An electron microscopic study of peripheral nerve damage in mice induced by repeated subacute exposure to endrin

by James John Walker

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences
Montana State University

Abstract:
Endrin is a chlorinated hydrocarbon insecticide that has been used worldwide. Although acute endrin exposure produces neurological symptoms, there is generally a lack of morphological data documenting its cellular effects along the neuromuscular axis. This study was designed to characterize the morphological effects of endrin on peripheral nerve, optic nerve and muscle. Mice were given 20 daily intraperitoneal injections of endrin in sesame oil at subacute doses that increased from 1.5 mg/kg to 4.0 mg/kg. Controls were given the same intraperitoneal volume without endrin. Animals were sacrificed after 4, 7, 14, and 20 days of exposure and 14 and 92 days after the last injection. Sciatic nerve, optic nerve, skeletal muscle and cardiac muscle tissue were examined using both light and electron microscopy. Daily behavioral/neurological tests were used to assess general effects on the nervous system. Animals exposed to endrin were hyperactive, hypersensitive to stimuli, had difficulty maintaining their position on a rod and displayed signs of piloerection. Optic nerve, skeletal muscle and cardiac muscle appeared unaffected as were myelinated nerve fibers from sciatic nerve also appeared unaffected. However, unmyelinated axons in the sciatic nerve of exposed animals showed various changes which included axonal swelling, dissolution of microtubules and neurofilaments, axonal and Schwann cell vesiculation, and axonal vacuolation. Some vesicles were present in scattered rows along the axon or within the axon while others were present in pockets which often appeared continuous with the periaxonal space and often invaginated the axolemma. Some such pockets contained an amorphous material as well as vesicles and some regions within the Schwann cell that had contained axons appeared completely filled by vesicles or flocculent background debris. The results of this study indicate that repeated subacute doses of endrin produce morphological alterations in unmyelinated peripheral nerve fibers and their associated Schwann cells and thus could probably cause changes in neurological functions such as pain perception and autonomic activities related to temperature and blood pressure regulation.

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by

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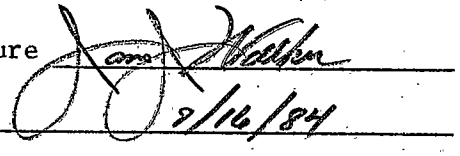
Signature 

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ABSTRACT

Endrin is a chlorinated hydrocarbon insecticide that has been used worldwide. Although acute endrin exposure produces neurological symptoms, there is generally a lack of morphological data documenting its cellular effects along the neuromuscular axis. This study was designed to characterize the morphological effects of endrin on peripheral nerve, optic nerve and muscle. Mice were given 20 daily intraperitoneal injections of endrin in sesame oil at subacute doses that increased from 1.5 mg/kg to 4.0 mg/kg. Controls were given the same intraperitoneal volume without endrin. Animals were sacrificed after 4, 7, 14, and 20 days of exposure and 14 and 92 days after the last injection. Sciatic nerve, optic nerve, skeletal muscle and cardiac muscle tissue were examined using both light and electron microscopy. Daily behavioral/neurological tests were used to assess general effects on the nervous system. Animals exposed to endrin were hyperactive, hypersensitive to stimuli, had difficulty maintaining their position on a rod and displayed signs of piloerection. Optic nerve, skeletal muscle and cardiac muscle appeared unaffected as were myelinated nerve fibers from sciatic nerve also appeared unaffected. However, unmyelinated axons in the sciatic nerve of exposed animals showed various changes which included axonal swelling, dissolution of microtubules and neurofilaments, axonal and Schwann cell vesiculation, and axonal vacuolation. Some vesicles were present in scattered rows along the axon or within the axon while others were present in pockets which often appeared continuous with the periaxonal space and often invaginated the axolemma. Some such pockets contained an amorphous material as well as vesicles and some regions within the Schwann cell that had contained axons appeared completely filled by vesicles or flocculent background debris. The results of this study indicate that repeated subacute doses of endrin produce morphological alterations in unmyelinated peripheral nerve fibers and their associated Schwann cells and thus could probably cause changes in neurological functions such as pain perception and autonomic activities related to temperature and blood pressure regulation.

INTRODUCTION

Endrin is a chlorinated hydrocarbon pesticide that has been used in the U.S. for agricultural and public health purposes since its introduction by the Velsicol Company in 1951. It is the most acutely toxic hydrocarbon insecticide known (Gaines,1969) and belongs to a group of structurally related cyclodiene compounds which includes aldrin, chlordane, chlordecone (kepone), dieldrin, heptachlor, isodrin, and telodrin. Although animals and humans acutely exposed to endrin display neurological symptoms which include hyperexcitability, hypersensitivity to stimuli, bradycardia, hypertension, increased rectal temperature, increased vascular resistance, tremor, and convulsions (Treon,1955; Emerson et al.,1964; Emerson and Hinshaw,1965; Reins et al.,1964; Coble et al.,1967; Weeks,1967), morphological documentation of such neurological symptomology is generally lacking. Since some of these symptoms are indicative of changes within the peripheral nervous system and since exposure to chlordecone has been shown to damage unmyelinated peripheral nerve fibers (Phillips and Eroschenko,1982), the present study was undertaken to examine peripheral nerve from animals exposed to endrin.

While endrin is toxic to all animals, the lethal toxicity to one half of a group of animals (LD50) varies depending on sex, method of

administration, and species variation. The acute oral toxicity of endrin and a number of related compounds is summarized in Table 1.

