Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
29 (5): 557-570, 2023
DOI: 10.9775/kvfd.2023.30161

Review

Thiamethoxam Toxicity: A Review in One-Health Perspective

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How to cite this article?
Qamar W, Shahid MU, Irfan M, Abbas RZ, Faraz A, Hussain R, Alvi MA: Thiamethoxam toxicity: A review in one-health perspective. *Kafkas Univ Vet Fak Derg,* 29 (5): 557-570, 2023.
DOI: 10.9775/kvfd.2023.30161

Article ID: KVFD-2023-29955
Received: 01.07.2023
Accepted: 06.09.2023
Published Online: 06.09.2023

ABSTRACT

Extensive and frequent use of pesticides has induced numerous abnormalities in target and non-target exposed organisms. Among different various pesticides, neonicotinoids are extensively employed in agro-production sectors. Thiamethoxam (TMX) plays an essential role in keeping the crop safe from insect attack, but on the other hand, it has been reported to induce adverse effects in both humans and animals. Previously, it was thought that neonicotinoids have low toxicity potential in mammals, but widespread use has made it evident that these pesticides have serious toxic effects on both invertebrates and vertebrates. Extensive applications of pesticides also pose serious eco-toxicological threats to aquatic and terrestrial organisms in the ecosystem. This review describes the chemistry, pharmacodynamics, and toxic effects of various TMX on living organisms. Moreover, this review summarizes the excretion/deposition of TMX in different tissues along with potentially adverse effects on production potential, immunity, blood parameters, and male/female reproductive systems. Though the pros of TMX surpass the cons, its reported intrinsic toxicity stresses the need to develop new pesticides that have high potency with little harm to humans and animals. Hence, there is a need for hours to address knowledge gaps related to TMX and design effective rational usage of TMX strategies to keep the ecosystem safe from the potentially harmful effects of TMX.

Keywords: Thiamethoxam, Pesticides, Neonicotinoids, Toxicity, Residues, Human, Animals

Introduction

The massive rise in the human population and livestock farming with food and fodder shortages has ultimately highlighted the need for improved agriculture production. Insecticides/pesticides/herbicides are extensively used in agriculture, public health management, and veterinary practice to control insects/pests, and parasites and to enhance crop yield [1-3]. Globally, there has been a massive rise in the usage of insecticides and pesticides to protect crops and preservation of agricultural products [4,5]. However, irrational and indiscriminate use leads to various harmful effects on the exposed organisms including humans and animals [6-8]. Nutrient swings, climate change, habitat loss, acidification, and biological invasions have seriously threatened public health. Even the labeled use of different synthetic chemicals in agriculture leads to aquatic ecosystem contamination and thus becomes dangerous to aquatic life [9]. These synthetic compounds induce oxidative stress that may lead to decreased reproductive efficiency, oxidative disorder, poor erythropoiesis, and genotoxic effects on their consumers [10-13]. Extensive usage of pesticides increases the chances of serious risk to the environment, animals, human beings, and birds living in the same ecology [7,14]. Pesticides are commonly used in agriculture systems to increase crop yield and eradicate a variety of parasites from livestock populations [15]. Previously, it was thought that neonicotinoids have low toxicity potential in mammals, but widespread use has made it evident that these pesticides have serious toxic effects on both invertebrates and vertebrates [16,17]. The European Union has banned neonicotinoid pesticides, based on the threat they pose to pollinators [18]. Extensive applications of pesticides also pose serious

eco-toxicological threats to aquatic and terrestrial organisms in the ecosystem [10,19,20]. Pesticides continue to bio-accumulate in aquatic organisms, depending on their Physico-chemical properties, and can therefore interact with tissues and cells to induce the process of biotransformation [21-23]. Over the last two decades, the extensive use of these synthetic chemicals has increased dramatically and tends to accumulate these chemicals in groundwater, agricultural products, soils, and surface water [24]. Other parameters are potentially affected, such as growth, reproductive capacity, and even survival of aquatic organisms [25-27]. Among different pollutants that induce carcinogenic and mutagenic effects, pesticides are the most hazardous compounds responsible for numerous tissue changes in target and non-target organisms. The objective of this review is to provide insights about thiamethoxam in the One-Health interface.

