

## Impact of Endosulfan on Male Reproductive functions - a review

Srinivasa Jayachandra

Address for correspondence: Dr. Srinivasa Jayachandra, Associate Professor, Department of Physiology, Fathima Institute of Medical Sciences, Kadapa, Andhra Pradesh, India.

Email: jayachandra.srinivasa@gmail.com

### ABSTRACT

Endosulfan is an organochlorine insecticide and acaricide used to control a broad range of insect and arthropod pests on a wide variety of crops in many agrosystems. It can cause acute and chronic toxicity. Endosulfan is a neurotoxin, haematotoxin, genotoxin and nephrotoxin. Toxicity of this insecticide on reproductive organs is confirmed. So this article reviews experimental and epidemiological studies of reproductive toxicity of endosulfan in males. The Literature search was carried on PubMed for the years 1994 -2014. Twelve experimental studies and two human studies on Male Reproductive Toxicity induced by endosulfan were identified. Based on the findings, it suggest that endosulfan can affect the male reproductive system and also that these effects are likely to be larger if exposure occurs during the developmental phase. Further investigation of endosulfan as reproductive toxicant is warranted.

**Keywords:** Endosulfan, Organochlorine insecticide, Male reproductive system

### INTRODUCTION

Chemicals released into the environment may have either overt toxic effects or more subtle effects that influence long-term survival and reproduction. Numerous occupational hazards to male reproductive function are known but exposure prevalence is hardly sufficient to play a role for reduced sperm count in the general male population<sup>1-4</sup>. Endosulfan is a chlorinated hydrocarbon insecticide of the cyclodiene subgroup, which acts as a poison to a variety of insects. It is used on a wide variety of crops including tea, coffee, cotton, fruits and vegetables, as well as on rice, cereals, maize, or other grains. It has been classified as a moderately hazardous (class II) pesticide [World Health Organization (WHO) 2002]. Neurotoxicity is the major end point of concern in acute endosulfan exposure in human beings and experimental animals. The subacute and chronic toxicity studies of endosulfan suggest that the liver, kidneys, immune system, and testes are the main target organs<sup>1</sup>. Endosulfan is banned in more than 75 countries and in some countries like Argentina, Peru and Chile, the demand for alternative pesticide substances increased after the ban. Recently, India witnessed a furore over

banning endosulfan, with reports appearing on endosulfan poisoning, and the subsequent political pressure. Moreover, Endosulfan was previously banned in Kerala and has been recently banned in Bihar<sup>5</sup>.

Endosulfan not only acts as a hormone disruptor, but it also affects neurotransmitter systems of many species such as rats, catfish, and bullfrog tadpoles. For example, endosulfan suppressed testosterone and 17 $\beta$ -estradiol concentrations in neonatal rats and also increased thyroxine (T<sub>4</sub>) levels in catfish, and induced neurotoxic effects including increased excitability, trembling, and deficits in operant learning performance via the disruption of cholinergic, dopaminergic, and serotonergic neurotransmitter systems in freshwater fishes, pigeons and rats<sup>1</sup>. Oral LD50 (lethal dose sufficient to kill 50% of population) endosulfan in rats is 80 mg/kg. With reference to impact of endosulfan on reproductive system, there is no review summarizing the studies related to occupational or experimental exposure of endosulfan on male reproductive functions. So this present paper was aimed to critically review experimental and epidemiological studies of reproductive toxicity of endosulfan in males to summarize current knowledge and to suggest possible further research in this field.

### For Search criteria

The Literature search was carried on most recent medical data base (PubMed with MeSH) for the years 1994 - 2014 with the following key words like: endosulfan: toxicity: male: reproduction: epidemiological studies: animal studies: human. Besides the data base search, the reference lists of the selected articles were screened for other articles that could be useful. After collecting the abstracts, these references were searched manually for relevant papers in the English language literature. 12 experimental studies on Male Reproductive Toxicity induced by endosulfan were identified. But there were only two evident studies on humans.

**For the human data**, practically we had the idea to select the articles concerning spermatozoal, histopathological, and hormonal aspects, and birth rates in workers exposed to pesticides. But we found only one human study of endosulfan on male reproductive functions which did not fully met our selection criteria.

