# Correlation of body tissue levels with aldrin and dieldrin exposure and onset of toxicity

by

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# INTRODUCTION

Since prehistoric times, man has undoubtedly been plagued by insect pests that were primarily a nuisance to his comfort and well-being. As man evolved to a more agrarian culture, the pests also became fierce competitors for his food, as well as transmitters of many diseases.

Through the centuries man has continually sought more effective methods of controlling the pests that plagued him. The first method of control was probably mechanical; that is by crushing the pest with his hand or foot, which in time led to the use of a stick or stone to extend his effective range. The mechanical means were quite effective in controlling an individual pest but had little effect in controlling the multitude of pests that abounded to compete with the emerging agrarian development.

As we look in retrospect, it is evident that man would eventually discover and develop some means other than mechanical to control insect pests. Thus, it happened that man eventually did discover some natural control methods. As early as 1848, T. Oxley suggested the use of a substance extracted from roots that the primitive Malayan people had used for years as a fish poison (O'Brien, 1967). We still use this toxicant known as rotenone, derris dust, or cube' root as a dust or liquid suspension. It is used to control external parasites of domestic animals and is used extensively by wildlife biologists to control rough fish in lakes and ponds. As early as 1846, the use of an infusion of tobacco leaves was recommended to control an insect pest of plants (Waite et al., 1925; O'Brien, 1967). This insecticide was eventually extracted and identified as nicotine sulfate and has been marketed for years as a pesticide. One of the popular brand names

is Blackleaf 40, which is a dark, viscid liquid that contains 40% nicotine sulfate and is now only sparingly used. The pyrethrins are another group of insecticides, of botanical origin, that have a long history of use and are still used extensively to control insect pests. The pyrethrins have perhaps the longest history of use. It is reported that Marco Polo brought the pyrethrins to Europe from one of his Asian trips (Buck et al., 1973).

The early discoveries of botanical extracts that had insecticidal characteristics and the work by the early chemists laid the groundwork for the development of myriad chemicals that are now available to control the various plant and animal pests that tend to disrupt man's comfort and compete for his food supply.

The chemical insecticides have been beneficial to man's well-being, but they also have the detrimental side effect of being relatively toxic to animals and man. The chlorinated hydrocarbon insecticides are very persistent chemicals that are being incriminated for polluting the environment and causing many problems in wildlife (Lehner and Egbert, 1969; Walker et al., 1969).

This study is concerned with two of the chlorinated hydrocarbon insecticides, aldrin and dieldrin, that are classified chemically in the group of cyclodienes (O'Brien, 1967).

The study reported in this thesis was conducted to determine the animal tissue that would have the most consistent level of residual insecticide that would correlate with exposure and death of the animal.

If a specific organ or tissue consistently contained residues of an insecticide in direct proportion to exposure level, this would greatly aid

the clinical veterinary toxicologist in rendering a diagnosis in suspected cases of poisoning.

It seemed apparent that the brain would have the most consistent level of insecticide that would correlate with the outcome as the clinical signs are primarily manifestations of central nervous system disturbances. Lipids of various types, such as lecithin, cholesterol, cephalin, and sphingomyelin, constitute up to 40-65% of the total solids of the brain (Best and Taylor, 1961; Klemm, 1970; Davison, 1970). These lipids would have an affinity for lipid soluble compounds such as chlorinated hydrocarbons.

A review of the literature confirmed the premise that brain levels of chlorinated hydrocarbon insecticides would be useful to establish whether or not bird deaths were caused by dieldrin (Stickel et al., 1966, 1968; Robinson et al., 1967; Linder et al., 1970). The purpose of this study was to determine if a similar relationship exists in the tissue of domestic animals and to determine if such a relationship would be an aid in diagnosing suspected poisoning cases.

Two species of domestic animals were utilized in this study, swine and chickens.

This study was conducted on the premises of the National Animal Disease Laboratory at Ames, Iowa, under the guidance of William B. Buck, Professor in charge of the Toxicology Section, Iowa Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa.

# REVIEW OF LITERATURE

Accidental poisoning of domestic animals by the chemical pesticides has been a significant problem since their introduction. One of the early chemical compounds, lead arsenate, was used as early as 1892 in the control of gypsy moths (Buck et al., 1973). Arsenical formulations have been used for years in dipping vats for cattle entering the United States from Mexico to control Texas Tick Fever (Cole and MacKellar, 1956; Hourrigan, 1970). Arsenic poisoning in livestock is still a major problem, but the cases have shifted from the accidental misuse of arsenic compounds to inadvertent access to old or discarded stocks that have been stored in buildings and discarded in junk piles.

The development and introduction during World War II of the synthetic organic insecticides such as DDT, aldrin, dieldrin, and lindane (hexachlorocyclohexane) gave man the means of controlling major insect pests.

DDT (p,p'dichloro-diphenyltrichloroethane), the first widely used chlorinated hydrocarbon insecticide, was first synthesized in 1874 by Othmar Ziedler as one of a series of organic chemicals (Zeidler, 1874). However, the insecticidal properties were not discovered until 1939 by Paul Muller working for the Geigy Company of Switzerland (O'Brien, 1967). It appeared to be an ideal insecticide as it was relatively safe to economic species, it was quite potent to insect pests, relatively cheap and easy to make, and it was stable and quite persistent in its activity when applied to plants or other surfaces (O'Brien, 1967). The persistence of DDT, which was at first heralded as a beneficial characteristic, was in time recognized as actually being detrimental through the accumulation of residues in the

environment and in the fatty tissues of animals and man. The buildup of environmental residues contributed to the steady increased resistance of the insect pests to the effect of DDT (Brown, 1961; O'Brien, 1967). This led to the widespread controversy that still rages concerning the use of persistent chlorinated hydrocarbon compounds.

The discovery of the insecticidal properties of DDT prompted intensive research for other chemical insecticides by investigators in all parts of the world. In a short time, many other insecticides with varying degrees of safety, persistence, and effectiveness were introduced for controlling insect pests.

Included in the newly introduced insecticides were the hexachlorocyclohexanes, exemplified by lindane, and the group of cyclodienes exemplified by aldrin, dieldrin, heptachlor, chlordane, isodrin, endrin, and others (O'Brien, 1967).

The insecticides of interest in this study were aldrin and its epoxide, dieldrin, that are classified as cyclodienes in the group of chlorinated hydrocarbon insecticides.

Aldrin and dieldrin were developed by Julius Hyman in 1945 while working with the Velsicol Laboratories in the United States (O'Brien, 1967). Shell Chemical Company ultimately obtained the patent rights for both of these chemical compounds and is at present the major producer and distributor of them (Stecher, 1968).

Technical aldrin by definition contains not less than 95% of HHDN (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo,exo-5,8-dimethanonaphthalene) with the empirical formula of $C_{12}H_8Cl_6$ (Stecher, 1968). HHDN has a molecular weight of 364.93 with over half (57-59%) of this

weight derived from chlorine. At room temperature, it is a non-flammable, tannish-brown, waxy, solid that is moderately soluble in paraffins and halogenated solvents. It is sparingly soluble in alcohol and practically insoluble in water (11 ppb at 20°C). Aldrin is non-corrosive to steel, brass, monel, copper, nickel, and aluminum. It is stable in the presence of ordinary organic bases, inorganic bases, and alkaline oxidizing agents. It is stable with dilute acids but reacts with concentrated mineral acids, phenols, and active metals (Shell Technical Data Bulletin, 1072a).

Aldrin is formulated as an emulsifiable concentrate, as an oil solution, and in dusts. It is compatible with most fertilizers, herbicides, fungicides, and insecticides (Stecher, 1968). Thus it can be applied simultaneously with other crop applications.

