THE EFFECTS OF DIELDRIN ON REPRODUCTION IN THE RAT

by

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B.Sc. (Honors), University of British Columbia, 1965

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in the Department
of
ZOOLOGY

We accept this thesis as conforming to the required standard.

THE UNIVERSITY OF BRITISH COLUMBIA

JULY 1970
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Department of Technology

The University of British Columbia
Vancouver B, Canada

Date August 1970
ERRATA

Throughout the text. For "homogenous" read "homogeneous".
Throughout the text. For "homogenity" read "homogeneity".
Throughout the text. For "consistant" read "consistent".
Throughout the text. For "consistantly" read "consistently".
Page 4, line 12. For "in" read "it".
Page 13, line 19. For "was" read "were".
Page 18, line 15. For "is" read "are".
Page 34, line 9. For "have" read "has".
Page 42, line 23. For "is" read "are".
Page 44, line 1. For "dehydrogenoses" read "dehydrogenases".
Page 45, line 15. For "affects" read "effects".
ABSTRACT

The effects of chronic ingestion of technical dieldrin\(^1\) on reproduction in the rat were studied by determining the number of litters, the number and the viability of young born to animals exposed to dietary concentrations of 0, 13, 25, 37 and 53 ppm. Dieldrin had no effect on the reproductive success of the males. In the females dieldrin had no effect on fertility and did not significantly reduce litter sizes but evidence is presented which suggests the litter size reduction is real. Exposure of the dam during lactation reduces the survival of suckling young. It is suggested that the major cause of this mortality is acute poisoning from dieldrin secreted in the milk, although the growth responses of the young indicate that starvation resulting from decreased lactation may also be involved. None of the mortality is caused by increases in maternal aggression or neglect. Exposure during gestation alone also reduces the post-partum survival of young. The probable physiological mechanisms involved in the various effects are discussed.

\(^1\) (85% 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 and 15% related compounds.)
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ACKNOWLEDGEMENTS

I should like to thank Drs. H.C. Nordan, J. Mary Taylor, J.E. Phillips and J.F. Bendell for their advice and criticism during the study and the preparation of this manuscript. I am particularly grateful to the Shell Chemical Corporation whose gift of dieldrin made this work possible. I extend special thanks to my parents, particularly my mother who typed the drafts of this paper. Most of all I wish to thank my wife Sheila for her encouragement and support.
I. INTRODUCTION

The organochlorine insecticides are now distributed throughout the ecosystems of the world such that dietary exposure to these compounds is probably common for most species. It is becoming increasingly apparent that such exposure can result in mutagenesis, carcinogenesis, synergistic responses to other pesticides and lowered rates of reproduction.

Of these effects, impairment of the normal reproductive process is likely to have the most serious consequences on a population of a wild species. Many investigators have suggested that the organochlorines are responsible for the decreased rates of reproduction shown by raptors and other birds and the subsequent decline in population levels (Hickey and Anderson, 1968). Recent laboratory work has confirmed that these compounds can decrease rates of egg production, fertility and hatchability and the survival rate of young (Bitman et al., 1969; Heath et al., 1969; McFarland and Lacy, 1969).

Most of the organochlorine insecticides are unlikely to significantly affect mammalian reproduction rates. The hexachlorocyclohexanes (lindane and BHC) are not apt to cause problems since their high cost prohibits large scale use. The poly-chlorinated biphenols (DDT and its analogs) are known to reduce the pregnancy rate of the house mouse and the survival rate of young mice and rats (Bernard and Gaertner, 1964; Ware and Good, 1967; Tayan and Kemeny, 1969) but only at exposure doses unlikely to be obtained in natural systems. For similar reasons the single ring cyclodiienes are not likely to cause problems; chlordane is known to reduce the pregnancy rate and survival rate of young in house mice and rats but only at very high doses (Ambrose et al., 1953; Conney et al.,
1967); telodrin, at ecological doses, had no effect on house mouse reproduction (Ware and Good, 1967). Endosulfan and heptachlor, the others in this group, will probably behave in a similar manner.

The double ring cyclodienes (aldrin, isodrin, dieldrin, endrin) do present a real potential hazard to mammalian populations. Their stability (Nash and Wilson, 1967) and large scale (Anon., 1963) use result in environmental levels (Saha, 1969), which in conjunction with their ease of absorption (Lehman, 1956), could result in doses sufficient to lower reproduction in some species.

Snyder (1963) has presented the only evidence that these compounds can reduce numbers in a population of mammals when he demonstrated that endrin reduced the reproduction rate of a population of meadow voles. There are other reasons to believe that the double ring cyclodienes are apt to be the compounds most harmful to mammalian populations.

It has been shown that low doses of these compounds adversely affect reproduction in some mammals. Deichmann and Keplinger (1966) demonstrated with the house mouse that dieldrin and aldrin at 25 ppm reduced the pregnancy rate, the litter size and the survival rate of young; later work (Good and Ware, 1969) showed that dieldrin at 5 ppm had no effect on the pregnancy rate but reduced litter size (survival was not studied). The house mouse is sensitive to these compounds as is the dog; doses in excess of 3.0 ppm reduced the survival of pups but had no effect on pregnancy rates or litter sizes (Kitselman, 1953). Good and Ware also showed that endrin at 5 ppm reduced litter sizes in the house mouse whereas Morris (1968) demonstrated that 7 ppm had no effect on litter size in the deer mouse (Peromyscus maniculatus); the latter species also showed a decreased survival rate in the young. The double ring cyclodienes clearly
affect mammalian reproduction but there are large differences in response between species such that generalizations cannot be made. There is a lack of data on the effects of these compounds in the rat. Ball et al. (1953), using a vaginal smear technique, noted that aldrin appeared to cause transitory changes in the estrous cycle but they conducted no breeding tests. Treon and Cleveland (1955), using the 3 generation breeding technique of Fitzhugh (1949), claim that aldrin and dieldrin in doses as low as 2.5 ppm decreased fertility in the first generation and the survival of young in all generations, while doses as high as 25 ppm had no effect on the litter size; however, they present no data or statistics to substantiate their claims. No other work has been done on the rat and in view of the apparently extreme susceptibility of this species there is a need for data on it. This susceptibility, if real, would make the rat an ideal animal for studying the physiological mechanisms of organochlorine induced reproductive failure.

The purpose of this study was to determine if chronic exposure to dietary dieldrin would cause decreases in fertility, litter size and survival rate of sucklings and to determine the amount of exposure required to induce these effects.
II. METHODS

1. Exposure of Animals to Dieldrin

Five groups of post-pubertal rats were fed food containing dieldrin in concentrations of 0, 13, 25, 37 or 53 ppm for 44 weeks. The animals were of the Wistar strain (UBC stock, randomly bred for approximately 16 generations) and were 25-27 weeks of age at the onset of the experiments. There were 18 males and 18 females in each group at the start. The sexes were housed separately in communal metabolism cages with 9 animals per cage. All the animals were fed the 0 ppm ration for 1 month after being put in the cages to allow them to adjust to the ground feed and to combat a respiratory disease which arose. Food and water were supplied ad libitum.

The food was prepared by grinding commercially prepared UBC rat ration in a crusher mill and spraying in with dieldrin dissolved in 95% EtOH. To ensure homogeneity of dieldrin distribution the spray was applied by a small hand-sprayer to 2 lb. portions of feed; several applications were made and the feed was thoroughly mixed between applications. The 0 ppm ration was sprayed with ethanol only. The food was fed in the ground state.

2. Measurement of Exposure to Dieldrin (Adults)

It was desirable to have 2 measures of the amount of exposure to dieldrin. One is the amount actually being absorbed from the gut, the second is the concentration in the various organs.

It can be calculated from the data of Heath and Vandekar (1964) that only 0.007% of the fecal dieldrin is compound which has been absorbed and excreted unchanged. Therefore if the amount of dieldrin ingested, the amount of feces produced and the dieldrin concentration in the feces are
known then the rate of absorption can be calculated.

The animals were fed from spill-proof feeders which were weighed before and after filling such that the feed consumption could be determined by subtraction. The amount of dieldrin ingested was calculated by multiplying the amount of feed consumed by the concentration of dieldrin in the feed. The animals were weighed once a week such that exposure could be expressed as mgm. of ingested dieldrin per kgm. of body weight.

The feces were caught on screens below the cages and were collected once a week, dried for 12 hours at 100°C, weighed and a sample stored for analysis. The animals were sampled by sacrificing 2 males and 2 females at each level of exposure 1 day before the start of a breeding test; the carcasses were frozen and stored for analysis.

The method of analysis is presented below.

3. **Measurement of Reproductive Success**

The animals were tested for reproductive capability after 10, 19, 28 and 39 weeks of exposure.

For the first 2 tests a factorial mating system was used thus:

1. treated male X treated female
2. treated male X untreated female
3. untreated male X treated female

Pairs were made up by randomly selecting animals from the metabolism cages and placing them in small mating cages. Four pairs were used for each cross and each cross was made for the 5 levels of exposure. The males were removed after 2 weeks and the females left in the cages throughout pregnancy and lactation. The untreated animals were obtained from the UBC stock colony and were approximately the same age as the treated animals.

The results from the first 2 tests indicated that dieldrin was
affecting the females but not the males. To enlarge the sample size of treated females and yet still obtain data on the treated males a different mating system was used in the last 2 tests thus:

1. treated male X treated female
2. treated male X untreated female

The females were not placed in mating cages but were kept in their metabolism cages and 2 males were placed in each cage. The males were changed every day. Mating was allowed for 2 weeks and the animals were retained on their experimental diets during this time. The pregnant females were placed in individual cages at about day 14 of gestation to give birth and raise their litters.