Table 1. Acute oral toxicity of selected chlorinated hydrocarbon pesticides in rats. (Reference: Gaines,1969)

Compound	LD50(mg/kg)		Lowest dose to kill a rat (mg/kg)	
	Males	Females	Males	Females
Aldrin	39	60	25	40
Chlordane	335	430	250	350
DDT(technical)	217	-	150	-
Dieldrin	46	46	30	30
Endrin	18	7.5	10	6.0
Heptachlor	100	162	50	100
Kepone	125	125	100	125

The excretion of endrin and its metabolites takes place primarily via the liver, bile and feces (Cole et al.,1968). Animals exposed to carbon fourteen labeled endrin excrete approximately 50-60% of the dose in feces within 24 hours (Cole et al.,1968; Ludwig,1966, as cited in Soto,1967; Hunter,1960, as cited in Soto,1967; Bedford et al.,1975a), while very little endrin or its metabolites are excreted in the urine (Hutson et al.,1975)

Endrin does not appear to accumulate in the tissues of exposed animals, rather, a plateau level of storage is reached after 6-10 days

(Brooks,1969; Korte,1967 and 1970, as cited in Donoso, 1979). Detectable levels of endrin are generally found only in animals receiving doses of 0.25 ppm or more, indicating that a threshold level of intake is necessary before the compound can be detected (Terriere al.,1958; Kiigemagi et al.,1958; Ely and Moore,1957; Moubry et al.,1968). In dogs acutely exposed to endrin there appears to be no relationship between the blood concentrations of the compound and those in the non-fat tissues (Richardson et al.,1967).

Humans do not tend to accumulate significant quantities of endrin. Endrin could not be detected in the blood of occupationally exposed workers except in those who had been over exposed (Jager,1970). The biological half life of endrin in human blood is about 24 hours (Jager,1970). Endrin was detected in the urine and blood of patients acutely poisoned by ingesting contaminated bread although blood and urine samples were normal within one week after poisoning (Curley et al.,1970; Coble et al.,1967).

Endrin is an environmentally persistent compound. Forty one percent an original application of technical endrin persisted in the soil for up to 14 years (Nash and Woolen,1967). Endrin is one of the least water soluble insecticides and is extremely persistent in an aqueous environment with as much as 80% of the initial endrin concentration (2 ppm) remaining in water samples after 16 weeks (Sharom et al.,1980).

Its acute toxicity and environmental persistence have led to limitation of endrin's use in the past several years. Massive fish kills in the Mississippi river system in the early 1960's led to the initial restricted use of endrin which culminated in the Environmental Protection Agency's ban of its use east of the Mississippi in 1979. It is still used in the Plains States for the control of cutworm in grain and in the Pacific Northwest for the control of voles in apple orchards. Heavy use of endrin in Montana in 1981 led to public and governmental concern over detected residue accumulation in wildlife, resulting in hunting season restrictions and further restrictions on its agricultural use.

Histopathological data from animals exposed to endrin is scarce. Diffuse degenerative changes have been reported in liver and kidneys (Treon,1955; Reins et al.,1964; Boyd and Stefec,1969; Reuber,1979), adrenal glands (Treon,1955; Reuber,1979), heart (Treon,1955; Reuber,1979), spleen (Reins et al.,1964; Boyd and Stefec,1969; Reuber,1979), brain (Treon,1955; Boyd and Stefec,1969; Reuber,1979), thymus (Boyd and Stefec,1969) and lungs (Treon,1955; Reins et al.,1964; Boyd and Stefec,1969) of laboratory animals exposed to endrin. Other histopathological changes included local irritation of the gastrointestinal tract, capillary venous congestion in the brain, heart and lungs, and depletion of secretions in the salivary glands (Boyd and Stefec,1969),

Neurological symptomology in endrin exposed animals, as

manifested by convulsions, tremor, increased salivation, and hypersensitivity, has led to the belief that endrin acts chiefly on the nervous system (Emerson et al.,1964; Hinshaw et al.,1966). Activation of the sympathetic and parasympathetic nervous systems by endrin has been proposed after studies of acutely exposed dogs (Emerson et al.,1964 and 1966; Reins et al.,1964 and 1966; Hinshaw et al.,1966). Bradycardia, copious mucoid salivation, hypertension, and convulsions following lethal exposure to endrin (10 mg/kg) suggest hyperactivity of both the sympathetic and parasympathetic nervous systems (Emerson et al.,1964). Endrin exposed animals develop increased renal resistance due to possible sympatho-adrenal stimulation and subsequent increase in circulating catecholamines (Reins et al.,1964) and increased venous return due to a massive sympathetic discharge leading to the release of blood stores from the liver and spleen (Hinshaw et al.,1966).

Other physiological changes induced by endrin exposure in dogs include, left heart failure, acidosis, hypoxia, increased rectal temperature, increased hemoconcentration, increased leukocyte concentration, increased peripheral resistance, decreased glomerular filtration, increased cerebral spinal fluid pressure and increased cerebral venous pressure (Emerson et al.,1964; Emerson,1965; Emerson and Hinshaw,1965; Reins et al.,1966; Hinshaw et al.,1966). The suggested physiological action of endrin in the dog is summarized in Figure 1. Most past studies dealing with endrin have examined

