ISSN: 2458-8989

Natural and Engineering Sciences

NESciences, 2023, 8 (2): 119-128 doi: 10.28978/nesciences.1338147

The Genotoxic Damage in *Cyprinus carpio* Exposed to Abamectin

Funda Turan *, Ayşegül Ergenler

Iskenderun Technical University, Faculty of Marine Science and Technology, Hatay, Türkiye.

Abstract

The pesticide abamectin, which is often used in agriculture, poses a threat to aquatic animals. Though its toxicity to fish has not yet been fully understood. In this study, we used the comet assay to examine the effects of being subjected to various dosages of abamectin on the genotoxic impact of abamectin in *Cyprinus carpio*. During 10 days, common carp were exposed to three different doses of abamectin (0.3, 0.6, and 0.9 mg L^{-1}) based on previously discovered levels in aquatic environments. Toward the completion of the investigation, the Comet assay was used to assess the damage frequency (%), Arbitrary unit (%), and Genetic damage index (%) in carp gill and liver cells. The greatest damage frequencies of % 74.333±0.577 and % 70.333±2.082 were significantly found in the 0.9 mg L^{-1} group in the gill and liver cells, respectively ($P<0.001$). Our results showed a considerable increase in DNA strand breaks in *C. carpio* after exposure to abamectin, suggesting the pesticide's capacity to be genotoxic to fish.

Keywords:

Abamectin, DNA damage, pesticide, comet assay

Article history:

Received 24 April 2023, Accepted 12 July 2023, Available online 07 August 2023

Introduction

The usage of pesticides in agriculture has increased the amount of food produced worldwide (Alexandratos & Bruinsma, 2012; Santos et al., 2023). Yet, as more and more pesticides are employed to boost agricultural production, the situation is growing more dangerous. Thus, there are concerns over the possibility of environmental pollution and its consequences on creatures other than the targets (Blahova et al., 2020). By faulty container handling, transportation problems, and direct application, pesticides can pollute land and water. The mechanisms of retention, transformation, and transportation all have a role in how pesticides behave in the environment (Carvalho, 2017). Pesticides can have a variety of biological impacts and can affect several

*Corresponding Author: Funda TURAN, E-mail: turanfunda@yahoo.com

biochemical processes that are universal to all species. (Turan & Ergenler, 2022; Tresnakova et al., 2022).

Additional environmental factors and pesticides can produce oxidative stress, which is caused by a balance between oxidative and anti-oxidative stress mechanisms at the cellular level. Antioxidant enzymes and non-enzymatic antioxidants function to absorb reactive oxygen species (ROS) and protect cells from oxidative stress damage (Liang et al., 2017; Ergenler & Turan, 2022). In addition, oxidative stress will increase the inflammatory response and affect how the cells respond to infections and toxic chemicals as sources of harmful aggravation.

A group of substances known as "abamectin" is employed for human consumption as a veterinarian insecticide and repellent. (Pitterna et al., 2009; Liu et al., 2020). In 1978, S*treptomyces avermitilis* and fermentative actinomyces were used to isolate the first chemicals in this class. (McCavera et al., 2007; Prichard et al., 2012). Abamectin is a mixture of avermectins (around 80% avermectin B_{1a} and 20% avermectin B_{1b}) (Yu et al., 2017) (Figure 1).

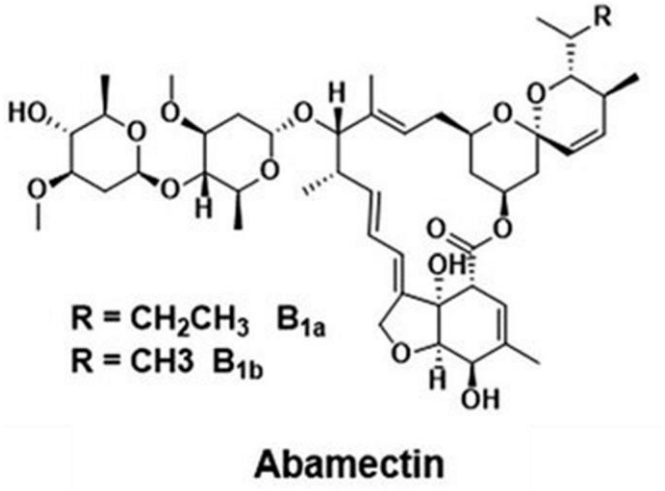

Figure 1. The chemical structure of abamectin (Yu et al., 2017).

