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Original Article

Isoprocarb induces acute toxicity in developing zebrafish embryos through vascular malformation

Hahyun Park[1], 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 Food and Nutrition, College of Science and Technology, Kookmin University, Seoul 02707, Korea

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***Correspondence**
Gwonhwa Song
E-mail: ghsong@korea.ac.kr

ORCID
https://orcid.org/0000-0003-2817-5323

***Correspondence**
Whasun Lim
E-mail: wlim@kookmin.ac.kr

ORCID
https://orcid.org/0000-0002-1328-0465

ABSTRACT In this study, the potential toxicity of isoprocarb was demonstrated using zebrafish embryos. We treated isoprocarb (0, 29, and 58 mg/L) to the zebrafish embryos for 72 h then, we estimated morphological changes and apoptotic cell numbers. The increasing extent of apoptosis from the anterior to posterior region of developing zebrafish larvae was correlated with toxicity in the overall development process, including growth and normal organ formation. The appearance of abnormalities in the isoprocarb-treated groups in comparison to normal developing zebrafish larvae was verified using quantitative image analysis based on ImageJ software program. The vascular system comprising a complex interconnection of blood vessels was visualized in vessel-fluorescent transgenic zebrafish (*fli1:eGFP*). The main vasculature was malformed on isoprocarb treatment, and this was also related to cardiac defects. Taken together, normal embryonic development in zebrafish was interrupted owing to the acute toxicity of isoprocarb.

Keywords: apoptosis, embryo toxicity, growth restriction, isoprocarb, vascular abnormalities

INTRODUCTION

Organophosphorus and carbamate compounds have been used extensively to control agricultural pests during the decades since they were developed in the 1940-1950s (Assis et al., 2011). Their sub-lethal toxicity in non-targeted organisms was indicated by the increased mortality in experimental vertebrates (Gallo et al., 1995). These compounds usually act as inhibitors of the enzyme acetylcholinesterase. Overstimulated cholinergic syndrome was observed in rats owing to the dysregulation of acetylcholinesterase, the major neurotransmitter acetylcholine in the nervous system, that is a target for these pesticides (Costa et al., 2008). Adverse potential risks of carbamate pesticides are also presented as dysfunctional mitochondria and disruption of enzymatic signaling in both *in vitro* and *in vivo* studies (Leung and Meyer, 2019). Reproductive toxicities, including abnormal sperm development, infertility, and infant diseases emphasize the need to monitor contamination from environmental pesticides (Frazier, 2007). Owing to public health concerns, ecotoxicological evaluations are required to prevent potential hazardous effects of these chemical compounds.

Isoprocarb, which is derived from methylcarbamic acid and 2-isopropylphenol, has been widely sprayed on rice, sugarcane, cocoa, and other vegetables to repel diverse chewing insects (Pesticide Properties DataBase). Isoprocarb belongs to the carbamate pesticides family and is known as a rapid-effective, short-residual insecticide in the field. Although there is a large deviation depending

on the season and the place, isoprocarb is easily detected in water with less hydrophobicity. For example, isoprocarb (0.1 to 16.6 ng/L) was collected from river samples in China and it accumulated in aquatic organisms and caused toxicity at a much higher concentration than the source of initial application (Saito et al., 1991; Xu et al., 2020). Previous studies have suggested that diverse carbamate pesticides unintentionally permeate into the environment and cause various toxic processes in organisms with toxicity. The risk of isoprocarb in hepatotoxicity, lethal-toxicity, and enzyme inhibitory activity has been investigated in the laboratory (Wang et al., 2009, Wang et al., 2012). Comprehensive toxicological information available from the material safety data sheet revealed that the lethal concentration of isoprocarb in rats is 403 mg/kg, effective concentration in algae is 21 mg/L, and its aquatic toxicology is also emphasized. However, the underlying toxic effects and concentration of isoprocarb remain largely unknown.

