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ANTIREPRODUCTIVE EFFECTS IN MALE RATS EXPOSED TO METHAMIDOPHOS

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ABSTRACT

This study examined the reproductive effects of methamidophos, an organophosphorus pesticide on male rats following sub-chronic oral administration (in 3 alternate days). The doses tested were 1.8750 and 0.9375 mg kg^{-1} day^{-1} and the reproductive effects were investigated upto 28 - 35 days post-treatment. The results show that both doses of methamidophos inhibited libido in a reversible fashion lasting upto 28 - 35 days. This effect on libido was accompanied by marked depressions in food and water intake, body weight and by cholinergic intoxication. Furthermore, the treatment was reprotoxic: decreased testicular interstitial fluid volume, seminiferous tubular diameter and reduction in motility and numbers of cauda epididymal sperm. Based on these data it is concluded that methamidophos could be detrimental to the sexuality and fertility of men who are constantly exposed to it.

INTRODUCTION

Over the last decade, the use of pesticides in agriculture has increased very rapidly. This has invariably resulted in greater exposure of young men, at their prime reproductive age, to these potentially hazardous chemicals. The results of a few studies conducted so far indicated that pesticides may cause deleterious effects on male reproduction and fertility (Kimmel, 1993). Thus, it becomes necessary to conduct further studies to investigate the possible effects of pesticides on male reproductive function.

The main aim of this study was to investigate the effects of methamidophos on male reproduction. This was carried out in male rats following sub-chronic oral administration using two sublethal doses (Worthing, 1983). Methamidophos is an organophosphrous pesticide (Worthing 1983) used widely in Sri Lanka (Senanayake and Johnson 1989).

METHODS

Cross bred healthy adult albino rats (males weighing 230-260 g and females weighing 200 - 250 g) selected randomly from the Zoology Department colony were used.

The animals were housed in plastic cages (4 per cage) under standardised animal house conditions (temperature: 28 - 31°C; photoperiod: approximately 12 h light and 12 h dark daily; relative humidity: 50 - 55%). Pelleted food (Oils and Fat Corporation, Seeduwa, Sri Lanka) and tap water were available continuously. Methamidophos was obtained from Lankem Ltd. Colombo, Sri Lanka and was dissolved in distilled water to obtain the required concentration in 1.0 ml solution (0.9375 mg kg^{-1} and 1.8750 mg kg^{-1}). Three groups of male rats were orally administered with 1.0 mL of distilled water (n=6) or 1.0 mL of methamidophos solution (the two concentrations; n=6/concentration) for 3 alternative days (between 9.00 - 9.30 h). The day of commencement of the treatment was designated as day 1 and the first day after completing the 3 day treatment programme was considered the first post-treatment day. Cage-side observations for overt signs of clinical toxicity were performed daily (between 9.30 - 10.30 h) throughout the period of study.

Libido (sexual drive), ejaculatory ability and fertility were assessed 7 days prior to the treatment and on days 3,14,21,28 or 35 post-treatment. Each male was paired (between 15.00 - 16.00 h) overnight individually with a pro-oestrous female rat. The precoital sexual behaviour of these paired rats was observed 2 h later.

On the following morning (between 8.00 - 8.30 h) vaginal smears were observed microscopically (at x 100) for the presence of spermatozoa (criterion for positive mating). If spermatozoa were present, their numbers in the vagina were estimated (in duplicate) using a haemocytometer (Neubauer improved type, BS 748, Weber, U.K.) after flushing the vagina with 0.05 ml of isotonic saline (0.9 % NaCl, w/v). This was used as a vaginal sperm count index. (10^6 ml^{-1}).

During the counting procedure the gross morphology of the spermatozoa was also noted (at x 100). If spermatozoa were absent in the vaginal smears of paired females, daily smearing was undertaken (at 8.00 - 9.00 h) from that particular individual to determine the occurrence of pseudopregnancy. The index of libido = (number mated/number paired) x 100 was then computed.

Fourteen days following mating, the females were laparotomised under ether (BDH Chemicals, Poole, U.K.) anesthesia, and the number of fetuses (both viable and dead) was counted to permit analysis of fertility. The diameter (along the horizontal axis of the intrauterine lumen) of the conceptus immediately proximal to the cervical end of each uterus was measured using a vernier caliper (Fisons Scientific Equipment, Leicestershire, UK) as an index of growth.