Abamectin can accumulate in animal tissues since it is known to be diffuse, slightly soluble in water, and soluble in organic solvents. (Prasse et al., 2009; Lumaret et al., 2012; Santos et al., 2023).The usage of pesticides in aquatic environments and the harmful effects of pesticides on animals have been investigated in several research trials (Novelli et al., 2012; Alm et al., 2017; Hong et al., 2020; Turan & Ergenler, 2022; Ergenler & Turan, 2022). Less is understood about their harm to aquatic habitats, particularly fish (Turan & Ergenler, 2022; Piriscila et al., 2023; Feng et al., 2023). Using the micronucleus test and comet assay, recent studies by Turan & Ergenler (2022), Ergenler & Turan (2022) revealed the genotoxic properties of acetamipridine and thiamethoxam in a common carp, *Cyprinus carpio*. Pesticides end up in the water when weeds in ecosystems and reservoirs of water are controlled. Since there is an increasing concern over the

presence of genotoxins in the aquatic environment, it is essential to develop methods for recognizing genotoxic chemicals. Additionally, it is unknown if interactions between aquatic organisms and abamectin lead to DNA damage (Feng et al., 2023).

Abamectin, like many other pesticides, can pollute regions outside of its targeted usage and harm creatures that are not their targets. Because of its brief half-life, abamectin is not commonly found in high concentrations in freshwater, but it is nonetheless very poisonous and also can affect aquatic creatures, including many fish species (Bai & Ogburn, 2006).

Fish is one of many aquatic organisms that serve as important biological monitors of aquatic ecosystems. Fish are the largest consumers and contribute significantly to the aquatic food chain by controlling the degree of pollution in aquatic ecosystems. Fish are directly exposed to many xenobiotics, making them ideal indicator organisms for measuring and monitoring water pollution. When foreign substances or carcinogens come into contact with fish, they trigger various interactions between the body's biological and chemical systems, ultimately resulting in biochemical abnormalities. Therefore, it is important to identify the mechanisms of pollution effects and all related countermeasures. To measure the quality of aquatic systems, fish can be used as biomarkers of water pollution (Bonomo et al., 2021). As a result of their capacity to metabolize and absorb contaminants in their systems, fish are among the most appropriate species for potential risk evaluation (Turan & Ergenler, 2019; 2022).

To minimize negative impacts on non-target creatures and public health, it is essential and critical to monitor for harmful effects and screen for various pesticides. Consequently, the purpose of this work was to use the comet test to investigate the genotoxic effects of abamectin in *Cyprinus carpio,* a model fish species.

Materials and Methods

Experimental Design

A total of 200 common carp (*Cyprinus carpio* Linnaeus, 1758), weighing an average of 2.50 g, were used in the experiment. The carp were acclimated for 15 days in a well-ventilated thirty-litre glass tank filled with dechlorinated water that had constant light periods (12:12 light/dark cycle). 24 hours passed between the last feeding and the time the animals were exposed to the pesticide. The specimens were fed commercial carp feed at a rate of 3% of their body weight. After acclimation, the fish were divided into two groups with n = 15 each: an experiment group and a group that served as a control. Three separate abamectin concentrations (0.3, 0.6, and 0.9 g L^{-1}) were chosen to symbolize an acute test lasting a week based on previously observed aquatic environmental values for ten days. There were 45 identical fish in each treatment group. At the end of the experiment, fish were given a dose of five milligrams per liter of quinaldine sulfates (Sigma Chemical Company, Germany) to put them to sleep (Yanar & Genç, 2004). When the specimens

stopped reacting to physical stimuli, they were only handled to extract tissue (liver and gills) for the Comet test (after around 1 to 2 minutes).

Comet Assay

An upgraded version of the Cavalcante et al., (2008) method employing gill cell suspension, cell pellet retention, and single-cell gel electrophoresis was used to conduct the comet experiment. Each slide was examined under a fluorescence microscope Image2M Zeiss at X40 magnification after being stained with ethidium bromide and neutralized with ice-cold 0.4 M Tris solution. Images of 100 cells from each sample were utilized to visually evaluate the nucleoids, which were divided into five groups. For comparison, the damage proportion (% DF), arbitrary values (AU), and DNA damage rating (GDI) were calculated.

