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Impact of Fungicides on Male Reproductive Health: A Review.

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ABSTRACT

Fungicides are pesticides that specifically inhibit or kill fungi underlying diseases important to man. Understanding mechanisms of fungicide action and toxicity is important because humans and domesticated animals encounter these pesticides through a wide variety of applications. The increasing knowledge of the reproductive toxicity of environmental chemicals has raised public concern. Fungicides disrupt structure and development of testis and epididymis along with decrease in male fertility, including a decline of sperm count, decreased sperm motility parameters and production of oxidative stress. The present article reviews the advances in the studies of male reproductive toxicity of fungicides in experimental animals, discusses the mechanism of male reproductive toxicity of pesticides and raises some problems concerning the evaluation of human reproductive hazards.

**Key words**: Fungicides; pesticides; reproductive toxicity; fertility; sperm count.

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INTRODUCTION

Pesticides enhance economic potential by controlling pests in agriculture sector but on the other hand they cause serious health implications for man and animal life [1]. The release of these chemicals into the environment creates a potential for unintended adverse health impacts to both humans and non-target wildlife including cases of severe acute and chronic human poisoning [2-7].

According to several studies, human semen quality and fecundity is declining day by day [8-13]. Volatile organic compounds (VOCs) [14], several heavy metals [15] or xenoestrogens like some polychlorinated biphenyls (PCBs) [16], phthalate esters (PEs) [17] and pesticides [18-21] may compromise reproductive male function.

Pesticides are one of the most fearful group substances, as far as biological communities and humans are concerned. These are mostly non-selective, widespread applied, possess toxic properties [22], and in some cases are very refractory. They include insecticides, acaricides, herbicides, fungicides and algaecides, indeed any chemical which is used to control an unwanted organism (except bacteria).
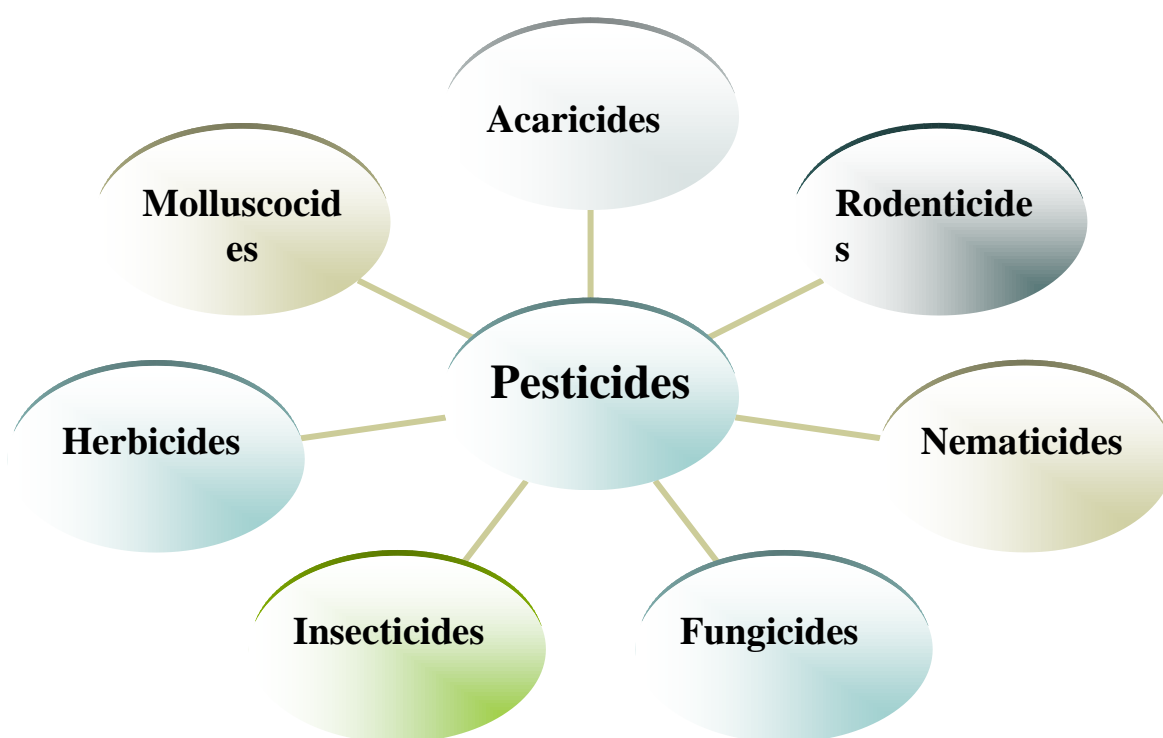
Different classes of Pesticides

