STUDIES IN EXPERIMENTAL DIELDRIN AND NITRATE TOXICOSES IN SWINE AND GUINEA PIGS

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S. D. Sleight, Professor
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ABSTRACT

STUDIES IN EXPERIMENTAL DIELDRIN AND NITRITE

TOXICOSES IN SWINE AND GUINEA PIGS

by Mba Uzoukwu

Seven experiments were designed in which dieldrin with or without $NaNO_2$ was utilized to study the toxicity of dieldrin to swine and guinea pigs. Some of the experiments were intended to provide evidence of transplacental transfer of dieldrin and any fetotoxic effects resulting from such transfers. The effect of a protein-deficient diet on dieldrin toxicity in swine was studied. The ultrastructural changes in the brain and liver in acute dieldrin poisoning were observed.

Parameters for evaluation of toxicity were death, abortion, body weight changes, gross and histopathologic lesions, and ultrastructural changes. Hematologic determinations revealed no significant deviations from normal values. The acute oral toxic dose (LD_{50}) of dieldrin for guinea pigs was calculated to be 45 mg./kg. Acute poisoning of guinea pigs with (75. mg./kg.) dieldrin resulted in convulsions and lesions were characterized submicroscopically by swelling of cerebellar and cerebral mitochondria with disintegration of associated cristae.

Administration of dieldrin with or without $NaNO_2$ to pregnant sows resulted in accumulation of the pesticide in maternal and fetal tissues, without causing abortion or any observable lesions in the fetuses. The amounts stored in the maternal tissues were not found to be proportional

to quantities given. However, there was a statistically significant difference ($P < 0.025$) in the amount of dieldrin stored in the fetal liver which appeared to depend on the total amount of the pesticide given to the dam. There was no evidence of synergism in the toxicoses of dieldrin and $NaNO_2$ in the pregnant sow.

Deaths but no abortions resulted from the administration of dieldrin (60 mg./kg.) to pregnant guinea pigs. Abortion but no deaths occurred when $NaNO_2$ (30 mg./kg.) was also given. However, dieldrin at 100 ppm in the feed caused 2 deaths and 2 abortions in 5 guinea pigs and nitrite (0.5% in H_2O) caused 3 out of 5 recipient guinea pigs to abort. Simultaneous administration of dieldrin (100 ppm) and nitrite (0.5%) caused 3 deaths and 6 abortions in 10 dosed guinea pigs. Administration of dieldrin at the level of 50 ppm or less with nitrite (0.25%) resulted in neither abortions nor deaths. There is, therefore, no evidence of potentiation of the toxicity of dieldrin by nitrite.

Adult guinea pig tissues stored dieldrin in proportion to the total amount administered and this proportionality was not affected by the simultaneous administration of $NaNO_2$. However, fetal storage did not appear to be in direct proportion to the quantity consumed by the dam unless nitrite was also administered.

There was no significant effect on food and water consumption, or on growth of guinea pigs fed dieldrin at 50 ppm or less for up to 53 days. Protein-deficient diet (4% casein) fed to pigs for 21 days appeared to enhance their susceptibility to the toxic effects of dieldrin, but was associated also with a reduced capacity on the part of the pig to accumulate the pesticide in its tissues.

The gross and histopathologic lesions were similar in both dieldrin and dieldrin/nitrite treated swine and guinea pigs. These lesions consisted of a generalized hyperemia of tissues and fatty metamorphosis of parenchymatous organs. Brownish discoloration of tissues due to methemoglobinization was evident in the nitrite-treated sows and guinea pigs. The most important uterine lesion in the pregnant guinea pigs consisted of thickening and vacuolar degeneration of the arterial media of small arteries with partial or total occlusion of the lumen.

STUDIES IN EXPERIMENTAL DIELDRIN AND NITRITE TOXICOSES IN SWINE AND GUINEA PIGS

By

Mba Uzoukwu

A THESIS

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Dedicated to

those who died to preserve

my freedom

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INTRODUCTION

Pesticides of the cyclodiene group to which dieldrin belongs are used commonly for agricultural, domestic, and public health purposes. Consequently, extensive studies have been made into the toxicoses associated with their use.

While much is known about the clinical signs and lesions that result from poisoning by these chemicals, the underlying mechanisms are not clearly established. Usually, these substances cause terminal convulsions. Some in the group have been associated with soft-shelling and reduced hatchability of eggs or increased mortality in the young of some species of birds even at low dietary concentrations. Evidence, however, indicates a strong species variability in susceptibility to poisoning by members of this group of pesticides. It is necessary, therefore, to determine minimum no-effect levels of each pesticide for each species investigated.

The rat has been the laboratory animal of choice for most investigators. The guinea pig has been used rarely. Of the farm animals, the pig has been investigated the least. The purpose of this study was to obtain some information on the effects of dieldrin on pregnancy, the fetus, life span, body weight, and tissues of guinea pigs and pigs following administration of the pesticide with or without sodium nitrite. In particular, the 2 species were selected for the study of the effects of toxicosis on pregnancy because it was conjectured that the differences in placentation will possibly provide varying degrees of resistance to

passage of the pesticide to the fetus. An attempt was made to show a correlation between tissue levels of dieldrin and any observed signs and/or pathologic changes.

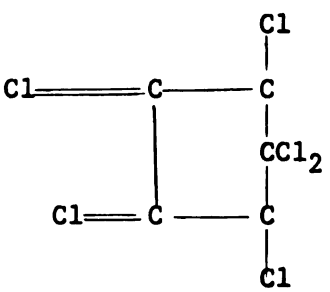
The mechanism of initiation of the terminal convulsions is not agreed upon; neither has any definite gross pathologic change been observed. This investigation included an electron microscopic study intended to obtain evidence of any damage to the central nervous system on the submicroscopic level.

REVIEW OF THE LITERATURE

General Principles

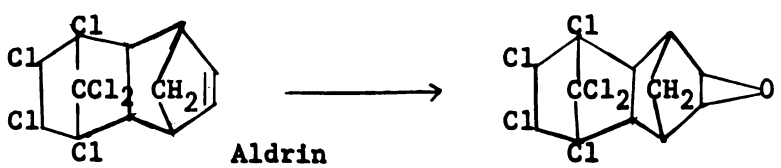
Dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethano-naphthalene) or HEOD is the oxidation product of a parent compound, aldrin. Both are chlorinated hydrocarbon compounds with strong pesticidal properties and rather long residual properties, hence the colloquial designation of "hard" or "persistent" pesticides.

The cyclodiene pesticides are the addition products obtained by condensation of chlorinated double-bonded compounds with hexachlorocyclopentadiene,



Hexachlorocyclopentadiene

Dieldrin is derived from aldrin by epoxidation of the double bond in the second ring as shown in the structural schematic representation:



Aldrin Dieldrin

and this is the mode of metabolism of aldrin in the mammalian body. That this epoxidation of aldrin may not be a detoxification but rather a toxification was suggested by Brooks *et al.* (1963a,b), who showed that oxygen

was required to produce the actual toxicant in houseflies treated with aldrin. Perry *et al.* (1958) had observed that although there was an adequate amount of heptachlor in the housefly during the latent period, the onset of signs of poisoning coincided with the appearance of heptachlor epoxide.

History

The cyclodiene pesticides are a relatively recent discovery. With the development of α-α-α-trichloro-β-β-bis (p-chlorophenyl)ethane (DDT) in 1939, an intensive search for other halogenated compounds with insecticidal properties was launched. Julius Hyman in the United States of America, and Reimschneider in Germany, working independently, are credited with the development of the first insecticide of the diene group known as chlordane or M410 in 1945 (O'Brien, 1967). Aldrin and dieldrin were both developed about 1948 by the Hyman Company. The most recent additions, Thiodan or Endosulfan in 1956 and Telodrin in 1957, were made in Germany. For more than a decade these compounds have been used extensively for agricultural, domestic, and public health purposes. Within this period, knowledge has also accumulated of their relative stability, photochemical products, and the hazards consequent on the virtual permanent contamination of soils, plants, and water with which they come in contact. Environmental contamination by these persistent pesticides has accordingly become a major concern of conservationists and public health officials in recent times.

Mechanism of Toxicosis

The toxicity of a contact insecticide is believed to be a net result of several factors, *viz.*, penetration of the tissues, enzymatic metabolism with formation of more or less toxic metabolites, elimination and storage

of unchanged insecticide or its metabolites. From the symptomatology and experimental observations of isolated nerves, the cyclodienes are primarily neurotoxicants. However, the exact mechanism of their intoxication is not clearly understood. Gowdey *et al.* (1952, 1954) and Gowdey and Stavraky (1955) first reported signs of toxicosis related to the autonomic nervous system in aldrin-poisoned cats. These signs were referable to peripheral sympathetic action and consisted of bradycardia, hypotension and decreased excretion of saliva by the submaxillary gland. These were not abolished by vagotomy, thus confirming the peripheral origin of the effects observed. Dieldrin, on the other hand, slowed the heart by a central mechanism which was abolished by vagotomy. Excessive excitability and convulsions caused by both chemicals are said to be related to their actions on the central nervous system.

Giannotti *et al.* (1956) reported on their experiments with cockroaches (*Periplanata americana*) in which both aldrin and dieldrin were used. They were able to produce convulsions in decapitated poisoned roaches. Moreover, decapitation after poisoning had no apparent effect on the development of signs. Nicotine, in low concentration, was an effective antidote, suggesting that the effects of aldrin/dieldrin poisoning are on synaptic transmission which appears to be enhanced. (Nicotine blocks synaptic but not axonal transmission in insects.) Lalonde and Brown (1954) obtained delayed short trains of impulses from the central nerve of *P. americana* after they crushed the ganglion and applied various cyclodiene insecticides. This confirmed the peripheral action of the insecticides.

Matsumura and Hayashi (1966) quoted the work of Yamasaki and Narashishi who found a direct relationship of the action of dieldrin to the central nervous system. These investigators had shown that dieldrin-

poisoned nerves of *P. americana* manifested spontaneous bursts of action potential. They also found that the latent periods between the application of dieldrin and observation of a discharge by the nervous system was much longer in dieldrin-resistant than susceptible houseflies.

