Chapter

Reproductive Toxicity of Insecticides

Mehtap Kara and Ezgi Öztaş

Abstract

Pesticides include several classes such as insecticides, herbicides, fungicides, and have widespread usage in agriculture. Different type of pesticides and their combinations affect dairy animals through their lifetime and the livestock industry. Under chronic exposure conditions, hormonal and cellular systems of animals, which play a role in reproduction, are affected dramatically. Some of the insecticides act as endocrine disruptors and impair reproductive hormone metabolic pathways via the hypothalamic-pituitary-gonadal (HPG) axis. Additionally, insecticides could have harmful effects on reproductive organs that may cause infertility. The aim of this chapter is review the toxic effects of insecticides on animal reproductive system focusing on molecular mechanisms.

Keywords: organophosphates, organochlorines, pyrethroids, male and female reproduction system, endocrine disruption

1. Introduction

Over decades, consumption of pesticides has slightly increased year by year; over 4 million tons of pesticides were used worldwide in 2017. Asia (52.8%) followed by USA (30.2%) and Europe (13.8%) were the highest amount of pesticide used obtain the most excessive amount of pesticide used continents. Insecticides, a subgroup of pesticides, constitute nearly 100ooo tons per year [1]; and, carbamates, chlorinated hydrocarbons, organophosphates and pyrethroids are most commonly used insecticides. Although these chemicals increase crop yields and provide economic benefits by reducing pest-borne diseases, their harmful effects on human health and environment still have the attention; and, considering these effects less toxic alternatives continue to be developed. Pesticide exposure alone or in mixture via environmental contamination could have important acute and chronic adverse effects on living organisms. Pesticide usage in agriculture is increasing every passing day and becoming a confusing issue due to the use of new chemical compounds that come into the market.

Chronic or delayed insecticide exposure exerts its toxicity on several systems such as nervous, immune, respiratory and reproductive. Reproductive toxicity of insecticides may affect either men or women; reduced fertility, spontaneous abortion, birth defects and developmental retardation have been linked to insecticide toxicity [2, 3]. For livestock industry, decreasing reproductive functions is rising problem; and, common problems can be listed as infertility, sub-fecundity, ovarian

cycle failures, decreased pregnancy rates, altered germ cell quality, reduced sperm motility as well as structural damage of testes or ovaries [4]. Furthermore, insecticides have important impacts on HPG axis and that qualifies them as endocrine disrupters. Endocrine-disrupting insecticides alter hormone synthesis or impair hormonal metabolic pathway by acting as hormonal receptor agonist or antagonists [5].

This chapter describes the reproductive system toxicity of commonly used insecticides based on each male and female; furthermore, it focuses on endocrine disruption.

2. The fundamentals of insecticides

Insecticides are described as "chemicals used to control insects by killing them or preventing them from engaging in undesirable or destructive behaviors" by United States Environmental Protection Agency (EPA) [6]. Insecticides provide substantial benefits during agriculture by controlling or preventing pests that could harm to crops and food causing nutritional and economic losses. Additionally, pests could damage wooden constructions and reduce the beauty and attractiveness of landscapes. Furthermore, insects could carry various diseases such as malaria [7, 8]. Insecticides play a crucial role in producing safe and quality food at affordable prices, home and gardening as well as controlling pest-borne diseases for public health.

Insecticides can be classified in varying ways such as their chemical structure, natural or synthetic origin, application requirement or mode of action. The chemical structure is particularly important for toxicology, since insecticides could exert similar toxicological effects due to their common chemical properties. Considering the chemical structure, insecticides could be divided into five groups: (i) organochlorines, (ii) organophosphates, (iii) carbamates, (iv) pyrethrins/pyrethroids and (v) nicotine/neonicotinoids.

