Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Antioxidant system in rat tissues after single exposure to triazole fungicides

S. V. Khyzhnyak, S. V. Midyk, V. M. Voitsitskiy, O. V. Machuskyy, O. M. Iakubchak

National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

Article info

Received 11.01.2025
Received in revised form 20.02.2025
Accepted 16.03.2025

National University of Life and Environmental Sciences of Ukraine, Heroyiv Oborony st., 15, Kyiv, 03041, Ukraine. Tel.: +38-050-696-92-23. E-mail: khs2014@ukr.net

Khyzhnyak, S. V., Midyk, S. V., Voitsitskiy, V. M., Machuskyy, O. V., & Iakubchak, O. M. (2025). Antioxidant system in rat tissues after single exposure to triazole fungicides. Regulatory Mechanisms in Biosystems, 16(2), e25054. doi:10.15421/0225054

The spread and accumulation in the environment of tebuconazole, which is a triazole fungicide, causes a variety of environmental problems and endangers human health. Despite numerous advances in studies of its toxicity, cellular mechanisms of influence have not been systematically studied. Wistar Han rats were administered *in vivo* a single oral dose of fungicide (600 and 1200 mg/kg), containing the active substance tebuconazole alone or in combination with triadimefon. Gas chromatography – mass spectrometry (GC–MS) revealed that the accumulation of tebuconazole in the liver prevailed over its accumulation in the kidneys. The features of the antioxidant protection system response (activity of antioxidant enzymes and sulfhydryl groups) of the liver, kidneys and myocardium were detected on the 14th day after a single exposure to the fungicide in rats. A decrease in the activity of superoxide dismutase and catalase, as well as a decrease in the content of both protein and non-protein sulfhydryl groups, was found in the liver. In the kidneys, with an increase in the administered dose of the studied fungicides, the activity of superoxide dismutase and the content of protein SH groups decreased, and the activity of catalase did not differ from the control. In the myocardium, only superoxide dismutase activity tended to increase, while the values of other indicators remained at the control level, which can be regarded as a compensatory reaction to the action of fungicides. No distinct differences in the action of fungicides containing tebuconazole alone or in combination with triadimephone were found, which requires further research. The changes detected in the antioxidant system of hepatocytes can be explained by oxidative stress mediated by the accumulation of tebuconazole in the liver. This may reflect metabolic changes and impairment of liver function under the influence of fungicides containing tebuconazole.

Keywords: tebuconazole; antioxidant enzymes; SH-groups; liver; kidneys; myocardium; rats.

Introduction

The widespread use of pesticides in agriculture creates an increasing threat of environmental pollution with toxic substances. The largest group of modern fungicides – triazoles belong to systemic compounds that are used to protect plants from a wide range of diseases (Culleres et al., 2009).

However, triazole fungicides can be toxic to target and non-target organisms (Cao et al., 2019), and have adverse effects on humans when migrating through the trophic (food) chain (Ahmad et al., 2024). Triazole fungicides are widely used both individually and in combination. Moreover, residual amounts of compounds of this chemical group are often found in increased concentrations in the environment (Gomez-Martinez et al., 2024). Thus, tebuconazole, which belongs to the third generation of triazoles, has a long half-life and its residues are stored in various environmental objects such as soil (Azhari et al., 2018) or water (Zubrod et al., 2019), as well as in raw materials and food products (Sannino et al., 2004). This requires further experimental studies of its toxic effects.

To assess the toxicity of pesticides used in agriculture and to establish the mechanisms of their toxic action, studies are conducted on non-target organisms, including rats. Despite the risks associated with triazole fungicides, studies of their effect on metabolic processes in animals are limited. In particular, modifications of the fatty acid composition of earthworms (Khyzhnyak et al., 2021) and total lipids in the liver of rats (Khyzhnyak et al., 2022) have been found after exposure to fungicides containing tebuconazole alone or in combination with other azoles. Morphological manifestations of the action of tebuconazole on rats are characterized by signs of hepatocyte dystrophy, damage to the tubular epithelium in the kidneys and microcirculatory disorders in the adrenal glands (Kornuta & Reshavs'ka, 2011). It is noted that tebuconazole-induced liver dysfunction may be associated with damage to the antioxidant defense system (Jonsdottir et al., 2016).

