Glutathione and its dependent enzymes’ modulatory responses to neonicotinoid insecticide sulfoxaflor induced oxidative damage in zebrafish in vivo

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Abstract
The use of neonicotinoid insecticides has progressively increased worldwide when compared with other insecticide groups. Due to this increase, non-target animal species such as fish are exposed to neonicotinoids from different sources, so they can be accumulated at trophic levels and cause various toxic effects by reaching humans. There are limited studies related to the toxic effects of neonicotinoid sulfoximine insecticides including sulfoxaflor on non-target species. The purpose of the present study was to evaluate the effects of sulfoxaflor on GSH-related antioxidants and to determine oxidative stress-producing effect of sulfoxaflor in the gill of zebrafish (Danio rerio). For this purpose, three sublethal concentrations of sulfoxaflor 0.87 mg/L (2.5% of 96 h LC50), 1.75 mg/L (5% of 96 h LC50), 3.51 mg/L (10% of 96 h LC50) of sulfoxaflor were exposed to zebrafish for 24, 48, and 96 h. GSH related antioxidants were evaluated by analyzing tGSH levels and GPx, GR, GST specific enzyme activities in the gill of zebrafish. The oxidative damage of sulfoxaflor on gill cells was determined by measuring TBARS levels. The results of this study demonstrated that sulfoxaflor activated GSH related antioxidants by increasing tGSH levels, GPx, GR enzyme activities and by diminishing GST enzyme activity in the gill of zebrafish. Sulfoxaflor also caused oxidative damage in the gill of zebrafish by increasing lipid peroxidation. In conclusion, this study indicated that sulfoxaflor led to oxidative stress and activation of GSH related antioxidants in the gill of zebrafish.

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Keywords
Neonicotinoids, sulfoxaflor, glutathione, oxidative stress, gill, zebrafish

Introduction
Neonicotinoids are the strongest class of neuroactive insecticides due to their neurotoxic effects on nicotinic acetylcholine receptors (nAChR). They have a higher affinity to insect nAChRs than to those of vertebrates, so they are selectively more toxic to insects. There are four generation neonicotinoids used in public health and animal health, especially conventional agriculture worldwide. These are; (1) klorapridils (imidacloprid, thiacloprid), (2) kloratiazols (Imidaclothiz, Thiamethoxam, Clothianidin), (3) frunils (dinotefuran), (4) sulfoximines (sulfoxaflor).

Neonicotinoid insecticides are highly soluble in water and leach easily to various waterbodies. This leaching potential of neonicotinoids is a very important concern about the applications on the fields for aquatic organisms in particular. It was also reported that neonicotinoids contaminate the environment and foods. This situation directly or indirectly increases the probability of exposure of the non-target organisms by neonicotinoids. Although neonicotinoids have a lower toxicity for mammalian compared to other organochlorine, organophosphorus and carbamate insecticides, the toxicity studies have shown that neonicotinoids have a potential risk for non-target organisms such as fish. Recent studies indicated that neonicotinoids induce reproductive toxicity, immunotoxicity, and multiorgan toxicity in model mammalian species and neurotoxicity in model mammalian fish species. In line with the researches carried out to date, neonicotinoid insecticides are considered as non-toxic to fish species such as Oncorhynchus mykiss and Cyprinus carpio. However recent studies show that neonicotinoid insecticides imidacloprid and thiamethoxam caused oxidative stress by affecting antioxidant system and oxidative stress parameters in fish species. The aforementioned toxicity of neonicotinoids could mostly originate from oxidative stress effects. It has been extensively investigated that pesticides cause oxidative stress by the generation of ROS that might cause lipid peroxidation, alternations in membrane functions, DNA damage and finally mutagenic and carcinogenic effects. Particularly gills in fish represent an important organ for the uptake of xenobiotics as they are in direct contact with pollutants and also are involved in the excretion of xenobiotics.