Organochlorines have chlorinated hydrocarbon structures with high lipophilicity and persistence in the environment. Most exert their effects by disrupting sodium/potassium imbalance and others affect γ-aminobutyric acid (GABA) receptors; eventually, they cause hyperexcitation in the nervous system. Organophosphates, as another major class of insecticides, are phosphoric acid esters that cause acetylcholine accumulation at neuromuscular junctions by irreversible acetylcholinesterase (AChE) inhibition [6, 9, 10]. The other AChE inhibitor insecticide group carbamates are carbamic acid derivatives and show their effects reversibly, unlike organophosphates [11]. Pyrethrins are isolated from the flowers of *Chrysanthemum cinerariaefolium*; and, pyrethroids are synthetic analogs of pyrethrins. Both keep open the sodium channels, cause hyperexcitation in peripheral and central nervous systems and ultimately lead to paralysis. Pyrethrins and pyrethroids have lower environmental bioaccumulation and mammalian toxicity [12, 13]. Nicotine and neonicotinoids, as a newer class of insecticides widely used all over the world, have selectively neurotoxic effects on nicotinic acetylcholine receptor (nAChRs) [14].

High levels of exposure to several insecticides due to lack of legislations, regulations and education with ignorant behaviors may cause serious consequences on the human health and environment. Many studies showed that the misuse or overuse of insecticides lead to harmful effects in various systems such as nervous, respiratory and reproductive. The rest of this chapter gives details of the effects of selected insecticides on the female and male reproductive systems.

3. Toxicity of insecticides on reproductive system

Toxic effects of insecticides on male and female reproductive system and HPG axis are shown in **Figure 1**.

3.1 Hormonal system disruption

Insecticides could be characterized as "endocrine disrupters" due to their adverse effects on reproductive hormone pathway [15]. The half-life of endocrine-disrupting insecticides changes from hours to months in the environment. Insecticides may have toxic effects on synthesis, secretion, transport, binding to target receptors, intracellular transmission and elimination processes of reproductive hormones. In addition, insecticides alter hormone-receptor binding via chancing receptor affinity or agonist/antagonist effects, since, they mimic hormones. Thus, many of insecticides have estrogenic, androgenic or anti-estrogenic and anti-androgenic effects. Furthermore, insecticides could bind several types of receptors such as membrane, nuclear, orphan and neurotransmitter receptors. Endocrine-disrupting insecticides also exert toxic effects via inducing cell death in reproductive system cells playing a role from hormone synthesis to germ cell axis. Different studies confirmed that insecticides irreversibly affect hypothalamic-pituitary axis due to their mimicking properties of hormones or undesired inhibition or activation of metabolic pathways [15–17].

Pyrethroids, synthetic esters of pyrethrins, widely used worldwide are important endocrine-disrupting chemicals. In animal studies, contradictory results were obtained about the effects of pyrethroids on HPG axis. It has been shown that permethrin, fenvalerate and cypermethrin exposure decreased serum testosterone levels and increased follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels. Lower levels of testosterone constitute negative feedback in HPG axis resulting in increased levels of FSH and LH. However, in another study, delthamethrin exposure caused increased levels of testosterone, FSH and LH [18].

Elbetieha et al. [19] demonstrated that cypermethrin exposure decreased the serum testosterone, FSH and LH levels in male rats. On the other hand, different studies reported that pyrethroids have no effects on hypothalamus functions and

