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Embryotoxicity of Diafenthiuron to Zebrafish (*Danio rerio*) After Advanced Oxidation Treatment

Menglan Su [1], Rongkai Bao [1], Bo Gao [1], Xiaobin Liao [2], Peng Xiao [3,*] and Wenhua Li [1,*]

[1] Key Laboratory of Fujian Molecular Medicine, Medicine and Molecular Diagnosis of Fujian Universities, Key Laboratory of Xiamen Marine and Gene Drugs, Key Laboratory of Precision, School of Biomedical Sciences, Huaqiao University, Xiamen 361021, China; 21014071051@stu.hqu.edu.cn (M.S.); 2134133001@stu.hqu.edu.cn (R.B.); 21013071004@stu.hqu.edu.cn (B.G.)

[2] Institute of Municipal and Environmental Engineering, College of Civil Engineering, Huaqiao University, Xiamen 361021, China; liaoxb@hqu.edu.cn

[3] National and Local Joint Engineering Research Center of Ecological Treatment Technology for Urban Water Pollution, Zhejiang Provincial Key Lab for Water Environment and Marine Biological Resources Protection, College of Life and Environmental Science, Wenzhou University, Wenzhou 325035, China

* Correspondence: pxiao@wzu.edu.cn (P.X.); wenhuali@hqu.edu.cn (W.L.)

Abstract: Diafenthiuron is a novel derivative of thiourea and is highly toxic to non-target organisms, necessitating its efficient removal from wastewater before discharge. This study compared diafenthiuron removal efficiencies at a target concentration of 1 μM using three methods: a 4 mg/L ozone (O_3) treatment; an ultraviolet (UV) light treatment, applying UV_{254} radiation with a fluence of 60 mJ/cm^2 for 10 min; and a combined O_3/UV treatment utilizing ozone and ultraviolet light. An acute toxicity assessment was conducted using a modeled zebrafish embryo (*Danio rerio*). The diafenthiuron removal efficiencies were 49.59%, 54.51%, and 68.90% for the UV light, O_3, and O_3/UV treatments, respectively. The treatments showed additional benefits of exerting no negative impacts on the survival rate, heart rate, or body length of the zebrafish larvae posttreatment. The survival and heart rates at 120 hpf, as well as the body length at 96 and 120 hpf, showed significant differences between the advanced oxidation and 1 μM diafenthiuron treatment groups. However, these parameters remained consistent with those of the control group. The three treatments alleviated the spatiotemporal downregulation of the liver-specific marker *fabp10a* caused by diafenthiuron exposure. The UV light and O_3/UV treatments were efficient at degrading diafenthiuron, causing decreased reactive oxygen species levels and increased *pomc* and *prl* expression levels. The O_3-treated diafenthiuron and 1 μM diafenthiuron treatments increased the reactive oxygen species levels and decreased the *pomc* and *prl* expression levels. The combined O_3/UV treatment showed the highest removal efficiency and the least toxicity, making it the most effective method for diafenthiuron degradation. This study provides valuable insights into the treatment of diafenthiuron-laden wastewater.

Keywords: diafenthiuron; toxicity analysis; zebrafish; advanced oxidation processes; wastewater treatment

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1. Introduction

Pesticides are used to reduce economic losses caused by pests, weeds, and crop diseases and to improve the quality and yield of crops during agricultural production; thus, they are crucial in agricultural production [1,2]. However, owing to their chemical stability in the environment, bioaccumulation capacity in organisms, and toxicity to non-target organisms, pesticides persist in the environment, contaminate human food, and are usually found in aquatic environments, including surface waters [1,3]. In 2021, global agricultural pesticide usage was 3.54 million tons, with insecticides comprising 0.758 million tons [4]. In pesticide use, only a minimal portion of the applied pesticides effectively reaches and controls the pests on the target plants [5]. Most of the pesticide proportions are dispersed

into the environment through pathways, including spray drift, surface runoff, adsorption onto soil particles, leaching into groundwater, and volatilization into the atmosphere [6,7]. These dispersed proportions can adversely affect non-target organisms, communities, entire ecosystems, and humans [8]. Therefore, treating pesticide-containing wastewater before discharge is essential to reduce the negative impacts of pesticides. Among these pesticides, diafenthiuron is significant owing to its dual characteristics, namely high efficacy against target pests and significant toxicity to aquatic organisms.

