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Toxic Effects Associated With Neonicotinoid Exposure on Non-target Organisms: A Review

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Abstract

The neonicotinoid class of insecticide is nicotine-like neuro-toxicants used to control the pests of agriculture crops and ornamental plants. They act as selective agonists of acetylcholine receptors in the central nervous system of insect pests and work by disrupting their nerve impulses. Some of the properties of this class of insecticides are a long half-life in soil, low volatility, and higher water solubility, leading to their accumulation in soil, underground water, and water bodies due to surface runoff. This, in turn, results in exposure to many beneficial non-target aquatic and soil fauna such as arthropods, fish, birds, mammals, etc. Although it has a selective mode of action for insects, some *in vivo* and *in vitro* investigations have also shown toxicity in non-target invertebrates and vertebrates. Initially, neonicotinoid toxicity was observed in honey bees, which are essential pollinators of crops. Later, studies reported the accumulation of neonicotinoid residues leading to the mortality of aquatic fauna, including salt marsh and freshwater mosquitoes, brine shrimp, fleas, and crayfish. Imidacloprid exposure led to disruption of larval development in the Mayfly larvae. Also, earthworms that play a crucial role in enhancing soil fertility were drastically affected by acetamiprid, clothianidin, imidacloprid, nitenpyram, and thiacloprid. Apart from these, toxicological impacts were also observed in vertebrates such as birds, where imidacloprid, clothianidin, acetamiprid, and thiacloprid caused reproductive, metabolic, and morphological alterations. Similarly, imidacloprid and acetamiprid caused gills, brain and liver dysfunction with embryo mortality. Even after the selective action of neonicotinoids, instances of mammalian toxicity were also reported in many *in vivo* studies. DNA damage and liver dysfunctions due to imidacloprid in rabbits were observed in various studies. In a recent study, imidacloprid exposure led to DNA damage and oxidative stress in bone marrow-derived mesenchymal cells of buffalo. Also, many instances of neurotoxicity, reproductive toxicity, immunotoxicity, genotoxicity and cytotoxicity in mouse and rat models were observed due to different neonicotinoids. Many *in vitro* studies using mammalian cell lines have also established potential risks of neonicotinoid exposure. This review, therefore, is a compilation of various toxicity studies of different types of neonicotinoid pesticides in both non-target invertebrates and vertebrates, including several kinds of toxicities caused in mammals with neonicotinoid exposure.

Keywords: Insecticide, Invertebrates, Pesticide, Toxicity, Vertebrates

1. Introduction

Neonicotinoids are a comparatively new class of insecticides with a specific and efficient mode of action that targets the acetylcholine receptor (nAChr) of the pests. They selectively bind to the insect nAChr in the insect's central nervous system[1]. They are actively used against various sucking pests such as whiteflies and aphids like dipterans, coleopterans, and lepidopterans species[2]. This class of pesticide shares

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structural and functional similarities to a well-known and commonly used compound called nicotine, a naturally occurring product present in the leaves of *Nicotiana tabacum*[1]. Neonicotinoid function by application on foliage by spraying and are also used to treat soil and seeds[3]. The Insect Resistance Action Committee (IRAC) has listed seven types of neonicotinoids: acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam[4]. All neonicotinoids are systemic in nature; therefore, they are easily absorbed by the roots or leaves of plants and are distributed throughout the plant. The insects then take up the insecticide while feeding on it, which is absorbed in their stomach. Due to the increase in the use of neonicotinoids for soil and seed treatment, there is a growing concern about their possible adverse effects on various non-target organisms, which are essential in maintaining the balance of the ecosystem[5]. Neonicotinoids have shown high toxicity to a wide range of non-target vertebrates and invertebrates, thus disturbing the ecological balance of the food web. They mainly affect the aquatic ecosystem and arthropods, affecting birds and other vertebrates feeding on these organisms[6].

Apart from their very efficient insecticidal activity against agricultural pests, the widespread and indiscriminate use of pesticides across the world has led to serious consequences. Analyzing and understanding their toxicity level is crucial to taking precautionary steps to mitigate their adverse effects. Therefore, the present review discusses the lethal and sublethal impacts of neonicotinoids on various non-target invertebrates such as brackish and freshwater arthropods, annelids, and some vertebrates such as fish and mammals.