Oxidative stress is believed to be the main mechanism that determines the potential toxicity of fungicides and results in disruption of the structural and functional activity of membranes, lipids and protein metabolism (Kaur et al., 2019). At the same time, the intensity of oxidative processes in cells, tissues and organs of the body is controlled by the antioxidant defense system, which is necessary for the regulation and maintenance of cellular homeostasis at the physiological level (Baraboi, 2016). Compartmentalization and functional activity of the components of the antioxidant (AO) system, which includes enzymatic and non-enzymatic components, has its own specificity for different organs of the body (Turrens, 2003).

This necessitates the study of the activity of this system in different organs under the action of triazole fungicides to establish the mechanisms of their toxic action. The key role in the antioxidant defense system is played by superoxide dismutase (SOD) and its synergist catalase, which are sensitive to external stimuli, allowing enzymes to be considered as an indicators of the overall state of the AO system (Nikolenko et al., 2012). The results of this study will therefore contribute to a better understanding of the potentially toxic effects of typical triazole fungicides on animal organisms.

The aim of the study is to compare the response of the antioxidant system in the liver, kidneys and myocardium of rats after a single exposure to fungicides containing tebuconazole alone or in combination with triadimefon.

Materials and methods

Experiments were conducted on sexually mature male Wistar rats weighing 210–240 g, which were kept in standard vivarium conditions (heat, humidity and light) of the Institute of Occupational Medicine of the National Academy of Medical Sciences of Ukraine. Animal manipulations were carried out in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), in accordance with the general ethical principles of animal experiments adopted by the Law of Ukraine "On Protection of Animals From Cruel Treatment" from 21.02.2006, No. 3447–IV as amended on 04.08.2017. All necessary animal interventions were carried out in compliance with the ARRIVE recommendations, without violating the guidelines

of Council Directive 2010/63/EU of 22 September 2010 regarding the protection of animals used for scientific purposes. The research program was approved by the Bioethics Committee of the National University of Life and Environmental Sciences of Ukraine.

This study used the systemic fungicides Azimuth Classic (containing the active ingredient, a.i. tebuconazole, 250 g/L) and Azimuth (containing two active ingredients, a.i.: tebuconazole, 125 g/L and triadimefon, 100 g/L) registered in Ukraine in the formulation of an emulsifiable concentrate (EC). They are widely used to protect agricultural crops. The active ingredients of the fungicides belong to the chemical class of triazoles.

The animals were kept in cages where they had unlimited access to food and water, and they were randomly selected for research. Rats were divided into five groups of 6 animals each: one control and 4 experimental. The animals were administered orally an aqueous emulsion of fungicide Azimuth (AZ) and Azimuth Classic (AC) at a single dose of 600 mg/kg (AZ 600 and AC 600) and 1200 mg/kg (AZ 1200 and AC 1200). The dose selection was based on the LD_{50} exceeding 2500 mg/kg calculated according to (Backhaus & Faust, 2012) for tested fungicides.

Toxicological studies were conducted according to the principles and criteria outlined in (OECD (2001) Guideline for testing of chemicals. Acute oral toxicity – acute toxic class method. 2001. No. 423. www.oecd.org/en/publications/test-no-423-acute-oral-toxicity-acute-toxic-class-method_9789264071001-en.html). The state of the animals was assessed daily during the experimental period, and their body weight was recorded at the beginning of the experiment and once a week. After 14 days of acute exposure to the drugs, the animals were decapitated under light ether anesthesia. Internal organs (liver, kidneys, and myocardium) were removed and perfused with chilled physiological saline. The tissue was minced, homogenized and centrifuged at 2500 rpm for 15 min, the resulting supernatant was filtered and used in the studies.

The activity of superoxide dismutase (SOD) (Partyka et al., 2012) and catalase (Aebi, 1984) was determined. To assess the content of SH-groups, a colorimetric method based on the reaction of 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) with sulfhydryl groups was used (Rahman et al., 2007). To determine the total content of SH-groups in the samples, the samples were incubated for 30 min. in the dark in the presence of 1 mM DTNB and 17.3 mM (0.5%) sodium dodecyl sulfate (SDS). The optical density was measured at 412 nm. To determine the content of non-protein SH-groups, chilled 10% trichloroacetic acid (TCA) was added to the sample and denatured proteins were separated. The supernatant was incubated with 0.1 mM DTNB in phosphate buffer (pH 7.7) in the dark for 30 min. The optical density was measured at 412 nm, the SH-groups content was calculated per mg of protein.