Diafenthiuron is a thiourea insecticide for controlling phytophagous mites, whiteflies, aphids, and diamondback moths [9–11] In addition to acting on the targets, it is highly toxic to aquatic organisms. The lethal concentration of 50% of diafenthiuron (96 h) for rainbow trout (*Oncorhynchus mykiss*) is 0.0007 mg/L (0.00182 μM) [12]. At 0.1 g/L, diafenthiuron causes mortality within 6 h in common carp (*Cyprinus carpio*) weighing approximately 10 g [13]. Similarly, diafenthiuron adversely affects the hematological, serum, and elemental concentrations in rohu (*Labeo rohita*) [14]. Our recent study showed that diafenthiuron exposure reduces the body length of larval zebrafish, inhibits the development of liver cells, and alters the expression of representative pituitary marker genes [15]. Moreover, according to the groundwater ubiquity score, diafenthiuron has a leaching potential index of 0.19, indicating its low leaching potential in soil [12] and, thus, a reduced risk of groundwater contamination. However, its common application threatens surface water bodies through runoff or untreated wastewater, which negatively affects aquatic organisms and other non-target species. Therefore, treating diafenthiuron-containing wastewater before discharge is essential.

Advanced oxidation processes (AOPs) are effective chemical methods for removing water-borne organic pollutants. These processes are based on chemical reactions with hydroxyl radicals and include photocatalytic, sonochemical, catalytic wet, electrochemical, O_3, Fenton, and Fenton-like oxidation [16–18]. AOPs effectively degrade various organic pollutants, including heavy metals, pesticides, and persistent organic pollutants [16,19–22]. To address the environmental concerns posed by pesticide contamination, this study aimed to explore and optimize AOPs for the degradation of diafenthiuron by comparing the efficiency of ozone (O_3), ultraviolet (UV) light, and their combined application (O_3/UV), evaluate the toxicity of their by-products on zebrafish embryos and larvae, and identify the most effective approach for achieving safe and efficient pollutant removal.

2. Materials and Methods

2.1. Zebrafish Husbandry and Exposure

The embryos used in this experiment were produced and collected from adult zebrafish (*D. rerio*, AB strain) and raised in a circulating culture system (Haisheng, Shanghai, China). The system contained dechlorinated and filtered water, with a conductivity of approximately 500 μS/cm, a pH maintained between 6.5 and 7.5, and dissolved oxygen concentrations (range, 6–7 mg/L), ensuring sufficient aeration through continuous circulation and mechanical filtration. The temperature was controlled at 28 ± 0.5 °C, and a 14-h light and 10-h dark cycle were maintained to support the zebrafish's natural circadian rhythm [23]. The adult zebrafish were fed with fresh-hatched *Artemia nauplii* thrice daily. Four male and one female zebrafish were housed in a breeding tank and separated with a middle divider. The middle divider was removed at the beginning of the next light cycle to allow the zebrafish to exhibit courtship behavior and lay eggs. Normal developing embryos were examined using a stereomicroscope (Leica, M205FA, Wetzlar, Germany) at 3 h post-fertilization (hpf) and were selected for subsequent toxicology analyses. Each exposure group was replicated thrice, with 70 embryos being placed in each Petri dish. The Animal Care and Welfare Committee of Huaqiao University approved all the experimental procedures in this study (Approval ID: A2021046).

The diafenthiuron (CAS No. 80060-09-9, purity ≥ 98.0%) used in this study was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO, Amresco, Solon, OH, USA) was used as a solvent. A concentrated 10 g/L (26,002 μM) stock solution

of diafenthiuron was prepared by dissolving it in DMSO. The control group was exposed only to 0.00381% DMSO without diafenthiuron, a concentration demonstrated to be safe for zebrafish embryos in previous studies [24,25].

2.2. Oxidation Treatment

A 500 mL nominal exposure concentration of diafenthiuron (1 μM) was subjected to three treatments, namely O_3, UV light, and a combined O_3 and UV light treatment (O_3/UV). The O_3 treatment proceeded as follows: a 10 g/h O_3 generator (Guolin, Qingdao, China) was used to introduce O_3 into a glass reaction vessel containing 500 mL of ultrapure water. Subsequently, 40 mL of the aqueous ozone solution was added to a 460 mL solution of diafenthiuron (1 μM); the O_3 concentration in the reaction system was carefully maintained at 4 mg/L, ensuring a uniform O_3 distribution and resulting in a single O_3-treated diafenthiuron-exposed solution. For the UV light treatment, a 500 mL solution of diafenthiuron (1 μM) was exposed to UV irradiation at an intensity of 60 mJ/cm^2 for 10 min. The UV lamp, operating at a wavelength of 254 nm, was directly immersed in the solution. For the O_3/UV treatment, the diafenthiuron (1 μM) solution was first treated with O_3, followed by irradiation with UV light. The parameter settings for each step were consistent with those used in the individual O_3 and UV treatments. All the treated exposure solutions were stored at 4 °C until use.

