



Review Article

INSIGHTS INTO THE ECOTOXICOLOGICAL EFFECTS ASSOCIATED WITH IMIDACLOPRID: A REVIEW

^{a,b,*}Stephy Rose K.V, ^aNiladri Sekhar Chatterjee, ^aSuseela Mathew, ^aAnandan Rangasamy

^aICAR-Central Institute of Fisheries Technology, Matsyapuri P.O., Cochin - 682029, Kerala, India

^bFaculty of Marine Sciences, Cochin University of Science and Technology, Cochin - 682016, Kerala, India

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ABSTRACT

Imidacloprid is a neonicotinoid insecticide contently used in agricultural fields with excellent systemic and contact activity, used in the largest volume worldwide against sucking pests of Diptera, Coleoptera and Lepidoptera in chilly, cotton, grapes, groundnut, okra, paddy, sugarcane, sunflower and tomato. It functions as an agonist at the acetylcholine receptors of the pest, affecting invertebrate movements, leading to palsy and mortality. It has an approbative toxicity profile, due to its poor penetration of the blood–brain barrier in vertebrates. Moreover, it does not exhibit any mutagenic, carcinogenic, teratogenic or immunotoxic properties. Besides these boons of imidacloprid, several studies reported the high leaching potential and persistence of imidacloprid in the ecosystem, creating threat to non-targeted organisms by altering their biochemical and reproductive processes.

Keywords: Imidacloprid, Properties, Environmental fate, Toxicity.

INTRODUCTION

Imidacloprid is the most renowned and widely used neonicotinoid registered for agricultural and residential usages since past three decades. It is contently used for crop protection, termite control and dermal application on animals. The excellent biological properties, mainly a low application rate, wide spectrum and quick uptake make imidacloprid more promising. Imidacloprid is marketed under trade names Advantage, Admire, Confidor, Hachikusan, Kohinor, Merit, Gaucho, Premise, Prothor, and Winner. The neonicotinoids were brought to light in the 1970s to control pests, by chemists at Shell Chemical Company, while evaluating the insecticidal potential of heterocyclic nitromethylenes (Schroeder and Flattum, 1984). The term “neonicotinoid” was introduced to distinguish from the nicotinoids, as the former is highly efficient as insecticides and less toxic to vertebrates than the later. In 1984, chemists at Nihon Bayer Agrochem discovered a moiety named imidacloprid (CAS 138261-41-3), with greatly increased insecticidal activity, maintaining its photostability, while working with a 3-pyridylmethyl group on the nitromethylene heterocycle structure (Shiokawa *et al.*, 1994). It was first enrolled as an

insecticide for agricultural application since 1994 in the U.S.

Structure

Neonicotinoid is structurally 6-chloro-3-methylpyridine with a pharmacophore. Neonicotinoids are either nitroguanidines (C = NNO₂), nitromethylenes (C = CHNO₂), or cyanoamidines (C = NCN) (Compounds with 3-tetrahydrofuranmethyl, 2-chloro-5-thiazolylmethyl and 6-chloro-3-pyridinylmethyl moieties are called tefuryl, chlorothiazolyls or thianicotinyls and chloropyridinyls or chloronicotinyls, respectively. The pharmacophore of the nitroguanidine insecticide imidacloprid is nitroiminoimidazolidine. Imidacloprid, known as chloropyridinyls or chloronicotinyls or 1- (6 –chloro- 3 -pyridylmethyl) N – nitroimidazolidin-2-ylideneamine is a nitroguanidine insecticide of neonicotinoid family, with a unique structure (Figure 1). It was acquired by the combining reaction of 2-chloro-5-chloromethyl-pyridine with the 2-nitro-imino-imidazolidine, in acetonitrile with potassium carbonate as the base. The nitrogen atom of the

*Corresponding Author: Stephy Rose K.V, ICAR-Central Institute of Fisheries Technology, Matsyapuri P.O., Cochin -682029, Kerala, India. Email: stephyrose512@gmail.com.

chloropyridine moiety of imidacloprid reacts with the hydrogen donor –nicotinic acetylcholine receptors (nAChR), and the nitrogen atom at the 1-position of the imidazolidine 5-ring reacts with a negatively charged domain (Okazawa *et al.*, 2000). Binding of imidacloprid to the nicotinic receptor is irreversible, which results in overstimulation of the neurons.

Several other chemical analogues of imidacloprid such as acetamiprid, nitenpyram, thiacloprid, etc. were also developed for commercial use soon after the discovery of imidacloprid. Later, a “second generation” of neonicotinoids were introduced by replacing the chloropyridinyl moiety with a chlorothiazolyl group to reduce its activity at mammalian receptors. The major members of the second generation include Thiamethoxam and Clothianidin. Further research on the compound led to the discovery of Dinotefuran with a characteristic Acetylcholine moiety without a pyridine ring, which started the era of “third-generation” neonicotinoids. Other members of the third generation neonicotinoids include Sulfoxaflor and Cycloxaprid.

