Proceedings of the



4-7 July 2000 - Valencia Spain

These proceedings were printed as a special issue of WORLD RABBIT SCIENCE, the journal of the World Rabbit Science Association, Volume 8, supplement 1

ISSN reference of this on line version is 2308-1910

(ISSN for all the on-line versions of the proceedings of the successive World Rabbit Congresses)

MORERA P., FAUSTO A.M., TADDEI A.R., MARGARIT R.

EFFECTS OF LINDANE ON RABBIT DOE REPRODUCTIVE PERFORMANCES

Volume A, pages 211-215

EFFECTS OF LINDANE ON RABBIT DOE REPRODUCTIVE PERFORMANCES

MORERA P^{*}, FAUSTO A.M.^{**}, TADDEI A.R.^{**}, MARGARIT R.^{*}

 * Unconventional Rabbit-Breeding Experimental Centre, Animal Production Institute, University of Tuscia, 01100 Viterbo, Italy
 ** Environmental Science Department, University of Tuscia, 01100 Viterbo, Italy

ABSTRACT

The effect of administration of Lindane (γ -hexachlorocyclohexane) on reproductive performances of rabbit has been studied on 30 pregnant Grimaud hybrid does. One mg/kg body weight of Lindane was orally administered daily for two weeks and each two days during the rest of gestation and lactation period. No differences on weight and reproductive parameters of does and weight of new-borns was observed in comparison to controls. Lactation curve showed a not significant difference in increase in the second week (+30.6% vs +24.3% for treated and control group respectively). But a significantly different trend was observed in the third week, when milk yield showed a marked decrease in does receiving Lindane (-17.9% vs +6.7%; P<0.05). The higher weight at weaning of rabbits born from mothers treated by Lindane, although not statistically different from the controls (736g vs 713g), appeared related with the higher milk yield in the first and second week of lactation. No difference was observed in values of estradiol-17ß and progesterone between the two tested groups. These results indicate that no negative physiologic effects were observed on reproductive parameters of does exposed at low dosage of Lindane.

INTRODUCTION

The γ -isomer of hexachlorocyclohexane (Lindane) is a chlorinated hydrocarbon insecticide largely employed to protect cultivations and to treat animal parasitic diseases (WATTAL, 1983; SAINI, 1990). Also rabbits are mentioned (EDITORIAL, 1996; 1998).

The molecule is considered toxic and scarcely degradable in the environment where it can easily accumulate and get in contact with humans and animals (HANIG, et al., 1976; LEE, et al., 1976; SOLOMON, et al., 1977).

It has been reported that the daily Lindane intake an adult person can receive from food is $0.14 \ \mu g$ in average (FERRELL, 1996). Drinking water contaminated with Lindane is another common exposure pathway, although Lindane concentration in drinking water is generally lower (0.05-0.1 ppb) (FERRELL, 1996).

Many damaging effects as neurotoxicity, carcinogenicity, tumorigenicity (ALLEN et al., 1989), and a possible impairment of reproductive functions due to the estrogenic action of Lindane are know (KUPFER and BULGER, 1982; COOPER et al., 1989). The effects of administration of the substance to rabbit does during pregnancy and lactation is here studied.

MATERIAL AND METHODS

Thirty Grimaud hybrid rabbit does, previously selected for same age (135 days old) and weight $(3.74 \pm 0.01 \text{ kg})$, were housed singularly in an industrial building and fed commercial pellets ad libitum. The does were artificially inseminated after synchronisation by 20 IU of PMSG (Ciclogonina, Prochena) given 72 h earlier. Each female received a dose of 0.5 ml of

sperm diluted in Tris-buffer and containing about 1×10^6 of spermatozoa. Ovulation was induced by injection of 20µg of GnRH (Gonadoreline, Fertagyl, Intervet Lab.).

Beginning on the 8th day after insemination a random group of 15 females received orally for 2 weeks a daily dose of 1mg/kg body weight of Lindane diluted in 0.5 ml of corn oil. This dose was slightly higher than the amount considered possible in natural conditions (MUCCINELLI, 1997).

The treatment was repeated every two days till the end of gestation and during all the lactation period per a total of 7 weeks. A control group of 15 does received the same volume of the solvent. Animals were weighted once a week to adjust doses for increasing body weight.

Blood samples for estradiol-17 β and progesterone assays were drawn from the marginal ear veins at -4, 11, 17, 23 and 28 days of pregnancy. The serum obtained by centrifugation at 4500 rpm for 10 minutes was stored at -20°C until analyses were performed. Concentrations of estradiol-17 β and progesterone were assayed by RIA as described by SEREN et al., 1974. At parturition fertility rate and live litter size were recorded. Milk yield at 7, 14, 21 days after parturition was measured by double weighting of the litters, before and after milking. Number and individual weight of weaned/parturition was also recorded at 35 days of age.

Experimental data of weight of does and litters, milk yield and concentration of serum steroid hormones were statistically analysed using a two-way analysis of variance with repeated measures by the general linear models procedure of SAS (SAS, 1996). Differences in some reproductive parameters as number of living new-born and weaned/parturition were assayed by the independent Student's t-test.

RESULTS AND DISCUSSION

Body weight of females during gestation and lactation is reported in figure 1. No difference was observed between the control group and does receiving Lindane. Also general health status showed no difference.