Some biochemical changes have been associated with dieldrin poisoning. Thus, Hossein and Proulx (1960) obtained extracts of brains from rats injected intraperitoneally with dieldrin. The extracts contained betaine esters, straight-chain compounds with a quaternary nitrogen at one end and a carboxyl group at the other, e.g., butyrobetaine $[(CH_3)_3\overset{+}{N}(CH_2)_2COOH]$, in what they called the supernatant A fraction instead of the B fraction. It was concluded that these must have been released from their normal cellular attachments in mitochondria by the convulsive agents, and this conclusion implied mitochondrial damage. These workers also found the esters to be indistinguishable from acetylcholine on assay preparations. However, doubt has been cast on this interpretation by the work of McLennan *et al.* (1963), who demonstrated that the chromatographic bond which had been claimed to be a betaine derivative by Hosein *et al.* (1962) was really the trichloroacetate of acetylcholine formed when the tissue was treated with trichloroacetic acid. Colhoun (1960) showed that neither gamma-butyrobetaine nor its esters appear in nerve cords of dieldrin-poisoned roaches. On the other hand, Hathway *et al.* (1965) have shown that substantial changes in intermediates of brain metabolism occur in rats poisoned with dieldrin and other chemicals. During acute dieldrin poisoning increases in lactic and pyruvic acid concentrations in the rat brain were associated with hyperactivity of the brain, whereas an increase in cerebral alanine concentration occurred before convulsions. Ammonia concentration in the brain fluctuated out of phase with the actual convulsions. Conversion

of ammonia into glutamine in the brain is regarded as the principal mechanism for protection against the toxic effects of ammonium ion. In this process glutamic and pyruvic acids and alanine are utilized in the ammonia-binding mechanism. In the later states of dieldrin-induced seizure free ammonia accumulates in cerebral tissues. Consequently, it has been suggested that dieldrin inhibits the ammonia-binding mechanism and this, in turn, results in inhibition of glutamine synthesis. O'Brien (1967) is of the opinion that these effects are more likely to be the result rather than the cause of the convulsions since comparable changes occur in poisoning by such unrelated convulsants as picrotoxin.

Metabolism of Dieldrin

Epoxides of the cyclodienes are formed only when the appropriate double bond exists. With dieldrin further epoxidation is not possible and it has been shown that insects seem to be unable to metabolize dieldrin (Cohen and Smith, 1961; Brooks, 1960). In confirmation, Earle (1963) demonstrated that dieldrin taken up by housefly larvae persisted through metamorphosis into the adult when it was slowly excreted as unaltered dieldrin.

In contrast to insects, mammals appear to be capable of degrading dieldrin. In 1964, Heath and Vandekar reported that 3% of the dieldrin dissolved in oil and administered orally to rats was eventually excreted, unchanged. Nine-tenths of the remaining unidentified metabolites was excreted in the feces as "metabolite 1" while the remaining one-tenth was excreted in the urine. The authors suggested that dieldrin was metabolized in the liver. Cueto and Hayes (1962) showed that men occupationally exposed to dieldrin had at least 2 metabolites of this chemical in their urine. Both metabolites were neutral, polar and chlorinated.

In 1963, Morsdorf *et al.* gave radioactive dieldrin (C^{14}-dieldrin) to rats and demonstrated that it was excreted primarily through the bile as a glucuronide composed mainly of an unknown polar product. In 1965, Korte and Argent administered C^{14}-dieldrin to rabbits by stomach tube and isolated 6 metabolites from the urine. These were characterized by thin-layer chromatography. Metabolite V, which was excreted in largest quantity (86%), was identified as one of the 2 enantiomorphic isomers of aldrin. The acute toxicity of this metabolite was found to be 1/12th to 1/6th that of dieldrin.

Perhaps of greater significance with regard to environmental contamination is the possibility of conversion of dieldrin on or in plants and the soil to more or less toxic products by sunlight. This has been adequately reviewed by Soto and Deichmann (1967). Mitchell (1961) had observed that strong artificial ultraviolet light could cause chemical changes in organochloride pesticides impregnated on filter paper. In 1963, Roburn first reported the presence of an unknown compound on grass previously treated with dieldrin. The same compound was recovered after ultraviolet irradiation of dieldrin on a glass plate. Rosen *et al.* (1966) and Robinson *et al.* (1966) identified this photoconversion product as the hexacyclo isomer of dieldrin or photoproduct VII. Rosen *et al.* (1966) determined that it has twice the toxic properties of dieldrin to houseflies and mosquitoes. They suggested that this may be due to a more rapid and thorough penetration of the cuticle. Brown *et al.* (1967) confirmed its greater toxicity and also agreed with Robinson *et al.* (1966) that it occurs in such negligible quantities in the environment that it does not represent any overall increase in the toxicologic significance of residues arising from the use of dieldrin. A pentachloro photoproduct, VIII, has also been obtained experimentally by irradiating

dieldrin-treated corn leaves with light of shorter wavelength than is found in sunlight (Henderson and Crosby, 1966), but this has not been shown to occur naturally. These authors also showed that dieldrin is 1.6 times as toxic as photoproduct VIII to flies by topical application, but that the photoproduct is 4.5 times more toxic than the parent compound to mice by oral administration.

Retention of Dieldrin in Body Tissues

Kitselman *et al.* (1950) reported that of the body tissues the peripheral fat of sheep and cattle fed aldrin-sprayed hay contained the largest amounts of aldrin, while the milk had no aldrin in it. However, in 1952 Bundren *et al.* reported finding dieldrin in the milk of cattle sprayed with a 0.1% dieldrin mixture at 3-week intervals. The maximum concentration in the milk (5.9 ppm) was achieved in the first 3 weeks; thereafter it decreased steadily despite continued application. Buck and Van Note (1968) obtained dieldrin in the milk of cows accidentally poisoned by ingested aldrin.

In 1959(b), Gannon *et al.* detected dieldrin in the fat of all animals fed various levels of dieldrin. The concentrations in other organs were proportional to the fat content of the tissues as well as to the rate of intake. This general pattern of distribution was also found by Fiserova-Bergerova *et al.* (1967) in humans. They also confirmed placental transfer of the pesticide in man. Kitselman *et al.* (1950) found that the amount of dieldrin in the liver and kidney of the rabbit varied with the amount fed per week but not with the duration of feeding.

According to Street (1964) and Street and Blau (1966), simultaneous administration of dieldrin and DDT to Sprague-Dawley rats resulted in a significant inhibition of dieldrin storage in the fat. Deichmann *et al.* (1969) could not demonstrate a similar effect in dogs.

Factors Predisposing to Dieldrin Toxicity

Diet. Dietary influences on toxicity have been demonstrated by several workers. Meyer (1936a,b) reported a modification of the toxicity of sodium cyanide and resistance to diphtheria toxin by the composition of the diet. Similar modification of arsenic trioxide toxicity to rats has been observed. Ambrose *et al.* (1943) observed that a high fat diet reduced the acute but enhanced the chronic toxicity of rotenone. McLean *et al.* (1965) reviewed the susceptibility of animals on a protein-deficient diet to poisons that affect the liver. They suggested that the resistance to carbon tetrachloride (CCl_4) intoxication during protein depletion may be due to loss of microsomal enzymes needed for metabolization of CCl_4. In 1966, McLean and McLean confirmed this observation in protein-depleted rats. Radeleff and Bushland (1962) found emaciated sheep on a low maintenance diet of poor hay 8 times more susceptible to benzene hexachloride poisoning than sheep fed a normal diet.

Boyd and DeCastro (1968) found that DDT toxicity to rats was not augmented by a low protein diet. Later, Boyd and Krijnen (1969) found that low protein diet augmented DDT toxicity and simultaneously reported that an excessive amount of protein in the diet of weanling rats also enhanced susceptibility which was manifested as a significant reduction in the LD_{50} of DDT to albino rats. The clinicopathologic signs were similar at all levels of dietary protein utilized. However, a similar low dietary protein significantly reduced the LD_{50} of malathion for male albino rats (Boyd and Tanikella, 1969).

Melvin *et al.* (1964), working with the laboratory rat, demonstrated decreased survival at both low and high protein levels if high concentrations (200 ppm) of dieldrin were also fed. However, with the dieldrin

concentration at 150 ppm increased mortality occurred only with the low dietary protein group. It was therefore concluded that high dietary protein conferred some protection. This was confirmed in 1965 by Krishnamurphy *et al.*, who showed that poor rice diets, low in protein, increased the chronic toxicity of dieldrin manifested as increased mortality in rats.

Stoewsand *et al.* (1969) found a curious interplay between nutrition, sex, and dieldrin toxicosis in rats. At the lowest level of protein (10% casein) utilized, all males died within 18 days on dietary dieldrin levels of 150 ppm, but at higher protein concentrations (25 and 50% casein) not only did fewer die but the survivors showed an adaptation to the toxicosis. In contrast, all females fed the 25 and 50% casein rations that received 150 ppm dieldrin died within 10 days and, by implication, lacked an adaptive response. Female rats fed 10% casein ration plus 150 ppm dieldrin had a lower cumulative mortality rate. These results correlated positively with the rates of metabolism and excretion of dieldrin in that males fed rations containing higher levels of protein had higher rates of metabolism and excretion and lower tissue concentrations of dieldrin. The low dietary protein levels in the females were accompanied by increased metabolism and excretion, thus, in part, accounting for the lower mortality rate.

Nitrates and nitrites, in addition to inducing a methemoglobinemia, directly affect the cardiovascular system by causing a relaxation of smooth muscle, especially of arterioles and postarteriolar vessels (Goodman and Gilman, 1965; Beckman, 1958). One of the main consequences of this vasodilation is a fall in blood pressure associated with vascular congestion and degenerative changes in visceral organs, especially the liver, the chief site of dieldrin detoxification. Pathologic changes

in nitrite toxicosis have been characterized (Sinha, 1968; Atallah, 1966; Case, 1957). Abortion and impairment of reproduction have been observed as features of nitrate/nitrite toxicosis in the cow (Crawford *et al.*, 1966), sow (Case, 1963), rat (Case, 1957), and guinea pig (Sleight and Atallah, 1968). In the opinion of the last quoted authors, most of the placental and uterine lesions developed after the fetuses had died. The effects of simultaneous administration of nitrite and dieldrin on pregnancy are not known.

Species and Age Susceptibility. There appears to be a definite species and age-dependent susceptibility to dieldrin toxicosis following oral administration of an acute lethal dose. Hodge *et al.* (1967) reviewed several reports and observed that of 12 species investigated, the cat and chicken are the most sensitive; the cow, rat, guinea pig, mouse, and monkey are intermediate; the dog, pig, and sheep are least sensitive. Estimating the lethal oral dose range for most species to lie between 20 and 70 mg./kg. body weight, Ressang *et al.* (1959) found the cat most sensitive to aldrin (LD_{50} 10-15 mg./kg.) but most resistant to dieldrin (LD_{50} 300-500 mg./kg.).

In species in which the age factor was considered, it was found that younger animals were usually more sensitive to poisoning than the old (Bundren *et al.*, 1952; Radeleff *et al.*, 1960; Iizuka, 1963). Other studies relating toxicity to the route of administration of dieldrin have revealed that the LD_{50} is lower for oral than for dermal application (Gaines, 1960; Conley, 1960; Jolly, 1954). For spraymen, inhalation is important (Deichmann and Radomski, 1968), but Hodge *et al.* (1967) produced evidence which indicated that dermal exposure is quantitatively more important than respiratory exposure.

Systemic Effects of Dieldrin

The Blood. All reported results of hematologic studies in dieldrin toxicosis have shown no changes. Relevant observations reported are the unaltered sedimentation rates in men occupationally exposed to dieldrin (Princi and Spurbeck, 1951); rat hemoglobin and erythrocytes which were not affected (Krishnamurphy *et al.*, 1965); and nonalteration of the serum protein content of ewes on chronic exposure (Harris *et al.*, 1966).

