ISSN : 2319-2607 (Print); 2583-0856 (Online) IISUniv.J.Sc.Tech. Vol.11(1), 23-31 (2022)
REVIEW ARTICLE

Hepatotoxicity of Neonicotinoids: A Review

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Abstract

A relatively new class of insecticides, namely neonicotinoid, works as neuro-toxicants and is extensively used to control the pests of agricultural crops and household plants. The insecticide class acts as a selective agonist of acetylcholine receptors in the central nervous system of insect pests. The insecticide properties like high water solubility and long half-life in agricultural soil led to its residual accumulation in the ecosystem. Many studies have shown neonicotinoid accumulation in various human samples along with some instances of DNA damage in blood samples of individuals related to agricultural sector. Since, the primary target organ for insecticide encounter and metabolism is the liver, it is highly susceptible for toxic injury. To validate the effects of neonicotinoid exposure in liver, many *in-vivo* studies have been conducted in different animal models. This review, therefore, is a compilation of hepatotoxic studies of different types of neonicotinoid insecticides in animal models, along with instances that demonstrates neonicotinoid accumulation in various human samples.

Keywords: Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Nitenpyram, Thiacloprid, Thiamethoxam.

Abbreviations: ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AOPP: Advanced oxidation protein products; AST: Aspartate aminotransferase; Bcl-2: B-cell lymphoma 2; CAT: Catalase; CLO: Clothianidin; CLO-dm: Desmethyl-clothianidin; GGT: Gamma glutamyl transferase; GPx: Glutathione peroxidase; GSH: reduced glutathione; GST: Glutathione-S-transferase; HDL-C: High-density lipoprotein-cholesterol; IL-6: Interleukin 6; LDH: Lactate dehydrogenase; LDL-C: Low-density lipoprotein-cholesterol; LPO: Lipid peroxidation; MDA: Malondialdehyde; NPSH: Non-protein sulfhydryl; PCO: Protein carbonyl; CaTNF: Tumor necrosis factor; SGOT: Serum glutamic-oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase; TAC: Total antioxidant capacity; TBIL: Total bilirubin; TEM: Transmission electron microscope; SOD: Superoxide dismutase; TGC: Triglycerides; TMX: Thiamethoxam; TMX-dm: Desmethyl-thiamethoxam

Introduction

In attempts to eradicate insect pests from the agricultural fields, the most exploited method is the use of chemical insecticides. Insecticide classes, namely organophosphates, organochlorines, carbamates, and pyrethroids, have been used extensively for several decades. Many studies have proved them to be highly toxic to the ecosystem including humans (Timoumi *et al.*, 2019). Neonicotinoid, a comparatively new class of insecticides, functions by targeting the acetylcholine receptors in the central nervous system of the insect pests by selectively binding to these receptors and disrupting the nerve impulses (Bass *et al.*, 2015). Seven types of neonicotinoids that are currently in commercial use are acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam (Ma *et al.*, 2019) (Table 1).

They were first developed and registered in the early 1990s and are currently used for approximately 140 crops (Morrissey *et al.*, 2015). This extensive use of neonicotinoids is due to their systemic activity, making them easier to absorb by plant roots or leaves and further distributed throughout the plant. Insects take up these pesticides while feeding on these plants. Due to their very effective insecticidal activity, the widespread and overwhelming use of these pesticides worldwide has led to contamination of the environment and adverse effects on the related ecosystems (Botias *et al.*, 2016).

In higher animals, the liver is the main organ that encounters all chemical compounds, drugs, and xenobiotic compounds and functions to neutralize them by its detoxification activity (Alarami, 2015). The detoxification process in the liver carried out to metabolize the incoming toxins is shown in Fig. 1

