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J. Environ. Biol.
33, 33-37 (2012)
ISSN: 0254-8704
CODEN: JEBIDP

Effect of methomyl formulation, a carbamate pesticide on ovarian follicular development and fertility in albino mice

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Publication Data

Paper received:
14 May 2010

Revised received:
02 December 2010

Re-revised received:
23 December 2010

Accepted:
08 January 2011

Abstract

Administration of 5 mg methomyl (40%) kg^{-1} b.wt. $mouse^{-1}$ (equal to 50% of LD_{50} dose), every day for 90 days to adult female mice resulted in a significant decrease in body weight, relative weight of the ovary, uterus and fallopian tube; mean number of small, preantral, antral and pre-ovulatory follicles and fertility compared to controls. On the other hand, total duration of the estrous cycle was significantly increased compared to controls. One month after the cessation of the treatment (a commercial methomyl preparation-lannate) the effect on estrous cycle and organ weight was not restored. Treatment of 2.5 mg or 1 mg lannate kg^{-1} b. wt., although did not alter duration of the estrous cycle; relative weight of the ovary, uterus, and fallopian tube and fertility, caused a significant decrease in mean number of small follicles compared to controls. All the groups of mice treated with lannate showed loss in body weight (15.15% in 1 mg, 6.61% in 2 mg and 12.16% in 5 mg treated groups) whereas controls showed a gain in body weight (20.02%) during the period of experimentation. The results indicate that 5 mg lannate kg^{-1} b. wt. causes loss of follicles and infertility, whereas lower dosages (2.5 and 1 mg) reduce the number of small follicles which might shorten reproductive life span of mice.

Key words

Carbamate pesticide, Fertility, Follicular development, Lannate, Methomyl, Mice

Introduction

Methomyl, a systemic and contact poison is used as a broad-spectrum insecticide and acaricide. Various commercial preparations of methomyl *viz.* lannate, methovin, methomex and nudrin are used to control a wide range of insects particularly aphids and lepidoptera, that infect a variety of vegetables, field crops and commercial plantings (Bonatti *et al.*,1994; Wei *et al.*,1997).

Methomyl, a carbamate insecticide acts as a reversible acetyl cholinesterase inhibitor of central nervous system (Baron, 1991).The reproductive toxicity of methomyl, especially on gametogenic activity in mammals has not been thoroughly studied. Though altered spermatogenesis (Saleh, 1996) and total spermatogenic cell destruction and reduced testosterone levels (Mahgoub and Medany, 2000) are reported following lannate treatment in rats, its effect on gametogenesis in females is not known. The ovarian follicular development is a continuous process, which is initiated during postnatal period. A few follicles leave the pool of non-growing follicles periodically and grow continuously, until they ovulate or degenerate. In contrast to spermatogonia, the germ cells in female mammals are not renewed (Greenwald and Roy, 1994; Bristol-Goull *et al.*, 2006). Hence, loss of follicular reserve (pool of non-growing follicles) might lead to serious consequence. For instance, loss follicular reserve due to exposure to ionizing radiation resulted in early reproductive senescence in rats (Guigon *et al.*, 2003). Hence, investigations on pesticide effects on female gametogenesis gain importance as alterations in follicular reserve might adversely affect reproductive potential of female mammals. In

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addition, earlier studies on methomyl focused on some reproductive endpoints whereas impact of methomyl induced changes in reproductive parameters on fertility was not studied. The objective of the present study was to investigate the chronic effects of sub lethal dosages of methomyl (40%) on weight and morphology of the ovary, ovarian follicular dynamics, estrous periodicity and fertility in mice.