Alimentary System, Body Weight, and Fat. No direct effects on the alimentary system have been reported except for occasional diarrhea in farm animals, which was observed by Ivey *et al.* (1961). This was not associated with any lesions. After a review of reported experimental results, Hodge *et al.* (1967) suggested that body weight or growth are not sensitive indices of the effects of aldrin and dieldrin. No marked changes in body weight of most species on nonlethal dietary levels of dieldrin have been reported, except for the young quail (DeWitt, 1955). However, at levels that cause weight loss, death occurs in most species (Treon *et al.*, 1955; Treon and Cleveland, 1955; Kitselman, 1951; Sherman and Rosenberg, 1954). Melvin *et al.* (1964) did not find a significant alteration in total body fat of rats at the levels of dieldrin given.

Reproductive System. The effects of dieldrin toxicosis on reproduction have been studied in several species. In no case has it been associated with teratogenesis, even when fetuses were stillborn. The criteria used for assessment include number of pregnancies, number of fetuses per litter, mortality amongst fetuses, egg production, fertility, hatchability, and chick mortality. Thus, reduction in the numbers of pregnancies and increased mortality of pups were obtained by feeding rats

diets containing 2.25 ppm of dieldrin (Treon *et al.*, 1954a). At a dietary level of 25 ppm, Kitselman (1951) observed increased mortality in pups of dogs. At a similar level of dieldrin, Harris *et al.* (1966) observed a decrease in survival of lambs. Ten parts per million was sufficient to induce decreased hatchability and increased mortality in quail chicks (Coulson *et al.*, 1962). Reduced egg production, hatchability, and increased chick mortality were not observed in pheasants on dietary dieldrin of less than 25 ppm (Rudd and Genelly, 1956).

Muscular System. Khairy (1960) described the impairment of muscular efficiency in the rat by dieldrin. The progressive deterioration was related directly to the amount of dieldrin administered. He also confirmed that dieldrin had no effect on body weight, food intake, and learning.

The Pathology of Dieldrin Toxicosis

The most important signs of dieldrin toxicosis are associated with the central nervous system and have been widely reported. These symptoms include convulsions, lethargy, salivation, teeth-grinding, and opisthotonos (Bundren *et al.*, 1952; Pearson *et al.*, 1958); and convulsions (Jolly, 1954; Conley, 1960). Changes in the electroencephalogram in exposed workmen (Spiotta and Winfield, 1952; Bell, 1960; Hoogendam *et al.*, 1962) are reversible in survivors. Most investigators report no corresponding gross lesions in the central nervous system. However, histopathologic changes in poisonings by both dieldrin (Radeleff *et al.*, 1955) and its parent compound, aldrin (Blaxter, 1959; Pearson *et al.*, 1958; Treon *et al.*, 1955; Ressang *et al.*, 1959) have been described. These changes consist of cloudy swelling and pericellular vacuolation

in the brain, and foci of degeneration in the forebrain involving oligodendroglia but not the neurons.

Changes described in other organs are, in general, nonspecific for dieldrin or aldrin since these are similar to lesions caused by other chlorinated hydrocarbon insecticides (Conley, 1960). The histopathologic changes consist of renal tubular degeneration; cloudy swelling, fatty metamorphosis and necrosis of the liver; congestion and edema of the lungs (Treon *et al.*, 1955; Ressang *et al.*, 1959; Kitselman, 1951; Anderson *et al.*, 1952). Ortega *et al.* (1957) observed centrilobular hepatic cell hypertrophy with peripheral migration of basophilic cytoplasmic granules in rats given relatively high doses of dieldrin or other chlorinated hydrocarbon insecticides. They also described distinctive cytoplasmic inclusion bodies which were referred to as "liposphères" and were regarded as a practical indication of chlorinated hydrocarbon effect on the liver cell. These authors found no significant differences in liver weights of test and control rats relative to their body weights, thus contradicting the findings of Borgmann *et al.* (1952) and Treon *et al.* (1951, 1955), who reported that dieldrin induced increases in liver:body weight (LW:BW) ratio in rats and dogs. The increases in LW:BW ratios have been confirmed by the Food and Drug Administration (1963).

Some reports on dieldrin toxicosis in cattle, sheep, goats, and pigs indicate no evidence of pathologic changes (Gannon *et al.*, 1959a,b,c), and no effect on milk production; but there are also numerous reports of histologic lesions similar to what are usually seen in other animals (Jolly, 1954; Radeleff *et al.*, 1955, 1960; Pearson *et al.*, 1958; Blaxter, 1959).

Other changes of toxicologic significance are decreased oxygen consumption in rats (Crevier *et al.*, 1954); unexplained alterations in concentrations of DNA in heart, spleen, kidney and brain of rats (Daugherty *et al.*, 1962); and depressed S wave of the electrocardiogram of sheep given dieldrin in the diet for a prolonged period (Harris *et al.*, 1966).

Ultrastructural Changes in Dieldrin Toxicosis

Ghazal *et al.* (1964) first applied electron microscopy to the study of dieldrin toxicosis. They demonstrated that dieldrin produced enlargement of the liver and hypertrophy of the smooth endoplasmic reticulum (SER), along with increase in microsomal protein and activities of drug-metabolizing enzymes. These findings were confirmed by Hutterer *et al.* (1968), who also demonstrated that in dieldrin-tolerant rats the SER was not only hypertrophic but hyperfunctional. If, however, such rats were given much higher doses of dieldrin, a phase of decompensation occurred and was associated with hypertrophic but hypofunctional SER. It was concluded that this may serve as a sensitive indication of toxicosis even before histopathologic changes are recognized.

MATERIALS AND METHODS

Pigs

Management. Sixteen young pigs and 9 pregnant sows were used in 3 experiments. All swine were housed in pens with concrete floors. Metal troughs were used as feed containers and automatic waterers provided drinking water *ad libitum*. Clinical observation was maintained.

Autopsy Procedure. The pigs were examined postmortem for gross pathologic changes. Specimens were obtained from the brain, heart, liver, kidney, adrenals, lungs, spleen, stomach, and intestines for histopathologic examination. Sections from these tissues were routinely stained with hematoxylin and eosin. Formalin-fixed sections of liver, kidney and adrenal were stained for fat with the oil red O stain.

Residual Dieldrin Determination. In all 3 experiments tissues were collected from the liver, kidney and abdominal fat, and additionally from the brain of some pigs in Experiment II. Fetal brain and liver, and sometimes kidney and fat, were also collected in Experiments II and III. These samples were used for determination of tissue dieldrin residue by gas chromatography after the tissues had been prepared according to Zabik's procedure.[1] A weighed amount of each tissue sample (usually 20 gm. or less) was ground in acetonitrile (CH_3CN) using a Waring blender

[1]Procedure used in Extraction of Residues from Fat and Tissue Samples. Laboratory Work Sheet, Entomology Department, Michigan State University, East Lansing, Mich.

for 3 minutes at "fast" speed. Fifty milliliters of CH_3CN plus 1 ml./gm. of tissue were used in the initial extraction. The mixture was allowed to settle and the supernatant fluid poured through a glass-wool filter into a separatory funnel. The extraction was repeated using 100 ml. CH_3CN, but the filtration was done without allowing the sediment to settle first. The dieldrin was extracted from the CH_3CN with 100 ml. of hexane (C_6H_{14}) by shaking the mixture vigorously 15 to 20 times and letting the 2 layers form. The acetonitrile was then washed out with 2 washes of about 200 ml. of 10% sodium chloride (NaCl) solution in the separatory funnel. The salt solution was rejected and the hexane layer dried with anhydrous sodium sulfate (Na_2SO_4). The following procedures were then carried out by the Analytical Laboratory of the Pesticide Research Center. The dry hexane extract was then introduced into a sodium sulfate--Florisil[1]/Celite[2] (5:1) column for cleanup. Elution with hexane was continued until about 300 ml. of the much diluted final sample was obtained. About 2 microliters (μl) of this sample was injected into the gas chromatograph,[3] along with a dieldrin standard, and readings were recorded and utilized for calculation of concentrations. Chromatograms were obtained using a 6 ft. x 1/8 in. stainless steel column packed with OV-17/QF-1 (11%) (1:1.3). Operating parameters were: inlet temperature 275 C, column 215 C, detector 275 C, and the carrier gas was helium at a flow-rate of 40 ml./mm.

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[1] Florisil. Fisher Scientific Co., Cleveland, Ohio.

[2] Celite. Johns-Manville Co., New York.

[3] Gas Chromatograph. Beckman GC-4 Discharge Electron Captive Detector. Beckman Instruments, Inc., Fullerton, California.

Blood was collected during exsanguination of the stunned pigs and allowed to clot. The serums were recovered, extracted with hexane, and subjected to the same procedures as given above for dehydration, cleanup and gas chromatography. The dieldrin residue values are expressed in parts per million (ppm) on a fresh weight basis.

Serum Protein Determination. Samples of the serums before extraction with hexane were used for total serum protein determinations by Kingsley's (1942) method. The serum proteins were also separated by the Gelman rapid electrophoresis method with Sepraphore III in Model 51101 Chamber[1] at room temperature. Beckman-prepared buffer, dye and fixatives were used, and the relative intensities of the fractions were determined with an analytrol.[2]

Guinea Pig Experiments

A total of 147 guinea pigs were utilized for 4 experiments. In this series laboratory investigations included autopsies on dead and euthanatized guinea pigs and fetuses, histopathologic examination of tissue samples collected at necropsy, and preparation of liver, kidney, fetuses and/or fat and brain samples for dieldrin residue analysis by gas chromatography. Samples from the lower treatment levels in Experiment VI were not subjected to chromatographic analysis.

All guinea pigs were housed in topless sheet metal cages that measured 1.5 x 2.5 x 1.0 feet. Generally, 4 were housed in each cage. Ceramic bowls were used to provide feed and water. The bedding consisted of wood shavings. Once a day cabbage was provided.

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[1] Gelman Instrument Co., Ann Arbor, Michigan.

[2] Spinco Model R. Beckman Instruments, Inc., Palo Alto, Calif.

EXPERIMENTAL PROCEDURE AND RESULTS

Pigs

Experiment I. The object of the experiment was to compare the toxicity of dieldrin for pigs of approximately the same age maintained after weaning on either a protein-deficient or normal diet.

Procedure. Sixteen pigs, 6 males and 10 females, between 7 and 8 weeks of age, were divided randomly into 2 groups of 8 each. Group I was fed a special low-protein (4% casein) piglet diet which was otherwise adequate (Table 1). Group II pigs were fed a normal pig diet obtained from the Michigan State University Swine Farm.

The pigs in Group I were gradually introduced to the protein-deficient diet over a 4-day period. Both groups were allowed 2 lbs. of the appropriate feed per pig daily and were provided drinking water from automatic waterers. The weights of the pigs were recorded at 0, 14, and 21 days (Figure 1). At the end of this period 4 randomly selected pigs from each group were dosed *per os* with dieldrin in a gelatin capsule at the rate of 30 mg./kg. body weight. The remaining 4 pigs in each group were maintained as nontreated controls. After a variable number of hours of observation, all pigs were killed by electrocution followed by exsanguination.

Table 1. Composition of the protein-deficient diet* for pigs in Experiment I

Ingredient	Proportion (%)
Alphacel	10.0
Dextrose	74.0
Lard	5.0
Corn oil	1.0
Casein (Vitamin Free)	4.0
Salt mixture	6.0
Vitamin Diet Fortification Mixture**	

*Nutritional Biochemicals Corporation, Cleveland, Ohio.