Physical and Chemical Properties

Understanding the physical and chemical properties of imidacloprid (Table 1) is important to know the biochemical interactions of imidacloprid with biotic and abiotic systems, which in turn helps in comparison of toxicological data and interpretation of future research.

Usage of Imidacloprid

Global annual trade of neonicotinoids reaches up to 1000 million dollars, contributing 11%–15% of the entire insecticide merchandise. Neonicotinoids are licensed for use in more than 120 countries, of which imidacloprid holds about 41% of the total neonicotinoid market. Imidacloprid is contently used for the pest in agricultural fields, flea control and termite control. The primary target of imidacloprid includes sucking pests (aphids, whiteflies, and leafhoppers). Excellent crop tolerance of imidacloprid allows its use on crops viz., chilly, cotton, grapes, groundnut, okra, paddy, sugarcane, sunflower and tomato (CSI, 2020). It is now considered a possible replacement for the insecticides under phase revocation due to its quick action on pests even at low doses. However, their large-scale use has raised growing concerns about their potential adverse effects on non target invertebrates. As a result, in December 2013, the European Union banned the use of imidacloprid for seed coating and soil treatment. Nevertheless, recently Environmental Protection Agency (EPA) announced that its use continues to be approved (US EPA, 2020). It used to prevent sucking insects on crops and seeds (Furlan & Tof- fanin, 1998). It used to prevent sucking insects on crops and seeds (Furlan & Tof- fanin, 1998).

Mode of application

Although imidacloprid could be applied by diverse methods viz., sprays, microcosm, glass plate treatment, leaf

dip of cotton, etc., the best mode of application is as a foliar spray. Imidacloprid is applied in paddy fields at a concentration of 0.003 ppm at a rate of 0.561 kg /ha. A major part of the imidacloprid applied is emitted to the environment causing toxic effects in non-targeted organisms (Naiel *et al.*, 2020). EPA categorizes neonicotinoids as both class II and class III toxicity agents labeled with “Warning” or “Caution” signs.

Neuromodulatory action of imidacloprid

Versatile properties of imidacloprid are increasing its usage than other insecticides. Imidacloprid is well-referred to as “chloronicotinyls” to highlight insecticidal activity of the chlorine atom. It functions as both contact and stomach poison. It binds to the $\alpha 4\beta 2$ subtype of the nAChRs (Figure 2.) in insects interfering with the nerve impulse resulting in twitching, muscle weakness and cramps, leading to paralysis, starvation due to impaired feeding and death (Alexander *et al.*, 2007). It was found highly effective on insects and less toxic to vertebrates especially mammals (Tomizawa and Casida, 2005). Imidacloprid is categorized as moderately harmful (Class II WHO; toxicity category II EPA).

Environmental dissemination

Pesticides could be released into the environment (Figure 3), as the ground water moves depending on their physical, chemical, and biological properties. Farm level studies by Tomizawa & Casida (2005) have shown that the application of imidacloprid into the crop field is imposing major threat to the environment as 80 to 98.4% of applied pesticide leaching into the surroundings. The high solubility of imidacloprid in water is directly proportional to its great leaching potential, which leads to incredible persistence in soils and aquatic sediments. Moreover, its half-life of up to one year poses a greater risk of contamination not only to riverine water bodies, but also a greater extent to groundwater system (Diaz *et al.*, 2017). The documentary reports of Environmental Protection Agency (1993) have indicated that imidacloprid is labelled as a category I pesticide along with 13 other turf insecticides due to its higher leaching potential from the soil environment into the aquatic system. Investigations by Rouchaud *et al.* (1994) had also demonstrated that around 97% of the imidacloprid applied on sugar beet seeds were found to be leached to the soil within 67 days after planting. A comparative study on the leaching potential of pesticides has indicated that imidacloprid is found to be the most leached pesticide into the environment as compared to other different pesticides, which includes chlorpyrifos, diazinon, diuron, etc. Other interest findings by Felsot *et al.* (1998) have shown that imidacloprid applied in a hop field drip irrigation system was detected at a maximum depth of 105 cm within 7 days after application.