Impacts of Pesticides on Terrestrial and Aquatic Life

Terrestrial Life

Pesticides lead to the induction of harmful effects on domesticated animals, plants, insects, and human beings. The severity of the toxic effects of pesticides depends upon the dose and the type of organism exposed [28]. Death of the target and non-target exposed species is the most obvious sign of toxicity that leads to a decline in number. The frequency of the mortalities is directly proportional to the route, and the dose of pesticide exposure. In addition, a fall in the number of members of one species in a community leads to a decrease in the population size of the other species with which the target species interact [29].

The forms of life are more often exposed to more than one pesticide at a time, which has either additive or synergistic toxic effects [30,31]. Pesticide toxicity also leads to weight loss in association to decrease food and water intake [32], memory loss, loss of aggressive behavior, and lack of mobility desire, making the affected animal prone to predation by various predators [33].

Malfunctioning of endocrine glands due to exposure to sublethal levels of pesticides can lead to impaired growth and development, and reproductive failures [34]. The thyroid hormone is crucial for metamorphosis and development. Studies have shown that exposure of birds to DDT, OP, carbamates, and pyrethroids [35,36], goldfinches to linuron, and amphibians and fishes to endosulfan [37] leads to developmental abnormalities as a result of decreased thyroid hormone levels. In many studies, the lower hatching success and impaired reproduction in bald eagles and alligators in Florida have been reported due to heavy contamination of agricultural lands with bifocal and DDT [38]. Moreover, in that area, the testicles of male alligators were found to be poorly organized and the morphological characteristics of female ovaries were abnormal [39].

Aquatic Life

Fishes are one of the important sources of proteins for human beings and occupy a vital place on the food web [40]. owever, contamination of the river and oceanic water due to different environmental pollutants, particularly insecticides, has induced adverse impacts on aquatic life by affecting growth, survival, and reproduction. Unfortunately, the extensive use of pesticides to improve crop production has led to deleterious effects on aquatic life [41,42]. Contamination of water bodies with pesticides is not only a serious threat to the food supply but also has a negative effect on the health of aquatic life [43]. Chronic toxicities with pesticides may lead to the extinction of endangered species due to loss of natural defense, induction of blindness, hyperexcitability, weakness, and sterility [44-46]. In addition, prolonged exposure of organophosphates to aquatic life has resulted to induce the abnormal swimming of fish and peroxidative damage to gills and brains [47,48]. Moreover, chromosomal aberrations and cellular hyperproliferation are the main cellular events that may appear as a result of exposure to pesticides [49-51]. The reduced protein level in muscle [52,53], poor feed utilization, and poor self-defense from predation are the other consequences that may appear due to pesticide exposure in various aquatic organisms [44,54]. According to [53], pesticides have been reported to have negative impacts on the reproductive potential of fish, causing abnormal sexual development, male feminization, abnormal sex ratio, and unusual mating habits. Some studies also indicate that long-term exposure to lufenuron causes DNA damage, increases oxidative stress, decreases the profile of enzymatic antioxidants, and causes histological lesions in the visceral organs of Nile tilapia [43]. A brief overview of the toxic effects of different pesticides on various forms of life is highlighted in *Table 1*, *Table 2*, *Table 3*, *Table 4*.

Synthesis of Neonicotinoids

The discovery of neonicotinoids was a breakthrough in pesticide research. Neonicotinoid pesticides are one of the fastest-growing classes of insecticides [94]. With no cross-resistance to the other conventionally used insecticides, this class started to replace other insecticides like organophosphates, pyrethroids, and carbamates [95].

During the 1980s, neonicotinoid insecticides were synthesized to regulate the pest population dynamics [96]. This class of chemicals is one of the most important classes of acetylcholine receptor inhibitors accounting for up to 25% of the commercial insecticides available in the market [97]. According to the classification by Hussain et al.[98], these insecticides can be grouped into three categories based on their chemical nature and generations. *Fig. 1* shows the representative members of each class along with the year of commercialization into

Table 1. Effects of different pesticides on Micro-Organisms and Soil Metabolism

Community	Pesticide	Adverse Effects	Reference
Microorganisms and Soil Metabolism	Benomyl and Captan	Reduces soil basal respiration	[55,56]
	2,4-D picloram and Glyphosate	Reduces soil basal respiration	[57, 58]
	Atrazine and Metolachlor	Alters the soil community structure Reduces methanotrophic bacteria	[59,60]
	Carbofuran insecticide	Increases nitrogen fixation by Azospirillum in rice paddies	[61]
	Glyphosate	Suppresses soil bacteria; Increases plants' susceptibility to pathogens; Inhibitory effect of phosphatase in the presence of glyphosate	[62,63]

