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Reproductive Effects of Technical and Formulated Tribenuron-Methyl on Male Albino Rats

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ABSTRACT

The present study describes the reproductive effects of technical and formulated forms of tribenuron-methyl on mail albino rats. Tribenuron-methyl was orally administered in single and repetitive doses. For single dose treatments of technical and formulated forms, testosterone concentration (ng mL^{-1}) was insignificantly affected ($P \leq 0.05$). While, in repetitive doses, such effects were more pronounced, also after treatment with 25 and 50 mg kg^{-1} formulated and 50 and 100 mg kg^{-1} technical tribenuron-methyl were significant at ($P < 0.05$), respectively. Also treatment with 100 mg kg^{-1} formulated tribenuron-methyl caused highly significantly increase in serum testosterone concentration (ng mL^{-1}) as compared with control group. The body and testis weight of animals groups were not affected with single dose, but in repetitive doses significantly decreased specially with formulated compound. There was no histopathological alteration in testis, except in male rat treated with 100 mg, repetitive dose of formulated tribenuron-methyl which caused degeneration in most seminiferous tubules and the lumenae of epididymis were mostly free from spermatozoa.

Key Word: Herbicide; Formulated; Technical; Rats; Testosterone; Testes; Histopathology

INTRODUCTION

Pesticides clearly have the potential to cause reproductive toxicity in animals, and several compounds are known to affect human reproduction (Mattison *et al*., 1990; Hileman, 1994). Also, some pathological effects of pesticides on the reproductive system of experiment animals were recorded by many authors (Abd-Elghaffar, 1989; Afifi *et al*., 1991; Abou Salem *et al*., 1997; Salem & Abd Elghaffar, 1998; Okamura *et al*., 2005; Presibella *et al*., 2005)

Tribenuron-methyl initially registered in EPA, 1989 and is commonly used as herbicide in Egypt, against broad leaf weeds in wheat Sabra *et al*. (1999), within IPM program of wheat in reclaimed area Soliman *et al*. (2000) and Sabra *et al*. (2003), and its side effect on wheat plant defense enzyme (Sabra & Housssien, 2003; 2004). By the very nature of their use in weed control, they are common contaminations of the environment, food, water and domestic structures. Although selective toxicity toward target organisms is a desirable quality, it is not absolute, and most pesticides are toxic to a greater or lesser extent toward non-target organisms, including humans (Ernest & Patricia, 1997). Our previous studies on male albino rat fig-out effect of the tribenuron-methyl on hematological parameter especially the formulated one with 100 mg (Marzouk *et al*., 2005).

However, many modern herbicides such this compound kill weeds selectively by impairing metabolic processes that are unique to plant life, through its effect on biochemistry branched chain amino acid synthesis (ALS or AHAS) as an inhibitor. For this reason, their systemic toxicities in mammals are generally low. Nonetheless, some herbicides pose a significant risk of poisoning if handled carelessly. Health professionals who may need to assess the consequences of prior exposure should understand the fate of these compounds after absorption by humans. Also, many formulations contain adjuvant (stabilizers, penetrants, surfactants) that may have significant irritating and toxic effects. Therefore, this study aimed to evaluate the toxopathological effects of sub-lethal doses of pure and commercial formulation of tribenuron-methyl using male white rats.

MATERIALS AND METHODS

Herbicides. Tribenuron-methyl: methyl 2-[[[[N-(4-methoxy-6-methyl-1, 3, 5-triazin- 2-yl) methylamino] carbonyl] amino] sulfonyl] benzoate, technical (95%) (Du Pont de Nemours & Company, Inc.), formulation (75% DF). Acute oral LD_{50} for rats > 5000 mg kg^{-1}. Non-observable effect level (NOEL) of 5 mg kg^{-1} day^{-1} (the established LD_{50} values (Anonymous, 2005).