2.3. Chemical Analysis

A high-performance liquid chromatograph (Agilent 1260, Waldbronn, Germany) was used to measure the diafenthiuron concentrations in the exposure solutions. A spectrophotometer (NanoDrop 2000, Thermo Scientific, Wilmington, DE, USA) and a total organic carbon (TOC) analyzer (Shimadzu, Kyoto, Japan) were used to measure the UV absorption spectra and TOC content of the liquid before and after treatment, respectively. All the measurements were repeated thrice. The degradation and TOC removal efficiency were calculated as follows: UV degradation efficiency (%) = $\frac{C_0-C_t}{C_0} \times 100\%$ and TOC removal efficiency (%) = $\frac{T_0-T_t}{T_0} \times 100\%$, where C_0 and C_t represent the initial and final concentrations, respectively, which were calculated using the UV-visible absorption values of diafenthiuron. T_0 and T_t represent the initial and final values of TOC, respectively [26].

2.4. Morphological Analysis

The larvae from 96 and 120 hpf were anesthetized using 0.03% MS-222 (Aladdin, Shanghai, China). In each exposure group, 7–12 larvae (n = 3 replicates) were observed, and their images were captured under a stereomicroscope (Leica, M205FA, Wetzlar, Germany). The body lengths of the larvae were determined using the Leica Application Suite X (LAS X) software (3.4.1.17822) of the stereomicroscope.

2.5. Whole-Mount In Situ Hybridization (WISH)

The primers (*pomc* F: GTTTCTGACTTCTTTCTGTGCAAAA, *pomc* R: TAATACGACTCACTATAGGGAGATAAGTGCTATTGTTAACATTGCCCA; *prl* F: AAACCTGTTCTAGTAATGGCTCAAG, *prl* R: TAATACGACTCACTATAGGGAGAAGGGTATATTTTGCCATGTGATTGT; *fabp10a* F: TTTACGCTCAGGAGAACTACGA, *fabp10a* R: TAATACGACTCACTATAGGGAGAAACGCTTCAGATCTTCTTGC) were designed for synthesizing anti-sense RNA probes targeting *pomc*, *prl*, and *fabp10a*. WISH was performed using the standard procedures with minor modifications [27]. Briefly, the larvae at 72 and 96 hpf were fixed in a 4% paraformaldehyde solution overnight at 4 °C. Subsequently, methanol was added to remove moisture, and the larvae were stored at –20 °C. The larvae (at least 15 larvae per group, n = 3 replicates) were hybridized overnight with the anti-sense RNA probes at 60 °C. Next, the larvae were washed before being incubated with blocking buffer and, subsequently, with anti-DIG antibody solution (Roche, Mannheim, Germany) overnight at 4 °C. After washing, the larvae were stained using a BCIP/NBT staining solution (Bey-

otime, Shanghai, China). Finally, the stained larvae were observed and recorded under a stereomicroscope (Leica, M205FA, Wetzlar, Germany).

2.6. Reactive Oxygen Species (ROS) Detection

Fifteen larval zebrafish (n = 3 replicates) at 72 hpf were collected, rinsed with phosphate-buffered saline solution thrice, and incubated with 20 mg/L 2′,7′-dihydro dichloro fluorescein diacetate (Nanjing Jiancheng Biotechnology Institute, China) for 30 min at 28 °C in the dark. The larvae were washed thrice with filtered zebrafish circulatory water and anesthetized with 0.03% MS-222. Lateral images of whole larvae were captured using a stereomicroscope (Leica, M205FA, Wetzlar, Germany), and the fluorescence intensity was measured using Image-Pro Plus software (version 6.0; Media Cybernetics, Rockville, MD, USA).

2.7. Data Analyses

Statistical analyses were conducted using PSS software (version 26.0; Chicago, IL, USA) for all the datasets. The differences among the control and each diafenthiuron treatment group were examined using a one-way analysis of variance (ANOVA) (body length at 96 hpf and the fluorescence intensity of ROS) or the nonparametric Kruskal–Wallis test (survival, hatching, heart rate, and body length at 120 hpf), depending on the normality of the data distribution. The data are expressed as the mean ± standard deviation. Statistical significance was set at $p < 0.05$.

