© Environmental Toxicology and Ecology
2025, Vol. 5 (2)
ISSN: 2757-9719

ETOXEC
Environmental Toxicology and Ecology

Research Article

Investigation of Genotoxic Mechanisms of Etofenprox: Cytotoxicity, Micronucleus and Comet Analyses

Faysal YILMAZ

ABSTRACT

Etofenprox is a synthetic pyrethroid pesticide widely used in agriculture, with significant ecotoxicological concern due to its potential for long-term persistence in the environment. This study aimed to evaluate the cytotoxic and genotoxic effects of etofenprox on Chinese hamster ovary (CHO) cells. Cytotoxicity was determined by measuring the cytokinesis-block proliferation index (CBPI) after exposure to various concentrations (1–800 µg/mL) of etofenprox. Genotoxicity was assessed using the micronucleus (MN) assay and alkaline comet assay. The results revealed significant reductions in CBPI, increases in MN frequency, and pronounced elevations in DNA damage parameters (tail length, % tail DNA, olive tail moment) at higher concentrations. These effects increased in a dose-dependent manner, particularly at concentrations ≥200 µg/mL, where the changes were statistically significant ($p<0.05$). The data indicate that at high doses, etofenprox may cause both clastogenic effects and DNA strand breaks. The findings suggest that, while the pesticide may pose a low risk at typical environmental exposure levels, high-dose exposure can induce adverse effects at cellular and genetic levels.

Article Info

Author e-mail:
faysal1774@gmail.com

Institution: [1] Bursa Teknik Unıversity, School of Graduate Studies

Article history

Received: 15/08/2025

Accepted: February 26/09/2025

Available online: 13/10/2025

Keywords:

Etofenprox, Genotoxicity, Cytotoxicity, Micronucleus assay, Comet assay

Anahtar Kelimeler:

Etofenprox, Genotoksisite, Sitotoksisite, Mikronükleus testi, Komet testi

How to Cite: F. Yılmaz "Investigation of Genotoxic Mechanisms of Etofenprox: Cytotoxicity, Micronucleus and Comet Analyses", *Environmental Toxicology and Ecology, c. 5, sayı. 2, ss. 72-81, 2025.*

DOI: 10.59838/etoxec.1765508

Etofenprox'un Genotoksik Mekanizmalarının İncelenmesi: Sitotoksisite, Mikronükleus ve Komet Analizleri

ÖZET

Etofenprox, tarımda yaygın olarak kullanılan sentetik bir piretroit pestisittir ve çevrede uzun süre kalabilme potansiyeli nedeniyle ekotoksikolojik açıdan önem taşımaktadır. Bu çalışma, etofenprox'un Çin hamsteri ovaryum (CHO) hücrelerinde sitotoksik ve genotoksik etkilerini değerlendirmek amacıyla gerçekleştirilmiştir. Sitotoksisite, farklı konsantrasyonlarda (1–800 µg/mL) etofenprox uygulaması sonrası hücrelerde çekirdek bölünme indeksi (ÇBİ) ölçülerek belirlenmiştir. Genotoksisite değerlendirmesi ise mikronükleus (MN) testi ve alkali komet testi ile yapılmıştır. Bulgular, yüksek doz uygulamalarında ÇBİ'de anlamlı azalmalar, MN frekansında artışlar ve DNA hasar parametrelerinde (kuyruk uzunluğu, kuyruk DNA yüzdesi, olive kuyruk momenti) belirgin yükselişler olduğunu göstermiştir. Bu etkiler doza bağımlı olarak artmış ve özellikle ≥200 µg/mL konsantrasyonlarda istatistiksel olarak anlamlı bulunmuştur ($p<0.05$). Elde edilen veriler, etofenprox'un yüksek dozlarda hem klastojenik hem de DNA zincir hasarına neden olabileceğini ortaya koymaktadır. Çalışma, bu pestisitin çevresel maruziyet seviyelerinde düşük risk taşıyabileceğini, ancak yoğun temas koşullarında hücresel ve genetik düzeyde olumsuz etkiler oluşturabileceğini göstermektedir.