2. Toxicity in Invertebrates

The toxic effects of various types of neonicotinoids were studied in multiple invertebrates, including aquatic arthropods, crustaceans, and non-target insects in agricultural crop fields. Some important invertebrates, such as bees and earthworms, were also drastically affected.

2.1 Toxicity in Bees

One of the first invertebrates to be tested for toxicity in this pesticide class was honey bees (*Apis mellifera*), which are fundamental pollinators in agricultural fields. In 2001, an acute toxicity study conducted by Suchail and co-workers reported the Lethal Dose values (LD50) of imidacloprid for bees. In a 48 h study, the LD50 was reported to be 60 ng/bee (600 µg/kg). Similarly, in 72 h and 96 h studies, it was reported to be 40 ng/bee (400 µg/kg). The concentration of the lethal dose of imidacloprid mentioned above was found to be far less than most of the other classes of insecticides like organochlorines, pyrethroids, and carbamates. While chronic exposure to insecticide at concentrations of 0.1, 1.0, and 10 µg/l for 10 days, 4000 times smaller than the LD50, was found to be enough to kill a bee within 10 days of consumption[7]. There was a decline in managed bee colonies in the US and European nations, reaching such a drastic low level that it was given the term "Colony Collapse Disorder" (CCD), where the loss of approximately 50% of colonies was seen. In a study conducted on 12 colonies of neonicotinoid-treated bees with a dose of 0.74 ng/bee/day, it was observed that 6 of these colonies died from symptoms of CCD[8]. Apart from the CCD, it was also demonstrated by Pettis and co-workers that neonicotinoid exposure increases the susceptibility of bee colonies to *Nosema caranae* infection. *Nosema caranae*, a microsporidian parasite, acts synergistically with neonicotinoids and targets the midgut of the bees, depriving them of nutrition and leading to premature death[9]. A combination of imidacloprid and thiamethoxam was tested for toxicity on *Bombus terrestris*. The pesticides were tested singly and in a constant combination ratio of imidacloprid and thiamethoxam (0.310:0.438). Individual toxicity was seen to be highest due to imidacloprid followed by thiamethoxam. The binary combination, on the other hand, produced additional effects[10].

2.2 Adverse Effects on Aquatic Fauna

The persistence of neonicotinoids in the water bodies increases due to their high-water solubility and easy application modes by seed treatment and foliar spray. Surface runoff from the agricultural fields also contributes to the accumulation of neonicotinoids in the water bodies[11]. Therefore, the fauna of these aquatic ecosystems is also largely susceptible to insecticide, as they share similar neurological and respiratory adverse effects as insects. The aquatic organisms also lack a proper detoxification system due to their primitive iso-enzyme cytochrome P450 and mono-oxygenase, which cannot degrade toxic compounds entering their bodies[12]. Song and colleagues studied the toxicity of imidacloprid and tebufenozide in four different aquatic arthropods. The arthropods under study included two aquatic mosquito species, namely *Aedes taeniorhynchus* (saltmarsh mosquito) and *Aedes aegypti* (freshwater mosquito). The other two arthropods were aquatic crustaceans, namely *Artemia* (brine shrimp) and *Daphnia magna* (freshwater flea). The LC50 values for all four arthropod species used

for imidacloprid exposure were in the range of 0.013-361.23 mg/l. Observations were made after 6, 12, 24 and 48 hours. It was observed that *A. taeniorhynchus* was most susceptible to imidacloprid, i.e., it was found to be three times more toxic to *A. aegypti*. Out of the two crustaceans, imidacloprid was more toxic to *D. magna* than to *Artemia*. In addition to this, mosquito larvae were more susceptible to imidacloprid than tebufenozide. It was concluded that the neurotoxic symptoms caused by imidacloprid exposure in mosquitoes were similar to those of other insect species. Also, it is more toxic than the two acetylcholine esterase inhibitors, imidacloprid and tebufenozide, used in the study[13].

In another study conducted in the southern USA, Barbee and Stout observed the effects of clothianidin and thiamethoxam on a crustacean, *Procambarus clarkii*, popularly known as crayfish. Large quantities of rice and crayfish were grown in a 2-year crop rotation system in the USA. It was seen that residual insecticide targeted against rice weevil in the same field where crayfish was produced decreased the crayfish yield. In this study, the organism was exposed to a dose of 59 µg Al/l of clothianidin and 967 µg Al/l of thiamethoxam. Out of the two, the mostly used neonicotinoid clothianidin was characterised as highly toxic to crayfish, whereas thiamethoxam was found to be moderately toxic. Thiamethoxam at sublethal concentrations caused very distinct behavioural changes in crayfish, including extreme aggressiveness, which resulted in up to 80% mortality. At higher concentrations, acute toxicity was also observed[14].