Materials and Methods

Laboratory bred adult (3 months old) female Swiss albino mice weighing 25-30 g were randomly divided into 4 groups. Mice in the first group (10 mice) received 0.1 ml distilled water mouse^{-1} and served as controls. Each mouse in second, third and fourth groups (15 in each group) received 1, 2.5 and 5-mg lannate kg^{-1} b. wt., respectively. These dosages correspond to 10, 25 and 50% of LD_{50} dose (*i.e.* 10 mg kg^{-1} b.wt.) of lannate, respectively. All the injections (ip) were given everyday. Lannate (granules) is a commercial preparation of a carbamate, methomyl, consisting of 40% methomyl and adjuvants and it was purchased form local market. Mice were provided with food (pallets) and water *ad libitum* and were maintained under 12 hrs light and dark schedule during the period of experimentation. The experimental protocol was approved by the Institutional Animal Ethics Committee and the guide lines of the committee were followed for the maintenance of the mice. Vaginal smear of each mouse in all the groups was observed everyday to record the stages of estrous cycle as per standard morphological criteria (Cooper *et al*., 1993). The body weight of each mouse was recorded weekly. After 3 months treatment in each group, five mice were autopsied, five mice were used for fertility test and remaining five (except controls) were kept without treatment for 1 month to study recovery if any.

At autopsy, weight of the body and that of the ovary, uterus and fallopian tube were recorded. The ovaries were fixed in Bouin's fluid for histological studies. Thick (5 μ) paraffin sections (serial) of the ovary were cut and stained with hematoxyline and eosin. Serial sections of the ovary were used to count the number of follicles in different stages of development, *viz*. small (primordial and primary follicles), pre-antral, antral and pre-ovulatory follicles (Pedersen and Peters, 1968). Number of follicles in each category in entire left ovary per mouse was counted under 400X magnification. Fertility of mice was tested by allowing each lannate treated and control female mouse with a healthy male (1:1 pairing), which had mated and produced a progeny earlier, chosen from the colony. Percentage of fertility was judged by number of females conceived out of total number of females bred in an experimental group. In addition length of pregnancy and litter size of each mouse was recorded and expressed as mean values of each group.

Group means of each parameter were computed considering data on five mice per group. Significant difference among group means of various parameters in different experimental groups was judged by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT).

Results and Discussion

In the present study, responses of mice in different groups to lannate treatment were not uniform. Mice treated with higher doses (2.5 or 5 mg kg^{-1} b.wt.) of methomyl (40%) showed more pronounced loss in body weight than 1 mg treated mice (Table 1).Further, duration of the estrous cycle was not affected by 1or 2.5 mg kg^{-1} b. wt. treatment, whereas it was significantly increased in 5 mg kg^{-1} b. wt. treated group (Table 2). The relative weight of the ovary, the uterus and the fallopian tube of 1 or 2.5 mg kg^{-1} b.wt. treated mice did not significantly differ from controls whereas 5 mg kg^{-1} bw treatment caused a significant decrease in relative weight of these organs (Table 3). Similarly, 1 or 2.5 mg treatment caused a significant decrease only in small and antral follicles whereas 5mg treatment caused a significant decrease in all four categories of follicles (Table 4). Lastly, fertility was 100% in mice treated with 1 or 2.5 mg, as all the treated mice became pregnant in these two groups whereas it was 20% in 5 mg treated mice (Table 5). These results reveal a dose dependent action of methomyl on reproductive physiology of mice. A dose dependent action of another carbamate, carbofuran on the ovarian follicular development in mice has been reported (Baligar and Kaliwal, 2004).

Estrous periodicity is controlled by cyclic secretion of pituitary and ovarian hormones, hence any variation in estrous cyclicity is an indication of hormonal imbalance. An increase in length of the cycle resulting in reduced number of estrous cycles following 5 mg lannate treatment in the present study is an indication of hormonal imbalance. Similarly, increase in the duration, especially diestrus phase of the cycle has been reported following treatment with organophosphates (Asmathabanu and Kaliwal, 1997; Math *et al*., 1998). On the other hand organochlorine pesticide, dicofol (Jadaramkunti and Kaliwal, 1999) cause persistent estrus and an increase in duration of the estrous cycle due to its estrogenic effects in rats. Since, lannate did not affect estrus phase it appears that it does not have estrogenic effects. It is to be noted that, biochemical effect of carbamates *i.e.* inhibition of acetylcholine esterase activity was reversible (Baron, 1991), whereas in the present study increase in the duration of the estrous cycle (Table 2) was not restored to normalcy after cessation of the treatment. The lack of reversibility might be due to irreversible effect on endocrine mechanisms or insufficient duration (1 month) for recovery. Studies on reproductive effects of other carbamates (Baligar and Kaliwal, 2002; 2004) reported earlier did not consider restoration to normalcy after cessation of treatment.

