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Monocrotophos impairs the fertility of male rats

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Abstract: Monocrotophos, an organophosphorous pesticide which is used extensively in Sri Lanka, was investigated for its effects on male reproductive function, using rats. Monocrotophos, was administered orally (3.5 mg kg^{-1} and 1.75 mg kg^{-1}) for three alternative days and these rats were subjected to serial mating (approximately at weekly intervals) from day 3 following treatment. Monocrotophos inhibited fertility (in terms of uterine implants and implantation index) from day 3 post-treatment and the fertility was restored by day 28 post-treatment. The antifertility effect was accompanied by reduced sperm content in the ejaculates (in terms of vaginal sperm count index) and in the cauda epididymis, impairment of motility of cauda epididymal sperm and an elevation of pre-implantation loss. In contrast, monocrotophos had no significant effect on libido, quantal pregnancy, fertility index, food and water intake, body weight, selected haematological parameter (RBC, WBC, PCV and ESR), systolic blood pressure or heart rate. It is concluded that monocrotophos is detrimental to male fertility. This effect stems mainly from oligozoospermia and increased pre-implantation losses.

Introduction: Methamidophos and monocrotophos are two organophosphate pesticides widely used in agriculture in Sri Lanka. There is increasing evidence that organophosphates hamper the reproductive potential of both males and females of several animal species [1]. Recently, we showed that monocrotophos impaired the libido of male rats in a reversible manner [2]. This anti-libido effect was accompanied by other reprotoxic effects on the epididymis and testes.

It is possible that monocrotophos may also have anti-reproductive effects on the male. However, there are no data in the literature on this question. We have investigated the possible effect in male rats using a dose and treatment regimen which we used previously to demonstrate the sedative and analgesic potential of monocrotophos [3].

Materials and methods: Healthy adult cross bred albino rats (initial body mass: males 250–280 g, females 225–250 g) were selected randomly from our own breeding colony. All rats were kept under standardised animal house conditions (temperature: 28–31°C; photoperiod: approximately 12 h light and 12 h dark daily; relative humidity: 50–55%) and had access to standard pelleted food (Oils and Fats Co. Ltd, Seeduwa, Sri Lanka) and tap water *ad libitum*.

Monocrotophos (Lankem Ltd, Colombo, Sri Lanka) was dissolved in distilled water to obtain the desired concentration in 1 mL solution (3.5 mg kg^{-1} and 1.75 mg kg^{-1}).

Three groups of male rats (each $n = 6$) were randomly selected and given 1.0 mL of distilled water ($n = 6$) or 1.0 mL of monocrotophos solution (at the two concentrations) for three alternate days (between 10.00 and 10.30 h). The first day was considered as day 1 of treatment. Observations for mortality and overt signs of clinical toxicity (changes in appearance and behaviour) were made twice daily (between 09.30 and 10.00 h and 15.00 and 16.00 h) throughout the study. Body weight, food consumption and water intake were determined daily up to day 14. In addition, the consistency of the faeces was noted, their weight determined and the colour of the urine recorded.

Libido, ejaculatory ability and fertility of treated and control rats were assessed 7 days before treatment and on days 3, 7, 14, 21, 28 and 35 post-treatment by pairing each male (15.00 h–16.00 h) with a pro-oestrous female (having had a regular 4 day oestrous cycle on at least in three occasions before pairing). The pre-coital sexual behaviour of the paired rats was observed 1–2 h later.

On the following morning between 08.00 and 08.30 h), vaginal smears of the females were obtained and examined microscopically (× 100). If spermatozoa were present (considered as the onset of pregnancy), their numbers were estimated (in duplicate) using a haemocytometer (Neaubauer improved type, BS 748, Weber, UK) after flushing the vagina with 0.05 mL of isotonic saline (0.9% NaCl, w/v). This was used as the vaginal sperm count index (10^6 mL^{-1}).