It is difficult to replace diverse vertebrates in the entire environment to identify specific detrimental effects and ecological implications of compounds that are used for industrial and agricultural purposes. The reported advantages of the ideal vertebrate model organism, zebrafish, including a short reproductive cycle, rapid developmental period, transparency for visualization, and ease of generating transgenic lines, has resulted in its utilization as a prominent animal model system for evaluating pollutants (Dai et al., 2014). Furthermore, as genetic homology to humans was found to be up to 70% in the genome, it is commonly used in disease modeling to mimic organ structure and targeted clinical study for drug discovery (Cassar et al., 2020). The use of experimental zebrafish models in the past few decades to evaluate immunotoxicity, developmental toxicity, abnormal behavioral responses, and intracellular damage has contributed to increase global concerns regarding environmental contaminants in living organisms that inhabit different environments (Bhagat et al., 2020).

Our study focused on the potential of isoprocarb to induce acute toxicity in aquatic organisms. The hypothesis was based on the high likelihood of isoprocarb seeping into areas surrounding agricultural fields and resulting in potential environmental effects in non-target organisms. To demonstrate the action of isoprocarb on zebrafish development, we first detected basal anatomical changes under acute isoprocarb treatment. Then, intracellular damage leading to apoptotic cell death and the development of vasculature in larvae were analyzed based on specific genes expressed in transgenic zebrafish. Our results revealed that embryonic abnormalities mediated by developmental toxicity were induced by isoprocarb during the early stages of zebrafish development.

MATERIALS AND METHODS

Maintenance and collection of zebrafish embryos

Ab strain wild-type and *fli1:eGFP* transgenic adult zebrafish, obtained from the Zebrafish Organogenesis Mutant Bank (KNRRCZOMB, Kyungpook National University, Korea) were reared in water maintained at a temperature of 28.5 ± 1℃ with appropriate salinity, UV sterilization, and pH. Zebrafish were reared under a controlled day-night 12 h light:dark photoperiod using an automatic timer as previously reported (Park et al., 2020). Male and female zebrafish were incubated in a breeding tank under dark conditions for 12 h to obtain embryos for use in all the experiments in this study. Using light stimulation, breeding was initiated in the fish and then, eggs were fertilized in water at a temperature of approximately 28℃. Fertilized eggs were washed thrice using fresh embryo medium and further maintained in an incubator at 28.5 ± 1℃ until treated with isoprocarb for each experiment.

Isoprocarb exposure

Isoprocarb (Cat. No. 45541) purchased from Sigma-Aldrich (St. Louis, MO, USA) was completely dissolved in dimethyl sulfoxide (Sigma-Aldrich). Isoprocarb treatment concentrations were estimated as 0, 29, and 58 mg/L based on pre-experimental dose-response observations. The isoprocarb stock solution was diluted with 1-phenyl-2-thiourea (Sigma-Aldrich, final concentration of 0.03%) in fresh embryo medium to inhibit pigmentation during embryonic development, as it could interfere with the visualization of developmental abnormalities arising in response to isoprocarb exposure. Treatment solution containing the same volume of only dimethyl sulfoxide as the isoprocarb treatment solutions was considered as the solvent control. The fertilized eggs (n = 12) were incubated for 72 h in 24-well plates with up to 1 mL volume of solution per well for each treatment. All treatment solutions were renewed every 24 h to avoid any possible contami-

nations, and the dead embryos and larvae were removed. All surviving embryos and larvae were rinsed with fresh embryo medium to examine the underlying toxic effects of isoprocarb at 72 hours post fertilization (hpf) in the treated zebrafish larvae. For analysis of toxic effects of isoprocarb, the experiments were performed in triplicate. We observed and captured pathological abnormalities using LEICA DM 2500 microscope (Leica, Germany). At least three captured images per larvae were used to analyze microscopic images.

