Acute Single and Joint Toxicity Effects of Imidacloprid and Thiamethoxam on Zebrafish (*Danio Rerio*)

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Abstract This study attempted to assess the lethal concentration (96-h LC_{50}) effects of imidacloprid (neonicotinoid pesticide), thiamethoxam (neonicotinoid pesticide), and their combination on juvenile Zebrafish (*Danio rerio*). Each set of trials contained a control (de-chlorinated tap water), and the experiments were repeated three times. The fish ($n = 10$) were randomly measured with an average length of (3.4 ± 0.34) cm and weight of (1 ± 0.1) g. The temperature was kept at 24 ℃. Experiments 1 and 2 were designed to investigate at the acute toxicity of imidacloprid and thiamethoxam on juvenile zebrafish (*Danio rerio*) respectively, whereas experiment 3 was aimed at the combined toxicity of IMI and THM on zebrafish. The tests followed the same study design, and each experiment used seven different logarithmic concentrations of imidacloprid insecticides (310.00, 317.08, 324.33, 331.74, 339.32, 347.07, 355.00 mg/L) and thiamethoxam (175.00, 185.52, 200.93, 215.30, 230.70, 247.20, 264.88 mg/L). The results show that THM is more toxic than IMI, with LC_{50} values of 190.34 mg/L for THM and 310.92 mg/L for IMI. Both individual toxicities showed a substantial positive connection ($P < 0.05$) with confidence limits of 321.50 – 300.68 mg/L for IMI and 199.91 – 181.21 mg/L for THM. The joint toxicity test was carried out using the 96-h LC_{50} values of imidacloprid and thiamethoxam obtained in the individual acute toxicity trials at a 1∶1 ratio. The Additive Index (AI) demonstrated that imidacloprid and thiamethoxam acted synergistically on *D. rerio*. As a matter of fact, more research is needed to better understand the impact of IMI and THM on other aquatic organisms and also create strategies to mitigate its harmful effects on aquatic life.

Key words Acute toxicity (LC_{50}); Joint toxicity; Imidacloprid; Thiamethoxam; Zebrafish (*Danio rerio*)
DOI: 10.19759/j.cnki.2164 – 4993.2025.03.009

Pesticides are widely used and disposed of, resulting in pesticide pollution in the atmosphere, soils, water, and the food chain. These pose a tremendous threat to both human health and the environment [1]. Pesticide overuse has negative repercussions for both human health and the ecosystem due to their high biological activity and persistence in the environment. The major causes of contamination or non-target harm are inaccurate pesticide application, spray or vapor drift, spillage, backflow, erosion, leaching, and improper chemical or container disposal. Pesticide contamination of the aquatic environment, both direct and indirect, can cause fish death, reduced fish productivity, and elevated levels of undesirable chemicals in edible fish tissues. However, numerous surveys have documented the impact of pesticides and raised concerns about the health risks associated with food and water contamination. Neonicotinoids are among the most frequently used insecticides, accounting for over 80% of seed treatments sold worldwide. Global monitoring programs have detected both neonicotinoids in surface waters, with IMI and THM usually found combined. The purpose of studying the single and joint acute toxicity of imidacloprid and thiamethoxam to zebrafish (*Danio rerio*) is to establish the lethal concentration (LC_{50}) that kills 50% of zebrafish in a particular time. Zebrafish was used for the experiment because they share about 70% of their genes with humans, therefore making zebrafish an excellent model for toxicity test. Temperature, pH, dissolved oxygen, and ammonia were measured daily as the primary water quality parameters, and a daily routine check was performed to assure good physio-chemical parameters. Throughout the test period, the 12 h day and 12 h light photoperiod was maintained and the OECD requirements was used.