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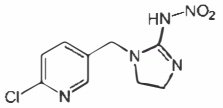
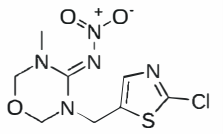
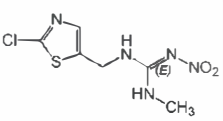
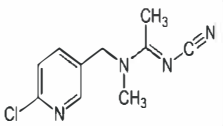
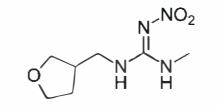
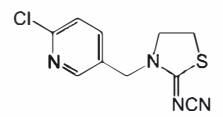
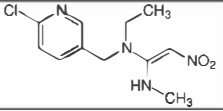
(Bhattacharya, 2015). The liver is also responsible for the crucial metabolism of carbohydrates, proteins, and fats in the body. These metabolic pathways use specific enzymes and produce certain end products that are greatly sensitive to any kind of malformation. Therefore, these enzymes and products are used as biochemical markers for any hepatic disorders. Some common markers to detect liver injury are bilirubin, alkaline phosphatase, alanine aminotransferase, lactate dehydrogenase, and aspartate aminotransferase (Gowda *et al.*, 2009).

Several studies have been conducted to understand the toxicological profile of neonicotinoids. It has been seen to cause a toxicological impact in liver, immune system, reproductive system and several others, in animal models. The subsequent sections discuss the lethal and sub-lethal adversities in the liver of different animal models due to exposure to various types of neonicotinoids. This review also includes instances that have indicated towards accumulation of neonicotinoids residues and damage caused in human samples.

Table 1. Different types of neonicotinoids used in agricultural practices (Watts, 2011)

Types of Neonicotinoids	Chemical Structure	Target Pests	Crops
Imidacloprid		Jassids, Aphids, Termites, Shoot fly, Mealybugs, Planthoppers, Whitefly	Cotton, rice, sugar, vegetables, oilseed rape, fruits, cereals
Thiamethoxam		Mealybugs, leafminers, Aphids, Stem borers, Gall midge, Jassids, Leaf folder, Termites, Planthoppers	Potato, cotton, tobacco, sorghum, vegetables, rice, fruits, cereals, tea, maize, ornamental
Clothianidin		Jassids, Woolly Aphids, Corn Rootworm, Brown Planthoppers, Whitefly	Potato, rice, cereals, cotton, oilseed rape, corn, sugar beets, maize
Acetamiprid		Aphids, Jassids, whitefly, Codling Moth, Diamondback Moth	Citrus fruits, grapes, vegetables, vines, potato, apples, cotton, ornamental plants
Dinotefuran		Soft scales, Thrips, Mealybugs, Aphids, Whitefly, Crickets, Cockroaches, Sawfly, Leaf miner	Leafy vegetables, rice, apples, sugar beets, potato, turf, cotton, fruits, home gardens
Thiacloprid		Aphids, Whiteflies, Thrips, Jassids, Bollworms, Codling moth, Diamondback moth, Pollen Beetle, Fruit Borer, Leafhoppers, Leaf miners	Cotton, cereals, ornamental plants, potato, apple, vegetables, oilseed rape, pears, rice
Nitenpyram		Fleas, Ticks	Rice, fruit, field crops, vegetables, tea

