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Occupational Risks of Pesticide Exposure for Females

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Objectives

The present project is designed to characterize some of the reproductive hazards that confront females engaged in occupations which subject them to potential exposure to pesticides. It will evaluate the effects of a known reproductive toxin on both the ovary and ovulatory ability of a non-pregnant female and on the embryological development of the ovaries of females exposed prenatally to the toxic agent. This study intends to determine how inhibition of normal reproductive activities is induced by such agents by ascertaining the effects of such chemicals on steroid hormone levels, specific follicle populations and cell surface characteristics of the different cellular components of the ovary.

Methodology

Virgin female CD-1 mice were employed in this study. Adults were randomly distributed into three treatment groups: a chlordecone-treated group, a group treated with estradiol-17B (E-17B), and sesame oil vehicle control group. Mice were exposed to these agents for two, four, six or eight weeks. Weekly procedures consisted of five consecutive daily exposures followed by two days of no treatment. This time-table was established to mimic an ordinary five-day work week which would represent the maximum weekly exposure to which a female working with such a compound, might be subjected. Chlordecone (0.062, 0.125 and 0.25 mg), estradiol-17B (0.1 mg) and sesame oil were administered in a 0.2 ml volume by oral gavage. During the final week of exposure exogenous gonadotropins were administered to determine whether the ovaries could elicit an ovulatory response to these gonadotropins. At the end of the prescribed time period, animals were sacrificed. Livers were removed from the mice and prepared for gas chromatographic analysis. Oviducts were flushed to tabulate the number of eggs ovulated in response to the gonadotropins. Ovaries were also removed to determine the number and ratio of the different follicle populations.

Prenatal studies consisted of exposing a pregnant female mouse to either chlordecone, E-17B or sesame oil between Day 6 and Day 15 of pregnancy. Animals were sacrificed at Day 18. Fetuses were weighed and sex was determined. Fetal livers were removed for gas chromatographic analysis of pesticide incorporation. Fetal ovaries were subjected to similar procedures as described above.

Progress and Accomplishments

Results obtained, thus far, in the adult mouse study reveal that chlordecone at a dosage of 0.25 mg causes a significant decrease in the ovulatory response of the ovaries following four and six week exposures when compared to both E-17B and vehicle controls. The absence of this response has been shown to be reversible within three weeks following cessation of exposure. In order to determine the reason for the lack of response of the chlordecone-exposed ovary, follicles and enclosed oocytes were tabulated in the ovary. Results revealed that there was an increase in the number of athletic large follicles following four-week exposure which meant that there was no ready pool of follicles to ovulate in response to the gonadotropins. Liver weights of chlordecone-exposed mice increased significantly and linearly from two to six weeks of exposure with a plateauing of values between 12-19% of body weight at six weeks. Similarly, incorporation of chlordecone by the liver rose dramatically at first and the leveled off at the longer exposure times.

Preliminary evidence obtained in the prenatal studies indicates that chlordecone does not induce any external malformations nor any deviation from the normal sex ratio. The female offspring displayed

vaginal openings significantly earlier than those of controls. In addition, when the mothers exposed during their first pregnancy were allowed to deliver a second litter, the time of vaginal opening in this second litter was also significantly advanced.

Significance

The decreased ovulatory response seen in adult females following chlordecone exposure suggests that since there are sufficient exogenous gonadotropins available to stimulate ovulation and since ovulation is drastically impeded in chlordecone-treated animals, the effect of the pesticide might be exerted at the ovarian level. Data also reveals that even though exposure occurs during a previous pregnancy, offspring from a subsequent pregnancy are affected. Data soon to be obtained on steroid hormone levels, cell membrane alterations of different cell types in the ovary will be useful in determining where, within the ovary, such a toxic agent might have its target.

Publications

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Unpublished Articles

Final Report, William J. Swartz, Occupational Risks of Pesticide Exposure for Females, November 25, 1988

Swartz WJ, Mall GM: Chlordecone-Induced Follicular Toxicity in Mouse Ovaries. Reproductive Toxicology, 1988. Submitted

FINAL PERFORMANCE REPORT

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List of Abbreviations

E-17B - estradiol-17B
FSH - follicle stimulating hormone
hCG - human chorionic gonadotropin
LH - luteinizing hormone
PMSG - pregnant mare's serum gonadotropin
PVE - persistent vaginal estrus
SEM - standard error of the mean

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A. Exposure of Non-Pregnant Adult Females to Chlordecone

Exposure of mature female CD-1 mice to a 0.25 mg dose of chlordecone for four and six weeks produced a significant and progressive decrease in the ovulatory response of the ovary to exogenous gonadotropins when compared to mice treated with E-17B or sesame oil. Exposure to lower dosages of chlordecone (0.062 and 0.125 mg) for similar time periods exerted no alterations in the ovulatory response. No significant changes in the ovulatory response was apparent after only two weeks exposure in any of the treatment groups.

The observed decrease in ovulatory response to exogenous gonadotropins seen in the mice treated with 0.25 mg chlordecone is apparently not linked to the potency of its estrogenic activity since E-17B treatments failed to induce a similar decrease in the ovulatory response. This appears to indicate that the mouse ovaries are sensitive to the pesticide and its inherent toxicity. It was also shown that, following four weeks of chlordecone exposure, this effect is reversible as soon as three weeks following cessation of exposure.

The reason for an absence or reduced number of ovulated oocytes from chlordecone-exposed animals could not be determined from the above experiment.

In a subsequent experiment mice were similarly treated with either chlordecone, E-17B or sesame oil. Following a four week exposure, the ovaries were removed and prepared for histological assessment and tabulation of the different sized follicles (small, medium and large). In this study the number of healthy, large antral follicles was greater in the E-17B treated mice than in the pesticide-exposed animals, whereas both groups contained a similar number of large atretic follicles. This coupled with the larger number of medium-sized follicles in estrogen-treated mice could explain the normal ovulatory response to exogenous gonadotropin observed in E-17B -treated mice.

Both chlordecone and E-17B caused an increase in atresia in the large follicles, which is probably due to the estrogenicity of these agents. However, the decreased number of healthy, large follicles and medium-sized follicles in chlordecone-treated mice appears to be due to the toxicity of this agent. As a result, the pool of oocytes available for ovulation is reduced, thereby reducing the response to exogenous gonadotropins.

In the above experiments the majority of chlordecone- and E-17B-treated mice displayed PVE by the end of the second week of exposure. With one exception, excluding vehicle controls, all mice remained in PVE for the duration of the experiment. Persistent vaginal estrus and decreased ovulation in animals after chlordecone treatment is a characteristic feature of premature ovarian failure or premature reproductive aging. Reproductive aging induced by a pesticide could be considered as another form of reproductive toxicity.

In addition, data obtained from liver tissue of exposed mice revealed that liver weights of chlordecone-exposed mice increased significantly and linearly from two to six weeks of exposure with a plateauing of values between

12-19% of body weight at six weeks. Similarly, incorporation of chlordecone by the liver (as measured by gas chromatography) rose dramatically at first and then leveled off with increased exposure. Incorporation decreased following withdrawal of the chlordecone for three weeks.

B. Exposure of Pregnant Females to Chlordecone

In this set of experiments the procedure involved exposure of the pregnant female to daily dosages of chlordecone, E-17B or sesame oil from Days 6-15 of pregnancy. Follow-up studies were performed on both the mothers and on the offspring.