### Experimental data of Endosulfan on Male reproductive function

In recent years, there has been growing concern about toxicity of a number of chemicals, including pesticides, on the male reproductive system<sup>7-8</sup>. The affects of endosulfan on the Male reproductive system in experimental animals have been variable, depending on age at exposure, dose, duration of exposure, and study end points. **Table 1** summarizes the animal (rat and mice) studies of endosulfan on male reproductive functions. Routine gross and histopathologic examination of the reproductive organs of male mice that consumed doses of 2,6 and 18 ppm/day for 2 years revealed no toxic effects<sup>9</sup>. Later on, more detailed studies in adult rats exposed to 2.5, 5, and 10 mg/kg/day endosulfan for 5 days per week for 10 weeks showed reduced intratesticular spermatid counts, sperm abnormalities, and changes in the marker enzymes of testicular activities, such as lactate dehydrogenase, sorbitol dehydrogenase, g-glutamyl transpeptidase and glucose-6-phosphate dehydrogenase, providing further evidence of effects on spermatogenesis<sup>10-11</sup>.

Exposure of younger animals (3 weeks old) showed marked depletion of spermatid count as well as decreased daily sperm production at a dose of 2.5 mg/kg/day<sup>15</sup>, which was earlier seen at the same dose in adult rats by the same investigators<sup>10</sup>. More studies have shown that exposure of pregnant rats to endosulfan at 1 mg/kg/day from day 12 through parturition leads to decreased spermatogenesis in offspring<sup>12</sup>. Dalsenter et al. (1999) reported similar observations at 3 mg/kg/day but not at 1.5 mg/kg/day<sup>13</sup> and they attributed this to strain variation<sup>16</sup>.

There are reports of testicular toxicity of endosulfan manifested as decreased spermatogenesis and testicular hormone synthesis (steroidogenesis), as evidenced by a decrease in spermatid count in testes and in sperm count in the cauda epididymis and by changes in marker enzymes for testicular steroidogenesis in a dose of 1.0 mg/kg body weight for 30 days in pubertal rats<sup>14</sup>. These effects were seen at much lower dosages and shorter durations if exposures occurred during the prenatal or prepubertal periods<sup>11-13</sup>.

Rao M et al (2005) studied the effect of endosulfan on postnatal exposure of testis in the rat. Endosulfan significantly affected the testicular function enhancing the incidence of abnormal spermatozoa, decreasing the sperm count and sperm motility in a dose dependent manner. This study also showed that L-ascorbic acid prevents the adverse effects considerably in the rat<sup>17</sup>.

Yet in a recent study, Endosulfan (2 µg/ml) had no significant effect on progeny production and on the expression of certain genes associated with reproduction. However,

exposed males performed worse in sperm competition, both as 1(st) and 2(nd) male competitors<sup>18</sup>. Wang N et al in year 2014 showed that testosterone propionate (TP) significantly prevented the declines of concentration and motility rates in sperm, reduced the rate of sperm abnormalities in epididymis; and antagonized the decreases in spermatogenous cell and sperm numbers in testes induced by endosulfan (0.8 mg/kg/d endosulfan)<sup>19</sup>.

### Epidemiological studies of Endosulfan on Male reproductive system

The results of the human study also showed a significant decrease sexual maturity rating (SMR) scores and serum testosterone levels and higher levels of serum LH in study group (Exposed school children aged between 5-19 years), the observations of low testosterone levels in male children conform with the animal studies<sup>6</sup>. Lower SMR scores appear to reflect lower serum testosterone levels for age. In their study, they were not able to confirm disturbed spermatogenesis observed in animal studies.

In a cross-sectional study on humans an inverse associations between Organochlorine (OC) pesticide concentrations (endosulfan) and testosterone in men was observed which suggests that these OC compounds may have triggered anti-androgenic effects in men and estrogenic effects in women in this population<sup>20</sup>.