Fungicides prevent and cure diseases that can have adverse effects on crop yield and quality. Fungicides prevent or mitigate damage caused by fungi to plants, including agricultural crops. They are developed from natural sources or are chemically synthesized [23]. The major fungicides are benzimidazoles, dithiocarbamates, phenylamides, chloronitriles, stobilurins, and triozoles. Triozoles and strobilurins are the most widely used fungicides due to their versatile utility and effectiveness against a number of fungal diseases. Fungicides are nearly always applied in the form of an aqueous solution or suspension, only to the produce. The fungicides market is growing at a Compounded Annual Growth Rate (CAGR) of 5.3% from 2014 to 2019. Geographically, the European region has the highest demand for fungicides in 2013. The Latin American region is the fastest-growing market for fungicides from 2014 to 2019.The market is estimated by segmenting it into micro markets, based on the share of each type, crop type, and geographical region. The market data is available from 2012 to 2019 with a forecasted CAGR from 2014 to 2019.
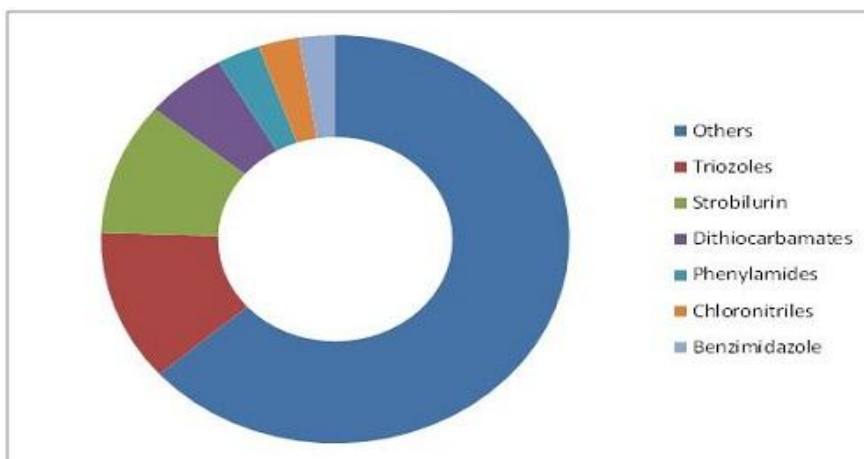
Fungicides Market Share (Revenue), by Type, 2013 ($Million)

(Source: MarketsandMarkets Analysis)

Increasing concern expressed about the declining sperm counts of humans in the last few decades. This is hypothesized to be as a result of the rising incidence of both testicular cancers and sub-fertility caused by exposure of the developing male embryo to certain potential environmental estrogenic agents that disrupt normal hormonal balance in the body [24-27]. Prolong exposure of pesticides affected the normal functioning of different organ system and produced many clinical effects [28-31].

Fungicides have toxic effects like reproductive, teratogenic, carcinogenic and mutagenic as well as on ecology, including non target plants and animals [32-35]. These chemicals could adversely affect male reproductive system by either disrupting the gonadal endocrine axis or the spermatogenesis process [36]. They also alter the reproductive function by altering sperm count and sperm shape, alter sexual behavior or increase infertility in animals and human beings [37-45].

Mode of action

Mode of action of the pesticides is important to understand so that we can use it to prevent development of pesticide resistance in the target pest(s). Using pesticides with the same modes of action contributes to this problem by killing the susceptible pests and leaving only those with resistance to the entire class of pesticides that work through similar mechanisms. Development of pest resistance can be delayed by rotating pest control chemicals that work through different modes of action [46, 47].