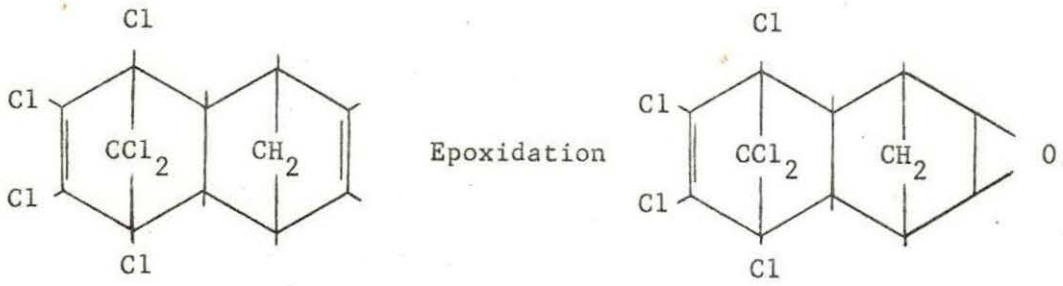
Aldrin, as shown by the structural formula (Figure 1), has two double bonds ("diene") in the naphthalene 2 ring skeleton, and the endomethylene bridges connect the ends of each ring. The endomethylene bridge that connects the ends of the fully chlorinated ring is also fully chlorinated which accounts for the six chlorine atoms in the aldrin molecule (O'Brien, 1967; Stecher, 1968; Shell Technical Data Bulletin, 1972a).

Aldrin undergoes bioactivation in biological systems by epoxidation of the unsaturated bond in the unchlorinated ring to form dieldrin (Dahm and Nakatsugwa, 1968; Brooks, 1966).

Technical dieldrin by definition contains not less than 100% of HEOD (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-dimethanonaphthalene), with an empirical formula $C_{12}H_8Cl_6O$. HEOD has a molecular weight of 380.93 with over half (55-56%) of this weight derived from chlorine. It is a non-flammable, buff to light brown, solid,

Aldrin - 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexhydro-1,4-endo,exo-5,8-dimethanonaphthalene.

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-dimethanonaphthalene.




Aldrin

Dieldrin

Figure 1. Aldrin and dieldrin structural formulas

dry flake. It is moderately soluble in halogenated solvents, sparingly soluble in alcohols, and is practically insoluble in water (110 ppb at 20°C). It is non-corrosive to steel, brass, monel, copper, nickel, and aluminum. It is stable in the presence of ordinary organic bases, inorganic bases, and alkaline oxidizing agents. It is stable with dilute acids but reacts with concentrated mineral acids, acid oxidizing agents, phenols, and active metals (Shell Technical Data Bulletin, 1972b).

The structural formula of dieldrin (Figure 1) is similar to aldrin with the addition of the oxygen radical at the 6 and 7 carbon positions (O'Brien, 1967).

The epoxide, dieldrin, is about equally as toxic as aldrin, the parent compound (Gaines, 1960). A major consideration is the increased persistence of the epoxide in the biological system and in the environment (Dahm and Nakatsugwa, 1968). Experimentally, it has been shown that the clinical signs of poisoning in house flies by exposure to aldrin coincides with the appearance of the epoxide, dieldrin (Dahm and Nakatsugwa, 1968; Brooks et al., 1963; Perry et al., 1964).

Aldrin and dieldrin have been highly effective in controlling plant-feeding pests. The relatively high toxicity to livestock and the persistence, of especially dieldrin, have prevented the safe use of these insecticides on livestock (Radeleff, 1970), although dieldrin has been recommended in England for control of fly strike in sheep by dipping in 0.05 to 0.1% concentrations (Clarke and Clarke, 1967). Oral doses of aldrin in excess of 2.5 mg/kg were toxic to young calves, while 25 mg/kg doses were lethal for adult cattle (Radeleff, 1970). Dieldrin produced toxicosis in young dairy calves at 10 mg/kg and in young pigs at 50 mg/kg (Radeleff

et al., 1960). The experimental data of Radeleff and co-workers (1970) indicates that dieldrin is not as toxic as aldrin but still would be considered to be highly toxic.

The clinical signs of poisoning by aldrin and dieldrin usually show a deep depression, which alternates with periods of hyperexcitability and convulsive seizures (Radeleff, 1970). Aldrin poisoning may induce clinical signs that are analagous to stimulation of the peripheral parasympathetic autonomic nervous system, such as slowing of the heart rate and increased salivation by the submaxillary salivary bland, whereas dieldrin does not elicit this autonomic response (O'Brien, 1967; Gowdey et al., 1952, 1954, 1955). The primary effect of both aldrin and dieldrin is on the central nervous system, resulting in a markedly altered behavior pattern in the affected individual animal. The clinical signs of poisoning within a given group of animals may vary from deep depression to hyperexcitability, as evidenced by muscle fasciculations, especially of the facial and cervical area, intermittent convulsive seizures, belligerence, and abnormal posturing which may proceed to either death or recovery. The rapidity of the onset and the severity of clinical signs are a poor criteria for predicting a prognosis. It has been the author's observation and personal communication with Dr. Buck that the clinical signs are very misleading to predict the outcome of a poisoning case in the individual animal. The animal that shows early, typical, clinical signs of toxicosis may proceed to recovery, while others that did not appear to be as severely effected may die. It is also possible that an apparently fully recovered animal may show typical, clinical signs of toxicosis as long as a month after the initial exposure (Radeleff, 1970).

Postmortem lesions are non-specific and are usually limited to skin abrasions, found primarily around the head and on the legs as a result of the convulsive seizures. The epicardium may show diffuse petechial hemorrhages and there may be an excessive amount of pericardial fluid. The lungs may be edematous, heavily congested, and dark in color. A mild gastroenteritis may be evident following the oral ingestion of either aldrin or dieldrin. In subacute and chronic cases, there is usually a loss of weight and condition accompanied by dehydration and loss of fat depots (Radeleff, 1970).

The lack of specific postmortem lesions and the variability of clinical signs associated with poisonings by chlorinated hydrocarbon insecticides makes the chemical analysis of tissue, feed, and other pertinent material very important to the clinical veterinary toxicologist in establishing a diagnosis for suspected cases of poisoning. It is well documented that chlorinated hydrocarbons have an affinity for fatty tissue (Baron and Walton, 1971; O'Brien, 1967; Radeleff, 1970; Deichmann et al., 1970). The highly persistent insecticides, such as dieldrin, are excreted very slowly from the fatty tissue (Radeleff, 1970; Dahm and Nakatsugwa, 1968). The knowledge of this phenomenon has led to the misconception that fat is the best tissue for chemical analysis in suspected cases of chlorinated hydrocarbon poisoning. The results of fat analysis are meaningful for historical and environmental evaluation of contamination but are of little, if any, value in diagnosing the acute or subacute cases of poisoning. The quantity of residual insecticide in the body fat will only determine that the animal has had a prior exposure to the detected chlorinated hydrocarbon. It will not indicate if it had any influence on the death of the animal.

Animals may have fat tissue levels of several hundred ppm without any signs of overt toxicosis (Radeleff, 1970).

# MATERIALS AND METHODS

## Experimental Animals

### Swine

The 24 swine used in this experiment were naturally farrowed SPF males and females obtained from the National Animal Disease Laboratory (N.A.D.L.) Animal Services at Ames, Iowa. They ranged from 98 to 114 days of age with an average age of 107 days.

The individual weights averaged 24.5 kg (54 lbs.), ranging from a high of 30 kg (66 lbs.) to a low of 20.5 kg (45 lbs.).

The 24 swine represented 5 different litters and were 15/16 Yorkshire and 1/16 Chester White with each litter having a different sire (Appendix Tables 1 and 2).

### Chickens

The 48 chickens used in this experiment were ten-week-old Rhode Island Reds, obtained from Animal Services at N.A.D.L.

The individual weights averaged 1.02 kg (2.23 lbs.), ranging from a high of 1.41 kg (3.1 lbs.) to a low of 0.77 kg (1.7 lbs.) (Appendix Tables 6, 7, and 8).

## Husbandry Practices

### Swine

The swine were housed in one of the modular units at N.A.D.L. with controlled temperature, humidity, and ventilation.

The floors were concrete with adequate drainage to a gutter leading to a trapped floor drain. The rear and side walls were solid to prevent com-

munication between adjacent pens. The front walls had a solid, removable panel that faced a service aisle.

The pens were cleaned and washed down thoroughly with a high pressure hose each morning prior to treatment and feeding.