### Mechanism of Action

The question of the biological mechanisms of endosulfan damage has also to be clarified. It is also difficult to say if the deposition of endosulfan in the tubules of the testis is important or not if it is the localisation or distribution of endosulfan in spermatozoa that determines the possible adverse effect. A theory about the blood-testis barrier suggests that the germinal epithelium is divided into two compartments: the basal compartment, related to spermatogonia, and the adluminal compartment, mainly related to the more differentiated cells. The different substances (nutritional or toxic) reach the first compartment more easily and seem to be excluded from the second by occlusive junctions, located in the lateral surfaces of Sertoli cells and immediately above the spermatogonia layer. Therefore, the interaction between endosulfan and germinal cells is easier for spermatogonia than for differentiated cell<sup>21-23</sup>. This could lead to damage of spermatogonia.

Another mechanism shown by Wang N (2012) et al includes that endosulfan could directly damage sperm structures by oxidative stress, leading to a decrease in sperm quantity and quality. It also could indirectly cause a decline in reproductive function by damaging the structure of

mitochondria, resulting in energy metabolism dysfunction, which could be one of the mechanisms behind the reproductive toxicity induced by endosulfan<sup>24</sup>. Furthermore, this mechanism exposure induces the apoptosis of spermatogenic cells via mitochondria-dependent pathway mediated by oxidative stress resulting in the damage of mitochondrial structure and mitochondrial dysfunction<sup>25</sup>.

#### Future Research

As there are less studies reported on humans, future studies on human male reproductive effects of endosulfan should consider by using several methodological issues—such as the standardisation of analytical procedures with strict quality controls within and between laboratories—the adoption well defined terms of the outcome to be measured, the use of concurrent control subjects, the analysis of semen with respect to the time variables, the adoption of standardized criteria for information and motivation of the participants, and exposure assessment. It is also important to study large cohorts to evaluate possible effects at the endosulfan concentrations currently encountered in occupationally exposed populations and to design such studies to characterise the dose-response relation and evaluate change of values over time. This will also enable establishment of a no effect level and proper biological limit values. The question of the indicator of exposure should also be clarified, taking into account that possible indicators—such as seminal endosulfan—may be more useful in fertility studies than the indicators of recent exposure. It should be underlined that interactions with other occupational and non-occupational exposures must be considered and every possible confounding factor should be controlled for. In this respect, integrated studies should be promoted which will evaluate not only seminal and endocrine end points, but also other aspects such as the time to pregnancy.

#### CONCLUSION

Endosulfan was classified by the WHO in the category of technical products that are moderately hazardous. It has been shown that endosulfan has estrogenic property and male rats are more sensitive to the chronic effect of endosulfan than female rats. From these studies it suggest that endosulfan can affect the male reproductive system and also that these effects are likely to be greater if exposure occurs during the developmental phase. These findings warrant further investigation and reinforce the need to minimize exposure to pesticides in occupational settings.

#### Acknowledgements

I would like to thank Dr CRV Murthy, Senior Lecturer, Department of Pathology, IMU, Malaysia for rendering his advice .