Acridine orange staining in zebrafish larvae

To investigate cell death in response to isoprocarb exposure in developing zebrafish, we used acridine orange (Cat. No, A3568, Life Technologies, Carlsbad, CA, USA) that shows green fluorescence in apoptotic cells. After 72 h treatment with different concentrations of isoprocarb (n = 12 for each treatment), we added 10 mg/mL acridine orange dye to the treated larvae at a final concentration of 5 μg/mL in 1 mL fresh embryo medium. The larvae were housed in a 28℃ incubator for 1 h, and then washed twice with fresh embryo medium to completely remove extant dye and treatment solutions. Anesthetized larvae from each isoprocarb treatment concentration were aligned under an upright fluorescence microscope and analyzed using green fluorescent channel and bright field image information, which could configure the entire structure. We captured images of larvae that expressed green fluorescence in apoptotic cells in the anterior (mainly in the brain and eyes) and posterior (caudal area) parts using Axiovision microscope (ZEISS). Images acquired from larvae exposed to 29 and 58 mg/L isoprocarb were compared with those of the controls, which showed no significant features of apoptosis, using ImageJ (NIH, Bethesda, MD, USA). At least three captured images per larvae were used to analyze microscopic images. The experiments were performed in triplicate.

Analysis of vasculature formation in *fli1:eGFP* transgenic zebrafish larvae

Fresh embryos (n = 12 for each treatment) from transgenic zebrafish with the *fli1* gene encoding green fluorescent protein (GFP), presenting normal blood vessels throughout embryogenesis, were incubated for 72 hpf with different concentrations of isoprocarb (0, 29, and 58 mg/L). Then, they were washed thrice with fresh embryo medium to remove the remaining isoprocarb solution, anesthetized, and transferred to a glass slide to obtain a clear visualization of vasculature formation. Using Axiovision microscope software (ZEISS), we captured images of the main vessels of the cardiovascular system along the dorsal part of the body, known as the dorsal longitudinal anastomotic vessel (DLAV) and sprouts of intersegmental vessels (ISVs), and arteries and veins in the caudal area connected to the aorta. The developing vasculature was highlighted using eGFP, whereas disrupted vessels revealed disconnected or vacant structures in the trunk of individual larvae. At least three captured images per larvae were used to analyze microscopic images. We used ImageJ software program to analyze the extent of damage according to isoprocarb exposure against that in the controls. The experiments were performed in triplicate.

Statistical analysis

Each well in the 24-well plates contained 12 embryos, and the total range of embryos used in this study were 36 embryos per concentration of isoprocarb (0, 29, and 58 mg/L) with triplicate. At least three captured images per larvae were used to analyze microscopic images of wild type strain, fluorescent images of dyed larvae and transgenic models for vascular development. The method of least-squares analysis of variance based on the general linear model (PROC-GLM) in SAS (SAS Institute, Cary, NC, USA) was used to validate all quantitative values in this study. A p-value < 0.05 was considered statistically significant.

RESULTS

Isoprocarb induced developmental toxicity at the early stage in zebrafish larvae

With 72 h isoprocarb treatment, developmental malformations, including reduced body length, edema in the heart and yolk sac, and curvature in the caudal area of early stage zebrafish were observed (Fig. 1A). Quantitative values supported the development of endpoints in response to isoprocarb exposure; body length was reduced by 10% and 20% in the 29 and 58 mg/L isoprocarb-treated zebrafish larvae, respectively (Fig. 1B). Edema generation was markedly increased to 170% (with 29 mg/L isoprocarb) and 220% (with 58 mg/L isoprocarb) in the yolk sac (Fig. 1C) and 180% (with 29 mg/L isoprocarb) and