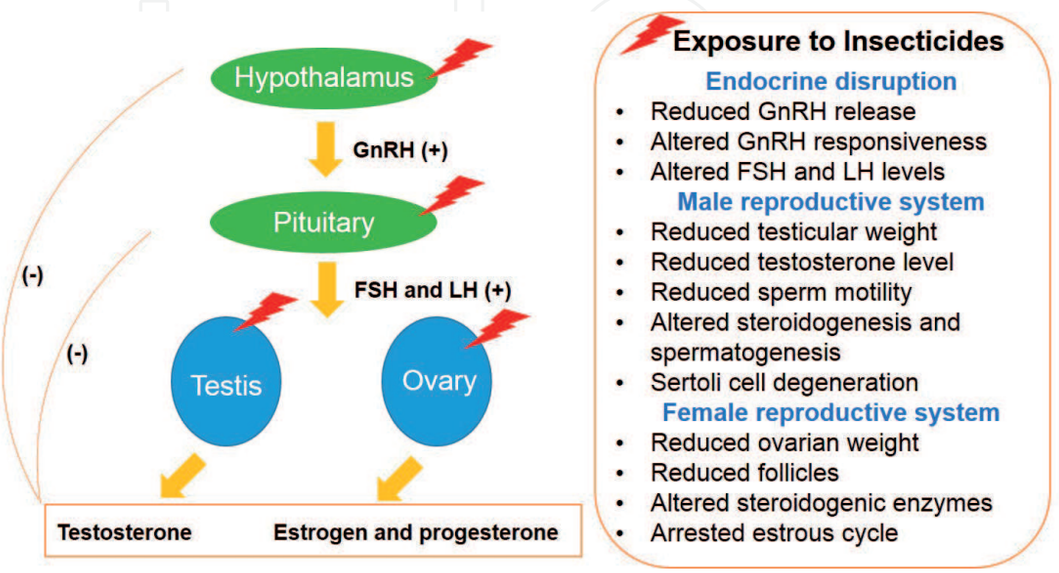

Figure 1.
Schematic representation of insecticides on male and female reproductive system via HPG axis (32).

gonadotropin releasing hormone (GnRH) levels. There are few studies demonstrating that gonadotropic cells' function and expression of LH and FSH coding genes have changed with pyrethroids exposure [20]. Dohlman et al. [21] reported that permethrin caused reduction in progesterone levels in beef heifers. Overall, it has been concluded that changes of hormone production due to exposure of pyrethroids depend on dose and duration of the exposure.

Soljjou et al. [22] demonstrated that thiacloprid, a neonicotinoid, and deltamethrin, a pyrethroid, exposure decreased GnRH, LH, FSH and testosterone serum levels in the hypothalamus in a dose-dependent manner; and, interfered with steroidogenesis in testicular tissues. Annabi and Dhouib [23] showed that imidacloprid, a neonicotinoid, affected the biochemical pathways of hypothalamic-pituitary-adrenal (HPA) axis via induction of oxidative stress.

Heptachlor, an organochlorine, may induce testosterone synthesis via 16-α and 16-β hydroxylases. Thiram, sodium N-methyldithiocarbamate and other dithiocarbamate insecticides inhibit the dopamine-β-hydroxylase activity and result in higher LH production, which prolonged proestrus stage. It has been reported that chlordimeform and amitraz interfere with the norepinephrine by binding to α2-andrenoreceptors and disrupt the GnRH release. Some other insecticides such as methoxychlor, DDT endosulfan, toxaphene, dieldrin, triadimefon, aldrin, methiocarb, chlordecone, malathion and sumithrin affect the HPA axis via binding receptors, mimicking the hormones and have shown estrogenic effects [24, 25]. In **Table 1** [3], selected insecticides and their endocrine-disrupting effects are listed.

3.2 Toxicity on male reproductive system

Dysfunction of male reproductive system represents a fundamental issue for livestock industry. Impairment of spermatogenesis, anti-androgenic effects, alterations in reproductive enzyme pathways, decreased sperm quality and motility

Pesticide	Hormone disruption effects
Aldicarb	17 beta-estradiol and progesterone inhibition
Aldrin	Androgen receptor binding
Bioallethrin	Estrogen-sensitive cells proliferation inhibition
Carbofuran	Estradiol and progesterone increase; testosterone decrease
Chlordane	Androgen receptor binding, estrogenic pathway inhibition
Chlorpyrifos-methyl	Androgen activity antagonism
Cypermethrin	Estrogenic effect increase
Deltamethrin	Estrogenic activity
Dieldrin	Androgen receptor binding, inducing estrogen receptor production in the cell
Endosulfan	Androgen receptor binding, inducing estrogen receptor production in the cell
Fenoxycarb	Testosterone metabolism disruption
Lindane	Luteal progesterone decrease, androgen, estrogen and progesterone receptor binding
Methoxychlor	Estrogenic effect, pregnane X cellular receptor binding
Parathion	Gonadotrophic hormone synthesis inhibition
Tetramethrin	Estrogen antagonism in females

Table 1.
Selected insecticides and their effects on endocrine system.

are key elements in insecticide-induced male infertility [5]. Insecticides exert their toxic outcomes on male reproductive system by directly affecting reproductive organs (testes, sertoli cells, leydig cells) and germ cells or impairing hormonal balance in secondary endocrine system [26].