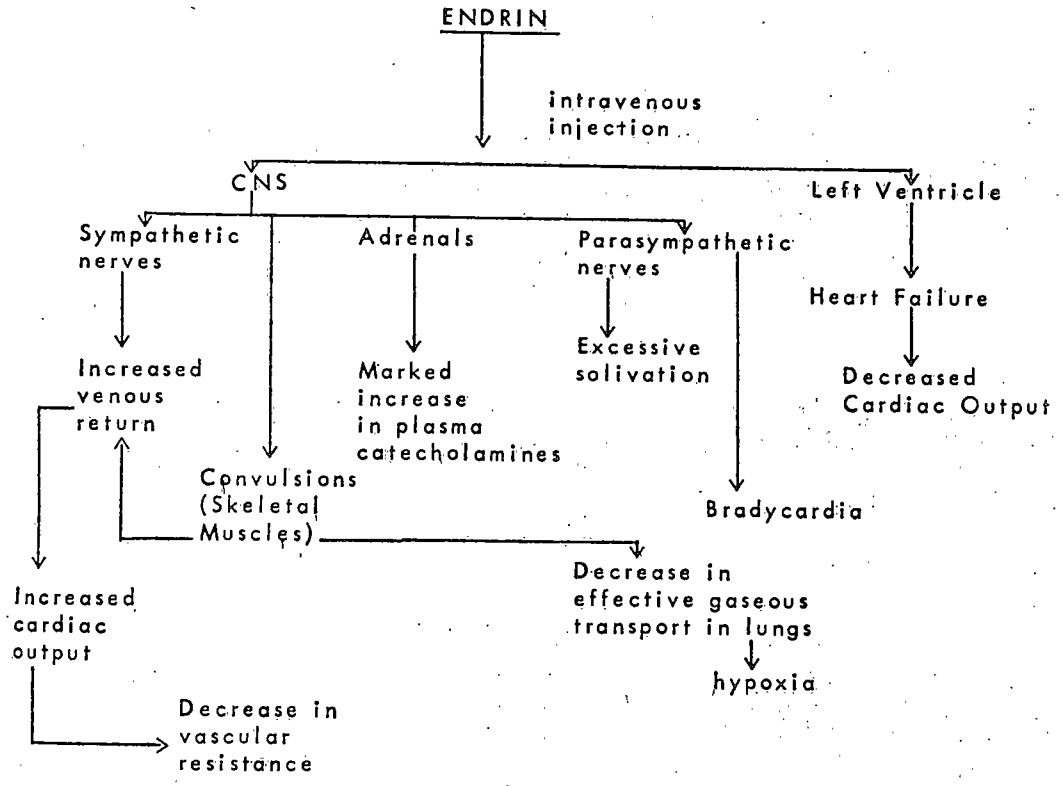


FIGURE 1. Summary of the effects of endrin in the dog.

metabolism, hepatic and renal damage, changes in the cardiovascular system, reproductive abnormalities and its acute and chronic toxicity. While much speculation exists in the literature concerning its effects on the mammalian nervous system, little morphological data exists to support such speculation even though the most profound symptoms are neurological. Most research up to this point involving the effects of endrin on the nervous system has been related to studying the physiological aspects of convulsions, hypertension, and bradycardia while the morphological basis for the development of such symptoms has been ignored. Thus, there is a need for morphological studies to characterize the effects of endrin along the neuromuscular axis. Because previous studies in this laboratory (Phillips and Eroschenko,1982) have shown that subacute exposure to the related compound chlordecone damages unmyelinated peripheral nerve fibers, and because endrin exposure has been speculated to cause autonomic dysfunction, it was of interest to determine the effects of endrin on peripheral nerve in animals exposed to repeated subacute doses of the compound. In addition, studies of some behavioral/neurological parameters were initiated to determine if changes similar to those observed in chlordecone exposed animals (Phillips and Eroschenko,1982; Jordan et al.,1981) and in animals acutely exposed to endrin (Treon,1955; Emerson et al.,1964; Reins et al.,1964) occurred in animals receiving subacute doses of endrin.

LITERATURE REVIEW

CHEMICAL AND PHYSICAL PROPERTIES OF ENDRIN

Endrin is the common name for 1,2,3,4,10,10,-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-endo-5,8-dimethano-naphthalene. It is a compound containing at least 92% of the endo-endo stereoisomer of dieldrin and is an epoxide of isodrin. Pure endrin is a white crystalline solid that is stable in the presence of wetting agents, emulsifiers, alkaline oxidizing agents and basic reagents but decomposes when treated with acids or heated above 200 C. Endrin, like other cyclodiene compounds is soluble in most organic solvents but is insoluble in water. It is unlike these compounds in that it has a much smaller partition coefficient (the tendency to distribute between polar and non-polar substrates), which limits its rate of decomposition and retention in exposed organisms (Montana Dept. of Agriculture,1983).

Pure endrin is stable in air and light but undergoes isomerization, rearrangement, or decomposition upon exposure to heat, acids or ultraviolet light (Soloway, et al.,1960; Burton and Pollard,1974; Rosen et al.,1966). These processes, plus microbial metabolism (Matsumura et al.,1971) are the primary means of degradation of the parent compound and play an important role in its environmental persistence. The principle products of such breakdown

are shown in Figure 2. Soto and Diechmann (1967) studied the acute toxicity of these metabolic products in rats and found the endrin aldehyde to be essentially non-toxic while the endrin ketone was approximately 1/4 as toxic as endrin itself. Susceptability of the endrin aldehyde appeared greater in females than males.

Photochemical degradation is an important factor in the persistence of endrin on the surface of plants. Harrison et al., (1967) showed that apple foliage sprayed with endrin retained only 13% of the initial treatment after one week and only 2% after 7 weeks. A monitoring program initiated by the Montana Department of Agriculture revealed that endrin residues on wheat foliage measured 2 days after application, had degraded by 99.9% after 10 weeks and that soil residues had degraded 67% during the same time. Detectable levels of the insecticide were still present 55 weeks after application (Montana Dept. of Agriculture,1983).

USES OF ENDRIN

Endrin has been used throughout the United States as an avicide, and rodenticide, but its major use has been as an insecticide. It was used throughout the 1950's on crops such as included alfalfa, eggplant, lettuce, potatoes, peppers, tomatoes, strawberries, corn, sorghum, sugar beets, cotton and small grains. It was used primarily in southeastern U.S. during the 1960's and 70's to control

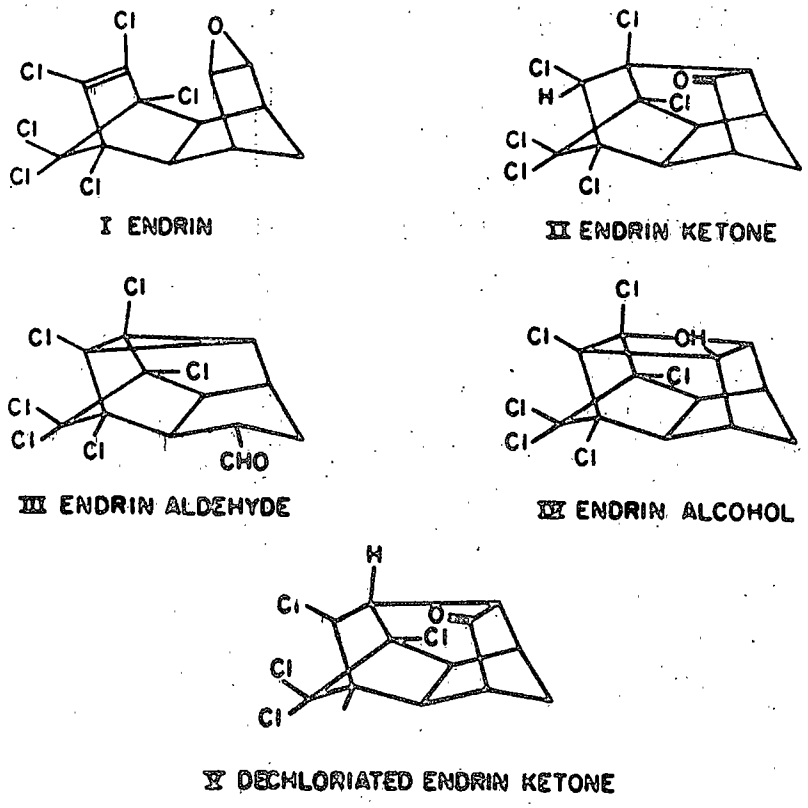


FIGURE 2. Products of endrin degradation.