The data obtained from these tests were the number of pregnancies, the litter size and sex ratio at birth, and the survival of the young. The latter was determined by examining the litters daily and noting the number and sex of the young present. In addition, the weight of the young born to females in the third and fourth tests was determined daily.

To determine if dieldrin had an effect on the estrous cycle of the female, vaginal smears were taken daily from animals at each level of exposure for the periods of 4-8, 23-28 and 36-38 weeks inclusive. Smears were obtained by aspirating the vagina with a drop of physiological saline and placing the aspirant on a glass slide. After the aspirant had dried the cells were fixed with absolute EtOH and stained for 10 minutes with a 0.015% Giemsa stain. The smears were examined and defined as estrus if 80% of the cells were cornified epithelial cells, diestrus if 80% were leucocytes and proestrus if 80% were nucleated epithelial cells.

In the analysis of the results the data from the treated male X treated female and untreated male X treated female crosses were combined to test female effects if there was no effect on the male.
4. Measurement of Exposure to Dieldrin (Young)

To clarify the cause of death of the young that died before weaning it was desirable to determine the amount of dieldrin in them. All young found dead were labelled, frozen and stored for analysis. In addition all young that survived until weaning were sacrificed, frozen and stored for analysis.

5. The Foster Nursing Experiments

The data from the reproduction tests indicated that dieldrin in the females' diet caused a decreased survival of her young. To determine whether the cause was due to pre-partum or post-partum exposure of the female, the young of 15 females exposed to 53 ppm in their diet during gestation were foster nursed on females exposed only to the 0 ppm diet; the reverse was also followed. This was done by replacing half the litter of a 53 ppm female with half the litter of a 0 ppm female and vice versa. The transfers were made within 6 hours of birth but after the young had received some colostrum from their own female. The young of these mixed litters were toe-clipped in order to identify their real dam. The survival and weight of the young were recorded daily.

To determine the effects of a foster mother on the survival and growth of young an experiment was run previous to that described above but identical in every respect except all of the females (10) were on a 0 ppm dieldrin diet.

6. Observations on Maternal Behavior

The foster nursing experiment indicated that there was both a pre- and post-partum component in the causation of the decreased survival of young. To elucidate the post-partum component of the cause(s) observations were made on the maternal behavior of females on the 0, 37 and 53 ppm
diets. These observations were made on females used in the fourth reproductive test and the foster nursing experiment. Previous observation suggested 12 behavior patterns were shown by females and in addition 3 others were added which \textit{a priori} could be defined as aggressive (see Appendix I). The 15 patterns could be fitted into one of 4 categories:

1. Maternal - care of young
2. Aggression - toward young
3. Maintenance - female's care of herself
4. Others - fitted none of above i.e. function debatable

Three 10 minute periods of observation were made on each animal per day. One period was chosen between 0800-1100 hours, one between 1400-1700 and the third between 1900-2200. Every 1.5 seconds the animal's behavior was noted. A metronome was used for timing.

7. **Measurement of Dieldrin Residues**

The method chosen to measure the amount of dieldrin in the feces and animal tissues was that originally described by Cueto (1960). He applied it to animal fat and it was later used by Cueto and Hayes (1962) to measure dieldrin in human urine.

The 3 steps in the estimation of the dieldrin content of a sample are:

1. extraction of the dieldrin
2. removal of contaminants from the extract i.e. clean-up
3. colour development of the extracted dieldrin

The colour reaction is the basic principle of the technique and depends on the fact that dieldrin forms a blue complex with diphenylamine in the presence of zinc chloride. The optical density (OD) of the reaction products in acetic acid solutions at 650 \textmu m follows Beer's Law such that an unknown amount of dieldrin can be determined from a standard curve.
This method was chosen because it is sensitive to microgram quantities of dieldrin and is very inexpensive in comparison to the gas chromatographic techniques. Two major problems were encountered, one of which was not solved and the technique could not be used.

The colour reaction as originally developed was used on dieldrin which was at least 99.5% pure. In this study technical grade dieldrin, which is the basis for commercial insecticides, was used and it contains 85% dieldrin and 15% related insecticidal compounds. Technical grade dieldrin is biologically the more important preparation, particularly since there is evidence that the properties of purified, recrystallized compounds may differ considerably from the commercial products (Ball et al., 1953; Treon et al., 1955). Major improvements were required in Cueto's technique before it had adequate sensitivity and precision for use on technical dieldrin.

The extraction of a pesticide from biological materials invariably results in the simultaneous extraction of materials which will interfere with the quantification of the pesticide. An adequate method for removing contaminants from an extract is essential and it is this process which is usually limiting in the effectiveness of any measurement procedure. In this study modifications of 3 clean-up procedures (those of Cueto, 1960; Kutney, 1969; de Faubert Maunder et al., 1964) were applied to extracts of both feces and tissues in an unsuccessful attempt to find one which would allow the use of the colour reaction.

To use the colour reaction it became apparent that there must be absolutely no contaminants in the final solution of dieldrin undergoing the reaction because their presence resulted in the formation of red or green materials which were insoluble in the reaction products solvent and which completely masked the diphenylamine-dieldrin complex. The degree of
purity required in the final solution to be analyzed is probably much higher than for gas chromatographic techniques.

The interfering materials in the feces were apparently carotenoid in nature (Kutney, 1969) while those in the tissues were lipoidal (i.e. ether soluble).

A full description of the technique is presented in Appendix II.
III. RESULTS

1. Exposure to Dieldrin and the Health of the Animals

The cumulative dose of dieldrin which the animals ingested is presented in Fig. 1. The females experience more exposure than the males at each dose and this is a result of their eating more food (Table I).

Table I. The total food intake (kgm. feed/kgm. body weight) of animals eating dieldrin contaminated food for 44 weeks.

<table>
<thead>
<tr>
<th>Dieldrin Concentration (ppm)</th>
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<tr>
<td>Sex</td>
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<tr>
<td>O</td>
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<tr>
<td>F</td>
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</table>

The difference in food intake is not large being about 0.5 gm./day for an average sized unexposed female and about 1.5 gm./day for an exposed female. The magnitude of the difference and the time at which it is first detectable appear to be dose-dependent (Fig. 1). The apparent exception of the 53 ppm animals to the time effect is due to the males eating an abnormally large amount of food for the first 4 weeks. These data suggest that dieldrin stimulates the female to eat more food but has no effect on the male.

The amount of exposure for females ranged from 5.3 mgm./kgm. body weight/week at 13 ppm to 20.4 at 53 ppm; this represents 12% to 47% of the acute LD50 of 43 mgm./kgm. for adult females (Borgmann et al., 1952b). The total exposure for females over the course of the experiment ranged
Fig. 1 The total exposure of the rats to dietary dieldrin.

Cumulative Dose (mg/kgm. body wt.)

Weeks of Exposure

- 93 ppm
- 57 ppm
- 25 ppm
- 15 ppm
- 10 ppm
- 5 ppm
from 56.6 mgm./kgm. body weight at 13 ppm to 859.3 mgm. at 53 ppm.

The amount of exposure for the males was slightly less, which in conjunction with the higher LD50 for males (47 mgm./kgm. Borgmann et al., 1952b), resulted in a much lower "effective dose" for this sex. Males on the 53 ppm diet for example, ingested 18.2 mgm./kgm. body weight/week or only 39% of the lethal dose.

The growth of either sex does not appear to be affected by dietary dieldrin (Fig. 2). Each point is the mean weight of 10-18 animals and range is such that none of the apparent differences are real. The sharp decline in weight shown by the males during weeks 30-34 was caused by an epidemic of a respiratory disease.

None of the animals were observed to show the classic symptoms of acute poisoning as described by Hodge et al. (1967).

Females on the 37 and 53 ppm diets were observed to show hyper-excitability to external stimuli, particularly sound and to be nervous and irritable during weeks 7-10 of exposure. No other symptoms of chronic poisoning were noted.

2. Statistical Analyses of the Data on Reproductive Performance

The enumeration data (% pregnant, % survival, sex ratios) was tested for significance by use of the x² test for independence in r x c tables (Steele and Torrie, 1961) except for the % survival data from the foster nursing experiment which was tested by the G test for r x c x t tables (Sokal and Rohlf, 1969). The litter size, life expectancy and age at death data were tested for significance by use of an analysis of variance (Steele and Torrie, 1961). All paired comparisons between doses were made regardless of the significance of the overall analysis of variance; the significance of these comparisons was tested by use of Scheffe's
The effect of dietary dieldrin on the post-pubertal growth of the rat.
procedure for multiple comparisons with unequal sample sizes (Scheffe, 1959).

3. Effects on the Reproductive Capacity of the Male

The pregnancy rate of untreated females mated with treated males is homogenous between doses ($X^2 = 8.182, \text{df} = 4, P = 0.084$) which suggests that the fertility of the male is unimpaired by dieldrin at doses as high as 53 ppm; note however, that the fertility rate of males on 25 ppm is much lower than those on the other doses (Table II). Similarly the size of litter a male sires is not affected by the dose of dieldrin he receives. (overall analysis: $F = 0.770, P>0.10$; none of the paired comparisons indicate significant differences).

The sex ratio of young sired by treated males is not homogenous between doses ($X^2 = 11.47, \text{df} = 4, P = 0.022$), but differs at the 0.05 level. However, at what dose(s) this apparent effect occurs is unclear, because removal of the data from any cell results in an insignificant $X^2$ when it is re-calculated. This effect is very difficult to explain and is probably spurious. The male determines sex and if the effect is real one would expect similar results from the young sired by exposed males and born to exposed females and this is not the case (Table IV). If the sex ratios from both Tables II and IV are combined the data are homogenous ($X^2 = 3.82, \text{df} = 4, P = 0.443$). There could of course be some form of compensatory mortality of embryos in the treated females which would mask the sex ratio effect but this would probably be reflected by a decrease in the litter size and this does not occur (Table IV).