1. Effects on the exposed mothers

There was a significant weight gain from Day 6 to Day 18 of pregnancy in pregnant females exposed to 0.25 mg chlordecone during pregnancy. This was not apparent in those mice exposed to 0.125 mg chlordecone or in those exposed to E-17B. Expressing the weight of the livers as percent body weight there was a significant increase in the weight of the liver in mothers exposed to 0.25 mg chlordecone when compared to controls. Uptake of chlordecone by the maternal liver was 9.5 ppm for the group receiving 0.25 mg chlordecone.

With respect to the number of offspring delivered following the different exposures, no significant differences were observed in any of the treatment groups when compared to controls. Exactly what the reproductive potential of these offspring will be could not be ascertained from the study.

Some of the mothers were mated again following weaning of their young. The previous exposure to chlordecone appeared to have little effect on mating and conceiving and delivering another set of offspring. Specific references to this set of young will be addressed in the next section.

2. Effects on offspring of exposed mothers

There were no significant differences in the weights and sex ratios of any of the offspring of mice treated with the different dosages of chlordecone and E-17B when compared to controls. In addition, there was no difference in the liver weights of these young when expressed as percent total body weight when compared to controls. It is important to note that at the dosages employed these offspring demonstrated no external malformations at birth.

The time of vaginal opening in the mouse has long been considered an indicator of the commencement of sexual maturity. Preliminary studies had shown that mice exposed to chlordecone prenatally demonstrated an advanced vaginal opening time when compared to controls. Cross-fostering studies were then devised to determine whether this effect on the time of vaginal opening was due to the prenatal exposure to chlordecone or to the postnatal exposure via lactation or whether both exposures were necessary. Results revealed that the prenatal exposure to chlordecone was the sole exposure necessary for speeding up the time of vaginal opening.

Some additional preliminary experiments were conducted using the same pregnant mothers who were exposed during gestation to either chlordecone, E-17B and sesame oil who had given birth to the prenatally-exposed offspring mentioned above. Following weaning these mothers were removed from their young and allowed to mate again. Almost all conceived and delivered a second set of offspring. The females of this second group of offspring were followed and the time of their vaginal openings recorded. It was interesting to note that mothers exposed to chlordecone during their first pregnancy retained some residual effects of this compound and conferred it to their second set of female offspring since the time of vaginal opening was still advanced in the 0.25 mg group. It was not advanced as much as previously, but still significantly advanced over controls.

Abstract

The proposed study is designed to further characterize the reproductive hazards that confront pregnant and non-pregnant females engaged in occupations which subject them to potential exposure to pesticides. Adult virgin female mice (7-10 weeks old) were subjected to either chlordecone, E-17B or sesame oil for two, four, or six weeks. During the final week of exposure the mice received a superovulatory regimen of PMSG and hCG to determine whether they could exhibit an ovulatory response to these exogenous gonadotropins. After four and six weeks of treatment with 0.25 mg chlordecone mice ovulated a significantly lower number of oocytes compared to the E-17B treated group and the vehicle controls. Histological examination of the ovaries revealed that there was a reduced number of healthy large and medium follicles in the pesticide-treated group. These serve as the immediate pool of follicles to be selected for ovulation. It thus follows that less oocytes are available for ovulation as a result in the reduction in this pool. Thus, exposure to such a toxicant can affect the fertility of the exposed adult female without inducing any visible neurotoxic effects. Exposure of pregnant female mice to different dosages of chlordecone did not elicit any visible morphological aberrations in the young nor any effect on the number, weight and sex ratio of the offspring. The pregnant females exposed to 0.25 mg chlordecone did have a significant increase in weight during the time of exposure when compared to controls. Female offspring exposed prenatally to 0.25 mg chlordecone demonstrated an advanced time for vaginal opening; however, it was not as advanced as that for the young exposed prenatally to E-17B. Cross-fostering studies revealed that the early onset of vaginal opening was entirely dependent on prenatal exposure to 0.25 mg chlordecone and not to postnatal exposure via lactation alone or in combination with prenatal exposure. Additionally, the mothers exposed during their first pregnancy who were allowed to mate again and those previously exposed to 0.25 mg chlordecone gave birth to a second set of offspring, the females of which still exhibited an earlier time of vaginal opening. Thus, exposure to chlordecone during a prior pregnancy can still elicit an effect on a reproductive parameter during a subsequent pregnancy. Unlike the apparent toxic effect exhibited by chlordecone on follicular populations in adult mice, this effect on vaginal opening appears to be due to the estrogenic potency of this agent since this is a well observed effect of estrogens. What the future reproductive potential of both sets of female offspring exhibiting early vaginal opening is, is not known. What can be ascertained from this study is that exposure to such estrogenic pesticide agents (e.g., chlordecone, DDT, methoxychlor) can induce effects attributable to both their toxic nature and their estrogenic nature.

INTRODUCTION

The manufacture of pesticides is a major industry in this country. Exposure to pesticides is a fact of life for many workers both skilled and unskilled. Research chemists formulating and synthesizing such toxic agents are vulnerable. Individuals involved in the mixing, packaging and transport of pesticides also face potential risks as do exterminators, farmers and the migrant worker population.

In the past, these jobs were performed almost entirely by the male segment of the population. However, today's economy has forced many women into the workplace and some are finding employment in areas where pesticide exposure is a distinct possibility. The potential hazards of such exposure, with regard to the female reproductive system have not been clearly elucidated. It is imperative that an in-depth look into the effects of specific pesticides on specific aspects of reproductive function be undertaken so as to better clarify and assess actual risks women face in such an environment.

This study was designed to provide a comprehensive evaluation of the reproductive toxic effects of chlordecone on the female reproductive system by examining its toxic effects on three physiologically distinct states of the female reproductive system:

1) the adult non-pregnant female
2) the pregnant female
3) the prenatal female

Additionally, this study seeks to provide additional information to both female worker and employer concerning the risks of pesticides on the reproductive integrity of the female so that solid judgements about their roles in positions where exposure to such agents is a possibility can be made.

MATERIALS AND METHODS

A. Exposure of the Adult Non-Pregnant Female

Virgin female CD-1 mice (Charles River Breeding Laboratories, Wilmington, MA) were used in this study. Mice, aged 7-10 weeks and weighing between 25 and 30 gms, were housed in animal quarters and exposed to a 14:10 light:dark cycle regimen. Food and water were provided ad libitum. After seven days of acclimatization, the mice were randomly distributed into a control group, an estradiol-17B group and three groups treated with different concentrations of chlordecone. The following chemical dosages were used: 0.1 mg estradiol-17B (E-17B) (Sigma Chemical Co., St. Louis, MO), 0.062 mg, 0.125 mg and 0.25 mg chlordecone (Kepone) (Chem Service, West Chester, PA). The 0.25 mg (8 mg/kg) dosage of chlordecone was the highest dose used since preliminary experiments indicated that this dosage did not induce tremors or mortality. The group treated with estradiol served as a positive control in verifying whether the effects observed in chlordecone-treated mice were due to the estrogenicity of the pesticide or to the inherent toxicity of the chemical itself. All chemicals were first suspended in sesame oil and administered by oral gavage in a 0.2 ml volume. The control group received the sesame oil vehicle only. The mice were treated daily for five consecutive days for a total of either two, four or six weeks. There was a minimum of six animals per treatment group at each of the time intervals. The number of mice in the 0.25 mg

chlordecone group and the estradiol group, at each of the time periods, ranged from 15-22 and for the sesame oil vehicle control group, the number ranged from 15-19. The number of animals in these groups were higher since these dosages were repeated in two other experiments.