Experimental investigations by Bonmatin *et al.* (2005) in different soil types showed that 97% out of 33 soil samples got exposure to imidacloprid coated seeds were found to retain the pesticide residues even after 1 or 2 years, indicating the persistence capability and non-

degradable nature of imidacloprid in the environment. Studies by Kreuger *et al.* (2010) on half-life of imidacloprid demonstrated that the shortest half-life of imidacloprid recorded was 107 days in Georgia in turf-covered soil. Interestingly, it was noticed that the concentration remained the same even after one year after treatment in cornfield soil in Minnesota (Kreuger *et al.*, 2010). Furthermore, a field study by Tyor & Harkrishan (2016) reported that 39% of the water samples collected from the greenhouse farming system of the experimental locations was found to be positive for the pesticide residue. Thus application of imidacloprid in the soil is a serious water contaminant concern, which resentfully influence whole aquatic ecosystem.

Factors affecting leaching property of imidacloprid includes its formulations, adjuvants, surfactants, etc. A comparative investigation by Gupta *et al.* (2002) on the leaching property of different formulations of imidacloprid had shown that soluble concentrate has the highest potential to leach through the soil than analytical grade and water-dispersible powder. A field study by Jemec *et al.* (2007) demonstrated that the distribution of imidacloprid in the environment is promoted by high alkalinity of water, low soil sorption, low octanol-water partition coefficient, hydrolysis and stable soil degradation. A recent study by Pang *et al.* (2020) revealed that the biodegradation (Figure 4.) is one of the most important processes controlling the fate and transformation of imidacloprid. Nevertheless, an earlier report by Tisler *et al.* (2009) had shown that the biodegradation of imidacloprid is relatively slow in aquatic environments.

Although imidacloprid is subjected to active photodegradation, Liu *et al.* (2006) had stated that this occurs only in well-sunlit water surfaces and the photodegradation and hydrolysis of imidacloprid produce imidacloprid-urea as an intermediate compound, which is a potent toxicant. Interestingly, imidacloprid-urea was found to be the predominant metabolite followed by olefine, nitrosimine, 6-chloronicotinic acid, 5-hydroxy and nitroguanidine. The use of imidacloprid in the agricultural fields had been deprived by the European Commission since 2013. According to the EFSA report, imidacloprid causes severe environmental hazards even at low concentrations, regardless of its boons, including its unique structure, target specificity and safety for its user.

Dissipation in the paddy field

Several researchers (Thuyet *et al.*, 2011a, 2011b; La *et al.*, 2015; Pereira *et al.*, 2017) have investigated the degradation of imidacloprid in the paddy fields under different environmental conditions. Dissipation of imidacloprid in paddy field was found to be biphasic first-order kinetics in the water, while single-phase first-order kinetics in soil (Thuyet *et al.*, 2011b). The highest recorded concentration of imidacloprid in surface soil and water collected from different paddy fields were 440 µg/kg and 60 µg/l, respectively. (Thuyet *et al.*, 2011a, 2011b; La *et al.*, 2015; Pereira *et al.*, 2017). Study by La *et al.* (2015) has indicated that around 21 - 68 % of the total applied mass of imidacloprid was leached into the surrounding aquatic system. The estimated concentration of imidacloprid was found to be 8.8 µg/L in the irrigation canal (Pereira *et al.*, 2017) and 83 µg /L in afflux of the stream (La *et al.*, 2015).

Environmental monitoring of imidacloprid and its metabolites by Akoijam & Singh (2014) has shown that residues of imidacloprid metabolites were found to be present upto 90 days, whereas imidacloprid could be detected only upto 60 days. The estimated DT₅₀ of imidaclopridin water and soil of paddy field ranged between 1.3-3.4days and 11.0-229 days, respectively (Thuyet *et al.*, 2011 a & b; Pereira *et al.*, 2017). Thuyet *et al.* (2011a) reported the biphasic dissipation of imidacloprid in water as 2.0 - 2.4 days during the initial phase (0 - 7 days) and 8.0 - 20.5 days in the later phase (7 - 35 days). Also Thuyet *et al.*, (2011b) recorded faster degradation of imidacloprid at a higher pH (pH 10) with a DT₅₀ of 44.7 days. Previously, studies by Fossen (2006) had reported the highest DT₅₀ value of 229 days for imidaclopridin soil. Experimental field study in rice plot conducted by Daam *et al.* (2013) revealed that application of imidacloprid induced 48 h-EC₅₀ immobility ¼84 mg/L for *Daphnia magna* and 6 d-EC₅₀ growth inhibition ¼0.01–0.015 mg/L for sediment-dwelling ostracod *Heterocypris incongruens*. This observation was in accordance with an earlier reported study (Sánchez-Bayo and Goka, 2006a), which recorded a significantly reduced abundance of both aquatic and terrestrial organisms in paddy fields with presence of imidacloprid residues at a level greater than 1 µg/L.

Table 1. Physical and chemical properties of Imidacloprid.