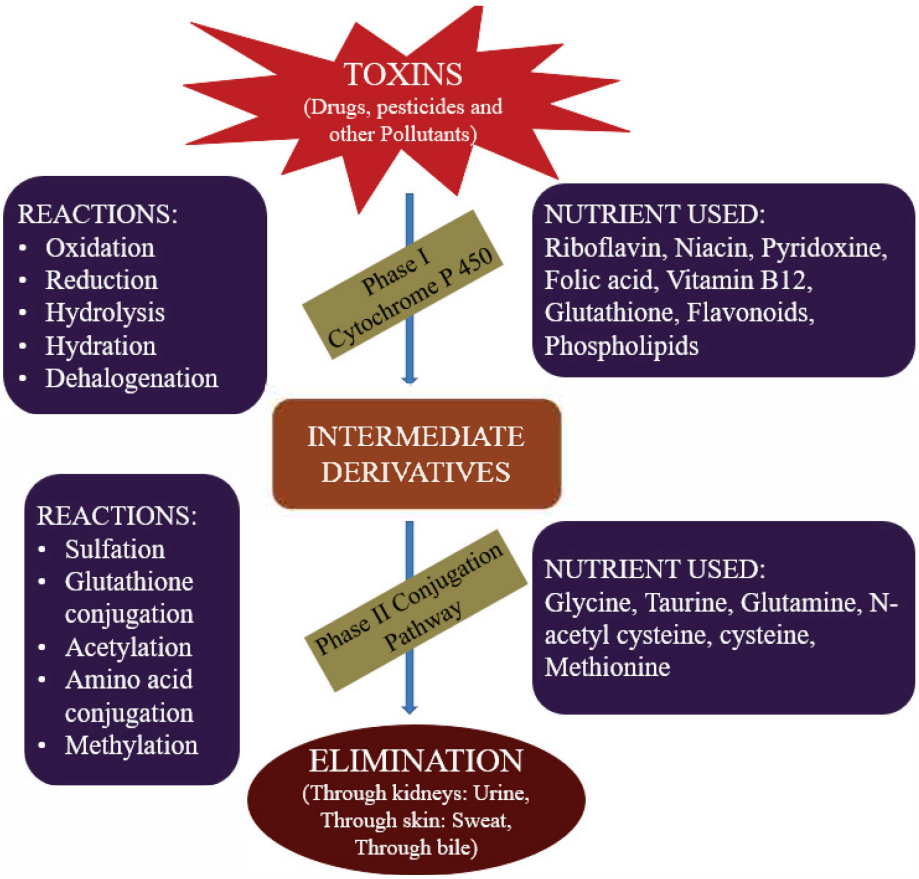


Fig 1. The detoxification process in the liver (Bhattacharya, 2015)

In-vivo Neonicotinoid Toxicity

Imidacloprid

A systemic neonicotinoid, imidacloprid is structurally related to nicotine and is frequently utilized to fight insect pests that cause severe threats to agricultural productivity (Arfat *et al.*, 2014). Imidacloprid protects crop and ornamental plants against sucking insects and mites. It is also used to control pests in households and veterinary applications against insect pests in poultry farms and fisheries (Simon-Delso *et al.*, 2015). Since imidacloprid was introduced as a safer alternative than other classes of insecticides, many studies were conducted to analyze the toxic effects of imidacloprid in various animal models.

Acute toxicity of imidacloprid was investigated at a concentration of 5 mg/kg when administered in male mice for 14 days. Significant elevation was observed in AST, ALT, and MDA levels, whereas there was a drastic dip in the activities of GSH, SOD, CAT, and GPx. Inflammation was also observed in the hepatic cells due to significant upregulation in TNF-α expression. Transmission electron microscopy revealed apparent swelling of the ER structure and the perinuclear cistern in the liver tissue (Shao *et al.*, 2020). Similar results were observed after administration of imidacloprid at three doses with concentrations 5, 10, and 15 mg/kg/day for 15 days in mice. At the concentration of 15 mg/kg/day, notable elevation was reported in serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), and total bilirubin (TBIL) levels in serum along with an increase in bilirubin after 15 days of exposure. Mild focal necrosis with swollen nuclei, cytoplasmic lesions, and hypertrophied blood vessels was also reported in the histopathological analysis (Arfat *et al.*, 2014). Comparable liver injury such as cell necrosis, vascular swelling, disordered hepatic cords, and the presence of a pyknotic nucleus were also reported in female KM mice at 20mg/kg/day ($1/7^{th}$ LD50) concentration of imidacloprid after 28 days of exposure. At the same concentration and duration, biochemical parameters confirmed hepatic injury and deterioration

as plasma ALT, AST, and ALP were seen to be notably elevated. Subsequently, piloerection, diarrhea, salivation and notable depletion of net body weight, were also reported in female KM mice when exposed to a repeated higher dose (20 mg/kg/day) of imidacloprid (Zheng *et al*., 2020).