lepidopterous larva in cotton crops. In 1971, over 75% of the endrin used was for treatment of cotton and 99% of this use was in the southeastern and delta states (Donoso et al.,1979).

During the period from 1957 to 1966, the lower Mississippi river system had the highest levels of endrin contamination of any surface waters in the U.S., primarily as a result of runoff from crops and fields in this area. Such contamination resulted in continual restriction of its use nationwide although in 1981 over 27,000 lbs. of endrin was used for the control of rodents in the state of Washington (Eaton,1982) while in the same year over 200,000 acres of grain was treated in Montana (Montana Dept. of Fish, Wildlife and Parks,1983).

Monitoring programs involving State and Federal agencies during the heavy use in Montana revealed widespread contamination of fish and wildlife (primarily documented in birds). Several years of controversy and studies by the Montana Department of Fish, Wildlife and Parks have finally culminated in a recent (July,1983) Montana Department of Agriculture prepared EPA Environmental Impact Statement which has resulted in the Department of Agricultures' recommendation to " suspend the sales and use of endrin and to cancel endrin registrations when alternatives are registered by the Environmental Protection Agency " (K. Kelly,1984, personal communication).

METABOLISM AND ELIMINATION

The metabolism of endrin in mammals has been well studied (Terriere et al.,1958; Cole et al.,1968; Bedford et al.,1975a and 1975b; Soto and Deichmann,1967; Brooks,1969; Hutson et al.,1975). Hutson et al.(1975) dosed rats of both sexes for 2 weeks and found endrin and its major metabolites in feces in the following proportions : endrin, 11%; anti-12-hydroxyendrin 83%; syn-12-hydroxyendrin, <0.01%; 3-hydroxyendrin, 5%; 12-ketoendrin, 1%; and delta-ketoendrin, <0.01%. Bedford et al.(1975b) reported that the oral administration of endrin to rats resulted in the production of three metabolites, all of which were more acutely toxic than the parent compound, with 12-ketoendrin being the most acutely toxic. Other investigators have reported that the metabolites of endrin are less acutely toxic than is endrin (Klein et al., 1968, as cited in Donoso,1979). The greater susceptibility of females appears related to the fact that they metabolize endrin more slowly than do males (Korte, 1967, as cited in Brooks 1969) and tend not to excrete 12-ketoendrin (Hutson et al.,1975).

The accumulation of endrin in the rat varies with the sex (Hutson et al.,1975). These authors found that the tissue residue retention was higher in females and was composed primarily of unmetabolized endrin while 12-ketoendrin was the main tissue residue found in males. They found 12-ketoendrin was the major metabolite in the fat, liver and kidneys of males while unmetabolized endrin was the main

component in fat and kidneys of females. Endrin and 12-ketoendrin were detected in the liver of females although 70% of the original radioactively labeled compound could not be accounted for in their analysis.

The metabolic fate of endrin in the rabbit is markedly different from that in the rat. Bedford et al.(1975a) reported that almost half of the administered dose of endrin in the rabbit is excreted in the urine, compared to only 2% in the rat. This study also found excretion to be almost complete within 24 hours and that the major fecal component was unchanged endrin. The metabolites of the two species is similar although 12-ketoendrin is not a significant excretion product in the rabbit.

Endrin tends to reach a steady state condition in the tissues of repeatedly exposed animals. Rats fed carbon fourteen labeled endrin for 12 days tended to reach a plateau level of storage after 9 to 10 days (Brooks, 1969) although in another study, oral administration of endrin to rats resulted in a steady state of storage after about 6 days (Korte,1967 and 1970, as cited in Donoso et al., 1979). Steers, lambs, and hogs fed endrin for 12 weeks had detectable levels within the tissues only in those animals receiving a dose higher than 0.25 ppm (Terriere et al.,1958). In animals allowed to recover for 6 weeks, steers had a 60% reduction in the endrin content in their fat while no detectable levels were found in lambs and hogs. In a similar study Kiigemagi et al.(1958) fed dairy cows endrin and reported

detectable concentrations in milk only from animals receiving doses of 0.25 ppm and greater. They also noted that increased dietary levels had no effect on the milk secretion concentration of endrin. Other studies also indicate that a threshold level of intake is necessary before measurable amounts of the compound can be detected (Ely and Moore,1957; Moubry et al.,1968). Richardson et al.(1967) demonstrated in dogs that, excluding fat there is no relationship between blood concentrations of endrin and those in the tissues.

Most endrin and its metabolites are excreted in the feces (Cole et al.,1968), while very little endrin or its metabolites are excreted in the urine (Hutson et al.,1975). Cole et al.(1968) administered carbon fourteen labeled endrin intravenously to rats with and without bile fistulas. They found that over 50% of the daily dose was excreted within 24 hours and that 90% of the excreted compound was in the feces. In the same study, in an isolated perfused liver, 50% percent of the labeled endrin was excreted in the bile within one hour. In pigs fed endrin, 47% of the administered compound was recovered from urine, feces, stomach, gut, liver, fat, and blood within 24 hours while 94% had been recovered after 6 days (Hunter 1960, as cited in Soto and Diechmann,1967). Intubated rats fed endrin for 8 days excreted 60-70% of each daily dose in the feces (Ludwig, 1966, as cited in Soto and Deichmann, 1967). The first day, 30% of the amount excreted contained pure endrin and 70% was metabolites. Cessation of dosing resulted in 93% of the amount excreted as

metabolites. He also reported that of the amount excreted in urine, 82% consisted of metabolites and 18% was endrin. Rabbits given two oral doses of carbon fourteen labeled endrin 14 days apart excreted 37.3% of the first dose via the urine and 49.6% in feces during days 1-13. The second dose was eliminated in a similar fashion (Bedford et al.,1975a). Subsequent recovery of 96.7% of the radioactivity in urine and feces was achieved by day 49.

