

Effects of Chlordane on Ova Implantation in the Mouse

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Treatment of mammals with halogenated hydrocarbon insecticides has been reported to increase levels of microsomal enzymes that hydroxylate androgens, estrogens and progestational steroids (Conney et al. 1967); Welch et al. 1971). The ability of these pesticides to enhance the hepatic metabolism of steroids is reflected in altered physiological activity of hormonal steroids. Treatment of immature male rats with chlordane augmented testosterone metabolism and lessened the growth promoting effect of exogenous testosterone and testosterone propionate (Levin et al. 1969). The anesthetic action of large doses of progesterone is inhibited in rats pretreated with DDT, chlordane and phenobarbital (Conney et al. 1966). When female rats are pretreated with chlordane for several days, whole body concentration of injected radioactively labeled estrone is decreased. Furthermore, the reduced amount of tritiated estrone found in the uterus is correlated with a marked inhibition of the uterotrophic response to the exogenous estrogen (Welch et al. 1971).

In consideration of the influence that chlorinated hydrocarbon insecticides may exert on reproductive physiology, the present investigation was undertaken to examine the effects of chlordane on ova implantation in the mouse.

MATERIALS and METHODS

BALB/c Crg1 virgin mice were maintained under controlled conditions of artificial illumination (12L:12D) at a room temperature of 24°C. Mice were fed Berkeley Mouse Breeder Diet and water ad libitum. Three weeks after acclimatization, animals were mated and insemination verified by the presence of the vaginal plug; day of occurrence of the vaginal plug was designated as day 1 of pregnancy. On day 1 of pregnancy mated females were randomly assorted into four experimental groups and one control group. On day 1 of pregnancy, animals were injected intraperitoneally with a single injection of 50 or 100 mg/kg body weight of alpha (cis)

or gamma (trans) chlordane.* Control females were injected with the corresponding volume of the solvent sesame oil, 0.01 ml/gm body weight.

On days 11-14 after mating, mice were sacrificed by cervical dislocation and the reproductive tracts were dissected out with the aid of a dissecting microscope. Corpora lutea, placentae and embryos were examined and counted; each placenta with or without a viable embryo was considered as an indication of an implantation site. The number of implantations were statistically treated using the student T test and the number of pregnant and nonpregnant animals were analyzed by the Chi-Square method.

RESULTS

Table 1 summarizes the results of the effects of chlordane on ova implantation in the mouse. A single 50 mg/kg injection of alpha chlordane was found to decrease the number of implanted ova ($P < .05$). The group of mice receiving a single 100 mg/kg injection of gamma chlordane also displayed a lower number of implantations, but the reduction was not statistically significant. Mice receiving 50 mg/kg gamma or 100 mg/kg alpha chlordane did not show reduced numbers of implantation.

TABLE 1

Implantation of ova in the mouse after administration of chlordane on day 1 of pregnancy.

Dosage	No. of Preg. Animals	No. of implantations	Mean No. of implantations	P value
Control sesame	20	207	10.35	
50 mg/kg alpha	13	105	8.08	<.05
50 mg/kg gama	17	180	10.59	
100 mg/kg alpha	14	142	10.14	
100 mg/kg gamma	12	101	8.4	.1

* Alpha and gamma chlordane with a purity of 100% and 99.3% respectively were kindly supplied by the Velsicol Chemical Corporation.

According to the data presented in Table 2, the number of nonpregnant mice tended to be higher in the groups receiving 50 mg/kg alpha, 50 mg/kg gamma and 100 mg/kg gamma chlordane; the latter group displayed statistically significant fewer pregnancies ($P < .05$).

TABLE 2

Number of pregnant females after administration of chlordane on day 1 of pregnancy.

Dosage	Total No. of Animals	No. of Animals Pregnant	% Pregnant	P value
Control (sesame oil)	30	21	70	
50 mg/kg alpha	29	14	48	
100 mg/kg alpha	27	14	52	
50 mg/kg gamma	25	17	68	
100 mg/kg gamma	29	12	41	<.05

All treated and control animals survived the term of the experiment and no gross fetal abnormalities were detected during the dissections.

DISCUSSION

A chlorinated hydrocarbon insecticide which stimulates hepatic metabolism of steroid hormones may potentially diminish fertility by interfering with a variety of reproductive processes including tubal transport, fertilization and implantation. For successful implantation to occur, a uterus properly primed by estrogen and progesterone is required for ovum implantation and decidual growth (Yoshinaga et al. 1966); Smith et al. 1968); Yochim et al. 1962).

Ambrose et al. (1971) reported that chlordane (technical grade*) chronically fed to rats before and

* Technical grade chlordane refers to a combination of alpha and gamma chlordane in addition to impurities resultant from the manufacturing process.

and after mating decreased the number of litters born and offspring surviving through weanling stage. Female mice injected i.p. with 25 mg/kg chlordane (technical grade) once a week for three weeks before mating gave birth to fewer litters as compared to the controls; however, the size of the litters remained unaffected by the chlordane pretreatment (Welch et al. 1971). On the other hand, female mice receiving a single injection of 50 or 100 mg/kg DDT (a chlorinated hydrocarbon) on day 1 after mating, displayed a fewer number of implantations (Lunberg and Kihlstrom, 1973). In our study, three out of four groups of mice receiving an injection of reference grade chlordane on day 1 after mating displayed a fewer number of pregnancies as compared with the control group; this reduction of mated mice becoming pregnant agrees with the observations of Welch et al. (1971).

In reviewing our results, differences in potencies between the different concentrations of the alpha and gamma isomers cannot be assessed; in only one of the treated groups (50 mg/kg alpha chlordane) was there any statistically significant differences on ovum implantation as compared to the controls.

When rodents are treated with a series of chlordane injections, the liver responds with enhanced steroid hydroxylase activity after several days (Conney et al. 1967); Welch et al. (1971). In our studies, a single injection of chlordane on day one after mating may not have immediately stimulated the hepatic response to result in increased steroid metabolism. Furthermore, other steroid producing glands may have adequately responded to any increased metabolism of steroids by synthesizing and/or secreting more hormonal steroids. Thus, implantation of ova on days 4-5 after mating may not be severely affected by a single insecticide administration on day 1 of pregnancy.

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REFERENCES

- AMBROSE, A.M., H.E. CHRISTENSEN, D.J. ROBBINS and L.J. RATHER: Arch. Ind. Hyg. 7, 197 (1953).
CONNEY, A.H., M. JACOBSON, W. LEVIN, K. SCHNEIDMAN and R. KUNTZMAN: J. Pharmacol. Exp. Ther. 154, 310 (1966).
CONNEY, A.H., R.M. WELCH, R. KUNTZMAN and J.J. BURNS:

Clin. Pharmacol. Ther. 8, 2 (1967).
LEVIN, W., R.M. WELCH and A.H. CONNEY: Steroids 13, 155 (1969).
LUNDBERG, C. and J.E. KIHLSSTROM: Bull. Environ. Contam. Toxicol. 9, 267 (1973).
SMITH, D.M. and J.D. BIGGERS: J. Endocrinol. 42, 1, (1968)
WELCH, R.M., W. LEVIN, R. KUNTZMAN, M. JACOBSON and A.H. CONNEY: Toxicol. Appl. Pharmacol. 12, 234 (1971).
YOCHIM, J.M. and V.J. DE FEO: Endocrinology 71, 134 (1962)
YOSHINAGA, K. and C.E. ADAMS: J. Reprod. Fert. 12, 583 (1966).