The survival rate of pre-weaning young sired by treated males is not homogenous ($X^2 = 40.30, \text{df} = 4, P<10^{-5}$) which suggests that dieldrin is affecting the sperm in such a way that the young are relatively inviable. "Surviving" is defined as being alive at weaning, 23 days of age. However, survival is a crude index of viability and it is difficult to determine
Table II. The reproductive success of unexposed females mated by males exposed to dietary dieldrin. The number in brackets is the sample size.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>13</th>
<th>25</th>
<th>37</th>
<th>53</th>
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<tbody>
<tr>
<td>% Pregnant</td>
<td>83</td>
<td>83</td>
<td>50</td>
<td>83</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>(18)</td>
<td>(18)</td>
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<td>(18)</td>
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<tr>
<td>Litter Size x±SE</td>
<td>10.5±0.54</td>
<td>11.6±0.39</td>
<td>10.9±0.74</td>
<td>10.9±0.65</td>
<td>11.1±0.75</td>
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<td></td>
<td>(12)</td>
<td>(14)</td>
<td>(9)</td>
<td>(13)</td>
<td>(12)</td>
</tr>
<tr>
<td>Sex Ratio at Birth</td>
<td>100</td>
<td>89</td>
<td>106</td>
<td>82</td>
<td>62</td>
</tr>
<tr>
<td>♂:100 ♀:s</td>
<td>(136)</td>
<td>(155)</td>
<td>(87)</td>
<td>(129)</td>
<td>(133)</td>
</tr>
<tr>
<td>% Survival to 23 Days</td>
<td>58.1</td>
<td>63.9</td>
<td>39.1</td>
<td>66.7</td>
<td>36.1</td>
</tr>
<tr>
<td></td>
<td>(136)</td>
<td>(155)</td>
<td>(87)</td>
<td>(129)</td>
<td>(133)</td>
</tr>
<tr>
<td>Expectancy of Life (days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at Day 1 of Life (♂)</td>
<td>16.0±1.1</td>
<td>17.5±1.0</td>
<td>12.3±1.2</td>
<td>18.9±0.9</td>
<td>14.9±1.2</td>
</tr>
<tr>
<td></td>
<td>(63)</td>
<td>(66)</td>
<td>(48)</td>
<td>(57)</td>
<td>(50)</td>
</tr>
<tr>
<td>Expectancy of Life (♀)</td>
<td>14.7±1.1</td>
<td>16.6±1.0</td>
<td>12.8±1.5</td>
<td>17.6±0.9</td>
<td>13.3±0.9</td>
</tr>
<tr>
<td></td>
<td>(64)</td>
<td>(84)</td>
<td>(34)</td>
<td>(72)</td>
<td>(81)</td>
</tr>
</tbody>
</table>
where the real differences lie using this parameter because of the large error term associated with binomial data. The life expectancy of the young is a much more sensitive index of viability and therefore was chosen as the parameter to compare between doses.

The life expectancy of the male offspring differs significantly between doses ($F = 5.36, P = 0.0004$). Bartlett's test indicates that the variances are homogenous ($P = 0.370$) and therefore paired comparisons are valid. Scheffe's test indicates that none of the treatments differ significantly from the control ($P > 0.05$). As such, exposure of the male to dieldrin does not affect the viability of the male offspring he sires. The sources of variance which cause significance in the overall analysis are from the doses with the high and low extremes in life expectancy and this can be attributed to chance; 37 and 53 ppm differ at $P < 0.01$ and 25 and 37 ppm differ at $0.01 < P < 0.05$.

Similarly, the life expectancy of the female young also differs significantly between doses ($F = 3.93, P = 0.0041$). Bartlett's test indicates the variances are homogenous ($P = 0.498$) such that paired comparisons are valid. Scheffe's test indicates that none of the treatments differ significantly from the control ($P > 0.05$). As such exposure of the male to dieldrin has no effect on the life expectancy of the female offspring he will sire. The significant variance arises from the doses with the two extremes of life expectancy; 37 and 53 ppm differ at $0.01 < P < 0.05$.

The life expectancy of the females is lower than the male at all doses. However, Fisher's t test indicates that the differences are not significant (maximum $t = 0.349$, $df = 00$).

4. Effects on the Reproductive Capacity of the Female

The effect of dietary dieldrin on the length of the estrous cycle and the number of positive estrus' is presented in Table III. The data from
weeks 23-32 and 36-38 have been combined to give a sample size comparable to that for weeks 4-8. The mean number of positive estrus' has been corrected for the time difference in the 2 periods.

There was a great deal of variation between individuals. Animals had cycles ranging from 2-14 days in length; many were acyclic, being in constant estrus, diestrus or proestrus. There was no correlation between the type of cycle exhibited by a female and her fertility. Moreover there is no reason to believe that either the cycle length or the number of positive estrus' differ between doses or times.

The fertility rate of the treated females and the size and sex composition of their litters is presented in Table IV. The data from all 4 breeding tests have been pooled as there were no apparent differences between them. Neither the pregnancy rate \( \chi^2 = 5.97, \ df = 4, \ P = 0.20 \), the litter size (overall \( F = 0.74, \ P > 0.10 \) and all paired comparisons are not significant), nor the sex ratio \( \chi^2 = 4.36, \ df = 4, \ P = 0.360 \) differ between doses.

For future reference it should be noted that the litter size of the treated females is consistently smaller than those of the controls. More important is the fact that the young of treated females were very frequently born with gross hematomas in the head and caudal region.

The survival rates of the young born to treated and control females (Fig 3) are not homogenous \( \chi^2 = 127.99, \ df = 4, \ P < 10^{-5} \) such that exposure of the female to dieldrin affects the viability of her offspring. The data in Fig. 3 fit a typical dose-mortality curve (see Appendix III) except that the lower plateau phase is missing.

Since survival is such a crude index of viability, life expectancy was used as the index to determine where the significant effects lay (Fig 3). Considering the males first, the analysis of variance indicates highly
Table III. The effect of dietary dieldrin on the estrous cycle of the rat.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Weeks of Exposure</th>
<th>Dietary Concentration (ppm)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 13 25 37 53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean No. of Positive Estrous' per Animal</td>
<td>4-8</td>
<td>3.8 3.3 3.7 3.1 3.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>23-38</td>
<td>3.4 3.9 3.2 3.2 3.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Cycle Length (days)</td>
<td>4-8</td>
<td>5.42 4.97 5.10 5.20 5.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>23-38</td>
<td>4.86 5.23 4.72 6.13 5.21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample Size</td>
<td>4-8</td>
<td>16 16 17 17 17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>23-38</td>
<td>16 17 17 17 17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table IV. The reproductive success of females exposed to dietary dieldrin.

The number in brackets refers to the sample size.

<table>
<thead>
<tr>
<th>Parametre</th>
<th>Dietary Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>% Pregnant</td>
<td>36.8</td>
</tr>
<tr>
<td></td>
<td>(38)</td>
</tr>
<tr>
<td>Litter Size (\bar{x} \pm SE)</td>
<td>11.3±1.0</td>
</tr>
<tr>
<td></td>
<td>(13)</td>
</tr>
<tr>
<td>Sex Ratio at Birth (\frac{\text{M}}{\text{F}})</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>(147)</td>
</tr>
</tbody>
</table>
Fig. 3 The effect of dieldrin in the diet of the dam on the survival and life expectancy of the young.
significant differences between doses \((F = 20.57, P<10^{-6})\).

Scheffe's test shows that:

\[
\begin{array}{ccc}
0 \text{ ppm} & \text{vs} & 13 \text{ ppm} & \text{ns. } P>0.05 \\
0 \text{ ppm} & \text{vs} & 25 \text{ or } 37 \text{ or } 53 \text{ ppm} & ** P<0.01 \\
13 \text{ ppm} & \text{vs} & 25 \text{ or } 37 \text{ or } 53 \text{ ppm} & * P<0.05 \\
\text{all comparisons between 25, 37 and 53 ppm} & & \text{ns. } P>0.05
\end{array}
\]

The results of the analysis on the life expectancy of the females are identical. The validity of Scheffe's test may be questioned because Bartlett's test indicates that the probability of homogenous variances is less than \(10^{-6}\). By Fisher's t test there is no difference between the life expectancy of males and females \((\text{maximum } t = 0.457, \text{ df } = 00)\). Considering these results it is clear that the dose received for a significant reduction in the viability of the offspring is between 13 and 25 ppm but the Threshold Dose is less than 13 ppm.

The age at death of those offspring dying before weaning is presented in Table V where the sexes have been pooled. An analysis of variance indicates that there are significant differences at the 0.05 level \((F = 3.49, P = 0.017)\). However Scheffe's test for multiple comparisons indicates that none of the treatments differ from the control \((F = 1.67 \text{ with } 4 \text{ and } 476 \text{ df})\) and the significant variance comes from the maximum and minimum doses \((13 \text{ and } 53 \text{ ppm})\) which differ at the 0.01 level \((F = 3.93)\). Bartlett's test indicates non-homogenity of variance at the 0.05 level \((P= 0.038)\).

The growth responses of the young are presented in Fig. 4. The mean body weight per surviving young was determined by weighing the litter of a female, dividing by the number of young in the litter and then averaging these weights for all females. There would appear to be no differences in growth. The sample, however, is very small and there is normally little growth in the first 5 days by which time the offspring of females on 25,
Table V. The age at death of those young dying before weaning whose dams were exposed to dietary dieldrin.

<table>
<thead>
<tr>
<th>Dietary Concentration (ppm)</th>
<th>Control</th>
<th>13</th>
<th>25</th>
<th>37</th>
<th>53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at Death</td>
<td>5.02±0.58</td>
<td>6.44±0.63</td>
<td>4.35±0.64</td>
<td>4.64±0.44</td>
<td>3.98±0.43</td>
</tr>
</tbody>
</table>
Fig 4 The growth response of young rats nursing on dams exposed to dietary dieldrin.

