J Anim Reprod Biotechnol 2022;37:176-182
pISSN: 2671-4639 • eISSN: 2671-4663
https://doi.org/10.12750/JARB.37.3.176

Original Article

JARB
Journal of Animal Reproduction and Biotechnology

Check for updates

Norflurazon causes developmental defects including cardiovascular abnormalities in early-stage zebrafish (*Danio rerio*)

Garam An[1], Hahyun Park[1], Taeyeon Hong[2], Gwonhwa Song[1,*] and Whasun Lim[2,*]

[1]Institute of Animal Molecular Biotechnology and Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Korea
[2]Department of Biological Sciences, Sungkyunkwan University, Suwon 16419, Korea

Received July 18, 2022
Revised August 27, 2022
Accepted August 30, 2022

***Correspondence**
Gwonhwa Song
E-mail: ghsong@korea.ac.kr
***Correspondence**
Whasun Lim
E-mail: wlim@skku.edu

Author's Position and Orcid no.
An G, Graduate student,
https://orcid.org/0000-0002-0065-793X
Park H, Research professor,
https://orcid.org/0000-0001-9876-070X
Hong T, Graduate student,
https://orcid.org/0000-0002-1827-7062
Song G, Professor,
https://orcid.org/0000-0003-2817-5323
Lim W, Associate professor,
https://orcid.org/0000-0002-1328-0465

ABSTRACT Norflurazon is widely used on agricultural lands and has a high potential to pollute water sources. However, its effects on fish have not been fully elucidated. The purpose of our study was to determine whether norflurazon adversely affects the developmental stage of zebrafish, which are frequently used as a model system to evaluate the environmental impact of pollutants. Norflurazon interfered with the hatching of zebrafish embryos and induced several sublethal deformities including body length reduction, increased yolk sac volume, and enlargement of the pericardial region. We further examined the cardiotoxicity of norflurazon in the *flk1:eGFP* transgenic zebrafish line. The vascular network, mainly in the brain region, was significantly disrupted in norflurazon-exposed zebrafish. In addition, due to the failure of cardiac looping, norflurazon-exposed zebrafish had an abnormal cardiac structure. These developmental abnormalities were related to the apoptotic process triggered by norflurazon. Overall, the present study demonstrated the non-target toxicity of norflurazon by analyzing the hazardous effects of norflurazon on developing zebrafish.

Keywords: apoptosis, cardiovascular toxicity, developmental toxicity, norflurazon, zebrafish embryo

INTRODUCTION

Herbicide use in agricultural production may potentially contaminate groundwater or surface water and adversely affect non-target species. Therefore, analysis of the ecotoxicological effects of herbicides is important to preserve the aquatic ecosystem. Norflurazon, which is categorized as a pyridazinone herbicide, has been applied to preemergent weed management since 1974 (Sathishkumar et al., 2016). Due to its water solubility (28 mg/L), norflurazon use on agricultural lands has the potential to leach to groundwater and surface water (Wilson and Koch, 2013). Wilson et al. (2007) reported that 8,480 mg of norflurazon escaped from the applied site when sprayed at 7.7 kg/ha. Moreover, around 6-8 months were required for norflurazon to be degraded to 50% of its original concentration in aquatic environments.

Considering the possibility of water pollution, some studies have analyzed the toxicity of norflurazon towards aquatic organisms. For example, the planarian *Polycelis felina* exposed to 200 μM norflurazon for 3 days showed lower survivability. In addition, locomotive defects and

abnormal morphologies such as depigmentation and acephalia were observed after 14 days of treatment with lower concentrations of norflurazon (0.2, 2, and 20 μM) (Horvat et al., 2005). Therefore, we hypothesized that norflurazon might also adversely affect fish and analyzed its effects on a zebrafish model to verify our hypothesis.

Toxicity assessment using zebrafish embryos has been suggested as a promising method to demonstrate the impact of environmental toxicants (An et al., 2021; Ha et al., 2021). Various studies have confirmed that reactivity to toxicants is highly similar between zebrafish and other fish species (Busquet et al., 2014; Birke and Scholz, 2019). In addition, because of its rapid development and transparency, experiments with zebrafish embryos provide time and cost benefits (Glaberman et al., 2017).