During counting, the gross morphology of the spermatozoa was noted. If spermatozoa were absent in the smear from a paired female, daily vaginal smearing was undertaken (8.00–9.00 h) from that particular female to determine the occurrence of pseudopregnancy.

14 days following mating, the females were subjected to uterotomy via a mid-line incision under ether anaesthesia (BDH Chemicals, Poole, UK) for the determination of conventional endpoints: the number of fetuses and their viability and resorption sites. The number and appearance of the corpora lutea in each ovary were also noted.

The following reproductive indices were computed: index of libido = (number mated/number paired) × 100; quantal pregnancy = (number pregnant/number mated) × 100; fertility index = (number pregnant/number paired) × 100; implantation index = (total number of implants/number mated) × 100; pre-implantation loss = [(total number of corpora lutea – total number of implantations)]/total number of corpora lutea × 100 and post-implantation loss = [(total number of implantations – total number of viable implantations)/total number of implantations] × 100.

In a second set of experiments, 18 male rats were randomly selected and treated with either the vehicle ($n = 6$) or 3.5 mg kg^{-1} ($n = 6$) or 1.75 mg kg^{-1} ($n = 6$) of monocrotophos as described earlier. On day 3 post-treatment, the blood pressure and heart rate of these rats were determined using a rat blood pressure manometer (MK-1000-S, Muromachi Kikai Co. Ltd, Tokyo, Japan). Then the animals were lightly anaesthetised with ether (BDH Chemicals) and weighed.

Blood was withdrawn from the tail vein using a microcap (Drammond Scientific Company, New Jersey, USA) and counts of red cells (RBC) and white cells (WBC), packed cell volume (PCV) and erythrocytic sedimentation rate (ESR)

were determined using techniques described by Cheesbrough and McArthur [4].

Immediately following blood collection, the animals were killed with an overdose of ether and their body weights determined using an electronic balance (Mp 600, Chyo Balance Corporation, Kyoto, Japan). Testes, epididymis, vasa deferentia and seminal vesicles, together with the coagulating glands, ventral prostate, liver, spleen, kidney and brain, were excised, defatted where necessary, and blotted free of any blood. The length between the two poles and the greater width of the left testis was determined using a vernier calliper (Fisions Scientific Equipment, Leicester, UK).

The weights of the left epididymis and vas deferens and ventral prostate, and the paired seminal vesicle together with their coagulating glands and liver, spleen or brain were determined using a Metler analytical balance (Model H-18, Metler Instruments Corporation New Jersey, USA). The weights are represented as percentages of body weight. Seminal vascular fluid was extracted and 1.0 mL mixed thoroughly with 9.0 mL of doubled distilled water. The *p*H of this solution was monitored using a *p*H meter (HM-30V TOA Electronics Ltd, Kyoto, Japan).

Six male rats were treated with 1.0 mL of distilled water, another six with 3.5 mg kg^{-1} monocrotophos and another six with 1.75 mg kg^{-1} monocrotophos as described previously.

On day 3 post-treatment, these rats were anaesthetised with ether. The left epididymis was removed, spermatozoa were extruded into isotonic saline and the numbers of motile spermatozoa (sperm showing any movement) counted and expressed as a percentage. The right cauda epididymis was weighed and the sperm count estimated in duplicate using a haemocytometer as described by Ratnasooriya and Sharpe [5] and expressed as 10^6 g^{-1} tissue.

The results are expressed as means ± SEM. Different statistical tests were employed for different forms of data as indicated in the results section. All statistical analysis were performed with the level of significance at $p < 0.05$.

Results: There were no treatment-related deaths or pronounced overt signs of clinical toxicity. However, marked piloerection was evident in all rats within 5–10 min of administration of monocrotophos. This cholinergic effect lasted up to 30–60 min. In addition, 0.5–1.0 h following administration of monocrotophos mild to moderate salivation (wet zone almost half the submaxillary area) and mild lachrymation were seen. In 90–95% of treated rats salivation was followed by moderate to mild reddening of the mucus membrane along the oral cavity and nose tip. These symptoms were short lived (lasted 3–4 h).