Oxidative stress occurs when the production of ROS overrides the antioxidant capacity in the cell, resulting in the damage of macromolecules such as nucleic acids, lipids, and proteins causing alterations in the cell function. Numerous studies have shown that radical reactions and oxidative damage are associated with cancer and pathology of many other diseases, especially neurodegenerative diseases, so clarifying the toxic effects of xenobiotics is pivotal in this direction. Environmental contaminants like pesticides contain different toxic action mechanisms. Pesticides display toxic effects causing oxidative stress and affecting...
antioxidants statues including enzymatic elements such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.21), glutathione peroxidase (GPx; EC 1.11.1.9), glutathione reductase (GR; EC 1.6.4.2), glutathione S-transferase (GST; EC 2.5.1.18), or non-enzymatic such as glutathione (GSH), ascorbic acid, tocopherol are being studied as potential biomarkers in environmental risk assessment together with lipid peroxidation products such as thiobarbituric reactive species (TBARS). GSH is the most abundant intracellular small molecule thiol and a water-soluble tripeptide composed of the amino acids glutamine, cysteine, and glycine. As an important antioxidant, GSH plays a role in the detoxification of a variety of electrophilic compounds and peroxides via catalysis by GST and GPx.

Sulfoxaflor is the fourth generation neonicotinoid insecticide including sulfoximine group [CAS: 946578-00-3] (methyl (oxo) {1-[6-(trifluoromethyl)-3-pyridyl] ethyl}-λ6-sulfanylidene) cyanamide) acts as nAChRs agonist in insects. Recently, sulfoxaflor was reported to be highly toxic to some aquatic organisms and especially to bees. Sulfoxaflor was found to cause extremity abnormalities in rats. In carcinogenicity studies on rats and mice, sulfoxaflor has been reported to cause carcinoma and adenoma in the liver tissues for both species.

Sulfoxaflor has some unique properties including low soil and sediment sorption, high solubility in water, long anaerobic half-life in soil (113–120 days) and in water (103–382 days), resistance to photolysis and hydrolysis in soil and water, and very high to high mobility in soil. Thus, sulfoxaflor might be a potential contaminant of the surface and underground water bodies. Research reports indicated that interactions between sulfoxaflor and the environment have not been well investigated. There are no studies in the literature that evaluate the environmental concentration in water bodies of sulfoxaflor or its metabolites until this time. Consequently, potential hazards and the toxic effects of sulfoxaflor to the aquatic ecosystems need to be studied in more detail.

Zebrafish (Danio rerio), selected as a model organism in the current study, are among the most extensively used model species in the life sciences and ecotoxicological studies. The toxicity testing of pesticides including herbicides, insecticides and fungicides has been increasingly conducted using zebrafish in recent years. The use of zebrafish has found broad application due to their small size, low-cost breeding, water quality tolerance as well as homology to mammals, robust, phenotypes, high-throughput genetic, and chemical screening. Zebrafish as a tropical freshwater fish inhabit rivers (Ganges mainly) of Himalayan region of South Asia especially India, Nepal, Bhutan, Pakistan, Bangladesh, and Myanmar. Zebrafish, referred to as the bony fish (teleost), belong to the family Cyprinidae under the class Actinopterygii. As an omnivorous, a zebrafish’s natural diet consists primarily of zooplankton and insects. After hatching, zebrafish have the most rapid growth rate during the first 3 months. The adult zebrafish reach sexual maturity very quickly and have a generation time of about 10 weeks.

Researches regarding the toxic effects of sulfoxaflor on fish species are still limited and there is no known study investigating the effects of sulfoxaflor on the
antioxidant system and its oxidative toxicity on fish. Therefore, the purpose of the present study was to determine the potential effects of sulfoxaflor on GSH-related antioxidants and lipid peroxidation in the gill tissue of zebrafish and to assess the potential sublethal toxic effects of sulfoxaflor on fish.