The superovulatory gonadotropin regimen was initiated on the second day of chlordecone, estradiol or sesame oil exposures during either the second, fourth or sixth week of treatment depending upon the length of the exposure period for the individual group. This regimen consisted of IP administration of 10 IU pregnant mare's serum gonadotropin (PMSG) followed 48 hours later by 10 IU human chorionic gonadotropin (hCG). It has been shown that sequential administration of PMSG and hCG stimulates a superovulatory response in mice within 12 to 15 hours following exposure to hCG. The animals were sacrificed by cervical dislocation 15 to 20 hours following the hCG exposure. Chlordecone, estradiol or sesame oil treatments continued during the gonadotropin exposure.

At sacrifice, the ampullae of the oviducts were punctured with a 25 gauge needle and the ovulated oocytes released into a dish containing physiological saline. Ovulated oocytes were counted and the average numbers of oocytes from each treatment group were compared for significant differences using the Student's t test. In addition, livers were removed from the mice and weighed and prepared for gas chromatography to determine amount of chlordecone uptake.

In another set of experiments adult female mice were exposed to 0.25 mg chlordecone, E-17B or sesame oil for four weeks. Animals were sacrificed by cervical dislocation 24 hours following the final exposure of the fourth week. Both ovaries were removed and were prepared for histological evaluation. Ovaries were fixed in Bouin's fixative for approximately 72 hours. Following alcoholic dehydration, tissues were embedded in paraffin, then serially sectioned at 8u and stained with hematoxylin and eosin. Sections were examined under a light microscope and the general histological appearance of the ovary was assessed. Follicles were classified according to the method of Chen et al.'s (1981) modification of Pederson and Peters (1968). This classification is as follows:

Small:	smallest oocyte still without follicle cells to an oocyte surrounded by no more than a single layer of follicle cells
Medium:	those containing growing oocytes surrounded by more than one layer of follicle cells and having no antrum
Large:	antral follicles including preovulatory ones.

These follicle counts were performed by examining every tenth section and tabulating only those follicles in which the nucleus of the oocyte was visible. These data were then compared and statistical significance was determined using the Student t test.

While compiling the above-mentioned data, it appeared as if there were a large number of large follicles undergoing atresia in the ovaries of chlordecone-treated mice. In order to statistically verify this observation, the ovaries was reexamined. This time each section of both serially sectioned ovaries was examined for the presence of large follicles over 300u in their widest diameter. Follicles were classified as healthy or atretic according to the characteristics described by Mandl and Zuckerman (1950) for antral

follicles. Specifically, this characterization consisted of seeing more than three pyknotic cells in the granulosa cell layer, a free-floating oocyte detached from the granulosa cells or an obviously degenerated oocyte. The percentage of atretic large follicles was determined and then compared among the three groups for statistical significance using the Student t test.

B. Experiments on the Pregnant and Prenatally Exposed Offspring

Pregnant females were exposed to either chlordecone (0.125, 0.25 or 5 mg), E-17B (0.05) or sesame oil by oral gavage daily beginning at Day 6 of gestation and ending at Day 15. Mothers were allowed to deliver and the following parameters were recorded:

1) number of offspring
2) weight of offspring
3) sex of offspring
4) externally visible malformations

Some mothers were sacrificed at Day 18 at which time their livers and ovaries were removed as well as those of their female offspring. Other mothers were allowed to wean their young. Female offspring from the different groups were identified as to the treatment of their mother during pregnancy and random cross-fostering of the offspring occurred within 48 hours after birth. The cross-fostering procedure consists of placing a female offspring exposed prenatally to a particular agent with a mother exposed to a different or the same agent during her pregnancy. Thus, mice exposed to one agent during pregnancy are allowed to lactate from a mother exposed to a different or the same agent during her pregnancy. This allows us to determine the relative importance of prenatal and/or postnatal exposure of chlordecone on observed toxic reproductive effects. The different groups that were formed from this cross-fostering procedure were as follows:

1) 0.5mg chlordecone/0.5mg chlordecone
2) 0.5mg chlordecone/sesame oil
3) 0.25mg chlordecone/0.25mg chlordecone
4) 0.25mg chlordecone/sesame oil
5) 0.125mg chlordecone/0.125mg chlordecone
6) 0.125mg chlordecone/sesame oil
7) sesame oil/0.5mg chlordecone
8) sesame oil/0.25mg chlordecone
9) sesame oil/0.125mg chlordecone
10) sesame oil/E-17B
11) E-17B/sesame oil
12) sesame oil/sesame oil

The first chemical listed signifies the prenatal exposure agent and the second indicates the exposure of the mother, during her pregnancy, from which the offspring is lactating. For example, an offspring who was exposed to 0.5 mg chlordecone prenatally and is placed after birth, with a mother who had been exposed to sesame oil during her pregnancy is indicated as 0.5mg chlordecone/sesame oil. The day of vaginal opening was recorded for each of the mice.

In addition, the mothers who were exposed to a chemical agent during their first pregnancy were allowed to mate again with a normal male. The second set of offspring were evaluated as to number, sex, weight and day of vaginal opening.

RESULTS AND DISCUSSION

A. Exposure of the Adult Non-Pregnant Female

There were no mortalities or significant differences in body weights in any of the treatment groups during the duration of the experiment.

The majority of chlordecone- and the E-17B-treated mice displayed persistent vaginal estrus (PVE) by the end of the second week of exposure (Table 1). The mice were considered to exhibit PVE when their vaginal smears contained keratinized and/or nucleated epithelial cells without leukocytes for four consecutive days. By the end of the third week all animals treated with 0.25 mg chlordecone exhibited PVE whereas by the end of the fourth week all chlordecone-treated mice displayed PVE. One of the eight mice in the estradiol group came out of estrus, temporarily. With this exception, all mice remained in PVE for the duration of the experiment. The sesame oil controls did not display any evidence of interrupted estrous cycles or signs of PVE (Table 1).

Two weeks of chlordecone treatment did not produce any significant differences in the ovulatory response to the exogenous gonadotropins (PMSG and hCG) between any of the groups. After four and six weeks of treatments, the groups receiving the lower two chlordecone doses (0.062 and 0.125 mg), exhibited increased variation in the mean number of ovulated oocytes. In fact, after six weeks of treatment, the 0.062 mg chlordecone dose induced a significantly higher number of ovulations than any of the other groups, experimental or control. On the other hand, 0.25 mg of chlordecone produced a consistent inhibition in ovulations during the experiment. At the end of four weeks there was a significant decrease in the number of ovulated oocytes in mice from this treatment group. After six weeks of treatment, the pattern of decreased ovulation in the mice treated with 0.25 mg chlordecone was repeated (Fig. 1). The actual mean number of oocytes ovulated during the experiment by all mice is tablulated in graph form in Figure 1 and in tabular form in Table 2.

The data indicate that exposure of adult mice to chlordecone for four or more weeks produced variable results in the number of oocytes ovulated in response to PMSG and hCG treatments. When compared to the control and estradiol-treated mice, the two lower chlordecone doses did not induce a reduction in the number of ovulated oocytes. Paradoxically, at the end of the experiment, the number of oocytes ovulated by the mice treated with the lowest chlordecone dose significantly exceeded all other groups. The only group that produced a consistent inhibitory effect on ovulation was the group treated with the highest chlordecone dose.