Monocrotophos did not significantly ($p > 0.05$; Mann-Whitney U-test) alter food or water intake (data not shown). There was also no significant ($p > 0.05$; Mann-Whitney U-test) growth impairment in treated rats although their weights were slightly diminished as compared with untreated animals (lower dose by 3.6% and higher dose by 6.7%). The appearance of the faecal boluses of treated rats was almost identical to that of control rats and there was no significant difference ($p > 0.05$; Mann-Whitney U-test) in the weights among the three groups (overall means: control = 289.1 ± 11.8 g; lower dose = 249.0 ± 15.5 g; and higher dose = 248.8 ± 9.0 g). The urine appeared slightly yellowish in both control and treated rats.

Monocrotophos treatment did not induce any significant change in the haematological parameters tested, in systolic blood pressure, heart rate or organ weight (Table 1). Furthermore, monocrotophos had no significant effect ($p > 0.05$; Mann-Whitney U-test) on the *p*H of the seminal vesicular fluid.

On the other hand, both monocrotophos doses cause marked and significant reductions ($p > 0.05$; Mann-Whitney U-test) in the motility of cauda epididymal sperm and in their numbers (sperm motility; lower dose by 45% and higher dose by 87% and sperm numbers; lower dose by 49% and higher dose by 50%). With the higher dose, a significant linear correlation (lower dose r = 0.81; $p < 0.05$ and higher dose r = 0.87; $p < 0.05$), was evident between cauda epididymal sperm motility and the numbers of uterine implants at day 3.

Table 2 summarises the data from mating experiments. The pre-coital chasing sexual foreplay (chasing, mounting, anogenital sniffing, attempted mounts and intromission with or without pelvic thrusting) of the monocrotophos-treated rats remained essentially similar to that of controls throughout the study. It was also in line with our in-house norms. Furthermore, the libido of treated rats was not significantly ($p > 0.05$, G-Test) affected.

Ejaculated sperm numbers (as revealed from vaginal sperm count index) of monocrotophos-treated rats declined markedly and significantly ($p < 0.001$; Mann -Whitney U-Test) up to day 14 post-treatment with the lower dose (by 48–82%) and up to day 21 with the higher dose (by 71–86%). None of

Table 1: Effect of monocrotophos on some parameters of rats (means ± SEM; n = 6).Weights of organs are expressed as mg/100 g body weight.

Parameter	Control	3.50 mg kg^{-1} monocrotophos	1.75 mg kg^{-1} monocrotophos
Cauda epididymal sperm count (10 mg^{-1})	24.6 ± 2.6	12.2 ± 2.3**	12.6 ± 2.0**
Motility of cauda epididymal sperm (%)	92.3 ± 1.7	12.1 ± 3.1**	50.8 ± 6.3**
Weight of cauda epididymys sperm (mg/100g body weight)	15.3 ± 4.1	12.1 ± 6.0	12.2 ± 5.0
Weight of testes (mg/100g body weight)	510.0 ± 9.1	610.0 ± 8.9	550.0 ± 9.4
Weight of vas deferens (mg/100g body weight)	89.9 ± 9.0	98.4 ± 3.0	98.8 ± 7.0
Weight of ventral prostate (mg/100g body weight)	42.6 ± 3.0	30.4 ± 3.0	31.4 ± 3.0
Weight of seminal vesicles (mg/100g body weight)	145.3 ± 3.0	188.9 ± 1.0	168.7 ± 5.0
*p*H of seminal fluid	6.4 ± 0.1	6.8 ± 0.1	6.8 ± 0.1
Weight of liver (mg/100g body weight)	1003.1 ± 9.8	1326.4 ± 6.8	1365.5 ± 9.6
Weight of kidney (mg/100g body weight)	239.4 ± 5.0	200.9 ± 8.0	256.6 ± 3.0
Weight of spleen (mg/100g body weight)	186.5 ± 2.0	180.7 ± 8.0	192 4 ± 2.0
Weight of brain (mg/100g body weight)	697.2 ± 3.0	593.4 ± 4.0	569.1 ± 6.0
Pack cell volume (%)	44.7 ± 1.5	44.3 ± 0.4	43.5 ± 2.1
RBC count (10 mm^{-3})	5.9 ± 7.3	6.7 ± 0.9	5.8 ± 1.6
WBC count (10^3 mm^{-3})	17.1 ± 1.6	14.6 ± 1.8	13.1 ± 0.5
ESR (mm h^{-1})	3.0 ± 1.1	3.7 ± 0.8	2.9 ± 0.7
Blood pressure (mm^3 Hg)	138.1 ± 6.5	138.0 ± 5.3	130.5 ± 4.5
Heart rate (min)	400.9 ± 9.3	389.9 ± 9.5	389.0 ± 9.8