© Environmental Toxicology and Ecology
2025, Vol. 5 (2)
ISSN: 2757-9719

ETOXEC
Environmental Toxicology and Ecology

Research Article

1. INTRODUCTION

Etofenprox is a synthetic pyrethroid insecticide widely used in agricultural production to control harmful insects. Due to its high lipophilicity and low vapor pressure, it has long-term environmental persistence [1] and possesses the potential to interact with biota in soil and aquatic ecosystems. The effects of pesticides on non-target organisms, particularly the potential damage they can cause to genetic material, constitute an important research area in ecotoxicology [2].

Genotoxicity refers to biological effects that lead to permanent changes in DNA, such as strand breaks, chromosomal aberrations, and mutations [3]. Such effects can, in the long term, contribute to the development of cancer, reproductive disorders, and hereditary diseases. Current data on the potential genotoxic effects of etofenprox on mammalian cells are limited, and most studies have been restricted to acute toxicity or ecotoxicological parameters [4,5].

In this study, the cytotoxic and genotoxic effects of etofenprox on mammalian cells were investigated in vitro using the Chinese hamster ovary (CHO) cell line. Cytotoxicity was evaluated through the cytokinesis-block proliferation index (CBPI), and genotoxicity was assessed using the micronucleus (MN) assay and alkaline comet assay. The results are expected to contribute to determining the potential risks of this pesticide at the cellular and genetic levels, and to environmental safety assessments.

2. MATERIALS and METHODS

2.1. Cell Culture

The Chinese hamster ovary (CHO) cell line was used in this study. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Experiments were conducted using cells in the logarithmic growth phase. All chemical solutions were prepared under sterile conditions.

2.2. Test Substance and Determination of Concentrations

Etofenprox (≥98% purity, Sigma-Aldrich) stock solution was prepared in dimethyl sulfoxide (DMSO) and diluted with the appropriate culture medium prior to experiments. Based on preliminary trials and clonogenic assay results, six concentrations were selected: 1, 10, 50, 200, 400, and 800 µg/mL. In all treatments, the DMSO concentration did not exceed 0.5%.

2.3. Cytotoxicity Analysis – Clonogenic Assay

The clonogenic assay was used to determine cell viability. A defined number of cells were seeded into each well, and after 24 h of etofenprox exposure, cells were washed with fresh medium and incubated for 7 days. At the end of incubation, colonies were stained with 0.5% crystal violet and counted using an image analysis system. The percentage of cell viability was calculated according to Equation (1) with respect to the control group:

$$\text{Viability (\%)} = \frac{\text{Number of colonies (treatment)}}{\text{Number of colonies (control)}} \times 100 \qquad (1)$$

© Environmental Toxicology and Ecology
2025, Vol. 5 (2)
ISSN: 2757-9719

ETOXEC
Environmental Toxicology and Ecology

Research Article

2.4. Micronucleus (MN) Assay

The MN assay was performed in accordance with OECD Guideline 487. CHO cells were exposed to selected doses of etofenprox for 24 h. At the end of this period, cytochalasin-B (3 µg/mL) was added to block cytokinesis, followed by an additional 20 h of incubation. Cells were harvested by trypsinization, centrifuged, and fixed with methanol:acetic acid (3:1). Prepared slides were stained with Giemsa, and micronucleus frequencies in binucleated cells were scored under a microscope.

The cytokinesis-block proliferation index (CBPI) was calculated using Equation (2):

$$\mathrm{CBPI} = \frac{M_1 + 2 \times M_2 + 3 \times M_3 + 4 \times M_4}{N} \qquad (2)$$

where M1M_1M1–M4M_4M4 represent the number of cells with 1, 2, 3, and 4 nuclei, respectively, and NNN is the total number of scored cells.

2.5. Comet (Single Cell Gel Electrophoresis) Assay

The alkaline comet assay was performed to determine DNA damage. Cells from each treatment group were embedded in low-melting-point agarose on pre-coated microscope slides. Slides were immersed in lysis buffer (pH 10) for 1 h, followed by alkaline electrophoresis buffer (pH > 13) for 20 min, and then subjected to electrophoresis at 25 V/300 mA for 20 min. After neutralization, slides were stained with ethidium bromide. Comet parameters, including tail length and olive tail moment, were analyzed using "KAMERAM" software under a fluorescence microscope.

