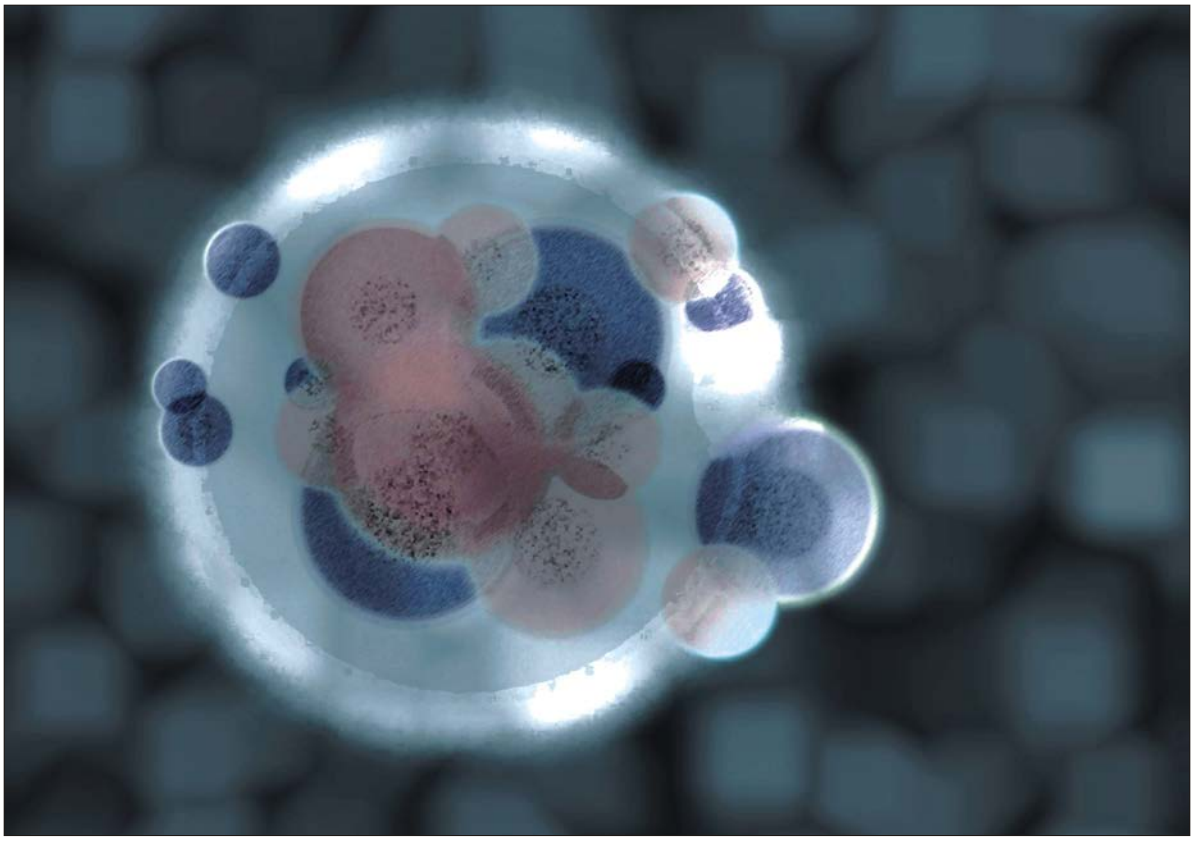

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Testicular Histopathological Alterations in Rats Treated with Sumithion® NP 25/2.5 EC, Insecticide

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Abstract: The histopathological changes induced by Sumithion® NP 25/2.5 EC, a newly formulated organophosphorous insecticide used in dengue fever vector control in Jeddah, Saudi Arabia, on rats testis were investigated. In addition to the control group, four experimental groups of male Wistar rats were daily injected intraperitoneally with two doses of Sumithion® NP 25/2.5 EC (1/10 and 1/4 the LD_{50}; 80 and 200 mg kg^{-1} of body weight, respectively) for two and four weeks. Histological examination revealed significant alterations in the testis of all treated groups including: Focal mild testicular damages, blood hemorrhage and vascular congestion, hypospermatogenesis, dilatation and tubular deformity, cellular vacuolated degeneration (necrosis), aspermatogenesis and tubular destruction and atrophy. The observed pathological changes were dose and time dependent. The testicular toxicity of Sumithion® NP 25/2.5 EC was proven and alternative harmless control strategies should be applied.