In the present study, the survival and hatching rates of zebrafish embryos were measured following norflurazon treatment. In addition, morphological endpoints in toxicity tests and cardiovascular abnormalities in norflurazon-treated zebrafish were examined. To determine which cellular response may contribute to the reduced viability and deformities of norflurazon-treated zebrafish, we investigated whether norflurazon triggers apoptotic cell death in developing zebrafish. Overall, we conducted a toxicity assessment of norflurazon using a zebrafish model and found that norflurazon may negatively affect the aquatic ecosystem.

MATERIALS AND METHODS

Experimental organism

Adult wild-type (AB strain) and transgenic (*flk1:eGFP*) zebrafish lines acquired from the Zebrafish Organogenesis Mutant Bank (Kyungpook National University, Korea) were maintained as previously reported (Park et al., 2021). Parental zebrafish were separately kept overnight and exposed to light to stimulate mating in the following morning. After 1 h following the onset of spawning, zebrafish embryos were collected, washed, and kept at 28℃ prior to subsequent experiments. All experiments using zebrafish were performed following approved guidelines and regulations of Institutional Animal Care and Use Committee at the Animal Ethics Committee in Korea University.

Herbicide exposure

After 6 h of fertilization, which is the beginning of gastrulation (D'Costa and Shepherd, 2009), normally developed embryos (n=12 for each concentration) were selected and exposed to norflurazon (catalog number: 34364; Sigma-Aldrich, St. Louis, MO, USA). Norflurazon powder was diluted in dimethyl sulfoxide and further diluted to 0, 50, and 100 μM in embryo medium containing phenylthiourea to observe zebrafish embryos without visual interference due to pigmentation. Experimental concentration of norflurazon was determined by referring its LC50 value in other fish species (Munn and Gilliom, 2001). The same volume of dimethyl sulfoxide (0.02%) was diluted in the same medium as a vehicle control. Embryos were transferred to each well of a 24-well plate containing the norflurazon solution. The solution was replaced daily to maintain a specific concentration.

Evaluation of toxicological endpoints

From 24 h to 96 h of norflurazon treatment, the number of live and hatched embryos was recorded. The coagulation of embryos or absence of a heartbeat was used as an indicator of dead embryos. After 96 h of treatment, other sublethal endpoints were evaluated using an optical microscope (DM 2500; Leica, Wetzlar, Germany). In brief, the image of each live larva was captured under the microscope following immobilization with tricaine (catalog number: A5040; Sigma-Aldrich) and mounting with methylcellulose (catalog number: K390; Amresco, Solon, OH, USA). Image analyses were conducted using ImageJ software (NIH, Bethesda, MD, USA).

Visualization of the vasculature in norflurazon-treated zebrafish

Transgenic *flk1:eGFP* zebrafish were examined following norflurazon treatment to analyze the effects of norflurazon on the cardiovascular system. Immobilization of zebrafish was carried out in the same method as evaluation of toxicological endpoints for the imaging of zebrafish exposed to norflurazon for 96 h. Each image was captured under an upright microscope (Axio Imager M1; Zeiss, Oberkochen, Germany) and further analyzed with ImageJ software.

Visualization of apoptotic cell death in norflurazon-treated zebrafish

After 96 h of treatment, norflurazon-treated zebrafish were stained with 5 μg/mL acridine orange (catalog

number: A3568; Life Technologies, Carlsbad, CA, USA) for 1 h to visualize apoptotic cells with green fluorescence. Immobilization of zebrafish was carried out in the same method as evaluation of toxicological endpoints, and Axio Imager M1 was used to capture images. Captured images were further analyzed with ImageJ software.

Statistical analysis

All graphs represent the mean and standard deviation of the experimental data from toxicity tests. Statistical analyses of the data were performed by one-way ANOVA based on the general linear model of SAS software (SAS Institute, Cary, NC, USA). A p value below 0.05 was defined as statistically significant.