3. Results and Discussion

3.1. Degradation of Diafenthiuron

Diafenthiuron was degraded using three methods, namely O_3, UV light, and O_3/UV treatments. The diafenthiuron degradation efficiencies are listed in Table 1. The TOC removal efficiency of diafenthiuron increased in the following order: UV < O_3 < O_3/UV. The diafenthiuron removal efficiencies for the UV light, O_3, and O_3/UV-combined treatments were 49.59%, 54.51%, and 68.90%, respectively. The TOC removal percentages relative to diafenthiuron removal for the UV light, O_3, and O_3/UV-combined treatments were 6.53%, 13.36%, and 18.55%, respectively. Additionally, the UV_{254} removal efficiency of diafenthiuron increased in the order O_3 (30.74%) < UV (33.84%) < O_3/UV (58.44%). The three treatments (O_3, UV light, and O_3/UV) increased the precursor, TOC, and UV_{254} removal rates from the aqueous solution, suggesting the notable effectiveness of these methods in removing diafenthiuron. However, the O_3/UV-combined treatment demonstrated significantly higher removal efficiency than the other two treatments, achieving precursor, TOC, and UV_{254} removal rates of 68.90%, 18.55%, and 58.44%, respectively. This might be attributed to the synergistic effects of O_3 and UV in producing ROS [28,29], which are more effective at degrading diafenthiuron's molecular structure than single treatments. This finding aligns with that of a previous study, which demonstrated that the O_3/UV-combined treatment enhances the degradation of organic compounds [30].

Table 1. Diafenthiuron, total organic carbon (TOC), and UV254 removal using ozone (O_3), ultraviolet (UV), and ozone with ultraviolet (O_3/UV) combined methods.

Oxidation	Diafenthiuron Removal (%)	TOC Removal (%)	UV254 Removal (%)
Ozone (O_3)	54.51 ± 4.29	13.36 ± 1.58	30.74 ± 1.87
Ultraviolet (UV)	49.59 ± 0.70	6.53 ± 1.67	33.84 ± 2.31
O_3/UV	68.90 ± 0.95	18.55 ± 1.13	58.44 ± 1.12

3.2. Effects of Advanced-Oxidation-Treated Diafenthiuron on Zebrafish

Diafenthiuron induced significant developmental toxicity in zebrafish embryos and larvae. Exposure to 1 μM diafenthiuron at 120 hpf causes decreased zebrafish survival,

a decreased heart rate, and a shortened body length [15]. To assess the detoxification effects of the three advanced oxidation methods used in this study, we used the zebrafish embryo–larval model to evaluate the toxicological endpoints. After exposure, the body length, as well as the survival, hatching, and heart rates, of the zebrafish embryos/larvae were observed (Figure 1). Between 48 and 96 hpf, no significant mortality was observed among the embryos/larvae of the control, 1 μM diafenthiuron exposure, O_3-treated diafenthiuron, UV-treated diafenthiuron, and O_3/UV-treated diafenthiuron groups. At 120 hpf, a significant decrease in the survival rate was observed in the 1 μM diafenthiuron exposure group (Figure 1A, Kruskal–Wallis $X^2 = 8.000$, $p = 0.005$). This decrease was significantly reversed with the O_3, UV light, and O_3/UV treatments, with each one significantly restoring (increasing) the survival rates to nearly that of the control (Figure 1A, 1 μM vs. O_3: Kruskal–Wallis $X^2 = 6.000$, $p = 0.035$; 1 μM vs. UV: Kruskal–Wallis $X^2 = 8.000$, $p = 0.005$; 1 μM vs. O_3/UV: Kruskal–Wallis $X^2 = 8.000$, $p = 0.005$). The hatching rates exhibited no significant differences after diafenthiuron exposure and diafenthiuron oxidation treatments (Figure 1B, $p = 0.089$). Between 48 and 96 hpf, the heart rates showed no significant differences among the control, the 1 μM-diafenthiuron exposure, O_3-treated diafenthiuron, UV-treated diafenthiuron, and the O_3/UV-treated diafenthiuron groups. However, the heart rate significantly decreased in the 1 μM diafenthiuron exposure group at 120 hpf (Con vs. 1 μM: Kruskal–Wallis $X^2 = 26.350$, $p = 0.000$). Notably, compared with that of the 1 μM diafenthiuron exposure group, the heart rates significantly increased in the three diafenthiuron oxidation treatment groups (O_3, UV light, and O_3/UV) (Figure 1C, 1 μM vs. O_3: Kruskal–Wallis $X^2 = 22.800$, $p = 0.001$; 1 μM vs. UV: Kruskal–Wallis $X^2 = 22.750$, $p = 0.001$; 1 μM vs. O_3/UV: Kruskal–Wallis $X^2 = 22.100$, $p = 0.001$).