Materials and Methods

Test organisms and acclimatization

This study used healthy juvenile adult zebrafish of both sexes, with an average length of (3.4 ± 0.34) cm and an average weight of (1 ± 0.1) g. Zebrafish were obtained from a fish farm hatchery in Shanghai, China. When the fish were transferred to the FFRC laboratory for acclimatization, OECD guidelines 203 were followed (OECD, 2019). The fish received continuous oxygenation during shipping. In the laboratory, fish were acclimated in plastic tank (200 L capacity) with de-chlorinated tap water. During the acclimatization period, 1% of the fish died. Aquarium air pumps provided continuous aeration at a temperature of 24 ℃ for 14 d. The zebrafish were fed commercial feed pellets once each day and feeding were stopped 24 h before the trial. A semi-static atmosphere was maintained, with water quality indicators evaluated on a daily basis during the acclimatization phase.

Test water

During the test period, temperature was maintained at 24 ℃. The study recorded average values of dissolved oxygen (DO) and pH at 9.2 and 9.6 mg/L and 7.5 and 7.8 respectively, with

Received: March 10, 2025 Accepted: May 12, 2025
Supported by the Central Public-interest Scientific Institution Basal Research Fund, CAFS (2025XT0902); the earmarked fund for China Agriculture Research System (CARS-46).
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ammonia recordings below 0.05 mg/L. The culture medium for acute toxicity test was appropriate and in conformance with the OECD guidelines (OECD, 2019).

Preliminary test

A preliminary range-finding experiment was carried out in line with established literature and after acclimatization. Concentrations 310, 325, 350, 400 and 150, 200, 250, 300 mg/L were used for the pretests in imidacloprid and thiamethoxam respectively. Zebrafish responses to each concentration were observed within 24 and 48 h to determine the correct ranges for the main test.

Acute test

The single acute toxicity values for imidacloprid and thiamethoxam were 310.00, 317.08, 324.33, 331.74, 339.32, 347.07, 355.00 mg/L and 175.00, 185.52, 200.93, 215.30, 230.70, 247.20, 264.88 mg/L, respectively. Each concentration was triplicated, and zebrafish responses were evaluated at 24, 48, 72, and 96 hours.

Joint toxicity

The concentrations for the joint toxicity for imidacloprid and thiamethoxam were 142.93, 153.16, 164.11, 175.85, 188.42, 201.90, 216.34 and 87.50, 93.76, 100.46, 107.65, 115.35, 123.60 and 132.44 mg/L respectively. Marking's Additive Index (AI) method was used to evaluate the joint toxicity of imidacloprid and thiamethoxam mixture to test organisms (Marking, 1977). The number of dead fish per group was recorded against the time of their death in a tabular form as specified in OECD (2019) guidelines. A joint toxicity test was carried out according to the ratio 1 : 1 of toxicity units of the two toxicants as follows:

$$\frac{A_m}{A_t} + \frac{B_m}{B_t}$$

$$AI = \frac{1}{S} - 1\ (S < 1),\ AI = 1 - S\ (\geqslant 1)$$

Wherein, S is biological activity of chemicals (imidacloprid and thiamethoxam mixture); A and B are toxicants; A_i and B_i are LC_{50} (mg/L) of A and B respectively in single toxicity test; A_m and B_m are LC_{50} (mg/L) of A and B respectively in joint toxicity test; AI is additive index; and $AI = 0$ means additive effect, $AI > 0$ means synergistic effect, and $AI < 0$ means antagonistic effect. Significant correlation level ($P < 0.05$) of coefficients was tested with Pearson's and regression statistical command in Excel 2013.