TOXICITY

Numerous studies have examined the toxicity of endrin in laboratory animals. Treon (1955) studied the toxicity of endrin in a number of laboratory animals utilizing various modes of administration. Continual application of endrin (dry powder) to the skin of rabbits for 24 hours resulted in development of convulsions in the most severly poisoned animals, while intermittent application for 2 hours on each of 5 days per week caused death in animals receiving as little as 19 applications. Symptoms displayed by these animals included convulsions, tremors, and facial twitching. Oral administration of 1 mg of endrin on each of 5 days per week to rabbits and rats of both sexes resulted in abdominal distention in rabbits, and hypersensitivity to stimuli in rats. In another experiment, the same author fed rats endrin at various doses over a period up to 2 years and reported convulsions and hypersensitivity to stimuli in

those animals fed diets with the highest concentration of endrin. Bedford et al.,(1975a) also reported signs of toxicity in rats such as ataxia followed by tonic convulsions. Treon (1955) also found that dogs given lethal injections of endrin regurgitated their food, became lethargic, salivated, developed respiratory distress and central nervous system symptoms that included hypersensitivity to stimuli, tremors, twitching, and severe convulsions.

Seizures developed in mice following intravenous injections of endrin (Walsh and Fink,1972). Graves (1965) gave intraperitoneal injections of endrin to mice and reported no mortality at doses of 1 or 2 mg/kg but all animals died at exposure levels of 10 mg/kg or more. Similar results were reported in bats exposed to various concentrations of endrin over a 28 day period (Luckens and Davis,1965). They found complete mortality at a dose of 12 mg/kg. Animals receiving 50 mg/kg developed tremors within one hour and died by 2 2/3 hours after exposure.

Endrin in also extremely toxic to birds and fish. Quail and pheasants develop symptoms of intoxication within 48 to 72 hours after being given endrin in their diets (DeWitt,1956). Initially these birds displayed a lack of muscular coordination, occasional tremors, and stiff-legged, hesitating movement in walking. Later they made spasmodic leaps and went into violent cartwheels. In another study (DeWitt,1955), it was reported that quail developed severe tremors, loss of muscular coordination and "extreme nervousness" within two

hours after being fed endrin. All birds exposed to endrin in this group died within 48 hours.

Numerous studies have documented the toxic effects of endrin on fish. Signs of intoxication include ineffective feeding (Grant and Merhle,1970), swimming in whirling patterns when disturbed (Hermanutz,1978), hypersensitivity to sudden noise (Grant,1976; Argyle et al.,1973), respiratory difficulties, sluggishness (Johnson,1968), and hyperexcitability (Grant and Mehrle,1973).

Symptoms of endrin poisoning in humans have been well documented. While death has been reported only in extreme cases, the initial symptoms after less severe exposures include headache, weakness, nausea, and abdominal discomfort while individuals more severely poisoned often exhibit convulsions, unconsciousness, and frothing at the mouth (Weeks,1967; Coble et al.,1967). Acutely poisoned infants displayed early symptoms such as tonic and clonic convulsions, slight trismus, unconsciousness, tachycardia, an elevated body temperature, respiratory distress and cyanosis (Jacobziner and Raybin,1959; Hayden et al.,1965). During the next several days these individuals developed symptoms such as vomiting, difficulty in swallowing, convulsions, limbs in a state of permanent contraction and pupilary dilation. Neurological examination of one child revealed signs of "diffuse encephalopathy with focal motor parietal discharge on the right side resulting in decerebrate rigidity and brain stem signs and vasomotor instability and hypertension due to involvement of

the cardiovascular centers in the medulla and pons" (Jacobziner and Raybin,1959). A hyperactive startle reaction and the alternating extension and flexion of the upper limb led these investigators to speculate extensive diencephalon involvement although movements due to basal ganglia involvement were not noted.

Four outbreaks of acute poisoning from endrin contaminated bread were reported in Saudi Arabia in 1967 (Weeks,1967). Within hours after ingesting contaminated bread, individuals developed abdominal pain, nausea, vomiting, lethargy, mental confusion, unconsciousness and convulsion. In many instances improvement was rapid, often within 2-5 hours. Seven people died in one incident within 12 hours after the onset of symptoms. Similar incidents in Egypt (Coble et al,1967) and Wales (Davies and Lewis,1956, as cited in Weeks,1967) have been described, all involving ingestion of endrin contaminated bread. Symptoms of intoxication included convulsions, facial contortions, frothing at the mouth, lethargy, headaches, mental confusion, beating the head on the floor, hyperactive reflexes and periods of semiconsciousness. Recovery in these poisonings was rapid, generally occurring within 1-7 days, depending on the severity of exposure.

CARCINOGENICITY

Endrin has been reported to be carcinogenic to rats (Reuber,1979). Rats exposed to concentrations as low as 0.1 ppm

developed significant incidences of carcinomas in liver, lung, thyroid, mammary gland, and adrenal cortex. Females tended to be more susceptible to the development of neoplasms in endocrine and reproductive organs. In other studies of male and female rats fed endrin in concentrations varying from 1 to 100 ppm over a period of 2 years no increased incidence of tumors was reported in experimental animals (Treon,1956, as cited in Jager,1970). Similar studies in mice and dogs were inconclusive (Reuber,1979). Reuber (1979) states that "sufficient documentation is available on qualitative extrapolation of animal data that one must conclude that finding of carcinogenicity in one mammalian species should be deemmed to have relevance in other mammalian species-including man".