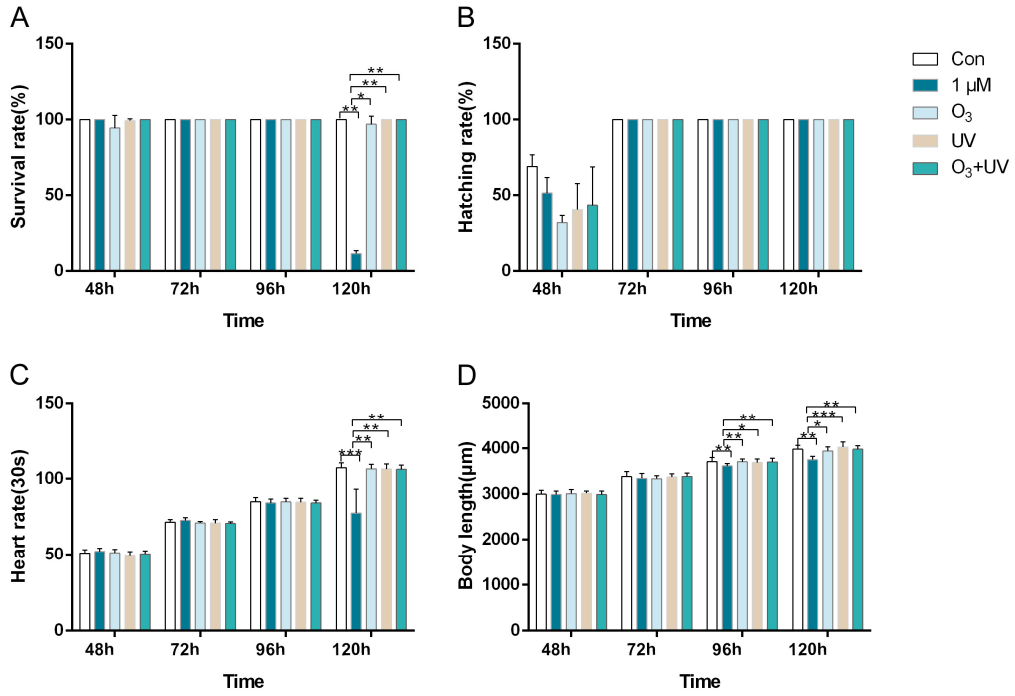

Figure 1. Effects of diafenthiuron on embryogenesis before and after oxidative treatment. (**A**) Survival rate ($n = 3$ replicates). (**B**) Hatching rate ($n = 3$ replicates). (**C**) Heart rate ($n = 7$–10 larvae). (**D**) Body length ($n = 7$–12 larvae). Con, larvae treated with dimethyl sulfoxide. The error bars denote the mean ± standard deviation (SD). * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

As aforementioned, a significant phenotype in zebrafish larvae after diafenthiuron exposure is characterized by a reduced body length [15]. Thus, subsequently, we assessed the effect of oxidative treatment on diafenthiuron-induced changes in body length. The larvae within the 1 μM diafenthiuron exposure group displayed a decreased body length at 96 and 120 hpf (Figures 1D and 2). However, subsequent oxidation treatments (i.e.,

O_3, UV light, and O_3/UV) significantly increased the body length. The average body lengths of the zebrafish larvae at 96 hpf in the control, 1 μM diafenthiuron exposure, O_3-treated diafenthiuron, UV-treated diafenthiuron, and O_3/UV-treated diafenthiuron groups were 3711.53 ± 86.51, 3614.32 ± 48.46, 3705.17 ± 61.39, 3690.02 ± 72.84, and 3701.96 ± 77.23 μm, respectively (Figure 2A–E; 1 μM vs. O_3: ANOVA $p = 0.003$; 1 μM vs. UV: ANOVA $p = 0.011$; 1 μM vs. O_3/UV: ANOVA $p = 0.004$). Correspondingly, at 120 hpf, the body lengths in the control, 1 μM diafenthiuron exposure, O_3-treated diafenthiuron, UV-treated diafenthiuron, and O_3/UV-treated diafenthiuron groups were 3982.98 ± 84.84, 3753.66 ± 68.98, 3941.74 ± 91.14, 4027.95 ± 117.08, and 3979.50 ± 79.57 μm, respectively (Figure 2F–J; 1 μM vs. O_3: Kruskal–Wallis $X^2 = 18.667$, $p = 0.014$; 1 μM vs. UV: Kruskal–Wallis $X^2 = 33.000$, $p = 0.000$; 1 μM vs. O_3/UV: Kruskal–Wallis $X^2 = 22.917$, $p = 0.003$).