#### REFERENCES

1. Park D, Hempleman SC, Propper CR. Endosulfan exposure disrupts pheromonal systems in the red-spotted newt: a mechanism for subtle effects of environmental chemicals. *Environ Health Perspect.* 2001; 109(7): 669-73.
2. Srinivasa J. The alternate approach to infertility. Are they safe? *Turk J Med Sci* 2005; 35: 195–6.
3. Srinivasa J, Maxim P, Urban J, D'Souza A. Effects of pesticides on male reproductive functions. *Iran J Med Sci* 2005; 30: 153–9.
4. Srinivasa J, Maxim P, Urban J D'Souza A. Decline in human sperm counts during the past 50 years? *Malta Med J* 2005; 17: 48–50.
5. Ezhil Vendan S. Endosulfan ban in India: for good or not?. *CURRENT SCIENCE* 2011; 101(11): 1398.
6. Saiyed H, Dewan A, Bhatnagar V et al. Effect of endosulfan on male reproductive development. *Environ Health Perspect.* 2003; 111(16): 1958-62.
7. Murray TJ, Lea RG, Abramovich D R et.al. Endocrine disrupting chemicals: effects on human male reproductive health. *Early Pregnancy.*2001; 5: 80-112.
8. Sharpe RM. Hormones and testis development and the possible adverse effects of environmental chemicals. *Toxicol Lett.*2001; 120: 221-232.
9. Hack R, Ebert E, Leist KH. Chronic toxicity and carcinogenicity studies with the insecticide endosulfan in rats and mice. *Food Chem Toxicol.* 1995; 33: 941-50.
10. Sinha N, Narayan R, Shanker R, Saxena DK. 1995. Endosulfan-induced biochemical changes in the testis of rats. *Vet Hum Toxicol.*1995; 37: 547-49.
11. Khan PK, Sinha SP. Ameliorating effect of vitamin C on murine sperm toxicity induced by three pesticides (endosulfan, phosphamidon and mancozeb). *Mutagenesis.*1996; 11: 33-36.
12. Sinha N, Narayan R, Saxena DK. Effect of endosulfan on the testis of growing rats. *Bull Environ Contam Toxicol.* 1997; 58: 79-86.
13. Dalsenter PR, Dallegrave E, Mello JRB, et al. Reproductive effects of endosulfan on male offspring of rats exposed during pregnancy and lactation. *Hum Exp Toxicol.*1999; 18: 583-589.
14. Chitra KC, Latchoumycandane C, Mathur PP. Chronic effect of endosulfan on the testicular functions of rat. *Asian J Androl* 1999; 1(4): 203-206.

15. Sinha N, Adhikari N, Saxena DK. Effect of endosulfan during fetal gonadal differentiation on spermatogenesis in rats. *Environ Toxicol Pharmacol.*2001; 10: 29-32.
16. Dalsenter PR, de Aranjó SL, Silva de Assis HC, et al. Pre- and postnatal exposure to endosulfan in Wistar rats. *Hum Exp Toxicol.*2003; 22: 171-175.
17. Rao M, Narayana K, Benjamin S, Baiyy KL. L-ascorbic acid ameliorates postnatal endosulfan induced testicular damage in rats. *Indian J Physiol Pharmacol.* 2005; 49(3): 331-336.
18. Misra S, Kumar A, Ratnasekhar Ch, Sharma V, Mudiam MK, Ravi Ram K. Exposure to endosulfan influences sperm competition in *Drosophila melanogaster*. *Sci Rep.*2014; 4: 7433.
19. Wang N, Xu Y, Zhou XQ, Wu YH, Li SL, Qiao X, Li YB, Sun ZW. Protective effects of testosterone propionate on reproductive toxicity caused by Endosulfan in male mice. *Environ Toxicol.* 2014 Jul 31. doi: 10.1002/tox.22029.
20. Freire C, Koifman RJ, Sarcinelli PN, Rosa AC, Clapauch R, Koifman S. Association between serum levels of organochlorine pesticides and sex hormones in adults living in a heavily contaminated area in Brazil. *Int J Hyg Environ Health.* 2014; 217(2-3): 370-8.
21. Naqvi SM, Vaishnavi C. Bioaccumulative potential and toxicity of endosulfan insecticide to non-target animals. *Comp Biochem Physiol C.* 1993; 105: 347-61.
22. Dixon RL, Nadolwey CH. Assessing risk of reproductive dysfunction associated with chemical exposure. In: Dixon RL ed. *Reproductive toxicology.* New York: Raven Press, 1985:329–39.
23. Srinivasa J, Urban JA D'Souza. Pre- and Postnatal Toxicity of Diazinon Induces Disruption of Spermatogenic Cell Line Evidenced by Increased Testicular Marker Enzymes Activities in Rat Offspring. *Journal of Environmental Pathology, Toxicology and Oncology* 2013; 32(1): 73-90.
24. Wang N, Qian HY, Zhou XQ, Li YB, Sun ZW. Mitochondrial energy metabolism dysfunction involved in reproductive toxicity of mice caused by endosulfan and protective effects of vitamin E. *Ecotoxicol Environ Saf.* 2012; 82: 96-103.
25. Xu Y, Wang N, Shi ZX, Li YB, Zhou XQ, Sun ZW. The mechanism study of endosulfan-induced spermatogenic cell apoptosis of mouse in vitro. *Toxicol Ind Health.* 2015 Jan 29. pii: 0748233714567525.