RESULTS

Norflurazon causes sublethal alterations in developing zebrafish

To determine whether norflurazon has sublethal effects on developing zebrafish, we treated zebrafish embryos with 0, 50, and 100 μM norflurazon and recorded the number of surviving embryos at 96 h post-fertilization (hpf). The survival rates following norflurazon exposure did not show statistically significant differences (Fig. 1A). However, the proportion of unhatched live zebrafish embryos was markedly increased following norflurazon exposure. At 72 hpf, 86.1 ± 9.6% of zebrafish embryos hatched following 0 μM norflurazon treatment, whereas only 19.4 ± 9.6% and 11.1 ± 4.8% of zebrafish embryos hatched following 50 μM and 100 μM norflurazon treatment, respectively (p < 0.001). The hatching rate of zebrafish embryos exposed to 100 μM norflurazon at 96

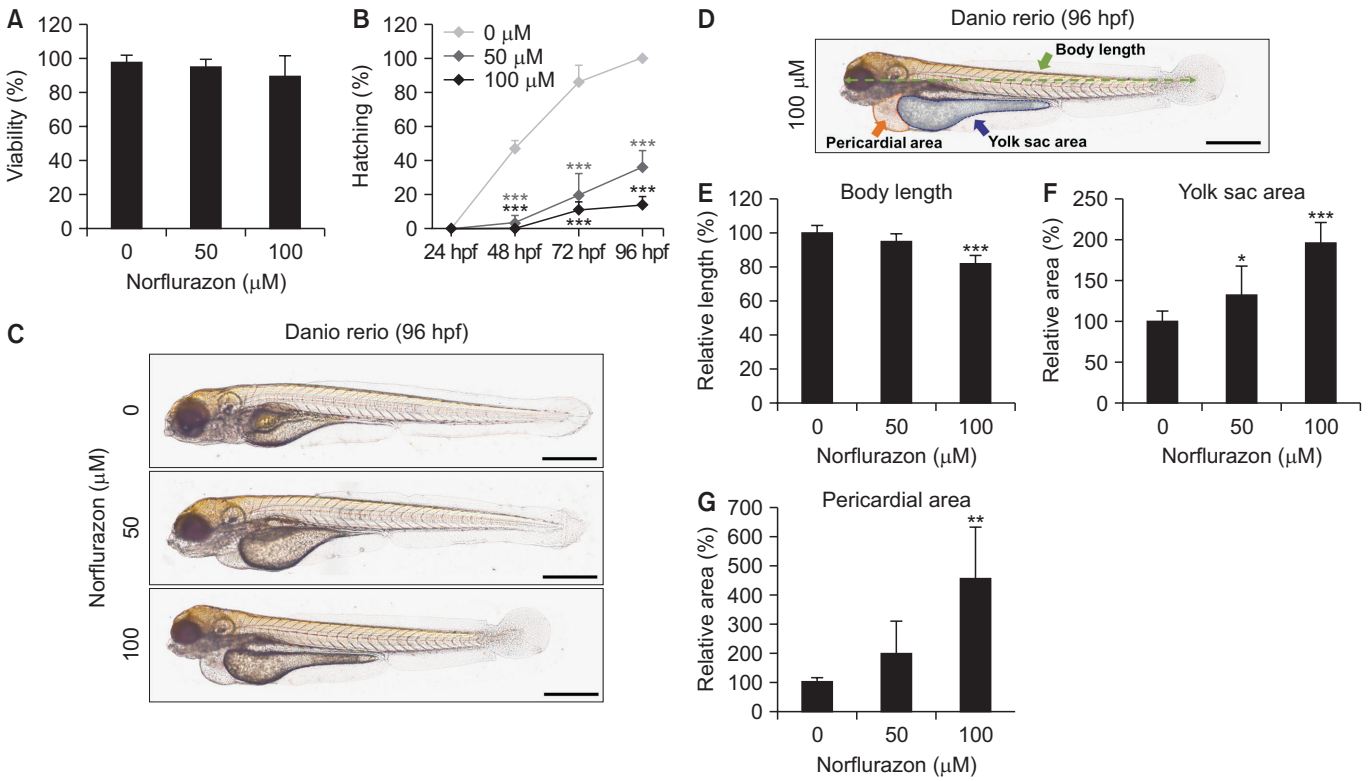
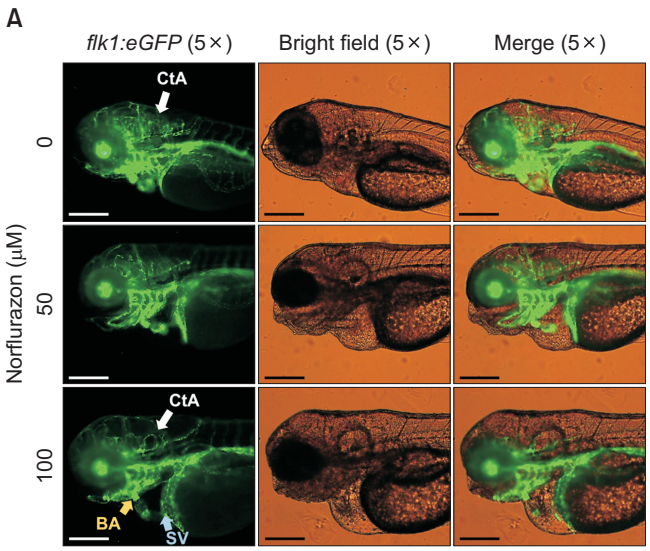