Drinking water was provided free-choice, and the standard N.A.D.L. 14% crude protein pelleted complete hog ration was fed twice daily in adequate amounts to maintain normal weight gain.

After being randomly selected, the swine were separated into groups of four per 4' by 12' pen according to their respective experimental procedure.

Chickens

The chickens were housed similarly as the swine with the exception that the group size was increased but did not exceed ten per 4' by 12' pen, and the ration was the standard N.A.D.L. 16% crude protein crumbled chicken ration.

## Experimental Insecticide

The insecticide was technical grade aldrin, Code No. Ac-23, obtained for experimental purposes from the Agricultural Division of Shell Oil Company at Modesto, California.

## Tissues for Analysis

The tissues collected from the swine for chemical analysis for aldrin and dieldrin residue levels included the entire brain, liver, one kidney, and about a pound of backfat. The tissues collected from the chickens included the entire brain and liver and both kidneys. The chickens did not have any visible gross body fat for collection.

The tissues, as they were collected, were placed in labeled, individual plastic bags and frozen until extracted for analysis.

The chicken and swine feeds were collected for analysis for residual levels of insecticides.

A sample of the gelatin capsules from the same lot as those used to administer the aldrin was also collected for analysis for residual levels of insecticides.

## Experimental Methods

### Swine

The swine were randomly selected by drawing cards that were numbered corresponding to the individual eartag numbers.

The swine were weighed individually and separated into groups of four according to their allotted experimental procedure. Four swine were allotted to each of the four exposure levels, and eight were maintained as untreated controls.

The swine on the subacute dosage levels were reweighed on the 10th and 20th days of the experiment to adjust the daily dosage.

### Acute dosage levels

75 mg/kg Four swine were given one oral dose of 75 milligrams (mg) of technical grade aldrin in a gelatin capsule per kilogram (kg) of body weight.

This group consisted of two males and two females with an average weight of 25.65 kg. The weight of the two males averaged 25.65, and the two females averaged 25.65 kg (Appendix Table 1).

The dosage level of 75 mg/kg is approximately equivalent to 1173 ppm of aldrin in the diet of swine this size.

150 mg/kg Four swine were given one oral dose of 150 mg of technical grade aldrin in a gelatin capsule per kilogram of body weight.

This group consisted of one male and three females with an average body weight of 24.4 kg. The weight of the one male was 23.1 kg, and the weight of the three females averaged 24.8 kg (Appendix Table 1).

The dosage level of 150 mg/kg is approximately equivalent to 2346 ppm of aldrin in the diet of swine this size.

Subacute dosage levels

4 mg/kg Four swine were given technical grade aldrin orally in a gelatin capsule daily at 4 mg per kg of body weight for 22 consecutive days.

This group consisted of two males and two females with an average weight of 24.5 kg. The weight of the two males averaged 25.5 kg, and the two females averaged 23.5 kg (Appendix Table 2).

The dosage level of 4 mg/kg is approximately equivalent to 63 ppm in the diet of swine this size.

10 mg/kg Four swine were given technical grade aldrin orally in a gelatin capsule daily at 10 mg per kg of body weight for 22 consecutive days.

This group consisted of two males and two females with an average weight of 24.2 kg. The weight of the two males averaged 22.5 kg. and the two females averaged 25.9 kg (Appendix Table 2).

The dosage level of 10 mg/kg is approximately equivalent to 156 ppm of aldrin in the diet of swine this size.

The seven swine that survived the full 22 days of treatment in the two subacute groups were euthanatized on the 24th day of the experiment (48 hours after receiving the last aldrin) with succinylcholine hydrochloride intramuscularly.

Swine controls

The eight control swine were also euthanatized with succinylcholine hydrochloride intramuscularly for necropsy, and tissue collection was performed in groups of two at the beginning and end of the subacute treatment period, with two groups also euthanatized at intermediate periods within the treatment period.

The negative controls consisted of five females and three males with an average body weight of 23.8 kg. The weight of the five females averaged 23.9 kg, and the three males averaged 23.6 kg (Appendix Tables 1 and 2).

Chickens

The chickens were randomly selected by drawing cards that were numbered corresponding to the individual wing tag numbers. They were then weighed individually and separated into five groups with each group confined to a 4' by 12' pen.

Acute dosage level

75 mg/kg Nine chickens were given one oral dose of technical grade aldrin in a gelatin capsule at the rate of 75 mg per kg of body weight.

This group consisted of three females and six males with an average weight of 1.04 kg. The weight of the three females averaged 0.91 kg, and the weight of the six males averaged 1.11 kg (Appendix Table 6).

The dosage level of 75 mg/kg is approximately equivalent to 882 ppm in the diet of chickens this size.

150 mg/kg Ten chickens were given one oral dose of 150 mg of technical grade aldrin in a gelatin capsule per kilogram of body weight.

This group consisted of seven males and three females with an average weight of 1.07 kg. The weight of the males averaged 1.13 kg, and the females averaged 0.91 kg (Appendix Table 6).

The dosage level of 150 mg/kg is approximately equivalent to 1764 ppm in the diet of chickens this size.

Subacute dosage level

4 mg/kg Ten chickens were given technical grade aldrin orally in a gelatin capsule daily at 4 mg per kg of body weight until one-half of them had either died or were showing overt signs of toxicosis. The daily treatment was discontinued at this time to enable the birds to metabolize and excrete the existing body burden of insecticide. This recovery period was intended to avoid prematurely sacrificing individuals that already had a sufficient body burden of insecticide that might be fatal. This group consisted of six males and four females with an average weight of 1.17 kg. The weight of the males averaged 1.27 kg, and the females averaged 1.02 kg (Appendix Table 7).

The dosage level of 4 mg/kg is approximately equivalent to 47 ppm in the diet of chickens this size.

10 mg/kg Ten chickens were given technical grade aldrin in gelatin capsules daily at 10 mgs per kilogram of body weight until one-half of them had either died or were showing severe clinical signs of toxicosis.

This group consisted of five males and five females with an average weight of 1.18 kg. The males averaged 1.33 kg, and the females averaged 1.03 kg (Appendix Table 7).

Three days after receiving the last aldrin, the surviving two chickens were sacrificed for necropsy and tissue collection.

The dosage level of 10 mg/kg is approximately equivalent to 118 ppm in the diet of chickens this size.

Untreated controls

The nine untreated control chickens consisted of four males and five females with an average body weight of 1.07 kg. The males averaged 1.23 kg, and the females averaged 0.95 kg (Appendix Table 8). The controls were sacrificed in 3 groups of 3 at 4-day intervals.

## Analytical Procedures

The liver, kidney, brain, and fat samples were extracted and clarified for electron-capture, gas-liquid chromatographic analysis by the acetonitrile-Florisil column method according to the official method of the Association of Official Analytical Chemists (AOAC), Volume 49, p. 222 (1966) as listed in the Department of Health, Education, and Welfare (DHEW), Food and Drug Administration (FDA) Pesticide Analytical Volumes I, II, and III.

The weighed tissue samples were finely ground with sea sand by a mortar and pestle, the excess moisture removed with sodium sulfate and the

sample taken up in 40 mls of ethanol. The sample was extracted into petroleum ether and partioned into acetonitrile and purified by passage through a Florisil column. It was concentrated in a Kuderna-Danish concentrator in preparation for injection in the electron-capture, gas-liquid chromatograph for detection and quantitation of the tissue levels of aldrin and dieldrin. The first gas-liquid chromatograph was a Hewlett-Packard,[1] Model 5750B with a $_{63}Ni$ detector. The column was a 1.5% OV-17+1.95% QF-1 on Chromasorb WHP 80/100 mesh with the column oven at 210°C.

The detector temperature was maintained at 220°C; the attenuation was set at 16 and the range at 10X. The purge gas was 10% methane in argon, and the helium carrier gas flow was maintained at 50 ml/min. The chart paper was operated at 0.25 in/min.