Hepatotoxic effects of imidacloprid were also observed in male albino rats. Imidacloprid at concentration of 0.21 mg/kg body weight (1/100th LD50) (Mohany *et al*., 2011) and 80 mg/kg body weight (Soujanya *et al*., 2013), when orally administered in male rats for a period of four weeks resulted in notable elevation in two major hepatic injury markers i.e., aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Apart from these markers, 0.21 mg/kg body weight imidacloprid caused significant increase in other parameters such as alkaline phosphatase (ALP) and Malondialdehyde (MDA). A dense congestion in the central veins and blood sinusoids in the liver tissue of rats were also observed. Presence of pyknotic nuclei were observed throughout the hepatic cells, as well as instances of leukocyte infiltration throughout liver tissue was seen (Mohany *et al*., 2011). Imidacloprid at 80 mg/kg body weight for four weeks also lead to significant decline in total protein levels and reduced glutathione (GSH) activity in rat liver. Histopathological analysis reported severe congestion of central and portal veins along with sinusoidal spaces was also reported in the histopathological analysis. Ultra-thin sectioning also disclosed varied size and shape of mitochondria, disrupted chromatin, rough endoplasmic reticulum, and swollen nuclei in the hepatic cells (Soujanya *et al*., 2013).

In the same year, a study reported hepatotoxicity due to imidacloprid in female albino rats aged three months in which they were administered with 45 mg/kg b.w. (1/10th LD50) and 9 mg/kg b.w. (1/50th LD50) imidacloprid over four weeks. The exposure to dose 1/10th LD50, i.e., 45 mg/kg b.w., caused a significant decline in the average feed intake, leading to a notable decrease in the growth rate and weight of the liver. Overall body weight also declined. At the same concentration, dose-dependent histopathological changes were also observed in liver tissue, such as the presence of pyknotic nuclei, dilation between the central vein and the sinusoid spaces followed by infiltration of leucocytic inflammatory cells. Levels of ALT and ALP were elevated in the plasma, and AST was elevated in the liver tissue (Toor *et al*., 2013).

Adverse impacts of neonicotinoids were also observed in birds in many countries like India, USA, and France, where neonicotinoids were used for the first time and faced major outbreaks in the migratory avian population (Mason *et al*., 2013). A study to observe the impact of imidacloprid exposure on Japanese quails (*Coturnix japonica*) was conducted by Emam and co-workers in 2018. Japanese quails are a substantial domesticated avian population used commercially for egg and meat production (Ahmed *et al*., 2015). Quails were fed with 3 mg/kg b.w. (1/10thLD50) imidacloprid for 30 days. The levels of AST and ALT elevated, whereas the relative liver weight significantly lowered. Lipid profile analysis showed an increase in triglycerides (TGC), LDL-C, liver tissue MDA, and a decrease in SOD, GSH, and HDL-C levels. In histopathological examination, hydrophobic vacuolar deterioration in the cytoplasm, vessel congestion, and sinusoidal disruption was also reported (Emam *et al*., 2018).

Thiamethoxam and Clothianidin

Thiamethoxam was introduced into the market in 1999, and it replaced imidacloprid in many parts of the world (Simon-Delso *et al*., 2015). It is a second-generation neonicotinoid and comes under the subclass thianicotinyl. Its slightly varying structure provides it the property of being a higher water-soluble compound (Fishel, 2005). Thiamethoxam is used commercially to destroy pests such as sucking and chewing insects in potato, rice, cotton, fruits, cereals, sorghum, maize, vegetables, tea, and many ornamental plants (Prabhaker *et al*., 2011).

However, some studies in the last two decades have reported toxicological effects of thiamethoxam in non-pest organisms. In a study conducted in 2018, severe hepatotoxicity of thiamethoxam in rabbits was reported by El-Okle and coworkers. There was a significant decrease in the absolute body weight and elevation in the relative and absolute weight of the liver after 3 ml/kg b.w. (equivalent to 250 mg thiamethoxam/kg b.w.) of exposure for a span of 90 days. Also, a drastic elevation was observed in all the serum enzyme parameters AST, ALP, ALT, GGT, and LDH, followed by increased bilirubin levels in the serum. At the same concentration oxidative stress in the liver was reported as levels of MDA were elevated with subsequent increases in CAT and GST activities. The potential pro-carcinogenic effects of thiamethoxam were also elucidated in this study. The results showed a 1.6-fold increase in the mRNA expression of pro-inflammatory cytokine IL-6 and a 1.8-fold increase in the mRNA expression of Bcl-2, which is a pro-apoptotic marker. The over expression of Bcl-2 may lead to lowering mRNA expression of tumor necrosis

factor (TNF-α), which can elevate the chances of toxicity and carcinogenicity in the hepatic cells of rabbits. Predominant fatty infiltration and fat cysts production with subsequent lymphocyte infiltration, narrowing of sinusoids, and fibrosis of the central vein were observed in the histopathological analysis (El-Okle *et al.*, 2018).