- litters increasing from birth weight until all litter members died
- litters maintaining birth weight until all litter members died
- litters decreasing from birth weight until all litter members died

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Litters</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td>(7)</td>
</tr>
<tr>
<td>13 ppm</td>
<td>(7)</td>
</tr>
<tr>
<td>25 ppm</td>
<td>(2)</td>
</tr>
<tr>
<td>37 ppm</td>
<td>(6)</td>
</tr>
<tr>
<td>53 ppm</td>
<td>(5)</td>
</tr>
</tbody>
</table>
37 and 53 ppm dieldrin are dead. Moreover the growth of the young is apparently a function of the female such that growth effects are masked by averaging. The body weight of surviving young of any female consistently increases, decreases, or remains constant and the proportion of females whose young grow decreases with increasing exposure to dieldrin (Fig. 4). The young that did not grow consistently had little or no milk in the gut.

5. Viability of Young - The Foster Nursing Experiment: Effects of Pre and Post-partum Maternal Exposure

The most efficient way of partitioning the causation of the decreased viability of young is by foster nursing as long as the effect of a foster dam per se on viability is known. The % survival, life expectancy and growth of foster young does not differ from those raised by their own dam (Fig 5). Since post-partum maternal factors do not influence viability all possible comparisons can be made between the controls (0-0) and the treatments (0-53, 53-0 and 53-53) - the first number refers to dose the female was receiving while the young were in utero and the second number refers to the dose received by the female on which the young were nursing.

Despite the apparent marked difference in survival (Fig. 6) between groups there is no statistical significance to the differences (G = 7.82, df = 4, P = 0.063). Similarly the G tests for paired comparisons indicate no differences.

However the life expectancy index does reveal significant differences in viability (Fig. 7). The overall analysis of variance is significant (F = 156.75, P<10^-6). Scheffe's test reveals that the life expectancy is different between all groups (minimum F = 7.81 which with 3 and 285 df results in P<0.01) except for the 0-53 and 53-53 comparison (F = 0.40, P>0.10) Bartlett's test casts some doubt on the validity of the comparisons as the probability of homogenous variances is less than 10^-6.
Fig. 5 The effect of a foster dam on the survival, life expectancy, and growth of young rats.

- % Survival
- Life Expectancy (days, \(\bar{x} \pm se\))

- Foster nursed
- Not foster nursed

Mean Body Weight (g) vs. Age (days)
Fig 6. The survival of young rats born to females exposed to 0 or 53 ppm dietary dieldrin during gestation (1st number) and nursed on dams exposed to the same doses during lactation (2nd number).
Fig. 7 The life expectancy of young rats born to females exposed to 0 or 53 ppm dietary dieldrin during gestation (1st number) and nursed on dams exposed to the same doses during lactation (2nd number)
The growth of the young in the four groups is presented in Fig. 8; the body weights were calculated as before. Fisher's t test indicates that on no day does the weight differ between groups despite the fact that the young of the treatment groups are consistently smaller than the controls (for comparisons between all four groups maximum t was found to be $t = 1.23$ df = 15 - for groups 0-0 and 53-53 on day 4 - and for groups 0-0 and 53-0 after day 7 maximum $t$ is $t = 1.11$, df = 23 on day 14). There are apparently no effects on the growth of young as was the case in the other experiment (Fig. 4).

However, as before there appeared to be differences in the growth responses of entire litters and it is clear the young of some litters grew while others did not (Table VI). The female then affects the growth of her litter.

The growth response of the young is not dependent upon the exposure of the dam during gestation but is greatly affected by the dam's exposure during lactation. Classifying the data into a $2 \times 2$ contingency table yields a $X^2 = 0.0$ because of the 0 in one cell. Moreover if the body weight data in Fig. 8 are pooled depending upon the post-partum exposure (i.e. 0-0 with 53-0 and 0-53 with 53-53) and t tests conducted the 2 groups differ from days 2 to 4 (minimum $t = 5.26$, df = 52, $P = 0.01$); the weight does not differ at birth or on days 5 or 6 but this latter effect is probably due to the small sample size of 53 ppm post-partum young because of mortality. The young being raised by females exposed to 53 ppm had little or no milk in their gut.

6. Behavior of Exposed Females during the Raising of Young

The % frequency with which the 15 behavior patterns (see Methods p 8 and Appendix I) were shown by exposed and unexposed females is presented in Table VII. There is no reason to believe that dieldrin caused the females
Fig. 8 The growth of suckling rats born to females exposed to 0 or 53 ppm dietary dieldrin during gestation (1st number) and nursed on females exposed to the same doses during gestation (2nd number).
Table VI. The growth responses of the litters of females exposed to 0 or 53 ppm dietary dieldrin during lactation.

<table>
<thead>
<tr>
<th>Dietary Concentration during Lactation</th>
<th>0</th>
<th>53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Concentration during Gestation</td>
<td>0</td>
<td>53</td>
</tr>
<tr>
<td>0</td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td>Net Growth of Litter Mates</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Litter Mates Maintained Birth Weight</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Net Decline in Body Weight of Litter Mates</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table VII. The frequency of occurrence of behavior patterns shown by females with young and exposed to dietary dieldrin.

<table>
<thead>
<tr>
<th>Behavior Pattern</th>
<th>% Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unexposed Females</td>
</tr>
<tr>
<td>Aggressive</td>
<td></td>
</tr>
<tr>
<td>Biting of Young</td>
<td>0.0 )</td>
</tr>
<tr>
<td>Kicking of Young</td>
<td>0.0 ) 0.0</td>
</tr>
<tr>
<td>Hitting of Young</td>
<td>0.0 )</td>
</tr>
<tr>
<td>Maintenance of Self</td>
<td></td>
</tr>
<tr>
<td>Grooming of Self</td>
<td>9.6 )</td>
</tr>
<tr>
<td>Drinking</td>
<td>1.7 ) 15.9</td>
</tr>
<tr>
<td>Feeding</td>
<td>4.6 )</td>
</tr>
<tr>
<td>Maternal</td>
<td></td>
</tr>
<tr>
<td>Nursing</td>
<td>69.0 )</td>
</tr>
<tr>
<td>Standing: over Young</td>
<td>3.8 )</td>
</tr>
<tr>
<td>Licking Anus' of Young</td>
<td>1.8 ) 76.4</td>
</tr>
<tr>
<td>Holding of Young</td>
<td>0.2 )</td>
</tr>
<tr>
<td>Smelling: Young or Nest</td>
<td>1.6 )</td>
</tr>
<tr>
<td>Others</td>
<td></td>
</tr>
<tr>
<td>Playing</td>
<td>3.0 )</td>
</tr>
<tr>
<td>Digging</td>
<td>1.7 )</td>
</tr>
<tr>
<td>Locomotion</td>
<td>0.1 )</td>
</tr>
<tr>
<td>Standing away from Young</td>
<td>2.8 )</td>
</tr>
</tbody>
</table>
to become aggressive towards their litters or to decrease the amount of maternal care. In particular there is no difference in the amount of Nursing. It is important to realize, however, that Nursing does not imply lactation or suckling; it merely means that the female was in the normal nursing position over the young.
IV. DISCUSSION

1. Exposure to Dieldrin and Health of the Animals

The rats in this study were exposed to 0, 13, 25, 37 or 53 ppm dietary dieldrin for 44 weeks with no apparent ill effects. Minor and transitory changes in the behavior of females on the two highest doses were noticed and the results suggest that the dieldrin may have stimulated an increase in food consumption in the female.

Gross body weight is a crude index of health commonly used in toxicological studies (Loomis, 1968) and the results of this study indicate that dieldrin in doses up to 53 ppm have no effect on the growth of either sex in the rat. Treon and Cleveland, (1955), Coulson and McCarthy, (1963), and Walker et al., (1969), employing similar doses of dieldrin and/or aldrin had identical results and Borgmann et al., (1952b), have shown that the rat is not affected by these compounds in doses as high as 150 ppm. However, Ball et al., (1953) reported the peculiar effect on an increased rate of growth in the female rat caused by 20 ppm aldrin. A similar effect was noted by Treon et al., (1955) who claim that growth in the male rat was decreased by 5 and 25 ppm endrin (the endo-endo stereoisomer of dieldrin) but unaffected by doses of 1 or 50 ppm; the female was unaffected at all doses. Contradictory effects of 25 ppm aldrin or dieldrin on the dog have been reported by Kitselman (1951) and Kitselman and Borgmann(1952).

Only rarely has the effect of an organochlorine insecticide on food intake been studied, probably because of the difficulty of obtaining valid data. Food intake in mammals is generally thought to be unaffected by low doses and decreased by high doses of organochlorines; for example, the rat does not decrease food intake until concentrations of dieldrin are over 100 ppm (Borgmann et al., 1952b) and the cow withstands dietary dieldrin at
75 ppm quite successfully (Gannon et al., 1959). However, the results suggest that dieldrin stimulates the female rat to increase food consumption in a dose-dependent manner but has no effect on the male. Increased food intake is unusual and has only been reported by Ball et al., (1953) in conjunction with increased growth. Moreover a sex difference has not been reported before although the growth effects described by Treon et al., (1955) can be explained by a similar mechanism.

Dietary dieldrin at concentrations of up to 53 ppm did not induce symptoms of acute poisoning. This is consistent with the work of Borgmann et al., (1952a,b) who demonstrated that 100 ppm is the minimum dose which will cause acute poisoning. Similarly endrin does not increase mortality at doses below 50 ppm (Treon et al., 1955). The rat is apparently the species most resistant to dietary double ring cyclodienes as the other species tested show acute poisoning in the concentration range of 5-25 ppm (monkey, mouse, dog, rabbit, - Hodge et al., 1967; deer mouse - Morris, 1968).