Fungicides may directly alter testicular function viz. germ cell depletion, or may alter Leydig cellular functions. The mechanism by which they induce Leydig cellular dysfunction remains obscure. One possible mechanism of fungicide toxicity is the disturbance of antioxidant balance by generation of reactive oxygen species (ROS). Tissue levels of lipid peroxide are proven an indicator of oxidative stress [48, 49]. Oxidative stress and lipid peroxidation (LPO) are established as a significant factor in the etiology of male infertility. Reactive oxygen species (ROS) formed in the body as a result of normal metabolic reactions, exposure to ionizing radiations, environmental pollution, alcohol toxicity, and by the influence of several xenobiotics are implicated in several diseases [50].

Among the ROS, hydroxyl radical is the most reactive species that could damage both macromolecules and small molecules. ROS damage DNA, proteins, carbohydrates and lipids and affect enzyme activity and the genetic machinery. However, biological systems possess a number of mechanisms to remove free radicals, the integrated antioxidant system which scavenges free radicals [51].

Reproductive toxicity of fungicides

Reproductive toxicity of some fungicides in male experimental models is discussed below:

Benomyl

Benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazole carbarnate] is a classic benzimidazole carbamate fungicide and nematocide that has been used for many years on a variety of food crops, ornamental plants, trees and grasses [52].
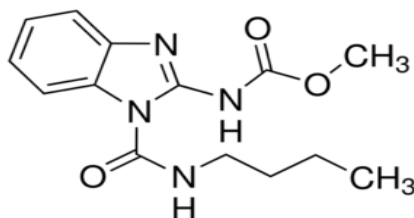

Benomyl

The U.S. Environmental Protection Agency regulated benomyl based partially on its reproductive toxicity, which was found to cause testicular atrophy and decreased fertility [53-54]. The germ cells were observed sloughing prematurely from the seminiferous epithelium and testicular sperm head counts were declining [55].

Its primary effects, at moderate to low dosages, are on the testis, where it causes sloughing of germ cells in a stage dependent manner. Sloughing is caused by the effects of the chemical on microtubules and intermediate filaments of the Sertoli cell. These effects spread to dividing germ cells and also lead to abnormal development of the head of elongating spermatids. At higher dosages, it causes occlusion of the efferent ducts, blocking passage of sperm from the rete testis to epididymis. The occlusion results in a rapid swelling of the testis and ultimately seminiferous tubular atrophy and infertility. Lesions in the male track that caused blockage may induce permanent testicular damage and a decrease in sperm production [56].

Benomyl at dose rates of 0, 100, 400 and 1000 mg/kg for 8 consecutive days was administered into crops of quails kept in different groups. A significant decrease in feed intake, body weight and relative weight of testes was observed in benomyl-administered quails in a dose related manner. The size of testes decreased in benomyl treated quails. Microscopically, seminiferous tubules of testes exhibited a decreased number of spermatocytes, necrotic spermatids and syncytial cell formation. The number of quails developing testicular alterations increased in a dose-related pattern [57]. Sakr and Okdah [58] studied the effect of benomyl fungicide on the testis of albino mice. Their results showed a degeneration of the spermatogenic cells, absence of sperm bundles and a significant reduction in the diameter of the seminiferous tubules and the height of the germinal epithelium.

Carbendazim

Carbendazim (Methyl *1H*-benzimidazol-2-ylcarbamate) is a member of the benzimidazole group with broad- spectrum nature. It is widely used as an agricultural and horticultural fungicide/pesticide around the world. Carbendazim was found to cause adverse effects in different mammalian systems including male reproduction such as sloughing of germ cells, inhibition of germ cell division, seminiferous tubular atrophy [59], and alterations in hormone concentrations [60]. Rehnberg et al. [61] found that carbendazim treatment resulted in severe seminiferous tubular atrophy and affected the functional capacity of Leydig cells to secrete testosterone.
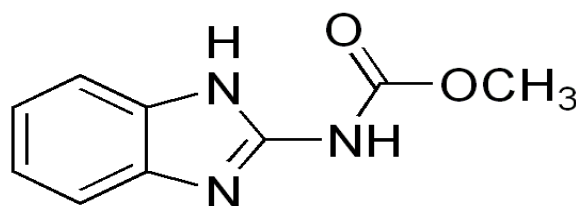