Greenwald (1987) has suggested that folliculogenesis can be applied to reproductive toxicology and Plowchalk *et al*., (1993) opined that quantitative assessment of follicular number is an indication of normal as well as toxic response of the ovary. Hence, counts of follicles in different categories fetch more information than mere gross histomorphological observation of the ovary sections. In the present study, administration of methomyl (5 mg kg^{-1} b.wt.) caused a significant decrease in mean number of follicles belonging to all 4 categories (Table 4). The damaging effect of treatment was such

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Table - 1: Effect of lannate (methomyl 40%) on the body weight of albino mice

Groups, treatment (mg kg^{-1} b.wt.)	% change compared to initial body weight				% change after cessation of treatment compared to body weight after 3 months treatment
	Months			One month after cessation of treatment	
	I month	II month	III month		
Controls	+ 8.46 ± 1.52	+17.79±1.25	+26.02±1.35	+30 ± 0.95	-
1	- 6.98 ± 0.73	-9.65 ± 1.59	-15.15 ± 1.50	-14.86 ± 1.53	0.29
2.5	- 4.61 ± 0.61	- 4.83 ± 0.19	-6.61± 0.38	- 7.35 ± 0.30	- 0.72
5	- 7.81 ± 0.92	-9.65 ± 0.35	-12.16 ± 0.46	-12.22 ± 0.07	- 0.06

Values are mean of five mice ± SE, + = Increase, - = Decrease

Table - 2: Effect of lannate (methomyl 40%) on duration of the estrous cycle of albino mice

Groups, treatment (mg kg^{-1} b. wt.)	Mean duration (days)	
	During treatment period	During recovery period
Controls	5.00 ±0.288^{a}	—
1	5.36±0.23^{a}	5.86±1.01 (NS)
2.5	5.64±0.183^{a}	5.70±1.01 (NS)
5	6.48±0.159^{b}	6.32±0.18 (NS)
ANOVA F value(df= 3,16)	8.12 (p< 0.05)	—

Values are mean of five mice ± SE. Values with different superscript letters are significantly different whereas those with same superscript letters are not as judged by Duncan's test . Mean values in each row were compared by Student's t test. df = Degrees of freedom; NS = Not significant

Table - 3: Effect of lannate (methomyl 40%) on the relative weight (weight in mg 100 g^{-1} b. wt.) of the ovary, uterus and fallopian tube in albino mice

Organ	Controls	Lannate treatment groups (mg kg^{-1} b.wt.)		
		1	2.5	5
Ovary	43.2 ± 1.56^{a}	41.00 ± 1.51^{a}	46.2 ± 1.37^{a}	35.6±0.18^{b}
Fallopian tube	44.00 ± 1.97^{a}	37.00 ± 1.70^{a}	32.00±1.37^{a}	22.6±0.81^{b}
Uterus	494.20 ± 9.85^{a}	651.40 ± 27.65^{a}	531.00±13.36^{a}	246.00±18.05^{b}

All values are mean of five mice ± SE. Values in each row with different superscript letters are significantly different whereas those with same superscript letters are not as judged by Duncan's test

Table - 4: Effect of lannate (methomyl 40%) on the number of ovarian follicles in albino mice

Types of follicles	Controls	Lannate treatment groups (mg kg^{-1} b.wt.)		
		1	2.5	5
Small follicles	306.6 ± 55.24^{a}	116.2 ± 3.69^{b}	121.80 ± 3.80^{b}	86.80±4.94^{b}
Pre – antral	235.20 ± 32.35^{a}	198.8 ± 26^{a}	161.80±23.81^{a}	142.2±15.85^{b}
Antral	24.00 ± 1.70^{a}	13.80 ± 1.24^{b}	10.80±2.80^{b}	8.80±1.15^{b}
Pre – ovulatoty	11.4 ± 1.12^{a}	8.20 ± 1.31^{a,b}	7.80 ± 1.52^{a,b}	4.6±1.02^{b}

All values are mean of five mice ± SE. Values with different superscript letters are significantly different and those with same superscript letters are not as judged by Duncan's test

Table - 5: Effect of lannate (methomyl 40%) on fertility of albino mice

Groups, treatment (mg kg^{-1} b.wt.)	No. of mice bred	No. of mice conceived	% of fertility	No. of mice delivered pups	Mean no. of pups ± SE	Duration of pregnancy (Mean no. of days±SE)
Control	5	5	100	5	8.4 ±0.24	20.2 ±0.37
1	5	5	100	5	6.2 ±0.37	21 ± 0.31
2.5	5	5	100	5	7.2 ± 0.37	19 ±0.44
5	5	1	20	1	4.0	17

Note: No SE for number of pups and duration of pregancy in 5 mg treatment as only one mouse concieved in this group.