In the past it was reported that chlordecone normally induced an increase in FSH and a decrease in serum LH levels in rodents (Huber, 1965; Uphouse et al., 1984). Recently, however, it was reported that in rats, low chlordecone doses had a positive feedback effect on the hypothalamic-pituitary axis and increased plasma LH levels. In contrast, estradiol treatments exerted a potent negative feedback effect on the postcastration rise of FSH and LH secretions (Reel and Lamb, 1985). This positive effect of chlordecone could explain the increased number of ovulated oocytes in mice treated with the lower doses of the pesticide. On the other hand, high chlordecone doses effectively inhibited pituitary secretions of both FSH and LH in a manner similar to that produced by estradiol. When comparing the overall results of chlordecone action, it was concluded that the pesticide exerted much weaker effects on gonadotropin synthesis and secretion than estradiol (Reel and Lamb, 1985).

The estrogenic potency of chlordecone is much weaker than that of estradiol (Hammond et al., 1979). However, our results show that the number

of oocytes ovulated after exposure to the highest chlordecone dose was much lower than that recorded after estradiol treatments. Since chlordecone is much weaker than estradiol and exhibits less of an effect on the hypothalamic-pituitary system, the action of the highest chlordecone dose implicates the pesticide in a direct deleterious effect on the mouse ovary resulting in decreased ovulatory responses following superovulatory stimulation. This is in addition to the previously reported inhibition of the pituitary secretions of FSH and LH (Huber, 1965; Uphouse et al., 1984; Reel and Lamb, 1985).

Although the highest chlordecone dose did not induce a total inhibition of ovulation, most treated mice ovulated fewer oocytes. This indicates that after heavy chlordecone exposure, the functional potential of the ovaries in mice was decreasing with time, even when supplemented with gonadotropin stimulation. This deleterious action of chlordecone on ovarian function was still apparent even after PMSG and hCG treatment which was shown to induce a normal superovulatory response in control and estradiol-treated mice.

Earlier studies reported decreased fertility and litter size in adult mice exposed to this chemical prior to pregnancy (Good et al., 1965; Huber, 1965). These impaired reproductive functions were believed due to chlordecone's interference with gonadotropin secretions. The reported reductions in fertility in chlordecone-treated mice would lend support to our present observations of reduced ovulations following chronic exposure to high chlordecone doses.

It is possible that in mouse ovaries, chlordecone may have altered gonadotropin receptors or was toxic to a specific population of granulosa cells, oocytes and/or follicles. Persistent vaginal estrus and decreased ovulation in animals after chlordecone treatment is a characteristic feature of premature ovarian failure or premature reproductive aging (Mobbs et al., 1985). Reproductive aging induced by a pesticide could be considered as another form of reproductive toxicity.

The observed decrease in ovulatory response to exogenous gonadotropins seen in mice treated with the highest chlordecone dose is apparently not linked to the potency of its estrogenic activity since estradiol treatments failed to induce a similar decrease in the ovulatory response. This could indicate that the mouse ovaries are sensitive to the chemical and its inherent toxicity.

In conclusion, this portion of the study indicates that in the adult mouse, the ovaries appear susceptible to impairment by sustained exposure of high levels of the estrogenic pesticide chlordecone. Whether the reduced number of oocytes actually ovulated by mice after chlordecone exposure were fertile and could be fertilized to develop into normal offspring is presently not known. However, the reported decrease in litter size following chlordecone treatments supports our argument that some of the released oocytes could be abnormal or damaged.

In tabulating the number of different sized follicles in the three different groups of animals, there were minimal differences in the total number of large follicles present (Table 3). With respect to medium-sized follicles sesame oil controls contained a mean of 116.2 ± 7.8 follicles which differed little from the chlordecone-treated mice (103.8 ± 11.8). The number

of medium-sized follicles in the E-17B group was significantly higher than both sesame oil controls and chlordecone-treated mice. In fact there were twice as many medium-sized follicles (231.9 ± 41.0) in the estrogen-treated animals as in each of the other two groups (Table 3).

A significantly reduced number of small follicles (190.1 ± 32.8) was observed in chlordecone-treated mice when compared to both E-17B and sesame oil control mice (Table 3). Although the number of small oocytes found in E-17B-treated mice was larger than that of sesame oil controls, it was not statistically significant. All of the values mentioned above and listed in Table 3 were obtained by examining every tenth section of serial sections of both ovaries.

In order to determine whether there was an increase in atresia in large preovulatory follicles in chlordecone-treated mice, all the ovaries examined in the first part of this study were re-examined. This time every section was examined and follicles larger than 300u in diameter were recorded and characterized as healthy or atretic.

When evaluating only large follicles it became readily apparent that there was a high percentage of large follicles that were atretic not only in chlordecone-treated mice (68.3%), but also in the E-17B-exposed mice (63.5%). Sesame oil control animals displayed a much lower percentage (51.1%) of visible atresia in their large follicles (Table 4). Since the actual numbers of large atretic follicles were quite similar in chlordecone and E-17B treated mice and since the total number of large follicles (healthy and atretic) present was greater in ovaries from E-17B treated mice, it follows that the mean number of healthy large follicles in mice treated with E-17B (25.4 ± 2.7) was greater than that of chlordecone-treated mice (18.5 ± 1.9) (Table 4). Thus, there are more viable large follicles available to respond to the gonadotropic stimulus in the estrogen treated animals.

The adult mouse ovary exposed to 0.25 mg chlordecone for four weeks fails to respond normally to exogenous gonadotropins. This diminished response could have its basis in an alteration of a specific reproductive process or a combination of several such functional activities. Possible explanations include a decrease in the pool of oocyte/follicle cell complexes able to respond to the gonadotropins, possibly due to an alteration in endogenous gonadotropin secretion during the chlordecone exposure. Chlordecone could also induce a physical impediment to ovulation such as occurs in the luteinized unruptured follicle syndrome where granulosa cells undergo premature luteinization (Marik and Hulka, 1978). This chlorinated pesticide could also alter receptor sites of PMSG and hCG on ovarian cells.

Chlordecone has been shown to possess estrogenic activity (Bulger et al., 1979). Chlordecone produces disturbances in reproductive parameters similar to those induced by estradiol-17B. Estrogenic substances have been reported to disrupt the ovulatory responses in laboratory animals when such animals are exposed either pre- or postnatally. Prenatal exposure of mice to diethylstilbestrol resulted in average numbers of follicles of all sizes; however, these females produced 70% fewer ova than controls when stimulated by gonadotropic hormones (McLachlan, 1977). Gellert (1978) reported that neonatal exposure of rats to chlordecone induced precocious vaginal opening and anovulation. The induced anovulation caused by these agents when administered to prenatal or newborn animals is a result of the disruption of

the normal differentiation of the hypothalamic-pituitary system which in turn alters or inhibits normal secretion of both FSH and LH, gonadotropins necessary for normal follicular development and ovulation (Gorski, 1971). However, very little information is available regarding exposure of adult mice to estrogenic compounds after the hypothalamic-pituitary axis is fully differentiated.

The data presented here show interesting differences when the numbers of the different sized follicles are compared between chlordecone-exposed mice and controls. Two types of controls were employed in this experiment: the first, a vehicle control consisting of sesame oil only; the second, an estradiol-17B (E-17B) control. The use of the latter group is imperative since chlordecone, like its predecessor, DDT, has been shown to induce effects similar to that of estrogen, such as persistent estrus, vaginal cornification and uterine hypertrophy (Gellert, 1978; McFarland and Lacy, 1969). Comparison of chlordecone- and E-17B-treated groups would serve to distinguish between effects resulting from the estrogenicity of the compound and those arising from its inherent toxicity.