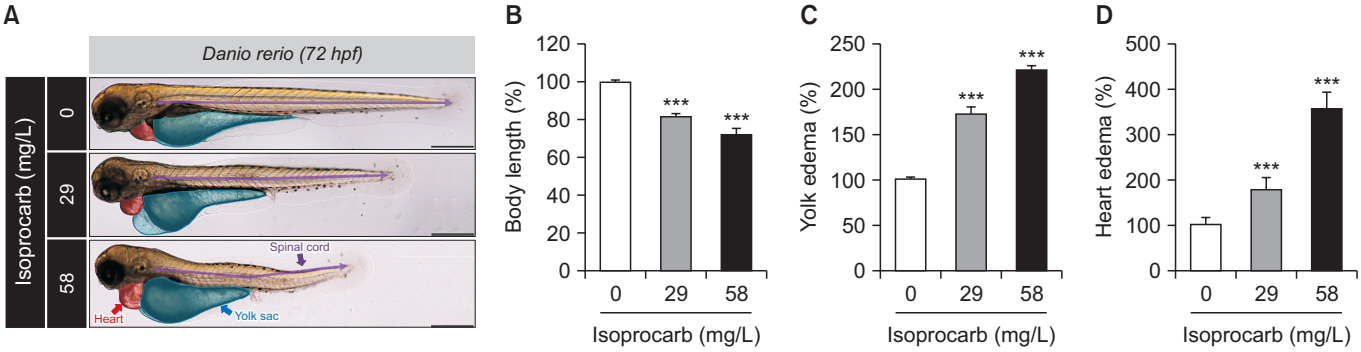

Fig. 1. Isoprocarb toxicity in developing zebrafish embryos. (A) Pathological alterations revealed abnormal embryonic development in zebrafish larvae at 72 hpf. The transparent body of the zebrafish embryo-larvae facilitates visualizing internal development. As compared with controls, distorted phenotypic changes including inflation of the heart and yolk sac to an unusual level, spinal curvature, and reduction in overall body length were detected (highlighted by colored box; heart - red and yolk sac - blue, line; curved body axis - purple). Scale bar: 300 μm. (B-D) Quantitative values of toxicity endpoints supporting those observed defects - (B) body length, (C) yolk edema, and (D) heart edema (***$p < 0.001$).

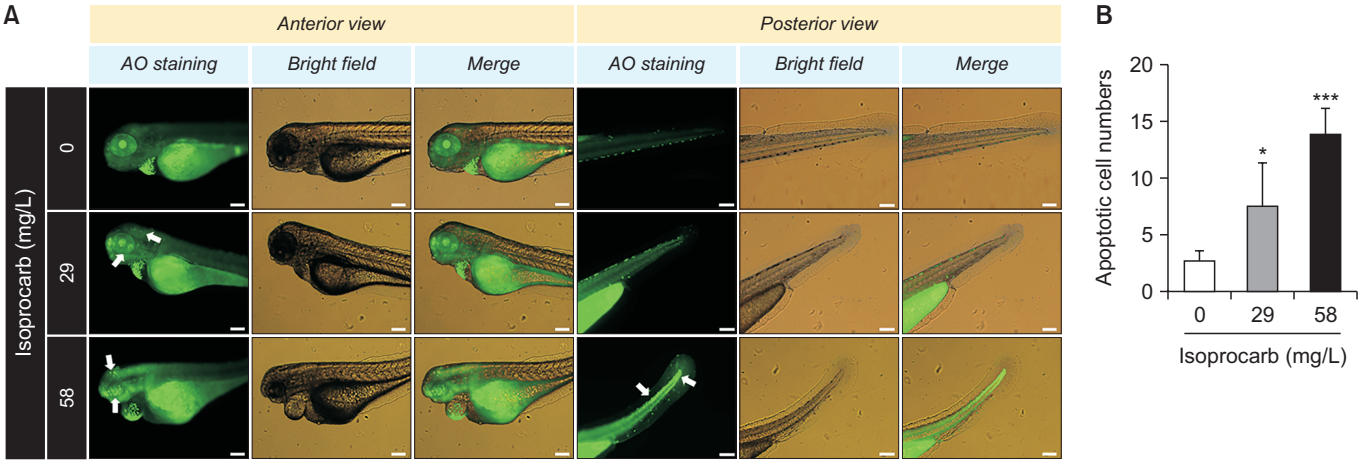

Fig. 2. Detection of apoptotic cell death in zebrafish larvae using acridine orange staining. (A) Evidence of developmental defects - acridine orange-positive apoptotic cells were highly increased in the eye and brain in the anterior part, and a significant number of green fluorescent cells were also observed in the curved tail in the posterior part of the body. Scale bar: 100 μm. (B) The number of apoptotic cells with each concentration of isoprocarb was counted at 72 h of development in the individual larva (*$p < 0.05$ and ***$p < 0.001$).

357% (with 58 mg/L isoprocarb) in the heart (Fig. 1D) as compared with 100% in the solvent controls. These results showed induction of developmental defects after exposure to different concentrations of isoprocarb.