Table 2. Effects of different pesticides on arthropods community

Community	Pesticide	Adverse effects	Reference
Arthropods	Aldrin, Dieldrin, Heptachlor, Chlordane and DDT	Reduced Springtails species *(Collembola)*, mites, and Myriapoda	[64-66]
	DDT, Endosulfan, Aldrin, Chordane and Heptachlor, Carbamate, Aldicarb, Carbofuran	Negative effect on predatory mites' population	[66-68]
	Gaseous pesticides	Destroys mite population; Decreases soil biodiversity	[69,70]
	DDT, Aldrin, Carbamates, Organophosphates	Detrimental to the centipede population	[71,72]
	Lindane, Carbamates and Organophosphates	Destructive to springtail population	[73,74]
	Simazine	Destroys Diptera larvae significantly; accumulation of dead organic matter	[75]
	Ivermectin	Reduction in the emergence of *Liatongus minutes* and flies from cowpats	[31,76]

the market. These chemicals irreversibly bind to nicotinic acetylcholine receptors very tightly [99,100], resulting in blockage of acetylcholine binding to the receptors leading to spastic paralysis (overstimulation of the cells) and death of cells and/or of individuals [101,102].

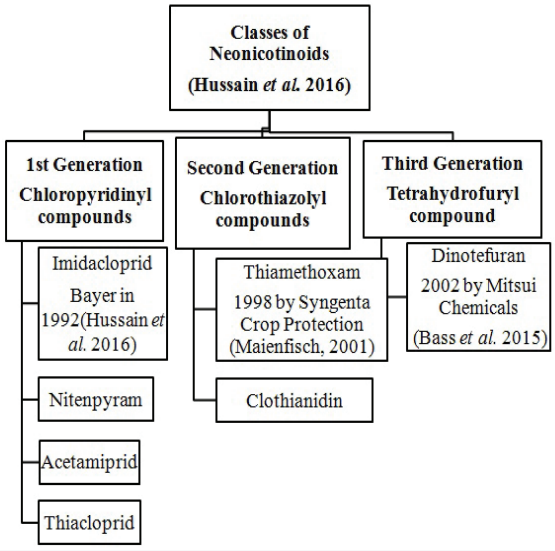

Fig 1. Classification of neonicotinoids (Created by Biorender)

Thiamethoxam

Thiamethoxam [3-(2-chloro-1, 3-thiazol-5-methyl) 25-methyl-4-nitroimino-perhydro-1,3,5-oxadiazine)] is a nitro-substituted second-generation neonicotinoid [103]. The empirical formula of TMX is $C_8H_{10}C_lN_5O_3S$ having a molecular weight of 291.7 g/mol. TMX is a crystalline powder with a slight cream color. The physicochemical properties of TMX are mentioned in *Table 5* [104].

Discovery and Synthesis

Thiamethoxam was first discovered and developed by Ciba Crop Protection in 1991 [105-107]. Since 1998, TMX has been marketed with different trade names like Actara® and Cruiser®. N-methyl-nitroguanidine treated with formaldehyde and formic acid leads to laboratory synthesis of thiamethoxam [108]. Alkylation with 2-chlorothiazol-5-ylmethyl chloride in N, N-dimethyl-formamide, and potassium carbonate as a base yield the active ingredient in good amounts [106].

Table 3. *Effects of different pesticides on other vertebrates community*

Community	Pesticide	Adverse Effects	Reference
Other Vertebrates	Lindane, Carbamate, and Organophosphates	Reduce saprophytic and beneficial nematodes	[59,75]
	Captan	Reduces fungal-feeding nematodes' population	[77]
	Thiobencarb and Simetryne	Increase plant-root parasites	[78]
	Chlormethoxyfen	Decimate predaceous mononchids	[78]
	Copper fungicides and Arsenates	Kill earthworms; increase avoidance behavior	[79,80]
	Imidacloprid	Adversely affect the burrowing activity of earthworm	[81]
	Chlordane, Heptachlor, Phorate, and Carbofuran	Toxic to all worms	[82]
	All fumigants	Kill earthworms	[75]
	Carbendazim	Harmful to earthworm	[83]
	DNOC, Chlorpropham, Atrazine, Simazine, Monuron	Rreduce earthworm populations	[83,84]

