first slow wave spindles were seen 4-6 hours after exposure except in one instance where they occurred 2.5 hours after the first exposure. Since the animals were not monitored continuously and because the occurrence of slow wave spindles is

Fig. 16. Increased occurrence of slow wave  
spindles in ECoG following exposure  
to dieldrin on day 2. Sheep 443;  
dosage: 25 mg/kg body weight

INCREASED SLOW WAVE SPINDLES



DIELDRIN-S443-25-DAY 2



irregular, it is probable that slow wave spindles occurred between recordings and thus the very first occurrence may have been missed.

As exposure to dieldrin continued there was an increased amplitude of the ECoG and before a convulsion occurred the ECoG consisted of high amplitude fast activity. Fig. 17 shows the progressive ECoG changes prior to the onset of convulsive seizures. In Fig. 18 the types of ECoG patterns that were recorded during convulsive seizures can be seen. More than one form occurred during a single convulsion.

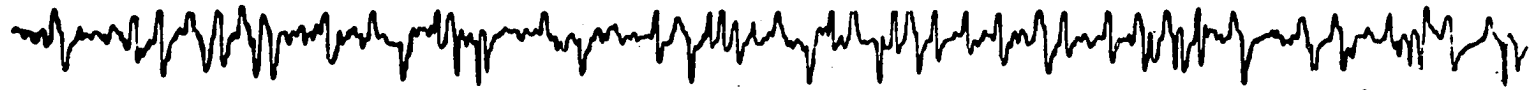
In Fig. 18 traces 2 and 4 are similar to the spike-dome wave form associated with petit mal epilepsy. Since petit mal epilepsy is associated with pathological "over-driving" from the thalamus (Ochs, 1965) it would appear that dieldrin may also be influencing thalamic function. Trace 3 is similar to the electroencephalographic activity associated with grand mal epilepsy.

Convulsive seizures terminated with the appearance of nearly isoelectric activity for 15-30 seconds which was followed by the appearance of an irregular slow wave pattern (Fig. 19).

The 4 sheep which received 20 mg dieldrin/kg body weight were also used on a behavioral task. Because of a limited amount of recording equipment these 4 sheep were not monitored as intensively. However, well defined slow wave spindles were observed on the first day of exposure in 2 of these and on the

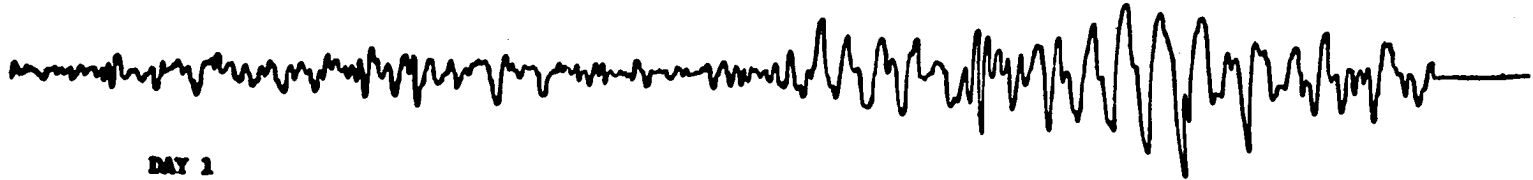
Fig. 17. Progressive changes in ECoG following exposure to dieldrin. All traces are from the left occipital electrode. Top trace: control, second trace: slow wave spindle on day 1, third trace: increased duration of slow wave spindle on day 3, bottom trace: prior to onset of convulsive seizure on day 3. Sheep 488; dosage: 25 mg/kg body weight

