Journal of
Fisheries and Aquatic Science

ISSN 1816-4927

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Journal of Fisheries and Aquatic Science
ISSN 1816-4927
DOI: 10.3923/jfas.2024.11.18

Research Article
Efficacy of a Neonicotinoid, Imidacloprid to *Clarias batrachus* Linnaeus (1758) in Relation to Selected Environmental Variables

[1]Nageshwar Wast and [2]Resham Rajput

[1]Department of Zoology, Jai Prakash University, Chapra, Bihar, India
[2]Department of Zoology, Government Girls PG College, Khandwa, Madhya Pradesh, India

Abstract
Background and Objective: The toxicity of pesticides is found to significantly vary with some environmental variables such as temperature, pH, hardness, etc. which adversely affect non-target organisms, particularly fishes and human beings. Therefore, the objective of the work was to compare the acute toxicity of a neonicotinoid insecticide (Imidacloprid) to a freshwater fish, *Clarias batrachus* (Linnaeus) in supply water and tube well water at two different selected pH strengths. **Materials and Methods:** There are a total of 720 acclimatized test fishes (5.0±0.5 cm) were taken for experiment in both supply water and tube well water at two different selected pH strengths and natural hardness. A full-scale static bioassay test has been carried out for a period of 96 hrs to analyze the LC_{50} values, 95% confidence limits by Probit analysis (SPSS, Version-16), presumable safe and safe dischargeable concentrations of Imidacloprid 70% WG for the test fishes. **Results:** The 96 hrs LC_{50} of Imidacloprid 70% WG were noticed to be 233.436 and 251.641 ppm ($p<0.05$) at pH 5.5±0.2 and 8.5±0.2 for supply water however, these values were reported as 343.779 and 429.947 ppm ($p<0.05$) for tube well water at pH 5.5±0.2 and 8.5±0.2, respectively. Moreover, any alteration in behavioural response in the exposed fishes has also been noticed during static bioassay. The safe or harmless concentrations of Imidacloprid 70% WG were estimated too high compared to safe dischargeable concentrations for both waters. **Conclusion:** The toxicity (LC_{50} values) of a neonicotinoid, Imidacloprid is found to be significantly altered with selected environmental variables and exposed fishes exhibit abnormal behavioral responses.

Key words: Acute toxicity, imidacloprid 70% WG, LC_{50} values, *Clarias batrachus*, environmental variables

Citation: Wast, N. and R. Rajput, 2024. Efficacy of a neonicotinoid, Imidacloprid to *Clarias batrachus* Linnaeus (1758) in relation to selected environmental variables. J. Fish. Aquat. Sci., 19: 11-18.

Corresponding Author: Nageshwar Wast, Department of Zoology, Jai Prakash University, Chapra, Bihar, India

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The pesticides adversely affect the growth, physiology, reproduction, nutritional quality and survival of aquatic life, particularly the non-target organisms including ichthyofaunal species due to their persistence in the environment[1,2]. Imidacloprid affects both target pests as well as non-target species such as insects that contain receptors through contact and stomach action[3].

Imidacloprid is a neonicotinoid insecticide that comes under Chloronicotinyl nitroguanidine chemical family[4], employed in agriculture to control insects, flea and termites on domestic pets and also to treat soil, crops and seeds[5]. It selectively acts on pests' nervous systems through nicotinic acetylcholine receptors and thus in case of human exposures, it has a favorable toxicological profile[6]. Imidacloprid is cleaved into water by photolysis[7], upon hydrolysis Imidacloprid produces the metabolite 1-[(6-chloro-3-pyridinyl)methyl]-2-imidazolidinone[8]. Nevertheless, it has been also reported that Imidacloprid is found to be stable against hydrolysis in neutral or acidic conditions and hydrolysis enhances with an increase in alkaline pH and temperature[8]. Imidacloprid is generally employed for non-agricultural purposes in urban areas for ant control of properties around homes, bed bug treatment, flea control on pets and management of lawn and garden insect[9,10]. Further, Imidacloprid was reported to be acutely toxic to adult fish at relatively high concentrations (over 80 ppm) however, the juvenile stage of the fish was found to be more susceptible to it. Recently, the use of Imidacloprid has become more controversial due to its potential effects on non-target organisms like aquatic animals[11]. Moreover, due to its large-scale toxicity to bees[12,13] and other non-target organisms[14,15], it has been restricted in a few countries. Since the non-target organisms, particularly fish are found to be vulnerable to the harmful effects of aquatic contamination[16], therefore an attempt has been made to elucidate the acute toxicity of Imidacloprid 70% WG to a freshwater fish, *Clarias batrachus* (Linnaeus, 1758) in supply water and tube well water to compare the toxicity strength at selected environmental variables to secure the health of the non-target organisms, particularly fishes.