Chemical formula	C ₉ H ₁₀ ClN ₅ O ₂
Molar mass	255.66
Molecular weight	255.7 g/mol
Appearance	Colourless crystals
Vapour Pressure	2x10 ⁻⁹ hPa at 20 °C
Melting point	136.4 to 143.8 °C
Density	1.54 g/cm ³
Solubility in water	0.514 g/L at 20 °C
Stability	Stable to hydrolysis at pH 5-11
Log K _{ow}	0.57

K_{oc}	260 mL/g
Leachability	Moderate
Half life in water	0-365 days
Half life in soil	17-6931 days

K_{ow} = octanol water partition coefficient; K_{oc} = organic carbon partition coefficient.

Ecotoxicology

Ecotoxicology serves an important role in addressing aquatic ecosystem health challenges in parallel with the rapid advancement in industry and technological gallops. Prolonged exposure to imidacloprid affects non-target organisms in aquatic ecosystems. Toxicity study by Zeid *et al.* (2019) demonstrated that the aquatic organisms are highly perceptive to imidacloprid contaminated ecosystems because of their propensity to accumulate contaminants in their body via their permeable skin, gills and other intrinsic sensitivities. An earlier research by Nyman *et al.* (2013) had also reported the potentiality of imidacloprid to cause

lethality even at small concentrations by impairing motility and feeding in aquatic organisms. Metabolism of imidacloprid in organisms (Figure 5) preliminarily involves its oxidative cleavage to imidazolidine and 6-chloronicotinic acid. The former product gets excreted through urine and the later gets excreted as hippuric acid conjugate after degradation. The second route of imidacloprid metabolism includes hydroxylation and formation of an unsaturated metabolite. About 90% of the imidacloprid ingested is eliminated via urine (80%) and feces (20%) within 24 h and the total elimination occurs within 48 h (Sheets *et al.*, 2016).

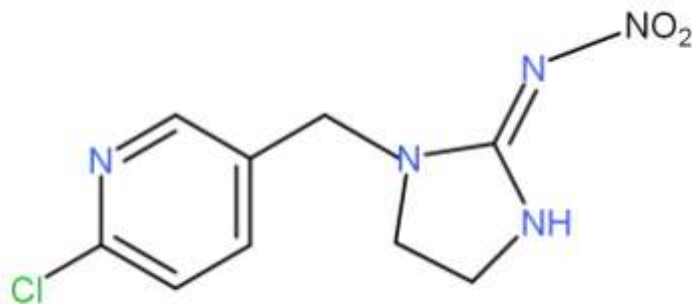


Figure 1. Structure of imidacloprid (Rose *et al.*, 2022) K_{ow} = octanol water partition coefficient; K_{oc} = organic carbon partition coefficient.

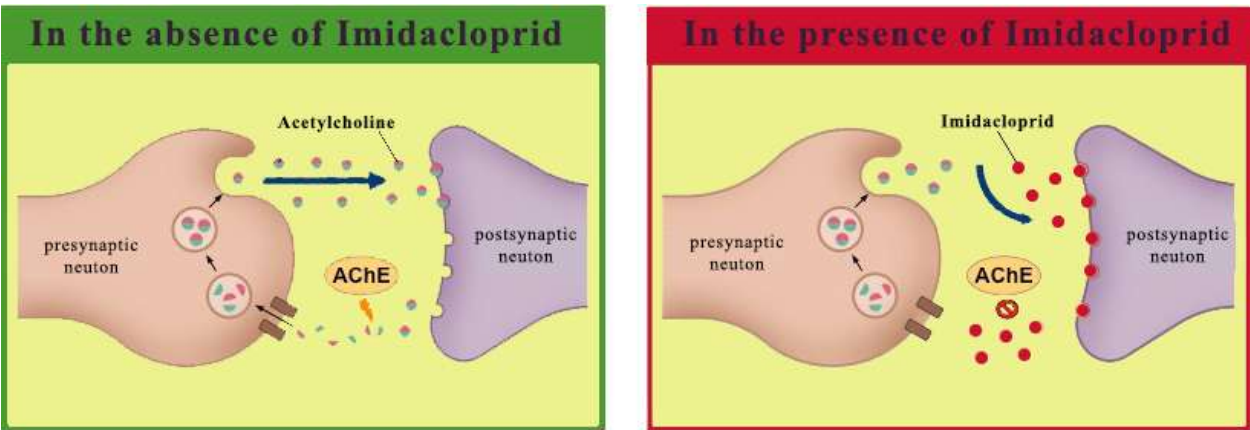


Figure 2. Neuromodulatory action of imidacloprid.