The females on the diets containing 37 or 53 ppm dieldrin showed an increase in excitability and activity during the week 7-10 of exposure; the males were unaffected. This sex difference is probably related to the greater ability of the male to detoxify dieldrin as evidenced by the differences in the acute LD50. The period of changed behavior is slightly later than the periods of peak tissue storage of dieldrin (Coulson and McCarthy, 1963) and probably reflects a temporary increase in the amount of dieldrin in the metabolic pool. Similar but permanent changes in behavior were caused in the CFW rat by 3 months exposure to 10 ppm dieldrin (Walker et al., 1969) and in the Carworth rat by 50 ppm endrin (Treon et al., 1955). Both these authors noted occasional convulsions in their animals which were not observed in this study.
In summary, exposure to dietary dieldrin in concentrations of up to 53 ppm did not appear to have caused any ill effects on the male and had minimal effects on the female, causing minor changes in nutritional physiology and temporary changes in behavior.

2. Effects on Reproductive Capacity

Reproductive capability can be considered as three empirically distinct phenomena:

(a) fertility, or the ability to produce young,
(b) fecundity, or the number of young produced,
(c) viability of the young produced.

The effects of chronic exposure to dieldrin on reproduction is best considered in terms of these phenomena because such an approach delineates the area(s) where reproduction is affected and allows inferences to be made as to the mechanisms involved.

The various mechanisms which can be the cause of decreased reproductive ability in any of the three areas are presented in Table VIII. Clearly, there are mechanisms which are very general and may cause failure in any or all of the three phenomena, for example, genetic damage or lowered levels of the sex hormones. On the other hand there are mechanisms which are very specific to one phenomenon, induced maternal aggression for example. Judicious interpretation of the combinations of phenomena which are affected and the severity of the effect(s) can suggest the mechanisms involved.

Let us consider for a moment the two mechanisms which may cause all of the phenomena. There is little reason to believe that dieldrin causes genetic damage. It has been claimed to cause some questionable changes in the DNA and RNA of rats and chicks (Daugherty et al., 1962) but these results have not been substantiated; similarly there is no evidence in the
Table VIII. The possible causation of dieldrin induced reproductive failure in rats

<table>
<thead>
<tr>
<th>Decreased Fertility</th>
<th>(1) Genetic damage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(2) Germ cell or oocyte mortality</td>
</tr>
<tr>
<td></td>
<td>(3) Decreased sex hormone levels</td>
</tr>
<tr>
<td></td>
<td>(a) Decreased production</td>
</tr>
<tr>
<td></td>
<td>(b) Increased metabolism</td>
</tr>
<tr>
<td>♀</td>
<td>As for ♂</td>
</tr>
<tr>
<td>♂</td>
<td>As for ♀</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Decreased Fecundity</th>
<th>(1) Decreased ovulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(a) Germ cell or oocyte mortality</td>
</tr>
<tr>
<td></td>
<td>(b) Decreased sex hormone levels</td>
</tr>
<tr>
<td></td>
<td>(c) Gonadotroph imbalance</td>
</tr>
<tr>
<td>♀</td>
<td>(2) Increased intra-uterine mortality</td>
</tr>
<tr>
<td></td>
<td>(a) Genetic damage</td>
</tr>
<tr>
<td></td>
<td>(b) Decreased sex hormone levels</td>
</tr>
<tr>
<td></td>
<td>(c) Acute poisoning of embryos</td>
</tr>
<tr>
<td>♂</td>
<td>(1) Genetic damage</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Decreased Viability of Pre-Weaning Young</th>
<th>(1) Genetic damage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(2) Starvation</td>
</tr>
<tr>
<td></td>
<td>(a) Maternal neglect</td>
</tr>
<tr>
<td></td>
<td>(b) Decreased lactation</td>
</tr>
<tr>
<td></td>
<td>(i) Decreased sex hormone levels</td>
</tr>
<tr>
<td></td>
<td>(ii) &quot;Blockage&quot; of lactogenic hormone or oxytocin</td>
</tr>
<tr>
<td></td>
<td>(3) Infanticide ie. increased maternal aggression</td>
</tr>
<tr>
<td></td>
<td>(4) Teratogenic defects</td>
</tr>
<tr>
<td>♀</td>
<td>(1) Genetic damage</td>
</tr>
<tr>
<td>♂</td>
<td>(1) Genetic damage</td>
</tr>
</tbody>
</table>
literature which suggests that this compound induces chromosomal breakage or interferes with chromosome mechanics. However, on the other hand it is very possible that dieldrin causes decreased hormone levels.

Decreased hormonal levels can result from a decrease in production and/or an increase in metabolism. Decreased production would be a complex phenomenon involving a direct attack on the hormone producing cells or an imbalance in gonadotroph levels; there is no evidence in the literature to suggest that either of these is occurring. This leaves increased metabolism.

It has long been known that drugs stimulate their own metabolism by inducing hepatic enzymes. Hart and Fouts (1963) suggested that the organochlorines would induce similar enzymes and this was subsequently shown to be true by Hart and Fouts (1965) and others. Trivus (1965) and Peakall (1967) demonstrated that the normal substrates for these enzymes were the sex steroids which suggested that small levels of the organochlorines could cause reproductive failure. This was confirmed in 1967 by Conney et al., who showed that chlordane caused a simultaneous reduction in litter size and an increase in steroid hydroxylase activity.

The threshold levels which cause enzyme induction have been determined for most of the common organochlorines and the levels for the double ring cyclodienes are the lowest (Street et al., 1969). Dieldrin has the lowest threshold of all and in the rat it is approximately 1.0 ppm (Gillet and Chan, 1968). There can be little doubt that the animals in this study were experiencing enzyme induction.

3. Effects on the Reproductive Capacity of the Male

The results indicate that exposing male rats to dietary dieldrin in concentrations up to 53 ppm does not affect their fertility, the size or sex composition of the litters they sire or the viability of the young they sire. These results suggest that dieldrin does not cause genetic damage,
net lowering of androgen levels, or germ cell or sperm mortality.

No work has been done on the effects of the double ring cyclodienes on reproductive capacity of the male of any species and little has been done on other organochlorines. The data of Ambrose et al. (1953) suggest that 320 ppm chlordane does not affect fertility in the male rat. Huber (1965), studying the effect of Kepone on the house mouse, found a decrease in the litter size of unexposed females bred by treated males but histological examination of their testes revealed normal spermatogenesis and interstitial cell content. McFarland and Lacy (1969) using Kepone on the Japanese quail found that it greatly enlarged the testes although the increase was insignificant statistically; histology revealed normal spermatogenesis and a slight increase in the number of interstitial cells. Albert (1962) showed that 1000 ppm DDT caused no histological changes in the testes of the chicken despite the fact that this dose precludes reproduction in this species (Ruben et al., 1947).

The present evidence suggests that the organochlorines do not disrupt the male reproductive system and therefore the disturbances these compounds are known to cause are mediated through the female.

4. Effects on the Reproductive Capacity of the Female

(a) Fertility

The results show that dietary dieldrin in concentrations of up to 53 ppm has no effect on the fertility or estrous cycle of the female; my data suggest that the rat is insensitive to the double ring cyclodienes. This is in direct opposition to the results of Treon and Cleveland (1955) who, using a 3 generation breeding trial, found that dieldrin at 2.5 ppm had a "slight effect" on the pregnancy rate which was "more pronounced" at 12.5 and 25.0 ppm. The validity of their results, particularly at the low doses, is questionable, however, since no statistical analyses were performed
and since the effect at 2.5 and 12.5 ppm "tended to disappear after the first generation".

Assuming that their effects are real, the dichotomy between our results probably is caused by a difference in the age of the animal at the time of exposure. Treon and Cleveland exposed their animals from week 3-28 of age and mine were exposed from week 25-69; similarly the breeding tests were conducted at different ages. The significance of a possible age effect is better appreciated when it is realized that young animals have a much lower threshold level for enzyme induction than do older animals (Kuntzman et al., 1964). Ball et al., (1953) found an aldrin caused transitory decrease in the number of positive estrus' which can also be explained as an age effect.

The significance of these various results is that even high doses of dieldrin and probably the other double ring cyclodienes will not reduce the pregnancy rate of a rat population to zero.

My results also suggest that dieldrin does not cause genetic damage, germ cell or ova mortality or a net decrease in the hormone levels before gestation.

(b) Size and Sex Composition of the Litter

There is no statistical evidence that dieldrin in doses up to 53 ppm alters the sex ratio or reduces the size of a litter a female will bear. This is in agreement with Treon and Cleveland (1955) who found that doses up to the maximum they used (25 ppm) had no effect on litter size. These data suggest that dieldrin does not increase intra-uterine mortality or decrease ovulation in the rat.

Despite the lack of significant results there is reason to believe that exposed females had lowered gestagen titres. The young of treated females were often born with gross hematomas in the head and caudal region,
the frequency and severity of which increased with increasing dose. Such a condition is known to result from hormonal deficiencies during gestation (Kroc et al., 1959) and is associated with a decrease in litter size; the litter size of the exposed females was consistently smaller than that of the control females. In view of this, the effect of dieldrin and the other double ring cyclodienes on litter size in the rat should be re-examined. Even if dieldrin does reduce the litter size, the effect is certainly small, even at high doses, and at the residue levels likely to be found in the wild the reduction would be insignificant. As such the fecundity of a real population is unlikely to be affected.

On the other hand the mouse shows a definite dose dependent decrease in the litter size caused by dieldrin and other organochlorines (Deichmann and Keplinger, 1966; Good and Ware, 1969). However, the mechanism is probably different from that of the rat.