2.3 Adverse Effects on Other Non-target Organisms

Neonicotinoids used for seed treatment were studied for toxicity in non-target insect species feeding on both crops and non-target plants on the margins of crop fields. First, residual insecticides were analysed in the foliage of non-target plants by comparing them with residual insecticides in treated crops, followed by studying the neonicotinoid exposure in non-target predatory insects and crop-pollinating insects. The insects were exposed to corn seedlings grown from thiamethoxam and clothianidin-treated seeds. Corn seeds were treated with 0.250 mg Al/seed for thiamethoxam and clothianidin. Feeding treated corn seedlings led to lethal toxicity in coccinellid larvae. Thiamethoxam and clothianidin concentrations were tested on *Harmonia axyrides* larvae. 93% of the 44 larvae showing neurotoxic symptoms died within 24 h when exposed to individual insecticides. When both insecticides were combined, the number of larvae with neurotoxic symptoms and dead larvae increased. Also, it was tested that clothianidin exposure resulted in higher neurotoxic symptoms and mortality of larvae than thiamethoxam[15]. Chloro-neonicotinoid, mainly imidacloprid, induced chronic toxicity in Mayfly larvae. For an acute dosage of 200 g/l, it was seen that Ephemeroptera (*Cloeon dipterum* and *Caenis horaria*) and Trichoptera (*Limnephilidae*) were most sensitive to imidacloprid. When the organisms were subjected to chronic imidacloprid exposure, *C. dipterum* showed the highest level of sensitivity, followed by *C. horaria*. Both of these species reported increased sensitivity compared to the other species tested[16].

Yet another invertebrate that was drastically affected was the earthworm (*Eisenia fetida*), which is very commonly found in agricultural fields and is an important organism for improving soil fertility and structure. They represent 60-80% of the total biomass in the agricultural soil. The accumulation of insecticide affects the organism and disturbs the higher trophic levels drastically. Five neonicotinoids (acetamiprid, clothianidin, imidacloprid, nitenpyram, and thiacloprid) and five other classes (pyrethroid, carbamate, etc., organophosphate, insect growth rate regulators, and antibiotic insecticides) of insecticide were tested for toxicity. The LC50 for neonicotinoids was taken in the range of 0.0088-0.45 µg/cm^2 and the results clearly showed that out of all the classes, neonicotinoids were more toxic to *E. fetida*[17]. A similar study on another earthworm species, *Aporrectodea trapezoide duges* was carried out and indicated that imidacloprid (155 mg/kg) was more toxic to earthworms than cyfluthrin (351 mg/kg), chlorpyrifos (390 mg/kg) and fipronil (>8550 mg/kg)[18]. Bandeira and co-workers studied the effects of clothianidin exposure in another earthworm species, namely *Eisenia andrei*. A 6.1 mg/kg concentration inhibited growth, and 15.7 mg/kg led to significant weight loss. They almost lost around 40% of their initial weight. This was followed by disruption in the reproduction process as a decline in the number of juveniles was observed at the lowest concentration of 1.5 mg/kg. The reduction reached up to 92% at 15.7 mg/kg[19]. Therefore, the use of neonicotinoids should be carefully evaluated to avoid severe damage to the beneficial earthworm species.

3. Toxicity in Vertebrates

Toxicological studies were also carried out to study neonicotinoid toxicity in different vertebrates, including mammals.