Identification and determination of tebuconazole content in the liver and kidney tissues was carried out by gas chromatography-mass spectrometry (GC–MS) using a single quadrupole detector. Chromatogram peak separation was performed on a HP-5 MS 15 m x 0.25 mm ID x 0.25 μm column. The analysis was conducted on a gas chromatograph Agilent 7890-MSD 5975C (Agilent Technologies, USA). Carrier gas pressure (helium) – 60.7 kPa.

For this purpose, tissue samples were homogenized to a homogeneous mass, acetonitrile was added and placed on an ultrasonic bath on ice for 20 minutes. After centrifugation, the supernatant was collected and acetonitrile was added to the tissue homogenate for subsequent extraction. Supernatants were pooled, acetone and anhydrous sodium sulfate were added, followed by centrifugation. The combined supernatant was concentrated and a dispersion purification was carried out on cartridges (clean-up kits 150 mg PSA/150 mg C18/900 mg $MgSO_4$). The purified sample extract was filtered through a 0.22 μm organic filter membrane into chromatographic vials for GC-MS analysis.

The obtained mass spectra were compared with the NIST database integrated with the DRS-AMDIS instrument software. Quantification was carried out using the analytics-Chrom program by the correlation method with the standard peak height (EVS-EN 15662:2008. Foods of plant origin – Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction / partitioning and cleanup by dispersive SPE–QuEChERS-method. www.evs.ee/products/evs-en-15662-2008).

Statistical processing of the obtained results was performed using one-factor analysis of variance (ANOVA), the data in the tables are presented in the form of x ± SD (mean ± standard deviation). Mean values in the groups were compared pairwise using the Tukey test, and the differences were considered significant at $P < 0.05$. When comparing enzymatic activities, Bonferroni correction was applied ($P < 0.005$).

Results

Single exposure of rats to the studied triazole fungicides caused toxic effects within the first hours after administration, most pronounced in the first four hours at a dose of 1200 mg/kg (AZ 1200 and AC 1200). It should be noted that toxic signs in the animals were manifested in their decreased activity, depression, ruffled fur, hunched posture, unkempt appearance, narrowing of the palpebral fissure, drooling, respiratory disorders, etc. The weight of animals in the experimental groups did not differ from the control. Toxic manifestations partially persisted until the second day and disappeared by the end of the studies (14 days).

At the end of the experiment, residual levels of tebuconazole in the liver and kidneys of rats were measured 14 days after the administration of fungicides. The results (Fig.1.) indicate that when the fungicide AZ was administered at a dose of 600 and 1200 mg/kg, the content of tebuconazole (mg/kg) in the liver was 0.42 ± 0.02 and 0.61 ± 0.01; when fungicide AC was administered in these doses, the content of tebuconazole (mg/kg) in the liver was 0.63 ± 0.01 and 0.88 ± 0.02, respectively. Accordingly, the accumulation of tebuconazole (mg/kg) in the kidneys for the fungicide AZ at doses of 600 and 1200 mg/kg was 0.20 ± 0.01 and 0.28 ± 0.01; and for the fungicide AC in the same doses, the content of tebuconazole (mg/kg) reached 0.33 ± 0.01 and 0.47 ± 0.03, respectively. That is, increasing the dose of tebuconazole increases its content in the kidneys and liver. Comparing the examined tissues, the accumulation of tebuconazole in the liver was approximately twice as high as the kidney level when rats were administered AZ or AC fungicides (Fig.1).

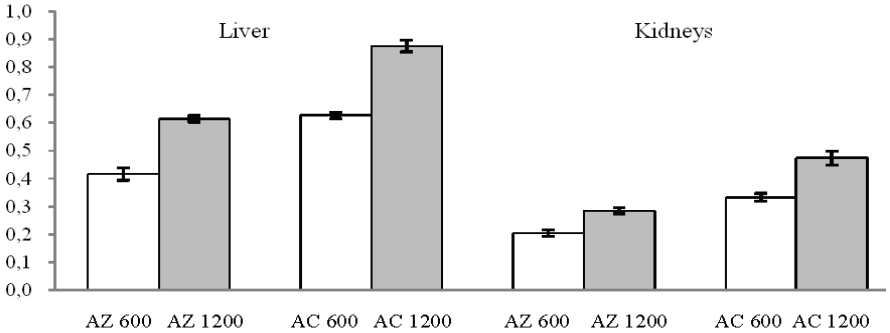

Fig. 1. The accumulation of tebuconazole in different tissues: determination of the tebuconazole content (mg/kg) in rat liver and kidneys by gas chromatography – mass spectrometry (GC–MS); data are expressed as the x ± SD (n = 6 rat/group); the values of tebuconazole (mg/kg) in the groups AZ 600, AZ 1200, AC 600, AC 1200 for the liver are compared for the corresponding groups in the kidney

The toxic effect of fungicides due to absorption and accumulation is associated with metabolic, in particular, oxidative processes in tissues. Therefore, indicators that are associated with the reaction to oxidative stress in response to the action of tebuconazole were determined. The effect of fungicides on the activities of the key scavengers of reactive oxygen species – SOD and catalase are presented in Table 1.