Reuber's findings (1979) have not been accepted by the Environmental Protection Agency's Carcinogen Assessment Group who reviewed studies of the U.S. Food and Drug Administration, the National Cancer Institute, the University of Cincinnati and the University of Miami. This group concluded that ".... the weight of evidence is that endrin is unlikely to be a human carcinogen" (Albert,1978, as cited in Montana Dept. of Agriculture,1983). Endrin is also not on the list of carcinogens reported by the World Health Organization (Spencer,1981, as cited in Montana Dept. of Agriculture,1983).

TERATOGENICITY and MUTAGENICITY

Endrin has been shown to be teratogenic in hamsters and mice as well as causing weight reduction and a significant increase in fetal mortality (Ottolenghi et al.,1973). Anomalies in the offspring of endrin treated hamsters included open eye, webbed foot, and cleft palate. In another study, Chernoff et al.(1979a and 1979b) reported fused ribs, meningoencephaloceles, reduced skeletal ossification, increased mortality and fetal weight reduction. In the offspring of exposed mice, there was no effect on survival or weight and only a few incidences of cleft palate, open eye, microcephaly and exencephaly (Ottolenghi et al.,1973).

Endrin has been shown to be mutagenic in rats following testicular injections (Dikshith and Datta,1973). They found chromosomal changes that included fragmentation and the formation of single and double bridges with acentric fragments, unequal distribution of anaphase chromosomes, and transformation of chromatin into an amorphous lump.

PHYSIOLOGICAL EFFECTS

Physiological studies involving endrin exposed animals have been well documented and while little data is available concerning its mode of action within the nervous system, pharmacological studies indicate

that it is not a cholinesterase inhibitor (Colvin and Phillips,1968).

Ryan and Shankland (1971) explored the synergistic action of DDT and endrin on the giant axons of the cockroach central nervous system. They found that when given individually neither compound produced physiological abnormalities over a period of 3-5 hours. However pretreatment of the axons with DDT followed by exposure to endrin led to instability followed by complete blockade of axonal conduction. They concluded that endrin alone had no toxic action on the axonal membrane of the cockroach and the that apparent synergistic action of these compounds cannot be attributed to either compound alone.

Joy (1976) studied the convulsive properties of a number of chlorinated hydrocarbon insecticides in the cat central nervous system. Animals injected with endrin at doses of 1-2 mg/kg developed spontaneous seizures within 5-15 minutes and at higher doses developed seizures in 0.5-2.0 minutes. Hypotension, which was followed by hypertension and cardiac arrythmias were observed in some animals. Following administration of endrin, any type of sensory stimulation, especially tactile or auditory, would evoke a seizure. The author speculated that the chlorinated hydrocarbon compounds act directly on the central nervous system and that they do not have to be converted to an active metabolite to produce toxic effects (Joy,1976).

Seizures were produced in all parts of the pigeon telencephalon after intravenous administration of endrin (Revzin,1966). The ectostriatum appeared to be particularly sensitive and because it is a visual projection area in birds, it is probable that such exposures could result in visual deficits.

MATERIALS AND METHODS

EXPOSURE AND TISSUE PREPARATION

Ten week old, male, ICR mice (Charles River) with an average weight of 34 grams (range 28.5-39.4) were divided into control and experimental groups. Experimental animals were given daily intraperitoneal injections of endrin in sesame oil (0.2 ml) at doses which increased from 1.5 mg/kg on days 0-3, to 2.0 mg/kg on days 4-11, and finally to 4.0 mg/kg on days 12-19. This dosing protocol (Figure 3) was employed in an attempt to maintain a maximum sublethal exposure, in animals that may have adapted to the previous level of exposure. The initial daily dose of 1.5 mg/kg represents approximately 27% of the acute LD50 for mice while the final daily dose represents approximately 71% of the acute LD50 (Graves,1964). The cumulative dose in those animals receiving 20 consecutive injections was 54 mg/kg (approximately 10 times the acute LD50). The solutions were prepared with endrin (99% purity) supplied by the Velsicol Company (Chicago). Control animals received the same volume of sesame oil without endrin. Four experimental and two control animals were sacrificed after 4, 7, 14, and 20 days of exposure (d o e) and after 14 and 92 days of recovery (d o r).

Each animal was weighed on a beam balance immediately preceding sacrifice. The animals were anesthetized with ether and, following