Materials and methods

Acute toxicity test

Adult zebrafish (*D. rerio*) of the wild-type, mixed sex, (0.58 ± 0.12 g weight) were acclimatized to laboratory conditions for 2 weeks before the experiments with 14/10 h of light/dark cycle at 28°C ± 1°C. Acute toxicity tests (24, 48, and 96 h) were performed on the fish with a renewal static system in accordance with American Public Health Association’s guideline.53 Water in glass aquaria (65 cm × 35 cm × 30 cm) was changed 24 h intervals by transferring fish to other aquaria. Experimental tanks contained 60 L of dechlorinated and gently aerated tap water (dissolved oxygen; 6.87 ± 0.75 mg/L, pH; 7.63 ± 0.5, temperature; 28.23°C ± 0.82°C, alkalinity; 245 ± 3.59 mg/L as CaCO3, and total hardness 252 ± 11.55 mg/L as CaCO3. Stock fish were fed twice a day with fish pellet and feeding was stopped 24 h before the toxicity test. Commercial formulation of sulfoxaflor (CAS number: 946578-00-3), 500 WG (50% w/w active ingredient), ([methyl (oxo) {1-[6-(trifluoromethyl)-3-pyridyl] ethyl}-6-sulfanylidene] cyanamide) was used for toxicity test. The randomly selected fish were divided into four groups each comprising 108 individuals; 36 fish for the 24 h exposure period, 36 fish for the 48 h exposure period, and 36 fish for the 96 h exposure period. Six zebrafish were considered as an experimental unit to collect the required amount of tissue samples. The measurements were repeated in six technical replicates (*N* = 6). Previously, 96 h LC50 value of sulfoxaflor was determined as 35.13 mg/L for zebrafish in our laboratory.54 Sulfoxaflor concentrations were selected based on toxicity symptoms (loss of balance, erratic swimming, rapid movement of gill) with the preliminary test. Fish were exposed to 0.87 mg/L (2.5% of 96 h LC50), 1.75 mg/L (5% of 96 h LC50), 3.51 mg/L (10% of 96 h LC50) of sulfoxaflor for 24, 48, and 96 h. Control fish was kept in clean tap water. Fish were removed from aquaria at the end of each exposure period, then weighed and euthanized by decapitation. Gills of the fish were carefully dissected out and examined on an ice plate by washing them with saline solution, weighing, and storing them at −80°C. A tissue pool was used for every six gill samples. All the experiments were conducted in accordance with the protocols approved by Cukurova University, Ethics Committee of the Faculty of Medicine Experimental Medicine Research and Application Centre (Approval date: 04.07.2018, Approval code:3).

Preparation of gill homogenates

A pool of six fish gills was homogenized with ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged (Hettich Micro 220, Tuttlingen, Germany)
at 13,000g for 60 min at + 4°C and the supernatants were used to determine GSH-Related parameters (tGSH levels, GPx, GR, GST enzyme activities), TBARS and protein levels with UV-Vis spectrophotometer (Shimadzu UV-Vis Spectrophotometer UV-1700). All chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and Merck & Co. Inc. (Merck, Darmstadt, Germany).

**Determination of tGSH levels**

Gill homogenates were mixed with 10% sulfosalicylic acid at a ratio of 1/0.5 (v/v) then centrifuged at 9500g for 5 min. tGSH levels were analyzed according to Anderson.55 The reaction medium contained 143 mM sodium-potassium buffer (contained 6.3 mM EDTA, pH 7.5), 0.3 mM NADPH, 6 mM DTNB, 50 units of GR; and the total volume was adjusted to 1 mL with distilled water. Absorbance was recorded at 412 nm at 30°C. The results were converted to concentration by using a standard graph that was prepared with GSH.

**Determination GSH-related antioxidant enzyme activities**

GPx-specific activity was analyzed by monitoring the consumption of NADPH by GR at 340 nm at 37°C. t-Butylhydroperoxide was used as substrate.56 Reaction medium contained 0.1 M GSH, 10 units of GR, 2 mM NADPH, and 7 mM t-butylhydroperoxide. GR-specific activity was assayed by monitoring oxidation of NADPH by GSSG at 37°C at 340 nm.57 Reaction medium included 100 mM phosphate buffer (pH 8), 0.12 mM NADPH, and 1 mM GSSG. GST-specific activity was determined by using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The conjugation rate of GSH (20 mM) with CDNB (1 mM) was monitored at 340 nm at 25°C.58 The reaction medium was adjusted to 1.2 mL with 100 mM, pH 7.4 tris buffer.

**Assay of lipid peroxidation**

The lipid peroxidation level was assessed using the TBARS assay, which measures the production of lipid peroxidation products that react with thiobarbituric acid.59 TBARS concentrations were converted from a standard curve of 1,1,3,3-tetramethoxypropane.

**Assay of protein levels**

Protein levels of gill homogenates obtained from gill tissues were determined using the method developed by Bradford.60 One hundred microliter of the diluted gill homogenates was added to 3 mL of Bradford reagent and incubated for 30 min at room temperature. Then, the absorbances were recorded at 595 nm using UV-Vis spectrophotometer (Shimadzu UV-Vis Spectrophotometer UV-1700). Protein levels were calculated by the standard graph prepared using bovine serum albumin.
Statistical analysis

Statistical analyses of data were carried out with SPSS software version 20 (IBM SPSS Statistics, Armonk, NY, USA). Data was tested for homogeneity of variance using Levene’s test. Then analysis of variance (One-way ANOVA) procedures were conducted and Duncan multiple comparison test was used to obtain the statistical differences among the control and test groups. In addition, Pearson’s correlation was applied to correlate between the parameters. The data were shown as mean ± standard error.