Isoprocarb triggered apoptotic cell death along the anterior–posterior axis of zebrafish larvae

To identify isoprocarb-related apoptotic events in developing zebrafish embryos, we detected the markers of apoptosis using acridine orange, a fluorescent dye. As seen in Fig. 2A, white arrows indicate cell breakdown owing to apoptosis in the brain and eyes in the anterior part and in the caudal area in the posterior part of zebrafish larvae (Fig. 2A). With increasing concentrations of isoprocarb, from 29 to 58 mg/L, a high level of apoptotic cell death was observed. We counted the number of cells showing apoptotic features at 29 and 58 mg/L isoprocarb exposure; the rate of increase was 2.7-fold and 4.9-fold higher than that in the non-treated control, respectively (Fig. 2B). These results suggested apoptotic cell death in zebrafish larvae.

Isoprocarb mediated vascular abnormalities in the developing zebrafish

As shown in Fig. 3A, defective vascular growth was observed in the main vasculature of zebrafish larvae at 72 hpf. In response to isoprocarb treatment, disrupted ISVs were observed; especially, the upper structure of the ves-

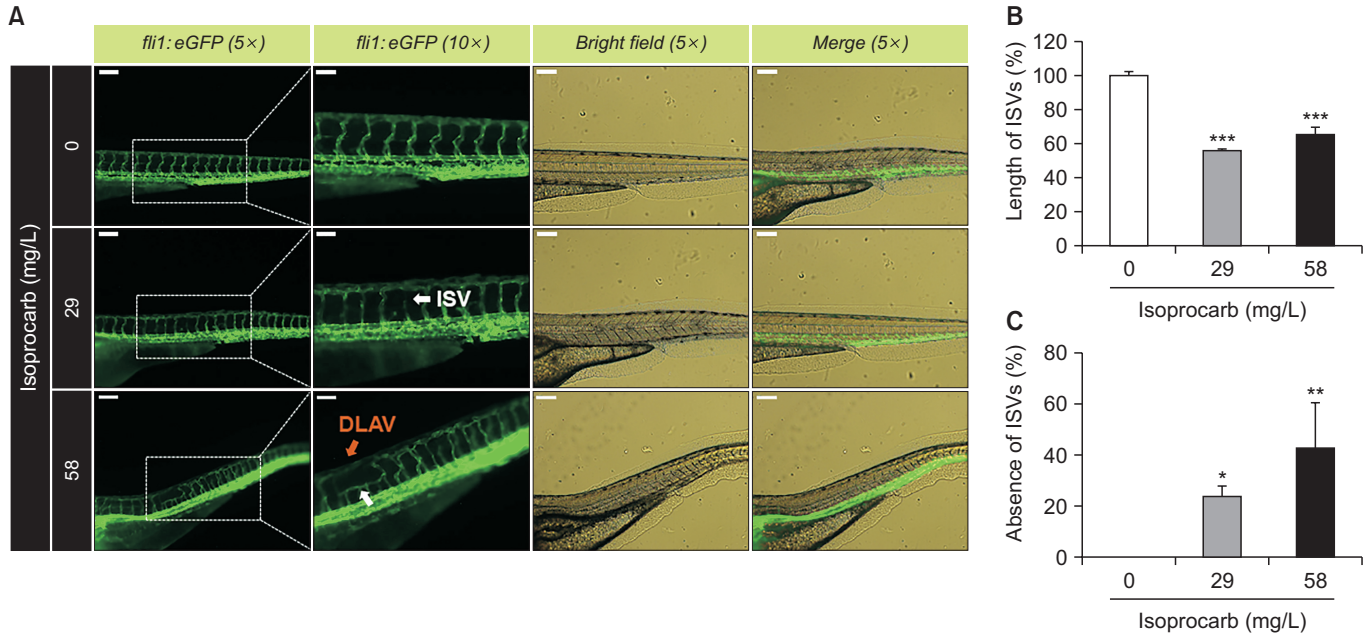

Fig. 3. Vasculature malformation displayed by representative larvae at the 72 hpf development stage. (A) Angiogenic process easily visualized in eGFP-expressed *fli1* transgenic zebrafish larvae throughout embryogenesis. As morphological variables predominated in response to isoprocarb exposure, newly formed ISV sprouts (white arrows) were undeveloped and DLAV (orange arrow) was expressed with unclear boundaries as compared with those in normal larvae in solvent controls. Scale bar: 100 μm for 5× magnification and 50 μm for 10× magnification. (B, C) Determination of the damage in ISVs connected with the cardiovascular system indicated that the length (B) and disruption (C) in ISV formation were affected in a concentration-dependent manner at 72 h of isoprocarb treatment (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