**Formulation

	grams/100 lbs. diet
Vitamin A Concentrate (200,000 units per gram)	4.5
Vitamin D Concentrate (400,000 units per gram)	0.25
Alpha Tocopherol	5.0
Ascorbic Acid	45.0
Inositol	5.0
Choline Chloride	75.0
Menadione	2.25
p Aminobenzoic Acid	5.0
Niacin	4.5
Riboflavin	1.0
Pyridoxine Hydrochloride	1.0
Thiamine Hydrochloride	1.0
Calcium Pantothenate	3.0
	mgms/100 lbs. diet
Biotin	20
Folic Acid	90
Vitamin B-12	1.35

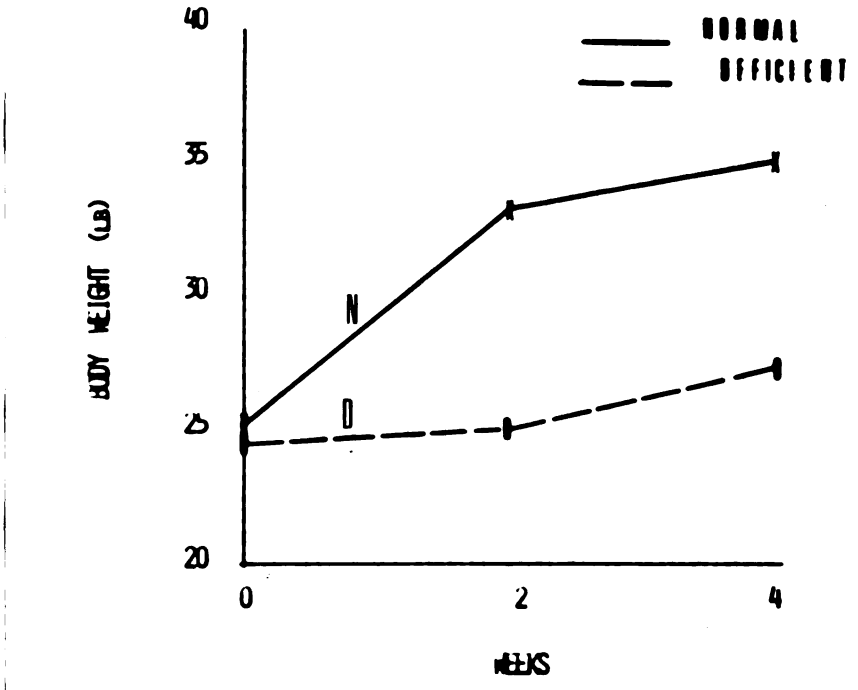

Figure 1. Group average weights of pigs fed normal (N) or protein deficient diet (D) for 21 days.

Results

Growth. The pigs fed low-protein diets manifested an overall reduction in rate of weight gain as compared with the pigs fed the normal diet (Figure 1).

Signs. No definitive signs of protein deficiency were observed. All pigs given dieldrin showed signs of toxicosis to variable degrees. Drowsiness, huddling, shivering, twitching of muscles, and occasionally prostration were observed. One pig manifested frothing at the mouth. The signs first appeared about 1 hour after administration but, in general, were most intense at the 2nd hour. Pigs of the protein-deficient group dosed with dieldrin were most severely affected.

Analyses

Hematologic determinations. The hemoglobin and hematocrit values were normal, as were the total and differential leukocyte counts.

Serum electrophoresis. The total protein values were within normal range, but there were alterations in component fractions which affected the albumin/globulin (A/G) ratio. In most of the samples there was a reduction in albumin associated with a relative increase in the globulins, especially α_2 fraction. The net result was a reduced A/G ratio, which was especially marked in deficient pigs 1 and 3 (Table 2).

Residual dieldrin. Dieldrin was demonstrated in all analyzed tissues from treated pigs (Table 3).

Table 2. Serum proteins--total protein, albumin, and α_2 globulin values, A/G ratios of representative pigs not given dieldrin in Experiment I

Feed Status	Pig No.	Sex		Total Protein (gm/100 ml)	Albumin (gm/100 ml)	α_2 (gm/100 ml)	A/G
Deficient	1	M	Initial	5.10	2.14	0.91	0.72
			Terminal	6.31	1.75	1.22	0.38
	2	F	Initial	4.86	2.10	0.85	0.76
			Terminal	5.97	2.52	1.21	0.73
	3	F	Initial	4.71	2.24	0.92	0.91
			Terminal	5.63	1.55	1.45	0.38
Normal	1	M	Initial	5.53	2.39	0.91	0.76
			Terminal	5.97	1.98	1.11	0.58
	2	M	Initial	4.56	1.96	1.01	0.75
			Terminal	5.46	1.95	1.09	0.56
	3	F	Initial	4.78	1.90	0.95	0.66
			Terminal	5.36	1.91	1.12	0.55

Table 3. Dieldrin concentrations (ppm) in tissues from representative treated pigs in Experiment I

Feed Status	Pig No.	Sex	Tissue Dieldrin Concentration (ppm)			
			Serum	Fat	Liver	Kidney
Deficient	1	M	0.66	8.71	2.41	0.96
	2	F	0.08	0.09	0.10	0.06
	3	F	0.04	0.13	n.d.*	n.d.
	4	F	0.30	0.25	0.01	0.26
Normal	1	M	0.56	2.27	3.67	1.45
	2	M	0.30	1.52	2.42	0.93
	3	F	0.26	1.27	2.31	1.74
	4	F	0.26	1.10	2.00	1.02

*n.d. = not done

Lesions. Grossly, the livers of the deficient pigs were of a dull gray color. Histologically, the pathologic changes consisted of sinusoidal dilatation, slight hyperemia and fatty metamorphosis with predominantly centrilobular distribution (Figure 2).

Kidney. The gross change was a paleness in the deficient group. There was histologic evidence of patchy vacuolar degeneration of proximal convoluted tubular epithelium.

Lungs. There were petechial hemorrhages in the lungs of all the pigs. These were characterized histologically as a more diffuse hyperemia involving alveolar capillaries. This change was regarded as agonal.

Experiment II. The experiment had as its objective the determination of toxicosis associated with dieldrin in sows during the immediate preparturient period. The experiment was also designed to demonstrate possible transplacental transfer of the chemical, the degree of dieldrin accumulation in the fetal liver, and any clinical effects and lesions resulting from such toxicosis.

Procedure. Dieldrin was administered *per os* in the mornings of alternate days at the rate of 15 mg./kg. body weight to 2 sows in the last 2 weeks of pregnancy using gelatin capsules and a metal balling gun. A total of 3 administrations was achieved before the sows were killed by electrocution and exsanguination. Using similar methods, 2 other sows were given dieldrin daily in the morning at a rate of 9 mg./kg. body weight for a total of 9 administrations before euthanasia. Two nontreated sows were used as controls (Table 4).

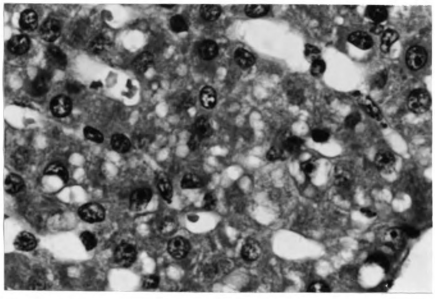

Figure 2. Liver. Lipidosis in pig fed protein-deficient diet and dosed with dieldrin. H & E stain. x 600.

Table 4. Duration of treatment and total dieldrin intake of sows in Experiment II

No. in Group	Duration (days)	Avg. wt. of sows (kg.)	Dieldrin		
			Dosage (mg/kg)	No. of Doses	Total Given (gm.)
2	*	133.8	0	0	0
2	5	133.3	15	3	6
2	13	166.6	9	9	13.50

*One control sow was sacrificed after 5 days to correspond with the sows in the second group; the other was sacrificed after 13 days to correspond with the sows in the third group.

Results

Signs. There were no obvious signs of intoxication. One of the control sows farrowed 1 dead and 7 live pigs before sacrifice. The other control sow had 1 dead and 11 live fetuses in the uterus when it was sacrificed. No fetal deaths were observed in either group of treated sows.

Food and water consumption. These remained normal throughout the duration of the experiment.

Analyses

Residual dieldrin concentration. Residual dieldrin was present in the fat, liver, kidney, and brain at appreciable levels (Table 5) in the treated sows. The brains and livers of randomly selected fetuses were also analyzed and found to contain fairly large quantities of dieldrin when compared to the fetuses from nontreated controls (Table 6).

Lesions

Liver. Grossly there was a dullness to the livers with some evidence of mottling in one sow's liver. Histologic changes consisted of hyperemia, some lymphocytic infiltration into the portal triads, and vacuolar degeneration of hepatocytes.

Kidneys. These were pale on gross examination. Microscopically, hyperemia of the glomeruli and vacuolar degeneration of the epithelium of proximal convoluted tubules in discrete areas were evident (Figure 3). As for the liver, histologic changes appeared to increase with increasing duration of treatment.

Table 5. Residual dieldrin concentration (ppm) in tissues of sows in Experiment II and total amounts of dieldrin given

Status of Sow	Total Dieldrin (gm.)	Sow No.	Tissue Dieldrin Concentration (ppm)			
			Fat	Liver	Kidney	Brain
Treated	6.0	1	5.02	3.59	1.28	n.d.*
	6.0	2	4.70	3.80	1.49	n.d.
	13.5	3	n.d.	4.68	1.23	1.18
	13.5	4	n.d.	2.96	0.87	0.90
Non-treated		1	0.23	0.01	0.007	n.d.
		2	n.d.	0.00	0.00	0.00

*n.d. = not done

Table 6. Residual dieldrin concentration (ppm) in fetal tissues from Experiment II

Fetus No.	Total Dieldrin Fed to Sow (gm.)	Tissue Dieldrin Concentration (ppm) Liver*	Brain
1	0	0	0
2	0	0	0
3	0	0	0
4	6.00	0.16	n.d.**
5	6.00	0.09	n.d.
6	6.00	0.08	n.d.
7	6.00	0.27	n.d.
8	6.00	0.14	n.d.
9	6.00	0.13	n.d.
10	13.50	1.51	0.43
11	13.50	1.05	0.26
12	13.50	1.14	0.47
13	13.50	1.35	0.27
14	13.50	0.60	0.26
15	13.50	0.41	0.25

*($P < .025$) for fetuses of 2 sows that were given totals of 6.0 gm. and 13.5 gm. dieldrin, respectively.

**n.d. = not done

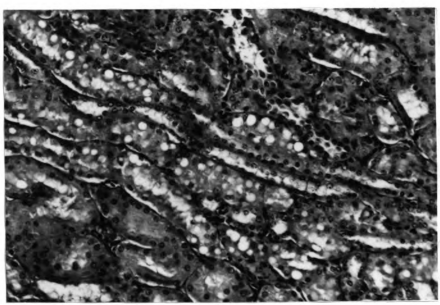

Figure 3. Kidney. Vacuolar degeneration of epithelium of proximal convoluted tubules of dieldrin-treated sow. H & E stain. x 150.