Acute toxicity

Acute toxicity studies are inevitable in evaluating the hazardous nature of environmental contaminants as it throws light on the health status of aquatic organisms in the contaminated ecosystem (Rose *et al.*, 2020). Nyman *et al.* (2013) had evaluated the toxicity of imidacloprid in aquatic crustaceans and recorded the LC_{50} values of *Hyaella azteca* and *Mysidopsis bahia* as 55 ppb and 37 ppb, respectively. Another study by Sánchez-Bayo and Goka, 2006b reported 48 h LC_{50} value of imidacloprid for cladocerans and ostracods as 65–133 mg/L and 301–715 μ g/L,

respectively and values of EC_{50} as 2–6 mg/L and 3–16 μ g/L, respectively. Chen *et al.*, 2010 reported LC_{50} of imidacloprid as 2.1 μ g/l in *Ceriodaphnia dubia*. Organisms that survived the exposure showed behavioral alterations such as, lethargy and loss of equilibrium. Records of EPA (1992) had shown that the exposure to imidacloprid at a very low concentration had reduced the growth rate, body size and fecundity in mysid shrimp. Another study reported a reduced number and diversity of invertebrate species in artificial ponds at a concentration of 5 ppb (EPA, 1992).

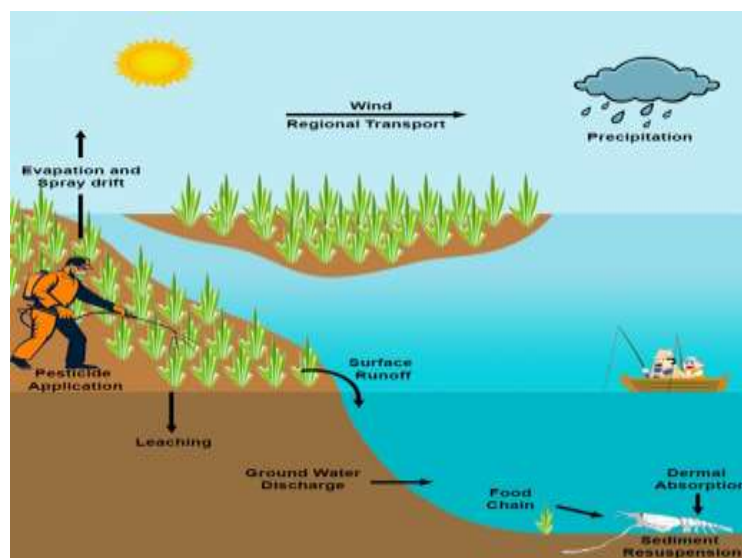


Figure 3. Environmental dissemination of imidacloprid.

The 96 h median lethal concentration of imidacloprid were determined for several fishes viz. *Labeo rohita* by Qadir *et al.* (2014) as 550 mg L^{-1} , *Tilapia* by Acar *et al.* (2018) as 141.42 mg L^{-1} , *Oncorhynchus mykiss* and *Cyprinus carpio* by Tisler *et al.* (2009) as 211 and 280 mg L^{-1} respectively. Su *et al.* (2007) investigated the toxicity of imidacloprid in *Paralichthys olivaceus* and revealed the median inhibitory concentrations (IC_{50}) of endpoint bioassays viz., neutral red (NR), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and total cell protein (TCP) as 41.86, 38.46, and 39.08 μ g/ml, respectively. They also found out that the mitochondria are the prime site of action of imidacloprid as the ultra structural observation of the gills cells exposed to 60 μ g/ml of imidacloprid for 48 h showed severely damaged mitochondria and normal nuclei and rough endoplasmic reticulum. Furthermore, Sanchez Bayo & Goka (2005) reported Stress syndrome in juvenile *Oryzias latipes*, Xia *et al.* (2016) demonstrated neurobehavioral disorders such as reduced swimming, loss of balance, etc. in *Misgurnus anguillicaudatus* and Crosby *et al.* (2015) studied toxicity of imidacloprid in various life stages of the *Danio rerio* viz., larvae, fingerlings and adult. Desai & Parikh (2013) illustrated that *Oreochromis mossambicus* and *Labeo rohita* exposed to sublethal levels of imidacloprid showed severe degeneration in the liver,

biochemical alterations in the gills, muscle and kidney. Besides, Tyor & Harkrishan (2016) reported that imidacloprid is potent to reduce the viability and hatchability of embryos of *C. carpio*, even in low concentrations (10% LC_{50}).