Table 4. *Effects of different pesticides on vertebrates community*

Community	Pesticide	Adverse Effects	Reference
Vertebrates	Organochlorines (OC)	Accumulate in tissues of all organisms and progressively released	[85]
	Consumption of invertebrates contaminated with OC insecticides	Causes the death of bats and other insectivorous birds	[86]
	OC insecticides, DDT	Reproductive impairment in birds and fish-eating birds	[87]
	Herbicides and Insecticides	Breeding failure; Chick starvation; Poor survival	[88,89]
	Routinely used pesticides	Adversely affecting the bird population, Pronounced teratogenic and histopathological effects in the liver were also observed in birds at higher dosages	[3]
	Combined effects of insecticides and herbicides	Negatively affect feeding patterns and growth of tadpoles; Sublethal effects due to promotion of trematode infection development	[90,91]
	Continuous use of herbicides in rice paddies	Reduces population of diving ducks	[92]
	Glyphosate sprays	Reduction in insectivorous and granivorous bird population	[93]

Mechanism of Action

TMX shows its action by binding to post-synaptic nicotinic acetylcholine receptors present inside the central nervous system and at neuromuscular junctions [109,110]. As a result of the irreversible binding of TMX to its receptors, nerve impulses are produced initially, followed by the collapse of the neurons to generate any further impulses [111]. Constant activation of these receptors appears due to the failure of acetylcholinesterases to break down TMX [112]. The potential of TMX binding to its receptors in insects is quite stronger as compared to the affinity for mammalian receptors [113,114].

Absorption

TMX is rapidly absorbed through the oral route and almost 90% is excreted in the urine of rats. TMX has low toxicity in experimental studies conducted on rats through oral, inhalation, or cutaneous routes. It is non-irritant to the eyes and skin with no sensitizing abilities [104,106]. TMX has very low dermal absorption both in humans and rats. After 6 h of exposure time to TMX, the systemic

*Table 5. Physicochemical properties of TMX**		
Color	**Slight Cream**	**Reference**
Physical state	Crystalline powder	[104]
Melting point	139.1	
Vapor pressure (at 25°C)	$6.6*10^{-9}$	
Solubility in water	4.1	
Solubility in methanol	10.2	
Dissociation constant pKa (at 20°C)	No dissociation in range pH 2–12	

absorption ranged between 0.4 and 2.7%. After 48 h of initial exposure, the systemic absorption was slightly increased ranging between 0.8 and 2.9% [107].

Metabolism

Studies have shown that 85-95% of TMX is excreted in the urine while only 2.5-6% is excreted in feces as the parent compound after 24 h of administration. Hydrolysis and N-demethylation are the metabolic pathways by which TMX is excreted out of the body. Hydrazine is produced as a result of hydrogenation of the N-nitro group which later on conjugates with acetic acid or 2-oxo-propionic acid resulting in the production of several metabolites. *Fig. 2* highlights the mechanism of action of TMX.

Animal and Human Toxicity

Due to heavy intoxication of TMX, clinical signs like hypersalivation, diarrhea, vomiting, muscular weakness, and ataxia are most seen in mammals [115]. Further, uncoordinated gait, reduced physical activity and tremors have also been reported due to TMX toxicity [116]. Signs of toxicity disappear soon but a very high toxic dose may lead to death within 24 h [117,118].

TMX is capable of producing Phenobarbital-like induction of enzymes in mouse liver. Repeated doses of TMX have led to affecting the liver and kidneys of rats and induced the production of liver tumors [104,119]. A yearlong study on dogs given TMX showed that TMX causes changes in blood biochemical parameters, alterations in testicular weights, and tubular atrophy at a toxic dose of 1500 ppm.