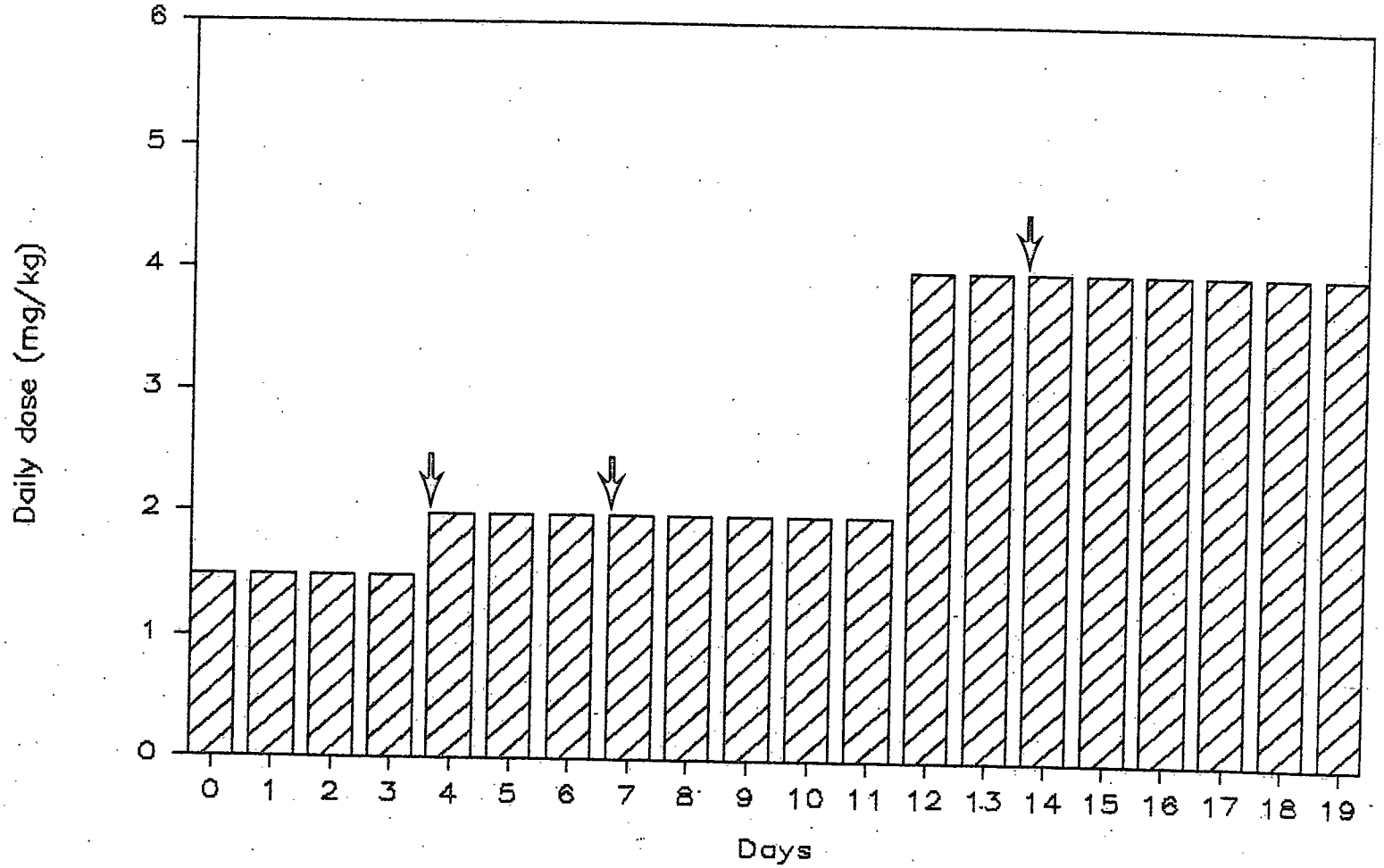


FIGURE 3. Dosing Protocol. Arrows indicate days of sacrifice.

lateral incisions, the rib cage was reflected superiorly. The pericardial sac was opened and a 21 gauge needle was inserted into the left ventricle. The right atrium was cut and the animal was perfused with 3% glutaraldehyde in 0.1M phosphate buffer (pH 7.3) at a pressure of 80-100 mm Hg for 5 minutes. Sciatic nerve, optic nerve, gluteal and cardiac muscle were removed, placed in fixative and diced into 2 millimeter cubes. Following 2-3 hours in cold fixative, the tissue was washed in buffer and stored overnight. The next day the tissue was post-fixed in buffered 1% osmium tetroxide, then dehydrated in a graded ethanol series and propylene oxide and embedded in Spurr's resin. Tissue sections 1-2 um (micrometers) thick were cut from several blocks of each animal and stained with basic toluidine blue (Meek,1976) for light microscopic examination and subsequent orientation of thin sections. Light micrographs were taken with an Olympus PM-6 camera mounted on an Olympus BH-2 light microscope. Thin sections were cut with glass knives on an LKB Ultratome III, mounted on uncoated copper grids, stained with alcoholic uranyl acetate (Weakley,1981) and lead citrate (Venable and Coggeshall,1965) and examined and photographed with a Zeiss 9S-2 electron microscope.

BEHAVIORAL/NEUROLOGICAL TESTS

Behavioral/neurological tests were performed within a 30 minute time span immediately preceding the dosing of the control and

experimental animals, excluding the days in which those animals were to be sacrificed. The tests were designed to assess changes in the animal's behavior approximately 24 hours after the previous injection. The tests were performed daily during the 20 days of dosing (days 0-19) and thereafter at 1, 8, 12, 14, 22, 29, and 92 d o r. Each animal was removed from their cage and tested with minimal space restrictions. Each animal was tested and observed as follows:

GAIT: The gait of individual animals was visually evaluated as they moved about a wooden surface. Animals were observed for any changes in gait such as splaying of either the front or hind limbs and for signs of unsteadiness and/or immobility.

TREMOR: Animals were observed for any sign of tremor.

TAIL FLICK: This test was used to evaluate each animal's response to noxious thermal stimuli (Janssen et al.,1963). Approximately one half of length of the animals tail was dipped in a water bath maintained at a temperature of 56-56.5 C and the time required for the animal to remove its tail completely from the water was recorded to the nearest tenth of a second.

STARTLE RESPONSE: The reactivity of each animal to a blast of air into their face was categorized as being hypoactive, normal or hyperactive. Each animal was tested using an aerosol can of compressed air, adjusted to the high setting and directed with an extension tube.

ACTIVITY: A subjective evaluation of activity was made based on visual observation of each isolated animal as they moved about a wooden surface. A general description was made of each animal (from extremely hypoactive to extremely hyperactive).

ROD TEST: This test checked for the animal's ability to maintain its position when placed on a fiberglass rod and was an indirect measure of balance and grip strength. A uniform fiberglass rod 43.0 cm long and 8.0 mm in diameter was suspended diagonally across a box 29.0 cm from the bottom. The animals were held by the tail and lowered onto the rod such that all 4 paws gripped the rod securely and the animal appeared stable. The animal was quickly released and the time interval between release and complete separation from the rod was recorded to the nearest tenth of a second.