PROGRESSIVE CHANGES IN EC<sub>06</sub>

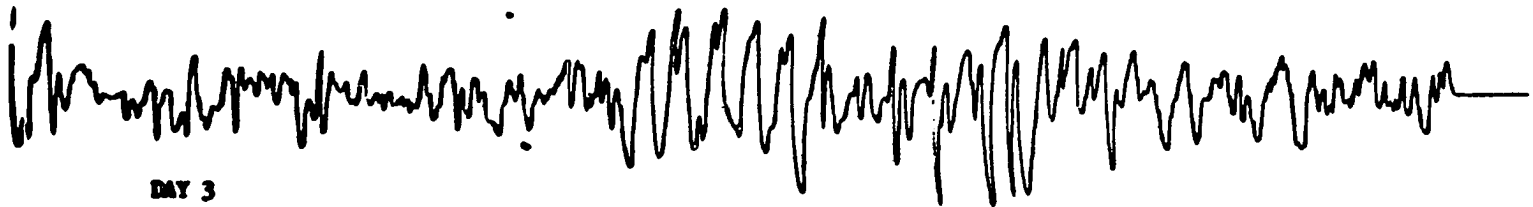


DAY 0

100  
1 SECOND



DAY 1



DAY 3

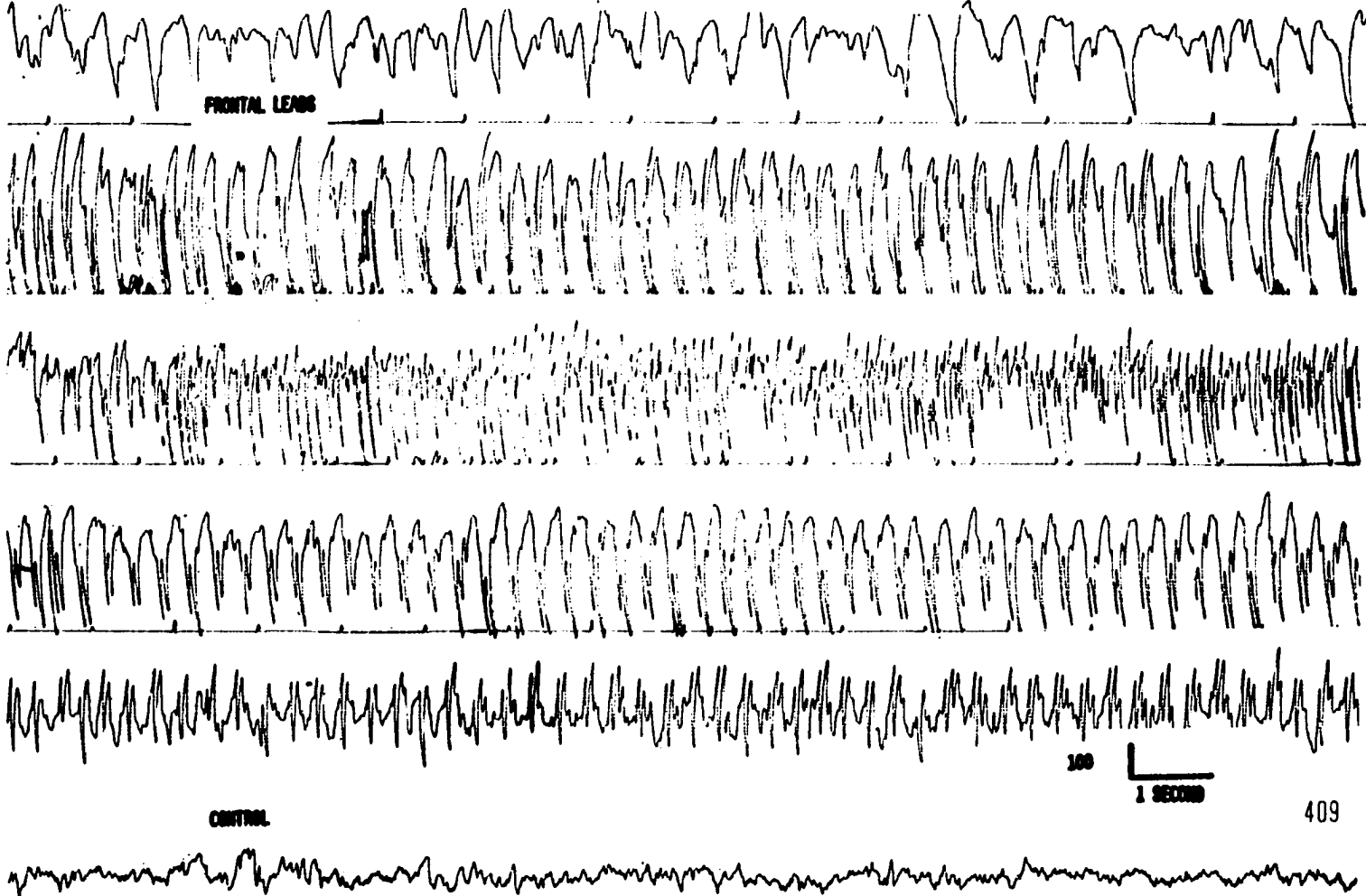


DAY 3

DIELDRIN-S488-25-DAY 0,1,3

Fig. 18. ECoG patterns recorded during diethyl ether induced convulsive seizures. Top trace: high amplitude slow wave, second and fourth traces: petit mal pattern, third trace: grand mal pattern, fifth trace: mixed ECoG with spikes superimposed on slow waves, bottom trace: control ECoG for comparison

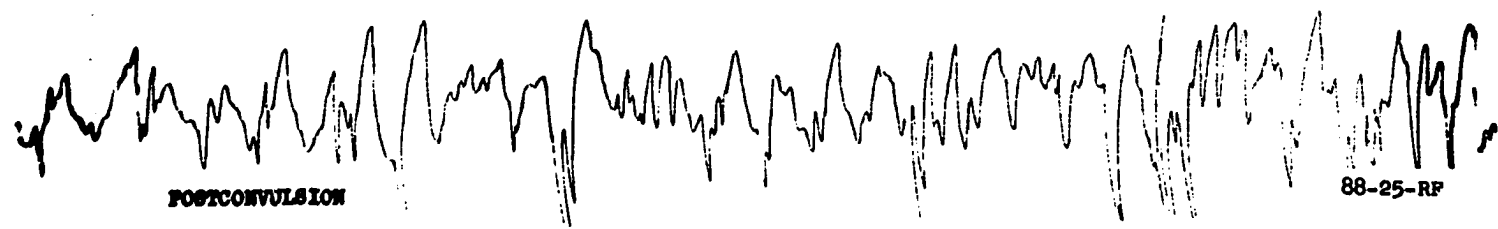
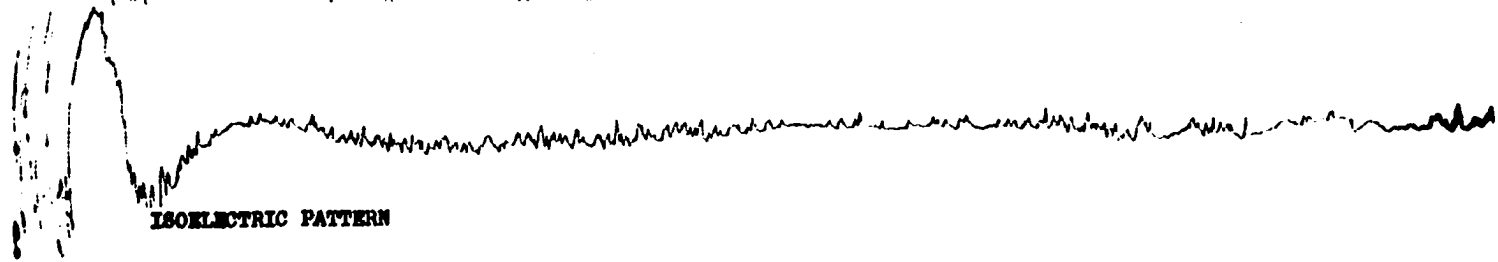
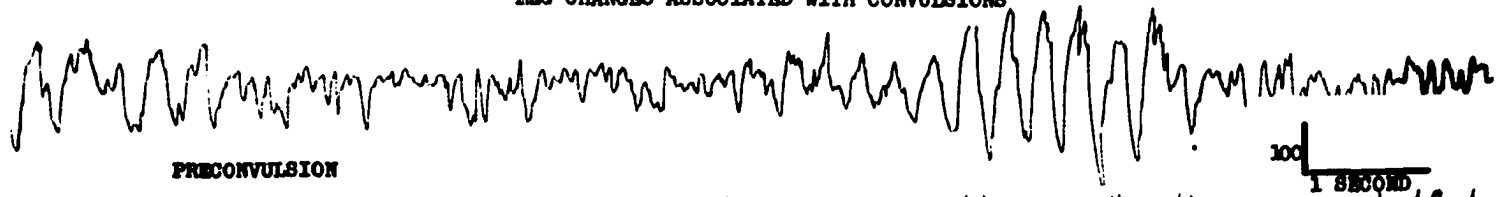
ELECTROENCEPHALOGRAMS ASSOCIATED WITH MELIBIEN TOXICITY



409

**Fig. 19.** ECoG activity before, during and after a dieldrin induced convulsive seizure. Trace 3 is the isoelectric pattern recorded at the end of the convulsive seizure. Sheep 488; dosage 25 mg/kg body weight

**EEG CHANGES ASSOCIATED WITH CONVULSIONS**



88-25-RF

second day in the other 2. All of these sheep became hypersensitive but none convulsed.

The sheep exposed to 10 mg dieldrin/kg body weight had slow wave spindle activity on the second day of exposure. By the tenth day of exposure a large amount of slow wave spindle activity occurred and spontaneous muscle spasms were observed coincident with the ECoG activity shown in Fig. 20. Since the sheep was clinically affected at this time ECoG recording was terminated, but exposure was continued until a convulsive seizure occurred on the forty-fifth day.

The two sheep receiving 5 mg dieldrin/kg body weight showed slow wave spindle activity on the second and third day of exposure. During this time the slow wave spindle activity occurred infrequently, but by the tenth day it was more prominent. On the twentieth day the sheep were visibly hypersensitive and nervous. One sheep experienced a transient period of nervousness and staggering on the twenty-eighth day. Exposure was stopped after 40 and 43 days without the occurrence of convulsive seizures.