*As compared with control, *p < 0.05, **p < 0.001.*

the treated rats became completely azoospermic. Furthermore, the gross morphology of the ejaculated sperm appeared normal during this period without much decapitation (about 10–15% as in control). Linear correlation analysis revealed a significant correlation between the number of uterine implants and ejaculatory sperm counts (lower dose, r = 0.86, $p < 0.05$; higher dose, r = 0.90, $p < 0.001$) up to day 7 with the lower dose and up to day 14 with the higher dose.

Monocrotophos caused a significant ($p < 0.05$; Mann-Whitney U-test) impairment in the number of uterine implants. This was evident up to day 7 post-treatment with the lower dose (by 61–72%) and up to day 14 post-treatment with the higher dose (by 52–77%). Both doses significantly impaired ($p < 0.05$; G-Test) the implantation index (the lower dose by 56–70% and higher dose by 53–76%). This effect, however, was evident up to day 7 post-treatment with the lower dose and day 14 with the higher dose. With the lower dose, none of the mated females were completely without implanted embryos. Embryos, when present, appeared normal in size. The mated females, irrespective of treatment, had normal numbers of reddish, rounded apparently healthy looking corpora lutea.

Monocrotophos modestly but significantly ($p < 0.05$; Mann-Whitney U-test) elevated pre-implantation losses: the lower dose; up to day 14 post-treatment, the higher dose; up to day 7. Linear correlation analysis revealed that a significant correlation between the number of uterine implants and pre-implantation losses (lower dose, r = –0.98, $p < 0.001$; higher dose r = –0.96, $p < 0.001$). In contrast, monocrotophos did not significantly ($p < 0.05$; G-Test) inhibit quantal pregnancy or the fertility index. In addition, monocrotophos had no significant ($p < 0.05$; Mann-Whitney U-test) effect on post-implantation losses.

Discussion: This study was designed to investigate the potential hazards of sublethal exposures of monocrotophos, an organophosphorous pesticide, on male reproductive function. The experimental subject was the rat, which is claimed to be a good model for humans in evaluating organophosphorous pesticide and nerve agent toxicity [6]. The doses and the treatment regimen were identical to those used previously to test the analgesic and sedative potential on monocrotophos [3].

The results clearly show that monocrotophos is detrimental to male reproductive performance. It inhibited fertility (expressed in terms of the number of the uterine conceptus and implantation index) in a dose-related fashion. However, monocrotophos did not impair fertility index or quantal pregnancy. This failure can be attributed to the inability of monocrotophos to inhibit totally blastocyst formation although it was able to impair the number of uterine conceptus. The monocrotophos-induced antifertility effect had two nota-

Table 2: Effect of monocrotophos on some reproductive parameters of rats (means ± SEM).