Fig. 1. Overall sublethal effects of norflurazon on zebrafish in the developmental stage. (A) Percentage of surviving zebrafish embryos at 96 h post-fertilization (hpf) following norflurazon treatment. Error bars represent standard deviations from triplicate data. (B) Percentage of hatched zebrafish embryos at 24, 48, 72, and 96 hpf. Error bars represent standard deviations from triplicate data. (C) Overall morphology at 96 hpf of zebrafish exposed to norflurazon. Scale bar represents 500 μm. (D) Representative image showing morphological abnormalities induced by norflurazon treatment. Green arrow: body length, quantified in (E). Blue arrow: yolk sac area, quantified in (F). Orange arrow: pericardial area with cardiac edema, quantified in (G). Bar graphs present the (E) relative body length, (F) relative yolk sac area, and (G) relative pericardial area of the treatment group compared with the vehicle group. Asterisks denote the significance level: *p < 0.05, **p < 0.01, and ***p < 0.001.

hpf was similar to the rate at 72 hpf (Fig. 1B). In addition, norflurazon induced several sublethal morphological changes (Fig. 1C). The relative length of the total body was reduced to 82.1 ± 4.7% in the 100 μM norflurazon group (Fig. 1E). The 50 μM and 100 μM norflurazon groups had a larger yolk sac size compared with that of the 0 μM norflurazon group ($p < 0.05$ and $p < 0.001$) (Fig. 1F). Enlargement of the pericardial area, which indicates edema, was observed in the 100 μM norflurazon treatment group ($p < 0.01$) (Fig. 1G).

The cardiovascular system of developing zebrafish is disrupted by norflurazon exposure

Considering that pericardial edema is one of the common characteristics of heart failure (Chen, 2013), we hypothesized that norflurazon might negatively affect the cardiovascular network. To confirm this hypothesis, the *flk1:eGFP* transgenic line was used to observe the cardiovascular structure under a fluorescence microscope. Irregularities in the brain vasculature and cardiac structure were clearly observed following norflurazon exposure (Fig. 2A). The density of the vasculature in the brain region was reduced to 69.1 ± 16.2% and 32.7 ± 9.7% in the 50 μM and 100 μM norflurazon groups, respectively ($p < 0.001$)