In the two sheep exposed to 1 mg dieldrin/kg body weight a slow wave spindle was recorded from one sheep on the first day of exposure (Fig. 21) 11 hours after administration. The other sheep showed some low amplitude slow wave spindles on the fourth, fifth and sixth days of exposure. Fig. 22 was recorded on the fourteenth day of exposure and shows a slow wave spindle



**Fig. 20. ECoG activity during a dieldrin induced muscle spasm following 10 days of exposure. Sheep 474; dosage 10 mg/kg body weight**

ECOG ACTIVITY DURING MUSCLE TREMOR

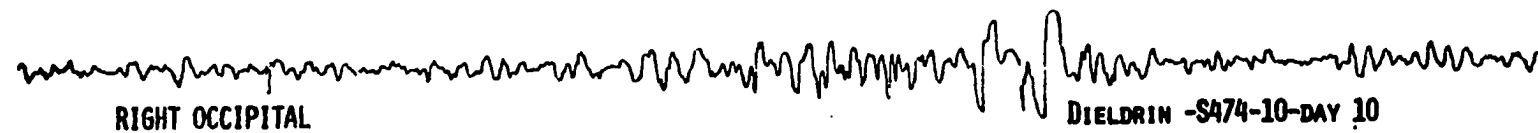
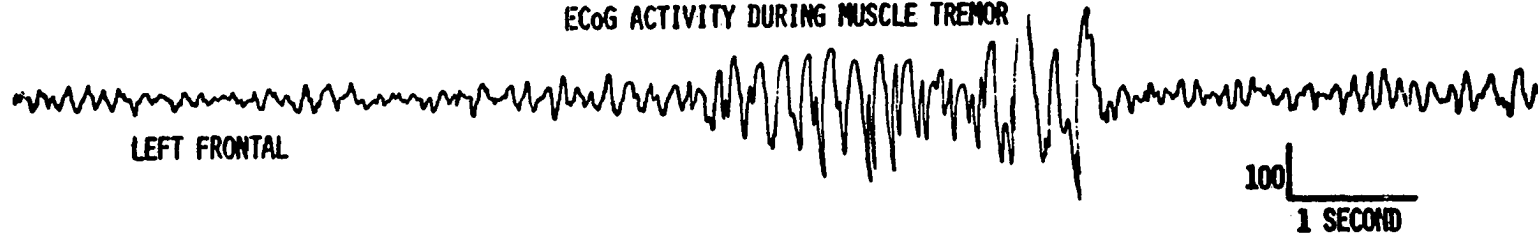


Fig. 21. Slow wave spindle in ECoG 11 hours  
after exposure to dieldrin. Sheep  
485; dosage 1 mg/kg body weight

SLOW WAVE SPINDLE - DAY 1



LEFT FRONTAL



LEFT OCCIPITAL



RIGHT FRONTAL



RIGHT OCCIPITAL

DIELDRIN-S485-1-11 HOURS

Fig. 22. Slow wave spindle in ECoG after 14 days  
of exposure to dieldrin. Sheep 485;  
dosage: 1 mg/kg body weight

SLOW WAVE SPINDLE - DAY 14



of 3.5 seconds duration. A slow wave spindle of 2.5 seconds duration recorded on the thirteenth day of exposure from the other sheep receiving the 1 mg/kg body weight exposure level is shown in Fig. 23. After 2 weeks of exposure slow wave spindles were recorded almost daily. No evidence of clinical toxicity was observed in these sheep during the exposure period.

Figs. 24 and 25 show slow wave spindles recorded on the first and eighth day of exposure from the sheep receiving 0.5 mg dieldrin/kg body weight. Other slow wave spindles were recorded on the seventh, eleventh and twenty-first days of exposure. Slow wave spindle activity in this animal did not occur as frequently as in the previous sheep. During the last 21 days of exposure ECoG records were made at staggered intervals on 7 days and no slow wave spindles were seen.

#### Effect of Ruelene<sup>®</sup> on the Electroencephalogram

Three sheep were exposed to Ruelene<sup>®</sup> (Table 2) and ECoG's recorded.

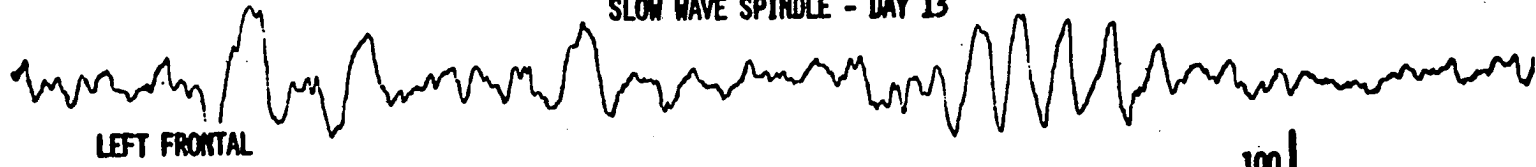
The results of the whole blood cholinesterase determinations are shown in Fig. 26.

For the pre-exposure period and first 15 days of exposure cholinesterase activity was determined once in the morning and again in the afternoon 2 hours after exposure to the insecticide. The two determinations were averaged. The remainder of the

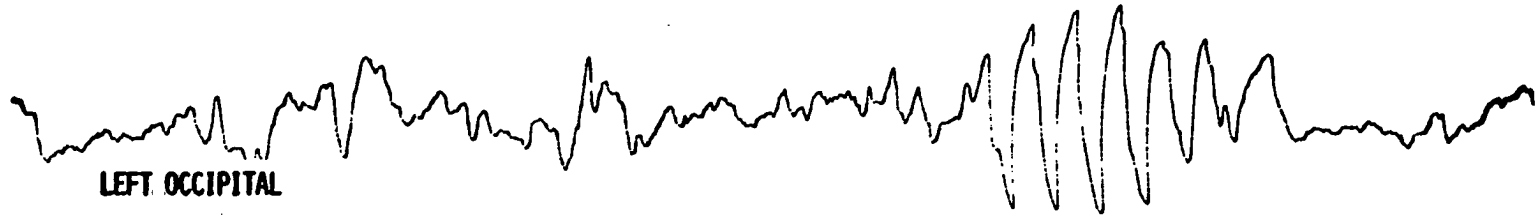
**Fig. 23. Slow wave spindle in ECoG after 13 days  
of exposure to dieldrin. Sheep 463;  
dosage: 1 mg/kg body weight**



SLOW WAVE SPINDLE - DAY 13



100  
1 SECOND



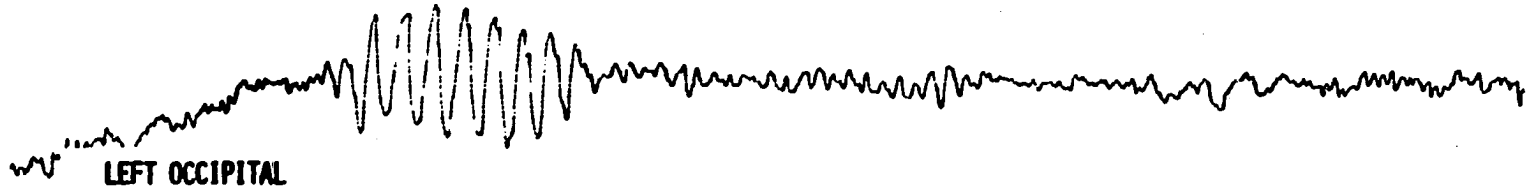
**Fig. 24. Slow wave spindle in ECoG 12 hours after exposure to dieldrin. Sheep 121; dosage: 0.5 mg/kg body weight**