Parameters	*n*	Treatment regime	Pre-treatment	Post-treatment Day 3	Day 7	Day 14	Day 21	Day 24	Day 35
Index of libido (%)		Monocrotophos (mg kg^{-1} day^{-1})							
	6	3.50	100	100	100	100	100	100	100
		1.75	100	100	100	100	100	100	100
		Control	100	100	100	100	100	100	100
Vaginal sperm count index (10^6 mL^{-1})	6	Monocrotophos (mg kg^{-1} day^{-1})							
		3.50	31.3 ± 51.2	6.2 ± 16.8**	4.4 ± 54.2**	7.0 ± 35.2*	9.1 ± 12.9*	25.0 ± 17.6	46.0 ± 53.6
		1.75	33.0 ± 51.2	17.0 ± 36.1*	6.0 ± 14.2**	11.0 ± 90.6*	29.0 ± 15.0	29.0 ± 70.1	43.0 ± 91.0
		Control	51.0 ± 61.1	39.0 ± 41.1	32.0 ± 17.2	37.0 ± 98.9	29.0 ± 18.9	45.0 ± 98.8	45.0 ± 68.6
Quantal pregnancy (%)	6	Monocrotophos (mg kg^{-1} day^{-1})							
		3.50	100	66.7	83.3	83.3	100	100	100
		1.75	100	100	100	100	100	100	100
		Control	100	100	100	100	100	100	100
Number of implants	6	Monocrotophos (mg kg^{-1} day^{-1})							
		3.50	10.7 ± 0.8	2.8 ± 1.1**	5.1 ± 1.4*	4.6 ± 1.3*	7.0 ± 0.9	9.7 ± 0.6	11.8 ± 1.1
		1.75	11.5 ± 1.1	3.2 ± 0.7**	4.5 ± 1.1*	9.3 ± 1.0	9.5 ± 1.1	9.8 ± 0.8	11.8 ± 0.8
		Control	11.2 ± 0.6	10.8 ± 0.5	11.8 ± 0.9	11.5 ± 1.2	10.7 ± 0.6	11.3 ± 1.3	9.2 ± 1.1
Implantation index	6	Monocrotophos (mg kg^{-1} day^{-1})							
		3.50	11.2	2.7**	5.2*	4.7*	7.5	10.5	11.8
		1.75	10.7	3.2**	4.7*	9.3	9.5	9.8	11.0
		Control	10.5	10.8	11.0	11.5	10.7	10.3	9.3
Fertility index (%)	6	Monocrotophos (mg kg^{-1} day^{-1})							
		3.50	100	66.7	83.3	83.3	100	100	100
		1.75	100	100	100	100	100	100	100
		Control	100	100	100	100	100	100	100
Pre implantation loss	6	Monocrotophos (mg kg^{-1} day^{-1})							
		3.50	1.4 ± 1.3	75.9 ± 8.5**	54.6 ± 10.3*	81.1 ± 18.0**	33.2 ± 8.9**	11.4 ± 4.04	2.7 ± 1.6
		1.75	2.8 ± 1.7	65.1 ± 7.6**	52.1 ± 9.2*	28.9 ± 4.3	19.5 ± 7.5	11.2 ± 1.21	3.2 ± 1.5
		Control	1.4 ± 1.3	1.2 ± 1.2	1.2 ± 1.2	2.6 ± 1.7	1.2 ± 1.0	2.3 ± 1.0	1.5 ± 1.0
Post implantation loss (%)	6	Monocrotophos (mg kg^{-1} day^{-1})							
		3.50	0	23.2 ± 12.1	15.7 ± 09.6	14.6 ± 9.1	16.7 ± 7.9	11.2 ± 4.5	5.7 ± 2.3
		1.75	0	20.8 ± 6.0	19.4 ± 10.0	16.5 ± 4.5	20.8 ± 10.6	10.8 ± 4.5	1.9 ± 1.0
		Control	0	0	0	0	1.5 ± 1.0	0	0

*As compared to controls, *p < 0.05, **p < 0.001.*

ble features: rapid onset and offset of action, and reversibility. The latter is suggestive of and extragonadal site of action.