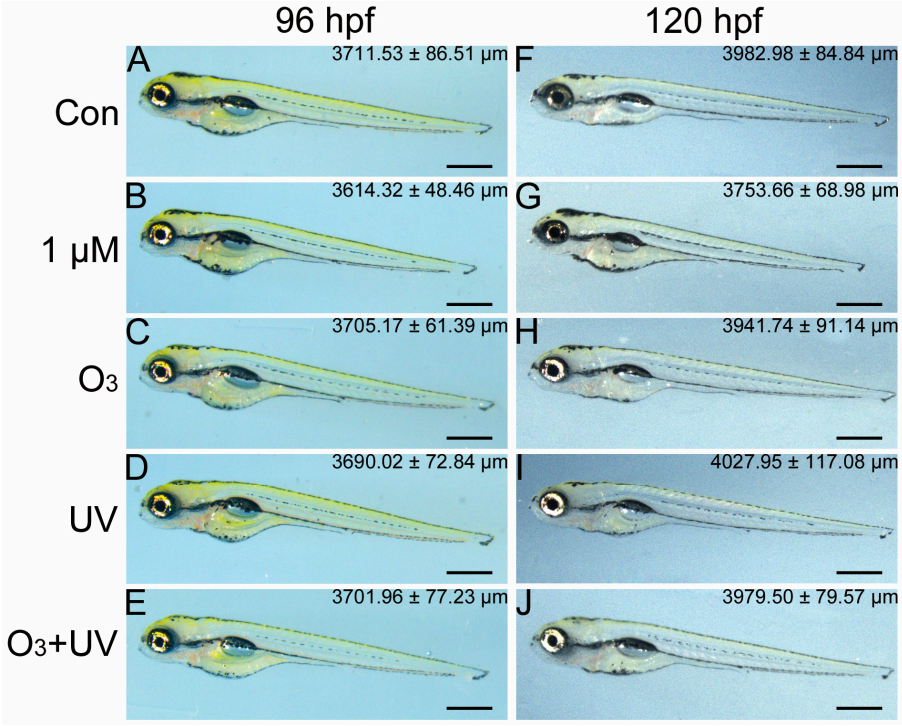

Figure 2. Effects of diafenthiuron on larval body length before and after oxidative treatment. (**A**–**E**) Lateral views of larvae at 96 hpf. (**F**–**J**) Lateral views of larvae at 120 hpf. Con, dimethyl sulfoxide-treated larvae. Scale bar = 500 μm.

The oxidative degradation of organic pollutants causes structural decomposition [31]. Based on the assessment of the survival rate, heart rate, and body length, the three advanced oxidation methods (O_3, UV light, and O_3/UV) demonstrated comparable detoxification effects on larval zebrafish (Figures 1 and 2). Although the removal of most insecticides is challenging owing to their structural complexity and stability [32], the data from Table 1 indicate that the parent compounds of diafenthiuron were partially removed using the three advanced oxidation methods, with the O_3/UV combination achieving the highest degradation rates. The partial removal of diafenthiuron effectively mitigated its adverse effects on the survival rate, heart rate, and phenotype in larval zebrafish. Despite the lower diafenthiuron removal rates observed for the O_3 and UV treatments alone than that of the combined O_3/UV treatment, these individual methods significantly mitigated the adverse effects of the compound on zebrafish development.

3.3. Effects of Diafenthiuron on ROS Production Before and After Oxidative Treatment

A study indicated that diafenthiuron exposure induces oxidative stress in zebrafish larvae [15]. Consequently, we investigated the possible impact on ROS production after diafenthiuron oxidation. After diafenthiuron exposure, ROS production in zebrafish lar-

vae was assessed at 72 hpf. Compared with that of the control, the diafenthiuron-treated group (1 μM) displayed pronounced fluorescence, indicating a significant increase in the intracellular ROS levels (Con vs. 1 μM: ANOVA $p = 0.000$) (Figure 3). The diafenthiuron O_3 treatment group also exhibited pronounced fluorescence (Con vs. O_3: ANOVA, $p = 0.000$). However, the fluorescence signal intensities in the diafenthiuron UV light and O_3/UV treatment groups were similar to those in the control group. This similarity suggests that both treatment groups exhibited effective detoxification of diafenthiuron on ROS production.