SLOW WAVE SPINDLE - DAY 1

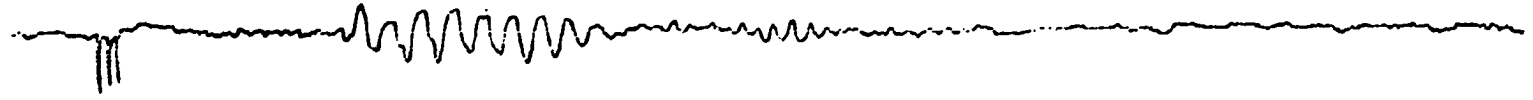


LEFT FRONTAL

100  
1 SECOND



LEFT OCCIPITAL



RIGHT FRONTAL



RIGHT OCCIPITAL

DIELDRIIN-S121-0,5-12 HOURS

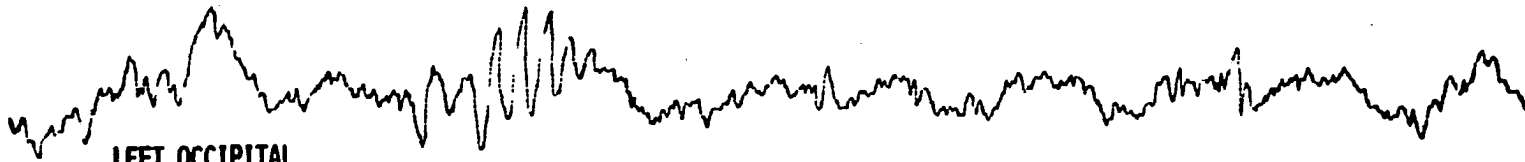
Fig. 25. Slow wave spindle in ECoG after 8 days  
of exposure to dieldrin. Sheep 121;  
dosage: 0.5 mg/kg body weight

SLOW WAVE SPINDLE - DAY 8



LEFT FRONTAL

100  
1 SECOND



LEFT OCCIPITAL



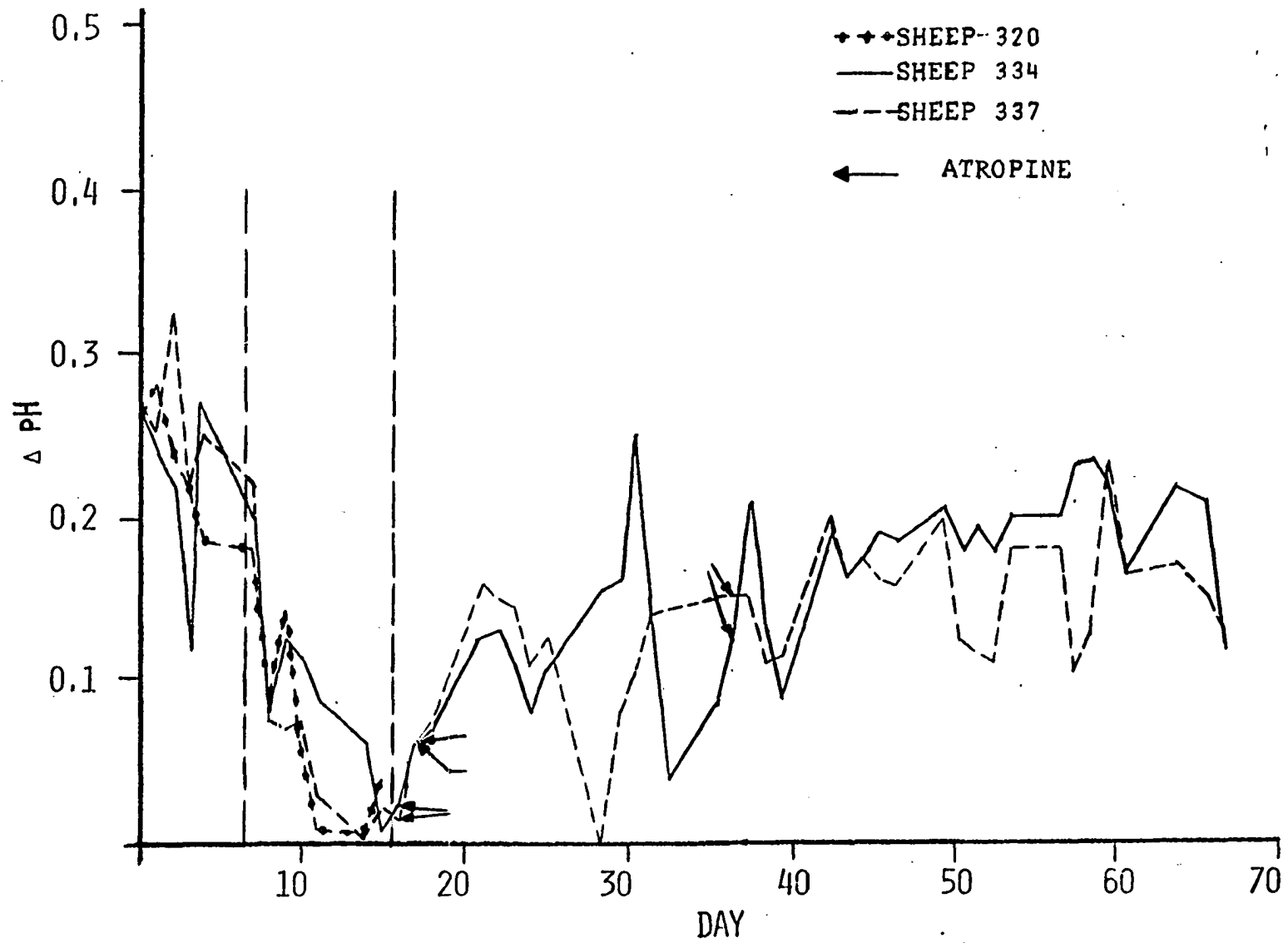
RIGHT FRONTAL



RIGHT OCCIPITAL

DIELDRIN-S121-0.5-DAY 8

Fig. 26. Whole blood cholinesterase activity before, during and after exposure to Ruelene®. The dashed vertical lines mark the beginning and end of the exposure period. The arrows indicate treatment with atropine



cholinesterase determinations were made in the morning only. The average whole blood cholinesterase activity for the 3 sheep during the 5 day pre-exposure period was 0.242  $\Delta$ pH. On the first day of exposure the whole blood cholinesterase activity decreased 17%, on the second day 68% and on the ninth day 90%. Twenty hours after the ninth and last exposure and before treatment was started the whole blood cholinesterase activity was decreased 92%.

Table 2. Exposure to Ruelene <sup>®</sup>

Sheep	Dosage	Days of exposure
334	150 mg/kg	9
337	150	9
320	150	8

Cessation of exposure to Ruelene <sup>®</sup> resulted in a partial recovery of whole blood cholinesterase activity, and during the first 15 days of the post exposure period there was considerable day to day fluctuation. On the fifteenth day of the post-exposure period sheep 334 had a 0.000  $\Delta$ pH cholinesterase activity. To verify that this was not a technical error a duplicate sample was analyzed with the same result. Blood samples from other non-exposed sheep were determined simultaneously and had normal activities (0.250  $\Delta$ pH). A second



sample was obtained from sheep 334 in the afternoon and duplicate samples yielded an activity of 0.005  $\Delta$ pH. Fig. 26 reveals that sheep 337 had a fall in cholinesterase activity a few days later. This can be interpreted as evidence for a delayed effect of Ruelene<sup>®</sup>. The average cholinesterase value for the last 30 days of the post-exposure period was 0.170  $\Delta$ pH.