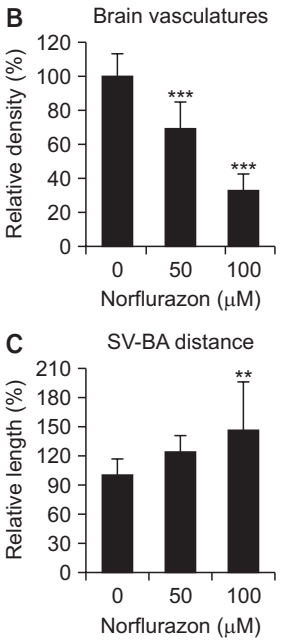
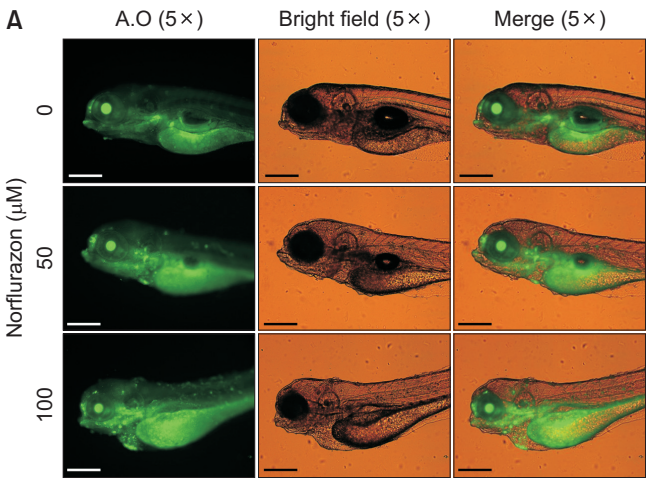


Fig. 2. Evaluation of the cardiovascular structure following norflurazon treatment. (A) Captured images of *flk1:eGFP* zebrafish at 96 h post-fertilization (hpf). White arrow: central artery (CtA). Yellow arrow: bulbus arteriosus (BA). Blue arrow: sinus venosus (SV). Scale bar represents 300 μm. (B) Density of the brain vasculature determined with an image of norflurazon-treated *flk1:eGFP* zebrafish. The 'Area' tool in ImageJ was used to quantify the area occupied by blood vessels. (C) SV-BA distance quantified with an image of norflurazon-treated *flk1:eGFP* zebrafish using the 'Length' tool in ImageJ. Asterisks denote the significance level: $^{**}p < 0.01$ and $^{***}p < 0.001$.



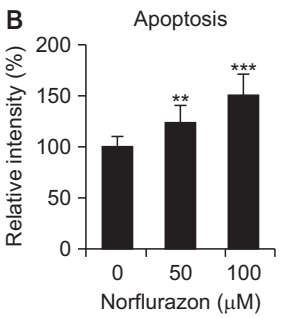


Fig. 3. Analysis of apoptotic cell death following norflurazon treatment. (A) Captured images of zebrafish stained with acridine orange (A.O) at 96 h post-fertilization (hpf). Scale bar represents 300 μm. (B) Green fluorescence intensity representing the number of stained apoptotic cells. The fluorescence intensity was quantified with an image of norflurazon-treated zebrafish incubated with acridine orange using ImageJ software. Asterisks denote the significance level: $^{**}p < 0.01$ and $^{***}p < 0.001$.

(Fig. 2B). In addition, the 100 μM norflurazon group showed a longer distance between sinus venosus (SV) and bulbus arteriosus (BA), implying an interrupted process of cardiac looping ($p < 0.01$) (Fig. 2C). Collectively, these results demonstrated the cardiovascular toxicity of norflurazon during zebrafish development.

Norflurazon triggers apoptotic cell death in developing zebrafish

To identify the cellular response responsible for the acute toxicity of norflurazon, we stained norflurazon-treated zebrafish with acridine orange, which intercalates with DNA strands and emits green fluorescence (Smirnova et al., 2021) (Fig. 3A). As several deformities were mainly observed in the posterior region of zebrafish previously, we focused on detecting apoptotic cell death in this region. The fluorescence intensity was 1.2-fold higher in the 50 μM norflurazon group compared with the vehicle group ($p < 0.01$), and it was 1.5-fold higher in the 100 μM norflurazon group ($p < 0.001$) (Fig. 3B). These results indicated that the acute toxicity of norflurazon might be mediated by the apoptotic process.

DISCUSSION

In the present study, we evaluated the toxicity of norflurazon towards zebrafish in the developmental stage. The hatching ability of zebrafish embryos exposed to norflurazon was significantly impaired. Moreover, several sublethal deformities and cardiovascular defects were observed in norflurazon-treated zebrafish larvae. The acute toxicity of norflurazon towards zebrafish was accompanied by apoptotic cell death.