In a second set of experiments, 18 male rats were randomly selected from the colony and were treated either with the vehicle (n=6) or with 0.9375 mg kg^{-1} (n=6) or 1.8750 mg kg^{-1} (n=6) methamidophos as described earlier. These animals were killed with an overdose of ether on day 8 post-treatment and weighed immediately. Testes, epididymides, vasa deferentia, seminal vesicles together with the coagulating glands, ventral prostate were excised, defatted and blotted free of any blood. The length between the two poles and the greatest width of the left testis were
determined using a vernier caliper and the weight was measured using an electronic balance (MP 600, Chyo Balance Corporation, Kyoto, Japan). The weights of the left epididymis and vas deferens and the ventral prostate, and the paired seminal vesicles together with their coagulating glands were also determined using a Mettler analytical balance (model H-18, Mettler Instruments Corporation, New Jersey, USA). The weights of these organs are represented as a percentage of body weight. Seminal vesicular fluid was extracted and 1.0 mL of it was mixed thoroughly with 9.0 mL of distilled water and the pH of this solution was monitored using a pH meter (HM-30V, TOA Electronics Ltd, Kyoto, Japan).

The right testis of these rats were fixed in Susa solution for 24 h and dehydrated in ethanol (70%, 80%, 90%, and 100%), cleared in xylene and embedded in paraffin wax. Sections of 4um were cut and stained with haemotoxylin-eosin and observed microscopically. The diameter of 10 - 15 seminiferous tubules of each animal were measured using an eye piece graticule (A.J. Cope & Sons Ltd, London, UK) and the mean tubular diameter was calculated.

Six male rats were treated with 1.0 mL of distilled water, another six with 0.9375 mg kg^{-1} methamidophos and yet another six male rats with 1.8750 mg kg^{-1} methamidophos as described previously. On day 3 post-treatment, these rats were anesthetised with ether, the left epididymis was removed, spermatozoa were extruded into isotonic saline and the number of motile spermatozoa (sperm showing any movement) was counted and was expressed as a percentage. The right cauda epididymis was weighed and the sperm count was estimated in duplicate using a haemocytometer as described by Ratnasooriya *et al.* (1980) and was expressed as 10^6 g^{-1} tissue. The testes were then removed and the interstitial fluid was collected from individual testis essentially as described by Sharpe (1981). Briefly, immediately after removal of the testis the caudal end of the testicular capsule was incised carefully and the testis was placed upright in an 83 x 13 mm polystyrene tube such that the testis was suspended 4 - 6 cm above the bottom of the tube. Fluid was then allowed to percolate from the testis for 18 - 20 h at 4°C. The testis was then removed and the interstitial fluid volume was measured by aspiration in 20 ul amounts using an ependoff pipette (Sherwood Medical Industries, Country Kildare, Ireland).

The results are expressed as means ± SEM. Different statistical tests were employed in different data forms as indicated in the results section. For all statistical procedures a p value of less than 0.05 was regarded as significant.

RESULTS

Methamidophos treatment caused marked erection of fur (within 15 - 20 min). Three to four hours following treatment salivation (wet zone almost upto half submaxillary area), lachrymation and mild convulsions (in the form of chewing) were evident. This was followed by development of bright deep red colouration in the mucous membranes (around oral cavity, nose tip and in cornea of eyes). Methamidophos also induced mild diarrhoea (between 3 - 4h) which lasted upto 4 - 7 h. Water and food intake of 11 out of 12 (98%) methamidophos treated rats appeared markedly suppressed from day of treatment upto 14 - 21 days. In addition, from days 3 - 21 post-treatment, methamidophos induced moderate to severe weakness in the animals.

A discharge of blood from the penis was evident between 7 - 14 days post-treatment in two rats treated with the lowest dose of methamidophos. One of these rats (No.8) died on day 8 post-treatment. The cause of the death was unknown. Compared to controls, the methamidophos treated animals displayed a lethargic locomotory activity upto 21-25 days post-treatment.