2.3. Statistical Analysis

All experiments were performed in triplicate, and data were expressed as mean ± standard error (SE). Statistical analysis was conducted using SPSS 22.0 software. Differences between groups were evaluated by one-way ANOVA followed by Dunnett's post-hoc test, with $p < 0.05$ considered statistically significant. Dose–response relationships were assessed by linear regression analysis.

3. RESULTS

3.1. Effect of Etofenprox on the Survival Rate (%)

To determine the concentrations of etofenprox to be used in the study, Chinese hamster ovary (CHO) cells were evaluated using the clonogenic assay, and the survival rates of the experimental groups were calculated (Figure 1). According to the formula, the IC_{50} value of etofenprox for CHO cells was calculated as 302 µg/mL. Based on this value, six concentrations (1, 10, 50, 200, 400, and 800 µg/mL) were selected for use in the genotoxicity assays.

© Environmental Toxicology and Ecology
2025, Vol. 5 (2)
ISSN: 2757-9719

ETOXEC
Environmental Toxicology and Ecology

Research Article

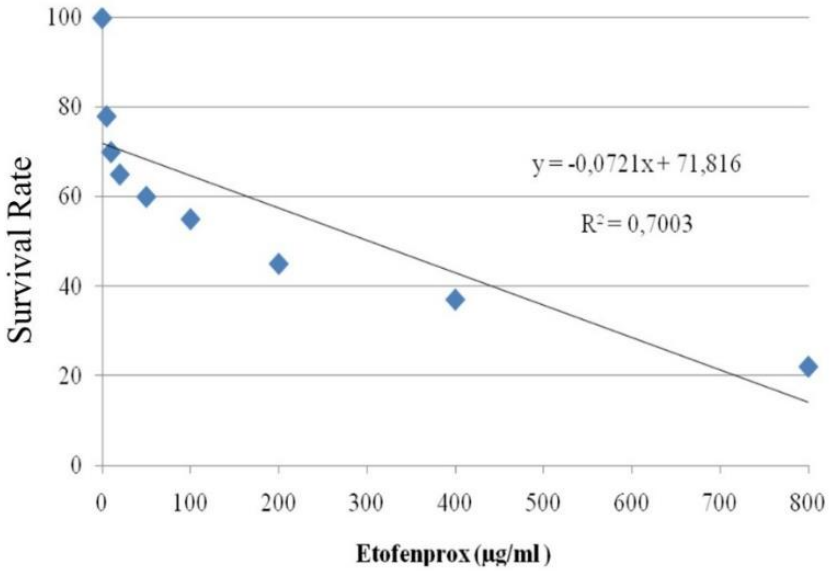

Figure 1. Survival rates (%) of CHO cells exposed to various concentrations of etofenprox, determined by the clonogenic assay.

3.2. Effect of Etofenprox on Micronucleus Formation

CHO cells were incubated for 24 hours with the determined concentrations, and micronucleus (MN) frequencies were calculated for each group according to the MN test procedure (Table 1, Figure 2). The data showed that, except for the 1 and 10 µg/mL doses ($p>0.05$), all other concentrations resulted in statistically significant increases in MN frequency. These increases were significant at $p<0.05$ for 50 µg/mL, $p<0.01$ for 200 and 400 µg/mL, and $p<0.001$ for 800 µg/mL. Regression analyses revealed that the increase in MN frequency was not dose-dependent ($p>0.05$).

Table 1. Micronucleus frequencies in CHO cells exposed to various concentrations of etofenprox.