MATERIALS AND METHODS

Study area and duration: This work was carried out in the Department of Zoology, Laboratory of Jai Prakash University, Chapra from August, 2020 to February, 2021.

Experimental fish and pesticide solution: There are numerous healthy freshwater fish, *Clarias batrachus* were collected from local sources and were acclimatized separately in a cemented tank of 500 L capacity for 21 days. Approximately, equal sizes of 720 test fishes (5.0±0.5 cm) were taken for the bioassay tests. A common stock solution for Imidacloprid 70% WG was prepared by standard formula:

$$N_1V_1 = N_2V_2$$

Where:

N_1 = Concentration of test pesticide
V_1 = Volume of test pesticide
N_2 = Required concentration of pesticide to be prepared
V_2 = Volume of solution required for application

Further, the different concentrations of selected toxicant were prepared by pouring the common stock solution (with the help of a micropipette) into the measured diluents supply water (natural hardness = 260±5 mg/L, pH = 7.0±0.2) and tube well water (natural hardness 540±5 mg/L, pH = 7.2±0.2). The water of selected pH strength, 5.5±0.2 and 8.5±0.2 of both supply water and tube well water were prepared by adding HCl and NaOH, respectively. The series of different concentrations of prepared water of both pH strengths were used in the full-scale static bioassay tests (up to 96 hrs), which are based on the progressive bisection of intervals on logarithmic scales, utilized by Wast *et al.*[17].

Preliminary/screening tests: The static bioassay test was conducted in a 5 L plastic container for the evaluation of acute toxicity (96 hrs) of Imidacloprid 70% WG to *Clarias batrachus*. The range between the highest and lowest concentrations (a critical concentration range) of selected pesticides at which most of the test fishes died or survived in time intervals of 24, 48, 72 and 96 hrs were determined by screening tests.

Full-scale static bioassay test: In full-scale bioassay, the test containers of 5 L capacity filled with 3 L toxicant solution were kept in three rows and each container was labeled with the details of the experiment viz; date, time, concentration and replicate number. There are 10 acclimatized tests fish were kept in each experimental plastic container along with controls. The fresh toxicant solutions were used after each 24 hrs and the experiments were continuously run for a period of 96 hrs. The number of test fishes died in time intervals of 24, 48, 72 and 96 hrs in each concentration of toxicant solution were noticed carefully and removed periodically. The LC_{50} values and 95% confidence limits for Imidacloprid 70% WG to different concentrations and time intervals were estimated statistically by the Probit analysis method documented by Garakouei *et al*.[18]. Further, presumable safe and dischargeable concentrations of Imidacloprid 70% WG for *Clarias batrachus* were calculated as per the standard formula utilized by Nwani *et al*.[19]. Behavioural changes in the exposed fishes were documented at each concentration of toxicant solutions.

Statistical analysis: The mortality data noticed at 24, 48, 72 and 96 hrs were subjected to the statistical software (IBM SPSS-Version 16) to analyse the LC_{50} values ($p<0.05$), Confidence Ratio (R) and Probit equation.

RESULTS

The 96 hrs exposure system and acute toxicity bioassay for different concentrations of Imidacloprid 70% WG to *Clarias batrachus* in supply water (hardness 260±5 mg/L) and tube well water (hardness 540±5 mg/L) at both pH levels, 5.5±0.2 and 8.5±0.2 were summarized in Table 1-8. Furthermore, the 24, 48, 72 and 96 hrs LC_{50} value of different concentrations of Imidacloprid 70% WG to *Clarias batrachus* were noticed as 318.872, 289.305, 256.461 and 233.436 ppm ($p<0.05$) at pH 5.5±0.2 (Table 2) and 325.141, 304.606, 278.157 and 251.641 ppm ($p<0.05$) at pH 8.5±0.2 (Table 4), respectively for supply water. Whereas, the 24, 48, 72 and 96 hrs LC_{50} value of different concentrations of Imidacloprid 70% WG to *Clarias batrachus* were reported to be 479.804, 414.325, 390.539 and 343.779 ppm ($p<0.05$) at pH 5.5±0.2 (Table 6) and 596.757, 540.007, 487.638 and 429.947 ppm ($p<0.05$) at pH 8.5±0.2 (Table 8) for tube well water, respectively. Moreover, safe or harmless concentrations of Imidacloprid 70% WG to *Clarias batrachus* were documented as 78.893 and 83.064 ppm for supply water and 107.334 and 146.596 ppm for tube well water at pH 5.5±0.2 and 8.5±0.2, respectively whereas, the safe dischargeable concentrations were reported too low for the toxicant as 1.048 and 1.033 ppm for supply water and 1.076 and 1.051 ppm for tube well water at pH 5.5±0.2 and pH 8.5±0.2, respectively (Table 9).