Considering the extensive usage of pesticides and the possibility of water contamination, several studies have investigated the hazardous effects of pesticides on aquatic non-target organisms. In this study, a zebrafish model was used to assess the toxicity of the herbicide norflurazon, which is considered as a potential water pollutant owing to its physicochemical properties (Horvat et al., 2005). For example, residual norflurazon was detected up to 3.9 mg/L in surface waters from south Florida (Schuler and Rand, 2008). In addition, 1,684 mg and 8,480 mg of norflurazon was runoff to non-target sites when 4.4 kg/ha and 7.7 kg/ha of norflurazon applicated in flatwoods (Wilson et al., 2007). While these concentrations not accurately reflect exact exposure concentration to fish. Thus, further research revealing exposure amount on fish should be conducted.

We found that norflurazon caused several developmental defects in zebrafish larvae. Norflurazon reduced the hatching rate of embryos. Unsuccessful hatching is frequently observed in response to extrinsic stressors and can be caused by reduced movement due to functional or structural malformation (Hill et al., 2005, Horzmann et al., 2020). This result cannot explain whether norflurazon only delays hatching period or induces lethality by hatching inhibition because hatching ratio was not measured after 96 hpf. However, belatedly hatched larvae usually have lower viability due to developmental deformities (Küçükoğlu et al., 2013, Zoupa and Machera, 2017). Thus, we suggest that norflurazon might also reduce viability of zebrafish by hatching inhibition. In addition, sublethal deformities such as body length reduction and yolk sac enlargement occurred following norflurazon treatment. A shorter length is mainly associated with the irregular development of the skeletal or muscular system (Zoupa and Machera, 2017). A larger yolk sac volume suggests that the normal utilization of nutrients in the yolk is hampered by norflurazon treatment (Sant and Timme-Laragy, 2018). In addition, excessive fluid accumulated in the pericardial area in norflurazon-exposed zebrafish.

Pericardial edema is one of the symptoms used to confirm the cardiovascular toxicity of chemicals (Zoupa and Machera, 2017). We revealed that norflurazon exposure induced not only massive pericardial edema but also an irregular brain vasculature and heart structure in zebrafish. During normal vascularization, migrated precursor cells form primordial hindbrain channels (PHBCs), and central arteries (CtAs) are subsequently sprouted from PHBCs (Gupta et al., 2021). In this study, an irregular cranial vasculature, mainly a reduction in CtAs, was observed following norflurazon treatment. This malformation might result from the altered transcriptional level of genes related to *vegf* signaling and chemokines, which are crucial for embryonic vascularization (Fujita et al., 2011). However, further studies are needed to elucidate the detailed mechanisms underlying vascular deformities. In addition, norflurazon-exposed zebrafish showed a longer SV-BA distance, which indicates a lack of cardiac looping. The correct bending of the heart tube, referred to as cardiac looping, leads to the asymmetrical arrangement of the ventricle and atrium (Desgrange et al., 2018). In the

current study, the linear alignment of the ventricle and atrium, quantified as the distance between the SV and BA, was increased by norflurazon treatment. This structural malformation can lead to various heart conditions (Tessadori et al., 2021). Collectively, our results suggest that norflurazon might adversely affect aquatic organisms by inducing the malfunction of the cardiovascular network.

Apoptosis is a cellular process induced by multiple external stimuli. As demonstrated in previous studies, various pesticides could trigger the apoptotic process through several mechanisms such as oxidative stress and metabolic alterations. For example, deltamethrin was found to impair defense systems against oxidative stress, resulting in reduced viability and malformation with apoptosis (Parlak, 2018). Paraquat exposure was also observed to trigger oxidative stress in a similar manner and induce cell death by modulating the transcriptional levels of genes associated with apoptosis (Wang et al., 2016). Changes in endogenous metabolites induced by imazalil and imazalil-M were found to be involved in apoptosis induction in developing zebrafish (Huang et al., 2022). Therefore, identifying the detailed mechanisms of norflurazon-mediated apoptosis might provide in-depth knowledge for predicting its effects on non-target organisms.