Statistical Analysis

Before doing any statistical calculations, the data were checked for normality and homogeneity. A one-way analysis of variance was then performed to see whether there were any significant differences between the treatment groups. The means were analyzed using a one-way ANOVA, and differences were considered statistically meaningful at ($P<0.05$). (Norusis, 1993).

Results

Table 1 presents averages and standard deviations for gill and liver cells of C. Carpio damage frequencies (% DF), arbitrary unit value (AU), as well as genetic damage index (% GDI).

Table 1. The averages and standard deviations for DNA damage in control and different concentrations of abamectin-treated carp gills and liver. (n=15).

Groups ($g\ L^{-1}$)	Damage Frequency (%)	Arbitrary Unit AU	Genetic Damage Index (%)
Liver			
Control	26.000 ± 3.472^{a}	49.333 ± 8.014^{a}	0.493 ± 0.814^{a}
0.3	61.000 ± 1.000^{b}	158.333 ± 1.528^{b}	1.583 ± 0.152^{b}
0.6	65.000 ± 1.000^{b}	173.667 ± 7.572^{c}	1.736 ± 0.752^{c}
0.9	74.333 ± 0.577^{c}	181.000 ± 3.606^{c}	1.810 ± 0.360^{c}
P	***	***	***
Gill			
Control	24.000 ± 0.577^{a}	67.333 ± 1.452^{a}	0.673 ± 0.145^{a}
0.3	56.333 ± 1.528^{b}	114.667 ± 8.145^{b}	1.146 ± 0.814^{b}
0.6	62.333 ± 2.309^{c}	159.000 ± 8.660^{c}	1.590 ± 0.866^{c}
0.9	70.333 ± 2.082^{d}	157.667 ± 6.506^{c}	1.577 ± 0.650^{c}
P	***	***	***

The information is presented as the numeric mean and standard deviation. Significant deviations are indicated by values with various superscripts in each section. Determine the level of significance between the three concentrations of abamectin and DNA damage in the gill tissues of carps taken from the control (*, $P<0.001$).

The damage frequency (%), arbitrary unit values (AU), and genetic damage index (%) in the gill and liver cells of *C. carpio* subjected to the control and three different concentrations of abamectin are reported together with means and standard deviations in Table 1. The DNA damage frequency, AU and GDI parameters are also impacted by abamectin therapy ($P<0.001$). The study's findings showed that the 0.9 mg L^{-1} group substantially had higher damage frequencies (%) at gill and liver cells, correspondingly, of 74.333±0.577 and 70.333±2.082 ($P<0.001$) (Table 1).

The lowest damage frequencies (%) were 24.000±0.577 and 26.000±3.472 discovered in this report's control group's liver and gill cells, accordingly. In addition, it was shown that additional damage markers (AU and GDI) were considerably greater ($P<0.001$) in the gill and liver tissues of the 0.3 and 0.6 mg L^{-1} group compared to the control group (Table 1). In this study, the control group notably had the least AU and GDI. The highest AU and GDI for gill were found to be 181.000±3.606 % and 1.810±0.360 % as a result of the study, while the highest AU and GDI for liver were found to be 157.667±6.506 and 1.577±0.650 % accordingly, in the 0.9 mg L^{-1} group for gill and liver cells ($P<0.001$). In this study, the DNA damage increased due to the increase in the concentrations of abamectin.

Discussion

Pesticides have widespread applications and are frequently employed to protect agricultural productivity. Yet, the consequences of the pesticides sprayed on agriculture might not only operate on target pest populations but additionally have effects on non-target species. Aquatic pesticide exposure is thought to come through runoff, leaching, spray drift, preferred migration, and via soil high porosity, or a mixture of such activities through agricultural regions. Pesticides are mostly transferred to aquatic environments via discharge, although the rate of movement is influenced either by different soil types, the physicochemical characteristics of the pesticides, the time and application rate, and the precipitation following pesticide application. Degradation of pesticides or adsorption to surfaces can be caused by abiotic mechanisms such as photodecomposition by sunshine or breakdown by water (Sumudumali et al., 2021). Yet, due to their indiscriminate and unregulated use, their use might have an impact on species that are not the target. Depending on the type of pesticide, its concentration, the length of exposure, and each one's vulnerability to other variables, the impacts of pesticides might show in different ways and to varying degrees. DNA damage and pesticide exposure have been linked to a wide range of issues. Pesticide exposure has been associated with DNA damage in many living things. Pesticide exposure causes changes in the genetic population, especially DNA damage (Valencia-Quintana et al., 2023).