3.1 Toxicity in Birds

Insects constitute a major part of the diet of insectivorous birds. The decline in the avian population became evident when it was observed that at concentrations of more than 20 ng/l, of imidacloprid insecticide, the avian population decreased approximately by 3.5% every year[20]. Pandey and Mohanty also demonstrated that imidacloprid exposure of 0.5% of the LD50 mg kg^{-1} BW d^{-1} (31 mg/kg BW/day) leads to disruption of the pituitary-thyroid axis in a wild bird, *Amandava amandava* (Red Munia). The plasma TSH levels decreased during the preparatory and breeding phases, indicating a lack of response to the Hypothalamic-Pituitary-Thyroid (HPT) axis. Due to this disruption, the reproductive and metabolic balance was altered in birds[21]. Imidacloprid also disrupted the HPT axis by directly inducing neurotoxicity in neurons[1]. Reproductive toxicity was observed when clothianidin was administered orally in different concentrations, 0.02, 1 or 50 mg CTD/kg BW in mature quails. Fragmented DNA in germ cells and their vacuolization were observed in the seminiferous tubules. It was also observed that when eggs of quails were treated with 1 and 50 mg/kg, BW declined, and the development of the embryo was delayed[22]. A combination of acetamiprid and thiamethoxam was tested in broiler chicken Ross 308, where 250 fertile eggs were treated with 100 µl/egg of acetamiprid with thiamethoxam at a concentration of 360, 540, 900, and 1800 ppm. Morphological analysis after 10 and 21 days of exposure revealed a reduction in the embryo's weight and a significant elevation in mortality. Clear abnormalities were observed, such as the wry neck, ectopic viscera, twisted cleft beak, abnormal limbs, delayed retraction of the yolk sac, and broken legs. Liver histology revealed necrosis and degeneration of liver cells and congestion of the central vein and sinusoids due to inflammatory cell infiltration[23].

3.2 Toxicity in Fishes

Imidacloprid, which has been included in toxicity class II and class III by the EPA has not only proved to be toxic to birds but to fish as well. Pathological changes and expression of the caspase-3, iNOS, CYP1A, and MT1 genes were observed in common carp (freshwater fish). Ozdemir and co-workers observed that imidacloprid exposure affected gills, brain cells, and the liver of fishes by causing severe histopathological lesions, oxidative stress, and inflammation. Upon imidacloprid exposure at low and high doses of 140 mg/l and 280 mg/l respectively, for 24, 48, 72, and 96 h, hyperplasia of mucous cells and secondary lamellar cells in the gills was observed. Also, there was hydrophobic degeneration of hepatocytes, which resulted in liver necrosis. There were alterations of caspase-3 expression when exposed for 72 and 96 h. MT1 gene expression in brain cells was up-regulated even with a low imidacloprid dose (140 mg/l) of imidacloprid when exposed for 96 h and also for a high dose (280 mg/l) for 48, 72, and 96 h[24]. Acetamiprid exposure in Zebrafish at a concentration of 374 mg/l resulted in high mortality of Zebrafish embryos, whereas at 760 mg/l led to complete mortality. Any embryo that survived had serious defects such as a bent spine, yolk sac edema, pericardial edema, and an uninflated swim bladder. Disruption in the nervous system was also reported, as there was a significant elevation observed in the spontaneous movement of the embryos[25].

3.3 Toxicity in Mammals

Imidacloprid was also studied for its genotoxicity in rabbits, where it was found to cause DNA damage. Genotoxicity was observed by measurement of binucleated cells with micronuclei (BNMN) and micronuclei (MN) in the lymphocytes of the group of rabbits exposed. The long-term exposure of 40 and 80 mg/kg/day resulted in significantly higher BNMN and MN in groups after exposure for 2 and 4 months, clearly indicating genotoxicity[26]. New Zealand male rabbits were tested for DNA damage after subjecting 30 mg/kg BW/day of imidacloprid for a span of 2 months. The tail intensity was elevated in the exposed groups by up to 70%, followed by an elevation in the occurrence of BNMN up to 126% in the imidacloprid-exposed group. Oxidative stress was also confirmed by a significant elevation in protein oxidation and a decline in lipid peroxidation[27]. Al-Arami and his coworkers examined the effects of imidacloprid in rabbit liver after 37 days of exposure. At concentrations of 45 and 90 mg/kg of imidacloprid, detrimental histopathological observations were made in the form of blood vessel congestion, inflammation, steatosis, necrosis, fibrosis, haemorrhage, and ballooned hepatocytes[28]. Effects of imidacloprid exposure were also studied in bone marrow-derived mesenchymal stem cells from buffalo. Imidacloprid at concentrations of 0.81, 1.61, and 3.22 µM caused a significant elevation in LDH, ALP and CK-MB, followed by an increase in the oxidative stress markers LPO, O^{2-} radicles, total ROS, apoptotic index, loss of mitochondrial membrane potential and DNA damage[29].