Carcinogenicity of thiamethoxam in male Tif:MAGf mice was observed by chronic exposure of thiamethoxam at concentrations of 200, 500, 1250, and 2500 ppm for a span of 50 weeks. The outcome showed clear instances of liver tumors in mice. After 20 weeks of exposure, elevation was reported in the hepatic cell replication rate at 500, 1250, and 2500 ppm doses. Subsequently, cancer bioassays showed hepatic tumors at the end of 50 weeks at these three concentrations (Green *et al.*, 2005). Oral exposure of thiamethoxam (20 mg/kg b.w.) for 35 days in non-target lizards demonstrated a notable decline of nuclei in the hepatic tissue and mild fibrosis in the histopathological analysis. Residual traces of thiamethoxam and its metabolites were detected in all the tissues of the lizards (Wang *et al.*, 2019). Thiamethoxam treatment in male Wistar rats at 100 mg/kg b.w. reported various preliminary symptoms of toxicity such as changes in appetite, mild tremors, diarrhea, and dyspnea. In addition, there was a notable decline in the body weight, but the relative and absolute liver weight increased significantly. Further analysis showed enzymatic changes in the liver as activities of AST, LDH, ALT, and GGT were higher as compared to the control. On the other hand, glucose levels, total protein, and albumin decreased drastically. Oxidative stress was also observed in the liver as significant elevation was observed in PCO, MDA, and AOPP and a tremendous simultaneous decline in the activities of CAT, GSH, NPSH, GPx, and SOD. Hepatic steatosis, hepatocyte vacuolization, and leukocyte infiltration were also observed in the liver cells (Feki *et al.*, 2019).

Clothianidin, just like thiamethoxam, is also a chlorothiazolylmethyl (Ford and Casida, 2006) and is categorized as "CAG 2a and 2b-hepatocellular hypertrophy and fatty changes". Its application in the agricultural fields raised severe concerns regarding adverse effects on bees to the extent that it was banned in Europe for outdoor use in the year 2018 (Alarcan *et al.*, 2020). Metabolism of thiamethoxam and clothianidin is deeply interlinked as clothianidin serves as an important metabolic intermediate in the breakdown pathway for thiamethoxam in insects, mammals, and even plants. A schematic reaction involving thiamethoxam to desmethyl-thiamethoxam or clothianidin to desmethyl-clothianidin has been reported to contribute to hepato-carcinogenicity seen in mice (Ford and Casida, 2006). An acute exposure of clothianidin in adult female Wistar rats resulted insignificant elevation in the absolute and relative liver weight, in the mid (225 mg/kg b.w./day), high (288 mg/kg b.w./day), and very high dose (350 mg/kg b.w./day) groups. Inflammatory cell infiltration, hepatocytic hypertrophy, and degeneration of cytoplasm were reported in liver tissue sections under the microscope (Alarcan *et al.*, 2020).

Acetamiprid

Acetamiprid is a third-generation choropyridinyl neonicotinoids (El-Bialy *et al.*, 2019), which is now utilized to target sucking type pests of leafy vegetables like cabbage, citrus fruits, soft-seeded fruits, various flowers, and ornamental plants. Due to the high-water solubility of acetamiprid, it is very likely to enter the human body through the plants as it easily gets absorbed by plants. After it is ingested, residual acetamiprid gets stored in high concentration inside major organs like the liver, kidney, thyroid, and adrenal glands (Karaca *et al.*, 2019).