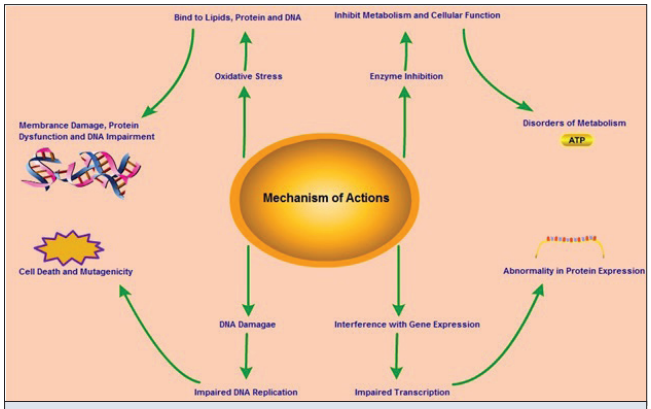

Fig 2. Mechanisms of actions of TMX toxicity (Created by Biorender)

According to previous investigations, under laboratory conditions, TMX degrades at slow rates and gets photolyzed in water very quickly. Moreover, TMX has low toxicity to birds and is non-toxic to fishes and mollusks through ingestion. Earthworms and green algae are insensitive to TMX [104]. TMX has a slight to moderate potential to cause harmful effects in beneficial insects and has proven to be safe for mites [107]. Moreover, TMX has no bioaccumulation abilities and degrades at a moderate to fast pace in the field [106].

The data related to TMX toxicity in humans is scarce. Fever, disorientation, dizziness, and vomiting are the most commonly seen side effects [103]. Tachycardia, hyperpnea, and profuse sweating have also been reported in cases of TMX toxicity [120]. Inhalation toxicity cases may lead to agitation, breathlessness, and disorientation in the affected human being [121]. Various toxicological properties and environmental toxicity profiles are mentioned in *Table 6* and *Table 7*.

Genotoxic Effect of TMX

After exposure to insects and birds, insecticides interact with DNA [122]. There are three possible ways of chemical-DNA interaction [123,124]. There may be electrostatic interactions between the chemical moieties of insecticide and charged phosphate backbone of DNA. Moreover, intercalative binding of chemicals within the stacked base pairs of DNA leads to disruption of conformation. Finally, groove binding interactions cause a significant change in

Table 6. Toxicological properties of TMX	
Study Type	**Comment**
Acute oral (LD50 mg a.i. /kg bw	4366
Acute dermal (24 h) (LD50 mg a.i. /kg bw)	>2000
Acute inhalation (4 h, aerosol) (LC_{50} mg a.i. /m^3 air)	3720
Skin irritation (4 h)	No irritation
Eye irritation	No irritation
Skin sensation	No skin sensation
a.i. active ingredient	

Table 7. Environmental profile of TMX	
Study Type	**Level**
Mallard duck (LC_{50} mg a.i. /kg diet)	>5200
Bobwhite quail (LD_{50} mg a.i. /kg bw)	1552
Rainbow trout (LC_{50} mg a.i. /l)	>125 (96 h)
Bluegill sunfish (LC_{50} mg a.i. /l)	>114 (96 h)
Water flea, *Daphnia magna* (EC_{50} mg a.i. /l)	>100 (48 h)

DNA conformations. As a result of the interaction between chemical exposure and subsequence reactions, genetic changes appear that influence biological parameters like fertility, fecundity, and longevity of the exposed organisms [19,125].

Oxidative Stress

TMX toxicity has been known to induce oxidative stress in different flora and fauna [126]. Chronic exposure to TMX has also been reported to cause oxidative damage in honey bees [127]. It is a fact that oxidative stress is one of the most important reactions to take place within the body after exposure to hazardous chemicals. As a result of oxidative stress, reactive oxygen species (ROS), which are normal products of cell metabolism, are released in very high amounts leading to cellular damage, and are considered to be the biomarkers of toxicity [128]. In order to prevent such destructions, cells activate different defensive mechanisms to counter the effects of ROS by releasing enzymatic and non-enzymatic antioxidants [129]. ROS causes cellular damage and necrosis through protein denaturation, lipid peroxidation, and DNA damage [130]. Superoxide dismutase (SOD), Glutathione-S-Transferase (GST), Peroxidases (POX), Catalases (CAT), and Ascorbate Peroxidases (APX) are the anti-oxidant enzymes while non-enzymatic antioxidants include ascorbic acid, thiols, and α-tocopherol which play their role in the process of detoxification. Oxidative stress leads to lipid peroxidation and the generation of hydroperoxides and various other free radicals which are efficiently removed by Glutathione-S4 Transferase (GST) [131,132]. Malondialdehyde (MDA), the end product of lipid peroxidation, causes injuries both at the cellular and subcellular levels [133,134]. TMX induces its toxic effects via different mechanisms. No exact report is available about the exact mechanisms of its toxicity. However, it is determined that TMX induces its adverse effects due to the induction of oxidative stress ensues leading to cell membrane damage and damage to DNA, and eventually cell death. Moreover, impaired transcriptional process as a consequence of TMX toxicity leads to abnormal protein synthesis and inhibition of various enzymatic reactions paving the way to abnormal metabolic processes.