Test substance	Doses (µg/mL)	MN frequencies		MN (‰) ± SE
		MONO	BI	
Control	---	10	1	2.52 ±0.002
SC (Ethanol)	50 µl	2	0	2.50 ±0.007
PK (H_2O_2)	200 µl	119	13	17.360 ±0.006
Etofenprox	1 µg/ml	6	0	3.100 ±0.066
	10 µg/ml	6	0	3.850 ±0.056
	50 µg/ml	10	1	5.255 ±0.087
	200 µg/ml	53	4	12.495 ±0.085
	400 µg/ml	57	11	14.250 ±0.017
	800 µg/ml	62	17	16.495 ±0.094

© Environmental Toxicology and Ecology
2025, Vol. 5 (2)
ISSN: 2757-9719

ETOXEC
Environmental Toxicology and Ecology

Research Article

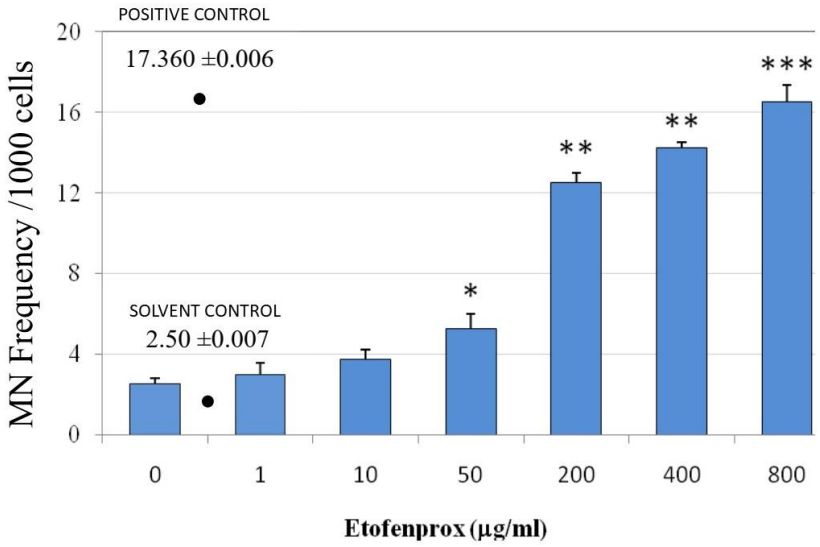

Figure 2. Micronucleus frequencies (‰) in CHO cells exposed to various concentrations of etofenprox (*p<0.05, **p<0.01, ***p<0.001).

3.3. Effect of Etofenprox on the Cytokinesis-Block Proliferation Index (CBPI)

Based on the MN test results, CBPI values were calculated and presented in Table 2 and Figure 3. No statistically significant changes in CBPI were observed at 1, 10, and 50 µg/mL doses compared to the control group (p>0.05). However, significant decreases were observed at p<0.05 for 200 and 400 µg/mL doses, and at p<0.01 for the 800 µg/mL dose. Regression analyses indicated that this decrease was not dose-dependent (p>0.05).

Table 2. CBPI values in CHO cells exposed to various concentrations of etofenprox.

Test substance	Doses (µg/mL)	Scored nucleus numbers				NDI ± SE
		1	2	3	4	
Control	----	498	3254	180	68	1.954 ±0.101
SC (Ethanol)	50 µl	1378	2253	247	122	1.931 ±0.097
PK (H_2O_2)	200 µl	1988	1953	51	8	1.213 ±0.226
Etofenprox	1 µg/ml	250	1634	104	39	1.960 ±0.121
	10 µg/ml	287	1612	102	31	1.941 ±0.095
	50 µg/ml	316	1553	106	20	1.910 ±0.187
	200 µg/ml	1222	2493	265	20	1.772 ±0.105
	400 µg/ml	1326	2496	164	14	1.712 ±0.167
	800 µg/ml	1708	2044	231	17	1.639 ±0.117