Reproductive parameters (table 1) remained in the range of normal farm values (COSTANTINI and CASTELLINI, 1990; RAMON et al., 1999) and no significant differences were recorded. The same was observed with reference to the weight of new-borns in the two tested groups.

	Control	Lindane
	Mean ±S.D.	Mean ±S.D.
Fertility (%)	100	73
Live litter size	9.8 ±1.9	10.0 ± 1.8
Weaned/parturition	6.9 ±2.6	7.6 ±2.5
Individual weight (g) at:		
7 days	125 ±11	129 ±12
14 days	216 ±23	240 ±24
21 days	295 ±38	286 ±29
35 days	713 ±88.7	736 ±69.9
•		

 Table 1. Reproductive parameters.

* All differences between the two tested groups were not significant

A significantly different trend was observed in the lactation curve (fig. 2). The pick of milk production was reached 14 days after parturition in the group receiving Lindane; the increase of milk yield was 30.6% in the treated group and 24.3% in the control group. In the untreated does the maximum was reached one week later when the increase of milk production was

+6.7% while the treated group showed a decrease (-17.9%). The difference of observed trend was statistically significant (P < 0.05).



Figure 1. Body weight of does during pregnancy and lactation (mean and SEM)



days after parturition Figure 2. Trend of milk yield (mean and SEM)



Figure 3. Trend of plasmatic concentration of estradiol-17ß in pregnant does (mean and SEM).



Figure 4. Trend of plasmatic concentration of progesterone in pregnant does (mean and SEM).

Plasma estradiol-17 β and progesterone profiles are reported in figures. 3 and 4. As it is shown Lindane did not affect the level of the studied ovarian hormones along all gestation period. Notwithstanding a higher dose of Lindane (+20%) and a more frequent initial administration to the does (every day vs 3 day/week) these results confirm those obtained by LINDENAU et coll., 1994.

Results indicate that ingestion of Lindane, at a concentration level depending on soil and water possible contamination, is not suitable to produce negative physiologic effects on reproductive traits of rabbit does.

ACKNOWLEDGMENTS

Support by the Italian Ministry of University and Scientific and Technological Research for this research project is appreciated.

REFERENCES

- ALLEN, J.R., HARGRAVES, W.A., HSIA, M.T.S., LIN, F.S.D. 1989. Comparative toxicology of chlorinated compounds on mammalian species. *Pharmac. Ther.* **7:** 513-547.
- COOPER, R.L., CHADWICK, R.W., REHNBERG, G.L., GOLDMAN, J.M., BOOTH, K.C., HEIN, J.F., MCELROY, W.K. 1989. Effect of Lindane on hormonal control of reproductive function in the female rat. *Toxicol. Appl. Pharmacol.* **99:** 384-394.
- COSTANTINI, F., CASTELLINI, C. 1990. Effetti dell'habitat e del management. *Riv. Coniglicoltura*, **27 (2):** 31-36.
- EDITORIAL 1996. Le lapin. *Test-Achats*. 385 (2): 32-37.
- EDITORIAL. 1998. La lucha contro la sarna. *Cunicutura*, 133-23 (6): 150.
- FERRELL, G.T. 1996. Lindane residues in the environment. Lindane in forestry: a continue controversy. USDA Forest Service General Technical Report: 6-10.
- HANIG, J.P., YODER, P.D., KROP, S. 1976. Convulsions in weanling rabbits after a single topical application of 1 per cent Lindane. *Toxicol. Appl. Pharmac.* 38: 463-468.
- KUPFER, D., BULGER, W.H. 1982. Estrogenic actions of chlorinated hydrocarbons. In Chambers, J.E. and Yarbrough, J.D. (eds). Effects of chronic exposure to pesticides on animal system. *Raven Press, New York*, pp. 121-146.
- LEE, B., GROTH, P., TURNER, W. 1976. Suspected reaction to gamma benzene hexachloride (letter). J. Am. Med. Assoc. 236:2846.
- LINDENAU, A., FISHER, B., SEILER, P., BEIER, H.M. 1994. Effects of persistent chlorinated hydrocarbons on reproductive tissues in female rabbits. *Human Reprod.* 9 (5): 772-780.
- MUCCINELLI, M. 1997. Prontuario dei fitofarmaci, Edagricole, Bologna, 208-209.
- RAMON, J., RAFEL, O., GOMEZ, E.A. 1999. Gestión técnico económica 1997. *Cunicultura*, **138** (4): 63-70.
- SAINI, L. 1990. La barbabietola da zucchero. Gli speciali di Terra e vita. *Edagricole, Bologna*: 6-8.
- SAS. User's Guide: Statistics, Version 6.11 Edition. 1996. SAS Inst., Inc., Cary, NC.
- SEREN, E., LEOPOLD, A., BOLELLI, G. 1974. Peripheral plasma levels of oestrogens and progesterone during the bovine oestrous cycle. *Arch. Vet. Ital.*, **25:** 1-20.
- SOLOMON, I. M., FAHRNER, L., WEST, D. 1977. Gamma benzene hexachloride toxicity. *Arch. Dermatol.* **113:** 353-357.
- WATTAL, B.L. 1983. The role and use of pesticides in health practices. Pestic. Inform. 9: 43.