Biochemical alterations

Biochemical changes have prognostic value as it precedes the clinical manifestations of a disease situation. It broadens our understanding of how alterations in the chemical aspects of biological processes are related to physiological alteration in the body of an organism (Figure 6). Iturburu *et al.* (2018) have elaborated on the biochemical aberrations in organisms during stress. They demonstrated that organisms exposed to imidacloprid could induce oxidative damage, hypoglycemia and genetic anomalies. Laboratory study by Shan *et al.* (2020) revealed significant increase in antioxidant enzymes and MDA content in the gills and digestive glands of *Corbicula fluminea*. Sanchez-Bayo and Goka (2005) reported physiological stress in juvenile *O. latipes* treated with imidacloprid. Moreover, Vieira *et al.* (2018) revealed that the exposure to imidacloprid could reduce immunity and result in massive infestation by ectoparasite, *Trichodina* spp. In addition, Bonmatin *et al.* (2005) reported that

imidacloprid and its degradation products could be hazardous even at lower concentrations during chronic intoxication. Jemec *et al.* (2007) explained alterations in biochemical and reproductive parameters in *Daphnia magna* after long-term exposure to Imidacloprid. Another study by Priya *et al.* (2012) in freshwater teleost *Channa punctatus* reported significant elevation in the serum

Glucose, Cholesterol, Creatinine and Creatine and decrease in the serum Protein, Albumin and Globulin after 96 h exposure to imidacloprid due to metabolic dysfunction in the fish. Qadir *et al.* (2014) had elaborated the sub-lethal effects of imidacloprid on the biochemical composition of *L. rohita*.

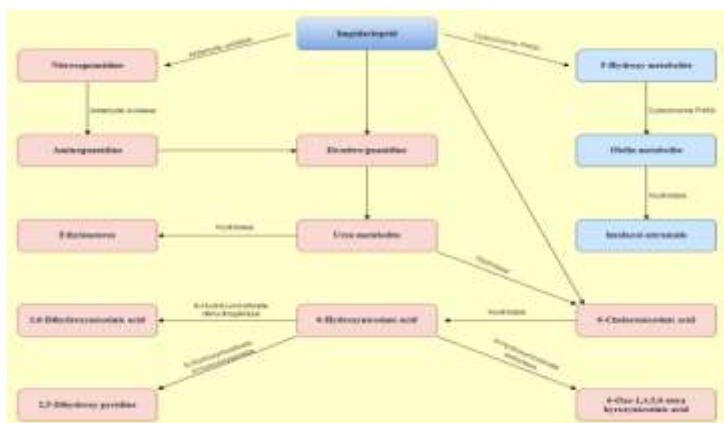


Figure 4. Biodegradation pathway of imidacloprid.

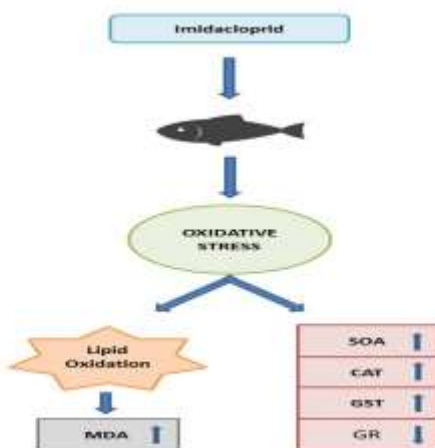


Figure 5.Metabolism of imidaclopridin organisms.

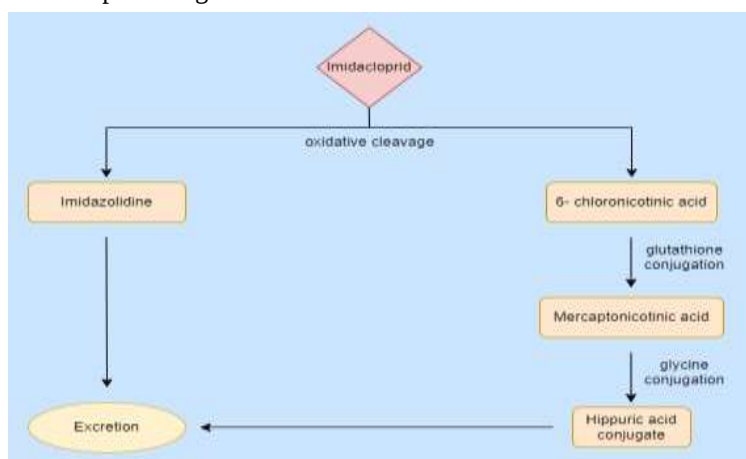


Figure 6. Oxidative stress caused by Imidacloprid.