The second gas-liquid chromatograph was a Packard,[2] 800 series with a tritium detector. The column was a 1.5% OV-17+1.95% QF-1 on Chromasorb WHP 80/100 mesh with the column and detector temperature at 200°C. The nitrogen carrier gas was maintained at a flow rate of 60 ml/min, and the chart paper was operated at 5 min/in.

The instruments were operated simultaneously. All the swine tissue samples were analyzed in the Hewlett-Packard, and the chicken tissue samples were analyzed in both instruments.

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[1] Hewlett-Packard Co., Avondale Div., Rt. 41, Avondale, Pa.

[2] Packard Instrument Co., Inc., 2200 Warrenville Road, Downers Grove, Illinois 60515.

# RESULTS OF EXPERIMENT

## Swine

Clinical signs of muscular fasciculations, incoordination, and jaw-champing convulsions were observed in the swine receiving the acute dosage level of either 75 mg/kg or 150 mg/kg of aldrin orally. This condition proceeded to lateral recumbency, aimless paddling primarily of the forelegs, and intermittent tetanic convulsions which led to a deep coma and death within a few hours after ingestion (six of the eight swine on the acute dosage level died within four hours following ingestion of the aldrin).

One of the four pigs (15389) in the 75 mg/kg group was in a deep coma at the end of 48 hours and was euthanatized with succinylcholine hydrochloride intramuscularly. This pig had a deceptively low level of insecticide in all tissues even though death appeared imminent. It is possible that this particular pig may have become nauseated and vomited, thus not retaining a lethal amount.

The eight swine receiving the subacute dosage level of either 4 mg/kg or 10 mg/kg orally lost condition and developed a roughened haircoat. The four swine on the 4 mg/kg level gained weight but at a slower rate than the controls. One pig in this group (15402) was observed in tetanic convulsions on the 24th day.

The three swine that survived the 10 mg/kg daily dose lost weight, as well as a general loss of condition, during the 24 days of the experiment (22 days of daily administration of aldrin and two days of excretion and recovery). One pig (15394) died after receiving 16 daily doses for a total aldrin intake of 160 mg/kg. This pig had been goose-stepping, wandering

aimlessly, and was apparently blind the day before dying. As will be noted in Appendix Table 4, the dieldrin levels in the tissues of this one pig that died while receiving the subacute level of aldrin were consistent with the levels found in the tissues of the swine that died in the acute dosage groups (Appendix Table 4).

The results of the analysis of the tissue, as shown in Appendix Table 3, show that the pigs that died peracutely from exposure to aldrin had significant levels of both aldrin and dieldrin in the tissue except in the body fat which, as expected, had no measurable level. Whereas the pigs that received a much lower amount of aldrin daily had significant levels of dieldrin but no significant level of aldrin in the tissues. They had a high level of dieldrin in the body fat, especially in the 10 mg/kg group (Appendix Table 4).

This finding is indicative of two physiological phenomenon, namely: (1) that the swine receiving the peracute level of aldrin did not have sufficient time to metabolize aldrin to its epoxide, dieldrin, and thus both aldrin and dieldrin were detected in significant amounts in the tissue, and (2) that swine receiving the peracute level of aldrin were essentially lacking any detectable amount of either aldrin or dieldrin in the body fat, while the swine receiving a low level of aldrin daily had a high amount of dieldrin in the body fat, up to a high of 471 ppm after 22 days of ingestion. This indicates that the animal had sufficient time to metabolize aldrin to dieldrin and to transport the lipid soluble chlorinated hydrocarbon to the body fat depots.

The results of the analyses shown on Appendix Table 5 are listed by increasing levels of insecticide in the brain. The first eight swine

listed survived the experimental exposure of aldrin and were all euthanatized with succinylcholine hydrochloride intramuscularly.

The levels of residual insecticide in the grain of these survivors ranged from a low of 0.49 ppm to a high of 2.75 ppm, with an average of 1.58 ppm, which would be considered below a diagnostically significant level.

The last eight swine listed in Appendix Table 5 all died from their respective intake of aldrin, with six of the eight dying peracutely (within four hours), one dying acutely (15 hours), and one subacutely (17 days). The levels of residual insecticide in the brain of this group ranged from a low of 5.11 ppm to a high of 16.54 ppm with an average of 7.59 ppm.

This data indicates a difference of 2.36 ppm between the highest brain level (2.75 ppm) of those that survived and the lowest brain level (5.11 ppm) of those that died. The difference of 6.01 ppm between the average brain level of 1.58 ppm of those that survived and the average brain level of 7.59 ppm of those that died is very significant from the viewpoint of the veterinary diagnostician.

## Chickens

The chickens receiving the acute dosage level of either 75 mg/kg or 150 mg/kg of aldrin orally were observed with varying degrees of incoordination, central nervous system disturbance, and convulsions. The convulsions were typically strychnine-like, tetanic spasms that could be induced by external stimuli, such as slamming a door or clapping one's hands. Touching or handling the chickens would elicit a tetanic convulsion. This response to external stimuli was not observed in the swine, although repeated attempts were made to elicit a similar response in them.

The chicken had a variable response to the acute dosage levels. In the 75 mg/kg group, one-third of the chickens were dead in less than four hours, with four of the remaining six dying between 12 and 20 hours after ingestion of aldrin. The remaining two were comatose and sacrificed 27 hours post-ingestion. In the 150 mg/kg group, one-half died within four hours, and the remaining one-half died between 12 and 21 hours post-ingestion (Appendix Table 10).

The two subacute dosage levels of either 4 mg/kg or 10 mg/kg daily dose of aldrin given orally in a gelatin capsule resulted in similar clinical signs as the total intake approached 40 mg/kg. There was a wide range in time to the onset of clinical signs and a more varied response of clinical signs. Some chickens became very belligerent, some became severely incoordinated, some walked backwards until hitting an obstruction then veered in another direction, and some pecked for variable periods of time at imaginary objects in the air. Typically, a strong external stimulus such as slamming a door, clapping one's hands, or a disturbance such as entering the pen would induce a tetanic convulsion in those observed with overt signs of toxicosis (Appendix Table 11).

It is interesting that when the total dose approached 40 mg/kg in either the 4 mg/kg or the 10 mg/kg groups, the onset of clinical signs occurred in over half the chickens (50% of the group receiving 4 mg/kg per day and 80% of the group receiving 10 mg/kg per day). This factor produced a logical point for discontinuing further treatment for both subacute groups. Thus, none of the chickens in the two subacute groups received more than 40 mg/kg total aldrin in this experiment.

The chickens having the higher brain level of residual insecticide in the acute dosage levels, as shown in Appendix Table 10, contain a significant amount of aldrin as well as its epoxide, dieldrin. This is similar to the finding previously shown in the swine in another part of this study. The chickens dying peracutely did not have sufficient time to metabolize aldrin to dieldrin.

The results of the analyses of the chicken tissue residues are not as clear cut as were the swine results. As shown in Appendix Table 12, the higher residue levels in the brain of those that survived, and were sacrificed two days after receiving the last aldrin, overlapped with the lower residue levels in the brain of those that died. However, when the average residue level of 1.65 ppm in the brain tissue of those that survived is compared to the average residue level of 4.39 ppm in the brain tissue of those that died, a significant difference is apparent.

It would be difficult to render a reasonably accurate diagnosis on the basis of a single analysis unless the results are either quite high or quite low and the clinical signs and history are compatible with the diagnosis. It is highly unlikely that the diagnostician working with a suspected poisoning in poultry would be limited to a single analysis. The husbandry practices employed in the poultry industry usually will result in a flock problem, and numerous animals are available for diagnostic evaluation.

The chicken and swine feeds did not contain any detectable levels of residue insecticides.

The sample of gelatin capsules did not contain any detectable levels of residual insecticides.

# DISCUSSION AND CONCLUSIONS

The literature abounds with data on the accumulative characteristics of lipid soluble chlorinated hydrocarbons in the fatty tissue of man and animals. This is an interesting phenomenon, but it does not appreciably aid the clinical veterinary toxicologist in positively diagnosing acute episodes of toxicoses that could conceivably involve litigation amounting to thousands of dollars.

The clinical veterinary toxicologist needs to know the tissue that will yield a level of the offending substance which consistently correlates with the death of the animal.