The comet test may be utilized to assess pesticide exposure. The single-cell gel electrophoresis test has been employed in the evaluation of food items in ecotoxicity, radioactive genetics, ecologic genotoxicity, and genetic toxicology. It has also been used to conduct studies and genetic toxicology brought on by exposure to potentially disruptive chemicals, at the level of

diagnosis, as a result of lifestyle decisions, or as a result of the interaction between diet and anti-oxidant substances use on carcinogenesis.(Liao et al., 2009; Hayat et al., 2018).

There aren't many studies on the toxicity abamectin causes in carp, even though its toxicological effects have been extensively researched. In this investigation, abamectin damage led to DNA damage in the liver and gill material of carp. Many studies have found that creatures exposed to pesticides develop cells with Strand breaks. Below are some studies on creatures that have been introduced to pesticides.

Abamectin damages several tissues and organs in aquatic organisms (Mohammed et al., 2018). In zebrafish investigations, exposure to abamectin damaged the structure and function of the gill tissues, eventually leading to oxygen starvation and mortality (Novelli et al., 2016). According to Feng et al. (2023) the carp gill filament structure was harmed with increasing doses of abamectin exposed, alongside distended and tissue necrosis gill epithelial cells and cells that were inflammatory, suggesting that abamectin hindered the integrity of the carp gill and impacted the carp's respiratory function.

Hepatocytes are being employed for the first time to assess the genotoxicity of Abamectin to *S. prenanti*. Just one day of treatment was necessary for DNA damage to manifest, and OTM levels increased in a dose- and time-dependent way. Even at a density (0.5 g/L) that was significantly lower than that of the environmental level, Abamectin produced substantial Genotoxicity in *S. prenanti* liver hepatocytes (Novelli et al., 2016), in addition to remaining densities 24 hours after implementation in aquatic species (Feng et al., 2023).

The aquatic organisms, such as Daphnia, were quite dangerous to Abamectin; for example, 48-h Abamectin EC50 measurements for *Daphnia similis* and *Daphnia magna* have been identified as 5.1 ng/L and 0.25 g/L, consequently (Novelli et al., 2012). The outcomes for fish were much better; for *Danio rerio*, the 48-h LC50 was 59 g/LV. Fish rates were fairly high. The numbers for fish were significantly higher; for example, the 48-hour LC50 for *Danio rerio* was 59 g/L (Alm et al., 2017). The toxicology of this substance varied depending on the variety; for example, the Abamectin LC_{50} values for the rainbow trout (*Oncorhynchus mykiss*), bluegill fishing sunfish (*Lepomis macrochirus*), channel catfish (*Ictalurus punctatus*), and carp (*Cyprinidae sp*.) were 3.2, 9.6, 24 and 42 g/L, accordingly (Novelli et al., 2012). Based on another study, the LC_{50} values for Abamectin on common carp and *Tilapia mosambica* were 0.475 and 6.828 respectively (Jasmine et al., 2008). Aquatic life was quite harmful to Abamectin. Abamectin was shown to be dangerous and to induce genotoxicity in living things within this inquiry once the gill and liver tissues were investigated. According to our research, abamectin exposure significantly increased the number of DNA breakages in *C. carpio*, indicating that the pesticide may be benign to fish. Our outcomes concur with those of previous studies.

In conclusions, we might recommend utilizing common carp as a model organism for eco-toxicological investigations after analyzing the sensitivity of this agricultural toxin to typical

reference contaminants. Hence, more research using different agricultural pesticides here on species is needed for the purpose of assessing their eligibility for toxicity detection, which is required for water environment monitoring tools.

Acknowledgments

Thanks to the Scientific &Technological Research Council of Turkey (TUBITAK-2211/C National PhD Scholarship Program for Priority Areas) and the Council of Higher Education for 100/2000 PhD scholarship program for A. Ergenler.

Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

F.T. performed all the experiments and drafted the main manuscript text. F.T. and A.E. collected samples and performed analysis.

References

Alexandratos, N. & J. Bruinsma, (2012). *World Agriculture towards* 2030/2050: the 2012 Revision. https://doi.org/10.22004/ag.econ.288998.

Bai, S. H., & Ogbourne, S. (2016). Eco-toxicological effects of the avermectin family with a focus on abamectin and ivermectin. *Chemosphere*, 154, 204-214. https://doi.org/ 10.1016/j.chemosphere.2016.03.113.

Agin, K., Hassanian-Moghaddam, H., Shadnia, S., & Rahimi, H. R. (2016). Characteristic manifestations of acute paint thinner-intoxicated children. *Environmental toxicology and pharmacology*, 45, 15-19. https://doi.org/ 10.1016/j.etap.2020.103370.

Bonomo, M. M., de Castro Sachi, I. T., Paulino, M. G., Fernandes, J. B., Carlos, R. M., & Fernandes, M. N. (2021). Multi-biomarkers approach to access the impact of novel metal-insecticide based on flavonoid hesperidin on fish. *Environmental Pollution*, 268, 115758. https://doi.org/10.1016/j.envpol.2020.115758.

Carvalho, F. P. (2017). Pesticides, environment, and food safety. *Food and Energy Security*, 6(2), 48-60. https://doi.org/10.1002/fes3.108.

Cavalcante, D. G. S. M., Martinez, C. B. R., & Sofia, S. H. (2008). Genotoxic effects of Roundup® on the fish *Prochilodus lineatus*. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 655(1-2), 41-46. https://doi.org/10.1016/j.mrgentox.2008.06.010.

Chen, L. J., Sun, B. H., ping Qu, J., Xu, S., & Li, S. (2013). Avermectin induced inflammation damage in king pigeon brain. *Chemosphere*, 93(10), 2528-2534. https://doi.org/10.1016/j.chemosphere.2013.09.058.

Collins, A. R. (2004). The comet assay for DNA damage and repair: principles, applications, and limitations. *Molecular biotechnology*, 26(3), 249-261. https://doi.org/10.1385/MB:26:3:249.

Dos Santos, K. P. E., Silva, I. F., Mano-Sousa, B. J., Duarte-Almeida, J. M., de Castro, W. V., de Azambuja Ribeiro, R. I. M., ... & Thomé, R. G. (2023). Abamectin promotes behavior changes and liver injury in zebrafish. *Chemosphere*, 311, 136941. https://doi.org/10.1016/j.chemosphere.2022.136941.

Feng, H., Zhou, P., Liu, F., Zhang, W., Yang, H., Li, X., & Dong, J. (2023). Abamectin causes toxicity to the carp respiratory system by triggering oxidative stress, inflammation, and apoptosis and inhibiting autophagy. *Environmental Science and Pollution Research*, 30(19), 55200-55213. https://doi.org/10.1007/s11356-023-26166-3.

Gibbons, D., Morrissey, C., & Mineau, P. (2015). A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. *Environmental Science and Pollution Research*, 22, 103-118. https://doi.org/10.1007/s11356-014-3180-5.

Muranli, F. D. G., Rasgele, P. G., Kekecoglu, M., Kanev, M., & Ozdemir, K. (2015). Potential genotoxicity of acetamiprid and propineb singly or in combination in cultured human peripheral blood lymphocytes by using MN assay. *Fresenius Environmental Bulletin*, 24(11), 3947-3955.

Hayat, K., Afzal, M., Aqueel, M. A., Ali, S., Khan, Q. M., & Ashfaq, U. (2018). Determination of insecticide residues and their adverse effects on blood profile of occupationally exposed individuals. *Ecotoxicology and Environmental Safety*, 163, 382-390. https://doi.org/10.1016/j.ecoenv.2018.07.004.

Hong, Y., Huang, Y., & Huang, Z. (2020). Oxidative stress, immunological response, and heat shock proteins induction in the Chinese Mitten Crab, *Eriocheir sinensis* following avermectin exposure. *Environmental Toxicology*, 35(2), 213-222. https://doi.org/10.1002/tox.22858.