Key words: Sumithion® NP 25/2.5 EC, histopathology, testicular toxicity, dengue fever

INTRODUCTION

Organophosphorous insecticides are widely used around the world, 5% of the world population (mainly agroworkers) is directly exposed to these insecticides, in 2001 for instant this population was calculated to be 2.6 millions persons (Bustos-Obregon and Gonzalez-Hormazabal, 2003). Therefore, the health hazards on human of this class of insecticides have attracted the attention of many investigators.

Sumithion®NP25/2.5EC {0,0-dimethyl-0-(3-methyl-4-nitrophenyl) phosphorothioate, 3,4,5,6-tetrahydrophthallmidomethyl,d,l-Cis, trans-chysanthemate} is an organophosphorous insecticide upgraded from the old Sumithion® formula {0,0-dimethyl-0-(3-methyl-4-nitrophenyl) phosphorothioate}, by Sumitomo Chemical Co. in Japan, has been lately used in Jeddah, Saudi Arabia, to control *Aedes aegypti* the dengue fever vector.

As any insecticide, the intoxication may be occurred either by direct inhalation exposure or by dermal direct contact, as it can be absorbed through the skin. Kageura *et al.* (1990) has detected high level of Sumithion® in blood plasma (5 ng mL^{-1}) of workers exposed to it. Previous study reported that long term exposure to the Sumithion® caused marked inhibition in the ovarian growth associated with significant inhibition of the acetylcholinesterase activity in experimental animals (Sreenivasula *et al.*, 1983). Methyl parathion, insecticide, acts as a reproductive toxicant in male rats and histologically it induced severe focal necrosis of the germ cells in the seminiferous tubules associated with tubular atrophy (Narayana *et al.*, 2006). Also at the testicular toxicity level, Turner *et al.* (2002) revealed that Fenitrothion, an active component of Sumithion®, acts as anti-androgenic compound characterized by its ability to induce significant decrease in testosterone level, disrupt and inhibit the spermatogenic cells maturation and differentiation and finally elicited serious testicular atrophy and causing male infertility.

Considerable attention has been given to the toxic effects of Sumithion® on crab (Sreenivasula *et al.*, 1983), on toads (Sakr and Hijji, 2000) and on fish (Bhuiyan *et al.*, 2001). To our knowledge, no information regarding the potential health risks of the newly formulated Sumithion® NP 25/2.5 EC is available in literatures. Therefore, the present study will evaluate the potential testicular toxicity of Sumithion® NP 25/2.5 EC on treated rats based on histopathological investigation.

MATERIALS AND METHODS

Dose preparation: Sumithion® NP 25/2.5 EC LD_{50} value for rats was already determined by the producer (Sumitomo Chemical Co., Japan) and found to be 800 mg kg^{-1} of body weight. Two sub-lethal doses (1/10

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and 1/4 of LD_{50} : 80 and 200 mg kg^{-1}, respectively) were prepared by dissolving Sumithion® NP 25/2.5 EC in 0.9% NaCl in a way similar to the method described by Bhuiyan *et al.* (2001). Animals were daily injected intraperitoneal (ip) with both doses for two and four week's period.

Experimental animals and procedures: Adult male Wistar rats (n = 30, weighting 140-145g) were obtained from the animal house King Abdul Aziz University, Jeddah. Animals were checked for external signs of injury or disease. Only healthy animals were involved in this experiment. All animals have been kept during the experiment in proper animal plastic cages under normal conditions of temperature, light and diet according to the method described by Ajarem (1987). Rats were randomly distributed into five groups (6 males in each group). Group I and II were daily injected with 80 mg kg^{-1} of the tested insecticide for duration of two and four weeks, respectively. Animals in group III and IV were daily treated with 200 mg kg^{-1} for same previous periods. Group V represents the control group, in which animals were daily injected (ip) with constant amount (0.3 mL) of physiological saline. At the end of the experiment, all treated and control rats were killed by neck dislocation, then testis samples were immediately collected from all groups.

Histopathology: Testis samples collected from all groups were immediately fixed in 10% buffered formalin fixative. A routine histological procedure was preformed according to the method of Culling (1974). All testicular cross sections were stained with haematoxylin and Eosin (H and E) and histologically examined by light microscope.