Results

TGSH levels in sulfoxaflor exposed gill of zebrafish

The effects of acute exposure to sulfoxaflor on tGSH levels in the gill of zebrafish are presented in Table 1. The sulfoxaflor significantly \( (p < 0.05) \) increased tGSH levels in the gill of zebrafish when compared to respective controls (from 38% to 96%). tGSH levels were increased \( (p < 0.05) \) 38% and 40% by 1.75 and 3.51 mg/L sulfoxaflor exposure respectively at 24 h. All applied sulfoxaflor concentrations caused a significant increase in tGSH levels at 48 h exposure period and increases were found as 96%, 68%, 69% by 0.87, 1.75, and 3.51 mg/L sulfoxaflor exposures, respectively. Similarly tGSH levels were increased \( (p < 0.05) \) 64%, 71%, 65% by 0.87, 1.75, and 3.51 mg/L sulfoxaflor exposures respectively at 96 h exposure period. tGSH levels were increased by sulfoxaflor in a time-dependent manner for all sulfoxaflor exposures. The increase in tGSH levels in a dose-dependent manner was determined in only a 24 h exposure period. There was a significant positive correlation in the gill tGSH levels and TBARS levels \( (r = 0.48, p < 0.01) \) (Table 2).

GPx, GR, GST enzyme activities in sulfoxaflor exposed gill of zebrafish

The effects of acute exposure to sulfoxaflor on selected GSH related antioxidant enzyme activities were shown in Table 1. Exposure to sulfoxaflor induced GPx enzyme activity of gill for all applied sulfoxaflor concentrations at 24 and 96 h. GPx specific activities in the gill of zebrafish were increased \( (p < 0.05) \) 66%, 62%, 121% by 0.87, 1.75, and 3.51 mg/L sulfoxaflor exposure, respectively at 24 h exposure period. Additionally, high sulfoxaflor concentration was the most effective on GPx activity at 24 h. On the contrary, the exposure to 3.51 mg/L sulfoxaflor caused a significant inhibition of 27% in GPx enzyme activity at 48 h. Sulfoxaflor exposures of 0.87, 1.75, 3.5 mg/L increased GPx enzyme activity 131%, 99%, 120% following 96 h of exposure, respectively (Table 1). Sulfoxaflor exposure of 0.87 mg/L increased GR enzyme activity 21% at the end of 24 h exposure period. GR specific activities in the gill of zebrafish were decreased 14% by 3.51 mg/L sulfoxaflor concentration following 48 h exposure. Similar to 24 h, sulfoxaflor exposure of 1.75 mg/L increased GR enzyme activity 19% at the end of the 96 h exposure period (Table 1).
All sulfoxaflor concentrations had no significant effects on GST specific activity at 24 h and 48 h exposure periods. On the contrary, the exposure to 1.75, 3.51 mg/L sulfoxaflor caused a significant decrease of 24% and 24% in GST enzyme activity at 96 h. GST specific activities were decreased by sulfoxaflor in a time-dependent manner for 1.75 and 3.51 mg/L sulfoxaflor exposures. However, there was a significant increase in GST specific activity depending on exposure periods (Table 1). Additionally, there was a significant negative correlation between the gill GST enzyme activity and TBARS levels ($r = 0.20, p < 0.05$) (Table 2).

**TBARS levels in sulfoxaflor exposed gill of zebrafish**

Table 3 shows the results obtained from TBARS levels in the gill of zebrafish. TBARS levels in the gill of zebrafish were increased 94% and 82% by 1.75, 3.51 mg/L sulfoxaflor concentrations following a 48 h exposure. Exposure to sulfoxaflor increased TBARS levels of gill for all applied sulfoxaflor concentrations at 96 h. Sulfoxaflor exposure of 0.87, 1.75, and 3.51 mg/L increased TBARS levels 36%, 32%, 42% at the end of 96 h exposure period.
Freshwater and terrestrial ecosystems could be at significant risk in the world due to the extensive use of neonicotinoids as insecticides. Direct fish mortality were not attributed to neonicotinoids in the aquatic ecosystem. Nevertheless, neonicotinoids could affect fish species due to loss in quantity or quality of prey or habitat modification in contaminated waters in freshwater ecosystems. Moreover, the high toxicity and persistence of neonicotinoid insecticides threaten aquatic ecosystems and these impacts can alter food web structure and dynamics and reach higher trophic levels, further affecting the higher-level consumers. Sulfoxaflor, neonicotinoid-sulfoximine insecticide has the potential to be transferred at trophic levels by entering the food web in the aquatic ecosystem due to its previously mentioned properties.