Lungs. Histologically there was hyperemia of the alveolar capillaries. The petechiae in the lungs were regarded as agonal changes in both categories of sows.

Uteri. These appeared edematous and congested grossly. No histologic changes other than the edema of the membranes and hyperemia were observed.

Experiment III. In a prior experiment with nitrite administered alone to a pregnant sow no effect on fetuses was demonstrated. Repeated dieldrin dosage to pregnant sows in another experiment did not produce any observable effect on the fetuses. The present experiment was designed to see if fetuses would be killed and aborted following repeated administration of dieldrin in conjunction with nitrite. It was also intended to determine if the vasodilatory effect of nitrite would produce a correspondingly greater accumulation of dieldrin in the fetal tissues.

Procedure. Three sows in the last trimester of gestation were utilized. Two sows were given dieldrin *per os* in gelatin capsules at the rate of 15 mg./kg. body weight, as well as sodium nitrite ($NaNO_2$) by subcutaneous injections of a 1% solution at the rate of 25 mg./kg. body weight. The third sow, inoculated with 1% $NaNO_2$ solution subcutaneously at the same rate, served as the positive nitrite control. These sows each received a total of 24 administrations of appropriate chemicals before death or euthanasia by electrocution and exsanguination (Table 7).

Results

Food and water consumption. Depression of appetite was observed in one of the sows receiving both dieldrin and nitrite. This occurred in the last 2 days before death.

Table 7. Duration of treatment and total dieldrin and $NaNO_2$ intake by sows in Experiment III

Number Represented	Duration of Experiment (days)	Dieldrin		$NaNO_2$	
		Daily Dosage (mg/kg)	Total Given (gm)	Daily Dosage (mg/kg)	Total Given (gm)
1	28	15.0	48.0	25.0	84.0
1	28	15.0	48.0	25.0	84.0
1	28	0.0	0.0	25.0	84.0

Signs. There was a slight brownish discoloration of all visible mucous membranes of the 3 sows. One dieldrin/nitrite-treated sow died in severe clonic convulsions. The nitrite control sow also died but the 2nd dieldrin/$NaNO_2$-treated sow remained normal until killed. Fetuses from all 3 sows appeared normal on gross examination.

Analyses

Residual dieldrin. Dieldrin was demonstrated in the fat, liver, kidney, and brain of sows given this chemical. The livers and brains of fetuses from these sows also contained dieldrin (Table 8).

Lesions

Liver. Some mottling was evident. Histologically, all the livers were hyperemic and manifested centrilobular fatty metamorphosis. The liver of the nitrite control sow was infiltrated in the portal triads, interlobular septa and parenchyma by neutrophils and a few eosinophils. The septa were thickened by edema fluid.

Kidney. Grossly the kidneys appeared dull gray but firm. Microscopic examination revealed hyperemia of the glomeruli with vacuolar degeneration of the proximal convoluted tubular epithelium in the dieldrin/nitrite treated sows (Figure 4). There was hyperemia with interstitial hemorrhage in the kidney of the nitrite control sow. Degeneration of tubular epithelium with neutrophilic and lymphocytic infiltration of the interstitium was present.

Spleen. The spleens were only slightly hyperemic.

Table 8. Dieldrin concentrations in tissues of sows and their fetuses in Experiment III

Sow No. and Status		Tissue Dieldrin Concentration (ppm)			
		Fat	Liver	Kidney	Brain
Dieldrin & Nitrite	1	54.60	4.26	1.27	1.24
	F1*	n.d.**	0.86	n.d.	0.41
	F2	n.d.	1.15	n.d.	0.47
	2	26.06	9.09	1.85	1.00
	F1	n.d.	0.16	n.d.	0.18
	F2	n.d.	0.17	n.d.	0.12
	F3	n.d.	0.17	n.d.	0.23
Nitrite	1	0	0	0	0
	F1	n.d.	0	n.d.	0
	F2	n.d.	0	n.d.	0

*Fetuses of sows numbered immediately above.

**n.d. = not done

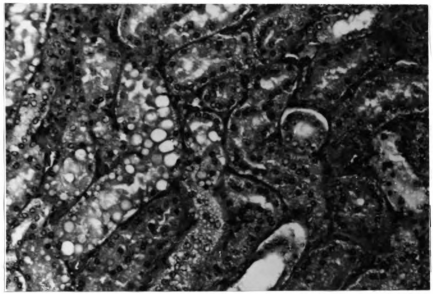

Figure 4. Kidney. Vacuolar degeneration of proximal convoluted tubular epithelium of dieldrin-nitrite treated sow. H & E stain. x 150.

Uteri. No abnormalities were observed either grossly or microscopically.

Fetuses. The fetuses appeared normal grossly. Histologically the livers were hyperemic, especially in the peripheral zone. There was an abundance of glycogen in the hepatocytes, which was confirmed by staining with Best's carmine. Megakaryocytes and nucleated erythrocytes were observed in fairly large numbers. Fetuses from all 3 sows manifested similar histologic appearance.

Guinea Pigs

A total of 147 guinea pigs was utilized for 4 experiments.

Experiment IV. The objects of the experiment were to investigate the toxicosis of dieldrin in guinea pigs and to obtain evidence of transplacental transfer of the chemical.

Procedure. Dieldrin was dissolved in cottonseed oil at a concentration of 60 mg./ml. Forty-six guinea pigs (30 pregnant females, 8 nonpregnant females, and 8 males) were given this preparation orally on a body weight basis as indicated in Table 9. Administration was repeated at about 5-day intervals until death or sacrifice by ether anesthesia. An additional 10 guinea pigs were maintained as controls and each received 0.5 ml. of cottonseed oil *per os* at 5-day intervals (Table 9).

Results

Signs. One of the dieldrin-treated guinea pigs farrowed 4 apparently healthy pups which, however, died 1 hour postnatally. The dam appeared to be normal before and after farrowing. Three males, 28 pregnant and 4 nonpregnant females died at various times in the course

Table 9. Groups and numbers of guinea pigs represented, sex, dosage, duration, and total quantity of dieldrin given in Experiment IV

Group	No. Represented*	Sex		Dose (mg/kg)		No. of Treatments	Duration Before Death (days)	Total Dieldrin Given (mg.)
		Male	Female	Initial	After			
1	2	1	1	60	-	1	3	60
2	10(4)	1	9	30	-	1	3	30
3	1		1	30	30	2	7	60
4	1		1	30	30	3	14	90
5	6	3	3	30	15	7	39	120
6	11(3)	2	9	15	15	6	26	90
7	15(5)	1	14	15	15	8	31	120
8	3	1	2	0	0	1	3	0
9	3	1	2	0	0	6	26	0
10	4	2	2	0	0	8	39	0

*Numbers in parentheses represent sacrificed guinea pigs.

of the experiment. Death in all instances was preceded by a period of depression, severe dyspnea and terminal convulsions. Epistaxis was observed in one nonpregnant guinea pig.

Frequently some of the guinea pigs became excited 2 or more hours after dieldrin administration. Such pigs often leaped high and performed gyrating movements, thus making it necessary to cover the cages with a grid metal top.

Analyses

Residual dieldrin. Dieldrin residues were present in tissues of treated guinea pigs. Dieldrin was also recovered in whole fetuses of the same guinea pigs (Table 10).

Lesions

Gross. The only consistent lesions were dull appearance of the livers and kidneys of all dieldrin-treated guinea pigs and petechiation of lungs of both the treated and the nontreated controls. However, a few amongst the dieldrin-treated pregnant guinea pigs manifested slight enlargement of the spleen and adrenal gland, gelatinous perirenal fat, and gastric congestion.

Histopathologic

Liver. Lipidosis, more commonly of centrilobular distribution, was observed and confirmed by staining with oil red O. This was in contrast to the abundant glycogen in livers of nontreated guinea pigs (Figures 5 and 6), confirmed by the Best's carmine stain. Hyperemia was a feature of the livers from dieldrin-treated guinea pigs.

Table 10. Groups and numbers of guinea pigs sampled, total dieldrin received, and average tissue and fetal concentrations (ppm) of dieldrin in Experiment IV

Group	No. of Samples	Total Dieldrin Received (mg.)	Average Dieldrin Concentrations (ppm)				
			Fat	Liver	Kidney	Brain	Fetus
2	2	30	1.95	29.06	n.d.*	n.d.	1.72
3	1	60	19.88	17.76	3.11	7.12	-
4	1	90	33.39	25.50	7.32	5.63	1.42
5	3	120	43.62	14.27	3.17	3.43	3.08
6	1	90	32.34	15.32	12.71	5.36	n.d.
7	3	120	40.50	29.19	5.32	6.94	n.d.
8,9,10	3	0	0.04	0	0	0	0

*n.d. = not done

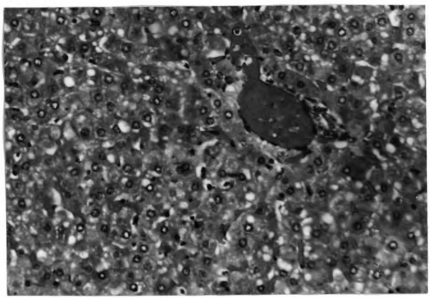

Figure 5. Liver. Lipidosis in dieldrin-treated guinea pig. H & E stain. x 150.

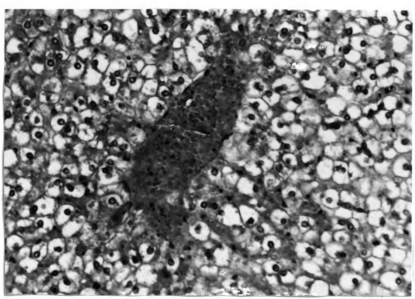

Figure 6. Liver. Control. Note presence of glycogen confirmed by the Best's carmine stain. x 150.

Kidneys. These were generally hyperemic. Focally, the proximal convoluted tubules manifested epithelial vacuolar degeneration (Figure 7) in the treated guinea pigs.

Spleen. Only the grossly enlarged spleens were hyperemic histologically.

Lungs. Hyperemia and atelectasis with compensatory alveolar emphysema were observed in the lungs of most treated guinea pigs (Figure 8).

Stomach. Hyperemia was confirmed microscopically in instances where there was gross congestion. There was gastric mucosal necrosis in some instances.

Uteri. The hypertrophic mucosae were edematous. In 2 instances mucosal necrosis, early mineralization and neutrophilic infiltration were observed. The 2 guinea pigs that manifested necrosis of uterine mucosa had the highest quantities of dieldrin.

Adrenal glands. Lipidosis was demonstrated in the grossly enlarged glands of treated guinea pigs.

Heart. Focal myocardial necrosis with mineralization was observed in only 1 instance.

Fetal livers. There were no demonstrable abnormalities in the livers of fetuses, even from animals that died shortly after birth.

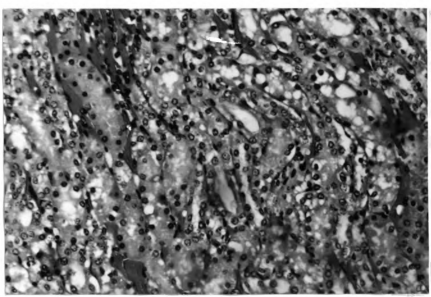

Figure 7. Kidney. Epithelial vacuolar degeneration in proximal tubules of dieldrin-treated guinea pig. H & E stain. x 150.