© Environmental Toxicology and Ecology
2025, Vol. 5 (2)
ISSN: 2757-9719

ETOXEC
Environmental Toxicology and Ecology

Research Article

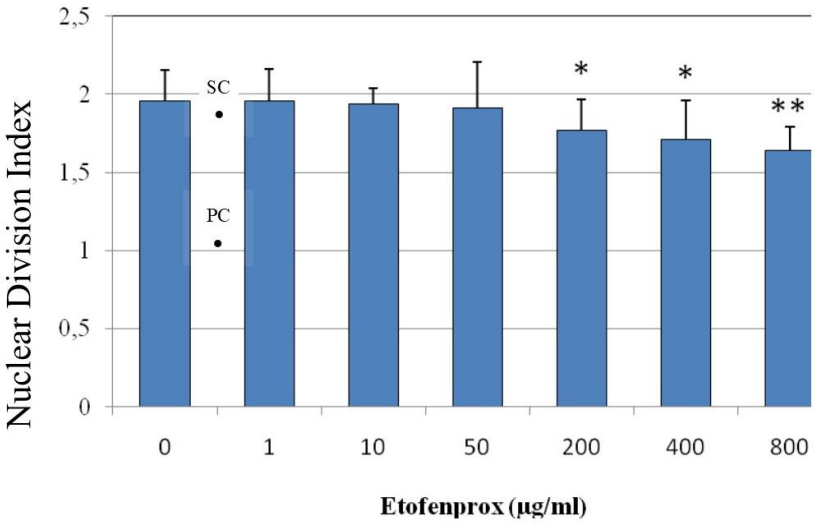

Figure 3. CBPI values in CHO cells exposed to etofenprox (*p<0.05, *p<0.01).

3.4. Effect of Etofenprox on Comet Formation

Comet measurements were performed using the "KAMERAM" software, and the DNA damage status in CHO cells is shown in Figure 4. The mean tail length and Olive tail moment values obtained are presented in Table 3, Figures 5 and 6.

The findings indicated a statistically significant increase in both tail length and Olive tail moment at 200 µg/mL ($p<0.05$) and at 400 and 800 µg/mL ($p<0.001$) doses. Regression analyses revealed that the increases in both parameters were dose-dependent (tail length: $R^2 = 0.88$; Olive tail moment: $R^2 = 0.87$; $p<0.05$).

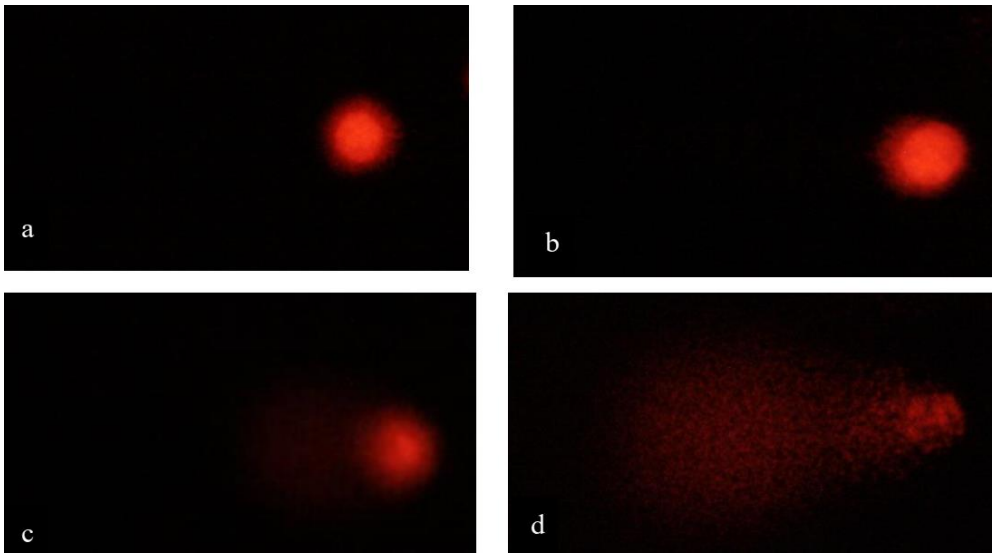

Figure 4. Comet images in CHO cells: a) undamaged DNA, b) slightly damaged DNA, c) moderately damaged DNA, d) severely damaged DNA (×400).

© Environmental Toxicology and Ecology
2025, Vol. 5 (2)
ISSN: 2757-9719

ETOXEC
Environmental Toxicology and Ecology

Research Article

Table 3. Mean tail length and Olive tail moment values in CHO cells exposed to various concentrations of etofenprox.