RESULTS

Animal's condition and mortality rate: With only one exception in group IV, all treated rats groups were found to be able to survive for four weeks. After two weeks of treatment, high mortality rate of 66% was noticed in the rats treated with 200 mg kg^{-1} of body weight (1/4 of the LD_{50}) for four weeks (group IV) and therefore this group was expelled from further analysis.

Histopathological investigations:

The control group: Normal histological structure of the testis was illustrated in Fig. 1a and b. Each seminiferous tubule lined with germinal epithelial layer. Various types of spermatogenic cells appeared in their normal shape including: spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and mature spermatozoa (Fig. 1b). Mature spermatozoa with their distinguished tails filled the lumen. Sertoli and Leydig cells with regular shape were observed (Fig. 1b).

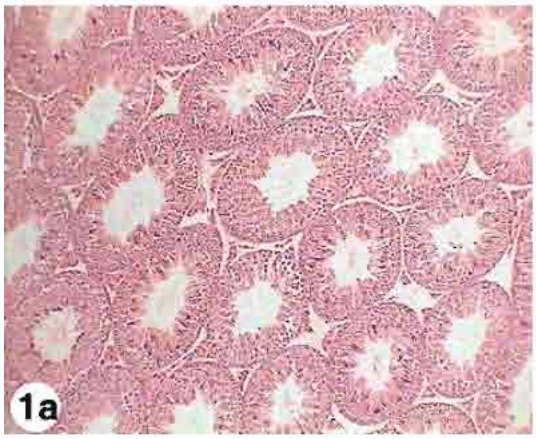

Fig. 1a: Testis normal structure of control rats (group V) (X100)

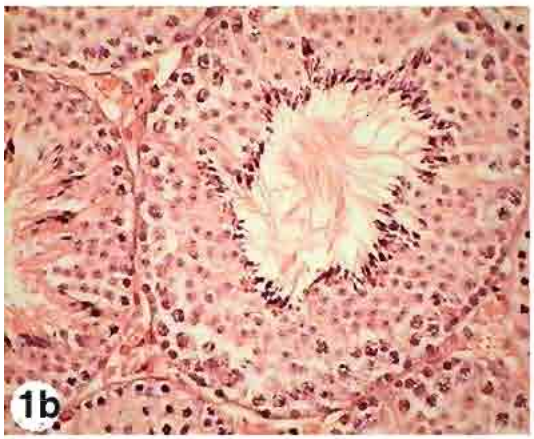

Fig. 1b: Large view in the previous section shows normal seminiferous tubule structure lined with different stages of germinal cells (X400)

Histopathological changes induced by the dose 1/10 LD_{50} (80 mg kg^{-1}):

Two weeks period: Mild focal testicular damage is observed in group (I) (Fig. 2a and b). That damage was characterized by 1) separation of spermatogenic cells from the germinal epithelial membrane 2) in decreasing number of the spermatogenic cells leading to hypospermatogenesis 3) degeneration of Sertoli cells (Fig. 2a). Other seminiferous tubules expressed vacuolated degeneration of their spermatogenic cellular. Only white empty vacuoles were present due to cell necrosis (Fig. 2b). Congested blood vessels were also observed between tubules (Fig. 2b).