The first clinical sign noticed was a transient muscle stiffness on the second and third day in two animals (334, 320). The third sheep did not show this phase of the toxicity syndrome. Salivation was noticed in all three sheep on the fifth or sixth day. Salivation was not as excessive as is sometimes seen in organophosphorus toxicity. During the third to fifth day of exposure the sheep were restless and appeared to have difficulty resting. During this time the sheep laid down for a few seconds and then got up and walked around the pen before lying down again. This cycle was repeated many times during the day and evening hours. After the fifth day the animals were somewhat depressed although again not as markedly depressed as is seen in some organophosphorus toxicities. Sheep 320 died on the morning of the eighth day of the experiment. This sheep's condition deteriorated rapidly between midnight of the seventh day and the morning of the eighth day. This animal was not treated with atropine.

On the tenth day both remaining sheep were weak and stumbled while walking. Sheep 334 had a watery diarrhea at this time which stopped on the eleventh day after 3 treatments with atropine. The remaining two sheep were given atropine (1.0 mg/kg body weight) twice on the tenth day at an 8 hour interval. The first two doses were administered 1/3 intravenously and 2/3 subcutaneously. A third dose of atropine (0.5 mg/kg body weight) was given subcutaneously on the eleventh day. A fourth dose was given on the thirtieth day.

By the fifth or sixth day the animals were partially anorectic. However, 30 minutes after the first administration of atropine the sheep began drinking large amounts of water and eating hay again.

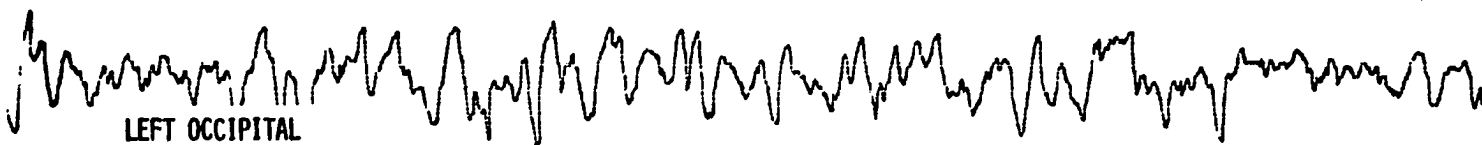
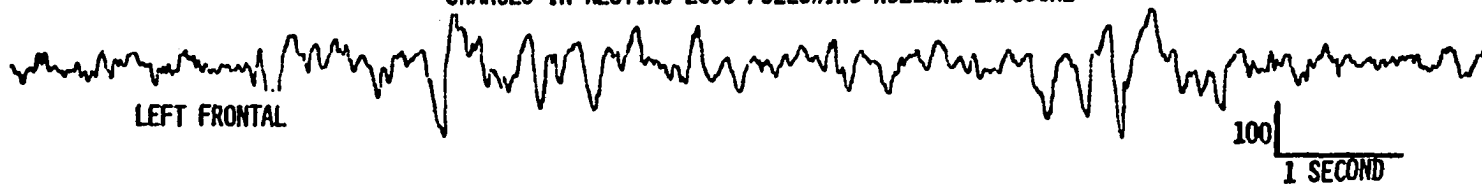
The post mortem examination of the sheep that died revealed no gross lesions in the brain, but considerable fluid was found in the lungs.

The ECoG findings were primarily a lack of slow wave activity. The ECoG tended to have a continuous arousal pattern and episodes of slow wave activity were infrequently seen even though the animals appeared to be resting or inattentive.

Sheep 334 and 337 had episodes of resting ECoG during the first 3 days of exposure. Sheep 320 showed arousal type ECoG during resting on day 2 (Fig. 27).

**Fig. 27.** Changes in ECoG in a behaviorally resting animal following exposure to Ruelene®. Top 2 traces: control resting ECoG, bottom 2 traces: second day of exposure. Sheep 320; dosage: 150 mg/kg body weight

CHANGES IN RESTING ECoG FOLLOWING RUELENE EXPOSURE



RUELENE-S320-150-CONTROL, DAY 2

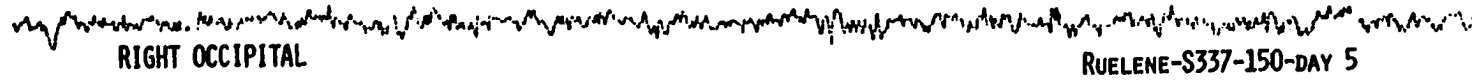
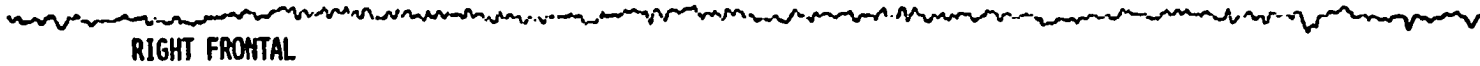
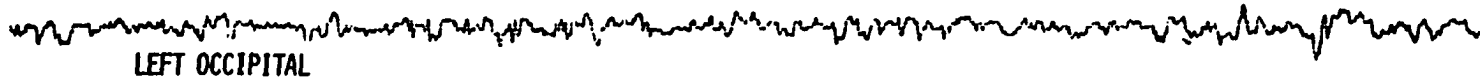
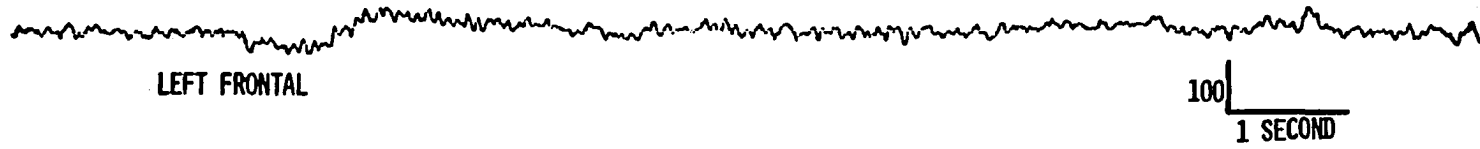
By the fourth and fifth days the slow wave resting ECoG was only occasionally seen. Fig. 28 shows the arousal type ECoG that was recorded nearly continuously during the day and evening on the fifth day of exposure. The only ECoG record during day 6 that differed from continuous arousal is shown in Fig. 29. The record in Fig. 30 is typical of the ECoG on the sixth day of exposure. This recording is from a continuous 6 minute epoch of ECoG which did not alter from what is shown here.

Fifteen days after the first exposure (6 days after the last exposure) low amplitude arousal patterns still dominated the ECoG. Fig. 31 is from a resting sheep on the 15th day. A small amount of slow wave activity was present but not as pronounced as in pre-exposure ECoG records. Twenty days after the first exposure normal periods of resting ECoG were recorded.