The results of the study of SOD and catalase activity in the liver and kidney indicate its decrease relative to the control with an increase in the administered dose of fungicides (Table 1). SOD activity in the liver was reduced by 19% for fungicide AZ 1200 and by 24% for fungicide AC 1200 relative to control. Liver catalase activity decreased more significantly by 34% and 37% for the fungicide AZ 600 and AZ 1200, and by 40% and 45% for the fungicide AC 600 and AC 1200, respectively, compared to the control.

Table 1

Superoxide dismutase (conditional units) and catalase (nmol/mg protein) activities in rats after administration (600 and 1200 mg/kg) of fungicides AZ (a.i.: tebuconazole 125 g/L and triadimefon 100 g/L) and AC (a.i.: tebuconazole 250 g/L), x ± SD, n = 6

Organ	Experimental conditions	Superoxide dismutase activity, conditional units	Catalase activity, nmol/mg protein
Liver	Control	3.61± 0.11^{a}	112.8 ± 10.6^{a}
	AZ 600	3.24 ± 0.14^{ab}	74.1 ± 7.6^{b}
	AZ 1200	2.93 ± 0.10^{b}	70.9 ± 9.2^{b}
	AC 600	3.12 ± 0.25^{ab}	68.1 ± 7.1^{b}
	AC 1200	2.75 ± 0.16^{b}	62.4 ± 4.6^{b}
Kidneys	Control	1.75 ± 0.1^{a}	95.6 ± 7.0^{a}
	AZ 600	1.63 ± 0.08^{ab}	94.4 ± 4.6^{a}
	AZ 1200	1.40 ± 0.06^{bc}	93.4 ± 5.7^{a}
	AC 600	1.51 ± 0.09^{ac}	93.1 ± 5.3^{a}
	AC 1200	1.35 ± 0.07^{c}	90.6 ± 12.5^{a}
Myocardium	Control	2.54 ± 0.13^{a}	121.7 ± 12.1^{a}
	AZ 600	2,52 ± 0.20^{a}	108.9 ± 4.6^{a}
	AZ 1200	2.88 ± 0.11^{a}	110.4 ± 8.1^{a}
	AC 600	2,78 ± 0.10^{a}	109.5 ± 6.0^{a}
	AC 1200	3.08 ± 0.21^{b}	117.4 ± 5.8^{a}

Note: in this and the following table: fungicide with a concentration of tebuconazole 125 g/L + triadimefon 100 g/L with a single administration at doses of 600 mg/kg (AZ 600) and 1200 mg/kg (AZ 1200); fungicide with a concentration of tebuconazole 250 g/L with a single administration at doses of 600 mg/kg (AC 600) and 1200 mg/kg (AC 1200). Different superscript letters indicate values which differ significantly one from another by comparison using the Tukey test and was considered significant at $P < 0.005$ (taking into account the Bonferroni correction).

For enzyme activities in the kidneys (Table 1) a significant decrease in SOD activity by 20% was found for the fungicide AZ 1200 and by 23% for the fungicide AC 1200 relative to the control. Under experimental conditions, catalase activity in the kidneys did not change relative to the control.

For the myocardium, a tendency to increase in SOD activity by 21% relative to the control was found after single exposure to the fungicide AC 1200. Catalase activity in the myocardium did not change compared to the control under experimental conditions (Table 1).

Important components of maintaining redox homeostasis in cells and tissues are thiol compounds, which are primarily subjected to oxidation. Studies of the content of non-protein and protein SH-groups in the liver (Table 2) indicate their decrease with increasing doses of fungicides. Thus, the content of non-protein SH-groups decreased by 25% compared to the control at administration of the fungicide AZ 1200. The content of protein SH-groups decreased significantly after administration of the fungicide AZ by 19% and 24% for AZ 600 and AZ 1200, respectively, relative to the control. For the fungicide AC the content of non-protein SH-groups decreased by 26% and 31% and protein SH-groups by 28% and 36% for AC 600 and AC 1200, respectively, in comparison with control levels. In the experimental conditions, a decrease in the content protein SH-groups was also observed in the kidneys. Thus, the content of protein SH-groups significantly decreased with the administration of the fungicide AZ 1200 by 17% and fungicide AC 1200 by 22% compared to the control. An insignificant decrease in the content of sulfhydryl groups relative to the control is noted in the myocardium (Table 2).