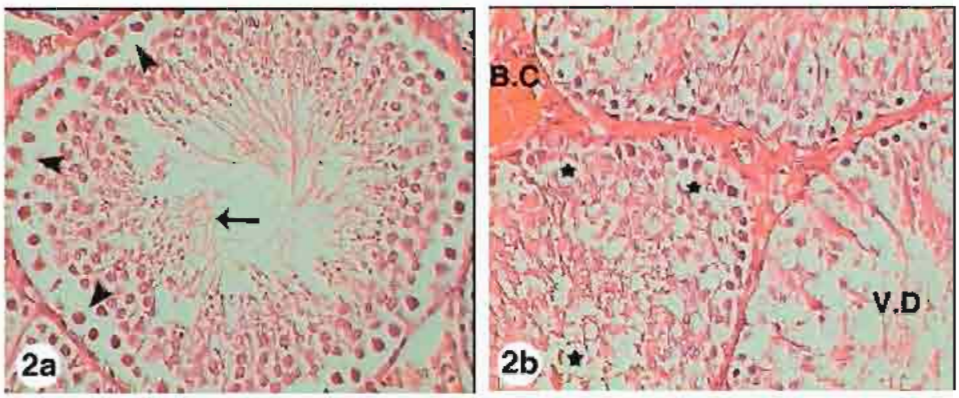

Fig. 2a: Section of rat testis treatid with 80 mg kg ^{1} for 2 weeks (group I), shows a separation of the spermatogenic cells (head arrows) away from the germinal epithelial layer associated with marked hypospermatogenesis. Also, notice the degeneration of some spermatozoa (arrow) (X400). (b): Section of rat testis from group I, shows blood congestion (B.C) in the intertubular space associated with massive vacuolated degeneration (V.D) of the spermatogenic cells, notice the vacuolated necrotic cells (stars) (X400)

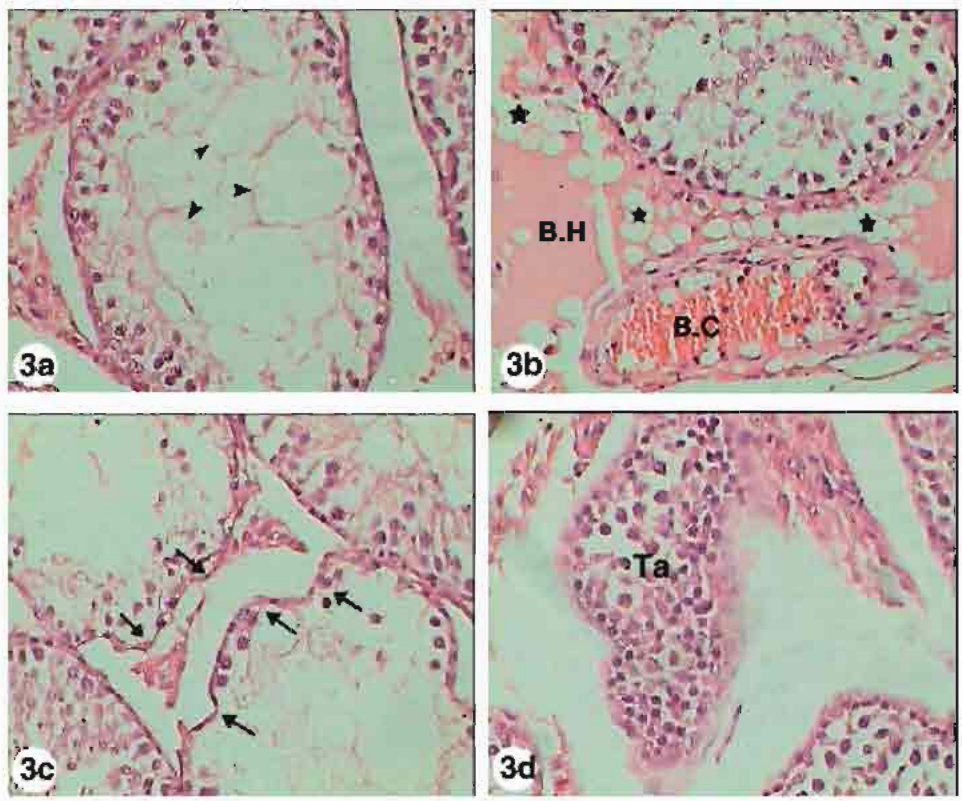

Fig. 3a: Section of rat testis treated with 80 mg kg^{-1} for 4 weeks (group II) shows severe massive vacuolated degeneration of the spermatogenic cells, nercotic cells appear as empty vacuoles (head arrows) (X400). (b): Section of rat testis from group (II), shows vacuolated degeneration of the interstitial cells (stars) associated with blood hemorrhage (B.H) and congestion (B.C) (X400). (c): Section of rat testis from group (II), shows marked dilatation and deformity of the tubules (arrows) associated with massive vacuolated degeneration of the spermatogenic cells and spermatozoa (X400) and (d): Section of rat testis from group (II), shows tubular atrophy (Ta) associated with degeneration of all the interstitial cells (X400)