Test substance	Doses (µg/mL)	Mean Tail Length ± SE	Mean Olive Tail Moment ± SE
Control	----	0.00767 ± 0.00019	0.00322 ± 0.00014
SC (Ethanol)	50 µl	0.00756 ± 0.00022	0.00291± 0.00012
PK (H_2O_2)	200 µl	0.05763 ± 0.00277	0.02550 ± 0.00139
Etofenprox	1 µg/ml	0.01063 ± 0.00048	0.00556 ± 0.00035
	5 µg/ml	0.01201 ± 0.00059	0.00626 ± 0.00035
	50 µg/ml	0.01063 ± 0.00032	0.00548 ± 0.00022
	200 µg/ml	0.02912 ± 0.00211	0.01326 ± 0.00107
	400 µg/ml	0.04660 ± 0.00275	0.02135 ± 0.00140
	800 µg/ml	0.05840 ± 0.00304	0.02633 ± 0.00149

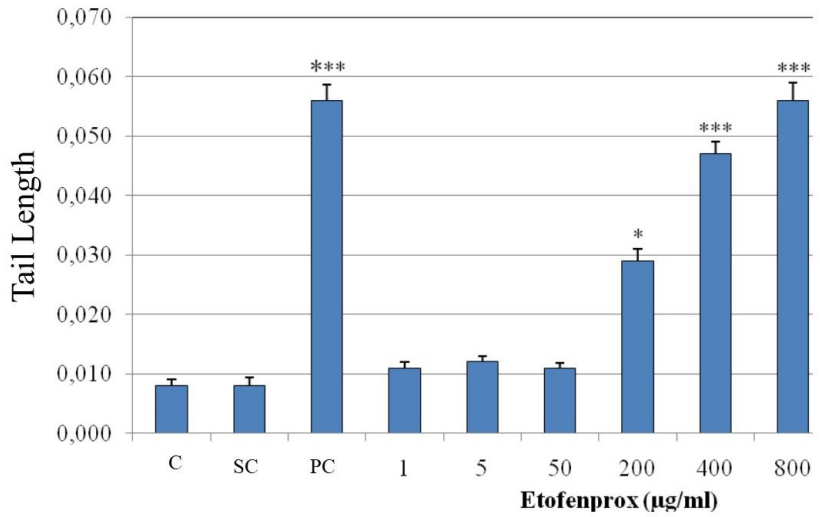

Figure 5. Mean tail length values determined by the comet assay (K: Control, SC: Solvent Control, PC: Positive Control) (*p<0.05, **p<0.01, ***p<0.001).

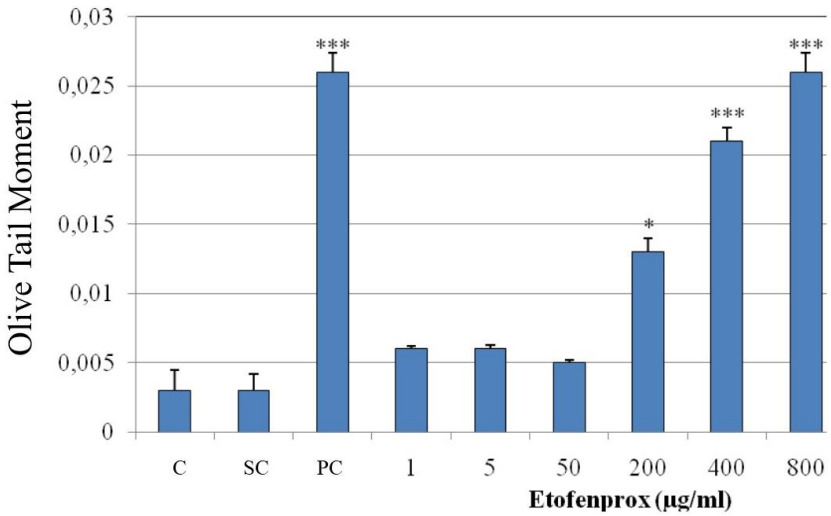

Figure 6. Mean Olive tail moment values determined by the comet assay (K: Control, SC: Solvent Control, PC: Positive Control) (*p<0.05, **p<0.01, ***p<0.001).