4. General Toxicity

Considering the involvement of neonicotinoids in invertebrate toxicity and disturbance in the trophic levels of the ecosystem, many studies conducted so far have revealed the potential for neonicotinoid toxicity in mammals. These include reproductive toxicity, genetic toxicity, neurotoxicity, hepatotoxicity, and in some cases, immunosuppression[30]. In a preliminary study, the exposure levels were evaluated, and seven neonicotinoids were detected in the urine samples of 52 people who had no history of using or spraying the insecticide. Four of the seven (imidacloprid, nitenpyram, thiacloprid, thiamethoxam, acetamiprid, and clothianidin) were present in high amounts. Thiamethoxam and dinotefuran were found in 100% of cases, whereas imidacloprid and clothianidin were found in 96% of cases. It was also discovered that some amounts of neonicotinoids were present in urine samples of 223 children aged 3-year-old[31]. In a field study conducted in Greece, genomic DNA from peripheral blood samples was extracted from farmers and 8-hydroxydeoxy-guanosine (8-OHdG) levels were determined. It was observed that with an increase in the frequency of application of neonicotinoids, there was an increase in oxidative damage to the DNA in the blood samples of farmers[32]. Apart from DNA damage, lung dysfunction was also observed when respiratory functions were measured and analyzed between farmers spraying pesticides and the non-spraying farmers in Spain[33]. Some imidacloprid metabolites have been reported to act as agonists on the human nAChRs in a similar pattern to nicotine. The metabolite desnitro-imidacloprid and imidacloprid-olefin were studied using Ca^{2+} imaging. Desnitro-imidacloprid was seen to activate the receptors potently at sub-micromolar concentrations compared to imidacloprid-olefin. It acted on human nAChR subtypes a7, a3β4, and a4β2, which are considered physiologically important. Imidacloprid and imidacloprid-olefin were also confirmed as agonists but with 2-3 orders lower than desnitro-imidacloprid[34].

4.1 Neurotoxicity

Neonicotinoids have been displayed to easily penetrate the blood-brain barrier in mammals[1]. Changes in behavioural patterns have been observed due to the neonicotinoid imidacloprid in male rats, where 150 and 300 mg/kg bw dosage of imidacloprid caused behavioural depression, leading to a decline in awareness, alertness, and grip strength. It was followed by a notable decline in both spontaneous and forced locomotor activity. Simultaneously, there was a decrease observed in response to sound, touch, and pinna reflexes. Significant elevation was also observed in the pentobarbitone-induced sleeping time without disturbing the start of the sleeping time[35]. Nakayama and co-workers conducted a study to analyse the impact of acetamiprid and imidacloprid (5 mg/kg/day) on the development of the hippocampal dentate gyrus in mouse neonates. A significant decline was observed in the neurogenesis in the hippocampal dentate gyrus of the neonates. The newborns were reported to have increased amoeboid-type and activated M1-type microglia. The exposure indicated impairment of neurogenesis and disruption in the microglial profiles in the mouse brain[36]. There was an immediate requirement to deduce and understand the potential risks of the neurotoxicity of neonicotinoids. They were observed to be developmental neurotoxicants due to their insecticidal action and structural and functional similarity to nicotine, an established mammalian neurotoxicant. The effects of imidacloprid and acetamiprid on cerebral neurons of neonatal rats were observed to validate the developmental neurotoxicity. Acetamiprid, imidacloprid, and nicotine were administered in rats at a concentration higher than 1 μM, at which proliferation, differentiation, and excitatory effects were observed in primary cultures of cerebral neurons[1]. In a study of 28 days, oral exposure to imidacloprid (45 and 90 mg/kg body weight) was administered to male rats. There was a significant decrease in antioxidant enzyme levels and a subsequent increase in lipid peroxidation. A notable decrease was observed in Spontaneous Locomotor Activity (SLA). Furthermore, pain thresholds decreased following exposure[37]. A single large gestational exposure of imidacloprid at a concentration of 337 mg/kg (0.75 × LD50) in corn oil was subjected to pregnant Sprague-Dawley rats. It led to the generation of serious neuro-behavioural deficits and elevated expression of Glial Fibrillary Acidic Protein (GFAP) in various regions of the brain in the offspring[38].