Acetamiprid exposure at concentration 2.14 mg/kg b.w. ($1/20^{th}$ LD50) in rats was tested after 45 days. Oxidative stress was reported in the liver as a notable increase in the levels of MDA and LPO was observed. The antioxidant system was disrupted in the liver tissues as GSH and CAT activities were lowered significantly. Insecticide also damaged the liver tissues, which showed necrosis, infiltrated with macrophages, and sinusoidal monocytosis (El-Bialy *et al.*, 2019). In another study, Chakroun and co-workers conducted 60 days of sub-chronic exposure to three doses of 10.8, 21.7, and 43.4 mg/kg (1/20, 1/10, and 1/5 of LD50) acetamiprid orally in rats. An increase in the activities of biochemical parameters such as ALT, ALP, LDH, and AST indicated hepatocellular damage. Notable decline in the SOD and CAT levels and subsequent elevation in the LPO clearly showed oxidative stress and disruption of the antioxidant system in the liver tissue. Vacuolization, dilatation of sinusoids, hypertrophy of the central vein, dilated portal triads, and necrosis was also reported in the hepatic cells (Chakroun *et al.*, 2016). Similarly, Karaca and co-workers conducted a sub-chronic study to assess the hepatotoxicity of acetamiprid in male Sprague Dawley rats for a span of 90 days. Three different concentrations of acetamiprid were orally administered to the rats, i.e., 12.5, 25.0, and 35.0 mg/kg/day. Elevation in the liver weight was observed, and a drastic decrease in cholesterol level was reported in the groups with

doses of 25.0 and 35.0 mg/kg/day. The decline in the GSH level and elevation in the LPO and MDA markers showed oxidative stress in the liver. Histopathology of liver tissues showed the presence of pyknotic nuclei, degenerative pattern of cells, acidophilic cytoplasm, and significant hepatic glycogenation in both higher dose groups (25.0 and 35.0 mg/kg/day) (Karaca *et al*., 2019). In yet another study, acetamiprid was administered intraperitoneally in rats for an acute exposure of one week at a dose of 5 mg/kg. Severe oxidative damage was observed after the dosage as GSH and Total Antioxidant Capacity (TAC) declined tremendously, followed by a notable increase in the GST, CAT, and LPO markers. A histopathological study further confirmed hepatic injury that reported extreme congestion, sinusoidal dilatation, bile duct hyperplasia, necrosis, and inflammatory cell infiltration (Khovarnagh and Seyedalipour, 2021).

Other Neonicotinoids

Thiacloprid is another neonicotinoid used as effective pest control in agricultural fields, ornamental plants, and flowering plants. To analyze the hepatotoxic effects of thiacloprid, a study was conducted in which the insecticide was fed to male rats at a concentration of 62.1 mg/kg ($1/10^{th}$ LD50) via oral gavage for 60 days. Elevation in the levels of MDA, 8-hydroxy-2-deoxyguanosine, and Nitric oxide (NO) was found to be significant. Also, considerable suppression was reported in CAT, GSH, and SOD activities. Notable elevation in the serum transaminases (AST and ALT) activities and decline of serum globulin, total protein, and albumin were observed, indicating hepatic injury. Expression of high mobility group box protein-1 gene and inducible nitric oxide synthase genes were significantly upregulated. Histopathological alterations such as hydropic degeneration, coagulative necrosis, congestion in hepatic sinusoids, and central and portal veins were observed (Abou-Zeid *et al*., 2021). Oxidative damage was reported in adult male rats when exposed to 22.5 mg/kg thiacloprid over a period of 30 days. AOPP (Advanced oxidation protein product) and MDA levels were found to be elevated, and subsequent significant depletion was observed in GSH levels. Liver tissue damage like necrosis, leucocytic infiltration, and hepatocyte vacuolization was reported after 30-day exposure to thiacloprid (Kammoun *et al*., 2019).

Nitenpyram is a nitromethylene neonicotinoid that is used to control ticks and fleas found in domestic pets and animals which are closely associated with humans. A study conducted on juvenile Chinese rare minnows; they are considered to be a new emerging animal model for studying aquatic toxicity. The fishes were exposed to nitenpyram and dinitrofuran at three concentrations of 0.1, 0.5, and 2.0 mg/l for a period of 60 days. Severe oxidative stress was observed in fish at all different doses as reported in table 2 (Tian *et al*., 2020).