CONCLUSION

Overall, the present results demonstrated the toxic effects of norflurazon on zebrafish in the developmental stage. Zebrafish exposed to norflurazon in the embryonic stage showed impaired hatchability, pathological phenotypes including a shortened body length, yolk enlargement, and severe edema in the cardiac region, and structural impairment of the cardiovascular system. These developmental defects were mediated by apoptotic cell death. Overall, our study demonstrated the effects of norflurazon on a zebrafish model, suggesting that it could be a threat to the aquatic ecosystem.

Author Contributions: Conceptualization, G.S., and W.L.; methodology, G.A., H.P., and T.H.; investigation, G.A., H.P., and T.H.; data curation, G.A., H.P., T.H., G.S., and W.L.; visualization, G.A.; writing-original draft, G.A.; writing-review and editing, G.S., and W.L.; funding acquisition, G.S., and W.L.; supervision, G.S., and W.L.; project administration, G.S., and W.L.

Funding: This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) grant funded by the Ministry of Education (2020R1A6A3A13075810 and 2019R1A6A1A10073079) and National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (2021R1A2C2005841). This study was also supported by the Institute of Animal Molecular Biotechnology, Korea University.

Ethical Approval: Not applicable.

Consent to Participate: Not applicable.

Consent to Publish: Not applicable.

Availability of Data and Materials: Not applicable.

Acknowledgements: None.

Conflicts of Interest: No potential conflict of interest relevant to this article was reported.

REFERENCES

An G, Park H, Song G, Lim W. 2021. Developmental toxicity of dimethachlor during zebrafish embryogenesis mediated by apoptosis and oxidative stress. J. Anim. Reprod. Biotechnol. 36:2-8.

Birke A and Scholz S. 2019. Zebrafish embryo and acute fish toxicity test show similar sensitivity for narcotic compounds. ALTEX 36:131-135.

Busquet F, Strecker R, Rawlings JM, Belanger SE, Braunbeck T, Carr GJ, Cenijn P, Fochtman P, Gourmelon A, Hübler N, Kleensang A, Knöbel M, Kussatz C, Legler J, Lillicrap A, Martínez-Jerónimo F, Polleichtner C, Rzodeczko H, Salinas E, Schneider KE, Scholz S, van den Brandhof EJ, van der Ven LT, Walter-Rohde S, Weigt S, Witters H, Halder M. 2014. OECD validation study to assess intra- and inter-laboratory reproducibility of the zebrafish embryo toxicity test for acute aquatic toxicity testing. Regul. Toxicol. Pharmacol. 69:496-511.

Chen J. 2013. Impaired cardiovascular function caused by different stressors elicits a common pathological and transcriptional response in zebrafish embryos. Zebrafish 10:389-400. (Erratum published 2014, Zebrafish 11:498)

D'Costa A and Shepherd IT. 2009. Zebrafish development and genetics: introducing undergraduates to developmental biology and genetics in a large introductory laboratory class. Zebrafish 6:169-177.

Desgrange A, Le Garrec JF, Meilhac SM. 2018. Left-right asym-

metry in heart development and disease: forming the right loop. Development 145:dev162776.

Fujita M, Cha YR, Pham VN, Sakurai A, Roman BL, Gutkind JS, Weinstein BM. 2011. Assembly and patterning of the vascular network of the vertebrate hindbrain. Development 138: 1705-1715.

Glaberman S, Padilla S, Barron MG. 2017. Evaluating the zebrafish embryo toxicity test for pesticide hazard screening. Environ. Toxicol. Chem. 36:1221-1226.

Gupta A, Rarick KR, Ramchandran R. 2021. Established, new and emerging concepts in brain vascular development. Front. Physiol. 12:636736.

Ha Y, Kim Y, Choi J, Hwang I, Ko JY, Jeon HK, Kim YJ. 2021. Evaluation of cytotoxicity, genotoxicity, and zebrafish embryo toxicity of mixtures containing *Hyssopus officinalis, Morus alba, Engraulis japonicus*, and 27 other extracts for cosmetic safety assessment. Mol. Cell. Toxicol. 17:221-232.