4.2 Reproductive Toxicity

Various studies in mice and rats have established reproductive toxicity. Reproductive parameters were tested in male Wistar rats due to imidacloprid exposure at concentrations of 45 and 90 mg/kg bw. A notable reduction was observed in the live and total epididymal sperm count, along with sperm mobility. Elevation was also observed in the activities of lactate dehydrogenase-x, sorbitol dehydrogenase, and gamma-glutamyl transpeptidase. However, a significant decline was reported in the GSH, SOD, CAT, GPx, and GST levels, along with 3β-HSD and 17β-HSD enzyme activation.

Whereas notable elevation was observed in the LPO levels. Along with these changes, histopathological alterations were also observed in the testis and epididymis of the rats[39]. It was observed that 90-day oral exposure to imidacloprid at doses of 5, 10, and 20 mg/kg/day in female rats caused significant toxicity in the ovary. Moreover, altered ovarian weight, along with pathomorphological alterations in atretic follicles, follicles and antral follicles. The generation of oxidative stress was also reported as notable changes were observed in levels of CAT, GSH, GPx, SOD and LPO. Imbalance in the hormonal levels of LH, FSH, and progesterone was also the result of imidacloprid exposure[40]. Similarly, after imidacloprid exposure of 0.5, 2.0, and 8 mg/kg in male mice, there was a decrease in the weight of the epididymis, sperm concentration, testosterone, and vesicular seminalis. There was a drastic weight gain and reduced levels of glutathione. Furthermore, lipid peroxidation and fatty acid concentration increased along with the increase in levels of abnormal sperms[41]. When administered for four weeks at concentrations of 10, 50, and 250 mg/kg/day in male mice, clothianidin demonstrated vacuolated seminiferous epithelia. There was a decrease in the immune reactivity of glutathione peroxidase 4, an antioxidant enzyme in the testis. Behavioural changes were also observed in mice, showing elevated anxiety-like behaviour[42].

4.3 Immunotoxicity

A second-generation neonicotinoid, clothianidin (100 ng/ml) disrupts the immune system by causing down-regulation and inactivation of nuclear factor-kappa B (NF-$_K$B) by inhibiting its activation. This down-regulation and inactivation disrupted the immune response, which produced pro-inflammatory cytokines in a study conducted by Prisco and co-workers on the human monocyte cell line THP-1. Down-regulation of a reporter gene under the control of NF-$_K$B promoter was reported along with reduced levels of TNF-α in human monocyte cell line THP1. Transcriptional levels of transcription factors TRAF4 and TRAF6 were also disrupted, which hampered NF-$_K$B signaling[43]. In thiamethoxam-treated mice with concentrations of 43.5 mg/kg BW and 87.1 mg/kg BW, a dose-dependent decrease was observed in total leukocyte count and an elevated lymphocyte count in both low and high doses. At a high concentration of 87.1 mg/kg BW, there was a notable depletion in total protein and globulin. The histopathological analysis at the higher dose revealed extramedullary hematopoiesis, followed by mild congestion and depletion of lymphocytes[44]. Whereas 90 days of acetamiprid exposure at concentrations of 27.5, 55, and 110 mg/kg BW in Wistar rats caused a notable decrease in lymphocyte proliferation, showing the inability of B-lymphocytes to produce an immune response, thus increasing the chances of sensitivity to infections. 15 to 28% depletion in nitrite production was observed, which is supposed to be an essential signal for an effective inflammatory response by the macrophages[45].

The role of imidacloprid in developmental immunotoxicity in pregnant female Wistar rats was determined by the oral administration of 10, 30 and 90 ml/kg BW up to the 21st gestation day. Age-dependent adverse effects were observed, and prolonged exposure throughout the development led to a compromised immune system[46]. In a similar study conducted in female BALB/c mice using imidacloprid, Badgujar and co-workers reported decreased cell-mediated immune response with significant histopathological changes in the liver and spleen at a high dose (10 mg/kg/day). At concentrations 5 and 10 mg/kg/day, immunosuppression was also observed due to the direct cytotoxicity of imidacloprid against T_H cells[47]. Thiamethoxam at a concentration of 1/10th of the LD50, i.e., 83.7mg/kg BW was administered orally in mice after immunisation with *Brucella abortus* culture filter antigen (CFB Ags) as a vaccine. The results showed humoral and cell-mediated immunity suppression even after initial immunization by the CFB Ags vaccine[48].

