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Evaluation of acute and subacute oral toxicity of tolfenpyrad in female Wistar rats

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Abstract

Tolfenpyrad (TFP) is a pyrazole-based insecticide that is widely used in agriculture, however information on its subacute toxicological effects remains limited. This study was designed to evaluate the effects of repeated oral exposure to tolfenpyrad in female Wistar rats over a 28-day period. Prior to subacute evaluation, the acute oral median lethal dose (LD₅₀) of TFP was determined using OECD Test Guideline 425 and was estimated to be 75 mg/kg body weight. For the subacute study, rats were orally administered tolfenpyrad at doses of 15, 7.5 and 3.75 mg/kg body weight daily for 28 days. Clinical signs, body weight, feed intake, and water consumption were monitored throughout the study period. No mortality was observed in any treatment group. However, rats receiving 15 mg/kg and 7.5 mg/kg exhibited dose-dependent clinical signs, including roughened fur, perioral and perianal erythema, hunched posture and altered fecal consistency. These groups also showed a significant reduction in feed intake, water intake and body weight gain compared with the control groups. In contrast, rats treated with 3.75 mg/kg did not exhibit any noticeable clinical alterations and remained comparable to the controls. These findings demonstrate that repeated exposure to higher subacute doses of tolfenpyrad adversely affects normal physiological functions in a dose-dependent manner, highlighting the potential toxicological risks associated with prolonged exposure and underscoring the need for further mechanistic and long-term safety evaluations.

Keywords: Tolfenpyrad, female Wistar rat, acute oral median lethal dose, feed, water intake and body weight gain

Introduction

Agriculture plays a pivotal role in India, providing employment to approximately 45% of the population and contributing nearly 18% to the nation's GDP. Annual crop losses of 20-30% owing to pests, diseases and weeds (Reddy *et al.*, 2024) [15]. While pesticides safeguard crop productivity, they pose significant risks to human health and the environment (Aktar *et al.*, 2009) [3]. Tolfenpyrad (TFP), a pyrazole-based insecticide and acaricide, is widely recognized for its broad-spectrum efficacy in protecting various crops, including cereals, legumes and vegetables. Chemically identified as 4-chloro-3-ethyl-1-methyl-N-(4-(p-tolyloxy)benzyl)-1H-pyrazole-5-carboxamide, TFP exhibits strong activity against diverse insect groups, including hemiptera, coleoptera, diptera, lepidoptera, thysanoptera, and acarina (Hikiji *et al.*, 2013) [8]. It has been shown to be effective against pest populations resistant to conventional pesticides such as organophosphates and carbamates, thereby providing an alternative pest management strategy (Bajpai *et al.*, 2013) [4]. Its mechanistic pathway involves inhibition of complex I in the mitochondrial electron transport chain, resulting in energy depletion and mitochondrial dysfunction, which enhances its potency against resistant pests (Wimer *et al.*, 2015; Yamaguchi *et al.*, 2012) [17, 18]. This distinctive mechanism suggests that TFP can be effective even in cases of pesticide resistance. Field trials also support its practical usefulness; for example, treatments with a 15% EC formulation at 125-150 g a.i./ha successfully controlled sucking pest complexes in okra without harming beneficial insects (Mallick *et al.*, 2016) [11]. However, the repeated use of leafy and other vegetable crops may lead to residue accumulation, posing potential risks to human and animal health (Dong *et al.*, 2018) [7]. Although data on human exposure are limited, several poisoning incidents have been documented (Yamaguchi *et al.*, 2012; Hikiji *et al.*, 2013) [18, 8], characterized by symptoms such as vomiting, mucosal irritation, dilated

pupils, respiratory depression and hypotension, which can be fatal in severe cases (Isaka *et al.*, 2016) [9]. Treatment is largely supportive, emphasising the need for safe handling. Against this background, the present study was undertaken to investigate the toxicological effects of sub-lethal oral doses of TFP in rats over a 28-day exposure period.