sels was undeveloped after exposure to 29 and 58 mg/L isoprocarb (white arrows). Likewise, the leakage of the DLAV was also measured after 58 mg/L isoprocarb treatment (indicted by an orange arrow). Images at 10× magnification showed a region 100 times larger than the actual pathological area in the trunk vasculature. We estimated a reduction of 45% and 35% in the entire length of ISVs in the zebrafish larvae exposed to 29 and 58 mg/L isoprocarb, respectively (Fig. 3B). With increasing concentrations of isoprocarb, the sprouts of ISVs gradually disappeared between the DLAV and dorsal aorta. The values were increased by 23% (29 mg/L isoprocarb) and 42% (58 mg/L isoprocarb) compared with that in the untreated larvae, which showed completely normal sprouting of ISVs (Fig. 3C). These results validated the toxic effects of isoprocarb on vasculature development during the embryonic stage of zebrafish.

DISCUSSION

The use of chemically processed compounds, such as pesticides and insecticides to ensure unhindered growth of agricultural crops has increased worldwide, and an increasing number of studies have reported the risk of these compounds in the soil and groundwater and further to living organisms. Our study identified morphological abnormalities with up to 59 mg/L of isoprocarb exposure, including generation of edema and growth retardation. Edema generation is regarded as a common feature of developmental toxicity. Reduced blood flow and irregular cardiac output related to problematic blood vessels because of certain contaminants caused pericardial edema and circulation failure; moreover, the inhibitory effect in the enzymatic pathway induced edema (Teraoka et al., 2014; Nijoukubo et al., 2020). During vertebrate development, functional heart differentiates with the help of cardiac progenitors into the myocardium, ventricles, and endocardium to establish cardiac contractility for nutrient delivery, oxygen supply, and nerves/lymphatic system stability, whereas structural loss in the conduction system precedes development abnormalities, ultimately causing lethality (Staudt and Stainier, 2012). The symptoms of heart failure are related to vascular insufficiency during isoprocarb exposure. Using vessel-specific fluorescent

transgenic zebrafish (*fli1:eGFP*), the elaborate connection of vessels in the circulatory structure in the trunk and tail was shown to be essential to embryonic survival. Angiogenesis of ISV sprouts between the dorsal aorta and DLAV achieve the somite-neural tube-notochord interface for proper cell migration and anteroposterior vascular construction (Childs et al., 2002). The degeneration of ISVs and related vessels with the formation of intercellular gaps caused changes including inhibited blood flow and endothelial barrier disruption, which may be negative for tissue engineering in embryonic development (Watkins et al., 2012; Li et al., 2019). An example of the importance of blood supply is the transportation of developmental signals to the proper target site of organic differentiation; in other words, the removal of main vessels interrupts the inductive expression of essential factors in organ development (Lammert et al., 2001).

Embryos are sensitive to ubiquitous contaminants in the environment, and previous studies on apoptotic pathways and mechanisms have been conducted to elucidate potential toxicity. For example, cypermethrin exposure adjusted the mitochondrial pathway consisting of the key molecules p53, caspase9, and Bak, and generated apoptotic cell death that resulted in immune deficiency in growing embryos (Jin et al., 2011). Chemoattractants released from apoptotic cells could induce an unexpected inflammatory response and post-apoptotic cell death, such as necrosis involved in the failure to construct robust durability in the developmental stage (Qu et al., 2007). As toxicological endpoints, increased apoptosis rate and genetic alteration during maternal-fetal interaction also have an adverse effect on fetal development (Barzi et al., 2020). The present study showed scattered apoptotic cell death increased by isoprocarb exposure in the early stage of zebrafish embryo development, which could be extended to developmental toxicity with general damage.