4.4 Genotoxicity and Cytotoxicity

Neonicotinoid poisoning has been reported in humans, which has raised the need to assess the genotoxic and cytotoxic impacts of these pesticides. Both *in vivo* and *in vitro* examinations have been carried out to evaluate the genotoxicity and cytotoxicity of neonicotinoids. Imidacloprid-administered female mice showed that medium (50% of LD50 = 75.5 mg/kg/BW) and high (75% of LD50 = 112.5 mg/kg/BW) dose-induced serious mitotic inhibition and chromosomal aberrations. Notable changes were observed in RBC, haemoglobin, and ESR (Erythrocyte Sedimentation Rates). The number of WBCs also increased drastically[49].

In two studies conducted on thiacloprid exposure in human peripheral blood lymphocyte culture, it was observed that, at all concentrations, there was a significant increase in micronucleus formation, chromosomal aberrations, and nucleoplasmic bridge formation. Also, a decrease was observed in the mitotic index, proliferation index, and nuclear division index. Nuclear bud formation has also been reported at concentrations of 150 µg/ml (48 hours) and 300 µg/ml (24 hours)[50]. In another study done

using thiacloprid concentrations of 112.5 mg/kg for 24 hours and 22.5 mg/kg for 30 days in bone marrow cells of rats, a significant decrease in mitotic index, binucleated cells, and an increase in chromosomal aberrations were observed. The cytokinesis block micronucleus assay (CBMN assay) using the cytochalasin B assay in bone marrow (*in vivo*) showed a drastic increase in micronucleus formation[51]. At the same time, genotoxicity and cytotoxicity of acetamiprid were only observed at high doses (4.6 mg/kg body weight given for 90 days) in somatic cells of male albino mice[52]. Calderon-Segura and co-workers (2012) demonstrated that even at low concentrations of thiacloprid, clothianidin, and imidacloprid, DNA damage was induced, which resulted in a significant increase in comet frequency and tail length[53].

5. Conclusion

Neonicotinoid is a broad-spectrum insecticide class comprising seven types of insecticide, namely acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram/nithiazine, thiacloprid, and thiamethoxam. They were developed as a safer alternative compared to other classes of insecticides like carbamates, organochlorines, and pyrethroids, which were being used globally. The toxic effects of neonicotinoids were initially seen on honey bees, leading to a large-scale wipeout of bee colonies, mainly in European countries. Therefore, various studies were conducted to indicate the potential damage to non-target vertebrates and invertebrates due to exposure to neonicotinoids. These are commonly used as foliar sprays, and surface runoff leads to their accumulation in the water bodies and thus affects the aquatic ecosystem the most. The involvement of neonicotinoid toxicity in invertebrates was observed. Though imidacloprid accumulation was found to negatively affect aquatic arthropods like shrimps and water fleas, residual amounts of clothianidin, thiamethoxam, and dinotefuran were found toxic to crayfish. Upon chronic exposure to imidacloprid, larval development was affected in mayfly larvae. All pesticides of the neonicotinoid class were found to be toxic to earthworms. Vertebrates such as birds and fish also tested positive for neonicotinoid toxicity, mainly imidacloprid and clothianidin. Some mammals were also tested for the harmful effects of neonicotinoid exposure. Various *in vivo* studies reported that acetamiprid and imidacloprid caused neurotoxicity in neonatal rats. Imidacloprid was also found to cause reproductive toxicity in mice. Various studies confirmed the immunotoxicity of thiamethoxam, acetamiprid, and imidacloprid. Similarly, acetamiprid, thiamethoxam, clothianidin, and imidacloprid were found to be cytotoxic and genotoxic in mice. However, the studies and investigations conducted to date are not enough and are still ongoing. Further, large-scale investigations need to be carried out to validate the harmful effects of neonicotinoids.

6. Abbreviations

ALP: Alkaline Phosphatase; CAT: Catalase; EPA: Environmental Protection Agency; FSH: Follicle-Stimulating Hormone; GPx: Glutathione Peroxidase; GSH: Reduced Glutathione; GST: Glutathione-S-Transferase; LC50: Lethal Concentration; LDH: Lactate Dehydrogenase; LH: Luteinizing Hormone; LPO: Lipid Peroxidase; ROS: Reactive Oxygen Species; SOD: Superoxide Dismutase; TNF: Tumor Necrosis Factor; TSH: Thyroid-Stimulating Hormone.

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