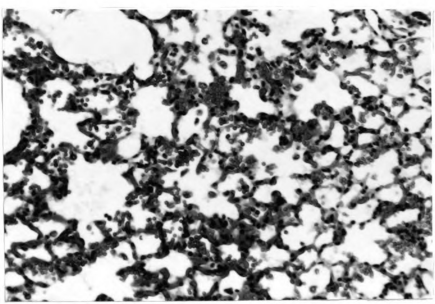

Figure 8. Lung. Hyperemia, atelectasis and alveolar emphysema in dieldrin-treated guinea pig. H & E stain. x 150.

Experiment V. The object of this experiment was to determine the combined toxicologic effect of dieldrin and nitrite in guinea pigs. Special emphasis was placed on the effects on pregnancy and upon fetal concentration of the pesticide.

Procedure. Twelve female guinea pigs, 7 pregnant and 5 nonpregnant, were given 15-45 mg. of dieldrin in cottonseed oil calculated on a dosage rat of 60 mg./kg. body weight. This was administered as drops in the mouth after appropriate individual doses had been measured with a 1 ml. syringe. The same guinea pigs were injected subcutaneously into the neck region with 1% $NaNO_2$ solution at the rate of 30 mg. (3 ml.) per guinea pig. Administration of both chemicals was continued on alternate days until the animals died or were euthanatized with ether.

A nitrite control was set up by administering $NaNO_2$ solution (1%) subcutaneously to 12 guinea pigs at the same rate of 30 mg./guinea pig. With another control group of 10 guinea pigs, each was given 1 ml. of cottonseed oil by mouth for the duration of the experiment, but no $NaNO_2$ solution was injected (Table 11).

Additional laboratory investigations in this experiment consisted of determination of hemoglobin and methemoglobin levels and the packed cell volume of randomly selected guinea pigs from all the groups except the nitrite group in the first 2 days of the experiment. Hemoglobin was estimated by the standard cyanmethemoglobin method. Evelyn and Malloy's method as described by Hawk *et al.* (1954) was adopted for methemoglobin determination while the capillary tube method was used for evaluating the packed cell volume. Blood for these determinations was obtained from the metacarpal vein as described by Uzoukwu and Sleight (1970).

Table 11. Numbers of guinea pigs represented, sex, total quantity of chemical given and duration of observation in Experiment V

Number Represented	Sex		Total Quantities Given (mg.)		Duration (days)
	Male	Female	Dieldrin	$NaNO_2$	
10	4	6	0	0	1-8
3*	1	2	60	0	3-7
12**	-	12	45	30-120	1-7
12	4	8	0	30-150	1-8

*Data extracted from Table 9, Experiment IV.

**Two abortions occurred.

Results

Signs. Slight brownish discoloration of visible mucous membranes was seen in animals of the nitrite treated group. Terminal convulsions and dyspnea were observed only in the 7 dieldrin and nitrite treated guinea pigs that died. Two guinea pigs given dieldrin and nitrite aborted.

Analyses

Hematology. There were no abnormal values for hemoglobin and hematocrit before or after treatment. However, there was a definite increase in methemoglobin in nitrite recipients usually within 1 hour of its administration.

Residual dieldrin. This was demonstrated in the tissues and whole fetuses analyzed (Table 12).

Lesions. No gross or histologic abnormalities were identified in the tissues of the untreated guinea pigs. In the other groups there was a variable amount of brown discoloration of tissues, the intensity of which appeared to depend on the time of death following nitrite administration. The perirenal fat was gelatinous in some guinea pigs.

Liver. There were no gross lesions. Histologically, centrilobular fatty metamorphosis was observed when the guinea pigs had been given dieldrin and nitrite for at least 4 days. Some degree of hyperemia was present in all instances.

Kidney. No gross lesions were observed but histologically hyperemia, and vacuolar degeneration of proximal tubules were discernible.

Table 12. Average tissue and fetal dieldrin concentration, total dieldrin and nitrite given and numbers of guinea pigs represented in Experiment V

Number Represented	Total Received (mg.) Dieldrin	Total Received (mg.) $NaNO_2$	Average Dieldrin Concentration (ppm) Fat	Liver	Kidney	Brain	Fetus
2	0	0	0	0	0	0	0
3	0	30-150	0	0	0	0	0
1*	60	0	19.88	17.76	3.11	7.12	0
2	45	30	29.99	4.82	4.17	2.38	4.95
2	60	60	30.32	14.27	7.32	3.05	1.66
2	60	120	70.20	29.26	12.07	8.94	n.d.**

*Data abstracted from Table 10, Experiment IV.

**n.d. = not done

In some cases there was slight lymphocytic infiltration of the interstitium.

Spleen. Some of the spleens in the dieldrin-nitrite treated guinea pigs were slightly enlarged. Microscopically, this enlargement was associated with hyperemia.

Adrenal. There was a histologically observable increase in cortical fat in guinea pigs given dieldrin and nitrite.

Uteri. Grossly the uteri of the guinea pigs that aborted were congested. Thickened arterial media with swollen and degenerated cells were seen microscopically (Figure 9). The epithelium was hypertrophic, vacuolate and necrotic in parts (Figure 10).

Lungs. Petechiation of the lungs was a constant feature in the dieldrin-treated guinea pigs. These lungs were observed in sections to be hyperemic, atelectatic, and emphysematous.

Experiment VI. Since dieldrin and nitrite could be either natural or accidental contaminants of animal feed and water, Part I of this experiment was designed to simulate this situation and study the combined toxicoses. The second part of the experiment was an attempt to determine effects of lower levels of the 2 chemicals used.

Procedure

Part I. Fourteen 1- to 2-month pregnant and 5 nonpregnant guinea pigs were fed for a variable period crushed guinea pig pellets[1]

[1] Rockland Laboratory Animal Diets. Tekland, Inc., Monmouth, Ill.

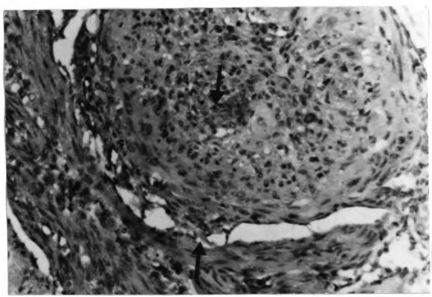

Figure 9. Uterus. Occlusion of small artery, thickening and vacuolation of arterial media (arrows) in dieldrin-nitrite treated guinea pig. H & E stain. x 150.

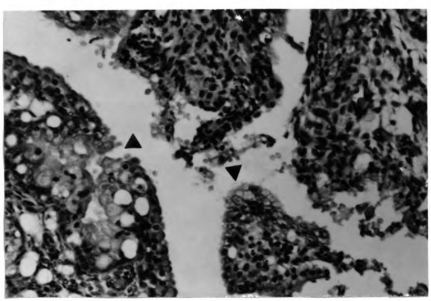

Figure 10. Uterus. Hyperplasia, vacuolation and necrosis (arrows) in guinea pig treated with dieldrin and nitrite. H & E stain. x 150.

that had been treated with dieldrin at a concentration of 100 ppm. They were also provided with 0.5% $NaNO_2$ solution for drinking *ad libitum*. In order to attempt differentiation of the action of dieldrin from responses to nitrite poisoning, controls were set up in which 5 guinea pigs drank 0.5% $NaNO_2$ solution in place of tap water but were fed normal crushed pellets, while 5 others ate dieldrin-treated feed but drank tap water (Table 13).

Part II. Three pregnant and 6 nonpregnant guinea pigs were fed diets treated with dieldrin at levels indicated in Table 14. They were allowed to drink 0.25% solution of $NaNO_2$ at will. Another group of 9 pregnant and 3 nonpregnant guinea pigs was fed dieldrin at similar levels but drank plain tap water. The dieldrin and nitrite control groups consisted respectively of 3 guinea pigs that were given neither dieldrin nor nitrite and 3 that drank 0.25% $NaNO_2$ solution only. Body weights were recorded at weekly intervals (Table 15).

Results (Part I)

Food and fluid consumption. After an initial reluctance to drink the $NaNO_2$ solution, the guinea pigs soon adjusted to it. Thereafter, consumption of the solution and the feed remained reasonably normal until the terminal stage. Refusal of food and fluid was consistently observed in the 24 hours preceding convulsions and death.

Signs. Abortion was commonly seen. Of the 14 pregnant guinea pigs that were given dieldrin and nitrite, 9 aborted and 3 died 2 to 5 weeks after the initiation of the experiment. Three died amongst the nonpregnant group of 5 that received the same treatment as above. Dieldrin alone caused 2 abortions and 2 deaths in a group of 5

Table 13. Number of female guinea pigs, concentrations of chemicals, and periods of observation in Experiment VI (Part I)

Number Represented	Concentration of Chemical Given*		Duration (days)
	Dieldrin (ppm)	$NaNO_2$ (%)	
14	100.0	0.5	30-41
5	100.0	0.0	37-41
5	0.0	0.5	21-45

*Dieldrin was fed in the food, while $NaNO_2$ was added to the drinking water.

Table 14. Concentrations of chemicals given in feed and water for 53 days, and groups of pregnant and nonpregnant guinea pigs in Part II of Experiment VI

Group No.	No. of Guinea Pigs	Concentrations of Chemicals	
		Dieldrin (ppm)	$NaNO_2$
1	4(3)*	50.0	0.0
2	4(3)	25.0	0.0
3	4(3)	10.0	0.0
4	3(1)	50.0	0.25
5	3(1)	25.0	0.25
6	3(1)	10.0	0.25
7	3(1)	0.0	0.25
8	3(1)	0.0	0.0

*Numbers in parentheses indicate numbers of pregnant animals.

Table 15. Mean body weights (gm.) of guinea pigs at about 6-day intervals during Part II of Experiment VI

Day of Experiment	Group No.	Mean Body Weight (gm.) 1 (4)	2 (4)	3 (4)	4 (3)	5 (3)	6 (3)	7 (3)	8 (3)
1		836	880	866	853	952	887	753	817
6		861	843**	870	830	925	893	742	835
12		888	853	895*	843	933	917	768	822
18		881	790*	791	835	898	882	753	806
23		940	803	791	867	893	957	792	813
30		915*	818	814	858	905	947	812	795
37		916	847	796	847	868	965	795	797
44		811*	855	781*	800*	830	898	790	780
53		725*	718**	764*	752	848	800**	732*	973**

*One farrowed and this explains the drop in subsequent mean body weight value.

**One died

guinea pigs, while nitrite alone induced abortion but no death in 3 out of 5. No signs of impending abortion were observed, but death in all cases was preceded by severe clonic convulsions.

Results (Part II)

Food and fluid consumption. In none of the groups was either food or fluid consumption severely affected after the period of adjustment by the nitrite-drinking guinea pigs. However, by comparison, the guinea pigs given water with or without dieldrin-treated feed drank more fluid than the nitrite group.