The mouse is known to have a poor induction response to the organochlorines in comparison to the rat (Kuntzmann et al., 1964) such that it will have relatively high concentrations of blood organochlorines when exposed to the same dose. Backstrom et al., (1965) have demonstrated that dieldrin readily crosses the mouse placenta such that in utero fetal poisoning could easily and probably does occur. The rat on the other hand with its greater enzyme systems probably detoxifies enough of the compound to prevent acute poisoning of the embryos but in doing so reduces the levels of progesterone and estrogen.

(c) Viability of the Young

The results indicate that the % survival of pre-weaning young born to exposed females is reduced in a dose-dependent manner, although this reduction is statistically insignificant. However, the life expectancy of these young
does demonstrate that the reduction in viability is statistically significant. The threshold dose for reduced viability is less than 10 ppm, the "LD50" is approximately 17 ppm and viability is effectively 0 at doses >25 ppm. The mean age at death is approximately 5 days and this does not occur at a time different from normal pre-weaning mortality. Moreover the mean age at death is much less than 14 days which is when the young start eating solid food such that they are not dying of acute poisoning from the feed. It is clear that exposure of the female to dieldrin during gestation and lactation reduces the viability of her offspring but it is not clear whether this is caused by the exposure during gestation (hereafter called "pre-partum"), lactation ("post-partum") or an interaction of the two. This decreased viability of pre-weaning young has been consistently reported by the few investigators who have looked for it. Every species examined regardless of the compound used and regardless of its effects on fertility or fecundity has shown this response. However, some of the organochlorines require doses unlikely to be obtained even in the diets of carnivores (eg. DDT at 250 ppm and chlordane at 100 ppm are required for mice; Deichmann and Keplinger, 1966) but this is not true of the double ring cyclodienes.

Treon and Cleveland (1955) reported that 2.5 ppm dieldrin (and aldrin) had "moderate effects" on the survival of young while 12.5 and 25.0 ppm had "severe effects". Similarly, Deichmann and Keplinger (1966) claimed that 25 ppm dieldrin (and aldrin) had "very severe effects" while 10 ppm had "some effect". My data and the data of these authors is comparable, suggesting little difference in response between the two species. The rat and the mouse appear to be more tolerant to these compounds than the dog where 3.0 ppm aldrin reduced survival by 60% (Kitselman, 1953). The deer mouse has been shown to suffer a significant 30% decrease in survival of young caused by 4.0 ppm endrin (Morris, 1968); whether the apparent
difference between the other two rodents and the deer mouse is a species
difference or a chemical difference is not clear.

It is widely known that all of the organochlorines are secreted in the
milk in quantities proportional to the amount ingested and it has been
assumed that the decreased survival of pre-weaning young is caused by acute
poisoning from the compound in the milk. There are however, other possible
post-partum causes and possible pre-partum causes as well (Table VIII). This
fact has also been recognized by Harris et al., (1965) whose unpublished data
has been summarized obscurely in Appendix 10 of Hodge et al., (1967). Harris
et al. found that lambs born to unexposed ewes died when nursed on ewes
receiving 25 ppm dietary dieldrin and conversely lambs born to exposed ewes
still died when nursed on dams receiving no dieldrin.

The effects of pre and post-partum exposure of the female to dieldrin on
the viability of her offspring were investigated by the foster nursing
experiment. Since the 0-0 and 53-0 groups differ there is a pre-partum
component in the causation. Since 0-53 and 53-53 do not differ the post-
partum component swamps the pre-partum and is therefore the more important.
The pre-partum causation is responsible for 30% of the total reduction in
survival at the 53 ppm dose used and as such the pre-partum effect is likely
to be experienced even at low doses.

The proximate cause of the pre-partum induced mortality must involve
either genetic damage, which is unlikely for the reasons previously
discussed, or a teratogenic defect (Table VIII). The latter probably
involves subtle disruptions of metabolism since no gross defects were
observed, the weight at birth is not affected and the induced deaths occur
at the same time as the normal deaths. The nature of the defect is com-
pletely unknown but could involve any of the following effects known to be
caused by dieldrin: altered N metabolism (Annau et al., 1952); altered
activity of carbonic anhydrase, several dehydrogenoses and several esterases (Verrett and Desmond, 1959; Hosein and Proulx, 1960; Gowdey and Stavraky, 1955); decreased O₂ consumption (Crevier et al., 1954); decreased transport in RBC's (Weikel et al., 1958); or abnormal transport systems (Daugherty et al., 1962).

The proximate cause(s) of the post-partum component of mortality could involve any combination of acute poisoning via the milk, starvation resulting from decreased lactation or maternal neglect, or infanticide from increased maternal aggression.

While dieldrin-induced changes in the maternal behavior of the rat can be postulated to occur, since the compound is known to disrupt the function of the CNS (Gowdey et al., 1954) and has been shown to change the behavior of sharptail grouse (McEwan and Brown, 1966) and sheep (Van Gelder et al., 1969), the results of this study indicate that such changes do not occur. Morris (1968) claims, without data, a similar situation exists in the deer mouse.

The results suggest that both acute poisoning from dieldrin in the milk and starvation from decreased lactation are operating. At the higher doses all the offspring die but they may or may not grow until death. This growth response is female dependent in the sense that the growth of littermates is consistant and the number of females whose litters grow decreases with increasing dose. Moreover, the young that do not grow show little or no milk in the gut. The death of the young who grow can only be explained by acute poisoning while decreased lactation probably plays at least a part in the mortality of the young who do not grow.

Deichmann and Keplinger (1966) have claimed that 10 ppm dieldrin resulted in a decrease in an undefined lactation index in the house mouse. Decreased lactation is also consistant with decreased sex hormone levels for which, as discussed previously, there is some evidence of occurrence.
In the rat, estrogen and progesterone are necessary for mammary gland development and the initiation of milk secretion from the alveolar cells (Cowie and Folley, 1961). As such dieldrin induced decreases in these hormones and could manifest itself in decreased milk production.

There is however the possibility that decreased lactation results from dieldrin directly affecting the brain. In the rat, lactogenic hormone is necessary for mammary gland development and the initiation and maintenance of milk secretion; oxytocin is necessary to induce release of the milk from the gland and may also cause increased milk secretion by stimulating the release of the large amount of lactogenic hormone effected at the onset of suckling (Cowie and Folley, 1961). The release of these pituitary factors is apparently under the control of complex CNS reflexes containing both adrenergic and cholinergic pathways and can be altered by foreign compounds affecting these pathways (Grosvenor and Turner, 1957 and '58; Meites, 1959). Dieldrin is cholinergic and mediates its affects through the brain (Gowdey et al., 1954). The implication is obvious.

In summary, the effect of small doses of dietary dieldrin on the reproductive capacity of the female rat is serious. The fertility and fecundity are unaffected by doses as high as 53 ppm but the viability of the young is reduced by a threshold dose of less than 13 ppm.
V. SUMMARY

(1) Groups of post-pubertal rats were exposed to 0, 13, 25, 37 or 53 ppm dietary dieldrin for 44 weeks. In the female this resulted in a dose dependent increase in food intake and a transitory hyperexcitability in those receiving 37 and 53 ppm; this latter effect coincided with the start of the decrease in the storage of dieldrin in the tissues. No other effects of chronic toxicity were observed in either sex. The animals were tested for reproductive success at 4 intervals during exposure. Other experiments were performed to elucidate the mechanisms of reproductive failure.

(2) Dieldrin had no effect on the reproductive success of the male. The number of litters sired by exposed males and the number, sex and viability of young in these litters did not differ from the controls. This suggests that dieldrin does not cause genetic damage, lowered androgen titres, or mortality of the sperm or germ cells.

(3) Dieldrin had no effect on the exposed females' estrous cycles, fertility or sex of offspring. This suggests that dieldrin does not cause genetic damage, mortality of the eggs or germ cells or decreases in the pre-gestational hormone titres.

(4) Dieldrin did not cause significant decreases in the size of litters born to exposed females. This suggests that dieldrin was not causing \textit{in utero} poisoning of the embryos or deficiencies of the gestational hormones. However the treatment litters were consistently smaller than the controls and the young had defects known to be associated with hormone deficiency such that the effect of this compound on litter size in the rat should be re-examined.
(5) Dieldrin decreases the viability of offspring born to exposed females in proportion to the dose received by the dam; the threshold dose is less than 10 ppm and the LD50 is 17 ppm. The mortality induced by dieldrin occurs at the same time as normal pre-weaning mortality.

(6) Exposure of the dam to dieldrin during gestation increases the mortality of the young after birth. This suggests that dieldrin causes some manner of defect and possibilities are discussed.

(7) Exposure of the dam to dieldrin during lactation increases the mortality of the young; the causation of this effect does not interact with the pre-partum defect. Dieldrin does not change the dams' behavior such that increased maternal aggression or neglect are not the causes of this effect. The growth responses of the young indicate that in at least some females dieldrin decreases lactation and starvation causes part of the mortality. It is suggested that acute poisoning from dieldrin secreted in the milk is the major cause of mortality.

(8) The probable physiological mechanisms of these effects are discussed.
VI. LITERATURE CITED


Coul


Kutney, J.P. 1969. Personal communication. Dept. of Chem. Univ. of B.C.