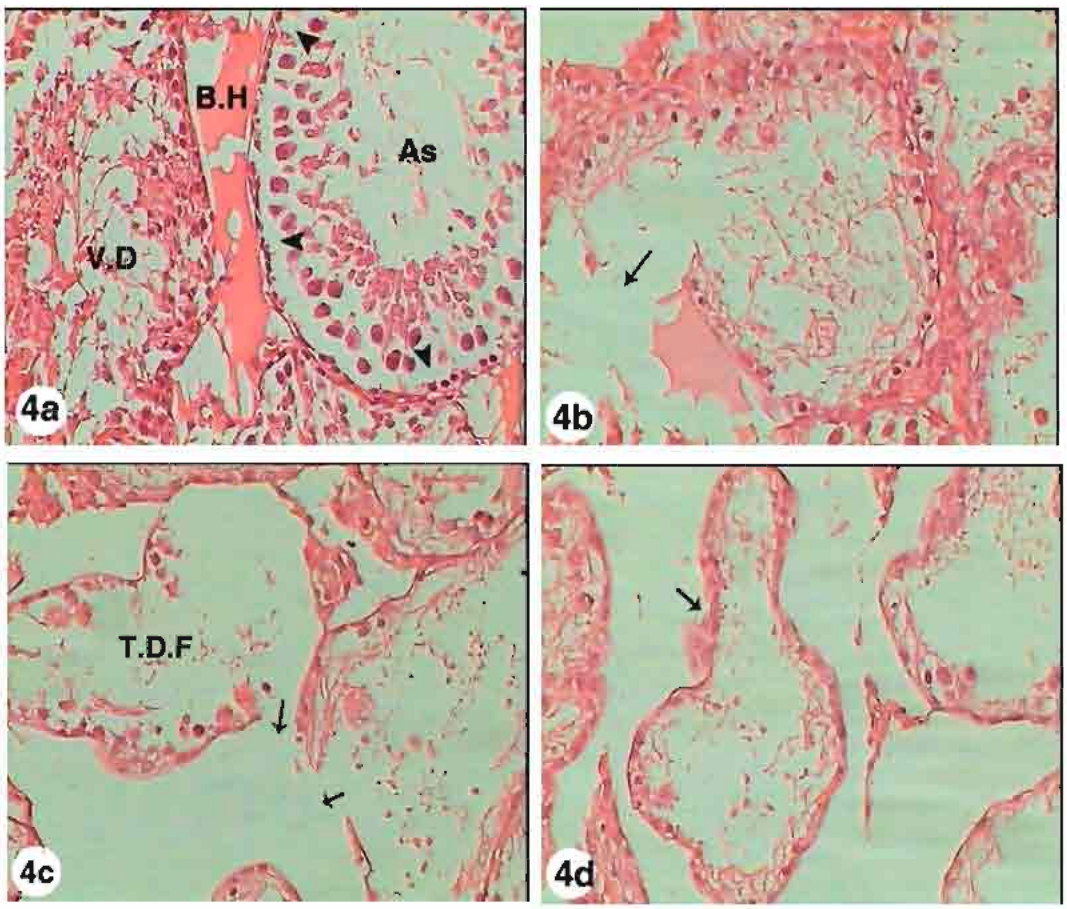

Fig. 4a: Section of rat testis treated with 200 mg kg^{-1} for 2 weeks (group III), shows the blood hemorrhage (B.H) with serious vacuolated degeneration (V.D) of the spermatogenic cells on right tubule, while left tubule shows a separation of spermatogenic cells away from the germinal layer (head arrows) associated with aspermatogenesis (As) (X400). (b) Section of rat testis from group (III), shows severe rupture of germinal epithelial layer (arrow) associated with degeneration of all cells inside the tubule (X400). (c): Section of rat testis from group (III), shows tubular deformity (T.D.F) associated with germinal layers rupture (arrows). Complete degeneration of all the spermatogenic cell, spermatozoa and Leydig cells are shown (X400). (d): Section of rat testis from group (III), shows tubular deformity (arrow) associated with massive degeneration of all the spermatogenic cells (X400)

Four weeks period: Increasing the treatment duration induced more severe testicular damages in group II rats (Fig. 3a-d).

These serious focal testicular damages appeared in various histopathological features can be summarized in; massive vacuolated degeneration of the spermatogenic cells associated with tubular dilatation and deformity (Fig. 3a), vacuolated degeneration in the intertubular spaces with blood hemorrhage and vascular congestion (Fig. 3b), abnormality in the tubular membranes with irregular malformed shape (Fig. 3c) and degeneration of Sertoli and Leydig cells with absence of spermatozoa inside the tubular lumens (aspermatogenesis) (Fig. 3c). Finally, remarkable seminiferous tubules atrophy (Fig. 3d).