Results and Analysis

Acute toxicity test of imidacloprid and thiamethoxam on zebrafish

The pesticides imidacloprid and thiamethoxam were tested on zebrafish using a single toxicity test. Individual toxicity tests for imidacloprid and thiamethoxam revealed LC_{50} values of 358.85, 344.27, 320.30, 310.92 and 263.65, 224.57, 203.30, 190.34 mg/L in 24, 48, 72, and 96 h, respectively. Both pesticides were found to be harmless using the acute toxicity evaluation system supplied by the United States Fish and Wildlife Service, which was based on El-Harbai (2014), despite the fact that zebrafish displayed comparable aberrant behavioral alterations in both studies. Over time, there was a substantial positive correlation ($P > 0.05$) between the LC_{50} values of both pesticides, indicating that the time it took for the fish to die was directly proportional to the concentration of both chemicals. Zebrafish were thoroughly evaluated for behavioral changes following exposure to both pesticides for varying amounts of time. Throughout the experiment, the control group remained active and showed no anomalous behavior, and all water quality measurements were taken. Dead fish were rapidly removed, and mortality was measured at 24, 48, 72, and 96 h. In general, behavioral changes over time were linked to higher concentrations and longer exposure durations. Abnormal signs included loss of stability, reduced swimming movements, and hyperactive behavior, were common in both pesticides. In reality, further research is required to better understand the impact of imidacloprid and thiamethoxam on other aquatic ecosystems and devise measures to alleviate its negative impacts on aquatic life. Adequate information on the single acute toxicity test is tabulated in Table 2 and Table 3 below.

Table 1 Toxicity results of imidacloprid on zebrafish

	Death rate//%			
Concentrations//mg/L	24//h	48//h	72//h	96//h
Control	0	0	0	0
310.00	0	20	40	50
317.08	10	30	45	60
324.33	25	35	50	65
331.74	30	40	65	75
339.32	35	45	75	80
347.07	40	50	80	90
355.00	40	60	90	100
Regression equation	$y = 19.075x - 43.735$	$y = 16.599x - 37.11$	$y = 26.209x - 60.668$	$y = 24.608x - 56.339$
Correlation coefficient	0.900	0.986	0.986	0.987 *
LC_{50}	358.85	344.27	320.30	310.92
95% confidence limit	374.69 – 343.68	360.46 – 328.81	329.76 – 311.11	321.50 – 300.68

Correlation coefficients (P-value) at 24 h = 0.01, at 48 h = 0.000 03, at 72 h = 0.000 04 and at 96 h = 0.000 2.

Table 2 Toxicity test results of thiamethoxam on zebrafish

	Death rate//%			
Concentration//mg/L	24 h//%	48 h//%	72 h//%	96 h//%
Control	0	0	0	0
175.00	0	0	0	20
185.52	10	15	30	50
200.93	20	40	50	70
215.30	30	50	60	85
230.70	30	50	80	100
247.20	40	60	90	100
264.88	50	80	100	100
Regression equation	$y=7.78806x-13.837$	$y=10.38x-19.407$	$y=14.799x-29.158$	$y=20.534x-41.808$
Correlation coefficient	0.972	0.943	0.994	0.992 *
LC_{50}	263.65	224.57	203.30	190.34
95% confidence limit	293.098 – 237.154	243.121 – 207.431	216.081 – 191.267	199.931 – 181.214

Correlation coefficients (*P*-Value) at 24 h = 0.001, at 48 h = 0.004, at 72 h = 0.000 5 and at 96 h = 0.008.

Joint toxicity test of imidacloprid and thiamethoxam on zebrafish

The study demonstrated that a combination of imidacloprid and thiamethoxam had a synergistic effect on exposure to *D. rerio*, suggesting that the mixture was more lethal than each pesticide used alone. The results recorded a positive correlation ($P>0.05$) which indicate a direct association between pesticide concentration and fish mortality time. It shows that the higher the concentration of pesticides, the greater the risk of mortality, which is consistent with earlier research. Both substances were found to cause neurotoxicity in zebrafish, and their exposure can result in neuronal oxidative stress, inflammation, apoptosis, and damage in the telencephalon of adult zebrafish brains. The effects were detected at relatively high pesticide concentrations, with longer exposure times resulting in more severe behavioral and neurological consequences. In summary, imidacloprid and thiamethoxam combined had broad neurotoxicological effects on zebrafish in acute toxicity tests, affecting locomotion, social behavior, brain structure, and gene expression, highlighting the potential risks these pesticides pose to zebrafish and non-target aquatic organisms. Furthermore, more research is needed to understand the cumulative effects of acute toxicity on different fish species, as well as to quantify these effects in each species. More information on the results of the joint toxicity test is shown in Table 3 and Table 4.