Tripathi and Singh (2004) demonstrated that an organism in pesticide contaminated ecosystem require high energy to detoxify the toxicants and to overcome the stress, physiological and histological alterations. Assessment of the biochemical contents, mainly protein could be used as an effective tool to understand the health status of an organism under stress (Prasanth & Arivoli, 2008). Experimental investigations by Xia *et al.*, 2016 in *Misgurnus anguillicaudatus* and Desai & Parikh, 2013 in *O. niloticus* demonstrated that the exposure to imidacloprid cause hepatotoxicity. They found out that the alterations in

the hepatic cell membrane permeability results in the loss of Alanine and Aspartate amino transaminases (ALT & AST) into the circulatory system, reducing the activation of aminotransferases in the liver. Several studies (Balint *et al.*, 1995 and Singh *et al.*, 2001) reported the increase in AST & ALT as a sign of tissue damage in the gill, kidney and liver. Hence, assessment of AST & ALT activity is of clinical and toxicological importance as its alterations are indicative of hepatic damage caused by pollutants or in diseased conditions.

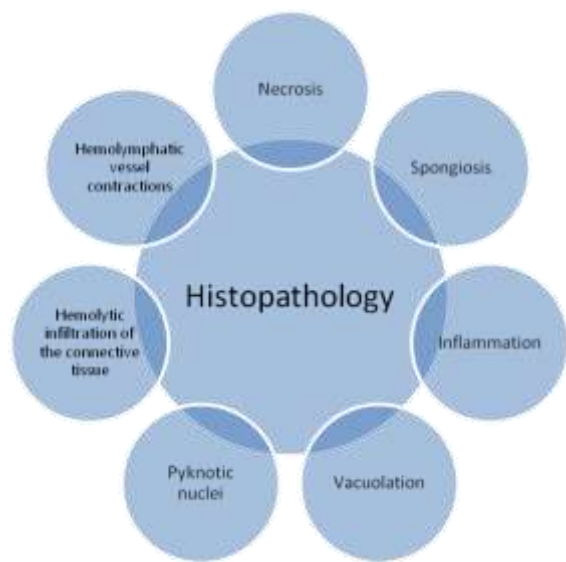


Figure 7. Histopathological alterations during imidacloprid toxicity.

Table 2.Bioconcentration factor of imidacloprid in aquatic organisms.

Common name Scientific name	Exposure time	Tissue ^a	Exposure concentration	Bioconcentration factor ^b	References
zebrafish	14 days	NR	0.5 mg/L	1.52 mg/L	Ding <i>et al.</i> , 2004
<i>Brachydanio rerio</i>	14 days	NR	5 mg/L	0.97 mg/L	Ding <i>et al.</i> , 2004
Cichlid fish	48 h	Brain	300 mg/L	0.9± 0.3 L/kg	Iturburu <i>et al.</i> , 2017
<i>Australoheros facetus</i>	48 h	Blood	300 mg/L	0.6± 0.4 L/kg	Iturburu <i>et al.</i> , 2017
	48 h	Gills	300 mg/L	0.4 ±0.1 L/kg	Iturburu <i>et al.</i> , 2017
	48 h	Muscle	300 mg/L	0.4 ±0.1 L/kg	Iturburu <i>et al.</i> , 2017
	48 h	Gut	300 mg/L	1.3 ±0.3 L/kg	Iturburu <i>et al.</i> , 2017
	48 h	Liver	300 mg/L	1.4 ±0.4 L/kg	Iturburu <i>et al.</i> , 2017
Freshwater oligochaete	1 day	NR	0.1 µg/L	66*	Contardo-Jara & Gessner, 2020
<i>Lumbriculus variegatus</i>	5 days	NR	0.1 µg/L	190*	Contardo-Jara & Gessner, 2020
	1 day	NR	1 µg/L	28*	Contardo-Jara & Gessner, 2020
	5 days	NR	1 µg/L	119*	Contardo-Jara & Gessner, 2020
	1 day	NR	10 µg/L	26*	Contardo-Jara & Gessner, 2020
	5 days	NR	10 µg/L	75*	Contardo-Jara & Gessner, 2020

^a Not reported (NR). ^b Unit not mentioned (*).

Researches (Pan & Dutta, 1998 and Huynh *et al.*, 2009) proposed acetylcholinesterase activity as a biomarker to assess the pesticide toxicity. Rao *et al.* (2003) reported the inhibition of acetylcholinesterase activity in the brain of fish exposed to pesticides. Van der Wal & Welling, 1988 demonstrated that the inhibition of acetylcholinesterase activity could result in mortality of the organism. A recent study by Guerra *et al.* (2021) reported increased Glutathione S-transferase (GST) activity & carbonyl protein (CP), AChE activity in the brain and decreased locomotory movements in zebrafish after 96 h exposure to imidacloprid. Kappus (1987) reported Cholinergic hyperactivity instigated by the restraint of the AChE initiates the aggregation of reactive oxygen species (ROS), resulting in oxidative stress and lipid peroxidation, which lead to cell injuries. Sies (1986) defines oxidative stress as the disparity between the production and the elimination of ROS by the antioxidant system. McCarthy and Shugart (1990) have prescribed the use of antioxidant biomarkers for environmental monitoring programs. In addition, Cajarville *et al.* (2000) have reviewed the use of antioxidant biomarkers in assessing the environmental contaminants in the field studies. Oakes *et al.* (2004) have also revealed the importance of lipid peroxidation (LPO) as a predictive biomarker in assessing pollution.