Jasmine, R. S., Kuttalam, S., & Stanley, J. (2008). Acute toxicity of abamectin to fishes, *Cyprinus carpio* Linnaeus and *Tilapia mosambica* (Peters). *Asian Journal of Bio Science*, 3(1), 127-129.

Li, B., Xia, X., Wang, J., Zhu, L., Wang, J., & Wang, G. (2018). Evaluation of acetamiprid-induced genotoxic and oxidative responses in Eisenia fetida. *Ecotoxicology and Environmental Safety*, 161, 610-615. https://doi.org/10.1016/j.ecoenv.2018.06.022.

Li, M. Y., Gao, C. S., Du, X. Y., Zhao, L., Niu, X. T., Wang, G. Q., & Zhang, D. M. (2020). Effect of sub-chronic exposure to selenium and astaxanthin on *Channa argus*: Bioaccumulation, oxidative stress and inflammatory response. *Chemosphere*, 244, 125546. https://doi.org/10.1016/j.chemosphere.2019.125546.

Liang, Y., Li, J., Lin, Q., Huang, P., Zhang, L., Wu, W., & Ma, Y. (2017). Research progress on signaling pathway-associated oxidative stress in endothelial cells. *Oxidative Medicine and Cellular Longevity*, 2017:7156941. https://doi.org/10.1155/2017/7156941.

Liao, W., McNutt, M. A., & Zhu, W. G. (2009). The comet assay: a sensitive method for detecting DNA damage in individual cells. *Methods*, 48(1), 46-53. https://doi.org/10.1016/j.ymeth.2009.02.016.

Liu, M., Panda, S. K., & Luyten, W. (2020). Plant-based natural products for the discovery and development of novel anthelmintics against nematodes. *Biomolecules*, 10(3), 426. https://doi.org/10.3390/biom10030426.

Lumaret, J. P., Errouissi, F., Floate, K., Rombke, J., & Wardhaugh, K. (2012). A review on the toxicity and non-target effects of macrocyclic lactones in terrestrial and aquatic environments. *Current Pharmaceutical Biotechnology*, 13(6), 1004-1060. https://doi.org/10.2174/138920112800399257.

McCavera, S., Walsh, T. K., & Wolstenholme, A. J. (2007). Nematode ligand-gated chloride channels: an appraisal of their involvement in macrocyclic lactone resistance and prospects for developing molecular markers. *Parasitology*, 134(8), 1111-1121. https://doi.org/10.1017/S0031182007000042.

Norusis M.J., 1993. *Advanced Statistics, SPSS for Windows*, Release 6.0, p. 578

Novelli, A., Vieira, B. H., Cordeiro, D., Cappelini, L. T. D., Vieira, E. M., & Espíndola, E. L. G. (2012). Lethal effects of abamectin on the aquatic organisms *Daphnia similis, Chironomus xanthus* and *Danio rerio*. *Chemosphere*, 86(1), 36-40. https://doi.org/10.1016/j.chemosphere.2011.08.047.

Novelli, A., Vieira, B. H., Braun, A. S., Mendes, L. B., Daam, M. A., & Espíndola, E. L. G. (2016). Impact of runoff water from an experimental agricultural field applied with Vertimec® 18EC (abamectin) on the survival, growth and gill morphology of zebrafish juveniles. *Chemosphere*, 144, 1408-1414. https://doi.org/10.1016/j.chemosphere.2015.10.004.

OECD (1992). Organisation for Economic Co-operation and Development. *OECD´s guidelines for the testing of chemicals*: *203 acute toxicity test for fish.* p.12, Paris, OECD Publishing

Pitarque, M., Creus, A., Marcos, R., Hughes, J. A., & Anderson, D. (1999). Examination of various biomarkers measuring genotoxic endpoints from Barcelona airport personnel. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 440(2), 195-204. https://doi.org/10.1016/S1383-5718(99)00026-1.

Pitterna, T., Cassayre, J., Hüter, O. F., Jung, P. M., Maienfisch, P., Kessabi, F. M., ... & Tobler, H. (2009). New ventures in the chemistry of avermectins. *Bioorganic & Medicinal Chemistry*, 17(12), 4085-4095. https://doi.org/10.1016/j.bmc.2008.12.069.