One-Health Perspective of Thiamethoxam (TMX)

TMX is amongst the most commonly used insecticides throughout the world against insect pests of different crops [135,136] because of its wide-spectrum insecticidal activity [137,138].

TMX is used as prophylactic protection of fruits, vegetables, rice, and cotton against aphids, beetles, and thrips [139]. In addition, to control the pest attack on crops like barley, cotton, maize, sorghum, and wheat, TMX is one of the most frequently used insecticides in poultry farms [140,141]. There are several ways in which TMX can be applied. Of them, foliar sprays, root drenches, and seed dressings are the most frequently used methods of application [142-144]. Once TXM is within the insect/plant, it is readily metabolized by cytochrome P450 enzymes via desulphuration and transformed into its metabolite clothianidin [101,145,146]. This metabolite is highly toxic to the insect and adds a continuing environmental hazard of TMX to poultry birds [147].

TMX is not readily degraded and remains in the soil as well as crops/food products for a long time posing serious risks to humans, animals, and birds [148-151]. Environmental contamination is a major drawback of TMX as its residues have been detected in cocoa farmlands [152], soils of parks, residential areas [153], and arable soils [154], with concentrations ranging from μg kg^{-1} to mg kg^{-1} [138,142]. Humans and animals get the TMX into their bodies either through dermal absorption, inhalation, or ingestion. *Fig. 3* gives a brief overview of the pharmacokinetics of TMX.

Animal and plants product with persistent pesticides is the main source of human exposure to pesticides. According to a study conducted in China, 94.90% of pesticide exposures are through ingestion whereas only 5.1% of exposures occur through inhalation or dermal contact [155]. Poultry meat and eggs are the major portions of the human diet as it meets the protein requirement with the least cost [156]. These pesticides in the human lead to chronic health effects by irritating eyes and skin, damaging the nervous system, causing asthma, affecting hormones leading to reproductive issues, fetal death, and neurodevelopmental issues [157-159]. Previous studies show that the daily intake of these pesticides by the chicken is excreted as its residues in the egg and meat [160].

TMX Setbacks to the Poultry Sector

Dusting with insecticides to control insect attacks on the birds within the premises of poultry farms is the most

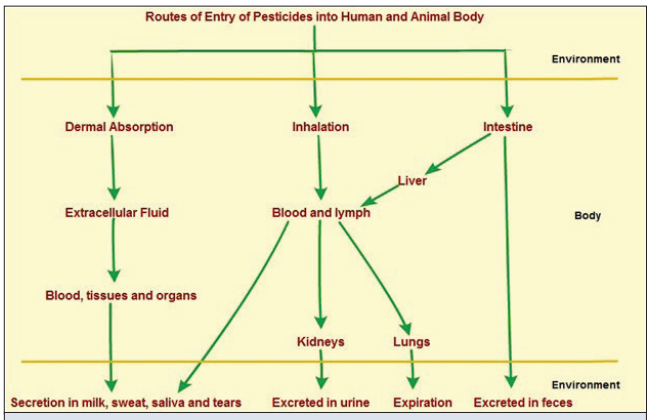

Fig 3. Mechanisms of actions of TMX toxicity (Created by Biorender)

Table 8. *Excretion/deposition of TMX and its metabolites in different animal species^*

Animal	TMX excretion/deposition	Other mMetabolites	
Rats	70-80% as TMX in urine	10% N- (2-chlorothiazol-5-ylmethyl)-N'-methyl-N"-nitroguanidine in urine	*Data collected from FAO/WHO Meeting on Pesticide Residues [161]
Goats	1% in milk	44-45% milk N- (2-chlorothiazol-5-ylmethyl)-N'-methyl-N"-nitroguanidine	
	36% goat fat	Metabolites in liver (up to 10%) N-(2-chlorothiazol-5-ylmethyl)-guanidine) N-(2-chlorothiazol-5-ylmethyl)-N'-methyl-guanidine 3-(2-chloro-thiazol-5-ylmethyl)-5-methyl- [1,3,5] oxadiazinan-4-ylidene]-Hydrazide 3-(2-chlorothiazol-5-ylmethyl)-5-methyl- [1,3,5] oxadiazinan-4-ylidineamine	
	51% muscles		
	22% kidneys		
Laying hens	Most dose excreted in droppings	Egg white 45% (N-(2-chlorothiazol-5-ylmethyl)-N'-nitroguanidine	
	Eggs 0.1%	Yolk 54% (N-(2-chlorothiazol-5-ylmethyl)-N'-nitroguanidine	
	Tissues 1-1.5%	Fat and skin 57%(N-(2-chlorothiazol-5-ylmethyl)-N'-nitroguanidine	
		Liver 39% N-(2-chlorothiazol-5-ylmethyl)-N'-methyl-N"-nitroguanidine	