Table 1: Mortality (%) and survival (%) rate of *Clarias batrachus* for Imidacloprid 70% WG during 96 hrs static bioassay

Imidacloprid 70% WG (ppm)	Number of exposed fishes	Duration of exposure 24 hrs	48 hrs	72 hrs	96 hrs	Mortality (%) (96 hrs)	Survival (%) (96 hrs)
Control (0.00)	10					00	100
180	10	0	0	1	1	10	90
210	10	2	3	3	4	40	60
240	10	2	4	6	7	70	30
280	10	3	4	6	7	70	30
320	10	5	6	7	9	90	10

*Each observation is the total of three replicates. For supply water of hardness 260±5 mg/L and pH 5.5±0.2

Table 2: LC_{50} value of Imidacloprid 70% WG to *Clarias batrachus* during 96 hrs static bioassay

Duration (hrs)	LC_{50} value of Imidacloprid 70% WG (ppm)	LCL	UCL	R	Probit equation (P = Intercept+BX)
24	318.872	279.801	519.727	1.857	-3.564+0.011X
48	289.305	254.927	391.431	1.535	-3.184+0.010X
72	256.461	220.577	304.007	1.378	-2.981+0.012X
96	233.436	201.345	259.748	1.290	-3.749+0.016X

For supply water of hardness 260±5 mg/L and pH 5.5±0.2, UCL: Upper confidence limits, LCL: Lower confidence limits and R = Confidence ratio (UCL/LCL)

Table 3: Mortality (%) and survival (%) rate of *Clarias batrachus* for Imidacloprid 70% WG during 96 hrs static bioassay

Imidacloprid 70% WG (ppm)	Number of exposed fishes	Duration of exposure 24 hrs	48 hrs	72 hrs	96 hrs	Mortality (%) (96 hrs)	Survival (%) (96 hrs)
Control (0.00)	10					00	100
210	10	0	1	2	2	20	80
240	10	2	3	4	5	50	50
280	10	4	4	6	7	70	30
320	10	4	5	6	8	80	20
370	10	7	6	8	10	100	00

*Each observation is a total of three replicates. For supply water of hardness 260±5 mg/L and pH 8.5±0.2

Table 4: LC_{50} value of Imidacloprid 70% WG to *Clarias batrachus* during 96 hrs static bioassay

Duration (hrs)	LC_{50} value of Imidacloprid 70% WG (ppm)	LCL	UCL	R	Probit equation (P = Intercept+BX)
24	325.141	293.619	388.297	1.322	-4.062+0.012X
48	304.606	270.032	358.406	1.327	-3.497+0.011X
72	278.157	229.771	324.930	1.414	-2.713+0.010X
96	251.641	215.118	277.074	1.288	-4.200+0.017X

For supply water of hardness 260±5 mg/L and pH 8.5±0.2, UCL: Upper confidence limits, LCL: Lower confidence limits and R: Confidence ratio (UCL/LCL)

Table 5: Mortality (%) and survival (%) rate of *Clarias batrachus* for Imidacloprid 70% WG during 96 hrs static bioassay

Imidacloprid 70% WG (ppm)	Number of exposed fishes	Duration of exposure				Mortality (%) (96 hrs)	Survival (%) (96 hrs)
		24 hrs	48 hrs	72 hrs	96 hrs		
Control (0.00)	10					00	100
280.00	10	0	1	1	2	20	80
320.00	10	2	3	3	4	40	60
370.00	10	3	4	5	7	70	30
420-00	10	3	5	7	8	80	20
490-00	10	5	7	7	9	90	10

*Each observation is a total of three replicates. For tube well water of Hardness 540±5 mg/L and pH 5.5±0.2

Table 6: LC_{50} value of Imidacloprid 70% WG to *Clarias batrachus* during 96 hrs static bioassay

Duration (hrs)	LC_{50} value of Imidacloprid 70% WG (ppm)	LCL	UCL	R	Probit equation (P = Intercept+BX)
24	479.804	420.138	796.219	1.895	-3.377+0.0070X
48	414.325	363.885	515.484	1.416	-3.137+0.0080X
72	390.539	339.863	455.536	1,340	-3.244+0.0080X
96	343.779	288.469	382.661	1,326	-3.601+0.010X