The work of Stickel et al. (1966, 1968) and Linder et al. (1970) with birds and pheasants indicated that the brain levels, although not being the highest of the tissues analyzed, were consistent in establishing whether the deaths were caused by chlorinated hydrocarbon insecticides. This is conceivably a sound hypothesis, as the solids of the brain are composed of 40 to 65% lipids of various types and would thus have an affinity for lipid soluble chemicals (Best and Taylor, 1961; Klemm, 1970; Davison, 1970). The clinical signs of toxicosis caused by chlorinated hydrocarbon insecticides are primarily manifestations of central nervous system disturbances. Thus, insecticide levels in this organ should be determined when one is attempting to establish a diagnosis of chlorinated hydrocarbon insecticide poisoning.

The acute dosage levels of aldrin given in this study were intended to simulate poisoning episodes of overwhelming proportions. Six of eight of the swine receiving the acute dosages orally died within four hours. One

died 15 hours post-administration, and the last one had been in a comatose condition for a full day before being euthanatized with succinylcholine hydrochloride intramuscularly.

The chickens that received the acute dosage of aldrin orally had a more varied response than the swine. Six of the 18 chickens died within four hours, nine died within 21 hours, one died 26 hours post-administration, and two that were comatose were euthanatized 27 hours post-administration.

The subacute dosage levels of aldrin given were intended to simulate an exposure of several days to several weeks with the possible death of some of the animals. The surviving animals were given a period of two days to metabolize and excrete a portion of the insecticide. This was to avoid the premature sacrificing of an animal that already had a sufficient amount to be fatal.

There was a major difference in the response between the swine and the chickens to the lower dose of aldrin even though they received the same total amount on a body weight basis. The swine received 22 daily doses of either 4 mg/kg or 10 mg/kg. This amounted to 88 or 220 mg/kg total intake for each respective group of swine. One pig receiving the 10 mg/kg dosage level died on the 17th day after having received 160 mg/kg total aldrin. The remaining swine lost condition and developed a roughened hair coat. The pigs remaining in the 10 mg/kg group also lost weight. One of the swine in the 4 mg/kg group had a series of convulsive seizures on the 24th day after starting on aldrin, two days after receiving the last dose.

The chickens had a remarkable sensitivity to aldrin plus a pronounced hyperexcitability, as external stimuli such as touch or sound induced minor clinical signs of toxicosis.

In the chickens, a total intake of 40 mg/kg of aldrin spread over a period of either four or ten days induced similar clinical signs of overt toxicosis. All administration of aldrin was discontinued when the total intake reached 40 mg/kg of body weight. In the 10 mg/kg dosage group, all but two of the ten chickens died after receiving 40 mg or less of total aldrin. In the 4 mg/kg dosage group, five of the ten chickens died on less than 40 mg/kg. The 15 chickens that lived after having received 40 mg/kg total aldrin were given two days of recovery without aldrin. During this period, eight of the survivors died, and the remaining seven were euthanitized two days after receiving the last dose. No overt clinical signs of toxicosis were observed in any of the survivors at the time of euthanasia.

As shown in Appendix Table 5, the brain levels of dieldrin and aldrin, in the eight surviving swine, had a range of 0.49 ppm to 2.75 ppm with an average of 1.58 ppm. The eight swine that died, seven peracutely and one subacutely, had brain levels of dieldrin and aldrin that ranged from 5.11 ppm to 16.54 ppm with an average of 7.59 ppm. There is a significant difference of 2.36 ppm between the highest level in the survivors (2.75 ppm) and the lowest level in those that died (5.11 ppm). There is a difference of 6.01 ppm when comparing the average of the eight swine that survived (1. 58 ppm) and the eight that died (7.59 ppm).

As shown in Appendix Table 13, when the discriminant function is computed, it is possible to statistically predict the probability of death or

survival of swine from the brain levels of dieldrin. As the number of analyses increase, the average level of residual insecticide will tend to either make the prediction more positive or to decrease the level of residue necessary to predict the same probability.

The results of kidney analyses for residual insecticide in swine, as shown in Appendix Table 5, shows a similar correlation as the brain with death or survival to aldrin exposure. The eight surviving swine had a range of 0.23 ppm to 1.88 ppm with an average level of 1.28 ppm. The eight swine that died had a range of 3.21 ppm to 14.7 ppm. There is a significant difference of 1.33 ppm between the highest kidney level of those that survived (1.88 ppm) and the lowest kidney level of those that died (3.21 ppm). This is not as significant as the difference in the brain levels, however, when the discriminant function is computed as shown in Appendix Table 13, the correlation is very similar in predicting the statistical probability of death or survival from the tissue levels.

The levels of dieldrin in the liver had an overlap of the higher levels of those that survived and the lower levels of those that died. This indicates that liver is not diagnostically as critical as the brain and kidney in swine.

The level in the fat, as was expected, indicated that an accumulation of dieldrin in the body fat was related to the duration of intake and would be of little value in diagnosing an acute episode of poisoning. The fat level is important in residue studies related to environmental pollution.

As shown in Appendix Table 12, the brain levels of insecticide in the seven surviving chickens had a range of 0.85 ppm to 2.59 ppm with an average of 1.65 ppm. The 31 chickens that died had a range of 1.25 ppm to 9.43

ppm with an average of 4.39 ppm. The highest levels of residual insecticide in brain tissue of those that survived overlaps with the lowest brain levels of those that died. There is, however, a difference of 2.74 ppm between the average brain levels of those that survived (1.65 ppm) and those that died (4.39 ppm).

The results of a single brain analysis would be difficult to evaluate if the level of dieldrin fell in the area where the residue levels overlap between those that survived and those that died. It is highly unlikely that the diagnostician would be committed to making a diagnosis in a flock problem on the basis of one analysis in poultry.

As shown in Appendix Table 13, when the discriminant function is computed, it is possible to statistically predict the probability of death or survival of a chicken from the brain levels. As the number of analyses increases, the average level of dieldrin will tend to either make the prediction more positive or to decrease the level of residue necessary to predict the same probability.

The kidney and liver levels of dieldrin did not correlate with death or survival in the chicken.

The results of this study indicate that brain levels of a chlorinated hydrocarbon, aldrin, and its epoxide, dieldrin, correlate with death or survival in the swine and chicken. In the swine an added dimension was established when the kidney levels of dieldrin also were found to correlate with death or survival of the animal as a result of aldrin exposure.

## SUMMARY

The level of dieldrin, the epoxide of aldrin, an insecticide of the chlorinated hydrocarbon group, was determined in various tissues in an attempt to correlate the tissue level with the effect on the animal.

Twenty-four young swine were divided randomly into six groups of four per group. Two groups were given a single dose of aldrin orally at either 75 mg/kg or 150 mg/kg estimated to be peracutely toxic. Two other groups were given daily doses of aldrin orally at either 4 mg/kg or 10 mg/kg which were estimated to be subacutely toxic. Two groups were maintained as untreated controls to check for background levels of insecticide.

A similar experiment was conducted utilizing 48 ten-week-old chickens given the same levels of aldrin.

The results of the chemical analyses on the tissues of the swine and chickens showed a definite statistical correlation that was diagnostically significant between the brain levels of dieldrin and the death of the animal.

The analyses of swine tissue also showed a statistical correlation between the kidney levels of dieldrin and the death of the animal. The same correlation with kidney levels was not true in the chicken.

From the viewpoint of the diagnostician, the brain levels of dieldrin have the greatest correlation and would be most helpful to render a diagnosis.