In the mating experiments, compared to control, methamidophos treated rats exhibited extremely diminished precopulatory sexual behaviour (chasing, nosing, anogenital sniffing, attempted mounts and intromissions with or without pelvic thrusting). Furthermore, upto day 28 post-treatment animals did not mate in 14 out of 21 pairings (66.7%) in the lower dose and 13 out of 24 pairings (54.2%) in the higher dose (Table 1) resulting in a significant suppression in the index of libido (at days, 3,21 and 28 post-treatment). However, the index of libido returned to normalcy by day 35 post-treatment with the lower dose (successful matings in 4 out of 5 pairings) and by day 28 post-treatment with the higher dose (successful matings in 5 out of 6 pairings).

At successful matings, there was no reduction in the number of ejaculated sperms in methamidophos treated rats as revealed from the vaginal sperm count index (lower dose: range; 6 - 18 million ml^{-1} and higher dose: range; 7.5 - 54 million ml^{-1} and control: range; 24-50 million ml^{-1} However, 3 successful matings with the lower dose of methamidophos were completely sterile (male Nos.7 and 9 on day 21 post-treatment and No.50 on day 28 post-treatment) although the vaginal sperm count index was within the normal range (male Nos. 7, 9, 50; 3, 18, 10 million ml^{-1} respectively). Furthermore, with methamidophos treatment mild to moderate (3 - 40%) degree of decapitation was evident in the ejaculatory sperm.

During the period of impaired libido (upto day 28 post-treatment with the lower dose and upto day 21 post-treatment with the higher dose) (Table 1), successful pregnancies had 8 - 11 foetuses with the lower dose and 4 - 14 foetuses with the higher dose. Further, the foetal diameter of treated rats was not significantly different ($p > 0.05$, Mann Whitney U-test) to those of controls (data not shown). The mean number of foetuses restored to normalcy by day 35 post-treatment with the lower dose and by day 28 post-treatment with the higher dose are shown in (Table 1).

As shown in Table 2, none of the methamidophos treatments had a significant effect ($p > 0.05$, ANOVA; followed by Least Significant Test) on the weights of the reproductive organs measured. Furthermore, the pH of the seminal vesicular fluid of the methamidophos treated rats was not significantly altered ($p > 0.05$, ANOVA; followed by Least Significant Test).

At the light microscope level there were no obvious changes in the seminiferous tubules or in the spermatogenesis of the testes of control rats. However, in the methamidophos treated rats seminiferous tubule diameter was reduced by 9-10%. This effect was statistically significant (ANOVA, followed by Least Significant Test, $p < 0.05$).

In the treated group 40% or more of seminiferous tubules had pyknotic and necrotic rounded spermatids. The interstitial tissue of the treated rats appeared almost similar to that of controls with seemingly normal clusters of Leydig cells. Compared to controls, methamidophos treatments significantly impaired ($p < 0.05$, Mann-Whitney U-test) the testicular interstitial fluid volume in both right (lower dose by 66.4%; higher dose by 23.7%) and left (lower dose by 42.7%; higher dose by 18.1%) testes.

Methamidophos treatments caused significant suppression ($p < 0.05$, ANOVA; followed by Least Significant Test) in the motility of the cauda epididymal spermatozoa (lower dose by 74.48% and higher dose by 60.70%). Although, quantitatively not measured, there appeared to be a marked decapitation of cauda epididymal spermatozoa. Compared to controls, the mean number of cauda epididymal spermatozoa was significantly reduced ($P < 0.05$, ANOVA; followed by Least Significant Test) in methamidophos treated rats (lower dose by 86.58% and higher dose by 68.92%).

Table 1

Effect of methamidophos on fertility of male rats, assessed as the number of foetuses resulting from each pairing

Treatment	Animal No.	Number of foetuses pre-treatment	post-treatment				
			Day 3	Day 14	Day 21	Day 28	Day 35
Control	98	09	12	09	08	09	09
	90	09	11	07	08	08	08
	99	12	09	06	08	09	06
	94	09	09	07	04	08	07
	95	13	11	08	09	11	09
	96	16	13	10	14	10	12
0.9375 mg kg^{-1} methamidophos							
	50	10	NM	NM	NM	00	08
	55	14	NM	NM	NM	NM	12
	08	11	NM	died	died	died	died
	09	13	NM	10	00	NM	06
	01	12	10	NM	NM	NM	07
	07	10	09	NM	00	11	NM
1.8750 mg kg^{-1} methamidophos							
	05	12	NM	NM	NM	01	NI
	04	12	NM	NM	NM	08	NI
	44	08	NM	NM	14	14	NI
	40	10	13	NM	NM	NM	NI
	45	14	06	13	04	09	NI
	60	10	09	NM	NM	10	NI