Table 2

Content (nmol/mg protein) of non-protein and protein SH-groups in the organs of rats after administration (600 and 1200 mg/kg) of fungicides AZ (a.i.: tebuconazole 125 g/L and triadimefon 100 g/L) and AC (a.i.: tebuconazole 250 g/L), x ± SD, n = 6

Organs	Experimental conditions	Non-protein SH-groups, nmol/mg protein	Protein SH-groups, nmol/mg protein
Liver	Control	9.92 ± 0.64^{a}	107.5 ± 6.3^{a}
	AZ 600	8.45 ± 0.51^{ab}	86.8 ± 6.9^{b}
	AZ 1200	7.48 ± 0.48^{b}	81.6 ± 8.1^{b}
	AC 600	7.39 ± 0.49^{b}	77.0 ± 3.5^{b}
	AC 1200	6.82 ± 1.05^{b}	69.1 ± 9.1^{b}
Kidneys	Control	11.81 ± 0.87^{a}	118.8 ± 4.5^{a}
	AZ 600	10.80 ± 0.78^{a}	106.0 ± 7.9^{ab}
	AZ 1200	10.03 ± 0.85^{a}	98.1 ± 7.7^{b}
	AC 600	10.11± 1.06^{a}	105.9 ± 7.7^{ab}
	AC 1200	9.60 ± 0.56^{a}	93.1 ± 5.2^{b}
Myocardium	Control	11.18 ± 1.08^{a}	112.4 ± 9.5^{a}
	AZ 600	10.09 ± 0.92^{a}	106.3 ± 4.4^{a}
	AZ 1200	9.94 ± 0.68^{a}	96.4 ± 7.0^{a}
	AC 600	11.10 ± 0.90^{a}	109.5 ± 6.0^{a}
	AC 1200	9.56 ± 0.59^{a}	96.4± 9.1^{a}

Note: letters indicate values that were significantly different according to the Tukey test ($P < 0.05$).

Discussion

The risks of triazole fungicides, especially those containing tebuconazole, are of global concern due to their widespread use, increased exposure through environmental accumulation, and toxicological effects (Boskovic et al., 2020; Ahmad et al., 2024; Gomez-Martinez et al., 2024). Therefore, research on the cellular mechanisms of their effects on non-target organisms continues.

The toxicological effect of a single exposure to fungicides, which are widely used to protect agricultural plants and contain tebuconazole individually AC (a.i.: tebuconazole 250 g/L) or in combination AZ (a.i.: tebuconazole 125 g/L and triadimefon 100 g/L) was investigated. Acute toxic manifestations were observed from the first hours after the administration of fungicides at doses of 600 and 1200 mg/kg and not detected in 14 days.

Considering the possible accumulation of triazole fungicides in the liver and kidneys, the level of tebuconazole accumulation in these organs under experimental conditions was determined. After 14 days, accumulation of the active substance tebuconazole in the liver of rats was detected when using both the fungicide AC and AZ. Moreover, the content of tebuconazole in the liver of rats was about 2 times higher than the kidneys. This confirms the fact that the liver is the main organ of accumulation of tebuconazole, as noted previously (Li et al., 2020; Marx-Stoelting et al., 2020).

The prerequisite for the study of the functional activity of the AO system, which controls the intensity of prooxidant processes, was consideration that the entry of fungicides in the body of animals can lead to the development of oxidative stress (Ku et al., 2021). The specific features of the response of the key AO defense enzymes, in particular SOD and catalase, were observed in the liver, kidneys, and myocardium of rats under the action of fungicides in our studies. Decreased SOD activity found in the liver and kidneys may be due to depletion of the enzyme pool or structural modification of the enzyme (Baraboi, 2016). Along with SOD, catalase activity was reduced mainly in the liver, which may indicate an increase in oxidative processes in the organ. At the same time, the tendency to increase in SOD activity in the myocardium upon the administration of the fungicide AC 1200 can be considered as an enhancement of the antiradical protection system of cells. The course of free radical processes is associated with redox transformations of sulfhydryl and disulfide groups of proteins and low molecular weight compounds, in particular, glutathione, which constitutes the majority of low molecular weight thiols in cells. In addition to antioxidant protection functions, glutathione affects physiological and biochemical processes, for example, mediating cell proliferation and apoptosis (Davis et al., 2001).