Significant differences were observed in the populations of different sized follicles. The significantly lower number of small follicles seen in the ovaries of chlordecone-treated mice could result from a high rate of induced atresia and subsequent disappearance of some of these small entities during the four week exposure period. This is similar to the toxic effects seen in mice exposed to a diet high in galactose where this sugar had a deleterious effect on small oocytes (Chen et al., 1981). Similarly, Mandl (1964) and Krarup (1979) found toxicity directed toward small oocytes by polycyclic aromatic hydrocarbons.

The number of medium-sized follicles in chlordecone-treated mice differed little from controls. However, a mean of more than twice as many were found in the E-17B-treated group. This large number of middle-sized follicles could have been due to either a stimulation of the pool of small follicles to undergo hyperplasia and/or an inhibition of further development of some medium-sized follicles into the large follicle pool. The tabulations of the total number of large antral follicles revealed a significantly smaller number of healthy follicles in the chlordecone-treated mice than in the E-17B and the sesame oil controls. The per cent of large atretic follicles in chlordecone- and E-17B-treated animals were similar. The fact that estrogen induces atresia in preovulatory follicles has been demonstrated by Clark et al. (1981) and Krey and Everett (1973). Ataya et al. (1988) demonstrated somewhat similar findings in animals exposed to cyclophosphamide. They found the total number of large antral follicles to be lower in treated groups than in controls and more atretic follicles were seen. However, in none of these studies was an attempt made to tabulate the number of healthy follicles present.

In the present study the number of healthy large antral follicles was greater in the E-17B-treated mice than in the pesticide-exposed animals. This coupled with the larger number of medium-sized follicles in estrogen-treated mice could account for the normally observed induced ovulatory response, thereby offsetting the high number of atretic large follicles found in the estradiol-treated ovaries.

Thus, both chlordecone and estradiol-17B cause an increase in atresia in the large follicles, which is probably due to the estrogenicity of these agents. However, the decreased number of healthy large follicles and medium-sized follicles in chlordecone-treated mice appears to be due to the toxicity of this agent. As a result, the pool of oocytes available for ovulation is reduced and response to exogenous gonadotropins is reduced. Whether this response is reversible once chlordecone exposure ceases is not known from these studies.

Throughout both the two and four week treatment groups there were no significant alterations in body weights among the chlordecone, E-17B and control groups. After the four week exposure period to either chlordecone or E-17B the mean weight change was 2.1 $\pm$ 0.2 gm (SEM) and 1.9 $\pm$ 0.4 gm compared to 2.2 $\pm$ 0.3 gm for the controls. These differences were not significant. Treated animals sacrificed three weeks after the cessation of treatment also did not differ significantly in body weight from controls.

When expressed as percent of body weight, the liver weights of chlordecone-exposed mice were found to be significantly larger than those of E-17B treated animals and controls as early as the end of the second week of treatment (Fig. 2). Livers of chlordecone-exposed animals comprised 7.4 $\pm$ 0.6% (SEM) of the total body weight compared to 5.6 $\pm$ 0.4% and 5.3 $\pm$ 0.2% for E-17B and control animals, respectively (Fig. 2).

After four weeks of experimentation, the disparity in liver weights of chlordecone-treated animals compared to the other groups is even more obvious. Livers of chlordecone-exposed mice ranged from 2.2 - 2.9 gm comprising a mean percent of body weight of 9.5 $\pm$ 0.2%. The livers of E-17B-treated mice comprised 5.5 $\pm$ 0.3% of total body weight and those of controls comprised 5.6 $\pm$ 0.1%. This was statistically significant at the $P < 0.05$ level (Fig. 2).

In order to assess whether the observed effects were reversible, groups of mice exposed to either the chlordecone, E-17B or sesame oil for four weeks were removed from this regimen for three weeks. After this time, the animals were sacrificed, livers removed and the same parameters investigated as described above.

In those animals that were continued on the experiments for three weeks following cessation of treatment, the mean percent of body weight that the livers of chlordecone-treated animals comprised decreased from the four week level to 7.3 $\pm$ 0.2%. E-17B and sesame oil control livers comprised 5.3 $\pm$ 0.1 and 5.5 $\pm$ 0.1%, respectively, of the total body weight. This is statistically different at the $P < 0.05$ level (Fig. 2).

Livers were assayed for chlordecone content to verify the incorporation of the pesticide into the liver. After two weeks of chlordecone exposure the amount of chlordecone found in the liver was 184.0 ppm (Table 5) and this value varied very little after four weeks (185 ppm). With the cessation of exposure after four weeks followed by a three week period of no exposure, the amount of chlordecone in the liver dropped to 70 ppm. At all time periods examined, both the E-17B- and sesame oil-treated animals possessed livers with background levels of chlordecone (Table 5).

The data here indicate that exposure to 0.25 mg chlordecone for as little as two weeks results in a significant increase in the size of the liver when

expressed as percent of total body weight when compared to that of E-17B-treated and control animals. The total cumulative dosage that the two week animals received was 2.25 mg and 4.75 mg for the four week exposed animals. These cumulative dosages are quite high; however, there were no deaths during any of the experiments. During the fourth week of the experiment the chlordecone-exposed animals did exhibit slight tremors and an increased reactivity to noises. By this time the animals had received a much larger cumulative dosage than Uphouse et al. (1984) had administered in a single exposure. These investigators found tremors in mice as early as 24 hours following a single exposure to 50 mg/kg of chlordecone. As would be expected, the lower multiple dosages of chlordecone and the two consecutive days of freedom from exposure each week, employed in this study, contributed to this delay in the external manifestation of toxicity.

There were no alterations in weight gain of chlordecone-exposed animals during the duration of the study when compared to both E-17B-treated animals and sesame oil controls. McFarland and Lacy (1969) reported no weight gain in mature female quail fed 200 ppm chlordecone for 30 days; however, they did find a significant weight gain in immature female quail fed chlordecone.

Huber (1965) found that liver weights doubled in 60-90 days when mice were fed a daily diet of chlordecone at 40 ppm. In the present study there was a significant increase in liver weight with as little as two weeks of exposure and the livers doubled their size within four weeks. Liver enlargement has not been observed in chicks fed chlordecone (Sherman and Ross, 1961).

The cause of this chlordecone-induced increase in liver weights remains unknown. Recent evidence indicates that chlordecone inhibits ATPase activity, ATP production and energy metabolism in mitochondria in the rat liver and that such inhibition alters cell function and reduces ionic transport across cell membranes (Desaiah et al., 1977). It has also been reported that, following ingestion of chlordecone by immature quail, the oviduct contained swollen epithelial cells which in turn contained swollen mitochondria with disrupted cristae (Eroschenko and Palmiter, 1980; Eroschenko, 1982). It may be that these abnormal increases in liver and oviduct sizes are the result of the inhibition of mitochondrial function which in turn reduces ionic balance within the cells and produces the abnormal cellular swellings (Eroschenko, 1982).

The accumulation of chlordecone within the liver reached its maximum within two weeks and continued administration of this pesticide for another two weeks increased this incorporation minimally. However, when the chlordecone exposure was halted for three weeks, the storage of this chemical in the liver was reduced by 60%. This rapid decrease in chlordecone residues in the liver after cessation of exposure was similar to that reported by Huber (1965) using mice exposed to chlordecone in the diet.