Prasse, C., Löffler, D., & Ternes, T. A. (2009). Environmental fate of the anthelmintic ivermectin in an aerobic sediment/water system. *Chemosphere*, 77(10), 1321-1325. https://doi.org/10.1016/j.chemosphere.2009.09.045.

Prichard, R., Ménez, C., & Lespine, A. (2012). Moxidectin and the avermectins: consanguinity but not identity. *International Journal for Parasitology*: *Drugs and Drug Resistance*, 2, 134-153. https://doi.org/10.1016/j.ijpddr.2012.04.001.

Prusty, J. K., & Patro, S. K. (2015). Properties of fresh and hardened concrete using agro-waste as partial replacement of coarse aggregate–A review. *Construction and Building Materials*, 82, 101-113. https://doi.org/10.1016/j.conbuildmat.2015.02.063.

Rejczak, T., & Tuzimski, T. (2015). A review of recent developments and trends in the QuEChERS sample preparation approach. *Open Chemistry*, 13(1), 980-1010. https://doi.org/10.1515/chem-2015-0109.

Sanches, A. L. M., Vieira, B. H., Reghini, M. V., Moreira, R. A., Freitas, E. C., Espíndola, E. L., & Daam, M. A. (2017). Single and mixture toxicity of abamectin and difenoconazole to adult zebrafish (*Danio rerio*). *Chemosphere*, 188, 582-587.

Sánchez-Alarcón, J., Milić, M., Kašuba, V., Tenorio-Arvide, M. G., Montiel-González, J. M. R., Bonassi, S., & Valencia-Quintana, R. (2021). A systematic review of studies on genotoxicity and related biomarkers in populations exposed to pesticides in Mexico. *Toxics*, 9(11), 272.

Sumudumali, R. G. I., & Jayawardana, J. M. C. K. (2021). A review of biological monitoring of aquatic ecosystems approaches: with special reference to macroinvertebrates and pesticide pollution. *Environmental Management*, 67(2), 263-276. https://doi.org/10.1007/s00267-020-01423-0.

Tišler, T., & Kožuh Eržen, N. (2006). Abamectin in the aquatic environment. *Ecotoxicology*, 15, 495-502. https://doi.org/10.1007/s10646-006-0085-1.

Tresnakova, N., Kubec, J., Stara, A., Zuskova, E., Faggio, C., Kouba, A., & Velisek, J. (2022). Chronic toxicity of primary metabolites of chloroacetamide and glyphosate to early life stages of marbled crayfish *Procambarus virginalis*. *Biology*, 11(6), 927. https://doi.org/10.3390/biology11060927.

Turan, F., & Ergenler, A. (2019). Assessment of DNA damage by comet assay in *Trachinotus ovatus* cells from Mersin Bay in the Northeastern Mediterranean. *Natural and Engineering Sciences*, 4(3), 25-31.

Turan, F., & Ergenler, A. (2022). Investigation of the genotoxic effect of acetamiprid in *Cyprinus carpio* using the micronucleus analysis and the comet assay. *Turkish Journal of Maritime and Marine Scie*nces, 8(2), 80-89. https://doi.org/10.52998/trjmms.1037906.

Valencia-Quintana, R., Milić, M., Bonassi, S., Ochoa-Ocaña, M. A., Campos-Peña, V., Tenorio-Arvide, M. G., ... & Sánchez-Alarcón, J. (2023). Effect of Pesticide Exposure over DNA Damage in Farmers from Los Reyes, Michoacan in Mexico. *Toxics*, 11(2), 122. https://doi.org/10.3390/toxics11020122.

Yanar, M., & Genç, E. (2004). Anaesthetic effects of quinaldine sulphate together with the use of diazepam on *Oreochromis niloticus* L. 1758 (Cichlidae) at different temperatures. *Turkish Journal of Veterinary & Animal Sciences*, 28(6), 1001-1005.

Yu, M., Yao, J., Liang, J., Zeng, Z., Cui, B., Zhao, X., ... & Cui, H. (2017). Development of functionalized abamectin poly (lactic acid) nanoparticles with regulatable adhesion to enhance foliar retention. *RSC advances*, 7(19), 11271-11280. https://doi.org/10.1039/C6RA27345A.