The obtained results indicate that under the action of fungicides AC (AC 600 and AC 1200) and AZ (AZ 600 and AZ 1200), a dose-dependent decrease in the content of non-protein SH-groups was observed in the liver, which confirms the intensification of oxidative processes. For the kidneys, this decrease was unreliable. The results of the studies indicate a decrease in the level of protein SH-groups in the rat liver with increasing doses of fungicides AC and AZ, and in the kidneys only at a higher dose (1200 mg/kg) of the fungicides AZ and AC. It is known that the oxidation of protein sulfhydryl groups is facilitated by the activation of oxidative processes. During the oxidation of SH-groups of cysteine residues, disulfide bridges are formed, which leads to structural modifications of proteins and disruption of their functional properties (Bhopatkar et al., 2020). In addition, SH-groups located on the surface of protein molecules can inactivate ROS by oxidation to disulfides, thus protecting the internal part of protein molecules from damage (Gebicki, 2016). Besides, the decreese in the content of SH-groups can be due to both an increase in the intensification of the free radical process and an increase in the involvement of these compounds in intracellular interactions, which can be an additional element of the regulation of biological processes. It is also known that oxidative modification of SH-groups, which leads to an increase in the number of disulfide groups, is a non-specific response of the body to extreme influences (Baraboi, 2016).

Thus, the accumulation of tebuconazole in the liver, detected in the experimental conditions, probably determines the most pronounced modification of the AO system in hepatocytes under the action of fungicides containing tebuconazole individually or in combination with triadimefon. A dose-dependent decrease in SOD activity and catalase activity, as well as a decrease in the content of protein and non-protein SH groups (especially under the individual effect of tebuconazole) was noted. Since the oxidation of sulfhydryl groups of proteins is facilitated by the activation of oxidative processes, this may affect biochemical processes and the intensity of metabolism. In addition, a decrease in the content of non-protein SH groups, mainly glutathione, which plays the role of a cysteine reserve and is an inhibitor of ROS, in turn indicates the activation of oxidative processes. Considering that the ability of the cellular AO system to provide ROS neutralization is complemented by the role of its individual components in the implementation of a number of signaling cascades, modification of the antioxidant defense system in hepatocytes can lead to liver dysfunction.

The liver is an important homeostatic organ of the body, and disruption of its functioning leads to an imbalance in protein and lipid metabolism, a decrease in the biotransformation of xenobiotics, resulting in intoxication of the body (Michalopoulos & Bhushan, 2021). The hepatotoxic properties of individual triazoles have been analyzed in toxicity tests (Tully et al., 2006). These studies indicate that high doses of triazoles cause an increase in liver mass, and in long-term studies, triazoles such as cyproconazole or epoxiconazole cause hepatocellular tumors (Hester et al., 2021). Despite the information about the hepatotoxic effect caused by individual triazole fungicides, there is a lack of *in vivo* studies of effects on the liver. Our previous studies have shown (Khyzhnyak et al., 2022) that the effect of fungicides containing tebuconazole individually or in combination with triadimefon leads to a redistribution of the fatty acid profile of total lipids in liver tissues. This is characterized, in particular, by an increase in the content of polyenoic fatty acids of the ω6 family, which, being endogenous bioregulators, may be involved in destructive and inflammatory processes in liver tissues (Khyzhnyak et al., 2022). Thus, the obtained results suggest the possibility of liver dysfunction due to the accumulation of tebuconazole containing fungicides, that may be associated with a modification of the antioxidant defense system.

At the same time, the data shows that it is impossible to unequivocally assert the existence of differences in the individual and combined effects of fungicides on the AO system. The interaction between pesticides in the mixture may vary depending on the percentage content (dose levels) of the individual components, the duration of exposure to the mixture and the biological target (Hernandez et al., 2017). This may also be due to a methodological problem: when analyzing the combined effects, there is a limited number of dose levels that cause effects. However, it is generally necessary to analyze several (at least five) effective doses for individual substances. Therefore, further research is needed to understand the nature of the effects of fungicide combinations.