The fact that the E-17B-treated mice failed to induce any alteration in the size of the liver following either two or four weeks of exposure, indicates that the observed increase in liver size induced by chlordecone is apparently not due to its estrogenic component but rather to the toxic nature of this compound. The present data provide the first indication that the liver can be significantly affected with a daily exposure to chlordecone for as little as two weeks. This pesticide is readily incorporated into the liver

and may be altering specific hepatic functions such as protein synthesis and steroid metabolism. Therefore, a person working in industry, where exposure to such an agent is a distinct possibility, may be subjecting himself to serious physical consequences. Whether there is a complete return to normal hepatic activities following cessation of chlordecone exposure cannot be ascertained from this study.

B. Exposure of the Adult Pregnant Female and Prenatally Exposed Offspring

Per Cent Weight Gain of Exposed Pregnant Females

Exposure to three dosages of chlordecone occurred between Day 6-15 of pregnancy. Animals were weighed daily from Day 5-18 of pregnancy. The mean per cent weight gain of sesame oil control females was 81.56 $\pm$ 4.7% (SEM). Pregnant mice exposed to E-17B increased 72.86 $\pm$ 6.1% over the period examined. This differed insignificantly from that of sesame oil controls.

Mice exposed to 0.25 mg chlordecone for ten days during their pregnancy increased in weight a mean of 99.11 $\pm$ 6.1% which was significantly higher than that of controls. There was no significant difference between the per cent of weight gain by pregnant mice exposed to 0.125 mg chlordecone (69.4 $\pm$ 10.8) and those animals exposed to 0.5 mg chlordecone (70.47 $\pm$ 7.3) when compared to controls.

Maternal Liver Weights Expressed as Per Cent Total Body Weight

The total body weight of the pregnant mouse is its total weight minus the weight of the delivered fetuses.

The livers of pregnant females exposed to sesame oil from Day 6-15 of pregnancy comprised a mean of 6.53 $\pm$ 0.24% of the total body weight. Estradiol-17B-treated animals possessed livers that made up a mean of 6.95 $\pm$ 0.35% of the total body weight which was very similar to the vehicle controls.

Exposure of pregnant mice to 0.25 mg chlordecone for ten days resulted in large livers which comprised a mean of 9.4 $\pm$ 0.43% of the total body weight which was significantly higher ($P < 0.0005$) than that of controls. Those mice receiving 0.5 mg chlordecone possessed livers whose weights represented a mean of 10.84 $\pm$ 0.27% of the total body weight which was also significant. The lowest dosage employed (0.125 mg) resulted in a mean liver weight of 7.13 $\pm$ 0.25% of total body weight which was not significantly different from controls.

Uptake of Chlordecone By Maternal Liver

The maternal liver exposed to 0.5 mg chlordecone contained 25.3 ppm chlordecone following the ten day exposure regimen. One-half of this dosage (0.25 mg) of chlordecone resulted in livers containing 9.5 ppm chlordecone. Animals receiving the E-17B or the sesame oil had trace or non-detectable amounts of chlordecone. The average recovery for this technique was 80% at 2 ppm (2 mg/ml).

The data on incorporation of chlordecone in mice receiving 0.125 mg or 0.062 mg. is presently being compiled at the US EPA laboratory in NSTL, MS.

Number of Offspring From Mothers Exposed to Chlordecone During Pregnancy

The number of offspring from these mothers was tabulated at birth. Sesame oil mothers delivered a mean of 10.1 $\pm$ 0.57 young. The mean number of offspring delivered by the group receiving estradiol-17B was 7.36 $\pm$ 1.1. This was significantly lower than controls ($P < 0.01$). Some members of the group exposed to estradiol experienced difficulty in coming to term in that five of the ten pregnant animals failed to deliver.

The mean number of young delivered by females exposed to all three dosages of chlordecone was not significantly different from that of controls. Exactly what the reproductive potential of these offspring will be cannot be ascertained from this study. It is important to note that no external malformations were visible in these offspring at birth.

Weight of Offspring Exposed to Chlordecone During Gestation

The mean weight of each offspring in the sesame oil control animals was 1.42 $\pm$ 0.05 gms while that of the E-17B-treated animals was 1.54 $\pm$ 0.07 gms which was not significant.

Offspring of mice receiving 0.125 mg chlordecone had a mean weight of 1.31 $\pm$ 0.04 gm. Increasing the dosage to 0.25 mg chlordecone had little effect on the weight of the offspring in that the mean weight of animals in this group was 1.49 $\pm$ 0.06 gm. Doubling this latter dosage to 0.5 mg had no significant effect on the mean birth weight of the offspring, the mean weight being 1.25 $\pm$ 0.08 gm.

Effects of Chlordecone on the Sex of the Offspring

There was no significant difference between any of the groups with respect to the sex ratio of the offspring following exposure to any of the dosages of chlordecone or E-17B when compared to the sesame oil controls. The mean percentage of females in the sesame oil controls was 44.69% in the E-17B-treated animals 40.89%, while that in the three successively higher dosages was 45.2, 53.1 and 55.4%, respectively. The standard errors were relatively large which attributed to the lack of significance of the data.

Liver Weight of Offspring Exposed to Different Dosages of Chlordecone

Livers of all offspring were weighed at 18 days gestation. There was no evidence of any increase in liver weight when expressed as percent of body weight in the offspring of chlordecone-treated mice. There is presently no data available for the 0.125 mg chlordecone dosage.

The livers of 18-day fetuses exposed to sesame oil had a mean percentage of total body weight of 6.27 $\pm$ 0.4. Estradiol-treated fetuses revealed a 5.73 $\pm$ 0.5% of total body weight by the liver. Fetal livers of mice exposed to 0.25 mg chlordecone during gestation comprised 7.64 $\pm$ 0.1% of the total body weight of the fetus. Thus, there were no significant alterations in this liver weight in the offspring at this age.

Uptake of Chlordecone by Fetal Livers Exposed During Gestation

Fetal livers from control, E-17B- and chlordecone-treated mice were analyzed for uptake by gas chromatography. No data yet is available for

E-17B-treated mice. Sesame oil-treated fetal livers revealed 0.04 ppm chlordecone. Fetuses from pregnant mice exposed to 0.125 mg chlordecone contained 0.12 ppm chlordecone in their livers. This amount was increased in those receiving 0.25 mg chlordecone (2.6 ppm). The amount of chlordecone incorporated into the livers of fetal mice whose mothers were exposed to 0.5 mg chlordecone during gestation was 23.0 ppm.

This certainly does indicate that chlordecone does cross the placental barrier and does become incorporated into the liver of the developing fetus during this time period.

Time of Vaginal Opening Following Prenatal Exposure and/or Postnatal Exposure to Chlordecone

Pregnant mice were exposed to one of three dosages of chlordecone, E-17B or sesame oil daily from Day 6-Day 15 of gestation. Following parturition, female offspring were randomly distributed among the mothers of all groups and cross-fostered. For example, some offspring of a chlordecone mother were placed with a mother who was exposed to sesame oil during gestation and some placed with another mother who was exposed to chlordecone during pregnancy. This cross-fostering was employed to determine whether the pre- or the postnatal exposure to chlordecone was most important in eliciting reproductive aberrations.