This study is the known first to report oxidative damage in the gill of zebrafish after acute sublethal sulfoxaflor exposure. The results of the present work disclosed that sulfoxaflor induced oxidative stress by causing lipid peroxidation and by affecting GSH related antioxidants in the gill of zebrafish and also showed that zebrafish was severely affected by the exposure to different sulfoxaflor concentrations.

### Table 2. Correlation coefficients ($r$) for the levels of gill TBARS and tGSH levels, GPx, GR, GST enzyme activities.

| Correlation coefficients ($r$) | tGSH | GPx | GR | GST |
|-------------------------------|------|-----|----|-----|
| TBARS                         | 0.48** | -0.117 | -0.05 | -0.20* |

*p < 0.05 = Statistically significant. **p < 0.01.

### Table 3. Effects of sulfoxaflor on TBARS levels (nmol/mg protein) in the gill of zebrafish.

| Periods (h) | TBARS |
|-------------|-------|
| **Experimental groups** | | | |
| SUL0 | SUL0.87 | SUL1.75 | SUL3.15 |
| 24 | 4.83 ± 0.27 abx | 5.50 ± 0.46 ay | 4.24 ± 0.44 bz | 4.32 ± 0.22 bz |
| 48 | 4.85 ± 0.15 bx | 4.40 ± 0.32 bz | 8.69 ± 0.21 ax | 8.15 ± 0.20 ax |
| 96 | 4.95 ± 0.23 bx | 6.63 ± 0.22 ax | 6.44 ± 0.24 ay | 6.92 ± 0.27 ay |

Values are expressed as mean ± standard error. Letters a and b show the differences between groups at the same duration, and letters x, y and z show the differences between groups at the same concentration. Data shown different letters are significantly different at the $p < 0.05$ level ($N = 6$).
TBARS is a marker of oxidative lipid damage and it is a major oxidative product of peroxidized polyunsaturated fatty acid.\textsuperscript{64} Previously, it was reported that neonicotinoid insecticides caused lipid peroxidation by increasing TBARS levels in fish species.\textsuperscript{14,65,66} In this study, all sulfoxaflor exposures caused lipid peroxidation by significantly increasing TBARS levels in gill of zebrafish.

The studies reported that pesticide exposure can disturb the balance between the ROS generation and antioxidant systems in fish\textsuperscript{67}; therefore, changes in the antioxidant system in cells suggest that an adaptive response to oxidative stress conditions could hinder the formation of organ damage.\textsuperscript{68} GSH is an important antioxidant, which prevents ROS damage and helps detoxification by conjugating with chemicals and also, it acts as an essential cofactor for antioxidant enzymes including GPx, GR, and GST.\textsuperscript{69,70} An alteration in GSH related antioxidants contributes to the elimination of ROS, which are induced by pesticide exposure. In this study, the sulfoxaflor significantly increased tGSH levels in the gill of zebrafish in all applied sulfoxaflor concentrations and exposure periods. Furthermore, the increase in tGSH levels was a concentration-dependent pattern in short-term exposure periods. GSH has curtailed the role in the antioxidant system by detoxifying hydrogen peroxide (H$_2$O$_2$) and lipid peroxides which are generated as a result of membrane lipid peroxidation.\textsuperscript{71} Consistent with this research, Shukla et al.\textsuperscript{65} reported that neonicotinoid imidacloprid caused a significant elevation in GSH levels in the brain, kidney and liver of zebrafish. Vieira et al.\textsuperscript{18} also showed that imidacloprid caused a significant increase in GSH levels in the tissues of \textit{P. lineatus}. Additionally, a significant positive correlation between gill tGSH levels and TBARS levels might indicate that a high production of ROS resulted in oxidative stress, as evidenced by the highest levels of TBARS, in this research. This relation between tGSH and TABRS levels coincides greatly with the results of the research results reported by Shukla et al.\textsuperscript{65} The authors showed that imidacloprid caused an increase in either TBARS levels or GSH levels in the liver of zebrafish.