Carbendazim

Nakai et al. [62] reported that these alterations occur with carbendazim treatment from disruption of the Sertoli cell cytoskeleton, propagating loss of germ cell adhesion). The effect of carbendazim on male reproduction was studied by many investigators. Yu et al. [63] reported that treating rats with carbendazim showed atrophic testis and epididymides, marked histopathological abnormality of the testis, reduced weight of the right testis and epididymis, and decreased sperm motility and counts in the left cauda epididymis. Gawande et al. [64] reported that carbendazim disrupt the development of sperm and damage testicular development in rats. Long term exposure of male animals with carbendazim revealed the decreased testicular, epididymal weights, altered sperm morphology, testicular atrophy and thus infertility [64, 65].

Metwally et al. [66] reported that carbendazim induced significant increase in malondialdehyde and significant decrease in the activity of SOD and glutathione peroxidase in testis of rat. Also results of Hamdy et al. [67] showed that carbendazim administration caused testicular dysfunction with an increase of malondialdehyde and reduced of SOD and glutathione peroxidase activity. Chronic low dose treatment of carbendazim is capable of inducing reproductive and endocrine toxicity. Rajeswary et al. [50] found that in carbendazim – treated rats, Leydig cellular activities of antioxidant enzymes SOD, CAT, GPx, GR, GST, gamma-GT, G-6-PDH and non-enzymatic antioxidants such as GSH, vitamins E, C and A were significantly diminished, whereas LPO and ROS were markedly elevated.

Treating rats with carbendazim induced significant decrease in testis weights, diameters and germinal epithelial heights of the seminiferous tubules. Histological results revealed degeneration of seminiferous tubules and reduction of spermatogenic cells. Moreover, carbendazim caused elevation of testicular malondialdehyde (MDA), and reduced the activity of the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) [68].

Epoxiconazole

Epoxiconazole{(2RS,3SR)-1-[3-(2-chlorophenyl)-2,3-epoxy-2-(4-fluorophenyl)propyl]-1H-1,2,4-triazole} is a fungicide active ingredient from the class of azoles developed to protect crops. It is extensively used as fungicides in cereals, grapes, and other crops in the world [69].
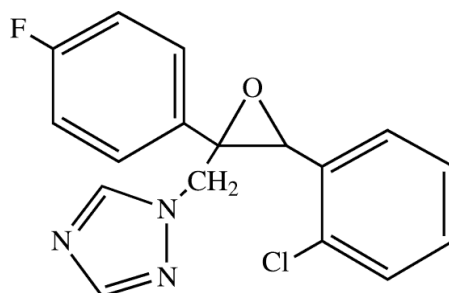

Epoxiconazole

Rats were given epoxiconazole (15 or 50 mg/kg bw/day) during pregnancy from gestational day (GD) 7 and continued during lactation until postnatal day (PND) 16. Epoxiconazole affected reproductive development in the offspring after exposure in utero. The high dose of epoxiconazole had marked fetotoxic effects, while the lower dose caused increased birth weights. The increased birth weights may be explained by a marked increase in testosterone levels in dams during gestation [70].

Hexaconazole

Hexaconazole (α-butyl-α-(2,4-dichlorophenyl)-1H-1,2,4-triazole-1-ethanol) (HC) is a systemic Triazole fungicide used in crop protection. It has outstanding activity against a wide variety of diseases and it's preventive, curative, systemic and antisporulant properties provide a useful addition to the range of commercial fungicides [71].
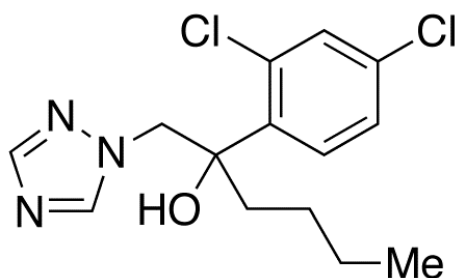
Hexaconazole

Hexaconazole treatment at a dose of 25 mg/kg body weight orally, for 60 days resulted in a significant decrease in the epididymal sperm count, motility and viability and increased incidence of sperm abnormalities indicates the impaired spermatogenic activity in the testis and maturation process of the sperms in the epididymis [72].