Tested animals and dosing. Seventeen groups (four rats/ group) of laboratory acclimatized male albino rats (*Rattus norvegicus* var. *albus*) weighing 80-100 g obtained from Animal Health Research Center (Cairo) were used as test animals. The rats of the first 8 groups received single oral

dose equals 5 (NOEL), 25, 50 and 100 mg kg^{-1} b.w. of tribenuron-methyl using corn oil and water as solvents for the technical and formulated forms, respectively. The rats of the second 8 groups were given (48 h. intervals) orally ten repetitive doses of 5, 25, 50 and 100 mg kg^{-1} b. w. Rats in control group (check group) were divided into four sub-groups and given orally the same volumes of water or corn oil (0.5 mL/rat) as used in single or repeated dose treatments. Also, in all experiments, animals were killed by decapitation 24 h. after last dosing.

Blood collection. Blood samples were collected in centrifuge tube and serum was obtained by centrifugation and assayed for the hormone parameters.

Testosterone measurement. Testosterone hormone determination was carried out according to the method reported by Granoff and Abraham (1979), Bricaire *et al*. (1991), Chen *et al*. (1991), Heinonen (1991), Yen (1991) and Tietz (1995) using International Immuno Diagnostics (ELISA) Kits. Also, absorbance was reading at 450 nm with a microtiter well reader using ELISA, GmbH model Jupiter. Also, animals were weighted before sacrifice, and then testes was removed and weighted.

Histological examination. After postmortem examination of sacrificed rats, testis were carefully separated and washed by water. Small pieces of testis organ were sampled and fixed in 10% neutral buffer formalin and in Bowan's fixative. The fixed samples were dehydrated in alcohols, processed and embedded in paraffin blocks. Sections of 5-7 µ were prepared. The sections were stained with heamatoxelin and eosin (Banchroft & Stevens, 1982).

Statistical analysis. The experimental design was a factorial CRD (Complete Randomized Design) with four replicates. Statistical analysis of data collected was carried out using a computer program (Cohort Software, 1986).

RESULTS AND DISCUSSION

Testosterone levels. Data in Table (I) indicated that the serum hormone levels in rats treated with technical and formulated tribenuron-methyl increased with increasing dose. After single oral dose treatment, serum testosterone concentration (ng mL^{-1}) of animals treated with 5, 25, 50 and 100 mg kg^{-1} b.w. of formulated and technical tribenuron-methyl not significant different than control animals. On the other hand, after repetitive oral doses treatments (Table II), animals that received 5 mg kg^{-1} formulated and 5 and 25 mg kg^{-1} technical tribenuron-methyl had a mean serum testosterone concentration (ng mL^{-1}), which was insignificant different than untreated control, while animal treated with 25 and 50 mg kg^{-1} formulated and 50 and 100 mg kg^{-1} technical tribenuron-methyl were significant at ($P < 0.05$) than control values.

Also, repetitive oral doses treatment with 100 mg kg^{-1} formulated tribenuron-methyl causes highly significantly increases (62.81%) in serum testosterone concentration (ng mL^{-1}) as compared with control group.

Effect on rat body and testes weight. The data in Table III represent the effect of repetitive dose of technical and formulate tribenuron-methyl when compared with control. From which the animals body weight were significantly decreased with 5 mg formulated and 50 and 100 mg technical tribenuron-methyl. And highly significant

Table I. Testosterone Hormone Concentration (ng/ ml) in the serum of rats treated with single oral dose of technical and formulated tribenuron-methyl

Doses	Formulated		Technical	
(mg / kg b.w.)	ng/ml	% increase	ng/ml	% increase
0	8.80 ± 0.26	0.00	8.82 ± 0.32	0.00
5	9.19 ± 0.15	4.43	8.92 ± 0.27	1.13
25	9.59 ± 0.63	8.97	9.08 ± 0.45	2.94
50	9.96 ± 0.87	13.18	9.63 ± 1.10	9.18
100	10.56 ± 1.27	20.00	10.38 ± 1.11	17.68

Values are mean ± S.E; statistical difference from the control:*significant at $P \leq 0.05$ &**highly significant at $P \leq 0.01$

$$\% \text{ Increase} = \frac{\text{Treatment - Control}}{\text{Control}} \times 100$$

Table II. Testosterone Hormone Concentration (ng/ml) in the serum of rats treated with repetitive oral dose of technical and formulated tribenuron-methyl