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that, compared to controls, about 1/3rd healthy, small, pre-antral and pre-ovulatory follicles and half of pre-antral follicles were found in the ovary of treated mice. Similarly, chlorinated pesticides, methoxychlor (Martinez and Swartz, 1991**)** in mouse and dicofol (Jadaramkunti and Kaliwal, 1999) in rat cause reduction in healthy, large and medium sized follicles. A significant decrease in the number of healthy follicles and increase in atretic follicles in dose dependent manner has been observed following treatment with organophosphates, methylparathion (Asmathabanu and Kaliwal, 1997; Dhondup and Kaliwal , 1997), edifenphos (Math *et al.*, 1998) in rats and monocrotophos (Rao and Kaliwal, 2002) in mice. Since the total number of follicles is fixed at the time of birth in mammals, (Greenwald and Roy, 1994; Bristol-Goull, *et al.,* 2006), reduction in follicular number might lead to sterility or reduced fertility or early reproductive senescence, depending on the type of follicles affected and the extent of damage. The studies mentioned above, although reported the reduction in the follicular number due to pesticide treatment, did not focus on impact of altered follicular dynamics on fertility, which is the end point of reproductive toxicological studies. Present study is the first attempt to correlate changes in follicular development and ovarian histomorphology, estrous periodicity, and accessory reproductive organs following a pesticide treatment with fertility and interesting facts emerge. A marked reduction (loss of about 2/3rd number of follicles compared to controls) of pre-ovulatory follicles, increased atresia, extended duration of estrous cycle and decrease in the weight of the uterus and fallopian tubes in 5 mg lannate treated mice were accompanied by a significant drop in fertility, whereas loss of about 1/3rd number of follicles (compared to controls) and unaltered duration of estrous cycle and the weight of the uterus and fallopian tubes in 1 or 2.5 mg lannate treated mice did not alter fertility (Tables 4,5). Infertility in 5 mg treated mice might be due to combined effect of impaired follicular development and accessory reproductive organ function. Further, earlier studies on lannate (Abdel-Aziz and Othman, 1996; Amer *et al.*, 1996) reveal toxic effects on various organs/systems. In the present study, consistent loss of body weight during the treatment period (Table 1) and its stabilization after cessation of the treatment in groups of lannate treated mice indicate general toxic effects and hence infertility might also be an indirect effect of unhealthy condition.

Low doses of lannate, which did not interfere with fertility, need not be considered safe. It is to be noted that 1mg as well as 2.5 mg lannate caused a significant drop in number of small follicles (Table 4), which included primordial and primary follicles. Another carbamate, carbofuran also caused reduction in number of small follicles in mice (Baligar and Kaliwal, 2002, 2004).Since the number of primordial follicles is fixed at birth, and from these other categories of follicles are derived, the loss of primordial follicles (follicular reserve) might adversely effect reproductive potential of animals. For instance, selective destruction of primordial follicles by irradiation in young rats, although did not interfere with onset of puberty, resulted in early reproductive senescence (Guigon *et al.*, 2003). It is further suggested that those follicles, which crossed primordial stage at the time of treatment, progressed and reached final stages of oogenesis and once they were exhausted, there were no follicles left in the ovary for continuation of cycle. Hence, low doses of lannate might cause early reproductive senescence, as they caused reduction in follicular reserve. Therefore, it is suggested that, despite normal fertility, the reproductive toxic effects of low doses of lannate (methomyl 40%) might have serious consequence.

Acknowledgments

First author is grateful to University Grants Commission, New Delhi, India for the award of Teacher Fellowship under faculty improvement program.

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