Male rats were administered hexaconazole per os daily at 0.0, 27.5, 55.0 and 110.0 my kg^{-1} body weight for 30 and 60 days. In medium and high doses of hexaconazole, treated groups there was a significant increase in seminiferous tubular number with concurrent decrease in tubular diameter which indicates the atrophy of testis in treated groups. The qualitative changes consisted of degenerative lesions in gonadal cells, detachment of seminiferous epithelium with impaired spermatogenesis and hyperplasia of Leydig cells. Hexaconazole has been reported to decrease serum testosterone levels in rats [73, 74].

Mancozeb

Mancozeb ([1,2-Ethaznediybis(carbamodithio)(2-)]manganese zinc salt), an inorganic-zinc dithiocarbamate, is a typical fungicide with a carbamate structure where sulphurs replace both oxygens in the amide functional group. It is chemically identified as ethylenebisdithiocarbamate (EBDC). Mancozeb has been shown to produce adverse effects in fertilization, damage to liver, kidney, central nervous system and chromosomes of bone marrow cells in mice [75]. Exposure to mancozeb affects the blood glucose, globulin levels and pathological changes in different organs of rats [76].
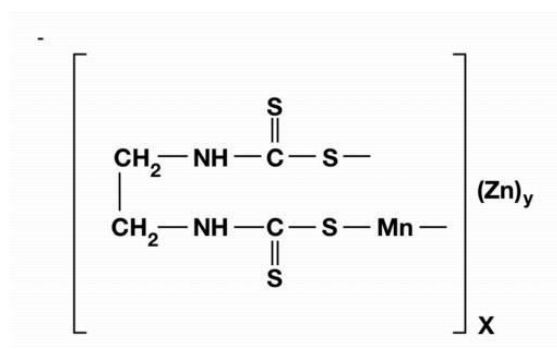
Mancozeb

Mancozeb was listed for male reproductive toxicity. The fungicide was administered to Wistar strain male albino rats orally at the dose level of 500 mg/kg b.wt./day for 30 days. Sex organ weight analysis, fertility, biochemical and enzymatic parameters and testosterone level were the criteria used to evaluate the toxicity of Mancozeb on treated rats. The weight of testis, epididymis, seminal vesicle, and ventral prostate decreased significantly. Mancozeb treatment also brought about marked reduction in epididymis and testicular sperm counts in exposed males. Pre- and post-fertility test showed 80% negative results after treatment. A significant reduction in the testicular glycogen and sialic acid was observed whereas a significant increase in the protein and cholesterol content of testis was noticed. Mancozeb also suppressed testosterone level significantly and exerts toxic effects on testis of rats [77].

Mancozeb when orally administered at doses of 500, 1,000 and 1,500 mg/kg body weight/day for 30, 90, 180 and 360 days showed signs of toxicity mortality pattern and loss in body weight in dose dependent manner. However, signs of intoxication and mortality pattern were more pronounced till the exposure of 90 days. A significant decrease in epididymis weight was associated with degeneration in epididymal tubules with loss of sperms. Sialic acid and protein content of testis and epididymis were also decreased in dose dependent manner. The study has thus indicated marked biochemical and pathological changes in gonads of male rats after chronic exposure to mancozeb [78].

In another study, mancozeb was orally administered at doses of 200, 400, 600 and 800 mg/kg/ day to male swiss albino mice for 30 days. Testes weight decreased significantly in all the mancozeb treated mice except 200 mg/kg/ day treated mice. Mice treated with 600 and 800 mg/kg/ day showed significant decrease in the number and diameter of spermatogenic cells and Leydig cells. Histological studies of the testis of the mice treated with high doses of mancozeb revealed spermatogenesis inhibition reflected by significant decrease in number of spermatogenic cells and sperms. Treatment with 800 mg/kg/ day mancozeb caused significant decrease in the levels of protein and glycogen and significant increase in the level of total lipids in the testis [79].