Doses	Formulated		Technical	
(mg / kg b.w.)	ng/ml	% increase	ng/ml	% increase
0	9.01 ± 0.48	0.00	9.12 ± 0.87	0.00
5	11.88 ± 0.63	31.85	11.06 ± 0.91	21.27
25	12.50 ± 0.05*	38.73	12.23 ± 1.34	34.10
50	13.50 ± 0.72*	49.83	12.98 ± 0.89*	42.32
100	14.67 ± 0.84**	62.81	13.76 ± 0.75*	50.87

Values are mean ± S.E; statistical difference from the control:*significant at $P \leq 0.05$ & **highly significant at $P \leq 0.01$

%Increase = [(((Treatment - Control)/Control)100)]

Table III. Effect of repetitive doses of technical and formulated tribenuron-methyl on body and testes weight of male rats

Doses	Formulated			Technical		
(mg/ kg b.w.)	Body weight (g)	Testes weight (g)	As % of b. w.	Body weight (g)	Testes weight (g)	As % of b. w.
0	139.5 ±2.3	2.23±0.1	1.59	142.08±2.5	2.26±0.05	1.59
5	116.43± 4.0*	1.84±0.2	1.58	119.65±3.2	1.89±0.04	1.57
25	112.17±1.0**	1.75±0.2	1.56	116.59±4.1	1.80±0.15	1.54
50	104.01±1.5**	1.62±0.3*	1.55	109.54±2.6*	1.66±0.28*	1.51
100	98.18±1.2**	1.50±0.1*	1.52	104.16±2.4*	1.56±0.15*	1.49

Values are mean ± S.E; statistical difference from the control:*significant at $P \leq 0.05$ & **highly significant at $P \leq 0.01$

$$\% \text{ of b.w} = \left[\left(\frac{\text{Testes weight}}{\text{Body weight}}100\right)\right]$$

decreased were recorded with 25, 50 and 100 mg formulated compound. Concerning the effect of this compound on testes weight, the significant decrease, were noticed with both technical and formulated forms at 50 and 100 mg. This finding is agreement with Oakes *et al*. (2002), they found that, tordon herbicide caused severe reduction in testicular weight in high dose animals and histological, small testes showed shrunken tubules with germ cell depletion. Also, Kniewald *et al*. (1998) reported that atrazine herbicide caused the same effect but, its dose dependent.

Effect on testes histopathology. The testes in group of rats kept as control was showing in Fig. 1. There was no histopathological alteration observed and the normal histology of the seminiferous tubules was recorded. Also, all tested doses from technical and formulated compound were not altered the structure of rats testicular. But, the repetitive dose of formulated tribenuron-methyl at high dose (100 mg) caused alteration. Degeneration was detected in most of the seminiferous tubules (Fig. 2), while the lumenae of the epididymis were mostly free from spermatozoa (Fig. 3). These symptoms were showed also by many workers, Jewell *et al*. (1998) and Ellis *et al*. (1998) with molinate herbicide.

Mammalian reproduction is a highly coordinated process in which almost all of the biologic resources are mobilized to achieve this critical function. Also, any toxic insults to tests can result in a multiplicity of effects. Since the testis is compartmentalized into spermatogenic (seminferous tubules) and steroidogenic (Leyding cells, interstitial) components, such effects can occur individually or in combination (Salem & Abd Elghaffar, 1998).

However, in males, testosterone is secreted primarily by the Leydig cells of the testes (Bricaire *et al*., 1991; Yen, 1991; Tietz, 1995). Mooradian *et al*. (1987) reported that the primary function of the Leydig cell is the biosynthesis and secretion of the testosterone hormone, also testosterone play an important role in the support of sexual behavior and

Fig. 1. Testes of rat in control group showing the normal histological structure of the seminiferous tubules