NM = mating did not occur at this pairing; NI = Not Investigated

Table 2

Effect of methamidophos (day 3 post-treatment) on some reproductive parameters of rats (mean ± SEM)

Parameter	Control	0.9375 mg kg^{-1} methamidophos	1.8750 mg kg^{-1} methamidophos
Caudal epididymal sperm count (x $10^6 mg^{-1}$)	2.98±1.50	0.40±0.90*	0.93±0.09*
Motility of cauda epididymal sperm (%)	88.63±5.57	22.62±8.56*	34.83±9.61*
Weight of cauda epididymis (mg/100g body weight)	0.05 ±0.00	0.06±0.00	0.07±0.00
Weight of testis (mg/100g body weight)	400.00±9.00	420.00±4.00	420.00±4.00
Length of testis (mm)	32.18±2.80	32.40±0.60	35.50±2.00
Diameter of testis (mm)	14.60±1.10	18.80±1.30	16.30±1.00
Left testicular interestitial fluid volume (uL)	47.15±5.40	27.00±5.10*	38.60±3.10*
Right testicular interstitital fluid volume (uL)	59.00±6.00	19.80±3.90*	45.00±2.90*
Diameter of seminiferous tubules (mm)	20.10±1.70	18.40±1.50*	18.03±1.30*
Weight of vas deferens (mg/100g body weight)	98.00±9.00	102.00±3.00	160.00±4.00
Weight of prostate (mg/100g body weight)	39.00±9.00	34.00±9.00	40.00±4.00
Weight of seminal vesicles (mg/100g body weight)	120.00±4.00	170.00±9.00	152.00±9.00
pH of seminal fluid	6.30±0.21	6.40±0.05	6.40±0.17

As compared with control, *$P<0.05$

DISCUSSION

This study examined the potential effects of methamidophos on male reproductive function. This was tested in male rats following subchronic oral administration (on three alternate days) of methamidophos in two sublethal doses (1.8750 and 0.9375 mg kg^{-1} day^{-1}). Acute oral LD_{50} of methamidophos for rats is reported to be 30 mg kg^{-1} day^{-1} (Worthing, 1983). The results show that methamidophos caused a rapid and a significant impairment in libido (measured in terms of precoital sexual behaviour and index of libido). This effect on libido was however, reversible (lasting only upto 28 - 35 days).

Several mechanisms can inhibit libido of males. A reduction in blood testosterone level (Hurbert, 1990), due to either a lower production rate or to a greater metabolic clearance rate, can suppress libido. However, such a mode of action seems unlikely with methamidophos as there was no significant reduction in the weights of the reproductive organs (seminal vesicles, ventral prostate, vas deferens and epididymis) and pH of seminal vesicular fluid: structural and functional integrity of these organs are testosterone-dependent (Neuman, 1977). This view is further supported by the histopathological evaluation of testes of methamidophos treated rats which showed apparently normal clusters of Leydig cells: over 95% of the total plasma testosterone concentration is secreted by Leydig cells (Hurbert, 1990).

Secondly, changes in the binding capacity of sex-hormone-binding globulin and/or testosterone receptors or their turnover rate can induce reduction in libido (Rommerts, 1990). This study, however, provides no evidence to offer in favour or against such potential mechanisms of action. An inhibition of libido can result from hyperprolactinaemia (Chambers and Phoenix, 1992) but this too seems unlikely to be operative in this study: in view of the potential stimulatory influence of prolactin on the growth and function of the accessory reproductive organs (Bartke *et al.* 1977) in rat, only long lasting hyperprolactinaemia is thought to disrupt libido (Bartke *et al.* 1977). Sedative drugs are known to suppress libido (Vermeulen, 1993) and in a previous study, we have shown that doses of methamidophos used in this study possessed marked sedative activity (Peiris *et al.* 1994). Thus, it is possible that this mode of action can account, at least partly, for the reduction in libido in this study. Methamidophos can pass through the blood brain barrier (Robinson and Beiergrohslein, 1980). Therefore, a possibility exists that methamidophos could inhibit libido through an action on the sexual center of the hypothalamus (Ganong, 1983). This action can account, at least partly, for the reduction in libido.