Nine female offspring whose mothers were exposed to sesame oil during pregnancy had a mean time of vaginal opening of 29.8 $\pm$ 0.85 days (Table 6). Those offspring whose mothers had received sesame oil during pregnancy and had been placed with mothers exposed to 0.25 mg chlordecone during pregnancy demonstrated a mean vaginal opening time of 28.0 $\pm$ 1.0 days which was not significantly different than that of controls. Prenatal exposure to chlordecone, whether it was followed by lactating from a mother previously exposed to chlordecone or sesame oil, advanced the time of vaginal opening significantly (27.1 $\pm$ 0.6 and 26.7 $\pm$ 0.76, respectively). Thus, the critical exposure time necessary for eliciting this effect was the *in utero* exposure and not the exposure resulting from lactation following birth.

Offspring of mothers exposed to E-17B during pregnancy and allowed to lactate with a similarly treated mother had a mean vaginal opening time of 26.0 $\pm$ 1.2 days which was significantly different than that of the controls. We do need some more data on the cross-fostering of E-17B-treated animals.

Employing the dosage of 0.125 mg chlordecone did not reveal any advancement in the time of vaginal opening when compared to controls. In fact, female offspring exposed both prenatally and postnatally to a mother receiving 0.125 mg chlordecone had an actual delay in the mean time of vaginal opening (31.92 $\pm$ 0.74 days).

Characterization of Second Litter of Females Exposed During the Previous Pregnancy

Number of Offspring

This portion of the study was designed to determine whether any residual toxic effects would be manifested in a subsequent pregnancy following a previous pregnancy during which exposure to chlordecone occurred.

Following weaning of their first litter who were exposed during gestation these females were mated again. In the group of nine females exposed to sesame oil during their first pregnancy, the mean number of young delivered in the subsequent pregnancy was 11 $\pm$ 0.44 young. Females receiving E-17B during their first pregnancy gave birth to a mean of 10.1 $\pm$ 1.1 offspring which was not significantly different from the controls.

The second set of offspring in mice receiving 0.25 mg chlordecone during their first pregnancy averaged a mean of 11.4 $\pm$ 1.1 young which was very close to the young of the control group.

Mothers exposed to 0.125 mg chlordecone during their first pregnancy had a statistically significant decrease in the mean number of their offspring resulting from their second pregnancy (8.2 $\pm$ 1.2). This may be misleading since there were only five mothers in this group, one of which only delivered two young. More data is presently being acquired for this group.

Time of vaginal opening

Interesting results were obtained when the time of vaginal opening of this set of offspring of mothers exposed during a previous pregnancy was recorded. The mean time of vaginal opening for offspring of mothers exposed to sesame oil during the previous pregnancy was 28.5 $\pm$ 0.5 days. The time of vaginal opening in the 0.125 mg chlordecone group was quite similar to the control group. Female offspring of mothers exposed to 0.125 mg during the previous pregnancy presented a mean of 28.11 $\pm$ 1.1 days for the time of vaginal opening. However, a significant difference was observed in the time of vaginal opening in female offspring of mothers exposed to 0.25 mg chlordecone during a previous pregnancy. The vaginae of these mice opened at 27.3 $\pm$ 0.3 days which was later than those of its young of the previous pregnancy exposed *in utero*, but still significantly earlier than controls ($P < 0.05$). Offspring of mothers exposed to E-17B during their first pregnancy still demonstrated an extremely early vaginal opening time (25.5 $\pm$ 0.6).

Thus, from this data it is apparent that chlordecone can remain in the maternal organism and elicit an effect on a subsequent pregnancy. How long such a effect can be exerted and what long-term repercussion this effect may have on the future reproductive potential of the offspring can not be determined from this study.

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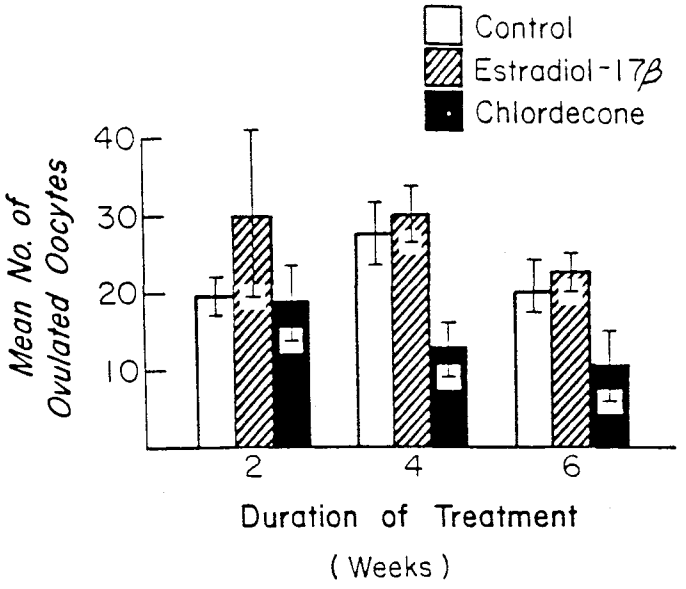

Fig. 1. Histogram depicting the mean number of oocytes ovulated in response to exogenous gonadotropins by mice exposed to one of the three treatment regimens for different weekly periods.

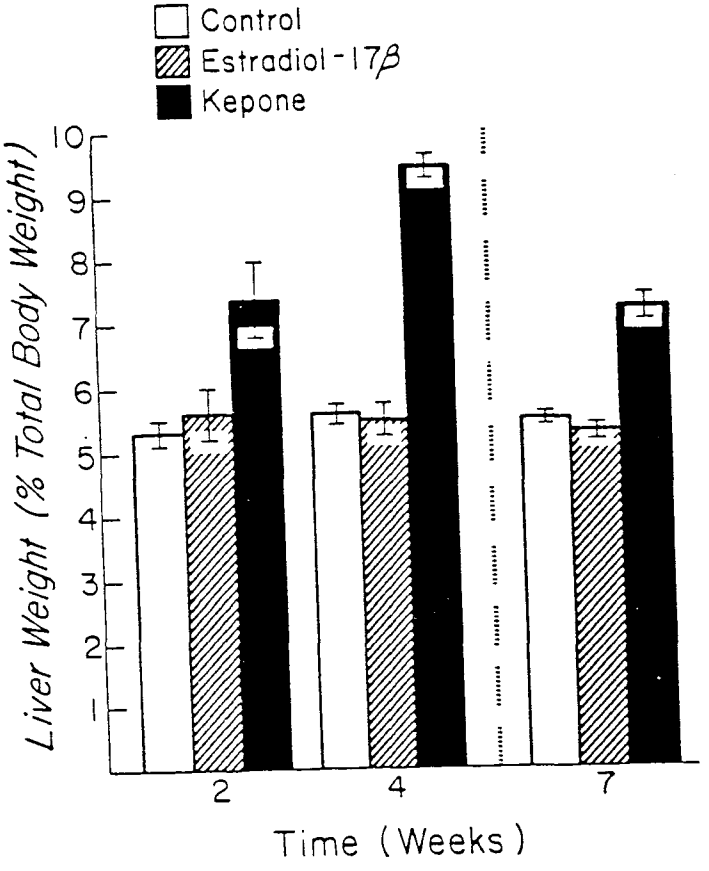

Fig. 2. Histogram depicting the liver weight expressed as percent body weight for Kepone, estradiol-17B and sesame oil control mice exposed for either two or four weeks. The seven-week data presented at the far right of the histogram represents that for the groups of mice exposed to the above agents for four weeks and then maintained for three weeks with no further exposure (seven weeks total).