The administration of atropine caused the return of slow wave activity (Fig. 32). The top trace shows the ECoG at the time of injection. Trace 2 was taken 8 minutes later and shows the return of slow wave activity. Two hours post-injection (trace 3) shows the atropine starting to lose its effect and by 9 hours (trace 4) the ECoG is tending toward continuous arousal again. At this time a second dose of atropine was given (Fig. 33). The top 2 traces were taken at the time of injection and the bottom 2 traces show the slow wave activity recorded 1 hour later. At this time the sheep was eating and drinking.

Fig. 28. Continuous arousal ECoG activity after 5 days of Ruelene® exposure. Sheep 337; dosage: 150 mg/kg body weight

CONTINUOUS AROUSAL PATTERN - DAY 5



**Fig. 29. The only slow frequency ECoG recorded on the sixth day of exposure. The bottom 2 traces are the continuation of the top 2 traces. Sheep 334; dosage: 150 mg/kg body weight**



ECOG DAY 6 OF RUELENE EXPOSURE

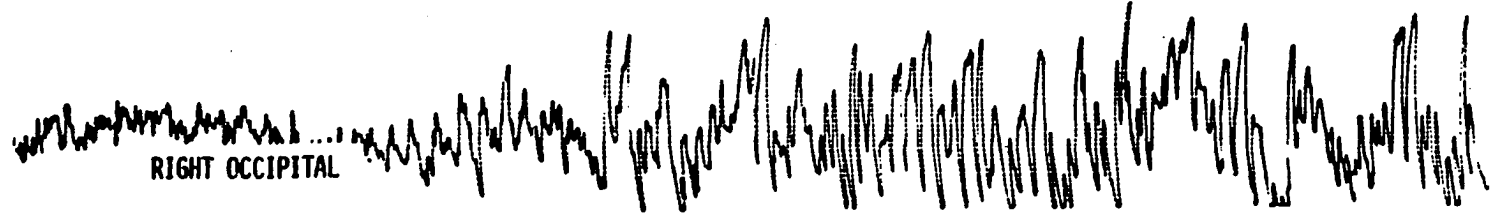


Fig. 30. Continuous arousal ECoG recorded after  
6 days of exposure to Ruelene®. Sheep  
334; dosage: 150 mg/kg body weight

AROUSAL ECoG - DAY 6

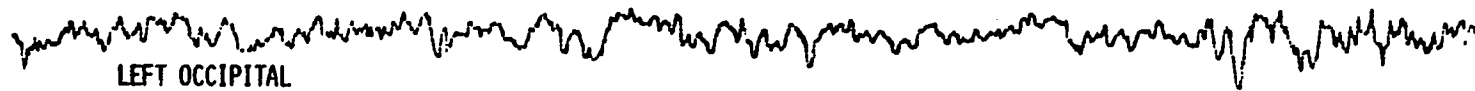


Fig. 31. Persistence of arousal ECoG during behavioral resting 6 days after termination of a 9 day exposure to Ruelene®. The bottom 2 traces were recorded 20 seconds after the top 2 traces. Sheep 334; dosage: 150 mg/kg body weight

PERSISTENCE OF AROUSAL ECoG

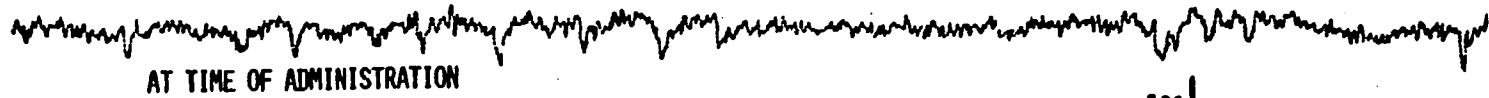


PERSISTENCE OF AROUSAL ECoG



Fig. 32. Effect of atropine on the ECoG of a sheep exposed to Ruelene® for 9 days. Top trace: at time of administration, second trace: 8 minutes after administration, third trace: 2 hours after administration, fourth trace: 9 hours after administration

EFFECT OF ATROPINE



100 |  
1 SECOND

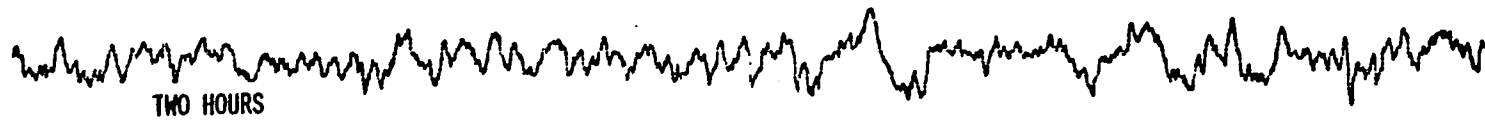
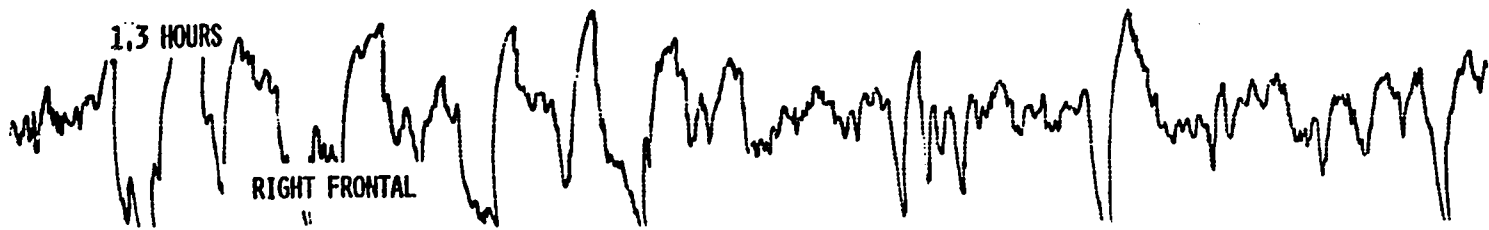




Fig. 33. Effect of second administration of atropine on ECoG of a sheep exposed to Ruelene<sup>®</sup> for 9 days. Top 2 traces: at time of injection, bottom 2 traces: 1.3 hours after injection. Sheep 334; dosage: 150 mg/kg body weight

EFFECT OF ATROPINE



### Effect of DDT on the Electrocardiogram

Two sheep were exposed to DDT (500 mg/kg body weight/day) and ECoGs recorded. One sheep (322) was exposed for 2 days and the other (111) for 3 days.