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# APPENDIX

Table 1. Swine - acute aldrin exposure

| Eartag no. | Sex | Wt. (kg) | Wt. (lbs) | Total aldrin (mgs.) | Age (days) |
|---|---|---|---|---|---|
| A. 75 mg/kg - single oral dose | | | | | |
| 15397 | M | 27.7 | 61 | 2077 | 116 |
| 15389 | M | 23.6 | 52 | 1770 | 100 |
| 15385 | F | 28.6 | 63 | 2145 | 105 |
| 15395 | F | 22.7 | 50 | 1702 | 104 |
| B. 150 mg/kg - single oral dose | | | | | |
| 15388 | F | 22.7 | 50 | 3405 | 100 |
| 15401 | M | 23.1 | 51 | 3465 | 116 |
| 15400 | F | 30.0 | 66 | 4500 | 116 |
| 15379 | F | 21.8 | 48 | 3270 | 104 |
| C. Untreated control swine | | | | | |
| 15382 | M | 23.2 | 51 | -- | 104 |
| 15392 | F | 24.1 | 53 | -- | 106 |
| 15384 | F | 22.7 | 50 | -- | 100 |
| 15381 | F | 24.5 | 54 | -- | 106 |

Table 2. Swine - subacute aldrin exposure

| Eartag no. | Sex | Wt. (kg) | Wt. (lbs) | Total aldrin (mgs.) | Age (days) |
|---|---|---|---|---|---|
| D. 4 mg/kg - daily oral dose - 22 days | | | | | |
| 15399 | M | 27.3 | 60 | 109.2 | 116 |
| 15387 | M | 23.6 | 52 | 94.4 | 106 |
| 15402 | F | 20.4 | 45 | 81.6 | 116 |
| 15391 | F | 26.6 | 58 | 106.4 | 105 |
| E. 10 mg/kg - daily oral dose - 22 days | | | | | |
| 15398 | F | 30.0 | 66 | 300 | 116 |
| 15396 | M | 21.3 | 47 | 213 | 104 |
| 15390 | M | 23.6 | 52 | 236 | 100 |
| 15394 | F | 21.8 | 48 | 218 | 106 |
| F. Untreated control swine | | | | | |
| 15380 | M | 22.7 | 50 | -- | 104 |
| 15383 | F | 23.6 | 52 | -- | 104 |
| 15386 | M | 25.0 | 55 | -- | 106 |
| 15393 | F | 24.5 | 54 | -- | 105 |

Table 3. Swine - acute aldrin exposure tissue levels

| Eartag | Aldrin-ppm | | | | Dieldrin-ppm | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | Kidney | Liver | Brain | Fat | Kidney | Liver | Brain | Fat | |
| A. 75 mg/kg - single oral dose | | | | | | | | | |
| 15397 | 0.58 | 0.47 | 1.1 | n[a] | 2.63 | 3.74 | 4.01 | 0.73 | Dead within 3 hrs.[b] |
| 15389 | n | n | n | n | 1.55 | 2.56 | 1.21 | n | Euth.-48 hrs. P.A. |
| 15385 | 1.76 | 11.08 | 5.4 | n | 3.14 | 13.1 | 11.14 | n | Dead within 3 hrs. |
| 15395 | 1.95 | 4.06 | 2.04 | n | 2.19 | 8.36 | 3.49 | n | Dead within 3 hrs. |
| | | | | | 2.3 | 6.9 | 4.9 | | |
| B. 150 mg/kg - single oral dose | | | | | | | | | |
| 15388 | 4.76 | 3.0 | 2.25 | n | 5.06 | 3.36 | 3.67 | n | Dead within 3 hrs. |
| 15401 | 3.11 | 2.82 | 1.95 | n | 5.33 | 11.62 | 5.93 | n | Dead within 3 hrs. |
| 15400 | 1.80 | 2.93 | 1.88 | n | 3.60 | 6.84 | 5.19 | 0.99 | Dead within 3 hrs. |
| 15379 | 2.21 | 2.61 | 2.51 | n | 4.55 | 9.15 | 4.20 | n | Dead within 15 hrs. |
| | | | | | 4.6 | 7.7 | 4.5 | | |
| C. Untreated controls | | | | | | | | | |
| 15382 | n | n | n | n | n | n | n | n | Euth.-2nd day |
| 15392 | n | n | n | n | n | n | n | n | Euth.-16th day |
| 15384 | n | n | n | n | n | n | n | n | Euth.-7th day |
| 15381 | n | n | n | n | n | n | n | n | Euth.-16th day |

[a] No detectable amount.

[b] Euthanatized, 48 hrs. Post-Administration.

Table 4. Swine - subacute aldrin exposure tissue levels

| Eartag | Aldrin-ppm | | | | Dieldrin-ppm | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | Kidney | Liver | Brain | Fat | Kidney | Liver | Brain | Fat | |
| D. 4 mg/kg - daily dose for 22 days | | | | | | | | | |
| 15399 | n[a] | n | n | n | 1.65 | 0.5 | 1.36 | 55.9 | Euth.-24th day[b] |
| 15387 | n | n | 0.03 | na[c] | 0.95 | 1.45 | 0.98 | na | Euth.-24th day |
| 15402 | n | n | n | n | 1.88 | 5.82 | 1.95 | 12.03 | Euth.-24th day Convulsions 24th day |
| 15391 | n | n | n | n | 0.23 | 1.25 | 0.49 | 19.8 | Euth.-24th day |
| E. 10 mg/kg - daily oral dose for 22 days | | | | | | | | | |
| 15398 | n | n | n | n | 1.63 | 3.28 | 1.44 | 121.6 | Euth.-24th day |
| 15396 | n | n | n | n | 1.24 | 2.97 | 2.44 | 149.8 | Euth.-24th day |
| 15390 | n | n | n | n | 1.15 | 5.7 | 2.75 | 471.4 | Euth.-24th day |
| 15394 | 0.86 | 0.34 | 0.35 | 1.82 | 13.84 | 7.09 | 5.67 | 254.7 | Dead at 17 days |
| | | | | | 4.4 | 4.7 | 3.0 | 248 | |
| F. Untreated controls | | | | | | | | | |
| 15380 | n | n | n | n | n | n | n | n | Euth.-7th day |
| 15383 | n | n | n | n | n | n | n | n | Euth.-21st day |
| 15386 | n | n | n | n | n | n | n | n | Euth.-2nd day |
| 15393 | n | n | n | n | n | n | n | n | Euth.-21st day |

[a] No detectable amount.

[b] Euthanatized.

[c] No analysis.

Table 5. Swine - combined aldrin and dieldrin tissue levels

| Eartag | Dosage (mg/kg) | Kidney | Liver | Brain | Fat | Remarks |
|---|---|---|---|---|---|---|
| 15391 | 4 | 0.23 | 1.25 | 0.49[a] | 19.8 | Euth.-24th day[b] |
| 15387 | 4 | 0.95 | 1.45 | 1.01 | na | Euth.-24th day |
| 15389 | 75 | 1.55 | 2.56 | 1.21 | n | Euth.-48 hrs. P.A. |
| 15399 | 4 | 1.65 | 0.5 | 1.36 | 55.9 | Euth.-24th day |
| 15398 | 10 | 1.63 | 3.28 | 1.44 | 121.6 | Euth.-24th day |
| 15402 | 4 | 1.88 | 5.82 | 1.95 | 12.03 | Euth.-24th day |
| 15396 | 10 | 1.24 | 2.97 | 2.44 | 149.8 | Euth.-24th day |
| 15390 | 10 | 1.15 | 5.7 | 2.75 | 471.4 | Euth.-24th day |
| 15397 | 75 | 3.21 | 4.21 | 5.11 | 0.73 | Dead-3 hrs. P.A. |
| 15395 | 75 | 4.14 | 12.42 | 5.53 | n | Dead-4 hrs. P.A. |
| 15388 | 150 | 9.82 | 6.36 | 5.92 | n | Dead-3 hrs. P.A. |
| 15394 | 10 | 14.7 | 7.43 | 6.02 | 256.52 | Dead-17 days |
| 15379 | 150 | 6.76 | 11.76 | 6.71 | n | Dead-15 hrs. |
| 15400 | 150 | 5.40 | 9.77 | 7.07 | 0.99 | Dead-3 hrs. |
| 15401 | 150 | 8.44 | 14.44 | 7.88 | n | Dead-3 hrs. |
| 15385 | 75 | 4.90 | 24.18 | 16.54 | n | Dead-3 hrs. |

[a]Listed in increasing order of brain level of insecticide.

[b]Euthanatized.