Administration of Mancozeb at the dose level of 300mg/kg body weight, orally for 60 days decreased the body weight, testicular weight and accessory sex organs weights compared to control rats. Mancozeb treatment also caused significant decrease in the specific activities of Acid Phosphatase and Alkaline Phosphatase in the testicular tissue of rats and increased the activities in the serum [80].

Sakr et al. [81] reported that mancozeb induced a significant decrease in the serum antioxidant superoxide dismutase and an increase in malondialdehyde which is lipid peroxidation marker in albino rats. Calviello et al. [82] confirmed the oxidative effect of mancozeb which caused post-apoptotic and necrotic alteration in cell membrane integrity. Therefore, it is suggested that testicular injury induced by mancozeb is mediated by depletion of antioxidants and elevation of lipid peroxidation.

Prochloraz

Prochloraz (PZ) (N-propyl-N-[2-(2,4,6-trichlorophenoxy)ethyl]imidazole-1-carboxamide) is an imidazole fungicide that displays multiple endocrine activities. It inhibits steroid synthesis via P450 modulation and acts as an androgen receptor (AR) antagonist [83, 84]. It is known to cause endocrine disruption through Effects on the hypothalamic-pituitary-gonadal (HPG) axis [85].
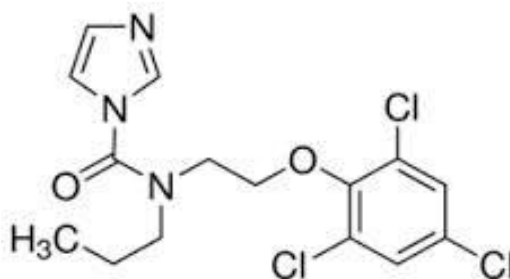
Prochloraz

In vivo, pregnant rats received PZ by gavage from gestational day 14 to 18 at doses of 31.25, 62.5, 125, and 250 mg/kg of body weight per day. PZ delayed delivery in a dose-dependent manner and resulted in pup mortalities at the two highest doses. In male offspring, anogenital distance and body weight were slightly reduced at 3 days of age. PZ causes reproductive malformations in androgen-dependent tissues of male offspring of exposed rats. PZ is the first imidazole fungicide shown to induce reproductive tract malformations [86].

Prochloraz (30 mg/kg/day) was dosed to pregnant Wistar dams from gestational day (GD) 7 until postnatal day (PND) 16. Some dams were taken for cesarean section at GD 21, and others were allowed to give birth. Results showed that prochloraz significantly reduced plasma and testicular testosterone levels in GD 21

male fetuses, whereas testicular progesterone was increased. Gestational length was increased by prochloraz. Chemical analysis of the rat breast milk showed that prochloraz was transferred to the milk. In males a significant increase of nipple retention was found, and the bulbourethral gland weight was decreased, whereas other reproductive organs were unaffected. Overall these results strongly indicate that prochloraz feminizes the male offspring after perinatal exposure, and that these effects are due to diminished fetal steroidogenesis [87].

Blystone et al., [88] reported that the fungicide prochloraz (PCZ) induced malformations in androgen-dependent tissues in male rats when administered during sex differentiation. They added that progesterone and 17alpha-hydroxyprogesterone production levels were increased significantly whereas testosterone levels were significantly decreased.