4. DISCUSSION and CONCLUSION

Exposure of CHO cells to etofenprox at concentrations ranging from 1 to 800 µg/mL resulted in a clear concentration-dependent reduction in cell viability, as determined by the clonogenic assay. The calculated IC_{50} value was 302 µg/mL, indicating substantial cytotoxicity at higher exposure levels. Statistically significant decreases in viability were observed at concentrations ≥ 200 µg/mL ($p < 0.05$), suggesting that etofenprox disrupts cellular proliferation and colony-forming ability under high-dose conditions.

Micronucleus (MN) assay results demonstrated a significant elevation in MN frequency at concentrations ≥ 50 µg/mL, with the maximum induction recorded at 800 µg/mL ($p < 0.001$). Despite these increases, regression analysis did not reveal a strictly linear dose–response relationship, implying that MN induction may occur through threshold-dependent or non-linear mechanisms.

The Nuclear Division Index (NDI) was stable at lower concentrations (≤ 50 µg/mL) but declined significantly at doses ≥ 200 µg/mL, indicating possible interference with mitotic progression and cell cycle dynamics.

Alkaline comet assay data revealed a robust, dose-dependent increase in DNA strand breaks, supported by strong correlations in both tail length and Olive tail moment parameters ($R^2 > 0.85$, $p < 0.05$). DNA damage became statistically significant at concentrations ≥ 200 µg/mL, suggesting that etofenprox can induce direct or oxidative DNA strand scission at high doses.

Taken together, these results provide strong evidence that etofenprox exerts both clastogenic and DNA-damaging effects in CHO cells. The observed genotoxicity becomes pronounced at concentrations substantially above typical environmental exposure levels, indicating a limited genotoxic risk for the general population under normal conditions. However, the significant cytogenetic and DNA damage effects at higher doses underscore the necessity for strict occupational safety measures and exposure monitoring, particularly in agricultural and industrial applications where etofenprox is extensively employed.

Acknowledgments

The author expresses sincere gratitude to Prof. Dr. Tolga Çavaş for providing the opportunity to conduct this study at the Department of Biology, Faculty of Science, Uludağ University, and to all members of the genotoxicity laboratory for their valuable assistance. The author also thanks the anonymous reviewers for their constructive comments and suggestions.

Funding

This study was supported by the Uludağ University Scientific Research Projects Unit under the project titled "Investigation of the Genotoxic Effects of Etofenprox on Chinese Hamster Ovary Cells Using Micronucleus and Comet Assays" (Project No: HDP(F)-2012/20).

The Declaration of Conflict of Interest/ Common Interest

The author declares that there is no conflict of interest or common interest associated with this work.

© Environmental Toxicology and Ecology
2025, Vol. 5 (2)
ISSN: 2757-9719

ETOXEC
Environmental Toxicology and Ecology

Research Article

The Declaration of Research and Publication Ethics

This study was derived from the author's Master's thesis titled "Etofenprox'un Genotoksik Etkilerinin Çin Hamsteri Ovaryum Hücrelerinde Mikronükleus ve Komet Testleri Kullanılarak Araştırılması" (Uludağ University, 2013). The thesis is registered in the National Thesis Center of the Council of Higher Education (YÖK) and has not been previously published in any academic journal. All procedures were carried out in accordance with international research, publication ethics, and citation principles. No fabrication or falsification of data occurred during the study, and all ethical responsibilities rest solely with the author.