Body weight. Individual guinea pigs manifested a fluctuation in their rate of weight gain mainly in relation to their pregnancy status. Nonpregnant guinea pigs gained weight with less fluctuation in body weight.

Signs. Amongst the 12 pregnant guinea pigs, 9 farrowed normally irrespective of the level of dieldrin given. The remaining 3 died. Two of these belonged to the group that was given 25 ppm of dieldrin and no nitrite. One of these died 6 days after the start of the experiment, while the other died acutely on the last observation day with a torsion of the uterine horn. The third guinea pig belonged to the group given 10 ppm dieldrin and 0.25% $NaNO_2$. Postmortem changes were too advanced to permit a diagnosis. One of the nontreated, nonpregnant controls died of an undetermined cause.

Lesions. In general, grossly there was a slight brownish discoloration of tissues in all guinea pigs that were given $NaNO_2$ with or without dieldrin. The livers appeared fatty, and the kidneys pale. Most

uteri were still large and edematous when the guinea pigs were sacrificed following abortion. The spleens of the test guinea pigs were slightly enlarged. No gross lesions were observed in other organs except the lungs, which were petechiated to varying degrees. Most of the nonpregnant guinea pigs had excessive abdominal fat of a normal consistency.

Histology

Liver. The histologic pattern was essentially similar in all treatment groups but varied in degree in relation to the duration of treatment. Sinusoids were dilated and contained blood, while hepatocytes had undergone fatty metamorphosis.

Kidney. There was a distinct hyperemia of the glomeruli. Patchy vacuolar degeneration of the epithelium of proximal convoluted tubules was marked in the dieldrin-nitrite group (Figure 11).

Uteri. Submucosal edema was evident. The uterine villi were hyperplastic and, in 2 instances, appeared to possess degenerated epithelium. Necrosis of the junctional epithelium of the placenta with neutrophilic infiltration was observed in one specimen from the dieldrin-nitrite group. Medias of small arteries were thickened and vacuolated; the changes sometimes caused partial occlusion of the lumina.

Other organs. The lungs were usually hyperemic. The spleen was oftentimes hyperemic. Increase in adrenal cortical lipid was not a constant finding. No appreciable changes were observed in the heart and gastrointestinal tract.

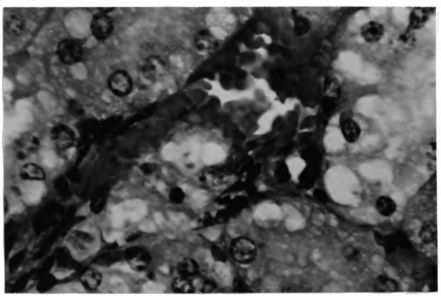

Figure 11. Renal hyperemia and vacuolar degeneration of proximal tubular epithelium in guinea pig treated with dieldrin and nitrite. H & E stain. x 600.

ULTRASTRUCTURAL STUDIES

Six 3-month-old guinea pigs were used in an experiment to determine the ultrastructural changes in the brain and liver in acute dieldrin toxicosis.

Procedure

Four randomly selected guinea pigs were injected intraperitoneally with dieldrin in cottonseed oil at the rate of 75 mg./guinea pig, irrespective of body weight, and kept under constant observation. Two of these were euthanatized with ether and exsanguinated at the peak of convulsions. Two were killed at the stage of pronounced depression which always preceded the convulsions. The remaining 2 guinea pigs were used as controls and were injected with 1 ml. of cottonseed oil intraperitoneally. These animals were killed and similar laboratory investigations were carried out for the dieldrin group.

Samples of brain and liver tissues were taken within 5 minutes after death and fixed in 4% glutaraldehyde in Sorensen's phosphate buffer (0.1M, pH 7.2) in an ice bath at 4 C. for 1 hour. They were then post-fixed in 1% osmium tetroxide (OsO_4) solution in Sorensen's phosphate buffer for 30 minutes at room temperature. The tissues were then washed in phosphate buffer and stored in cold (4 C.) 0.2M sucrose solution buffered at pH 7.4 until they were embedded. Later, each tissue was placed in a drop of OsO_4 fixative on a wooden spatula and diced to produce small pieces measuring about 1 cmm. The pieces were picked up with a

flat toothpick and deposited in a glass tube containing 2 ml. of the OsO_4 fixative. Dehydration with graded alcohols (50, 70, 95% and absolute) followed and the specimens were cleared in propylene oxide (Luft, 1961) and infiltrated for 1 hour in a 1:1 mixture of propylene oxide and Epon.[1] A modified Lufts (1961) method was adopted for embedding the selected specimens in Epon using gelatin capsules. An accelerator, 2,4,6-tridimethylaminomethylphenol (DMP-30)[2] at a concentration of 1.5% was added to the resin mixture just before use, and the resin was allowed to polymerize in an oven at 60 C. for 36 hours. Final preparation for electron microscopic examination of the specimens involved cutting sections on a Sorvall "Porter-Blum" ultramicrotome, Model MT-2[3] at 400-500A thickness and mounting these on uncoated grids. The sections were then stained by adopting a slight modification of the method described by Pease (1964) in which uranyl acetate was used as a primary stain and lead citrate, instead of lead hydroxide, secondarily. Sections were examined and photographed in the electron microscope, Philips Model 100B.[4]

Results: Ultrastructural Lesions

Brain. Some of the mitochondria were swollen to several times their normal size. There appeared to have been imbibition of fluid into the swollen mitochondria with resulting disintegration of the cristae (Figures 12 and 13). There was no obvious breach of the mitochondrial membrane. The endoplasmic reticulum and nucleus appeared normal.

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[1] Epon 812. Shell Chemical Corp., San Francisco, California.

[2] Rohm and Haas Co., Philadelphia.

[3] Ivan Sorvall, Inc., Norwalk, Connecticut.

[4] Philips, Mount Vernon, New York.

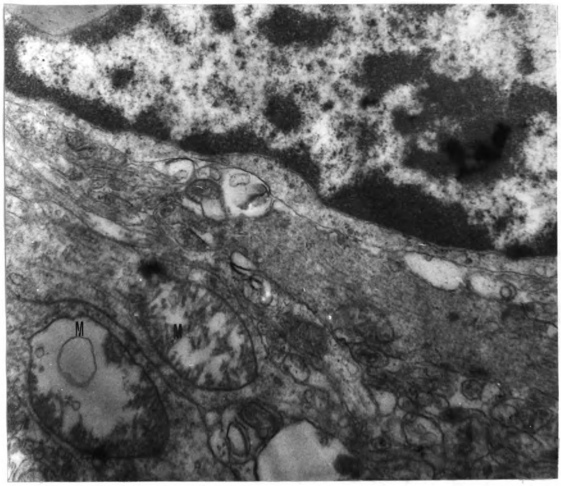

Figure 12. Note swollen mitochondria (M) with disruption of cristae in cerebral cells. Glutaraldehyde-osmic acid fixation, uranyl acetate-lead citrate stain. x 20,000.

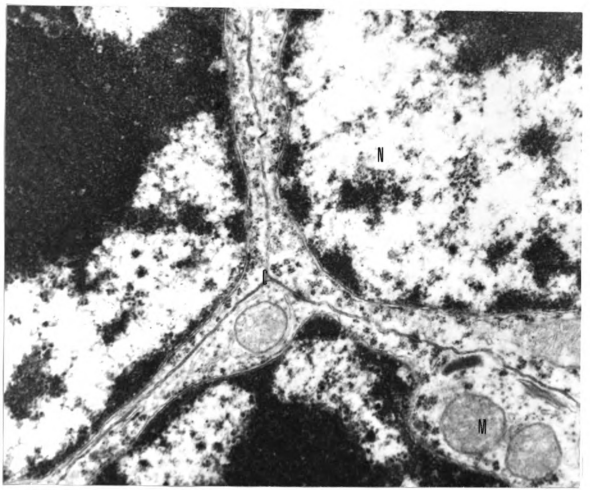

Figure 13. Control. Junction of three cerebellar cells, nuclei (N), cell membranes (C) and normal mitochondria (M). Glutaraldehyde-osmic acid fixation, uranyl acetate-lead citrate stain. x 20,000.

Liver. There was an abundance of "myelin bodies" in the liver sections of both treated and nontreated control guinea pigs. However, neither the mitochondria nor the endoplasmic reticulum appeared to have undergone any alterations (Figures 14 and 15) in the treated guinea pigs.

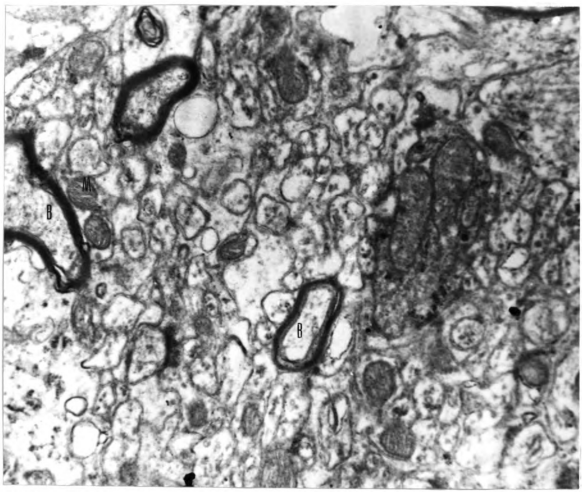

Figure 14. Part of a hepatocyte from a dieldrin-treated guinea pig. Note myelin bodies (B), normal mitochondria (M) and normal endoplasmic reticulum (arrows). Glutaraldehyde-osmic acid fixation, uranyl acetate-lead citrate stain. x 20,000.

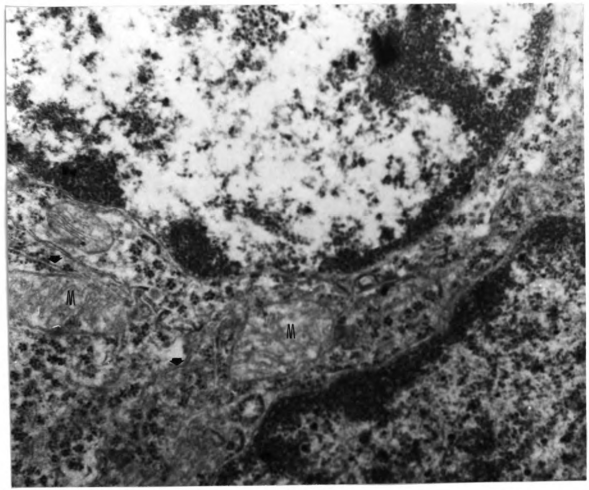

Figure 15. Hepatocytes in a dieldrin-treated guinea pig. Note the normal appearance of the mitochondria (M) and the endoplasmic reticulum (arrows). Glutaraldehyde-osmic acid fixation, uranyl acetate-lead citrate stain. x 25,000.

DISCUSSION

Ample clinical evidence implicates dieldrin essentially as a neurotoxicant to various species of animals. However, it shares with other chlorinated hydrocarbon pesticides the property of inducing nonspecific degenerative and congestive lesions in most parenchymatous organs. Thus, fatty metamorphosis and congestion were prominent in the livers and kidneys of pigs and sows that were fed the chemical. The degree of change observed appeared to be directly related to the duration of treatment.