Table 6. Chickens - acute aldrin exposure

| Wingtag | Sex | Wt. (kg) | Wt. (lbs) | Total aldrin (mg) |
|---|---|---|---|---|
| A. 75 mg/kg - single oral dose | | | | |
| 5188 | M | 1.18 | 2.6 | 88.6 |
| 5200 | F | 0.91 | 2.0 | 68.3 |
| 5145 | M | 1.13 | 2.5 | 84.75 |
| 5213 | M | 1.09 | 2.4 | 81.75 |
| 5202 | F | 1.0 | 2.2 | 75 |
| 5215 | M | 0.91 | 2.0 | 68.3 |
| 5198 | M | 1.23 | 2.7 | 92.25 |
| 5204 | F | 0.82 | 1.8 | 61.5 |
| 5163 | M | 1.09 | 2.4 | 81.75 |
| B. 150 mg/kg - single oral dose | | | | |
| 5191 | F | 0.77 | 1.7 | 115.5 |
| 5168 | M | 1.18 | 2.6 | 177 |
| 5186 | F | 0.95 | 2.1 | 142.5 |
| 5219 | M | 1.23 | 2.7 | 184.5 |
| 5167 | M | 1.04 | 2.3 | 156 |
| 5199 | F | 1.0 | 2.2 | 150 |
| 5155 | M | 1.13 | 2.5 | 169.5 |
| 5179 | M | 1.09 | 2.4 | 163.5 |
| 5176 | M | 1.18 | 2.6 | 177 |
| 5151 | M | 1.09 | 2.4 | 163.5 |

Table 7. Chickens - subacute aldrin exposure

| Wingtag | Sex | Wt. (kg) | Wt. (lbs) | Total aldrin (mg) |
|---|---|---|---|---|
| C. 4 mg/kg - daily oral dose - 10 days maximum | | | | |
| 5172 | F | 1.09 | 2.4 | 4.36 |
| 5217 | M | 1.36 | 3.0 | 5.44 |
| 5203 | F | 1.04 | 2.3 | 4.16 |
| 5154 | M | 1.09 | 2.4 | 4.36 |
| 5180 | M | 1.41 | 3.1 | 5.64 |
| 5220 | F | 0.95 | 2.1 | 3.8 |
| 5181 | M | 1.27 | 2.8 | 5.08 |
| 5171 | M | 1.27 | 2.8 | 5.08 |
| 5177 | F | 1.0 | 2.2 | 4.0 |
| 5211 | M | 1.23 | 2.7 | 4.92 |
| D. 10 mg/kg - daily oral dose - 4 days maximum | | | | |
| 5143 | F | 0.95 | 2.1 | 9.5 |
| 5175 | F | 1.18 | 2.6 | 11.8 |
| 5210 | F | 1.0 | 2.2 | 10 |
| 5147 | M | 1.36 | 3.0 | 13.6 |
| 5187 | F | 0.86 | 1.9 | 8.6 |
| 5193 | M | 1.36 | 3.0 | 13.6 |
| 5190 | M | 1.32 | 2.9 | 13.2 |
| 5169 | F | 1.18 | 2.6 | 11.8 |
| 5139 | M | 1.23 | 2.7 | 12.3 |
| 5142 | M | 1.36 | 3.0 | 13.6 |

Table 8. Chickens - untreated controls

| Wingtag | Sex | Wt. (kg) | Wt. (lbs) | Euthanatized (days after start) |
|---|---|---|---|---|
| 5152 | F | 0.91 | 2.0 | 2 |
| 5138 | M | 1.14 | 2.5 | 2 |
| 5207 | M | 1.18 | 2.6 | 2 |
| 5185 | F | 0.91 | 2.0 | 2 |
| 5149 | F | 0.95 | 2.1 | 2 |
| 5157 | M | 1.18 | 2.6 | 12 |
| 5146 | F | 0.86 | 1.9 | 12 |
| 5192 | F | 1.13 | 2.5 | 12 |
| 5162 | M | 1.41 | 3.1 | 12 |

Table 9. Chickens - tissue levels of untreated controls

| Wingtag | Aldrin-ppm | | | Dieldrin-ppm | | |
|---|---|---|---|---|---|---|
| | Kidney | Liver | Brain | Kidney | Liver | Brain |
| 5152 | n[a] | n | n | n | n | n |
| 5138 | n | n | n | n | n | n |
| 5207 | n | n | n | n | n | n |
| 5185 | n | n | n | n | n | n |
| 5149 | n | n | n | n | n | n |
| 5157 | n | n | n | n | n | n |
| 5146 | n | n | n | n | n | n |
| 5192 | n | n | n | n | n | n |
| 5162 | n | n | n | n | n | n |

[a]No detectable amount.

Table 10. Chickens - acute exposure tissue levels

| Wingtag | Aldrin-ppm Kidney | Aldrin-ppm Liver | Aldrin-ppm Brain | Dieldrin-ppm Kidney | Dieldrin-ppm Liver | Dieldrin-ppm Brain | Remarks |
|---|---|---|---|---|---|---|---|
| A. 75 mg/kg - single oral dose of aldrin | | | | | | | |
| 5188 | 0.26 | tr[a] | tr | 4.64 | 6.54 | 1.25 | Dead-21 hrs. after dose |
| 5200 | 4.26 | 2.6 | 2.0 | 12.1 | 8.53 | 6.04 | Dead-3 hrs. after dose |
| 5145 | n[b] | n | 0.34 | 6.57 | 5.38 | 1.11 | Dead-21 hrs. after dose |
| 5213 | n | n | n | 3.99 | 5.22 | 3.12 | Dead-21 hrs. after dose |
| 5202 | 0.48 | 1.1 | 0.62 | 1.65 | 5.93 | 2.59 | Dead-4 hrs. after dose |
| 5215 | 0.19 | 0.21 | 0.26 | 7.74 | 14.36 | 4.78 | Dead-21 hrs. after dose |
| 5198 | n | n | n | 4.2 | 5.8 | 1.33 | Euth.-27 hrs. comatose[d] |
| 5204 | n | n | n | 5.34 | 5.79 | 4.41 | Euth.-27 hrs. comatose |
| 5163 | n | n | 0.35 | 6.93 | 11.26 | 2.95 | Dead-21 hrs. after dose |
| B. 150 mg/kg - single oral dose of aldrin | | | | | | | |
| 5191 | n | n | n | 9.42 | 13.5 | 4.92 | Dead-21 hrs. after dose |
| 5168 | n | n | 0.29 | 8.83 | 5.64 | 2.44 | Dead-21 hrs. after dose |
| 5186 | 0.78 | 0.50 | 1.08 | 8.64 | 7.75 | 5.96 | Dead-3 hrs. after dose |
| 5219 | 1.36 | 0.98 | na[c] | 5.77 | 9.64 | na | Dead-3 hrs. after dose |
| 5167 | n | n | n | 9.29 | 10.35 | 4.59 | Dead-21 hrs. after dose |
| 5199 | 3.78 | 1.37 | 0.77 | 15.88 | 7.2 | 2.53 | Dead-3 hrs. after dose |
| 5155 | n | n | n | 5.77 | 17.8 | 2.0 | Dead-21 hrs. after dose |
| 5179 | 0.80 | 0.96 | 1.49 | 4.6 | 7.95 | 6.32 | Dead-3 hrs. after dose |
| 5176 | n | n | n | 6.2 | 9.03 | 9.5 | Dead-3 hrs. after dose |
| 5151 | 1.43 | 1.35 | 1.38 | 8.14 | 11.84 | 8.05 | Dead-26 hrs. after dose |

[a] Trace amount.
[b] No detectable amount.
[c] Analysis not run.
[d] Euthanatized.