Unlike mellamilophos [2], monocrotophos treatment did not impair food or water intake, or body weight gain. Neither did it induce marked signs of behavioural abnormalities, blood toxicity (in terms of haematological parameters) or other obvious overt signs of clinical toxicity. However, as with methamidophos [3, 7] some cholinomimetic side-effects (such as lachrymation and salivation) were evident, to a mild to moderate degree, 0.5–1.0 h following administration of monocrotophos. Other serious cholinomimetic side-effects such as tremors, convulsions, bradycardia, hypotension and diarrhoea were not evident. Collectively, these observations indicate that the antifertility effect may not be secondary to general toxicity and/or specific cholinomimetric side-effects of organophosphates.

Monocrotophos caused severe oligozoospermia (measured in terms of vaginal sperm count index). However, its rapid onset suggests that reduced sperm output is likely to be due to an ejaculatory dysfunction and unlikely to result from inhibition of spermatogenesis. Unimpaired testicular size and weight provide additional support for this notion.

Powerful correlations between ejaculated sperm count and fertility, as in this study, have been reported previously [8]. Further rat ejaculates with sperm counts less than $5–8 \times 10^6$ have been reported to reduce the number of embryos [9]. In this study, during the period of subfertility, the sperm numbers in the ejaculate were between 4 and 11×10^6. Taken together, these observations strongly suggest that oligozoospermia in monocrotophos-treated rats by itself could account for the impaired fertility.

To become pregnant, female rats need a minimum number of penile intromissions (usually 3–6) of sufficient strength [10]. Patients using sedative and antihypertensive drugs often experience erectile dysfunctions as side-effects [11]. We have shown that monocrotophos possessed potent sedative action [3]. Thus monocrotophos-induced impaired penile erection may cause suboptimal vaginocervical stimulation, leading to suppressed fertility.

A significant and a profound elevation in pre-implantation loss was evident with monocrotophos. This is indicative of impaired fertilising ability of sperm, as a result of morphologically abnormal and/or functionally defective sperm. The external appearance of the ejaculated sperm of monocrotophos treated rats was essentially similar to that of controls. Thus an antifertility action in the production of morphologically abnormal sperm seems unlikely although we cannot completely rule out ultrastructural changes of sperm. It is more likely that the latter mechanisms is operative and played a heavy contributory role in the observed antifertility action as motility of cauda epididymal sperm was impaired. Organophosphates inhibit *in vitro* sperm motility [12] and *in vitro* fertilisation of mouse gametes by affecting sperm capacitation [1]. Successful fertilisation is dependent on both optimal capacitation and motility of sperm [13].

Monocrotophos also caused a reduction in cauda epididymal sperm numbers in addition to inhibiting motility. This reduction had also been reported with methamidophos [12]. It could be a direct spermatotoxic effect [14] or may result from the activation of disposal or scavenger cells in the epididymis [15]. Alternatively, reduction of epididymal sperm numbers may be a consequence of spermatorrhea [16]. In the rat, it appears that epididymal sperm counts have to be reduced by more than 90% to inhibit fertility [17]. Thus the effect on monocrotophos on these counts by itself is unlikely to inhibit fertility.

However, if the data on cauda epididymal sperm numbers are relevant to humans, then fertility might be seriously impaired since males have a smaller reservoir of sperm available per unit of production by the testis [15]. Already, there have been reports indicating reductions in sperm numbers of human ejaculates over the last decade. The trend is claimed to be due to increased exposure to pollutants [18] and the number per human ejaculate is typically only two-to four-fold higher than the number at which fertility is significantly reduced [19].

Unlike methamidophos [2], monocrotophos did not inhibit libido (in terms of pre-coital sexual behaviour or index of libido). Since libido was uninhibited and sexual accessory glands remained normal (in wet weight and appearance), it is presumed that monocrotophos had no marked effect in blood testosterone level.

In conclusion, this study demonstrates for the first time that monocrotophos can impair the reproductive competence of male rats, as we showed previously with methamidophos [2]. However, the antireproductive mechanisms were very different to those of methamidophos [2]. If the rat data are applicable to humans, then this is a matter for concern in Sri Lanka where many reproductively active men with a desire to father children apply both methamidophos and monocrotophos in agriculture, often without adhering to recommended procedures.

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