### VII. APPENDIX I

**Behavior Patterns of Female Rats with Young**

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biting of Young</td>
<td>These were the patterns which were not seen but added to the list as being <em>a priori</em> aggressive.</td>
</tr>
<tr>
<td>Kicking of Young</td>
<td>They were also not seen during the experiment which since their meaning is obvious anyway makes further discussion unnecessary.</td>
</tr>
<tr>
<td>Hitting of Young</td>
<td></td>
</tr>
<tr>
<td>Grooming of Self</td>
<td>The cleaning of the pelage, tail, limbs etc. with the paws and/or mouth. Also includes scratching with the paws.</td>
</tr>
<tr>
<td>Drinking</td>
<td>Licking the spout of the water bottle to obtain water.</td>
</tr>
<tr>
<td>Feeding</td>
<td>Eating from the jar of food.</td>
</tr>
<tr>
<td>Nursing</td>
<td>This refers to the posture and position of the female relative to the young and implies nothing about lactation or suckling. The typical posture of the female was in a loosely curled foetal position but lying more on the ventral surface than on the lateral surface. Two variations of this were noted. In one the female was in a &quot;sprawled&quot; position, lying completely stretched out on the ventral surface; in the other she was lying on the lateral surface and almost completely stretched out. Regardless of the posture the female was typically asleep. In the former two positions</td>
</tr>
</tbody>
</table>
the young were hidden from sight underneath the female while in the latter position the young could be seen along her ventral surface.

**Standing over the Young**
The female would be standing straddling the nest and the young. The young could be seen to be suckling on occasion. This position was typically observed at the end of a bout of Nursing.

**Anus' Licking of Young**
The female would hold the young, head down, in her fore-paws and lick the anus of the young. The licking probably induces defecation in the young.

**Holding of Young**
This includes 3 patterns. The first was a fright response to external stimuli where the female picked the young up in her mouth and either ran from the nest or stood nervously by the nest. The second was a maternal response where the female picked up a young who had strayed from the nest and returned it to the nest by use of her mouth. The third pattern is of unknown function and merely involved a female picking up the young with her fore-paws, handling it, and putting it down.

**Smelling Nest or Young**
The female, when standing about the nest was frequently observed to lower her head and "smell" the nest and young.

**Playing**
This is a loose category for apparently functionless patterns. The most frequent pattern was holding the food dish tray in the mouth and dragging it
about the cage; a similar pattern involved batting the tray about the cage with the forepaws. Chasing the tail was the other most frequent pattern.

**Digging**

This includes 2 patterns. The first involves the female standing in one position, usually at/or near the nest, and pulling the litter away from one spot by use of the forepaws. In the second the female runs rapidly about the cage with the head lowered and often ploughing in the litter and with the forepaws "flicks" laterally the litter ahead of her.

**Locomotion**

Walking or running about the cage.

**Standing Away from the Young**

Usually an interruption on a bout of locomotion.
VIII. APPENDIX II

The Measurement of Dieldrin Residues

1. The Colour Reaction and the Preparation of a Standard Curve

The colour reaction was originally applied to dieldrin which was at least 99.5% pure (Cueto, 1960). In this work technical grade dieldrin, which is the basis for commercial insecticides and is biologically the more important, was used. Technical dieldrin contains 85% dieldrin and 15% related insecticidal compounds (ie. aldrin, endrin, isodrin). It was necessary to make many refinements to improve the precision of the colour reaction on this material before a reasonable standard curve could be produced. The refined technique is discussed below.

The reagents and their conditions required for the colour reaction are:

Diethyl ether, Certified ACS. Ether containing peroxides is unsatisfactory and cannot be used. The presence of free iodine as detected by the starch test after shaking a small quantity of ether with aq. KI is indicative of peroxides.

Hexane, Certified ACS. This solvent must be re-distilled at least twice before use.

Diphenylamine reagent. 0.25% diphenylamine in hexane. The diphenylamine must be Certified ACS and re-crystallized at least twice from hexane. This reagent should be used within 2 hours of preparation.

Zinc chloride reagent. 0.25% zinc chloride in ether. The zinc chloride must be certified ACS and must be stored and measured under conditions of absolute dessication. This reagent should be used within 0.5 hours of preparation and cannot be used after 4 hours.

Solvent for reaction products. 10% Certified ACS acetic anhydride by volume in reagent grade glacial acetic acid.
The procedure for the colour development is outlined below.

The hexane containing the dieldrin is placed in a 22 x 175 mm test tube and taken just to dryness in a water bath at 68°C. The evaporation rate under these conditions is about 0.33 ml/hour. Allowing the tube to stay in the water bath after all the hexane has evaporated results in loss of dieldrin.

Two ml of the diphenylamine and zinc chloride reagents are added to the tube and taken to dryness under the same conditions as before. This will take about 2 hours. A colourless, dry residual film should appear in the tube. This film should be left in the undeveloped condition for the minimum amount of time for best results.

To develop the colour the tube is placed in a test tube heating block for exactly 3.0 minutes at 205°C. The tubes are then cooled for exactly 5.0 minutes in running tap water. The white residual film will become clear where dieldrin is not present and a deep blue where it is. The reaction products are apparently unstable even in the dry condition and they should be dissolved and the OD determined immediately.

The reaction products are dissolved in 3.0 mls of the glacial acetic acid solvent and placed in a 4 ml capacity, 10 mm light path absorption cell and OD determined at 650 μm using the solvent as a reference. The OD must be determined within 10 minutes of the addition of solvent before the colour begins to fade. The spectrophotometer used was a Unicam SP 500.

The diphenylamine and zinc chloride reagents have ODs which vary tremendously between batches and within batches between times such that it is absolutely necessary to run a reagent blank through the entire process above and correct the ODs of the dieldrin samples with it.

The standard curve was prepared by determining the OD of known amounts of dieldrin. Standard solutions of 2.5, 5.0, 10.0, 20.0, 50.0, and 100.0μg/ml
of dieldrin (re-crystallized twice from hexane) in triple-distilled Certified ACS hexane were prepared and suitable aliquots were subjected to the colour reaction process. The range of the standard curve is from 5.0 to 150 \( \gamma \). Attempts to increase the sensitivity in the range of 0 to 20 \( \gamma \) by dissolving the reaction products in 1.0 mls of solvent and using a 0.8 ml capacity, 20 mm light path absorption cell were unsuccessful. The standard curve of gm of dieldrin vs optical density of the reaction products is presented in Fig. 9; the OD's have been corrected for the optical density of the reagents. Since there is no good linear relationship over the entire range of observations two regression lines have been fitted, one for 0-70 \( \gamma \) dieldrin and the other for 70-150.

The line for 70-150 \( \gamma \) has been fitted by the least squares method and the equation is \( Y = 10^{-3} (3.80 + 2.59 X) \).

There are however two possible lines for the 0-70 \( \gamma \) range. One, which is not shown on the figure, was fitted by the least squares method and has the equation \( Y = 10^{-3} (1.71 + 3.31 X) \); the other, which is shown, was also fitted by the least squares method but was forced through the origin and has the equation \( Y = 3.34 \times 10^{-3} X \). The reason for putting the line through 0, 0 is theoretical: if there is no dieldrin the optical density must be zero. In theory the lower limit of sensitivity should be extended by this line.

The latter two lines differ in their slope (\( F = 664, \text{df} = 1 \text{ and } 156, P < 0.01 \)) such that they cannot be considered estimates of a common line. This implies that only one or the other is the "true" line and that only one can be used. In reality there is little difference in the estimates of the amount of dieldrin (\( \hat{x} \)) calculated from the two lines (Table IX). Moreover the confidence limits on \( \hat{x} \) are wide such that it is really inconsequential which line is used (Table IX). For computational ease the fixed line is preferable.
Fig. 9 The relationship between the Optical Density of the dieldrin–diphenylamine reaction products and the amount of dieldrin involved in the reaction.
Table IX. A comparison of the usefulness of the 3 regression lines in predicting the amount of dieldrin.

<table>
<thead>
<tr>
<th>Line</th>
<th>OD (Y)</th>
<th>Dieldrin (X)</th>
<th>95% Confidence Limits on $\hat{X}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\gamma$</td>
</tr>
<tr>
<td>$Y = 10^{-3} (1.71+3.31X)$</td>
<td>0.050</td>
<td>14.59</td>
<td>12.84 - 53.02</td>
</tr>
<tr>
<td></td>
<td>0.100</td>
<td>29.69</td>
<td>22.81 - 33.73</td>
</tr>
<tr>
<td>$Y = 3.34 \times 10^{-3}X$</td>
<td>0.050</td>
<td>14.95</td>
<td>12.97 - 53.13</td>
</tr>
<tr>
<td></td>
<td>0.100</td>
<td>29.95</td>
<td>23.18 - 34.22</td>
</tr>
<tr>
<td>$Y = 10^{-3} (3.80+2.59X)$</td>
<td>0.300</td>
<td>101.10</td>
<td>4.01 - 162.01</td>
</tr>
</tbody>
</table>
The equations for calculating the 95% confidence limits on an estimate of the amount of dieldrin calculated from either of the three lines is presented in Table X; they have been derived from Sokal and Rohlf (1969).

The CL are not symmetrical about \( \hat{x} \) because in calculating \( \hat{x} \) from \( Y_1 \), all of the assumptions made in originally regressing \( Y \) on \( X \) are violated and the necessary mathematical corrections distort the CL. As is typical the CL are widest at the ends of the lines (Table X). In general the CL are narrowest when \( Y_1 = \bar{Y} \). Therefore the most accurate estimates are obtained when the amount of dieldrin present is about 30\( \gamma \) and the lower lines is used. The upper line is virtually useless since the CL are so wide even when \( Y_1 = \bar{Y} \). For practical use the colour reaction should be performed on a sample containing about 30\( \gamma \) dieldrin even though this necessitates the use of a pilot sample.

The minimum quantity of technical dieldrin which can be measured is about 2.5\( \gamma \).

2. The Extraction of Dieldrin from Feces and Animal Tissues

Three different extraction processes corresponding to the three clean-up procedures were used on the tissues but only one, a modification of Zweig (1964), was used on the feces. This was to avoid the possibility of converting fecal dieldrin metabolites into dieldrin.

The details of the extraction and clean-up procedures are presented below.