Residues from industrial chemicals also exist for long periods in the ground and their cumulative effects along with those of insecticide, herbicide, or pesticide residues that are ingested through agricultural crops threaten the health of ecosystems through oncogenic risk, reproductive failure, and metabolic defects (Mesnage and Antoniou, 2018). In the case of aquatic pollutants, investigations of pesticidal residues have revealed the inhibition of enzymatic reactions, increase in mortality, and abnormal behavior as well as synergistic toxicity occurring with combination treatment (El-Nahhal, 2018). Isoprocarb residues have also been detected in agricultural products; thus, there should be further study on the residual toxicity in ecosystems and combinatorial effects with other ingredients using crop materials (Zhang et al., 2019). In the present study, we suggested the potential toxicity of isoprocarb based on alterations in zebrafish embryos that caused developmental retardation. Likewise, hepatotoxicity and hematologic toxicity by isoprocarb induced alterations in vital signs, such as decrease in body weight (Rahman et al., 1990). In addition to those providing evidences, mechanistic interpretation of isoprocarb focused on disrupting endocrine system should be discussed as other carbamates are powerful endocrine disruptors in environments including humans in further (Zhou and Fang, 2015).

CONCLUSION

The negative impact of isoprocarb on an organism in a non-target environment was demonstrated in this study. Toxicological assessments of isoprocarb showed edema generation, decreased body growth, and debilitation of the main vascular system. The observation of apoptosis in cells during the course of overall development supported the toxicity of isoprocarb. Overall, isoprocarb toxicity during embryogenesis highlights the damage that can be accumulated and the unpredictable contamination of ecosystems as potential environmental stressors in environment.

CONFLICTS OF INTEREST

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

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AUTHOR CONTRIBUTIONS

Conceptualization: G.S. and W.L.
Methodology and Investigation: H.P.
Data curation: G.S. and W.L.
Visualization: H.P.
Funding: G.S. and W.L.
Writing-original draft preparation: H.P.
Writing-review and editing: G.S. and W.L.
Supervision: G.S. and W.L.

AUTHOR'S POSITION AND ORCID NO.

H Park, Graduate Student,
https://orcid.org/0000-0001-9876-070X
G Song, Professor,
https://orcid.org/0000-0003-2817-5323
W Lim, Assistant Professor,
https://orcid.org/0000-0002-1328-0465

REFERENCES

Assis CRD, Bezerra RS, Carvalho LB Jr. 2011. Fish cholinesterases as biomarkers of organophosphorus and carbamate pesticides. In: Stoytcheva M (Ed.), Pesticides in the Modern World-Pests Control and Pesticides Exposure and Toxicity Assessment. InTech, London, pp. 253-278.

Barzi NV, Eftekhari Z, Doroud D, Eidi A. 2020. DNA methylation changes of apoptotic genes in organogenesis stage of mice embryos by maternal chlorpyrifos induction. Environ. Toxicol. 35:794-803.

Bhagat J, Zang L, Nishimura N, Shimada Y. 2020. Zebrafish: an emerging model to study microplastic and nanoplastic toxicity. Sci. Total Environ. 728:138707.

Cassar S, Adatto I, Freeman JL, Gamse JT, Iturria I, Lawrence C, Muriana A, Peterson RT, Van Cruchten S, Zon LI. 2020. Use of zebrafish in drug discovery toxicology. Chem. Res. Toxicol. 33:95-118.

Childs S, Chen JN, Garrity DM, Fishman MC. 2002. Patterning of angiogenesis in the zebrafish embryo. Development 129:973-982.

Costa LG, Giordano G, Guizzetti M, Vitalone A. 2008. Neurotoxicity of pesticides: a brief review. Front. Biosci. 13:1240-1249.

Dai YJ, Jia YF, Chen N, Bian WP, Li QK, Ma YB, Chen YL, Pei DS. 2014. Zebrafish as a model system to study toxicology. Environ. Toxicol. Chem. 33:11-17.

El-Nahhal Y. 2018. Toxicity of some aquatic pollutants to fish. Environ. Monit. Assess. 190:449.