Conclusion

The intake by rats of fungicides containing tebuconazole alone or in combination with triadimefon, belonging to the chemical class of triazoles, is characterized by the peculiarities of the reaction of the AO defense system of the liver, kidneys and myocardium. The accumulation of tebuconazole in the liver is accompanied by changes in the activity of the antioxidant protection system, which indicate the intensification of oxidative processes and may be the basis of metabolic modifications, as well as the cause of dysregulation processes, changes in the efficiency of the implementation of intracellular regulatory mechanisms. The findings provide new insights into the molecular mechanisms of metabolic changes caused by triazole fungicides containing tebuconazole and contribute to a better understanding of the environmental hazards of these fungicides.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

The authors declare that they have no potential conflict of interest.

References

Aebi, H. (1984). Catalase *in vitro*. Methods Enzymology, 105, 121–126.

Ahmad, M. F., Ahmad, F. A., Alsayegh, A. A., Zeyaullah, Al-Shahrani, A. M., Muzammil, K., Saati, A. A., Wahab, S., Elbendary, E. Y., Kambal, N., Abdelrahman, M. H., & Hussain, S. (2024). Pesticides impacts on human health and the environment with their mechanisms of action and possible countermeasures. Heliyon, 10(7), e29128.

Azhari, N. E., Dermou, E., Barnard, R. L., Storck, V., Tourna, M., Beguet, J., Karas, P. A., Lucini, L., Rouard, N., Botteri, L., Ferrari, F., Trevisan, M., Karpouzas, D. G., & Martin-Laurent, F. (2018). The dissipation and microbial ecotoxicity of tebuconazole and its transformation products in soil under standard laboratory and simulated winter conditions. Science of the Total Environment, 637–638, 892–906.

Backhaus, T., & Faust, M. (2012). Predictive environmental risk assessment of chemical mixtures: A conceptual framework. Environmental Science and Technology, 46, 2564–2573.

Baraboi, V. A. (2016). Bioantioksidanty [Bioantioxidants]. Kniga Plus Publisher, Kyiv (in Ukrainian).

Bhopatkar, A. A., Uversky, V., N., & Rangachari, V. (2020). Chapter 9 – Disorder and cysteines in proteins: A design for orchestration of conformational see-saw and modulatory functions. Progress in Molecular Biology and Translational Science, 174, 331–373.

Boskovic, N., Brandstatter-Scherr, K., Sedlacek, P., Bílkova, Z., Bielska, L., & Hofman, J. (2020). Adsorption of epoxiconazole and tebuconazole in twenty different agricultural soils in relation to their properties. Chemosphere, 261, 127637.

Cao, F., Souders, C. L., Li, P., Pang, S., Qiu, L., & Martyniuk, C. J. (2019). Developmental toxicity of the triazole fungicide cyproconazole in embryo-larval stages of zebrafish (*Danio rerio*). Environmental Science and Pollution Research, 26, 4913–4923.

Culleres, D. B., Boesten, J., Bolognesi, C., Boobis, A., Buchert, A., Capri, E., Coggon, D., Hardy, A., Hart, A., Koepp, H., Liess, M., Luttik, R., Meyer, O., Michaelidou-Canna, S., Montforts, M., Moretto, A., Muller, M., Ossendorp, B., Steurbaut, W., Tasheva, M., & Vleminckx, C. (2009). Scientific opinion on risk assessment for a selected group of pesticides from the triazole group to test possible methodologies to assess cumulative effects from exposure through food from these pesticides on human health. European Food Safety Authority Journal, 7(9), 1167.

Davis, W. J., Ronai, Z., & Tew, K. D. (2001). Cellular thiols and reactive oxygen species in drug-induced apoptosis. Journal of Pharmacology and Experimental Therapeutics, 296, 1–6.

Gebicki, J. M. (2016). Electrons initiate efficient formation of hydroperoxides from cysteine. Free Radical Research, 50(9), 987–996.

Gomez-Martinez, D., Selvin, M. A., Nilsson, A. K., Carmona, E., Ngou, J. S., Kristiansson, E., Nilsson, R. H., & Corcoll, N. (2024). Concentrations of the fungicide tebuconazole alter microbial biodiversity and trigger biofilm-released transformation products. Chemosphere, 261, 127637.

Hernandez, A. F., Gil, F., & Lacasana, M. (2017). Toxicological interactions of pesticide mixtures: An update. Archives of Toxicology, 91(10), 3211–3223.