All of the trials on feces were performed on 50 g. (dry weight) samples from unexposed animals. The samples were ground to a flour in a Waring blender before extraction.

The feces were extracted by placing the 50 g sample in a 500 ml flask with 200 ml of hexane and shaking vigorously for 5 minutes. Approximately
Table X. The 95% confidence limits on an estimate of x from the regression lines of Optical Density vs Dieldrin.

<table>
<thead>
<tr>
<th>Regression Line</th>
<th>Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y = 1.71 \times 10^{-3} + 3.31 \times 10^3x$ for $0 &lt; x &lt; 70$ (floating)</td>
<td>$CL = 36.88 + 331(Y_1 - 0.125) \pm 97.5\sqrt{0.00376 + (Y_1 - 0.125)^2}$</td>
</tr>
<tr>
<td>$Y = 3.34 \times 10^{-3}x$ for $0 &lt; x &lt; 70$ (fixed through 0)</td>
<td>$CL = 36.88 + 327(Y_1 - 0.125) \pm 98.5\sqrt{0.00385 + (Y_1 - 0.125)^2}$</td>
</tr>
<tr>
<td>$Y = 3.80 \times 10^{-3} + 2.59 \times 10^{-3}x$ for $70 &lt; x &lt; 150$</td>
<td>$CL = 110.0 + 1038(Y_1 - 0.336) \pm 529\sqrt{0.0227 + (Y_1 - 0.326)^2}$</td>
</tr>
</tbody>
</table>
90 ml of hexane could be recovered and a 75 ml aliquot of this was centrifuged for 20 minutes at 2500 rpm to remove particulate matter. The 75 ml of extract was then concentrated to 5 ml by use of a water bath at 68°C. The concentrate was deep green in colour and subjected to the column chromatography of the clean-up procedures discussed below.

The trials on animals' tissues were performed on whole body homogenates of unexposed pre-weaning young. The carcasses were homogenized by grinding them, while still frozen, several times in a hand grinder.

A modification of the technique of Cueto (1960) was tried first.

(a) A 50 g sample of tissue was refluxed for 2 hours in 75 ml of 95% ETOH and 15 ml of 50% aq. KOH and the mixture allowed to cool. This process hydrolyzes organochlorine insecticides other than dieldrin, cleaves the peptide bond of proteins and the ester bond of lipids.

(b) Fifteen ml of the reflux mixture and 25 ml of hexane were mixed in a Waring blender for 1 minute and the mixture was then transferred to a 1000 ml separatory funnel. This was repeated until all of the reflux mixture had been extracted.

(c) The contents of the separatory funnel were mixed by vigorous shaking and the layers allowed to separate. This was repeated 3 times and after the third time the flask was allowed to sit for 30 minutes and the polar layer was then drawn to waste.

(d) The extract was neutralized with 1 N HCl and washed once with 75 ml distilled HOH and twice with 20% aq. NACL. The washings were performed by shaking the mixture vigorously and allowing the layers to separate; this was repeated three times. The polar layers were discarded.

(e) The final traces of HOH were removed from the hexane extract by
adding anhydrous Na$_2$SO$_4$ and allowing it to sit overnight.

(f) A 75 ml aliquot of extract was concentrated to 2 ml in a water bath at 68°C and subjected to column chromatography.

(g) The chromatography columns were prepared by adding small quantities of unactivated dry alumina (80-200 mesh) and tapping the column (10 x 330 mm) to ensure packing until the column of absorbant is 10 cm high.

(h) The concentrated extract is transferred to the column with the aid of a few ml of 10% ether in hexane (the same criteria of purity as in the colour reaction) and the column is eluted with 10% ether in hexane at a flow rate of 2 ml/minute.

(i) One hundred ml of elutant are collected in 10 ml fractions and each fraction is subjected to the colour procedure.

To determine which fractions would contain the dieldrin 100 γ were placed on a column and eluted. There was an average recovery of approximately 90% with the fractions 2, 3, 4, 5, 6, and 7 containing 15, 20, 25, 25 and 5 γ dieldrin.

All ten fractions of the elutant from a fecal blank contained interfering materials. The green contaminants apparently consisted of two major fractions; one with a very low R$_f$ which was picked up in fractions 1-7 and another which was picked up in 8-10.

Similarly all ten elutant fractions from a tissue blank contained interfering materials; the peak was in fractions 4-7.

The inadequacy of this clean-up procedure precluded its use with the colour reaction.

The second extraction-clean-up procedure which was tried was that of Kutney (1969).

(a) A 50 g sample of tissue was placed in a 250 ml flask and 100 ml
of hexane was added. The flask was then shaken vigorously for 5 minutes. A 75 ml aliquot was then concentrated to 5 ml by use of a water bath at 68 °C. Feces were extracted as previously described.

(b) Chromatography columns were prepared by filling the column half full of hexane and pouring a slurry of unactivated, 80-200 mesh alumina into it. The excess hexane was drained off and the procedure repeated until the desired height of alumina was obtained. The column sizes used were 360 x 25 mm, 300 x 25 mm, 240 x 25 cm and 180 x 25 mm. The extracts were transferred to the column by use of a few ml of hexane and eluted with hexane at a flow rate of 2 ml/min. The various size columns were evaluated for their effectiveness in cleaning up the concentrated extracts.

To determine if the 360 x 25 mm column could be used 2000 γ of dieldrin were placed on it and eluted with 600 ml of hexane; the elutant was collected in 10 ml fractions. These fractions were subjected to the colour development process. The compound was found in fractions 40-51 with the majority of it in fractions 41-46. Total recovery was about 20%. The elutants of fecal extracts showed no contaminants in the fractions 40-51. Therefore a concentrated fecal extract plus 100 γ of dieldrin were added to a column in order to estimate recovery at relatively low concentrations. Under these conditions it was found that recovery was 0% even when the fractions 40-51 were pooled. Subsequent trials with various amounts of dieldrin showed that 1000 γ is the minimum level which results in detectable levels in the elutant. As such this size column is not useful for detecting microgram quantities in biological materials.

The 180 x 25 mm column was the next tested by methods identical to those above and it was found that contaminants were found in the elutant
fractions containing the dieldrin. Similar work with the 2 intermediate sized columns showed that they were also unsuitable because of poor recovery or clean-up.

The final method which was tried was that of de Faubert Maunder et al., (1964). This is the most effective extraction and clean-up procedure which has been devised for pesticide residue analysis using the gas chromatograph.

(a) A 25 g sample of homogenized tissue was ground in a mortar with 25 g of quartz and 100 g of anhydrous Na₂SO₄ until a free flowing powder resulted.

(b) This was transferred to 250 ml flask and 100 ml of hot hexane was added and the flask shaken vigorously for 5 minutes.

(c) The hexane was decanted off and allowed to cool.

(d) A 25 ml aliquot of hexane and 10 ml of dimethylformamide (DMF) saturated with hexane were shaken vigorously for 5 minutes in a 100 ml separatory funnel. The funnel was allowed to sit for 10 minutes and the polar DMF layer was run into another funnel; any emulsion was left in the first funnel. The extraction of the hexane with 10 ml DMF soln. was repeated twice more. The DMF extracts were combined and washed with 10 ml of hexane saturated with DMF to remove the last traces of fat. The 10 ml of hexane were separated and washed with 10 ml of DMF which was added to the original 30 ml of DMF extract. This procedure was repeated on another 2 aliquots of hexane and all the DMF extracts were combined.

(e) The combined DMF extracts were shaken briskly for 2 minutes with 600 ml of 2% aq. Na₂SO₄ in a 1000 ml separatory funnel. The funnel was allowed to sit for 30 minutes while the hexane, previously held in solution, separated out. The polar layer was then run to waste
and the hexane was collected.

(f) The hexane was concentrated to 2 ml and subjected to the column chromatography.

(g) The chromatography columns were prepared by half-filling a 10 x 330 mm column with hexane and pouring a hexane slurry of 10g of 80-200 mesh alumina (activated by heating to 200°C for 4 hours) into it. The hexane was drained off until the alumina was just covered. A 5 cm layer of anhydrous Na₂SO₄ was added on top of the alumina and this layer was just covered with hexane.

(h) The extract was applied to the top of the column and washed on with three successive 2 ml portions of hexane. The column was eluted with 100 ml of hexane and the elutant was collected in 10 ml fractions.

To determine which fractions would contain the dieldrin 100γ were placed on a column and eluted. There was an average recovery of 90% with the fractions 2, 3, 4, 5, 6, and 7 containing 15, 20, 25, 25 and 57 dieldrin.

As with the chromatography of Cueto (1960) all ten fractions of fecal and tissue blanks contained materials which interfered with the colour reaction although the amount was reduced several fold.

It was concluded after trying these three techniques that Cueto’s spectrophotometric method of dieldrin quantification is not applicable to either feces or animal tissues.
IX. APPENDIX III

The Relationship between Dose and Response

An ideal dose response curve is presented in Fig. 10. Frequently, empirical curves are lacking the top and/or bottom plateau phase.

The AB segment of the curve represents the doses where all members of the population can detoxify all the compound they absorb. These animals may or may not differ in the amount they absorb. The BC segment represents the doses where the highly susceptible members of the population die; these animals have very poor mechanisms of detoxification and/or very "good" mechanisms of absorption. Dose B is defined as the Threshold Dose (i.e. minimum dose producing effect) and the so-called No-Effect Dose is slightly less than B. The CD portion of the curve includes the doses where the "normal" members of the population undergo mortality. The DE portion of the curve represents the doses to which only a very few, highly resistant members of the populations will not respond; these animals have very good mechanisms of detoxification or very poor mechanisms of absorption.

It is possible to calculate an LD50 from this curve or portions of it (Litchfield and Wilcoxon, 1949).
Fig. 10 The relationship between dose and response.