For tube well water of hardness 540±5 mg/L and pH 5.5±0.2, UCL: Upper confidence limits, LCL: Lower confidence limits and R: Confidence ratio (UCL/LCL)

Table 7: Mortality (%) and survival (%) rate of *Clarias batrachus* for Imidacloprid 70% WG during 96 hrs static bioassay

Imidacloprid 70% WG (ppm)	Number of exposed fishes	Duration of exposure				Mortality (%) (96 hrs)	Survival (%) (96 hrs)
		24 hrs	48 hrs	72 hrs	96 hrs		
Control (0.00)	10					00	100
370	10	0	1	2	3	30	70
420	10	2	3	4	5	50	50
490	10	3	5	6	7	70	30
560	10	4	5	6	8	80	20
650	10	6	7	8	10	100	00

*Each observation is a total of three replicates. For tube well water of hardness 540±5 mg/L and pH 8.5±0.2

Table 8: LC_{50} Value of Imidacloprid 70% WG to *Clarias batrachus* during 96 hrs static bioassay

Duration (hrs)	LC_{50} value of Imidacloprid 70% WG (ppm)	LCL	UCL	R	Probit equation (P = Intercept+BX)
24	596.757	534.295	771.140	1.443	-3.797+0.0060X
48	540.007	469.847	665.468	1.416	-2.986+0.0070X
72	487.638	402.803	569.846	1.414	-2.711+0.0060X
96	429.947	348.362	477.921	1.371	-3.668+0.0090X

For tube well water of hardness 540±5 mg/L and pH8.5±0.2, UCL: Upper confidence limits, LCL: Lower confidence limits and R: Confidence ratio (UCL/LCL)

Table 9: Safe or harmless and safe dischargeable concentrations of Imidacloprid 70% WG to *Clarias batrachus* for both water

Concentrations (ppm) (Imidacloprid 70% WG)	Supply water (hardness 260±5 mg/L)		Tube well water (hardness 540±5 mg/L)	
	pH (5.5±0.2)	pH (8.5±0.2)	pH (5.5±0.2)	pH (8.5±0.2)
Safe or harmless (C))	78.893	83.064	107.334	146.596
Safe dischargeable (S)	1.048	1.033	1.076	1.051

DISCUSSION

The current investigation exhibits 96 hrs LC_{50}'s of Imidacloprid 70% WG as 233.436 and 251.641 ppm at pH 5.5±0.2 and 8.5±0.2 for supply water however, for tube well water these values were recorded as 343.779 and 429.947 ppm at pH 5.5±0.2 and 8.5±0.2, respectively.