Hester, S., Moore, T., Padgett, W. T., Murphy, L., Wood, C. E., & Nesnow, S. (2021). The hepatocarcinogenic conazoles: Cyproconazole, epoxiconazole, and propiconazole induce a common set of toxicological and transcriptional responses. Toxicological Sciences, 127(1), 54–65.

Jonsdottir, O., Reffstrup, T. K., Petersen, A., & Nielsen, E. (2016). Physicologically based toxicokinetic models of tebuconazole and application in human risk assessment. Chemical Research in Toxicology, 29, 715–734.

Kaur, R., Mavi, G. K., & Shweta, R. (2019). Pesticides classification and its impact on environment. International Journal of Current Microbiology and Applied, 8(3), 1889–1897.

Khyzhnyak, S., Midyk, S., Polishchuk, S., & Velinskaya, A. (2021). Effect of triazole fungicides on fatty acid contentin *Eisenia fetida*. Polish Journal of Natural Sciences, 35(3), 113–124.

Khyzhnyak, S. V., Midyk, S. V., Velinska, A. O., Arnauta, O. A., & Kalachniuk, L. H. (2022). Fatty acid profile of the liver lipids under acute fungicide action and intake of a biologically active preparation in rats. Ukrainian Biochemical Journal, 94(4), 47–53.

Kornuta, N. O., & Reshavska, O. V. (2011). Morphologichni doslidzhennya pry vivchenny vplyvu pestitsidiv na organism vagitnih samitz i rozvitok plodu [Morphological studies in the study of the impact of pesticides on the body of pregnant females and fetal development]. Bulletin of Problems in Biology and Medicine, 2(2), 132–135 (in Ukrainian).

Ku, T., Zhou, M., Hou, Y., Xie, Y., Li, G., & Sang, N. (2021). Tebuconazole induces liver injury coupled with ROS-mediated hepatic metabolism disorder. Ecotoxicology and Environmental Safety, 220, 112309.

Li, S., Jiang, Y., Sun, Q., Coffin, S., Chen, L., Qiao, K., Gui, W., & Zhu, G. (2020). Tebuconazole induced oxidative stress related hepatotoxicity in adult and larval zebrafish (*Danio rerio*). Chemosphere, 241, 125129.

Marx-Stoelting, P., Knebel, C., & Braeuning, A. (2020). The connection of azole fungicides with xeno-sensing nuclear receptors, drug metabolism and hepatotoxicity, Cells, 9, e1192.

Michalopoulos, G. K., & Bhushan, B. (2021). Liver regeneration: Biological and pathological mechanisms and implications. Nature Reviews Gastroenterology and Hepatology, 18(1), 40–55.

Nikolenko, A. G., Saltykova, E. S., & Gaifullina, L. R. (2012). Molecular mechanisms of antioxidant protective processes in honeybee *Apis mellifera*. In: Farooqui, T., & Farooqui, A. A. (Eds.). Oxidative stress in vertebrates and invertebrates: Molecular aspects on cell signaling. John Wiley & Sons, Hoboken. Pp. 279–293.

Partyka, A., Lukaszewicz, E., & Nizanski, W. (2012). Lipid peroxidation and antioxidant enzymes activity in avian semen. Animal Reproduction Science, 134(3–4), 184–190.

Rahman, I., Kode, A., & Biswas, S. K. (2007). Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nature Protocols, 1, 3159–3165.

Sannino, A., Bolzoni R., & Bandini, M. (2004). Application of liquid chromatography with electrospray tandem mass spectrometry to the determination of a new generation of pesticides in processed fruits and vegetables. Journal of Chromatography A, 1036(2), 161–169.

Tully, D. B., Bao, W., Goetz, A. K., Blystone, C. R., Ren, H., Schmid, J. E., Strader, L. F., Wood, C. R., Best, D. S., Narotsky, M. G., Wolf, D. C., Rockett, J. C., & Dix, D. J. (2006). Gene expression profiling in liver and testis of rats to characterize the toxicity of triazole fungicides. Toxicology and Applied Pharmacology, 215(3), 260–273.

Turrens, J. F. (2003). Mitochondrial formation of reactive oxygen species. The Journal of Physiology, 552(2), 335–344.

Zubrod, J. P., Bundschuh, M., Arts, G., Bruhl, C. A., Imfeld, G., Knabel, A., Payraudeau, S., Rasmussen, J. J., Rohr, J., Scharmuller, A., Smalling, K., Stehle, S., & Schulz, R. B. (2019). Fungicides: An overlooked pesticide class? Environmental Science and Technology, 53(7), 3347–3365.