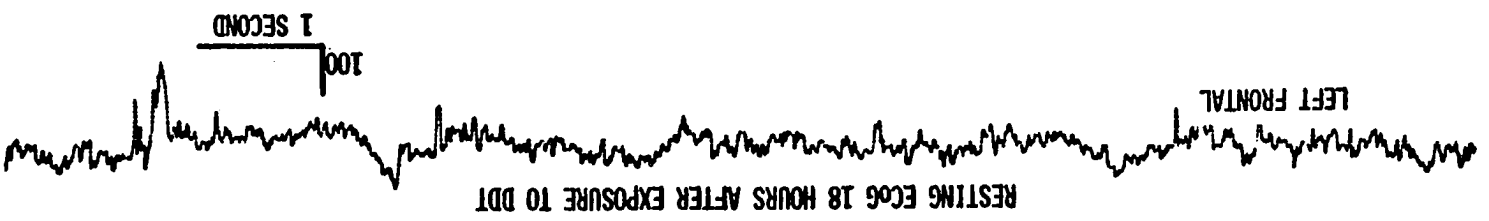
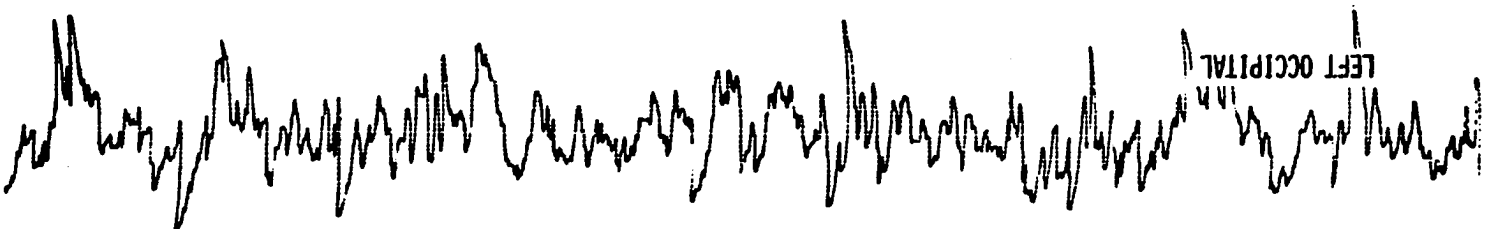
Both sheep showed muscle tremors and nervousness within the first 24 hours after exposure started. One animal was severely affected after 2 days and exposure was terminated.

Both sheep showed a continuous muscle tremor on the third day of the experiment. The sheep would stand and continually shift their weight from side to side giving an appearance of extreme nervousness. When the animals were handled they felt as though they were vibrating. Convulsive seizures were not observed at any time during the experiment.

The primary effect of DDT on the ECoG was an apparent increase in fast frequency components and an increase in amplitude. As with Ruelene<sup>®</sup> exposure, there was a noticeable lack of normal slow wave activity in the resting ECoG.

Fig. 34 is an ECoG record from a sheep during apparent rest 18 hours after the first DDT exposure. Although, there was some slow wave activity there was also a large amount of fast frequency components present. The sheep appeared to have difficulty in resting as they tended to get up shortly after lying down.

Fig. 34. Resting ECoG 18 hours after first exposure to DDT. Sheep 111, dosage: 500 mg/kg body weight



An arousal ECoG taken 6 hours after the first exposure showed an increase in amplitude (Fig. 35).

By the third day the ECoG tended to be composed mainly of arousal type activity (Fig. 36). This ECoG was taken during the occurrence of muscle tremors. Fig. 37 is from the sheep given 3 daily doses and represents ECoG activity 4 days after the last DDT administration. The fast frequency components show an increase in amplitude.

The progressive changes in the arousal ECoG of the sheep given two daily doses are shown in Fig. 38. The top trace is control arousal ECoG, the second trace represents activity on the second day, the third trace on the third day and the fourth trace represents activity six days after the start of exposure. ECoG's recorded 17 days after the first exposure still showed evidence of increased fast frequency components.

**Fig. 35. Increased amplitude in ECoG 6 hours after exposure to DDT. Top 2 traces: control, bottom 2 traces: 6 hours after administration. Sheep 11; dosage: 500 mg/kg body weight**

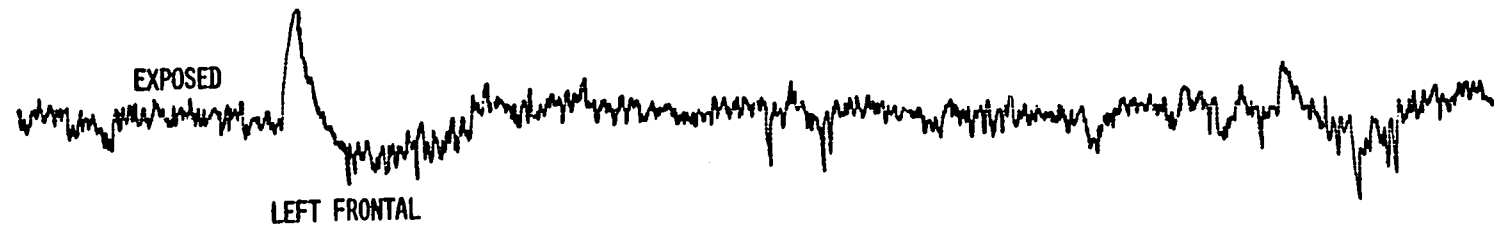
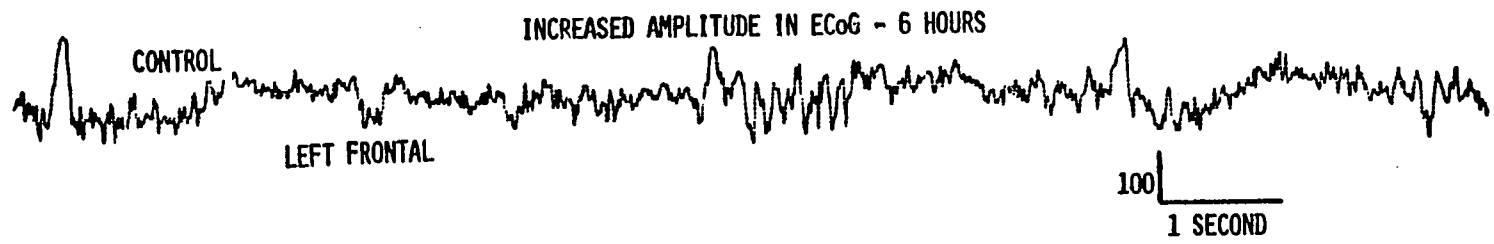




Fig. 36. Low amplitude-fast frequency ECoG recorded during DDT induced muscle tremors on day 3. Sheep 311; dosage: 500 mg/kg body weight

ECOG DURING MUSCLE TREMORS - DAY 3



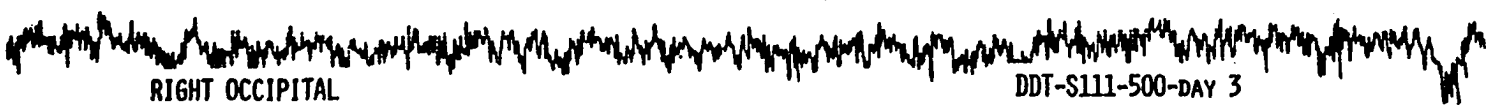
LEFT FRONTAL



LEFT OCCIPITAL



RIGHT FRONTAL



RIGHT OCCIPITAL

DDT-S111-500-DAY 3

Fig. 37. Changes in arousal ECoG 4 days after last exposure to DDT. Top 2 traces: control, bottom 2 traces: 4 days after last exposure. Sheep 111; dosage: 500 mg/kg body weight