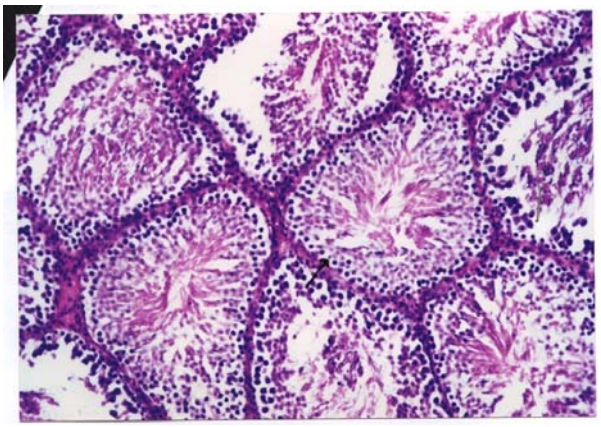


Fig. 2. Testes of rat in group administrated 100 mg formulated tribenuron-methyl (repetitive dose) showing degeneration of the seminiferous tubules and spermatogenial series with absence of spermatozoa in most tubular lumenae

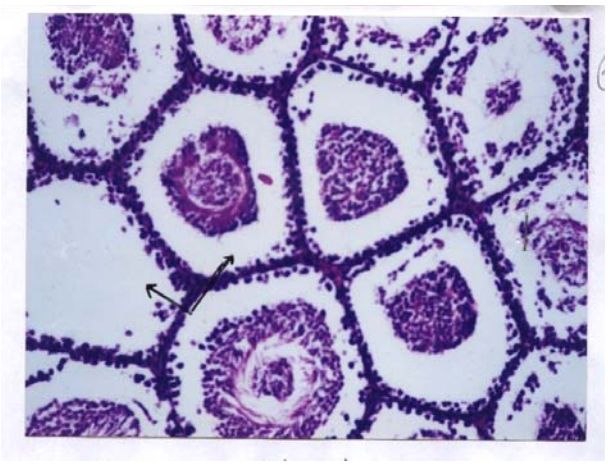
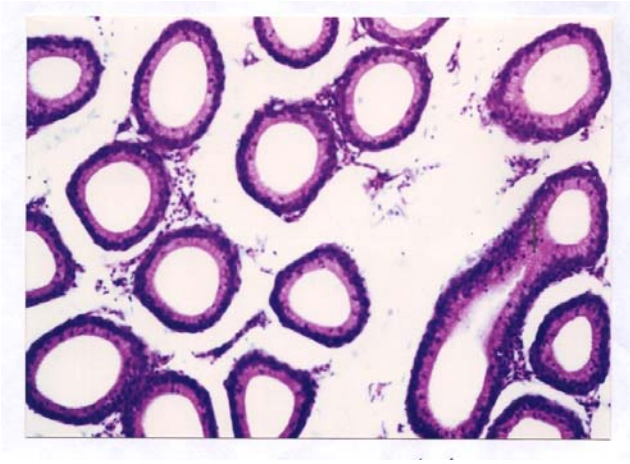


Fig. 3. Epididymis in group administrated 100 mg formulated tribenuron-methyl (repetitive dose) showing absence of the spermatozoa from the epididymol tubular lumenae



maintenance of spermatogenes. Increase of testosterone level may be incriminated to have an indirect bad effect on the spermatogenesis (Meineck & Mcdonald, 1961).

Our study revealed that after repetitive oral doses treatments, tribenuron-methyl cause significant increases in the concentration of testosterone in the serum of animals treated with 25 and 50 mg kg^{-1} formulated and 50 and 100 mg kg^{-1} technical tribenuron-methyl and high significant increased in animals treated with 100 mg kg^{-1} formulated tribenuron-methyl, respectively.

Our finding on these sex hormones are in agreement with those of Rosenberg and Malley (1997) who reported that benomyl, carbaryl, carbon disulfide, dinoseb, ethylene oxide, fenchlopose, molinate, triphenyl-tin, have

reproductive toxicity in male animals. Padungtod *et al*. (1998) found that exposure to methyl parathion; ethyl parathion and methamidophos in Chinese pesticide factory workers had a small effect on male reproductive hormones. Salem and Abd Elghaffar (1998) reported that increase of testosterone level in male rats treated groups with 1/10 or 1/20 LD_{50} nuvacron. Also, Kamijima *et al*. (2004) found an increase in serum concentrations of both FSH and testosterone in the insecticide sprayers in comparison to their corresponding controls.

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