TABLE 1

ONSET OF PERSISTENT VAGINAL ESTRUS IN MICE FOLLOWING EXPOSURE TO CHLORDECONE

	Length of treatment (Weeks)		
Treatment	2	3	4
Chlordecone			
0.062 mg	6/8[a]	6/8	8/8
0.125 mg	5/9	7/9	9/9
0.25 mg	8/9	9/9	9/9
Estradiol-17B	7/8	8/8	7/8
Sesame oil	0/9	0/9	0/9

[a]Number of mice displaying PVE/group.

TABLE 2

OVULATORY RESPONSE TO EXOGENOUS GONADOTROPINS
FOLLOWING EXPOSURE TO CHLORDECONE

		Number of Ovulated Oocytes		
		Weeks		
Treatment	Dosage	2	4	6
Chlordecone	0.062	26.7 ± 3.2(10)*	22.9 ± 4.3(7)	32.4 ± 3.8(7)
	0.125	19.2 ± 3.2(10)	27.1 ± 5.0(6)	21.0 ± 5.8(7)[1]
	0.25	17.7 ± 4.5(15)	14.1 ± 2.4(22)[1]	14.5 ± 3.5(16)[1]
Estradiol-17B	0.1	30.2 ± 11.8(6)	29.7 ± 3.3(11)	22.1 ± 2.5(9)
Sesame Oil	--	19.9 ± 2.4 (15)	28.4 ± 2.9(22)	23.7 ± 2.4(16)

*Number of animals in group
[1]Statistically significant ($P<0.05$)

Table 3

MEAN NUMBERS OF FOLLICLES PRESENT IN OVARIES FOLLOWING 4-WEEK EXPOSURE TO CHLORDECONE

	Types of follicles[a]		
Treatment	Small	Medium	Large
Chlordecone	190.1 ± 32.8[b,c,d]	103.8 ± 11.8[d]	27.5 ± 3.2
Estradiol-17B	368.0 ± 47.5	231.9 ± 41.0[c]	28.0 ± 8.3
Sesame Oil	279.2 ± 39.6	116.2 ± 7.8	21.3 ± 2.5

[a]Tabulations made on every tenth section

[b]Standard error of the mean (S.E.M.)

[c]$P < 0.05$ (chlordecone or estradiol-17B vs. sesame oil)

[d]$P < 0.05$ (chlordecone vs estradiol-17B)

Table 4

TOTAL NUMBER AND CONDITION OF LARGE FOLLICLES IN MICE EXPOSED TO CHLORDECONE FOR 4-WEEKS

Treatment	Large follicles			
	Total	Healthy	Atretic	% Atretic
Chlordecone	58.7 ± 5.8[a]	18.5 ± 1.9[b,c]	40.1 ± 5.1	68.3
Estradiol-17B	69.6 ± 6.7	25.4 ± 2.7	44.2 ± 4.3[b]	63.5
Sesame Oil	58.1 ± 7.3	28.4 ± 6.0	29.7 ± 3.4	51.1

[a]Standard error of the mean (S.E.M.)

[b]$P < 0.05$ (chlordecone or estradiol-17B vs. sesame oil)

[c]$P < 0.05$ (chlordecone vs. estradiol-17B)

Table 5

ACCUMULATION OF CHLORDECONE IN MOUSE LIVERS (PPM) AFTER EXPOSURE FOR TWO AND FOUR WEEKS AND THREE WEEKS AFTER TREATMENT CEASED

Treatment	Duration of experiment (weeks) 2	4	7
Chlordecone	184.0	185.0	70.0
Estradiol-17B	0.09	0.18	0.23
Sesame oil (control)	0.32	0.02	0.91

Table 6

MEAN DAY OF VAGINAL OPENINGS IN MICE EXPOSED TO EITHER CHLORDECONE, E-17B OR SESAME OIL PRENATALLY AND CROSS-FOSTERED AFTER BIRTH WITH A SIMILAR OR DIFFERENT TREATED MOTHER

Treatment		(N)	Mean day of vaginal opening	P value
	SO/SO	9	29.78	--
	E/E	5	26.0	< 0.0125*
	SO/E	2	32	< 0.15
	E/SO	2	30	< 0.475
0.5	K/K	2	29.0	0.0
0.25 mg Chlordecone	K/K	19	27.1	< 0.01*
	K/SO	10	26.7	< 0.01*
	SO/K	8	28.0	< 0.1
0.125 mg Chlordecone	K/K	13	31.9	< 0.05*
	K/SO	5	29.2	< 0.35
	SO/K	6	29.8	<0.487

APPENDIX

Published Data Resulting From Grant

PUBLICATIONS

Swartz WJ, Eroschenko VP, Schutzmann RL: Ovulatory Response of Chlordecone (Kepone)-Exposed Mice to Exogenous Gonadotropins. Toxicology 51: 147-153, 1988.

Swartz WJ, Mattison, DR: Galactose Inhibition of Ovulation in Mice. Fertility and Sterility, Vol 49: 522-526, 1988.

Swartz WJ, Schutzmann RL: Liver Weitht Response to Extended Chlordecone Exposure. Bull Environ Contam Toxicol 39: 615-621, 1987.

Swartz WJ, Schutzmann RL: Reaction of the Mouse Liver to Kepone Exposure. Bull Environ Contam Toxicol 37: 169-174, 1986.

Swartz WJ, Mattison DR: Benzo(a)pyrene Inhibits Ovulation in Mice. Anat Rec 212: 268-276, 1985.

Swartz WJ: Effects of Carbaryl on Gonadal Development in the Chick Embryo. Bull Environ Contam Toxicol 34: 481-485, 1984.

Swartz WJ: Effects of 1,1-Bis(p-chlorophenyl)-2,2,2-trichlorethane (DDT) on Gonadal Development in the Chick Embryo: A Histological and Histochemical Study. Environ Res 35: 33-345, 1984.

ABSTRACTS

Swartz WJ, Schutzmann RL: Long-term Kepone Exposure Reverses Effects on Ovarian Function Induced by Short-term Exposure. Anat Rec 214: 129, 1986.

Swartz WJ: Effects of Kepone Exposure on Ovarian Function in the Mouse. Anat Rec 211: 188A, 1985.

Paper Submitted

Swartz, WJ, Mall, GM: Chlordecone-induced follicular toxicity in mouse ovaries. Submitted to Reproductive Toxicology.

Papers in Preparation

I have several papers in preparation dealing with the effects of chlordecone on the activity of certain enzymes in the ovary and others involving the effects of prenatal exposure to chlordecone on both the offspring and exposed mother.

Equipment Inventory

The following two items were acquired under this project with a unit acquisition price of $1,000 or more. Both items were purchased solely with Federal funds.

Nikon DS-EPI-FL Epi-Flourescence Double Stain Microscope Accessory including Excitation filter and Barrier filter for viewing of fluorescein tagged lectins.

Serial No.:	09238308
Acquisition Date:	5/27/86
Cost:	$2,883.00

Centra-4B Multipurpose Centrifuge with Rotors, Trunian Rings and Carriers.

Serial No.:	23730143
Acquisition Date:	7/30/86
Cost:	$3,048.00

I wish to retain both of these items as they will be necessary to continue my studies funded by the National Institute for Occupational Safety and Health.