Materials and Methods

Chemicals

Tolfenpyrad (TFP) (analytical grade, 97% purity) was procured from Tokyo Chemical Industry (India) Pvt. Ltd., (CAS RN: 129558-76-5).

Animals

Healthy adult female Wistar albino rats, weighing 180-220 g, procured from the Committee for the Control and Supervision of Experiments on Animals (CCSEA) approved by the Laboratory Animal Facility (LAF), College of Veterinary and Animal Sciences, Pookode, were used in the study. All animals were housed under standard conditions at 24 °C and 50-60% relative humidity and were fed standard laboratory feed and water *ad libitum*. The daily cycle alternated between 12 hours of light and 12 hours of darkness. Seven days before the start of the trial, animals were acclimated to the laboratory environment. The study was conducted over a period of 28 days. All experimental protocol were approved by the Institutional Animal Ethics Committee (IAEC) of College of Veterinary and Animal Sciences, Pookode (IAEC/COVAS/PKD/24/10/2025) and were provided *ad libitum* feed and water throughout the duration of the study (CCSEA, 2018) [6].

Acute Toxicity Study

A total of 10 female Wistar rats were taken for acute toxicity study, the median lethal dose (LD₅₀) of tolfenpyrad was determined in accordance with OECD-TG 425 using the AOT 425 Stat PGM software. A single animal was used for each dose level based on the slope factor specified in the

guideline. Both immediate and delayed mortalities were considered for maximum likelihood estimation. Dosing was carried out at fixed time intervals of 48 hours and the study was terminated upon meeting any one of the following predefined stopping criteria: a) three consecutive animals survive at the upper bound. b) 5 reversals occur in any of the six consecutive animals tested. c) At least 4 animals have followed the first reversal and the specified likelihood-ratios exceeded the critical value (OECD, 2022) [13].

Sub-Acute toxicity studies

This study was conducted in accordance with the OECD Guidelines for the Testing of Chemicals, No. 407 (OECD, 2025) [14]. Thirty female Wistar rats were randomly divided into six groups with six animals in each group. Group I (control) and Group II (vehicle control) received olive oil at a dose of 4 mL/kg body weight. Groups III, IV and V were administered TFP at 15 mg/kg, 7.5 mg/kg and 3.75 mg/kg body weight, respectively. Prior to dosing (12h), the feed withdrawn and water was provided *ad libitum*. TFP was administered orally each day at 10:00 a.m. and feed provided three-hour post-dosing. Animals were observed daily for 30 min, every hour up to 4 h, 6 h and every 24 h following drug administration to check for clinical signs.

Parameters

Clinical signs, initial (0th day) and final (28th day) body weights, feed and water consumption of the rats in the control and treatment groups were recorded.

Statistical analysis

One-way ANOVA followed by Tukey's multiple comparison was used to determine which groups showed significant differences. Statistical analyses were performed using GraphPad Prism V 8.0.2. Results are expressed as the mean ± standard error of the mean (SEM), n = 6.

Results

AOT425StatPgm
 New Test Load Data Save Data Get Report Options About AOT425 Exit

Test / Substance: TOLFENPYRAD FEMALE WISTAR ALBINO RATS

Test Type: Main

Limit Dose: 2000

Assumed values at start of the main test
 LD50: Default Sigma: 0.5

Test Seq.	Animal ID	Dose mg/kg	Short-term Outcome	Long-term Outcome	Program's Data Entry Messages
1	HEAD	88	X	X	
2	BODY	64	0	0	
3	TAIL	88	X	X	
4	HEADBODY	64	0	0	
5	BODYTAIL	88	X	X	
6	TAILHEAD	64	0	0	
7			Stop Dosing		
8					
9					
10					
11					
12					
13					
14					
15					

The main test is complete.

WARNING: The dose sequence and short-term outcomes are not consistent with OECD 425 procedure. See report for details.
 Stopping criteria met: 5 reversals in 6 tests. LR criterion.
 Estimated LD50 = 75.05 (Based on an assumed sigma of 0.5). Approximate 95% confidence interval is 64 to 88.