GPx is responsible for the reduction of H$_2$O$_2$ and lipid hydroperoxides in cells.\textsuperscript{72} GPx enzyme activities were induced by all applied sulfoxaflor concentrations in the gill of zebrafish in short term exposure periods. This result is in agreement with many authors. The authors demonstrated that imidacloprid increased GPx enzyme activity in \textit{O. mykiss} and \textit{P. lineatus}.\textsuperscript{14,18} Additionally, high sulfoxaflor concentration was initially the most effective on GPx activity in this study. Although there was no statistically positive correlation between GPx activity and tGSH levels, GPx induction and increase in tGSH levels occurred at the same exposure periods and for the same sulfoxaflor concentrations. Inductions occurring in both parameters showed that the GSH needed by the GPx for protecting the cells from H$_2$O$_2$ and lipid peroxides could be provided by denovo synthesis of GSH or the reduction of GSSG by GR enzyme activity. On the contrary, the exposure to high sulfoxaflor concentrations caused a significant inhibition in GPx enzyme activity at the 48 h exposure period. This inhibition caused a significant increase in the lipid
peroxidation product. The result showed that GPx might be the most important enzyme for the protection of membrane by diminishing lipid peroxides. GPx detoxifies peroxides with GSH acting as an electron donor in the reduction reaction, producing GSSG as an end product. The reduction of GSSG is catalyzed by GSH reductase (GR) in a process that requires NADPH created by enzyme Glucose-6-phosphate dehydrogenase (G6PD).GPx requires secondary enzymes such as GR and G6PD and cofactors including GSH, NADPH, and glucose 6-phosphate to function. GR is considered secondary antioxidant enzymes, because they do not act on ROS directly but enable GPx to function. Previously, it was reported that pesticides may cause induction in GR activity response to oxidative damage triggered by ROS generation in fish. In this study, low and median concentrations of sulfoxaflor caused an increase in GR enzyme activities, however high sulfoxaflor concentration caused a decrease in GR enzyme activity. The increase in GR activity by sulfoxaflor exposure in the gill of zebrafish indicates an attempt to maintain normal levels of GSH. The maintenance of GSH levels by GR might contribute to the reduction of oxidative toxicity produced by sulfoxaflor. Additionally, it was considered that the decrease in GR activity may be related to acting with GPx activity in the gill of zebrafish.

GSTs, another antioxidant enzyme family, inactivate secondary metabolites, such as unsaturated aldehydes, epoxides, and hydroperoxides. GSTs also have an important role in the detoxification of electrophilic toxicants such as some groups of pesticides. Sulfoxaflor had no significant effects on GST enzyme activity at 24 and 48 h in this study. However, GST enzyme activities were inhibited by median and high sulfoxaflor concentrations at 96 h. While low sulfoxaflor concentration caused a significant increase in GST enzyme activity compared to short-term exposure periods, median and high sulfoxaflor concentration caused a significant diminishing in GST enzyme activity. Additionally, there was a significantly negative correlation between the gill GST enzyme activity and TBARS levels. There is no evidence of the role of GST enzyme in the detoxification process of sulfoxaflor in fish. In this study, the changes in GST enzyme could result from the antioxidant effect of GST depending on TBARS levels. As a matter of fact, a negative correlation between GST enzyme activity and TBARS levels in the gill of zebrafish was found. Supporting these results, imidacloprid exposure reduced GST activity in the gill and kidney of P. lineatus. Demirci and Gungördü reported that acetamiprid caused an increase in GST enzyme activity in Gambusia holbrooki. Thiometoxam significantly increased GST activity in the liver of zebrafish.

**Conclusion**

In conclusion, the results of this study indicated that sulfoxaflor caused oxidative stress by affecting biochemical functions and suggested that sulfoxaflor could be toxic to zebrafish by inducing lipid peroxidation and by altering GSH related antioxidant parameters in the gill. Therefore, data may disclose one of the molecular pathways that play a role in sulfoxaflor toxicity in the gill of zebrafish. The
investigations of toxic effects of newly developed pesticides such as sulfoxaflor on non-targeted organisms without increasing the usage rates of nature are considered to be very important for the health of humans, animals, and nature.

**Acknowledgements**

The authors would like to thank the Department of Food Engineering (Enzymology Laboratory, Biotechnology Laboratory), Faculty of Agriculture and The Faculty of Fisheries, Cukurova University.

**Declaration of conflicting interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The financial support for this project (FBA201810503) from Cukurova University Scientific Research Commission is gratefully acknowledge.

**Ethics approval**

All the experiments were conducted in accordance with the protocols approved by Cukurova University, Ethics Committee of the Faculty of Medicine Experimental Medicine Research and Application Centre (Approval date: 04.07.2018, Approval code:3).

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