Frazier LM. 2007. Reproductive disorders associated with pesticide exposure. J. Agromedicine 12:27-37.

Gallo D, Merendino A, Keizer J, Vittozzi L. 1995. Acute toxicity of two carbamates to the Guppy (*Poecilia reticulata*) and the Zebrafish (*Brachydanio rerio*). Sci. Total Environ. 171:131-136.

Jin Y, Zheng S, Fu Z. 2011. Embryonic exposure to cypermethrin induces apoptosis and immunotoxicity in zebrafish (Danio rerio). Fish Shellfish Immunol. 30:1049-1054.

Lammert E, Cleaver O, Melton D. 2001. Induction of pancreatic differentiation by signals from blood vessels. Science 294:564-567.

Leung MCK and Meyer JN. 2019. Mitochondria as a target of organophosphate and carbamate pesticides: revisiting common mechanisms of action with new approach methodologies. Reprod. Toxicol. 89:83-92.

Li YF, Canário AVM, Power DM, Campinho MA. 2019. Ioxynil and diethylstilbestrol disrupt vascular and heart development in zebrafish. Environ. Int. 124:511-520.

Mesnage R and Antoniou MN. 2018. Ignoring adjuvant toxicity falsifies the safety profile of commercial pesticides. Front. Public Health 5:361.

Nijoukubo D, Adachi H, Kitazawa T, Teraoka H. 2020. Blood vessels are primary targets for 2,3,7,8-tetrachlorodibenzo-p-dioxin in pre-cardiac edema formation in larval zebrafish. Chemosphere 254:126808.

Park S, Song G, Lim W. 2020. Anti-developmental effects of imazosulfuron on zebrafish embryos during development. J. Anim. Reprod. Biotechnol. 35:28-34.

Qu X, Zou Z, Sun Q, Luby-Phelps K, Cheng P, Hogan RN, Gilpin C, Levine B. 2007. Autophagy gene-dependent clearance of apoptotic cells during embryonic development. Cell 128:931-946.

Rahman MF, Siddiqui MK, Mahboob M, Mustafa M. 1990. Haematological and hepatotoxic effects of isoprocarb in chicken. J. Appl. Toxicol. 10:187-192.

Saito H, Iwami S, Shigeoka T. 1991. In vitro cytotoxicity of 45 pesticides to goldfish GF-Scale (GFS) cells. Chemosphere 23:525-537.

Staudt D and Stainier D. 2012. Uncovering the molecular and cellular mechanisms of heart development using the zebrafish. Annu. Rev. Genet. 46:397-418.

Teraoka H, Okuno Y, Nijoukubo D, Yamakoshi A, Peterson RE, Stegeman JJ, Kitazawa T, Hiraga T, Kubota A. 2014. Involvement of COX2-thromboxane pathway in TCDD-induced precardiac edema in developing zebrafish. Aquat. Toxicol. 154:19-26.

Wang C, Lu G, Cui J. 2012. Responses of AChE and GST activities to insecticide coexposure in Carassius auratus. Environ. Toxicol. 27:50-57.

Wang C, Lu G, Cui J, Wang P. 2009. Sublethal effects of pesticide mixtures on selected biomarkers of Carassius auratus. Environ. Toxicol. Pharmacol. 28:414-419.

Watkins SC, Maniar S, Mosher M, Roman BL, Tsang M, St Croix CM. 2012. High resolution imaging of vascular function in zebrafish. PLoS One 7:e44018.

Xu L, Granger C, Dong H, Mao Y, Duan S, Li J, Qiang Z. 2020.

Occurrences of 29 pesticides in the Huangpu River, China: highest ecological risk identified in Shanghai metropolitan area. Chemosphere 251:126411.

Zhang X, Liu L, Cui G, Song S, Kuang H, Xu C. 2019. Preparation of an anti-isoprocarb monoclonal antibody and its application in developing an immunochromatographic strip assay. Biomed. Chromatogr. 33:e4660.

Zhou Q and Fang Z. 2015. Graphene-modified TiO2 nanotube arrays as an adsorbent in micro-solid phase extraction for determination of carbamate pesticides in water samples. Anal. Chim. Acta 869:43-49.