Table 2: Oxidative stress due to thiacloprid exposure in juvenile Chinese rare minnows (Tian *et al*., 2020)

Oxidative Markers	Nitenpyram (mg/l)	Dinotefuran (mg/l)
SOD	Increased at 0.5 and 2.0	Increased at 0.5 and 2.0
CAT	Dose-dependent decrease at 0.1, 0.5, and 2.0	Decrease at 0.1 and 2.0
MDA	Increased at 0.1	Decreased at 0.5 and 2.0
GSH	Increased at 0.1, 0.5, and 2.0	Increased at 0.1 and 2.0

Impact of Neonicotinoid in Humans

Hepatotoxic impacts of neonicotinoids have been extensively observed in various animal models as discussed above. However, there have been several studies which have shown the residual accumulation of neonicotinoid class of insecticides in different human samples such as serum, urine and hair (Table 3). Correlation between hair sample of people and soil sample of the same locality were analyzed for presence of neonicotinoid residue. Concentration of thiamethoxam was well correlated with the soil in that locality and hair residues of people. Thiacloprid residues were detected in 15% hair samples but were not detected in soil samples. These findings indicated that there is an alternate route of thiacloprid exposure among the people, possibly via intake of agriculturally based eatables and drinks (Bonmatin *et al*., 2021). A team from Japan demonstrated the presence of eight neonicotinoids and three metabolites of acetamiprid in human urine and serum samples (Yamamuro *et al*., 2014). Whereas, oxidative damage was observed in whole blood DNA of 80 different farmers who had been handling and spraying neonicotinoids (mainly imidacloprid and thiacloprid) in agricultural fields. The occurrence and extent of DNA damage was seen to be directly related to the persistence of application (Koureas *et al*., 2014). Disruption of respiratory function was observed in neonicotinoid spraying 89 farmers as compared to the 25 non -spraying farmers. Decline in total lung capacity,

residual volume and functional residual capacity was observed in the spraying farmers (Hernandez *et al.*, 2008).

Table 3. Various studies conducted to evaluate neonicotinoid impact in humans

Type of Neonicotinoids	Impact of Neonicotinoid	Type of Human Sample	Reference
Thiamethoxam and Thiacloprid	Residual accumulation	Hair	Bonmatin *et al.*, 2021
Acetamiprid, Clothianidin, Dinotefuran, Flonicamid, Imidacloprid, Nitenpyram, Thiacloprid and Thiamethoxam	Residual accumulation	Urine and Serum	Yamamuro *et al.*, 2014
Imidacloprid and Thiacloprid	Oxidative damage	Blood	Koureas *et al.*, 2014
Neonicotinoids (type not specified)	Respiratory dysfunction	Lungs	Hernandez *et al.*, 2008

Conclusion

The widespread utilization of neonicotinoids poses an additional consequential threat to the environment and organisms due to their properties like high water solubility and high persistence. The broad application of neonicotinoids has also led to widespread human exposure. There is evidence of neonicotinoid accumulation in human samples like hair, urine, and serum along with some damage to the whole blood DNA and respiratory system. The metabolism or breakdown of these chemical pesticides occurs in the liver upon encounter, and the possibility of hepatic injury increases to a greater extent. Therefore, studies were carried out to assess the hepatotoxicity of various neonicotinoids in animal models. Imidacloprid, the most utilized neonicotinoid, was found to cause hepatotoxicity in rats, mice, and birds. The second most used neonicotinoid, namely Thiamethoxam and Clothianidin, a major metabolite of thiamethoxam, was found to be potent hepatotoxic and hepatocarcinogen in rats. Hepatotoxicity of thiamethoxam was also observed in rabbits. Acetamiprid and thiacloprid exposure also led to hepatotoxicity and oxidative stress in rats. Whereas nitenpyram and dinotefuran led to severe oxidative stress in the fish liver, indicating the impact of the insecticide on aquatic fauna. However, the investigations carried out to date are not sufficient and are still in progress. Further, large-scale studies need to be conducted to confirm the adverse impact of neonicotinoids.

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