Table 3 Death rate of zebrafish under joint toxicity test of imidacloprid and thiamethoxam

Imidacloprid	Thiamethoxam	Death rate//%			
μg/L	μg/L	24 h	48 h	72 h	96 h
Control	Control	0	0	0	0
142.93	87.50	0	0	15	30
153.16	93.76	0	10	30	50
164.11	100.465	10	25	50	70
175.85	107.65	15	40	70	90
188.42	115.35	20	50	85	100
201.90	123.60	30	65	95	100
216.34	132.44	40	75	100	100

Table 4 Joint toxicity test effect of IMI and THM on zebrafish

Pesticide	Parameter	24 h	48 h	72 h	96 h
Imidacloprid	Regression equation	$y=8.466x-15.039$	$y=12.3577x-23.103$	$y=17.485x-33.69$	$y=19.426x-37.378$
	Correlation coefficient	0.997 1	0.989 5	0.999 6	0.995 1
	LC_{50}	232.81	188.04	163.21	151.88
	95% Confidence	259.00 – 207.26	201.01 – 1 755.91	171.09 – 155.69	156.04 – 144.19
Thiamethoxam	Regression equation	$y=8.5995x-13.518$	$y=12.552x-20.883$	$y=17.761x-30.549$	$y=19.732x-33.888$
	Correlation coefficient	0.315	0.312	0.999	0.995 *
	LC_{50}	142.36	115.36	100.35	93.50
	95% Confidence limit	168.11 – 128.173	123.19 – 108.03	102.75 – 95.80	98.41 – 88.84
Joint effect	S	1.188 7	1.059 8	1.003 2	0.979 7
	AL	0.841 2	0.943 5	0.996 9	0.020 7
	Conclusion	Synergistic	Synergistic	Synergistic	Synergistic

Discussion

The joint acute toxicity test showed a correlation between pesticide concentration and fish mortality rate. The synergistic effect of imidacloprid and thiamethoxam (IMI + THM) can be attributed to pesticide interactions. The acute LC_{50} values (95% confidence limit) for imidacloprid and thiamethoxam in the (IMI + THM) mixture were found to be 151.88 and 93.50 mg/L, respectively, with confidence limits of 156.04 – 144.19 mg/L and 98.41 – 88.84

mg/L, respectively. A statistically significant positive relationship was found between these values ($P < 0.05$). Additionally, the biological activity (S) at 24, 48, 72, and 96 h were determined to be 1.1887, 1.059 8, 1.003 2, and 0.979 7, respectively. Furthermore, the additive index (AI) values at 24, 48, 72, and 96 h were found to be 0.841 2, 0.943 5, 0.996 9, and 0.020 7, respectively. It is vital to note that a synergistic effect was seen, as evidenced by computed AI. To counteract these effects, improved regulation, education, and potentially decreased use of harmful chemicals in delicate ecosystems will be required. Furthermore, more research is needed to understand the cumulative effects of acute toxicity on different fish species, as well as to quantify these effects in each species.

Conclusions

These findings confirmed the neurotoxicity of IMI and THM in zebrafish. The results of this study revealed that IMI and THM in the aquatic environment may represent possible dangers to fish fitness and survival in the long run. Sustainable farming practices that encourage biodiversity can reduce pesticide consumption by improving natural pest control mechanisms. These offer more environmentally friendly alternatives to traditional chemical pesticides. There are several agricultural environmental regulations and farm management practices that enhance environmental benefits. When considering alternatives to traditional pest control methods that are more environmentally friendly, several options emerge as effective and sustainable choices. These alternatives range from natural substances to integrated pest management strategies, offering a safer approach to controlling pests without harming the environment or human health. Every nation's government has a significant responsibility to play in ensuring that the environment is safe and devoid of harmful pollutants. In order to address public, scientific, and governmental concerns that chemical exposures are linked to human diseases and disabilities, as well as to protect the public from the health effects of chemical exposures, laws such as the Toxic Substances Control Act should be passed. These laws would give the Environmental Protection Agency (EPA) the authority to require testing and reporting, as well as to impose restrictions on the manufacture and use of chemicals and mixtures. To address this mission, the Environmental Protection Agency (EPA) and other agencies should be involved in collecting data and conducting studies, as well as using the best available scientific data to prevent and stop exposures, performing testing of chemicals currently in use, setting new risk-based safety standards, requiring protection for vulnerable populations, and increasing public transparency for chemical information to ensure community health.