However, the 96 hrs LC_{50} values of Imidacloprid were reported as 21 mg/L for rainbow trout, *Oncorhynchus mykiss* and 237 mg/L for golden orfe, *Leuciscus idus*[4]. The 96 hrs LC_{50} values of Imidacloprid 70% WG for juveniles, males, females and mixed population of *Poecilia reticulata* were estimated as 68.443, 90.056, 180.593 and 117.614 ppm, respectively[20]. Further, the 96 hrs LC_{50} values of technical Imidacloprid (99.2% pure) and Admire (a commercially available formulated product) were reported as 65.43 and 17.44 µg/L for *Hyalella azteca* and 5.75 and 5.40 µg/L for *Chironomus tentans*, respectively[21]. It has been noticed that Imidacloprid causes significant adverse effects on invertebrates and may produce small effects on the decomposition of organic matter in aquatic ecosystems if exposed to $\geq$0.02 µg/L[22]. The LC_{50} value of Imidacloprid was recorded as 227.33 mg/L in a common freshwater fish, *Pethia conchonius* (Rosy barb) and reported higher DNA damage and nuclear abnormalities than the control[23]. Imidacloprid® treated (96 hrs) neotropical fish, *Astyanax altiparanae* exhibits neurotoxic effects in muscle, oxidative damage of lipids in the gills, muscle and increased abnormalities in erythrocyte nucleus[24]. A significantly reduced AChE and BChE enzyme activities in the muscle, brain and serum has been reported in *Labeo rohita* when exposed to one-third of LC_{50} concentration of both neonicotinoids Imidacloprid (66.6 mg/L), clothianidin (30 mg/L) and its combined formulation (range of 33.3+15 mg/L) for 42 days[25]. The 96 hrs LC_{50} of Imidacloprid for *Cyprinus carpio* larvae was found to be 1292.6 µg/L. Also, there are several malformations in embryos and larvae of common carp have been noticed due to exposure to 300 and 1000 µg/L of Imidacloprid[26]. It has been observed that the nutritional quality of *L. rohita* may be adversely affected when exposed to sublethal concentration of Imidacloprid (120 mg/L) for 64 days[27]. There are changes in the histology of liver and gill tissue and also oxidative stress parameters along with lipid peroxidation (LPO) have been noticed after exposure to 50 and 100 mg/L sub-lethal concentrations of Imidacloprid in *O. niloticus*[28]. The LC_{50} (48 hrs) for fertilized eggs of *C. carpio* was found to be 78 ppm[29], an oxidative stress has been observed in freshwater fish *Australoheros facetus*, exposed to environmentally relevant concentrations (10-1000 µg/L) of Imidacloprid[30]. A neurotoxic effect has been seen in rainbow trout after 21 days exposure of Imidacloprid (5, 10 and 20 mg/L) due to a decrease in AChE enzyme activity (relatively at high concentration), increase in 8-OHdG activity and an oxidative stress alteration[31]. The 96 hrs LC_{50} value of Imidacloprid was estimated to be 35.59 mg/L for *Cnesterodon decemmaculatus* (Pisces: Poeciliidae) on acute exposure to environmentally relevant concentrations and also increased in genetic damage index (DNA damage) and activity of catalase has been reported due to exposure of sublethal concentrations (0.175, 0.35 and 1 mg/L) of Imidacloprid for 96 hrs[32]. Further, the LC_{50} values were analyzed in the ranges from 0.423 (24 hrs) to 0.270 mL/L (96 hrs) during the study of acute toxicity bioassay for Imidacloprid on zebrafish (*Danio rerio*)[33]. The consequence of the current investigation in the context of LC_{50} values is found to be concurring with the literature cited by above mentioned authors. In the present work, the safe or harmless and safe dischargeable concentrations of Imidacloprid 70% WG to *Clarias batrachus* ranged from 78.893-146.596 and 1.033-1.076 ppm, respectively in both water (Table 9), however these values were ranges between 21.948 to 56.079 ppm and 1.048 to 1.084 ppm for juveniles, males, females and mixed population of *Poecilia reticulata*[20]. Moreover, the approximate patterns of safe or harmless and safe dischargeable concentrations of Imidacloprid 70% WG withdrawn from the current investigation were also in agreement with the various species of organophosphate exposed fishes[34-36].

Furthermore, the abnormal behavioral response in Imidacloprid 70% WG treated *Clarias batrachus* such as erratic swimming, restless movement, lack of coordination, jerky movements, upside belly with whitish slimy substance (after death) and reddish colour of gill were documented at high concentration at low pH in both supply water and tube well water in the current study. Whereas, different sublethal concentrations (7.8, 15.6 and 23.4 ppm) of Imidacloprid treated *C. carpio* for 21 days illustrated abnormal behavior like loss of equilibrium, restlessness, hyperactivity, erratic swimming, jerky movements and reduced skin pigmentation[29] which are also in accordance to literature cited by Wast *et al.*[20]. It has been found that Imidacloprid exposure causes significantly decreased swimming activity of zebrafish (*Danio rerio*) larvae at both doses (45 or 60 µM) however, it significantly decreased in novel tank swimming and enhanced the sensorimotor response to scare stimuli at the low dose in adolescent and adult zebrafish[37]. The impaired visual behavior (visual toxicity) in adult zebrafish (*Danio rerio*) was noticed after exposure to Imidacloprid at 10 and 100 µg/L concentration (for 21 days) due to disruption of opsin and phototransduction genes[38]. Approximately, a more or less similar behavioral response has been recorded in current work and was also corroborated with another organophosphate exposed to different test fishes[39-41]. It has been previously reported by Clasen *et al.*[42] that contaminants can enter fishes through the gills, skin and food through biomagnification along the food chain in aquatic bodies. Human beings are exposed to environmental contaminants (accumulated pesticides) mostly through the consumption of fish[43-45] and chemically formulated pesticides are found to be harmful to human health and the environment[46,47]. Therefore, the use of pesticides may exert severe adverse effects on fish bodies which causes potential risks of health hazards to human beings.

CONCLUSION

It has been concluded that the toxicity of Imidacloprid 70% WG is found to be higher at low pH and natural hardness as compared to high pH and natural hardness in both water and also Imidacloprid exposed fish exhibit abnormal behavioral response. Moreover, awareness in the context of the health condition of fish becomes very important, since world most food is being supplied from fisheries sources, which consequently affects human health. Further, it has been recommended that users focus their attention on the concentration of toxicants utilized in particular environments since their toxicity significantly alters with selected environmental variables.