Table 11. Chickens - subacute exposure tissue levels

| Wingtag | Aldrin-ppm | | | Dieldrin-ppm | | | Remarks |
|---|---|---|---|---|---|---|---|
| | Kidney | Liver | Brain | Kidney | Liver | Brain | |
| C. 4 mg/kg - daily dose - maximum 10 days | | | | | | | |
| 5172 | n[a] | n | n | 5.57 | 13.5 | 2.59 | Euth.-2 days after last dose[b] |
| 5217 | n | n | n | 5.32 | 8.56 | 5.46 | Dead-9th day |
| 5203 | n | n | n | 8.86 | 18.15 | 6.67 | Dead-11th day |
| 5154 | n | n | n | 5.21 | 7.38 | 1.84 | Euth.-2 days after last dose |
| 5180 | n | n | n | 4.11 | 4.99 | 1.7 | Euth.-2 days after last dose |
| 5220 | n | n | n | 5.95 | 19.84 | 5.73 | Dead-8th day |
| 5181 | 0.24 | 0.51 | 0.34 | 3.87 | 5.16 | 1.99 | Euth.-2 days after last dose |
| 5171 | n | n | n | 6.53 | 21.21 | 3.77 | Dead-10th day |
| 5177 | n | n | n | 7.24 | 7.13 | 1.11 | Euth.-2 days after last dose |
| 5211 | n | n | n | 4.99 | 13.99 | 5.37 | Dead-8th day |
| D. 10 mg/kg - daily dose - maximum 4 days | | | | | | | |
| 5143 | n | n | n | 8.82 | 7.12 | 2.37 | Dead-7th day |
| 5175 | n | n | n | 4.79 | 5.06 | 1.4 | Dead-5th day |
| 5210 | n | n | n | 4.88 | 5.79 | 3.33 | Dead-4th day |
| 5147 | n | n | n | 4.63 | 9.30 | 2.84 | Dead-6th day |
| 5187 | n | n | n | 6.38 | 4.94 | 5.23 | Dead-5th day |
| 5193 | n | n | n | 5.04 | 6.98 | 0.85 | Euth.-2 days after last dose |
| 5190 | n | n | n | 5.69 | 6.43 | 5.18 | Dead-4th day |
| 5169 | n | n | n | 3.13 | 7.30 | 1.14 | Euth.-2 days after last dose |
| 5139 | n | n | n | 4.98 | 3.09 | 4.14 | Dead-4th day |
| 5142 | n | n | n | 6.59 | 7.19 | 2.43 | Dead-5th day |

[a]No detectable amount.

[b]Euthanatized.

Table 12. Chickens - combined insecticide tissue levels

| Wingtag | Mg/kg dose | Total dose (mg/kg) | Aldrin + Dieldrin-ppm Kidney | Liver | Brain | Remarks |
|---|---|---|---|---|---|---|
| 5193 | 10 | 40 | 5.04 | 6.98 | 0.85[a] | Euth.-2 days after last dose[b] |
| 5177 | 4 | 40 | 7.24 | 7.13 | 1.11 | Euth.-2 days after last dose |
| 5169 | 10 | 40 | 3.13 | 7.3 | 1.14 | Euth.-2 days after last dose |
| 5188 | 75 | 75 | 4.90 | 6.54 | 1.25 | Dead-21 hrs. after dose |
| 5198 | 75 | 75 | 4.2 | 5.8 | 1.33 | Euth.-after 27 hrs., comatose |
| 5175 | 10 | 40 | 4.79 | 5.06 | 1.4 | Dead-5th day |
| 5145 | 75 | 75 | 6.51 | 5.38 | 1.45 | Dead-21 hrs. after dose |
| 5180 | 4 | 40 | 4.11 | 4.99 | 1.7 | Euth.-2 days after last dose |
| 5154 | 4 | 40 | 5.21 | 7.38 | 1.84 | Euth.-2 days after last dose |
| 5155 | 150 | 150 | 5.77 | 17.8 | 2.00 | Dead-21 hrs. after dose |
| 5181 | 4 | 40 | 4.11 | 5.67 | 2.33 | Euth.-2 days after last dose |
| 5143 | 10 | 40 | 8.82 | 7.12 | 2.37 | Dead-7th day |
| 5142 | 10 | 40 | 6.59 | 7.19 | 2.43 | Dead-5th day |
| 5172 | 4 | 40 | 5.57 | 13.5 | 2.59 | Euth.-2 days after last dose |
| 5168 | 150 | 150 | 8.83 | 5.64 | 2.73 | Dead-21 hrs. after dose |
| 5147 | 10 | 40 | 4.63 | 9.30 | 2.80 | Dead-6th day |
| 5213 | 75 | 75 | 3.99 | 5.22 | 3.12 | Dead-21 hrs. after dose |
| 5202 | 75 | 75 | 2.13 | 7.03 | 3.21 | Dead-4 hrs. |
| 5163 | 75 | 75 | 6.93 | 11.26 | 3.30 | Dead-21 hrs. |
| 5199 | 150 | 150 | 19.66 | 8.57 | 3.30 | Dead-3 hrs. |
| 5210 | 10 | 40 | 4.88 | 5.79 | 3.33 | Dead-4th day |
| 5171 | 4 | 36 | 6.53 | 21.21 | 3.77 | Dead-10th day |
| 5139 | 10 | 30 | 4.98 | 3.09 | 4.14 | Dead-4th day |
| 5204 | 75 | 75 | 5.34 | 5.79 | 4.41 | Euth.-after 27 hrs. |
| 5167 | 150 | 150 | 9.29 | 10.35 | 4.59 | Dead-21 hrs. |
| 5191 | 150 | 150 | 9.42 | 13.5 | 4.92 | Dead-21 hrs. |
| 5215 | 75 | 75 | 7.93 | 14.57 | 5.04 | Dead-21 hrs. |
| 5190 | 10 | 40 | 5.69 | 6.43 | 5.18 | Dead-4th day |
| 5187 | 10 | 40 | 6.38 | 4.94 | 5.23 | Dead-5th day |
| 5211 | 4 | 28 | 4.99 | 13.99 | 5.37 | Dead-8th day |

[a] Listed by increasing brain level of total insecticide.

[b] Euthanatized.

Table 12. (Continued)

| Wingtag | Mg/kg dose | Total dose (mg/kg) | Aldrin + Dieldrin-ppm Kidney | Liver | Brain | Remarks |
|---|---|---|---|---|---|---|
| 5217 | 4 | 32 | 5.32 | 8.56 | 5.46 | Dead-9th day |
| 5220 | 4 | 32 | 5.95 | 19.84 | 5.73 | Dead-8th day |
| 5203 | 4 | 36 | 8.86 | 18.15 | 6.67 | Dead-11th day |
| 5186 | 150 | 150 | 9.42 | 8.25 | 7.04 | Dead-3 hrs. |
| 5179 | 150 | 150 | 5.4 | 8.91 | 7.81 | Dead-3 hrs. |
| 5200 | 75 | 75 | 16.36 | 11.13 | 8.04 | Dead-3 hrs. |
| 5151 | 150 | 150 | 6.2 | 9.03 | 9.5 | Dead-26 hrs. |
| 5176 | 150 | 150 | 9.57 | 13.19 | 9.43 | Dead-3 hrs. |

Table 13. Statistical discriminant functions

| Brain (ppm) | Kidney (ppm) | Statistical probability to live | $\bar{x}$ of 3 brain (ppm) | $\bar{x}$ of 3 kidney (ppm) |
|---|---|---|---|---|
| Dieldrin (aldrin) in swine | | | | |
| -- | -- | .995 | 1.7 | -- |
| -- | -- | .99 | 2.0 | 1.6 |
| 1.6 | 1.6 | .95 | 2.36 | 1.89 |
| 2.3 | 1.92 | .90 | 2.50 | 1.98 |
| 3.2 | 2.51 | .50 | 2.82 | 2.2 |
| 3.8 | 2.91 | .10 | 3.09 | 2.38 |
| 3.96 | 3.03 | .05 | 3.17 | 2.44 |
| Dieldrin (aldrin) in chickens | | | | |
| -- | | .98 | 1.5 | |
| -- | | .95 | 1.9 | |
| 1.5 | | .90 | 2.07 | |
| 2.72 | | .50 | 2.42 | |
| 3.2 | | .14 | -- | |
| -- | | .10 | 2.68 | |
| 3.3 | | .09 | -- | |
| 3.4 | | .06 | -- | |
| -- | | .05 | 2.75 | |
| -- | | .01 | 2.9 | |