It is believed that animals on protein deficient diets are most susceptible to poisons that affect the liver (McLean *et al.*, 1965). It has been demonstrated, however, that protein depleted rats are more resistant to the lethal effects of carbon tetrachloride (CCl_4) because of a drastic reduction in enzyme activity of the liver (McLean and McLean, 1966). The clinical signs in pigs reported in Experiment I suggest that dieldrin toxicosis could be enhanced in protein depleted pigs. The deficient group of pigs also manifested a decreased capacity to accumulate dieldrin in various tissues, with the exception of Pig #1 in this group. The reason for this reduced ability to accumulate dieldrin is not clear, but 2 possibilities are suggested. The resulting impairment of growth may have also reduced the fat content of the various tissues which is said to influence the amount of dieldrin that can be stored. It is possible, too, that there is impaired absorption of dieldrin from the gastrointestinal tract of protein deficient pigs. Impaired

absorption of dieldrin from the gastrointestinal tract had been observed in starved rats (Heath and Vandekar, 1964). Consequently these authors suggested that dieldrin absorption is controlled by the amounts of some unidentified naturally occurring materials in the gut and not by the quantity of dieldrin present. Such impairment can result in lower storage levels since it has been reported that the amount stored is proportional to the rate of intake (Gannon *et al.*, 1959).

Transplancental transfer of dieldrin was demonstrated in the 2nd experiment. The dieldrin concentrations in the sows' tissues did not correlate either with the doses or the total quantities given. However, examination of the results of analyses for dieldrin in fetal livers (Table 6) reveals a statistically significant difference ($P < 0.025$ by the t test) between the 2 dosage levels relative to the total amounts of chemical given to their dams. Two factors may be important in explaining this observation. The less active microsomal enzymes of the fetal liver may not metabolize dieldrin fast enough to prevent accumulation which will, therefore, vary with the rate of intake. The dose dependent accumulation in the fetus may also be a reflection on the virtual absence of large fat deposits. In the adult, the fat serves as the predilection site of storage for dieldrin. The concentrations attained by the fetuses apparently caused no harmful effects.

A greater than 3-1/2 fold increase in the total amount of dieldrin (from 13.5 to 48 gm.) administered simultaneously with $NaNO_2$ over a period of time did not cause malformation, abortion, or death *in utero* of the fetuses. This is in agreement with reports in other species (Harris *et al.*, 1966; Kitselman *et al.*, 1950). However, it caused the death of one sow. As the nitrite alone also killed a sow, this aspect of the study is inconclusive with regard to dieldrin alone. Nevertheless,

the study indicates that a pregnant sow may be given up to 48 gm. dieldrin over a period of 28 days without ill effects.

Since the concentration of dieldrin in a tissue depends on the fat content of that tissue, this may account for the wide difference between the concentrations in the 2 sows' livers which histologically manifested different levels of lipidosis. This differential concentration was not so marked in the kidneys or brains, the normal fat contents of which are relatively constant. Amongst the fetuses from each sow, there was a relative uniformity in the amounts of dieldrin stored either in the brain or the liver, indicating not only equal exposure to dieldrin but also a probable similarity in the metabolic states of these featuses, as all fetuses were alive and appeared normal. The nonoccurrence of abortions in these experiments may be associated with the absence of fetal and uterine pathology as indicated in previous reports (Gannon *et al.*, 1959).

Transplacental transfer of dieldrin was also achieved in the guinea pig experiments. In the first experiment, dieldrin did not cause deformity or prenatal death of the fetuses, but may have caused the early death of a litter of 4 neonatal guinea pigs. Abortions did not occur even though some pregnant guinea pigs died at all treatment levels. The fetuses in the dead or euthanatized guinea pigs manifested no gross or microscopic lesions in spite of the significantly high concentrations of dieldrin in them (Table 10).

The generalized hyperemia of tissues and degenerative lesions in parenchymatous organs of adult guinea pigs are in agreement with previously described lesions in other mammalian species (Borgman *et al.*, 1952; Kitselman, 1951). The significance of the necrosis and mineralization observed in uteri of 2 dieldrin-treated guinea pigs is not clear.

Myocardial necrosis and mineralization was evident in one of these guinea pigs. Heart failure may have been responsible for the death in this instance.

The lack of deviations from normal ranges in the hematologic studies confirms the uniformly negative results reported by other workers (Treon *et al.*, 1955; Borman *et al.*, 1952; Princi and Spurbeck, 1951).

Confirmation was obtained for the proportionality between intake and storage of dieldrin (Bundren *et al.*, 1952). However, this seemed to apply to the adult tissues, especially the fat and kidney, but not the fetuses (Table 12). With the total amount of 60 mg. each of dieldrin and nitrite or less, there appeared to be no enhancement of storage or diffusion of dieldrin to the fetuses. This lack of enhancement changed appreciably at higher total levels of nitrite (120 mg.) administration and may be explained by a possible increased vasodilatation, decreased blood flow rate and consequent longer contact of dieldrin in the blood with storage tissues and fetuses.

The histopathologic changes present in the organs have been separately associated with dieldrin and nitrite. Objective quantitative estimation of any synergism in the development of these changes was not possible. The observed arterial degeneration and occlusion were probably due to the nitrite. These changes as well as the nitrite-induced methemoglobinemia may have caused uterine and fetal hypoxia and consequent abortion (Sinha, 1968).

Sodium nitrite given as 0.5% solution alone caused abortion in 3 of 5 pregnant guinea pigs. Dieldrin at 100 ppm in the feed caused 2 abortions and 2 deaths in a group of 5 while a combination of both chemicals induced 6 abortions and 5 deaths amongst 14 recipient guinea pigs. These results appear to indicate that at these levels nitrite is

not an important potentiator of the toxic effects of dieldrin. They also indicate that at the level of 100 ppm, dieldrin is very toxic to most guinea pigs. However, levels of dieldrin at or below 50 ppm administered with or without 0.25% $NaNO_2$ appear to have no acute toxic properties as estimated by body weight gain, abortion, or death.

The acute oral toxicity (LD_{50}) of dieldrin to guinea pigs was estimated from Table 16 to be 45 mg./kg. This is less than the figure of 59 mg./kg. reported by Borgman *et al.* (1952), higher than Jolly's (1954) figure of 20 mg./kg., but in agreement with Gaines' (1969) finding of 46 mg./kg.

Ultrastructural Effects

Hypertrophy of the smooth endoplasmic reticulum (SER) was demonstrated in rat livers after administration of small doses (2 mg./kg.) of dieldrin (Hutterer *et al.*, 1968). This was usually associated with initial hyperfunction and later hypofunction of drug-metabolizing enzymes. In another report, Hutterer *et al.* (1969) indicated that the decompensation in metabolizing enzyme activities was associated with mitochondrial injury and reduction in glucose-6-phosphatase activity. The hypertrophy with hyperfunction was greatest 14 days after dieldrin administration was initiated, decompensation commencing after a temporary steady state was achieved.

In the present acute experiment, neither hypertrophy of the SER nor mitochondrial injury was observed in the liver because there was little time for the lesions to develop. However, it is suggested that the mitochondrial swelling in the brain which was similar to the change in a decompensating liver can be accounted for by the comparatively small drug-metabolizing capability of the brain cells. The very high dose of

Table 16. Acute oral toxicity of dieldrin to guinea pigs--numbers, doses and mortality*

Number Dosed	Dose (mg./kg.)	Died Within 24 Hours	Survived
7	0	0	7
25	30	8	17
9	60	3	6
4	75	4	0

*LD_{50} = 45 mg./kg. (Reed and Muench, 1938).

dieldrin given caused a high concentration of the nonmetabolized chemical to accumulate in the brain, and may have induced a depression of mitochondrial metabolic activity and alteration of mitochondrial membrane permeability with resulting swelling. That there may be a disruption of biochemical reactions in brain cells, especially of glutamine synthesis, in dieldrin poisoning was suggested by O'Brien (1967). The question of the ultimate cause of the convulsions is not settled. It is suggested, however, that dieldrin induced the initial changes in the mitochondria.

SUMMARY AND CONCLUSIONS

Pigs and guinea pigs were used in 7 experiments to study the toxicity of dieldrin. The main objectives of the study were to determine (a) the transplacental transfer of dieldrin and the resulting fetotoxic effects, if any; and (b) the ultrastructural changes in the brain and liver associated with dieldrin toxicosis. The possible roles of a protein-deficient diet and $NaNO_2$ toxicosis in enhancing the toxic effects of dieldrin were also investigated.

From the experimental results, the following observations and conclusions could be made:

1. The pesticide, dieldrin, crossed the placental barrier in the sow and accumulated in the fetal livers in proportion to the total amounts administered to the dam.

2. There was no observable fetotoxic effect, and no abortions.

3. Dieldrin induced nonspecific degenerative and congestive lesions in parenchymatous organs that were directly related to the duration of treatment of the sow.

4. Clinically, dieldrin toxicosis in pigs was enhanced by protein-deficient diets.

5. Protein-deficient pigs accumulated less dieldrin in their tissues than pigs fed a normal diet.

6. Sodium nitrite did not appear to influence the toxicity of dieldrin to sows.

7. Transplacental transfer of dieldrin occurred in the guinea pig.

8. Abortions in guinea pigs were caused by dieldrin at high doses. Similar doses also caused death without inducing abortion.

9. Sodium nitrite at high levels enhanced the accumulation of dieldrin in tissues and fetuses of pregnant guinea pigs.

10. Dieldrin storage in adult guinea pig tissues, especially the fat, was proportional to the intake. This was not influenced by $NaNO_2$ administration.

11. The amount of dieldrin stored in fetuses was not correlated with the quantity administered to the dam.

12. The acute oral toxicity (LD_{50}) of dieldrin to guinea pigs was calculated to be 45 mg./kg.

13. Body weight loss was not useful in estimating dieldrin toxicity.

14. Acute dieldrin toxicosis was manifested submicroscopically as swelling of mitochondria in the cerebellum and cerebrum with disintegration of cristae. The absence of hepatic injury reflected the acute nature of the toxicosis. Further work is required to delineate more clearly the association between the ultrastructural changes in the brain and liver and the often observed convulsions in the terminal stages of dieldrin toxicosis.

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VITA

Mba Uzoukwu was born the last of five children in Umuahia, Biafra, on March 26, 1931. He graduated from Methodist College, Uzuakoli, in 1950.

From 1951 to 1953 he attended the Federal Veterinary School, Vom, Nigeria. He spent the next two years, 1954 to 1956, as a student in the Nigerian College of Technology, Ibadan. His professional training was received, from 1957 to 1962, in the University of Glasgow Veterinary School, Glasgow, Scotland, from which he graduated B.V.M.S. and M.R.C.V.S.

In the years 1962 to 1964 he worked in the Federal Department of Veterinary Research, Vom, Nigeria. He transferred to the Veterinary Department of the University of Biafra in the summer of 1964, from where he came to Michigan State University in the summer of 1966.

He received the M.S. degree from the Department of Pathology, Michigan State University, in June, 1968, and remained in the same department as a candidate for the Ph.D. degree.

The author has published or co-authored three scientific papers.