Fig 1: Estimation of LD₅₀ by using AOT 425 Stat PGM software

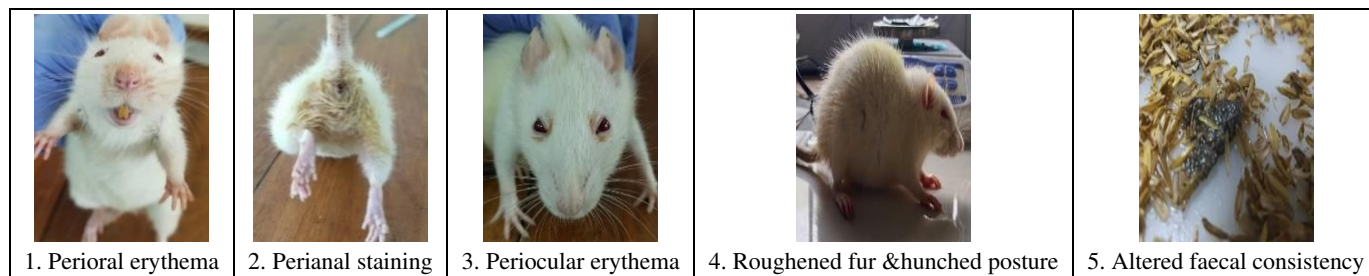


Fig 2: Clinical signs exhibited by TFP treated rats includes

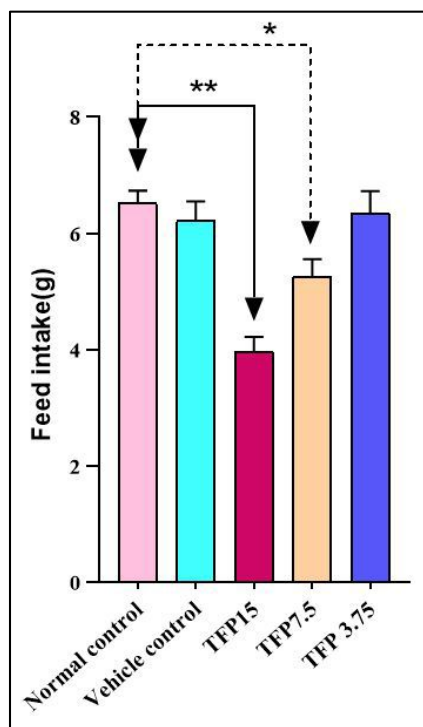


Fig 3: Graphical representation of feed Intake (g) in Wistar rats following sub-acute exposure of tolfenpyrad

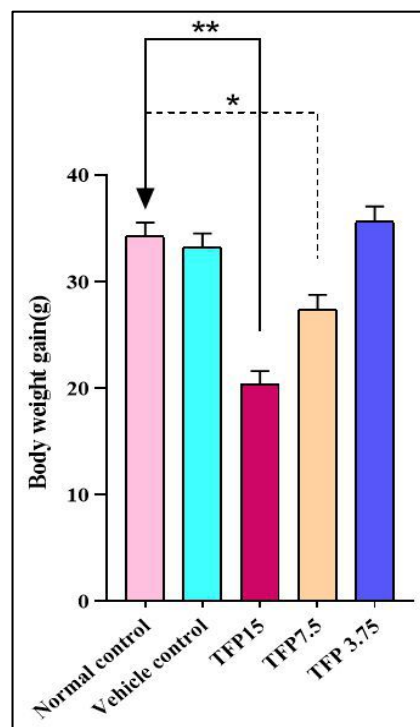


Fig 5: Graphical representation of Body weight gain (g) in Wistar rats following sub-acute exposure of tolfenpyrad