Hill AJ, Teraoka H, Heideman W, Peterson RE. 2005. Zebrafish as a model vertebrate for investigating chemical toxicity. Toxicol. Sci. 86:6-19.

Horvat T, Kalafatić M, Kopjar N, Kovacević G. 2005. Toxicity testing of herbicide norflurazon on an aquatic bioindicator species--the planarian Polycelis felina (Daly.). Aquat. Toxicol. 73:342-352.

Horzmann KA, Portales AM, Batcho KG, Freeman JL. 2020. Developmental toxicity of trichloroethylene in zebrafish (Danio rerio). Environ. Sci. Process. Impacts 22:728-739.

Huang S, Huang M, Tian S, Meng Z, Yan S, Teng M, Zhou Z, Diao J, Zhu W. 2022. Imazalil and its metabolite imazalil-M caused developmental toxicity in zebrafish (Danio rerio) embryos via cell apoptosis mediated by metabolic disorders. Pestic. Biochem. Physiol. 184:105113.

Küçükoğlu M, Binokay US, Pekmezekmek AB. 2013. The effects of zinc chloride during early embryonic development in zebrafish (Brachydanio rerio). Turk. J. Biol. 37:158-164.

Munn MD and Gilliom RJ. 2001. Pesticide Toxicity Index for Freshwater Aquatic Organisms. U.S. Department of the Interior, U.S. Geological Survey, Sacramento, pp. 34.

Park H, Song G, Lim W. 2021. Isoprocarb induces acute toxicity in developing zebrafish embryos through vascular malformation. J. Anim. Reprod. Biotechnol. 36:17-24.

Parlak V. 2018. Evaluation of apoptosis, oxidative stress responses, AChE activity and body malformations in zebrafish (Danio rerio) embryos exposed to deltamethrin. Chemosphere 207:397-403.

Sant KE and Timme-Laragy AR. 2018. Zebrafish as a model for toxicological perturbation of yolk and nutrition in the early embryo. Curr. Environ. Health Rep. 5:125-133.

Sathishkumar P, Mangalaraja RV, Rozas O, Vergara C, Mansilla HD, Gracia-Pinilla MA, Anandan S. 2016. Sonophotocatalytic mineralization of Norflurazon in aqueous environment. Chemosphere 146:216-225.

Schuler LJ and Rand GM. 2008. Aquatic risk assessment of herbicides in freshwater ecosystems of South Florida. Arch. Environ. Contam. Toxicol. 54:571-583.

Smirnova A, Mentor A, Ranefall P, Bornehag CG, Brunström B, Mattsson A, Jönsson M. 2021. Increased apoptosis, reduced Wnt/β-catenin signaling, and altered tail development in zebrafish embryos exposed to a human-relevant chemical mixture. Chemosphere 264(Pt 1):128467.

Tessadori F, Tsingos E, Colizzi ES, Kruse F, van den Brink SC, van den Boogaard M, Christoffels VM, Merks RM, Bakkers J. 2021. Twisting of the zebrafish heart tube during cardiac looping is a *tbx5*-dependent and tissue-intrinsic process. Elife 10:e61733.

Wang Q, Liu S, Hu D, Wang Z, Wang L, Wu T, Wu Z, Mohan C, Peng A. 2016. Identification of apoptosis and macrophage migration events in paraquat-induced oxidative stress using a zebrafish model. Life Sci. 157:116-124.

Wilson PC, Boman B, Foos JF. 2007. Norflurazon and simazine losses in surface runoff water from flatwoods citrus production areas. Bull. Environ. Contam. Toxicol. 78:341-344.

Wilson PC and Koch R. 2013. Influence of exposure concentration and duration on effects and recovery of Lemna minor exposed to the herbicide norflurazon. Arch. Environ. Contam. Toxicol. 64:228-234.

Zoupa M and Machera K. 2017. Zebrafish as an alternative vertebrate model for investigating developmental toxicity-the triadimefon example. Int. J. Mol. Sci. 18:817.