^ *Food and Agriculture Organization Joint Meeting on Pesticide Residues*

important source of insecticides toxicities in the broilers and layers. According to the estimates, nearly 670 poultry birds acquire direct toxicities from pesticides in the USA throughout the year while the mortality rate is around 10% in severe toxicity cases. These figures can be much higher as mortality rates are complicated to estimate since the death of birds may occur away from the site of contact with pesticides. Moreover, the presence of scavengers in/around the farm also has a role in the underestimation of mortality rate figures [162]. The use of insecticides has a one-health perspective as poultry food products may contain pesticide residues that would be unfit for human use [163].

Various other toxic effects of TMX include cellular damage, genotoxicity, and immunosuppression in birds [164,165]. Studies have shown that TMX affects different facets of bird physiology in a dose-dependent manner. The probable impacts of TMX on birds are increasing as they are at risk to be exposed in multiple ways and have been recognized to show adverse effects even at sublethal concentrations [166]. Behavioral changes and mortality have been observed in pigeons and partridges following TMX toxicity. Accumulation of toxic levels of chemicals in the liver and kidney along with weakened locomotory ability were also found in pigeons and partridges [167,168].

Effects on Production and Immunity

Ingestion of seeds and crops treated with pesticides is the main route of exposure and induces mortality. Acute toxicity in sub-lethal doses can produce various clinical manifestations including lethargy, decreased production, and dropped immunity leading to the emergence of different infections in exposed species [169]. In addition, TMX toxicity has been reported to cause drop in egg production and thinning of the eggshell [170-172]. Neonicotinoids insecticides like TMX adversely affect cell-mediated immunity by lowering type-IV hypersensitivity reactions and lowering the T-lymphocyte stimulation index to phytohemagglutinin [169,173]. Furthermore, TMX toxicity may lead to multiple infection susceptibility in the birds as it lowers the phagocytic activity of macrophages and turn down lymphoproliferative activity leading to failure of the mounting effective immune response [174]. Previously, TMX has been regarded as the safest neonicotinoid but the recent studies covering biochemical, hematology, and behavioral aspects of laboratory animals have shown that even the sub-lethal doses can cause toxic effects in the birds [169,175].

Adverse Effects of TMX on Blood Cells and Biomarkers of Liver and Kidneys

In laboratory animals like rats and guinea pigs, the adverse effects of TMX on hematological, biochemical, and behavioral parameters have been reported. The oral administration of TMX causes a decline in erythrocyte and leukocyte count, and low hematocrit and hemoglobin value [140,169,175]. A recent study was performed to check the

Table 9. *Toxicological monograph for risk assessment of TMX toxic effects*

Species	Trial Period	Effect	NOAEL*	LOAEL**	
Mouse	3-month study	Toxicity	100 ppm	1250 ppm	***Data collected from FAO/WHO Meeting on Pesticide Residues**
	18-month study	Toxicity	20 ppm	500 ppm	
		Carcinogenicity	20 ppm	500 ppm	
Rat	Single-dose study	Toxicity	100mg/kg bw	500 mg/kg bw	
	3-month study	Toxicity	250 ppm	1250 ppm	
	24-month study	Toxicity	1000 ppm	3000 ppm	
		Carcinogenicity	3000 ppm	-	
	2 generations study	Reproductive toxicity	2500 ppm	-	
		Parental toxicity	1000 ppm	2500 ppm	
		Offspring toxicity	30 ppm	1000 ppm	
	Developmental toxicity study	Maternal toxicity	30 mg/kg BW	200 mg/kg BW	
		Embryo and fetal toxicity	200 mg/kg BW	750 mg/kg BW	
Rabbit	Developmental toxicity study	Maternal toxicity	15 mg/kg BW	50 mg/kg BW	
		Embryo and fetal toxicity	50 mg/kg BW	150 mg/kg BW	
Dog	3-month study	Toxicity	250 ppm	1000 ppm	