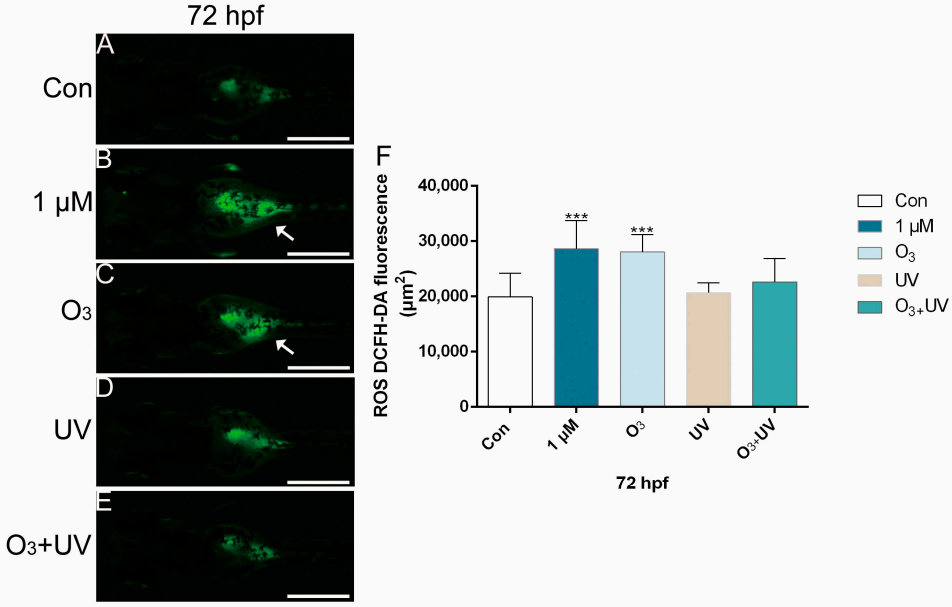

Figure 3. Effects of diafenthiuron on reactive oxygen species (ROS) production before and after oxidative treatment at 72 hpf. (**A**) Control larvae, (**B**) diafenthiuron treated larvae, (**C**) O_3-treated diafenthiuron-treated larvae, (**D**) UV-treated diafenthiuron-treated larvae, (**E**) O_3/UV-treated diafenthiuron-treated larvae, (**F**) Fluorescence intensity of the larvae. Con, larvae treated with dimethyl sulfoxide. The white arrows indicate the locations of intense fluorescence. Scale bar = 500 μm. The error bars denote the mean ± standard deviation (SD) ($n = 7$ larvae). *** $p < 0.001$.

From a phenotypic perspective (Figures 1 and 2), the O_3 treatment exerted no effects on the development of zebrafish embryos/larvae; however, it caused higher ROS levels than those in the control group (Figure 3C,F). However, when assessing the three removal results, the O_3 treatment did not exert the least effect. This finding aligns with those of Weng et al. [33] and Cambero et al. [34], who reported that TOC reduction during ozonation might not entirely reflect toxicity.

3.4. Effects of Diafenthiuron on Pituitary Marker Genes and Liver Development Before and After Oxidative Treatment

Our recent study has demonstrated that diafenthiuron exposure reduces the expression of the pituitary (*pomc* and *prl*) and liver (*fabp10a*) marker genes in larval zebrafish [15]. Pro-opiomelanocortin (*POMC*) was identified as a key gene in the hypothalamic–pituitary–adrenal axis of vertebrates [35]. In the pituitary gland, the *POMC* cell line includes cells that produce corticotropin (ACTH), melanocyte-stimulating hormone, and various endorphins [36,37]. Prolactin (PRL) is a hormone secreted by the anterior pituitary gland and performs various functions [38,39]. The fatty acid-binding protein Fabp10a is highly expressed in zebrafish liver, indicating that this protein may play a significant role in the liver by participating in fatty acid transport, metabolism, detoxification, and the maintenance of liver homeostasis [40]. To further explore the oxidative detoxification effects of diafenthiuron, the expression of these genes was assessed in treated larval zebrafish (Figure 4). The expression levels of the pituitary marker genes, *pomc* and *prl*, were significantly downregu-

lated in the diafenthiuron-treated and the diafenthiuron O_3 treatment groups compared with those in the control group (Figure 4A–C,F–H). Conversely, the expression levels of *pomc* and *prl* in the diafenthiuron UV light and O_3/UV treatment groups were similar to those in the control group (Figure 4D,E,I,J). The expression levels of liver marker genes, such as *fabp10a*, displayed pronounced downregulation in the diafenthiuron-treated group compared with those in the control group (Figure 4K,L). However, the expression levels of *fabp10a* in the diafenthiuron UV light, O_3, and O_3/UV treatment groups were comparable to that in the control group (Figure 4M–O). Although ozonation effectively oxidizes and decomposes organic compounds, it may not produce a hydroxyl radical concentration sufficient for the total degradation of all organic substances [41]. The O_3 treatment group exhibited increased and intensified fluorescence, along with decreased expression levels of *pomc* and *prl* (Figure 4), indicating that the O_3 treatment of diafenthiuron may not produce a detoxification effect as complete as those exerted by the other two treatments (UV light and O_3/UV). Similarly, we hypothesized that some intermediate products might persist in the solution during the O_3 reaction, and these intermediate oxidation products might exhibit relatively weaker toxicity than the parent compound does. Nevertheless, further studies are required to identify the structures of these intermediates.