It has been demonstrated in laboratory animals that carbamates have toxic effects on male reproductive system. Alterations of testicular weight and male accessory gland morphology, degeneration of seminiferous tubules and epididymis, spermatogenesis arrest, abnormalities of sperm motility and number, impairment of serum hormone and total proteins levels and estrogen receptor expressions were observed in several studies. However, detailed underlying molecular mechanisms of carbamate toxicity on male reproductive organs are still unclear [26–29].

Organophosphates could alter the spermatozoon chromatin structure, DNA, acrosome, motility and, have toxic effects on HPG axis. Reduced levels of testosterone were measured with organophosphate exposure due to inhibition of testosterone synthesis, which possibly occurs via reduction of steroidogenic enzymes' expression levels [5]. Organophosphates have dose-dependent detrimental effects on the morphology of testis and seminiferous tubules by causing atrophy and inducing germ cell death [26]. Additionally, organophosphate exposure is associated with decreased levels of sialic acid, glycogen alkaline phosphatase activity and increased levels of total protein, cholesterol and acid phosphatase. These imbalances could lead to induction of oxidative stress in male reproductive system by triggering inflammation, mitochondrial deficiency, DNA fragmentation and apoptosis [30, 31]. In wild birds such as parakeets and munias, organophosphate administration resulted in testicular dysfunctions [32]. Organophosphate insecticides induce DNA damage in sperm chromatin and that alters spermatogenesis pathway and causes infertility in male animals. Germ cell genetic material is protected by structure of male reproductive organs; however, it has been demonstrated that organophosphate insecticide disrupted the germ cell DNA integrity [32].

DDT, methoxychlor, chlordane, heptachlor, aldrin, dieldrin, endrin, toxaphene, mirex and lindane are commonly used organochlorines. Organochlorines have shown their toxic effects via inducing oxidative stress in the epididymis and decreasing antioxidant defense. It has been demonstrated that endosulfan caused abnormal sperm maturation in the epididymis. In addition, organochlorines disrupt male reproductive maturation in adolescence. TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), the most dangerous compound in world history, causes reduced fertility, delayed puberty and reproductive organ weights alterations, and also induces oxidative stress resulting in abnormal sperm morphology, motility and sperm number decrease [26].

Pyrethroids are generally accepted as safe; however, their weak toxic effects on reproductive system were demonstrated in limited studies. Pyrethroids have adverse effects such as reducing sperm count and motility, aneuploidy in germ cells, reducing sex hormone levels and reducing semen quality and sperm morphological abnormalities in human [33].

3.3 Toxicity on female reproductive system

Toxic effects of insecticides on female reproductive system were shown in different studies; and, it is concluded that insecticides disrupt female endocrine system and cause alterations in reproductive organs and germ cells [24]. Insecticides disrupt ovarian physiology. This is a two-way street as altering organ functions causes hormone secretion changes and this endocrine changes mostly affect the female reproductive system and result with dysfunctions via HPG axis.

Disrupted hormone synthesis, altered follicular maturation, disrupted ovarian cycle, pregnancy time prolong, stillbirth and infertility are linked to oxidative imbalance in the cells, and eventually lead to DNA damage, inflammation and apoptosis induction [34].

It has been speculated that pesticides have important role in slaughtering buffaloes reproductive defects. This could be associated with follicle membrane permeability features that permit xenobiotics entrance to the system. Higher concentrations of insecticides including DDT, eldrine, endosulphan and butachlor were detected in ovary than serum. This could make a way for follicular wall alterations and more insecticide entrance to the cellular system. In addition, insecticides could affect germ cells at primordial phases resulting in infertility in adult stage [5].

In wild birds such as female bobwhite quail (*Colinus virginianus*), parathion exposure caused reduction of egg production, impairment of follicular cycle, and reduction of LH and progesterone levels. Organophosphate (methyl parathion/*phosphamidon/quinalphos) administration of white-throated munia (Lonchura malabarica) caused inhibition of two important enzymes: Δ5-3β-hydroxysteroid dehydrogenase (3βHSD) and 17β-hydroxysteroid* dehydrogenase (17βHSD), playing key role in estrogen and progesterone production inhibition [32].