RESULTS

SCIATIC NERVE

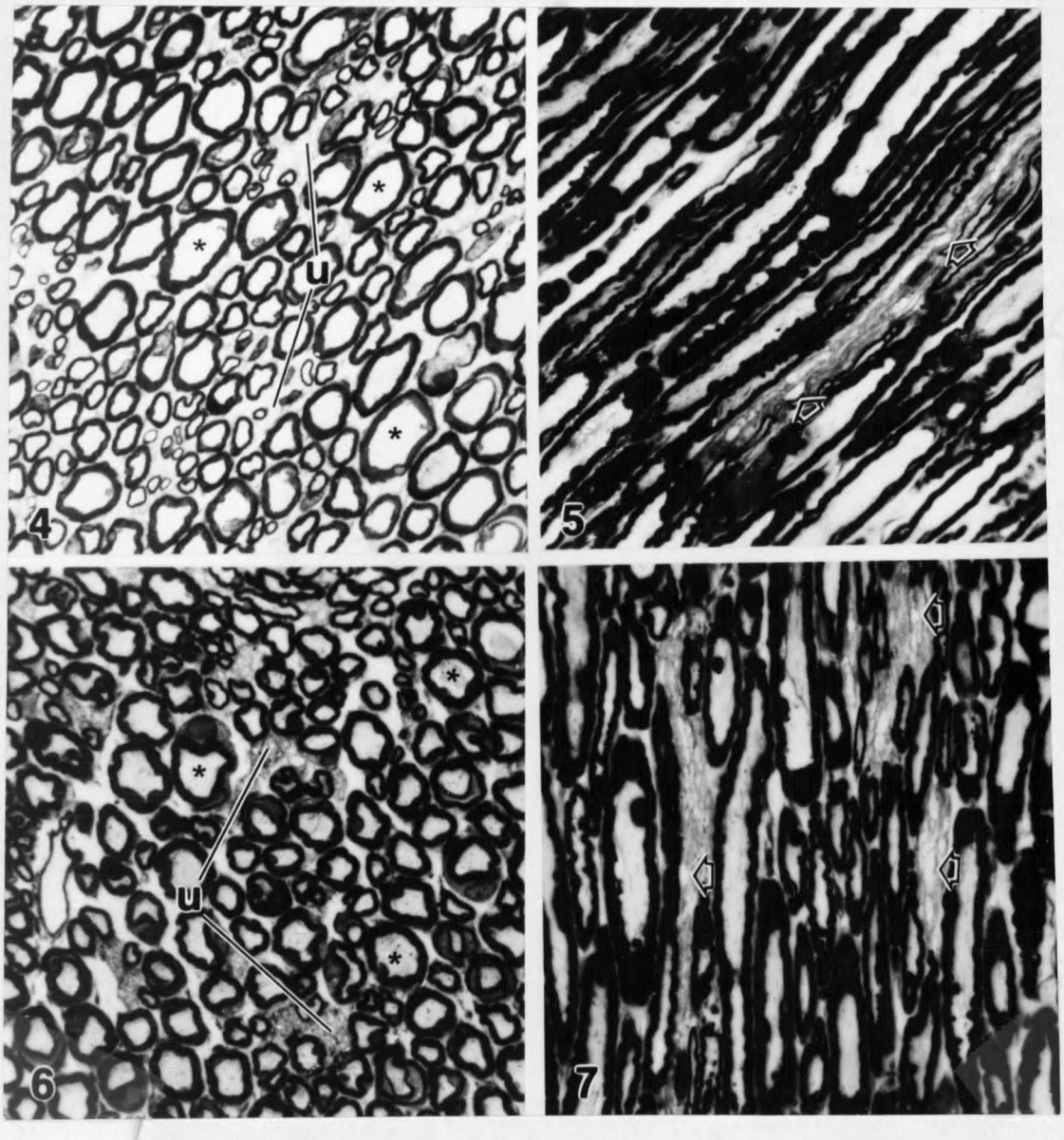
Myelinated peripheral nerve fibers, myelin, and myelin producing Schwann cells in endrin exposed animals appeared similar to those in control animals at the light (Figures 4-7) and electron microscopic levels (Figure 8) and resembled those described as normal in the literature (Peters et al.,1976; Landon and Hall,1976). In both control and experimental tissue, preparative artifact was commonly observed as focal interperiod swellings within the myelin (Figures 8 and 9).

Unmyelinated peripheral nerve fibers from control animals appeared similar to normal fibers described in the literature (Ochoa, 1976; Peters et al.,1976). These fibers were found throughout the entire cross-sectional area of the sciatic nerve and the actual number of unmyelinated axons always outnumbered the myelinated fibers. The unmyelinated axons were generally 0.5-0.6 um in diameter with an axonal membrane (axolemma) 7-8 nm thick. The axonal cytoplasm (axoplasm) contained an accumulation of microtubules (20-25 um in diameter), neurofilaments (10 um in diameter), mitochondria, and sparce smooth endoplasmic reticulum (Figures 8, 10, 11). The mitochondria were often thin and elongate in longitudinal section and almost round in cross section (Figures 8 & 10). They were often several micrometers long and contained cristae that typically

paralleled the length of the axon. The smooth endoplasmic reticulum was located between the microtubules and neurofilaments and was usually represented by a few irregular vesicles or tubules that also paralleled the length of the axon. While vesicles in unmyelinated axons are not typically described in the literature other than near the axon terminal, occasional vesicles were observed in unmyelinated fibers from control animals in this study. Such vesicles were round to hexagonal in shape, 0.1-0.2 um in diameter, and they generally occurred in groups of 2 or 3.

Unmyelinated peripheral axons were invested by Schwann cell cytoplasm, and it was not uncommon to find 10-15 axons per Schwann cell (Figures 8 & 10). The Schwann cell cytoplasm contained a variety of cellular organelles, most of which were near the perinuclear area. Typically microtubules, neurofilaments, and mitochondria were seen elsewhere in transverse sections. A basal lamina enclosed the Schwann cell (Figures 10 and 11). Collagenous fibers were frequently encountered as part of the endoneurium between the Schwann cell bundles of unmyelinated axons (Figures 8, 10, 11).

Changes were apparent in unmyelinated nerve fibers from endrin exposed animals and were most obvious after 4 days of exposure. At that time approximately 40% of the unmyelinated axonal profiles examined showed morphological changes that commonly included dissolution of microtubules and neurofilaments, axonal swelling and vesicle accumulation. It was not unusual to find all of these changes



FIGURES 4-7. Light micrographs of sciatic nerve tissue from 4 and 20 day control and experimental animals showing fields of myelinated (*) and unmyelinated (u) axons. x1500. FIGURE 4. 4 day control animal. FIGURE 5. 4 day experimental animal. Some swelling can be seen in unmyelinated axons (arrows). FIGURE 6. 20 day control animal. FIGURE 7. 20 day experimental animal. Note the swelling in some of the unmyelinated axons (arrows).