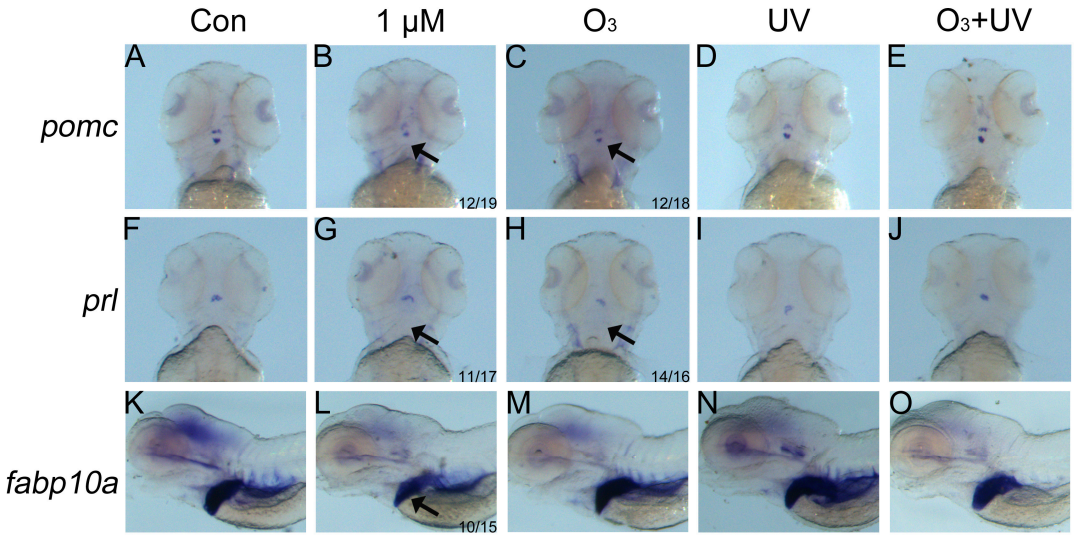

Figure 4. Effects of diafenthiuron on pituitary and liver marker genes before and after oxidative treatment. (**A**–**E**): The WISH data for gene *pomc* at 72 hpf. (**F**–**J**): The WISH data for gene *prl* at 72 hpf. (**K**–**O**): The WISH data for gene *fabp10a* at 96 hpf. Con, larvae treated with dimethyl sulfoxide. The black arrows highlight the sites of downregulated gene expression. (**A**–**J**): Dorsal views. (**K**–**O**): Lateral views.

Pesticide wastewater treatment aims to effectively remove or neutralize pesticide residues and contaminants from wastewater generated during pesticide manufacturing, formulation, application, and related activities [42,43]. Although the current method cannot completely degrade diafenthiuron because of its stable structure (Table 1), oxidation treatments (O_3, UV light, and O_3/UV) have shown notable effective detoxification effects. Considering the phenotype, ROS production, and marker gene expression in larval zebrafish, the most effective treatment was O_3/UV. Existing studies have demonstrated that in the O_3/UV treatment, the degradation of organic compounds is coupled with a concurrent reduction in their biological toxicity, resulting in highly efficient organic compound removal [44–46]. Owing to the generation of radicals with the highest oxidation potential, the O_3 and UV light treatments exhibited enhanced effectiveness in oxidizing organic and inorganic compounds, ensuring more efficient oxidation of the target substances. The formation and action of hydroxyl radicals further contribute to this heightened efficacy [45].

Therefore, the O_3/UV treatment is the optimal method for detoxifying diafenthiuron-containing wastewater.

The O_3/UV treatment has been demonstrated to be the most effective method for detoxifying diafenthiuron-containing wastewater under laboratory conditions; however, practical considerations should be addressed. For example, the relatively high energy demands of the O_3/UV process [47] may impact its scalability and cost-effectiveness for large-scale applications. Therefore, although the O_3/UV treatment shows great promise, further evaluations are required to assess its feasibility in real-world settings, including considerations of operational costs, infrastructure requirements, and the management of potential by-products.

4. Conclusions

These results suggest that the O_3, UV light, and O_3/UV treatments of diafenthiuron influence the toxicological endpoints in zebrafish. Among these treatments, the O_3/UV approach was the most effective, achieving superior diafenthiuron removal efficiency from aqueous solutions without harming zebrafish embryos/larvae, including effects on the phenotype, ROS generation, and marker gene expression. In contrast, the O_3 treatment group showed increased ROS levels and decreased expression levels of *pomc* and *prl*, suggesting that this method is not suitable for diafenthiuron detoxification. These findings establish the O_3/UV-combined method as an environmentally friendly and highly effective approach for diafenthiuron removal, with promising applications in advanced water treatment technologies.

Author Contributions: Data curation: M.S., R.B. and B.G.; Formal analysis: M.S. and R.B.; Investigation: M.S.; Methodology: M.S. and X.L.; Visualization: R.B., B.G. and X.L.; Writing—original draft: M.S.; Writing—review and editing: P.X. and W.L.; Supervision: P.X. and W.L. All authors have read and agreed to the published version of the manuscript.

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