Young-Lai *et al.* (1991) recommended the osmoregulatory capacity of crustaceans as a potential tool that forecasts their physiological status during stress. Na^+/K^+ ATPase plays an important role in branchial epithelial ion transport. Maintenance of a steady Na^+/K^+ gradient is necessary for the metabolic uptake of glucose & amino acids, transmembrane movement of Calcium ions during muscle stimulation, regeneration of transmembrane potential and safeguard of osmotic equilibrium in cells via controlled transcellular ion movements. De La Torre *et al.* (1999) reported inhibition of Na^+/K^+ ATPase activity in fishes during short-term exposure to pollutants in the laboratory as well as field conditions. An investigation by Blažič *et al.* (2005) in *Porcellio scaber* observed feeding impairment and altered GST activity after 2 weeks of exposure to imidacloprid. Another experiment conducted in *P. scaber* by Drobne *et al.* (2008) reported alterations in survival rate, feeding rate, weight gain, GST, total protein content, and epithelial thickness of the digestive gland. Lukančič *et al.* (2010) investigated physiological responses of *Asellus aquaticus* and *Gammarus fossarum* to imidacloprid and reported increased respiration and decreased electron transport system activity.

Histopathology

Histopathology is a potent tool to analyze and describe the biological effects (Figure 7) of a toxicant on an organism. Histopathological alterations increase with the concentration of toxicant and exposure period. Shan *et al.* (2020) elaborated the histopathological alteration caused by imidacloprid in gills and digestive glands of *Corbicula fluminea*. Severe hemolymphatic vessel contractions and adhesions, moderate epithelial cellular swelling and damage of cilia were reported in gills. Furthermore,

hemolytic infiltration of the connective tissue, severe degeneration of digestive tubules and necrosis of epithelial cells were observed in digestive glands of *C. fluminea*. The findings of Harkrishan *et al.* (2020) depicted several histopathological degenerations in the eyes (ruptured lens tissue and declined retinal pigmentation), gills (ruptured arterial wall, uplifted sclerotic layer and degenerated lamellae) and brain including necrosis, spongiosis, vacuolation, fragmented nuclei, pyknotic nuclei and mononuclear infiltration in hatchlings of *C. carpio*. Similar alterations were also observed by Naiel *et al.* (2020) in *Oreochromis niloticus* and Xia *et al.* (2016) in *Misgurnus anguillicaudatus*. Moreover, Qadir *et al.* (2014) reported moderate-to-severely damaged gills, heart, liver and kidney of *L. rohita*.

Bioconcentration

Bioconcentration refers to the direct transfer of chemicals from the surrounding environment into the organism via gills or other permeable membranes. The Bioconcentration of pesticides in aquatic organisms causes severe ecological problems. Hence bioconcentration is one of the essential tools in the pesticide toxicity assessment in aquatic organisms. Bioconcentration of several pesticides has been studied in different organisms (Sancho *et al.*, 1998; Amrani & Pena - Abaurrea, 2012; Zhang *et al.*, 2020). Bioconcentration factors give an estimate of the relative uptake of the chemicals from the environment by organisms. Bioconcentration factors for imidacloprid (Table 2.) had been calculated in several organisms including *Brachydanio rerio* (Ding *et al.*, 2004), *Australoheros facetus* (Iturburu *et al.*, 2017) and *Lumbriculus variegatus* (Contardo-Jara & Gessner, 2020).

CONCLUSION

Review on the ecotoxicological aspects of imidacloprid includes the baseline information regarding the neonicotinoid insecticide imidacloprid, its versatile uses, environmental fate and its toxicological effects on non-targeted organisms. Imidacloprid, being contently used globally at a large scale, with higher leaching potential than other widely used pesticides and persistence of more than a year causes potential risks for non-target organisms, especially aquatic invertebrates. Many researches portrayed the toxicity of imidacloprid on several beneficial species. This paper provides a critical revision of toxic sequel caused by imidacloprid in the biota and recommends to reduce the application of imidacloprid in agricultural fields by developing a better pest management practice that supports the sustainable development of biodiversity, to quench the increasing global food demand.

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