REFERENCES

[1] Ahamad, A., & Kumar, J. (2023). Pyrethroid pesticides: An overview on classification, toxicological assessment and monitoring. *Journal of Hazardous Materials Advances*, 10, 100284. https://doi.org/10.1016/j.hazadv.2023.100284

[2] Chu, Y., You, F., Wassick, J. M., & Agarwal, A. (2015). Integrated planning and scheduling under production uncertainties: Bi-level model formulation and hybrid solution method. *Computers and Chemical Engineering*, 72, 255–272. https://doi.org/10.1016/j.compchemeng.2014.02.023

[3] Hojo, Y., Shiraki, A., Tsuchiya, T., Shimamoto, K., Ishii, Y., Suzuki, K., Shibutani, M., & Mitsumori, K. (2012). Liver tumor promoting effect of etofenprox in rats and its possible mechanism of action. *Journal of Toxicological Sciences*, 37(2), 297–306. https://doi.org/10.2131/jts.37.297

[4] Malhat, F., Abdallah, H., & Nasr, I. (2012). Estimation of etofenprox residues in tomato fruits by QuEChERS methodology and HPLC–DAD. *Bulletin of Environmental Contamination and Toxicology*, 88, 891–893. https://doi.org/10.1007/s00128-012-0627-6

[5] Hayashi, K., Nakae, A., Fukushima, Y., Sakamoto, K., Furuichi, T., Kitahara, K., Miyazaki, Y., Ikenoue, C., & Toda, T. (2010). Contamination of rice by etofenprox, diethyl phthalate and alkylphenols: effects on first delivery and sperm count in mice. *Journal of Toxicological Sciences*, 35(1), 49–55.

[6] International Programme on Chemical Safety (IPCS). (1993). *Bifenthrin.* In *Pesticide Residues in Food. Evaluations 1992. Part II – Toxicology.* Joint FAO/WHO Meeting on Pesticide Residues (JMPR). Geneva: World Health Organization (WHO/PCS 93.34), pp. 79–102.

[7] International Programme on Chemical Safety (IPCS). (1994). *Etofenprox.* In *Pesticide Residues in Food. Evaluations 1993. Part II – Toxicology.* Joint FAO/WHO Meeting on Pesticide Residues (JMPR). Geneva: World Health Organization (WHO/PCS 94.4), pp. 215–232.

[8] International Programme on Chemical Safety (IPCS). (1996). *Cypermethrin and λ-Cypermethrin.* In *Toxicological Evaluation of Certain Veterinary Drug Residues in Food.* Prepared by the Forty-seventh Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva: World Health Organization (WHO Food Additives Series No. 38), pp. 133–170.

[9] International Programme on Chemical Safety (IPCS). (1997). *λ-Cypermethrin.* Geneva: World Health Organization (Environmental Health Criteria No. 142).

[10] International Programme on Chemical Safety (IPCS). (2001). *Deltamethrin.* In *Pesticide Residues in Food. Evaluations 2000. Part II – Toxicological.* Joint FAO/WHO Meeting on Pesticide Residues (JMPR). Geneva: World Health Organization (WHO/PCS 01.3), pp. 79–110.

[11] World Health Organization (WHO). (2005). *Safety of Pyrethroids for Public Health Use.* Geneva: WHO/CDS/WHOPES/GCDPP/2005.10.

[12] Environmental Protection Agency (EPA). (1985). *Guidelines for the Registration and Control of Pesticides.* EPA, 29 pp.

[13] United Nations Environment Programme (UNEP). (2007). *Stockholm Convention on Persistent Organic Pollutants Persistent Organic Pollutants Review Committee Third Meeting*, Geneva, 19–23 November 2007. UNEP/POPS/POPRC.3/12.

[14] Bootman, J., & May, K. (1985). *MTI-500 α-CO: Assessment of its Mutagenic Potential in Amino-Acid Auxotrophs of Salmonella typhimurium and Escherichia coli.* Life Science Research Limited, Eye, Suffolk, England. LSR Report No. 85/MT0020/433.

[15] Foster, D. E., & Wintersteen, W. K. (1986). *W.K. Insectic Acaric Tests.* Stalk Borer Control, 11, 230–231.

[16] Seeberg, A. H. (1985). *Unscheduled DNA synthesis in human cells, cell line: HeLa S3, test substance MTI-500.* Life Science Research Roma Toxicology Centre, Italy. Report No. 162003-M-05785.

[17] Demir, E., Kocağoğlu, S., & Kaya, B. (2008). Genotoxicity testing of four benzyl derivatives in the *Drosophila* wing spot test. *Food and Chemical Toxicology*, 46(3), 1034–1041.