*Toxicology*, 51 (1988) 147–153
Elsevier Scientific Publishers Ireland Ltd.

# OVULATORY RESPONSE OF CHLORDECONE (KEPONE)-EXPOSED MICE TO EXOGENOUS GONADOTROPINS

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(Received May 27th, 1987)
(Accepted February 4th, 1988)

SUMMARY

The present study assessed the ability of the murine ovary to ovulate in response to exogenous gonadotropins following exposure to an estrogenic pesticide chlordecone (Kepone). Sexually mature virgin female CD-1 mice were exposed by oral gavage to either 0.062 mg, 0.125 mg or 0.25 mg (2, 4 or 8 mg/kg, respectively) chlordecone for 5 consecutive days for 2, 4, or 6 weeks. Control groups received either 0.1 mg estradiol-17$\beta$ (E-17$\beta$) or the sesame oil vehicle for the same period. During the final week of exposure all experimental and control animals were treated with a superovulatory regimen of PMSG and hCG. The results revealed that the lower 2 chlordecone doses (0.062 and 0.125 mg) had highly variable effects on the ovulatory responses. The highest chlordecone dose (0.25 mg), however, produced a significant and progressive decrease in the ovulatory responses when compared to both E-17$\beta$ and vehicle controls. Since ovulation was progressively impeded in mice exposed to 0.25 mg chlordecone, this high chemical dose may have exerted a direct effect on the ovary since sufficient exogenous gonadotropins were available to stimulate ovulation.

*Key words:* Chlordecone; Kepone; Ovulation; Gonadotropins; Ovary

## INTRODUCTION

There is a continuing concern and interest in the effects of various environmental chemicals on the reproductive system. The ovary is a sensitive organ

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0300-483X/88/$03.50

which can become highly vulnerable to adverse effects of xenobiotic compounds. These adverse effects can alter the endocrine and/or gamete functions of the ovaries in exposed animals.

Numerous studies have demonstrated reproductive abnormalities in female avian and mammalian species exposed either prenatally or neonatally to an estrogenic pesticide chlordecone [1—5]. Chlordecone is a pesticide with a weak affinity for estrogen receptors; in mammalian uterus, the pesticide competed for and bound to these receptors both in vivo and in vitro [6,7]. Treatment of adult laboratory rodents with chlordecone produced interrupted estrous cycles, constant vaginal cornification and anovulation [8,9]. Exposure of neonatal mice to chlordecone produced accelerated vaginal cornification, ciliation and secretory activity in oviductal and uterine cells, a condition similar to that induced by estrogen [5].

The reproductive abnormalities induced by chlordecone in fetal and newborn rodents may be due, in part, to either the alteration of the hypothalamic differentiation during perinatal development or the later disruption of hypothalamic mechanisms that regulate gonadotropin secretion [10]. Relatively few studies have examined the effects of chlordecone exposure on adult ovary vs. effects on the fully differentiated hypothalamic-pituitary-gonadal axis.

Chlordecone is no longer used as a pesticide in this country; however, its estrogenic activity allows it to serve as an important chemical model in assessing the effects of other environmental chemicals with estrogenic action on reproductive functions. The purpose of this study was to assess the ability of the murine ovary to ovulate in response to exogenous gonadotropins following a prolonged chlordecone exposure. The introduction of exogenous gonadotropins should induce a superovulatory response as it has been shown to do in adult mice [11] even during the suggested disturbance in the endogenous gonadotropin secretion [12]. Recording ovulated oocyte numbers in chlordecone-treated mice would directly assess ovulation and serve as a quantitative indicator of ovarian toxicity.

## MATERIALS AND METHODS

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 g, were housed in animal quarters and exposed to a 14:10 light/dark cycle regimen. Food and water were provided ad libitum. After 7 days of acclimatization, the mice were randomly distributed into a control group, an estradiol-17$\beta$ group and 3 groups treated with different concentrations of chlordecone. The following chemical dosages were used: 0.1 mg estradiol-17$\beta$ (E-17$\beta$) (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 5 consecutive days for a total of either 2, 4 or 6 weeks. There was a minimum of 6 animals per treatment group at each of the time intervals. The number of mice in the 0.25 mg chlordecone group or vehicle control group, at each of the time periods, ranged from 15 to 22 and for the sesame oil vehicle control the number ranged from 15 to 19. The number of animals in these groups were higher since these dosages were repeated in 2 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 i.p. administration of 10 IU pregnant mare's serum gonadotropin (PMSG) followed 48 h 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 12–15 h following exposure to hCG [11]. The animals were sacrificed by cervical dislocation 15–20 h 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.

## RESULTS

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-17$\beta$-treated mice, displayed persistent vaginal estrus (PVE) by the end of the second week of exposure (Table I).

TABLE I

ONSET OF PERSISTENT VAGINAL ESTRUS IN MICE FOLLOWING EXPOSURE TO CHLORDECONE

| Treatment | Length of treatment (weeks) | | |
|---|---|---|---|
| | 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-17$\beta$ | 7/8 | 8/8 | 7/8 |
| Sesame oil | 0/9 | 0/9 | 0/9 |

[a]Number of mice displaying PVE/group.

TABLE II

OVULATORY RESPONSE TO EXOGENOUS GONADOTROPINS FOLLOWING EXPOSURE TO CHLORDECONE

| Treatment | Dosage | Number of ovulated oocytes | | |
|---|---|---|---|---|
| | | 2 weeks | 4 weeks | 6 weeks |
| 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) |
| | 0.25 | 17.7 ± 4.5 (15) | 14.1 ± 2.4 (22)* | 14.5 ± 3.5 (16)* |
| Estradiol-17β | 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.
*Statistically significant ($P < 0.05$).

The mice were considered to exhibit PVE when their vaginal smears contained keratinized and/or nucleated epithelial cells without leukocytes. 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 8 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 I).

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 4 and 6 weeks of treatments, the groups receiving the lower 2 chlordecone doses (0.062 and 0.125 mg), exhibited increased variation in the mean number of ovulated oocytes. In fact, after 6 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 4 weeks there was a significant decrease in the number of ovulated oocytes in mice from this treatment group. After 6 weeks of treatment, the pattern of decreased ovulation in the mice treated with 0.25 mg chlordecone was repeated. The actual mean number of oocytes ovulated during the experiment by all mice is given in Table II.

## DISCUSSION

Our present data indicate that exposure of adult mice to chlordecone for 4 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 2 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 chlorde-

cone 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 [12,13]. 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 [14]. 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 [14].

The estrogenic potency of chlordecone is much weaker than that of estradiol [7]. 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 [12–14].

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 superovulatory response in control and estradiol-treated mice [11].

Earlier studies reported decreased fertility and litter size in adult mice exposed to this chemical prior to pregnancy [8,12]. These impaired reproductive functions were believed due to chlordecone's interference with gonadotropin secretions [12,13]. 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 [15]. 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. This could indicate that the mouse ovaries are sensitive to the chemical and its inherent toxicity. In comparison, estradiol treatments failed to induce a similar decrease in the ovulatory response since the ovulation in the estradiol-treated mice remained similar to that of the sesame oil controls.

In conclusion, this 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 [8,12] supports our argument that some of the released oocytes could be abnormal or damaged.

ACKNOWLEDGEMENTS

This study was supported by Grant No. 0H00835 awarded to William J. Swartz by the National Institute for Occupational Safety and Health. The authors thank Archibald Fobbs of LSU and Aubry C. Dupuy, Jr. and Robert Maxey of the EPA Environmental Chemistry Laboratory for their valuable assistance.

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