Finally, the fungicide prochloraz disrupts androgen action by inhibiting the conversion of progesterone to testosterone and by antagonizing the androgen receptor [83, 86, 87, 89]

Procymidone

The pesticide procymidone (3-(3,5-dichlorophenyl)-1,5-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione) is used as a fungicide and was shown to be present in fruit products prepared for human consumption [90, 91]. It is a typical anti-androgen, competitively inhibiting the binding of androgens to the human androgen receptor and thereby preventing androgen-induced gene expression [90].
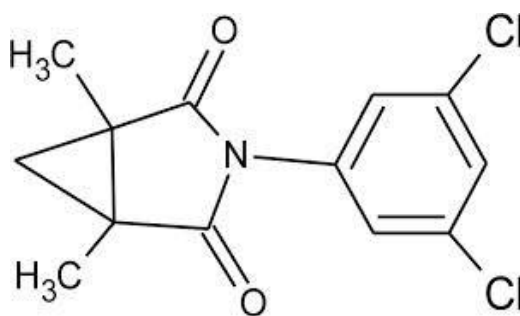

Procymidone

In a Hershberger assay using castrated immature testosterone treated male rats, vinclozolin and procymidone (0, 25, 50 and 100 mg/kg/day) alone or in combination inhibited testosterone induced growth of androgen dependent tissues in a dose-additive fashion [92].

A significant increase in both serum testosterone and LH was observed in the early stage at high dietary concentrations of procymidone in male Sprague-Dawley rats without any lesion in gonadal systems in histopathology [93].

The long-term dietary administration of procymidone (21·1 mg/animal per day) to rats exerts different effects on the pituitary– gonadal axis in vivo and on Leydig cell steroidogenesis ex vivo. As a result of disruption of hormonal feedback control due to its anti-androgenic action, procymidone activates this endocrine axis, thereby causing hypergonadotropic activation of testicular steroidogenesis [94].

Tebuconazole

Tebuconazole ((RS)-1-p-chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol) is a systemic fungicide used on crops such as barley, wheat, peanuts, and orchard fruits. Its mechanism of fungicidal activity is inhibition of a-lanosterol demethylase, which decreases ergosterol biosynthesis [95, 96].
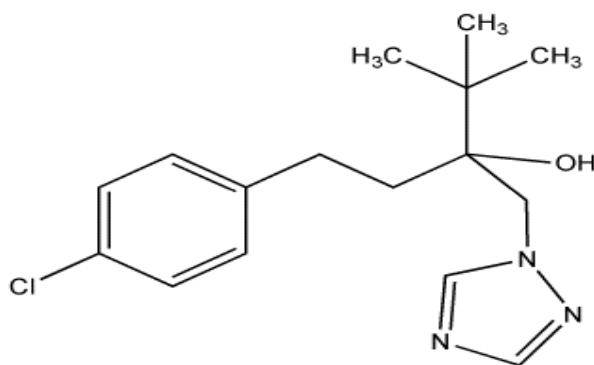
Tebuconazole

One developmental toxicity study in vivo showed that exposure of pregnant rats to at dose levels around the lowest observed effect level [97] resulted in disturbance of the reproductive system in the offspring. The males had reduced weight of epididymides [98].

Rats were dosed with tebuconazole (50 or 100 mg/kg bw/day) during pregnancy from gestational day (GD) 7 and continued during lactation until postnatal day (PND) 16. Tebuconazole affected reproductive development in the offspring after exposure *in utero*. Tebuconazole had a feminizing effect on male offspring as shown by increased nipple retention. This effect was likely caused by the reduced testosterone levels seen in male fetuses. Tebuconazole increased the testicular concentrations of progesterone and 17α-hydroxyprogesterone in male fetuses, indicating a direct impact on the steroid synthesis pathway in the Leydig cells [70].

CONCLUSION

Occupational exposure to fungicides has been associated with an increase in reproductive toxicity. Fungicides can cause the abnormalities in male reproductive system. In the recent years, more and more evidence indicates that fungicides can reduce sperm count and motility, cause deformity of the sperm head, increase the count of abnormal sperm, damage sperm DNA and induce its aneuploidy rate, as well as affect sex hormone levels and produce reproductive toxicity. The evidence of reproductive toxicity resulting from the intensive use of pesticides stresses the needs for educational programmes for farmers in order to reduce the use of chemicals in agriculture and to implement protection measure. Therefore, the sprayers should use proper protective devices while spraying the pesticides to reduce the pesticides exposure, and regular monitoring is essential to avoid further ill effects through pesticides exposure. Thus it is concluded that fungicides are highly toxic to reproductive function and alter the fertility of animals.

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