**No Observed Adverse Effect Level; **Lowest Observed Adverse Effect Level; ^ Food and Agriculture Organization Joint Meeting on Pesticide Residues (Page 318-319)*

hematological and biochemical changes in chickens. In birds, a significant decline in red blood cells, white blood cells, packed cell volume, and hemoglobin concentrations has been reported. Albumin, globulin, creatinine, urea, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were also noticed which showed that high dose of TMX leads to a significant decrease in the albumin globulin and creatinine value whereas a rise in the urea, ALT, and AST were seen [140,176]. Such alterations in renal and hepatic functioning can lead to debilitation [177], poor performance, and reduced feed conversion in poultry birds and these findings suggest conducting clinical trials on dairy animals to ascertain the role of TMX on these parameters.

Toxic Effects of TMX on the Reproductive System

TMX has been found to affect male fertility by either causing direct damage to spermatozoa or altering the functioning of Leydig or Sertoli cells. In addition, TMX also has the potential to disrupt the endocrine functions at a few stages of hormonal regulation [178]. Sperm nuclear proteins are changed by TMX toxicity leading to male infertility [179]. Epidemiology-based studies have indicated that the semen quality (sperm morphology, viability, motility, and fertilization capability) of agricultural workers is poor where TMX is frequently used [180].

Optimum female reproductive health determines profit maximization in the dairy sector. Poor reproductive performance has long-lasting effects on livestock farm economies worldwide. In-vitro studies have revealed that TMX has the potential to cause harmful effects on the developing embryo [97]. Sub-lethal doses of TMX have shown negative effects on the female reproductive system the disruption of ovarian structure, delayed sexual maturity, and reduced egg production [181]. This deposition of toxin within the eggs means that developing embryos are prone to be affected more easily by toxic effects being more sensitive than adult birds. Granivorous birds have shown a number of abnormalities after ingestion of seed coated with TMX. Reduced chick survival and lower egg fertilization rates in partridges [182], reduced clutch size, weight loss, and lower chick survival in northern bobwhite quail [166], and shorter embryos, testicular abnormalities, and increased rates of DNA damage in Japanese quail [183] have been reported. There is also evidence that neonicotinoid residues present in the environment can be deposited into eggs. A recent investigation showed that 24 clutches (out of 52) of Patridge collected from a habitat contained 15 different pesticides in trace amounts including thiamethoxam [184]. Such exposure has been reported to lead to changes in organogenesis [185-187] and anatomical abnormalities [188,189]. Damage to cellular structure and genotoxic effects can affect both growth as well as development in unpredictable ways. *Table 9* summarizes the results of TMX toxicity studies conducted with no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL).

Conclusion

The use of pesticides to keep save crops from insect attacks is advantageous as it boosts the economy of the agricultural

sector. Though, irrational, unwise, and extensive use of chemicals like TMX has deleterious effects on both animal and human health. The presence of toxic levels in the environment leads to the cellular level of harmful changes as well as many grossly observed alterations in metabolism, productivity, reproduction potential, and general health status of the exposed animals/humans. This review highlighted many injurious consequences of TMX toxicity including oxidative stress, hematological alterations, hepatic and renal parameter changes, and thrashes to the reproductive health of both males and females. Thus, we recommend that future research on determining acceptable exposure levels should be undertaken in One-Health Interface. Further, policy-making about the cogent use of TMX and awareness programs among farmers on the grass-root level about TMX toxic effects should be designed.

Acknowledgments

Authors are thankful to Dr. Shahbaz ul Haq for providing linguistics corrections.

Availability of Data and Materials

The datasets analyzed during the current study are available from the corresponding author (Mughees Aizaz Alvi) on reasonable request.

Funding Support

This research did not receive external fund.

Competing Interests

The authors declare that they have no conflicts of interest.

Authors' Contributions

Conceptualization, methodology, formal analysis, investigation, resources, data curation, writing-original draft preparation, W.Q., M.US., M.I., M.M.A., A.F. and R.Z.A.; writing-review and editing, W.Q. and M.A.A.; project administration, R.Z.A. and R.H. All authors have read and agreed to the published version of the manuscript.

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