**Table 1: Studies on Histopathological and Spermatozoal end points in animals**

Reference	Strain	Dosage	Age at Start	Duration of Exposure	Mode of Dosage	Signs of Systemic intoxication	Observed effect (n)
<b>Rat</b>							
Sinha et al. 1995 <sup>10</sup>	NS	2.5, 5, 10 mg	Mature/Adult	70 Days	Oral	None	Decrease in sperm count in the Cauda epididymis and reduced intratesticular spermatid counts associated with elevation in the activities of specific testicular marker enzymes were seen in all the endosulfan dosed groups
Khan & Sinha. 1996 <sup>11</sup>	NS	2.5,5,10 mg	Mature	70 Days	Oral	None	Reduce in intratesticular spermatid counts, sperm abnormalities and elevated levels of testicular marker enzymes were seen.
Sinha et al. 1997 <sup>12</sup>	NS	2.5	Mature	21 Days	Oral	None	Reduction of Spermatid count as well as decreased daily sperm production

Dalsenter PR et al. 1999 <sup>13</sup>	Wistar	1.5,3	From day 15 of pregnancy to postnatal day 21 of lactation. Male offsprings were investigated at Puberty and adulthood	7 Days	Oral	None	The age of testis descent and preputial separation was not affected on the male offsprings. The daily sperm productivity was permanently decreased in the high dosage group (3mg/kg) after puberty and adulthood The daily sperm productivity was permanently decreased in the low dose group (1.5 mg/kg) only at puberty
K.C. Chitra et.al 1999 <sup>14</sup>	Wister	1.0 mg/kg body	Puberty	30 Days	Oral	None	There were a reduction in the body weight and the weights of testis and accessory sex organs, a decrease in the testicular lactate and pyruvate activities, and in the testicular DNA and RNA concentrations, whereas the testicular protein concentration was slightly increased; the specific activity of testicular steroidogenic enzyme, 3 $\beta$ -OH-steroid dehydrogenase and the ascorbic acid level were decreased.
Sinha et al. 2001 <sup>15</sup>	NS	1,2 mg/kg	From day 12 of gestation through parturition, the male offspring were investigated at 100 days of age	10 Days	Oral	None	Elevation of testicular marker enzymes. Reduction of spermatid count in testis and sperm count in Cauda epididymis,, reduction of weights of testis, epididymis and seminal vesicle in treated groups
Dalsenter PR et al. 2003 <sup>16</sup>	Wistar	0.5, 1.5	21 days prior to mating, during mating, pregnancy and lactation. The male offsprings were investigated at adulthood (140th day)	42 Days	Oral	None	Pre and Postnatal exposure to low doses of endosulfan (0.5 & 1.5 mg/kg) did not induce significant adverse effect on sex organ weights, daily sperm production, spermatid number, sperm transit, sperm morphology and testosterone level There were a reduction in the body weight and the weights of testis and accessory sex organs

Rao M et al 2005 <sup>17</sup>	Wister	3, 6, 9 and 12 mg/kg	From postna- tal day 7 to 60	53 Days	Oral	None	Endosulfan significantly af- fected the testicular func- tion enhancing the inci- dence of abnormal sperma- tozoa, decreasing the sperm count and sperm motility in a dose dependent manner.
<b>Mice</b>							
Hack et al 1995 <sup>9</sup>	NM RI	2, 6 and 18 ppm	Nature	2 years	Oral	None	Routine gross and histo- pathologic examination of the reproductive organs of male mice that consumed showed no toxic effects
Wang N et al 2014 <sup>19</sup>	NS	0.8 mg/kg/ day	Mature	4 weeks	Oral	None	Decrease in concentration and motility rates of sperm and increase in the rate of sperm abnormalities in epi- didymis

NS= not specified

**Please cite this article as:** Jayachandra S. Impact of Endosulfan on Male Reproductive functions - a review. Perspectives in medical research 2016;4:2:44-49.

**Sources of Support:** Nil, Conflict of interest: None declared