CHANGES IN AROUSAL ECoG

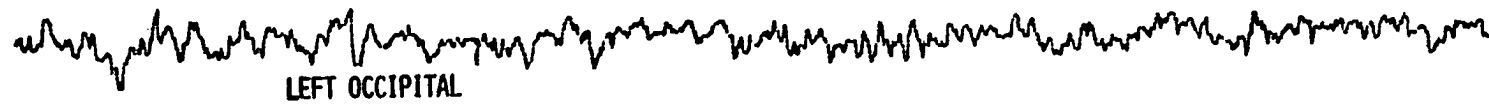
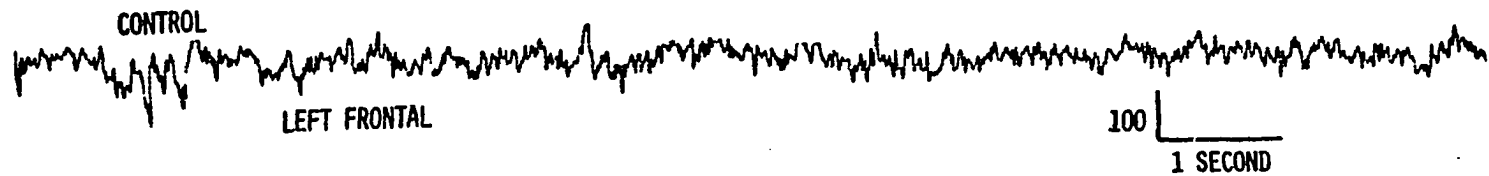
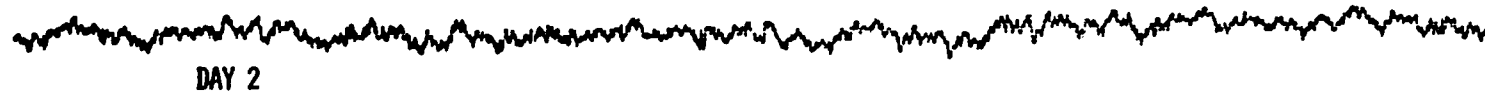
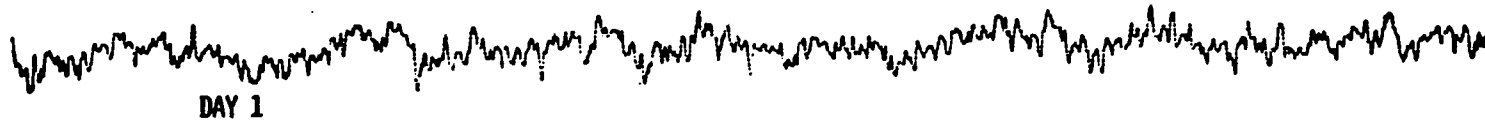


Fig. 38. Progressive changes in ECoG after exposure to DDT.  
Top trace: control, second trace: day 2, third  
trace: day 3, fourth trace: 4 days after last  
exposure. Sheep 322; dosage: 500 mg/kg body  
weight

PROGRESSIVE CHANGES IN AROUSAL ECoG



## CONCLUSIONS

The simultaneous use of radiotelemetry for the recording of electrophysiological data and closed circuit television for observation of the physical state of the experimental animal proved to be a reliable and useful technique for studying sheep exposed to insecticides. At all times it was possible to obtain records without physically disturbing the animals and it was also possible to obtain good records during physically violent convulsive activity.

The use of electroencephalography or in this case more specifically electrocorticalography is at best a qualitative method. As such it is a descriptive process which may best be called an art rather than a science. However, it does have a usefulness in helping to determine effective dosage levels and providing information on the effect of drugs or in this case selected insecticides.

In sheep receiving 25 or 20 mg dieldrin/kg body weight the slow wave spindles were usually observed 4-6 hours after the first exposure and always within the first 2 days of exposure. Continued exposure resulted in the appearance of more slow wave components in the ECoG. Convulsive ECoG patterns recorded during dieldrin induced convulsive seizures appeared similar to grand and petit mal epilepsy which indicates thalamic involvement in dieldrin toxicosis. This is an area for future study with specific placement electrodes.

Ten daily exposures of 5 mg dieldrin/kg body weight resulted in the occurrence of numerous slow wave spindles. Numerous slow wave spindles occurred after 2 weeks of exposure to 1 mg dieldrin/kg body weight, although, occasional slow wave spindles occurred prior to this. Slow wave spindles were observed less frequently in the one sheep receiving 0.5 mg dieldrin/kg body weight.

The sheep receiving the 1 and 0.5 mg dosages did not show the hypersensitivity exhibited by the other sheep.

Exposure to the organophosphorus insecticide Ruelene<sup>®</sup> for 8 days resulted in the death of 1 of 3 sheep. Whole blood cholinesterase activity was reduced by 92% after 9 days of exposure. Two sheep were treated with atropine and subsequently recovered over a period of several weeks. The ECoG findings were a nearly continuous arousal pattern after 5 or 6 days of exposure. Six days after the last exposure the arousal ECoG still predominated. Eleven days after the last exposure normal ECoG records were recorded. The arousal type ECoG agrees with results obtained with eserine and physostigmine. However, convulsive patterns were not seen as has been reported with DFP. Atropine was shown to reverse the ECoG effects of Ruelene<sup>®</sup>.

The increased frequency and amplitude following administration of DDT to sheep agree with those reported in the white rat. These effects persisted for at least 2 weeks after exposure



to 3 daily doses of 500 mg/kg body weight. These two sheep were clinically affected by the DDT exposure, although, convulsive seizures did not occur.

## SUMMARY

A study was conducted to investigate the effect of dieldrin, DDT and Ruelene<sup>®</sup> on the electrocorticogram of sheep.

Electrocorticograms were recorded using radio telemetry from sheep with surgically implanted dural electrodes. The animals were observed via closed circuit television.

Sixteen sheep were given technical dieldrin, a cyclodiene chlorinated hydrocarbon insecticide, at dosages ranging from 25 to 0.5 mg/kg body weight/day. In all animals bilateral hypersynchronous slow waves (slow wave spindles) were observed. The slow wave spindles were observed more frequently in the sheep receiving the higher exposures. Convulsive seizures occurred at the higher exposure levels. The ECoG activity during the convulsive seizures resembled electroencephalographic patterns of grand and petit mal epilepsy. This similarity in electrical activity may mean that dieldrin affects thalamic functioning, since epilepsy is thought to involve the thalamus.

Three sheep were given Ruelene<sup>®</sup>, (150 mg/kg body weight/day), an organophosphorus insecticide, for 8 or 9 days. The sheep were clinically affected and whole blood cholinesterase activity was reduced 92% when treatment with atropine was initiated. One sheep died before treatment was started. The electrocorticographic pattern was a continuous low voltage pattern similar to the arousal electrocorticogram. Slow wave

activity associated with resting was not observed during the latter part of the exposure period. Electrocardiograms recorded 11 days after the termination of exposure were normal.

Two sheep were given DDT (500 mg/kg body weight/day), a chlorinated hydrocarbon insecticide, for 2 or 3 days. The sheep were clinically affected after the first day of exposure. An increase in voltage and frequency was observed in the electrocardiogram which persisted for at least 2 weeks.

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