SIGNIFICANCE STATEMENT

Awareness in the context of the health condition of fish become very important since, world most food is being supplied from fisheries sources, which ultimately affects human health. Therefore, the study has been focused on making people aware of the health hazards of fish and other aquatic fauna, which are caused by to indiscriminate use of Imidacloprid pesticides in the field. Since the toxicity of Imidacloprid significantly alters with selected environmental variables, therefore, it has been recommended to the users of Imidacloprid that they should be aware while using its environmentally safe concentrations to prevent the deterioration of aquatic resources, particularly during aquaculture.

REFERENCES

1. Hayat, S., M. Javed and S. Razzaq, 2007. Growth performance of metal stressed major carps viz. *Catla catla Labeo rohita* and *Cirrhina mrigala* reared under semi-intensive culture system. Pak. Vet. J., 27: 8-12.
2. Abasali, H. and S. Mohamad, 2010. Effect of plant extracts supplemented diets on immunity and resistance to *Aeromonas hydrophila* in common carp (*Cyprinus carpio*). Agric. J., 5: 119-127.
3. Capowiez, Y. and A. Bérard, 2006. Assessment of the effects of imidacloprid on the behavior of two earthworm species (*Aporrectodea nocturna* and *Allolobophora icterica*) using 2D terraria. Ecotoxicol. Environ. Saf., 64: 198-206.
4. Tomlin, C., 2006. The Pesticide Manual: A World Compendium. 14th Edn., British Crop Protection Council, Alton, England, ISBN: 9781901396140, Pages: 1349.
5. Ensminger, L.G., 1964. Farm chemicals handbook. J. Assoc. Off. Agric. Chem., 47: 792-792.
6. Tomizawa, M. and J.E. Casida, 2005. Neonicotinoid insecticide toxicology: Mechanisms of selective action. Annu. Rev. Pharmacol. Toxicol., 45: 247-268.
7. Liu, W., W. Zheng, Y. Ma and K.K. Liu, 2006. Sorption and degradation of imidacloprid in soil and water. J. Environ. Sci. Health Part B, 41: 623-634.
8. Zheng, W. and W. Liu, 1999. Kinetics and mechanism of the hydrolysis of imidacloprid. Pestic. Sci., 55: 482-485.
9. Jeschke, P., R. Nauen, M. Schindler and A. Elbert, 2011. Overview of the status and global strategy for neonicotinoids. J. Agric. Food Chem., 59: 2897-2908.
10. Hladik, M.L. and D.W. Kolpin, 2015. First national-scale reconnaissance of neonicotinoid insecticides in streams across the USA. Environ. Chem., 13: 12-20.
11. Smit, C.E., C.J.A.M. Posthuma-Doodeman, P.L.A. van Vlaardingen and F.M.W. de Jong, 2015. Ecotoxicity of imidacloprid to aquatic organisms: Derivation of water quality standards for peak and long-term exposure. Hum. Ecol. Risk Assess.: Int. J., 21: 1608-1630.
12. Godfray, H.C.J., T. Blacquière, L.M. Field, R.S. Hails and G. Petrokofsky *et al.*, 2014. A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. Proc. R. Soc. B., Vol. 281. 10.1098/rspb.2014.0558.
13. Lundin, O., M. Rundlöf, H.G. Smith, I. Fries and R. Bommarco, 2015. Neonicotinoid insecticides and their impacts on bees: A systematic review of research approaches and identification of knowledge gaps. PLoS ONE, Vol. 10. 10.1371/journal.pone.0136928.
14. Vignet, C., T. Cappello, Q. Fu, K. Lajoie and G. de Marco *et al.*, 2019. Imidacloprid induces adverse effects on fish early life stages that are more severe in Japanese medaka (*Oryzias latipes*) than in zebrafish (*Danio rerio*). Chemosphere, 225: 470-478.
15. Thunnissen, N.W., L.S. Lautz, T.W.G. van Schaik and A.J. Hendriks, 2020. Ecological risks of imidacloprid to aquatic species in the Netherlands: Measured and estimated concentrations compared to species sensitivity distributions. Chemosphere, Vol. 254. 10.1016/j.chemosphere.2020.126604.

16. Vieira, C.E.D., M.R. Pérez, R.D. Acayaba, C.C.M. Raimundo and C.B. dos Reis Martinez, 2018. DNA damage and oxidative stress induced by imidacloprid exposure in different tissues of the Neotropical fish *Prochilodus lineatus*. Chemosphere, 195: 125-134.
17. Wast, N., A.K. Gupta, M.M. Prakash and S. Gaherwal, 2014. Toxic effect of alphamethrin 10 EC on freshwater fish, *Poecilia reticulata* (Peters, 1859). World Appl. Sci. J., 30: 782-786.
18. Garakouei, M.Y., Z. Pajand, M. Tatina and H. Khara, 2009. Median lethal concentration (LC_{50}) for suspended sediments in two sturgeon species, *Acipenser persicus* and *Acipenser stellatus* fingerlings. J. Fish. Aquat. Sci., 4: 285-295.
19. Nwani, C.D., D.O. Ugwu, O.C. Okeke, G.C. Onyishi, F.N. Ekeh, C. Atama and L.O. Eneje, 2013. Toxicity of the chlorpyrifos-based pesticide Termifos®: Effects on behaviour and biochemical and haematological parameters of African catfish *Clarias gariepinus*. Afr. J. Aquat. Sci., 38: 255-262.
20. Wast, N., M.M. Prakash, A.K. Gupta and S. Gaherwal, 2016. Acute toxicity assessment of imidacloprid 70 % WG to a Guppy, *Poecilia reticulata* (Peters). Global Vet., 17: 245-249.
21. Stoughton, S.J., K. Liber, J. Culp and A. Cessna, 2008. Acute and chronic toxicity of imidacloprid to the aquatic invertebrates *Chironomus tentans* and *Hyalella azteca* under constant- and pulse-exposure conditions. Arch. Environ. Contam. Toxicol., 54: 662-673.
22. Merga, L.B. and P.J. van den Brink, 2021. Ecological effects of imidacloprid on a tropical freshwater ecosystem and subsequent recovery dynamics. Sci. Total Environ., Vol. 784. 10.1016/j.scitotenv.2021.147167.
23. Dutta, D., A. Ray, B. Ghosh and M. Bahadur, 2024. Assessment of imidacloprid induced genotoxicity in *Pethia conchonius* (Rosy barb), a common freshwater fish of India. Drug Chem. Toxicol., 47: 101-114.
24. Almeida, É.C., L.S. Passos, C.E.D. Vieira, R.D. Acayaba and C.C. Montagner *et al*., 2021. Can the insecticide imidacloprid affect the health of the neotropical freshwater fish *Astyanax altiparanae* (Teleostei: Characidae)? Environ. Toxicol. Pharmacol., Vol. 85. 10.1016/j.etap.2021.103634.
25. Gajula, S.K., S.K. Kanthala, M.R. Narra and V.D. Vanamala, 2023. Individual and interactive biochemical profile damages in *Labeo rohita* (Cyprinidae) by imidacloprid and clothianidin. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol., Vol. 271. 10.1016/j.cbpc.2023.109689.
26. Islam, M.A., M.S. Hossen, K.A. Sumon and M.M. Rahman, 2019. Acute toxicity of imidacloprid on the developmental stages of common carp *Cyprinus carpio*. Toxicol. Environ. Health Sci., 11: 244-251.
27. Qadir, S., R. Bukhari and F. Iqbal, 2015. Effect of sub lethal concentration of imidacloprid on proximate body composition of *Labeo rohita*. Iran. J. Fish. Sci., 14: 937-945.
28. Günal, A.Ç., B. Erkmen, E. Paçal, P. Arslan, Z. Yildirim and F. Erkoç, 2020. Sub-lethal effects of imidacloprid on Nile tilapia (*Oreochromis niloticus*). Water Air Soil Pollut., Vol. 231. 10.1007/s11270-019-4366-8.
29. Bhardwaj, J.K., Harkrishan and A.K. Tyor, 2020. Imidacloprid induced alterations in behavioral and locomotory activity of fingerlings of common carp, *Cyprinus carpio*. Toxicol. Int., 27: 158-167.
30. Iturburu, F.G., L. Bertrand, J.R. Mendieta, M.V. Amé and M.L. Menone, 2018. An integrated biomarker response study explains more than the sum of the parts: Oxidative stress in the fish *Australoheros facetus* exposed to imidacloprid. Ecol. Indic., 93: 351-357.
31. Topal, A., G. Alak, M. Ozkaraca, A.C. Yeltekin and S. Comakli *et al*., 2017. Neurotoxic responses in brain tissues of rainbow trout exposed to imidacloprid pesticide: Assessment of 8-hydroxy-2-deoxyguanosine activity, oxidative stress and acetylcholinesterase activity. Chemosphere, 175: 186-191.
32. de Arcaute, C.R., M.R. Laborde, S. Soloneski and M.L. Larramendy, 2023. Do environmentally relevant concentrations of the neonicotinoid insecticide imidacloprid induce DNA damage and oxidative stress on *Cnesterodon decemmaculatus* (Jenyns, 1842)? Environ. Toxicol. Pharmacol., Vol. 100. 10.1016/j.etap.2023.104123.
33. Yadav, V., S. Ahmad and K. Zahra, 2020. Imidacloprid toxicity and its attenuation by aqueous extract of *Moringa oleifera* leaf in zebra fish, *Danio rerio*. Int. J. Curr. Pharm. Res., 12: 32-38.
34. Wast, N., A.K. Gupta, M.M. Prakash and S. Gaherwal, 2014. Acute toxicity of chlorpyriphos 50% + cypermethrin 5% EC to the guppy, *Poecilia reticulata* (Peters, 1859). Global Vet., 12: 393-398.
35. Wast, N., K. Tiwari, A.K. Gupta, M.M. Prakash and S. Gaherwal, 2015. Assessment of acute toxicity of chlorpyriphos 20% EC to the guppy, *Poecilia reticulata* (Peters, 1859). Global Vet., 14: 239-243.
36. Wast, N., R. Rajput and S. Verma, 2021. Chlorpyriphos 20% EC induced alteration in haematological parameter of freshwater fish, *Clarias batrachus* (Linnaeus, 1758). J. Fish. Aquat. Sci., 16: 19-25.
37. Crosby, E.B., J.M. Bailey, A.N. Oliveri and E.D. Levin, 2015. Neurobehavioral impairments caused by developmental imidacloprid exposure in zebrafish. Neurotoxicol. Teratol., 49: 81-90.
38. Fu, R., H. Liu, Y. Zhang, L. Mao and L. Zhu *et al*., 2024. Imidacloprid affects the visual behavior of adult zebrafish (*Danio rerio*) by mediating the expression of opsin and phototransduction genes and altering the metabolism of neurotransmitters. Sci. Total Environ., Vol. 910. 10.1016/j.scitotenv.2023.168572.

39. Wast, N., R. Rajput and M.M. Prakash, 2020. Toxicological evaluation of dimethoate 30% EC to a freshwater fish, *Heteropneustes fossilis* (Bloch) at two different selected levels of pH. World Appl. Sci. J., 38: 329-334.
40. Prakash, M.M., R. Rajput, N. Wast and R. Verma, 2020. Comparative assessment of acute toxicity of two organophosphates (Monocrotophos 36% SL and Dimethoate 30% EC) to a freshwater fish, *Poecilia reticulata* (Peters) at two different selected level of pH. World Appl. Sci. J., 38: 153-161.
41. Wast, N. and R. Rajput, 2021. Monocrotophos toxicity to freshwater fish, *Clarias batrachus* (Linnaeus, 1758) at two different pH levels. J. Fish. Aquat. Sci., 16: 12-18.
42. Clasen, B., V.L. Loro, C.R. Murussi, T.L. Tiecher, B. Moraes and R. Zanella, 2018. Bioaccumulation and oxidative stress caused by pesticides in *Cyprinus carpio* reared in a rice-fish system. Sci. Total Environ., 626: 737-743.
43. Storelli, M.M., 2008. Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: Estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). Food Chem. Toxicol., 46: 2782-2788.
44. Mahmood, A., R.N. Malik, J. Li and G. Zhang, 2014. Human health risk assessment and dietary intake of organochlorine pesticides through air, soil and food crops (wheat and rice) along two tributaries of River Chenab, Pakistan. Food Chem. Toxicol., 71: 17-25.
45. Buah-Kwofie, A., M.S. Humphries and L. Pillay, 2018. Bioaccumulation and risk assessment of organochlorine pesticides in fish from a global biodiversity hotspot: Isimangaliso Wetland Park, South Africa. Sci. Total Environ., 621: 273-281.
46. Halwart, M. and M.V. Gupta, 2004. Culture of Fish in Rice Fields. WorldFish, Penang, Malaysia, ISBN: 9789832346333, Pages: 77.
47. El-Sherif, M.S., M.T. Ahmad, M.A. El-Danasoury and N.H.K. El-Nwishy, 2009. Evaluation of diazinon toxicity on Nile tilapia fish (*O. niloticus*). J. Fish. Aquat. Sci., 4: 169-177.