Endosulfan, an organochlorine, triggered apoptosis via oxidative stress induction in the follicle cells. Moreover, it induced the expressions of steroidogenic acute regulatory protein (StAR), CYP19A1a and aromatase, causing improper ovarian maturation. DDT exposure caused ovulation time alterations via inhibiting CYP450-side chain cleavage enzyme, progesterone receptor, estrogen sulfotransferase, cyclooxygenase-2 (COX-2) and epidermal growth factor (epiregulin) [34].

In female reproductive system, chlorpyrofos cause alterations in uterine weight and morphology via inducing surface epithelium and myometrium thickness [35]. In addition, chlorpyrofos could qualify as an ovotoxic and embryotoxic agent while mimicking estrogen and altering embryonic hatching, cell proliferation and apoptosis in zebrafish. Furthermore, chlorpyrifos reduces the levels of serum sex hormones such as LH, estrogen and progesterone [36, 37].

Toxic effects mechanisms of insecticides in female reproductive system are schematized in **Figure 2**.

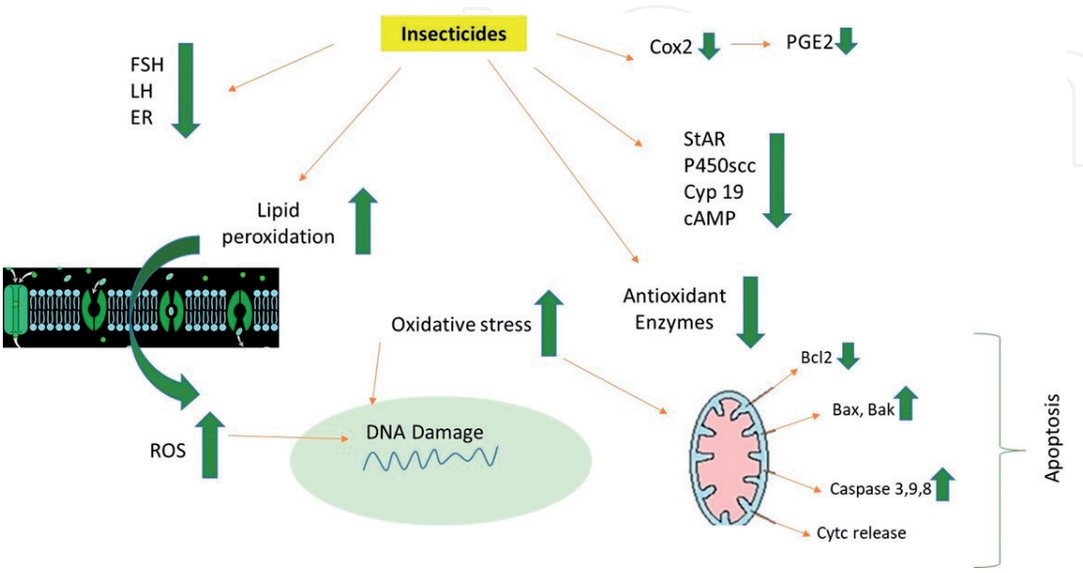

Figure 2.
Toxic effects mechanisms of insecticides in female reproductive system (FSH; follicle-stimulating hormone, LH; luteinizing hormone, ER; estrogen, ROS; reactive oxygen species, Cox2; Cyclooxygenase-2, StAr; Steroidogenic acute regulatory protein) [34].

4. Conclusions

Due to fact that insecticides may affect directly either male or female reproductive system as well as alter endocrine balance, eliminating or reducing the usage of insecticides is still a major concern. Considering literature data, many of insecticides caused infertility or developmental abnormalities by several pathways, and it is urgent to create awareness. Since, a huge amount of the data was obtained based on the rodent studies, further studies are needed to enlighten the toxic effects of insecticides on livestock. Furthermore, it would be possible to develop more effective and reduced-cost of stockbreeding by the clarification of possible molecular mechanisms of the insecticides.

Conflict of interest

The authors declare no conflict of interest.

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