Histopathological changes induced by the dose 1/4 LD_{50} (200 mg kg^{-1}):

Two weeks period: In comparison to the control group, the most sever testicular damages induced by Sumithion® NP 25/2.5 EC were found in group (III) (Fig. 4a-d). These damages include; vacuolated degeneration of the spermatogenic cells, presence of residual parts of the necrotic cells inside the degenerative tubule, absence of spermatozoa (aspermatogenesis) and blood hemorrhage in the intertubular space (Fig. 4a). Sertoli and Leydig cells also degenerated (Fig. 4a). Severe destruction of seminiferous tubule with multiple ruptured of the germinal epithelial layers (Fig. 4b). Massive degeneration of the spermatogenic cells (Fig. 4b). Severe tubular deformity

associated with tubular ruptured and complete spermatogenic cells degeneration (Fig. 4c and d).

DISCUSSION

Histopathological changes have been widely used as significant biological markers for environmental pollutants toxicity (Meyers and Hendricks, 1985; Hinton *et al*., 1992; Bhuiyan *et al*., 2001). The present study reported the testicular toxicity of Sumithion® NP 25/2.5 EC. The significant of this research comes from the lack of information about the toxicity of this upgraded formula. Various testicular damages were observed during this study include: severe vacuolated degeneration, massive spermatogenic necrosis, tubular dilatation and tubular deformity, rupture of the germinal epithelial layer surrounding the seminiferous tubules, tubular atrophy, aspermatogenesis, intertubular blood hemorrhage and vascular congestion, Leydig cells degeneration. The recorded symptoms were similar to those reported in previous studies on rat's testicular toxicity by Clos *et al*. (1994); Tamura *et al*. (2001), Turner *et al*. (2002), Hernandez *et al*. (2006). Manna *et al*. (2005) recorded edematous fluid accumulation between the tubules and focal formation within the tubules in rats treated with repeated dose of deltamethrin. No fluid accumulation was observed in the present study. The severity of the reported histopathological changes in this study was higher in rats treated with higher does and for long duration time. This dose and time-dependent also has been previously confirmed by Mishra *et al*. (1998) and Sakr and Hijji (2000).

The mechanisms that explain the toxic and degenerative cellular effects of insecticides generally and on the testicular cells in particular are not well known. Sheweita *et al*. (2004) suggested that Sumithion® induced remarkable inhibition of cytochrom P-450 activity in the liver decreasing drug metabolism and detoxification. This inhibitory action can also be considered as a possible cellular mechanism in which Sumithion® NP 25/2.5EC induce the observed testicular damages in this study. Where, testis and other body organs will be subjected to more accumulated toxins in the blood due to the detoxification disorder. Meanwhile, other studies reported that accumulation of the metabolites of Fenitrothion, an active component of Sumithion®, in blood may exert these testicular damages (Kohriyama, 1990). In another study, Fenitrothion considered as an anti-androgenic compound blocks the testosterone receptors on the target cells causing complete inhibition of testosterone action (Turner *et al*., 2002). As reported by Bustos-Obregon and Gonzalez-Hormazabale (2003) in their study that Sertoli cells necrosis is directly responsible for spermatogenesis disruption and depletion. Overall, all these mechanisms collectively or separately might be the logical explanations for the observed histopathological alterations in our study.

Furthermore, the genotoxicity effect is really a mater of concern. Bustos-Obregon and Gonzalez-Hormazabale (2003) mentioned the genotoxicity effect of malathion on mice newborns, this study also pointed that Sumithion® NP 25/2.5 EC could have an additional potential risk at the genes level especially, in mild testicular toxicity cases where abnormal spermatozoa carrying DNA alterations and mutations is produced and serious malformation in the offspring are expected. The presence of such teratogenic effects in newborn rats when mothers exposed during pregnancy to these insecticides was previously confirmed (Khera *et al*., 1978; Sreenivasula *et al*., 1983).

In conclusion, the Sumithion® NP 25/2.5 EC testicular toxicity has been clearly proved by the histopathological results. Therefore, we strongly recommend minimizing the usage of this insecticide and applying other harmless control strategies such as the biological control programs. For the meantime, more protective precautions should be applied among the workers during the performance of the dengue fever vector control in Jeddah, Saudi Arabia.

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