The main mechanism of inhibition of libido by methamidophos, however, is likely to be due to its general toxicity. In agreement with our previous observation (Peiris *et al.* 1994), methamidophos in this study caused marked suppression in food and water intake, body weight (although not quantified here) and the development of signs of mild to moderate cholinergic intoxication (salivation, lachrimation, convulsions or diarrhoea) and lethargic behaviour. It is obvious that such a stressful state would inhibit libido.

At pairings where successful mating did occur ejaculatory competence (measured in terms of vaginal sperm count index) and fertility (in terms of uterine implants) remained apparently normal. It is noteworthy that this unimpairment in fertility was inspite of several reproductive effects: reduction in seminiferous tubular diameter, impairment in testicular interstitial fluid output and necrosis of rounded spermatids reduction in cauda epididymal sperm count and impairment of cauda epididymal sperm motility. This is because normal males of most species of mammals produce sperms in numbers that generally exceed the minimum requirements for fertility as evaluated in protocols (Working, 1988) used in this study. In humans, however, sperm counts in ejaculates are closer to the threshold for the number of normal sperm needed to ensure full reproductive competence (Working, 1988). Therefore, a decrease in number of cauda epididymal sperm of this magnitude is very likely to inhibit fertility.

Being an organophosphorus pesticide (Worthing, 1983) methamidophos can enter into human body through the skin. Furthermore, in general, experimental animal data are predictive of reproductive effects in humans. Thus, the results of this study indicate that the sexuality and fertility of men (especially those without protective gear as is common in Sri Lanka) constantly exposed to methamidophos may be at a risk. This is a matter for concern.

REFERENCES

Bartke, A., Smith, M.S., Michael, S.D., Person, F.G. and Dakterio, S. (1977). Effects of experimentally induced chronic hyper prolactinemia on testosterone and gonadotrophin levels in male rats and mice. *J. Endocrinol.* **49:** 311-316.

Chambers, K.C. and Phoenix, C.H. (1992). Sexual behaviour and serum levels of prolactin, testosterone and estradiol in young and old Rhesus males. *Physiol. Behav.* **52**: 13-16.

Ganong, W.F. (1983). In *Review of Medical Physiology,* (11th ed.), pp 200-211, Lange Medical Publication, California.

Hurbert, W. (1990). In Nieschlag, E and Behre, H.M. (eds.) *Testosterone Action Deficiency Substitution,* pp 51-71, Springer-Verlag, Berlin.

Kimmel, C.A. (1993). Approaches to evaluating reproductive hazards and risks. *Environ Health Perspect.* **101**: 137-143.

Neuman, F. (1977). Pharmacology and potential use of cyproterone acetate. *Horm. Metab. Res.* **9:** 1-13.

Peiris, L.D.C., Jayatunga, Y.N.A. and Ratnasooriya, W.D. (1994). Analgesic and sedative effects of methamidophos in rats. *Med. Sci. Res.* **22**: 293-295.

Ratnasooriya, W.D., Gilmore, D.P. and Wadsworth, R.M. (1980). Effects of local application of sympathomimetic drugs to the epididymis on fertility in rats. *J. Reprod. Fert.* **56:** 643-651.

Robinson, C.P. and Beiergrohslein, D. (1980). Cholinesterase inhibition by methamidophos and its subsequent reactivation. *Pesticide Biochem. Physiol.* **13**: 267-273.

Rommerts, F.F.G. (1990). In Nieschlag, E, and Behre, H.M. (eds.) *Testosterone Action Deficiency Substitution,* 1-22, Springer-Verlag, Berlin.

Senanayake, N. and Johnson, W. (1989). In Fernando, R. (ed.), *Pesticides in Sri Lanka,* pp 179-182, Friedrich-Ebert, Stiftung, Colombo.

Sharpe, R.M. (1981). The importance of testicular intestitial fluid in the transport of injected hCG to the Leydig cells. *Int. J. Androl.* **4:** 64-74.

Vermeulen, A. (1993). Environment, human reproduction, menopause and andropause. *Environ. Health Perspect.* **101**: 91-100.

Working, P.K. (1988). Male reproduction toxicology: comparison of the human to animal models. *Environ. Health Perspect.* **77**: 37-44.

Worthing, C.R. (1983). *The Pesticide Manual,* British Crop Protection Council Publications, London.