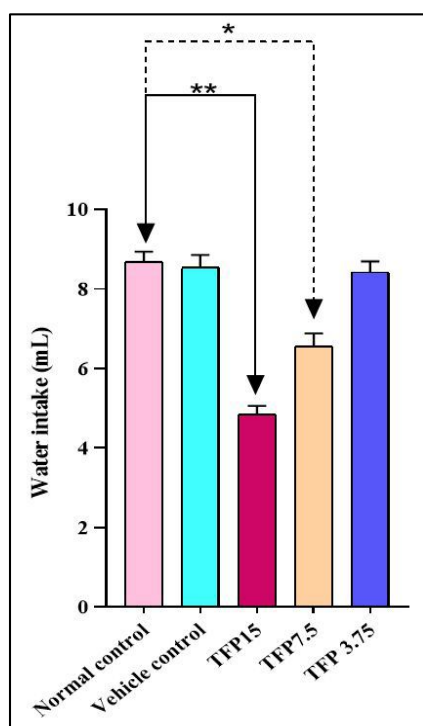


Fig 4: Graphical representation of water Intake (mL) in Wistar rats following sub-acute exposure of tolfenpyrad

Discussion

The present study demonstrated that repeated oral exposure to tolfenpyrad at sub-lethal doses for 28 days produced dose-dependent clinical and physiological alterations in female Wistar rats without causing mortality. The estimated LD₅₀ value of 75 mg/kg body weight indicated moderate acute oral toxicity of TFP in rats.

Although overt toxicity was not observed, the appearance of clinical signs such as roughened hair coat, erythema, hunched posture, altered fecal consistency, and reduced feed and water intake suggests treatment-related stress. The reduction in feed and water intake observed in this study is consistent with previous toxicological reports. The Joint FAO/WHO Meeting on Pesticide Residues (JMPR, 2013) [10] documented mild ocular and dermal irritation following TFP exposure in rabbits, supporting the clinical signs observed in the present investigation.

Subacute pyrazole toxicity has been reported to show patterns similar to those observed in a 90-day dietary study in Wistar albino rats, wherein tolfenpyrad caused marked reductions in feed intake and body weight at higher dietary concentrations (Mansour *et al.*, 2008; Bhardwaj *et al.*, 2010) [12, 5]. The significant reduction in body-weight gain observed in the high-and moderate-dose groups in the present study may be attributed to diminished metabolic energy availability resulting from reduced feed intake and

disruption of the physiological pathways essential for nutrient utilization and growth (Bhardwaj *et al.*, 2010) [5].

Mechanistically, appetite suppression and growth retardation in pyrazole-exposed rats may involve mitochondrial dysfunction leading to impaired energy metabolism, along with possible interference in central neural pathways regulating feeding behaviour, mechanisms have also been proposed for abamectin toxicity (Abdel-Razik and Hamed, 2015) [1]. Similar effects have been reported with other pesticides, including imidacloprid, which cause significant reductions in body weight and feed intake in female rats (Bhardwaj *et al.*, 2010; Vohra *et al.*, 2014) [5, 16]. Additionally, exposure to cymoxanil has been shown to significantly decreases body weight in rats (Ahmed *et al.*, 2020) [2], indicating that pesticide-induced alterations in body-weight gain are common toxicological findings.

Overall, the findings of the present study suggest that subacute exposure to tolfenpyrad induces dose-dependent reductions in feed intake, water intake and body-weight gain, which may contribute to its toxicity profile during repeated exposure.

Conclusion

The present study demonstrated that repeated oral exposure to tolfenpyrad for 28 days induced clear dose-dependent physiological and clinical alterations in female Wistar rats. Although no mortality was observed, higher doses of tolfenpyrad (15 mg/kg and 7.5 mg/kg body weight) resulted in significant reductions in feed intake, water consumption, and body weight gain, along with observable clinical signs such as roughened fur, erythema, hunched posture, and altered faecal consistency. These findings indicate that subacute exposure to tolfenpyrad can disrupt normal metabolic and physiological processes, likely through interference with mitochondrial energy metabolism and neurophysiological pathways that regulate appetite and growth. In contrast, the lowest dose tested (3.75 mg/kg) did not produce any adverse effects, suggesting a threshold for subacute toxicity. Overall, this study highlights the potential health risks associated with repeated exposure to tolfenpyrad and emphasizes the importance of cautious use and further investigations to elucidate the underlying mechanisms of toxicity and establish safer exposure limits for animals and humans.

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