References

[1] CHUNG KT, CHEN LW, TSENG HW, *et al.* Neonicotinoid imidacloprid affects the social behavior of adult zebrafish by damaging telencephalon neurons through oxidation stress, inflammation, and apoptosis[J]. Life, 2023, 13(6):1-7.

[2] HASAN M, AHMED K, SIDDIQUEE MAM, *et al.* Thiamethoxam affects the developmental stages of banded gourami (*Trichogaster fasciata*)[J]. Toxicology Reports, 2022, 9: 1233-1239.

[3] HLADIK ML, MAIN AR, GOULSON D. Environmental risks and challenges associated with neonicotinoid insecticides[J]. Environmental Science and Technology, 2018, 52(6): 3329-3335.

[4] JENINGA AJ, KOOIJ N, HARRAHY E, *et al.* Binary mixtures of imidacloprid and thiamethoxam do not appear to cause additive toxicity in fathead minnow larvae (*Pimephales promelas*)[J]. Frontiers in Toxicology, 2023, 5: 1-11.

[5] RASHID H, BOSMA RH, RAHMAN S, *et al.* Effects of imidacloprid on the ecology of sub-tropical freshwater[J]. Environmental pollution (Barking, Essex_1987), 2018, 236: 432-441.

[6] SAMOJEDEN CG, PAVAN FA, RUTKOSKI CF, *et al.* Toxicity and genotoxicity of imidacloprid in the tadpoles of *Leptodactylus luctator* and *Physalaemus cuvieri* (Anura: Leptodactylidae)[J]. Scientific Reports, 2022: 1-12.

[7] SÁNCHEZ-BAYO F, GOKA K, HAYASAKA D. Contamination of the aquatic environment with neonicotinoids and its implication for ecosystems [J]. Frontiers in Environmental Science, 2016, 4: 1-14.

[8] VERDEGEM M, BUSCHMANN AH, LATT UW, *et al.* The contribution of aquaculture systems to global aquaculture production[J]. Journal of the World Aquaculture Society, 2023, 54(2): 206-250.

[9] WERNER I, SCHNEEWEISS A, SEGNER H, *et al.* Medium-sized streams of Switzerland [J]. Toxics, 2021, 9(4): 1-15.

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU

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[22] ZHANG BB, HE HF, ZHANG ZL, *et al.* Research and control advances in thrips-vectored viruses of cucurbits and vegetables[J]. Zhongguo Gua-cai, 2021, 34(6): 1-10. (in Chinese).

[23] TONSOMROS Y. Heritability, phenotypic and genotypic correlations of peanut bud necrosis virus (PBNV) reaction parameters in peanut[J]. Songklanakarin Journal of Science& Technology, 2006, 28(3): 469-477.

[24] HASEGAWA DK, HLADKY LJ, WINTERMANTEL WM, *et al.* First report of impatiens necrotic spot virus infecting lettuce in Arizona and southern desert regions of Californias[J]. Plant Disease, 2022, 106(8): 2274.

[25] KHAN RA, SEAL DR, ZHANG S, *et al.* Distribution pattern of thrips (Thysanoptera: Thripidae) and tomato chlorotic spot virus in South Florida tomato fields[J]. Environmental Entomology, 2020, 49(1): 73-87.

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU