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RESEARCH PAPER

# Acute Toxicity Evaluation of the Disinfectant Containing Percarbonate and Tetraacetylethylenediamine by Measuring Behavioral Responses of Small Fish Using Image Analysis

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**Abstract** Disinfectants containing percarbonate and tetraacetylethylenediamine (TAED) has been developed as an effective and relatively safe disinfectant to destroy viruses and bacteria in animals and humans, however it is known that most disinfectants can cause danger to living organisms including humans. In the current study, acute toxicity of the disinfectant composed of percarbonate and TAED was assessed by measuring behavioral responses as well as lethal concentrations of aquatic organisms such as medaka and zebrafish when they were exposed to it. First, the breeding water properties were determined by measuring dissolved oxygen (DO) and pH changes over time up to 96 h in acute toxicity tests using the medaka, and the lethal concentration 50% (LC50, 88.39 ppm) was calculated using the lethality rate of the fish. This experiment was conducted in compliance with traditional OECD guidelines. Second, the assessment of behavioral responses (locomotive activity and swimming speed) with the zebrafish were assessed by the image analysis to capture the images per second for three hours, and the collected data were processed using image analysis to calculate the locomotive activity and swimming speed. Finally, the LC50 (135.76 ppm) of the disinfectant to the fish was also measured after three hours. Overall, the data revealed that LC50 of the disinfectant may be affected by the pH of the water exposed to the disinfectant, not by the DO in the water. In addition, the results from the image analysis indicated that the behavioral responses of the fish can further assess the acute toxicity of the disinfectant at concentrations below the LC50 and there was a relationship ($R^2 = 0.85$) between the behavioral responses and the survival rate of the fish.

**Keyword:** disinfectant, percarbonate, acute toxicity, image analysis, behavioral response

## 1. Introduction

Disinfectants are generally composed of various ingredients such as halogens (*e.g.*, bromine, hypochlorite, and iodophors), quaternary ammonium compounds ("quats"), aldehydes (*e.g.*, formaldehyde, glutaraldehyde, and *o*-phthalaldehyde), peroxy acids or peracids [1,2]. They have been used for sterilization or removal of viruses as a defense in veterinary, and food processing against the spread of diseases such as Covid-19, avian influenza, African swine fever and foot-and-mouth disease, and in operating rooms for sterilization work such as surgical suits and surgical instruments [3]. The global market for disinfectants was valued at $3.4 billion in 2019 and is expected to grow at an annual growth rate of 6% by 2027 [4]. Many chemical compounds including disinfectants, are sprayed into objects or air, and the remaining substances can eventually flow into rivers or oceans by rain or snow, causing toxicity to aquatic organisms [5]. Identification of environmental toxicity indicators of many chemical compounds including disinfectants, is essential because aquatic pollution has a negative effect on water, an essential component of all living organisms including humans [6].

Among various disinfectants, peroxy acids or peracids are organic compounds whose chemical structure includes the -OOH moiety and they are very strong oxidizers. Their principal biocidal mode of action is the oxidation of thiol

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moieties in the cysteine and methionine residues of both enzymatic and structural proteins. The most extensively applied organic peroxy acid is peroxyacetic acid, also known as peracetic acid (PAA). One method to synthesize PAA is to react between tetraacetylethylenediamine (TAED) and a peroxy compound such as percarbonate, whose preferred compound is sodium percarbonate (SPC), also known as washing soda, which has been extensively used as a laundry detergent component, particularly in Europe, often formulated with TAED [7]. The SPC and TAED produce PAA and they readily biodegrade into sodium carbonate, ammonia, carbon dioxide, acetic acid, oxygen, and water. The peroxide can be generated from the percarbonate as follows;

$$2Na_2CO_3 \bullet 3H_2O_2 \rightarrow 4Na + 2CO_3^{2-} + 3H_2O_2$$

However, in addition to killing the virus, most disinfectants can cause danger to living organisms including humans and animals [8,9]. In fact, studies have shown that disinfectants have increased human cancer incidence and caused respiratory symptoms such as irritation of the eyes and skin, coughing, and asthma [10,11]. Therefore, the various harmful effects of disinfectants should be properly assessed. In general, SPC and TAED are relatively nontoxic, easily biodegradable in water and considered as 'eco-friendly' compounds, thus their environmental toxicological effects of this formulation may be minimal [12,13]. However, it is noteworthy that the detailed acute toxicity evaluation of the formulation may be needed for its more widely acceptance as a real eco-friendly disinfectant, which is by nature potentially harmful or even toxic to animals or humans as mentioned earlier.

While a recent study revealed wide spectrum of biocidal and virucidal efficacy of the formulation containing SPC and TAED, the acute toxicity evaluation data was not shown in the paper [7]. In general, the acute toxicity tests using rodents or rabbits to identify toxic chemical compounds are costly and time consuming. Therefore, an efficient acute toxicity evaluation method needs to be carried out at a time when up to 1,000 new chemical compounds are introduced into the market each year [14]. In this study, small fish were chosen as a model organism due to time and cost saving as compared to vertebrates [15]. The two selected fish species, zebrafish *(Danio rerio*) and medaka (*Oryzias latipes*), were selected for the experiments. The fish are standard test species widely used in biomedical experiments that follow the standardized protocols such as OECD guidelines, and the selected small organisms that can be bred in the laboratory [16,17]. In addition, a study revealed that there was a strong correlation between the lethal concentration 50% (LC50) value of zebrafish and the LD50 value of rodents [14]. They have been widely used as a test organism for various biological studies due to their short life span [18,19].

Extensive toxicological studies have focused on acute lethal concentration (*e.g.*, LC50) and survival rate as indicators to assess the toxicity of chemical compounds to environmental effects [20]. However, as the scope of the research conducted on small animals gradually widened, it became difficult to analyze the effects of many chemical substances as a passive method of collectively identifying dead objects. Therefore, small animal imaging technologies have been devised, which are used to capture images and process data at intervals of time through a specific device [21-24]. By using this method, we can identify the quantitative differences in movement of animals over time at concentrations where animals do not die. Other studies have also shown that animal imaging techniques are used to measure the body length, swimming speed, and life span of exposure to different nanomaterials using *Caenorhabditis elegans* [25]. Moreover, high-throughput image analyses using small animals such as zebrafish have been utilized for drug discovery screening [26-28]. As such, research using small animal imaging techniques continues to help to analyze the toxicological effects of chemical compounds on animals more efficiently [29]. The purpose of this study is to evaluate the acute toxicity of the disinfectant formulation containing SPC and TAED in a wide range of concentrations (0.1-10,000 ppm) via a newly developed quantitative image analysis as well as lethal concentrations by using aquatic organisms. The lethal concentrations of the disinfectant were determined by using the medaka according to OECD guideline and the behavioral responses (locomotor activity [pixel$^2$] and swimming speed [mm/s]) of the zebrafish exposed to the disinfectant were measured by newly developed image analysis as well as lethal concentrations after three hours of exposure.

## 2. Materials and Methods

### 2.1. Materials

The experimental disinfectant (sample 1 or S1) used in acute toxicity experiments, consist of SPC and TAED at the weight ratio of 9 to 1. The water used for the experiment was stored at room temperature using the test water supplied by Hoseo University Toxicological Research Center. This test water itself was used as a control group in acute toxicity tests. For behavioral test for the zebrafish, the device used in the experiment used Logitech's brio 4k as a camera for real-time image capture, and PhenoCapture (www.phenocapture.com) was used as a program to collect images. The samples used in the experiment are an experi-

mental disinfectant (S1) and a commercially available disinfectant (S2, commercial name: Virkon S; Bayer Korea, Seoul, Korea), whose main active ingredient is potassium peroxymonosulfate, as a comparison to the sample (S1).

### 2.2. Fish maintenance

The species of fish used in this test was the medaka (*Oryzias latipes*) and the zebrafish (*Danio rerio*). To maintain the number of the fish, the water temperature was maintained at 21-25ºC. The photoperiod was set at 16 h. A transparent water tank covered with glass, which is easy to observe was used as the breeding tank. The feed was supplied with commercial fish feed (Topmeal; Tabia Inc., Korea) once a day except weekends and holidays (the feed was not supplied during weekends and holidays). The test water supplied to the building aerated in the storage tank for 24 h and was used after breeding water filtered by sequential filters (microfilter [10 μm], block carbon filter [10 μm], and hi-clean filter [1 μm]).

The zebrafish were grown at 28°C in water tanks and a customized Arduino-based automatic watering system was used to supply purified water daily and maintained the water temperature. For the experiments, healthy zebrafish with similar sizes were chosen from the larvae, which were grown up to 20 to 30 days after birth. At the end of the experiment, animals were euthanized at low temperature according to the Animal Care and Use Committee (IACUC) of Peachchem Co. Ltd. approved for chemical exposure experiments using zebrafish under the protocol (P-20190507-1).

### 2.3. Acute toxicity test

Acute toxicity of fish was evaluated in accordance with the OECD 203 (1992) guideline for testing of the formulations. The concentrations of test formulations were determined by referring to the preliminary test results. To prepare the test solution, the concentrate of the S1 was taken (15.6, 31.3, 62.5, 125.0, and 250.0 mL) and filled the water tank with 5 L of test water. The fish (medaka) were exposed to the S1 for acute toxicity testing for 96 h. After the fish were exposed all test tanks were observed to check the fatality of the fish every 24 h. When observed, not only the fatality rate but also other abnormal symptoms of fish were observed such as loss of equilibrium, abnormal swimming behavior, abnormal skin pigmentation, abnormal ventilatory function, *etc*. They were considered dead if there was no movement when the fish was touched by a glass rod. The hardness of test water was measured using a water hardness tester (HI93735; Hanna Instruments Inc., Seoul, Korea) to investigate the water quality of the exposed environment. The properties of the water (pH, dissolved oxygen [DO] and temperature) were measured once a day during the acute toxicity testing using the instrument for measuring (Orion 4-Star plus 710A+; Thermo Fisher Scientific, Waltham, MA, USA). LC50 with 95% confidence limit of the test solution were calculated using the moving average angle method.

### 2.4. Fish behavior test

The serially diluted test samples were exposed to zebrafish at concentrations of 10,000, 5,000, 1,000, 100, 10, 1, 0.1 ppm. In addition to each sample, the control, which did not contain test samples, was also used. Each fish was located at 15 well-plate with a sample and had a zebrafish larvae population of five or more per concentration. The temperature of the imaging indoor environment was maintained at 27 to 28°C using the radiator, and the imaging equipment consisting of camera, light sources and plates were used to capture and analyze behavioral images at every second for 180 min. The LC50 of the fish was also calculated based on the surviving animals.

### 2.5. Image analysis

Time-lapse images were taken at one-second intervals and then analyzed with the Activity Analyzer and ZebraTracker software. To compute the locomotion activity, the original color images were converted to gray images, and differential images (absolute difference in gray pixel values) were generated from two consecutive time-lapse gray images. The differential images were then binarized using a threshold value. The locomotion activity was then calculated by multiplying the total number of white pixels by the average distance between the white pixels in individual wells in the binary image [14,16]. Meanwhile, to compute the swimming speed of the animal between two time-lapse images, the original color images were converted to gray images and an adaptive thresholding was applied to convert the gray images to binary images [30]. In the binary image, an animal was represented as an object with white pixels, and the x and y coordinates of the animal's centroid were calculated. The distance between the centroids of the animals in the time-lapse images was calculated and divided by the elapsed time. The centroids of the time-lapse images were connected to create animal trajectory paths. The calculated activity and swimming speed could be visualized in the heatmap using analysis software [26].

### 2.6. Statistical analysis

Statistical analysis was performed using the SigmaPlot program and one-way analysis of variances (ANOVA) test followed by a Duncan's post hoc test was performed to assess the significance between conditions. Statistically significant values were denoted as $*p < 0.05$, $**p < 0.01$, and $***p < 0.005$.

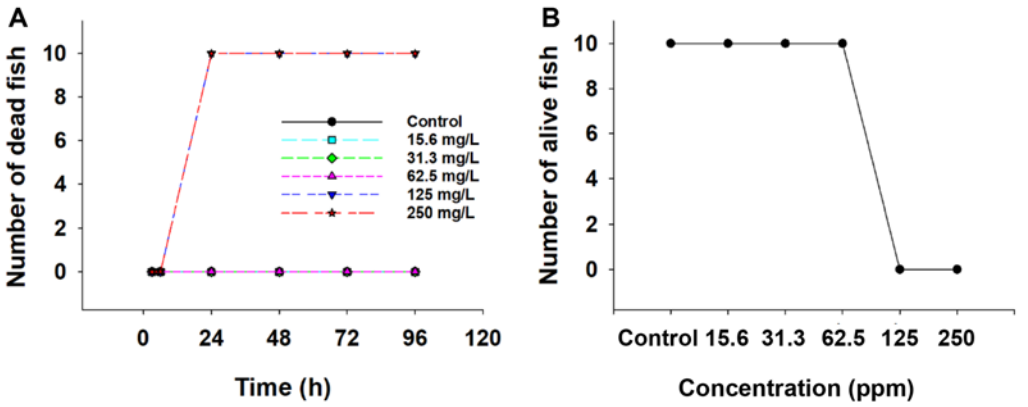


**Fig. 1.** (A) The number of dead fish (medaka) exposed to the disinfectant (S1) at different concentrations over time. (B) The number of alive fish after 96 h of exposure to the disinfectant at different concentrations.

## 3. Results

### 3.1. Acute toxicity test of the medaka

#### *3.1.1. Lethality rate and abnormal behavior*

First, acute toxicity of the tested disinfectant (S1) to the medaka was assessed to measure LC50 and survival rate (Table S1). No lethal and abnormal behavior was observed in the control group. Dead fish was not observed at 25.6, 31.3, and 62.5 mg/L after 24, 48, 72, and 96 h, whereas the fish did not survive at 125.0 and 250.0 mg/L after 24 h (Fig. 1). In this study, no visible abnormal symptoms (*e.g.*, loss of equilibrium, abnormal skin pigmentation, abnormal ventilatory function, *etc.*) of fish were identified.

#### *3.1.2. Calculation of the LC50*

During the testing process, the LC50 and 95% confidence limits for 24, 48, 72, and 96 h were calculated using the moving average angle method. After 96 h, LC50 was 88.39 mg/L (88.39 ppm). To reduce the calculation error, a logarithmic function was introduced into the concentration, and LC50 was calculated by a linear interpolation method based on the logarithmic concentration.

#### *3.1.3. Breeding water characteristics*

The hardness of the breeding water measured was 55 mg $CaCO_3$/L. The average temperature of the breeding water was almost constant at 21.4 ± 0.2°C. The mean DO concentration was 8.04 ppm (Fig. 2A). The average pH of each breeding water was 8.59 (minimum 7.25 to maximum 10.57) during the test period (Fig. 2B). As the concentration of the sample increased, the pH also increased due to the alkaline property of the sample, which can affect the behavior and lethality of the fish. The pH of the water appeared to decrease over time throughout tested concentrations likely due to the degradation properties of SPC and TAED, which eventually degrade into $CO_2$, $O_2$, and $H_2O$.

### 3.2. Behavior test data for the zebrafish

#### *3.2.1. Heat map of behavioral analysis*

The heat map of the mean locomotor activity and mean

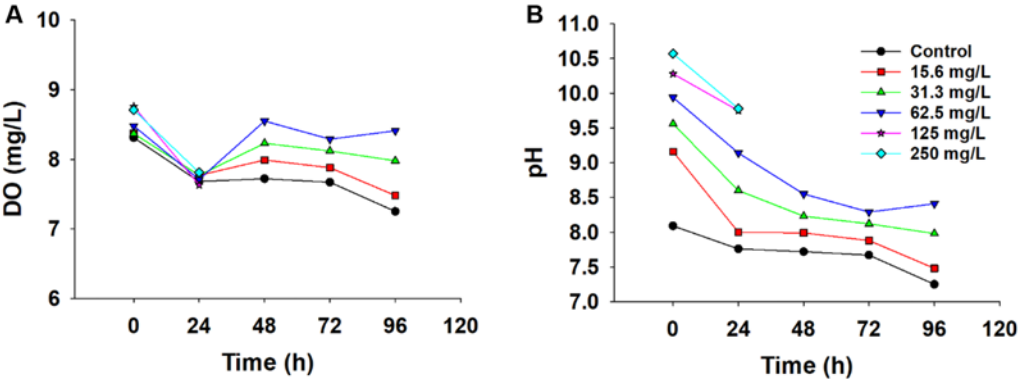


**Fig. 2.** Characteristics of breeding water over time. (A) Dissolved oxygen (DO) concentrations and (B) pH change of the water (all fish were dead after 24 h at 125 and 250 mg/L).

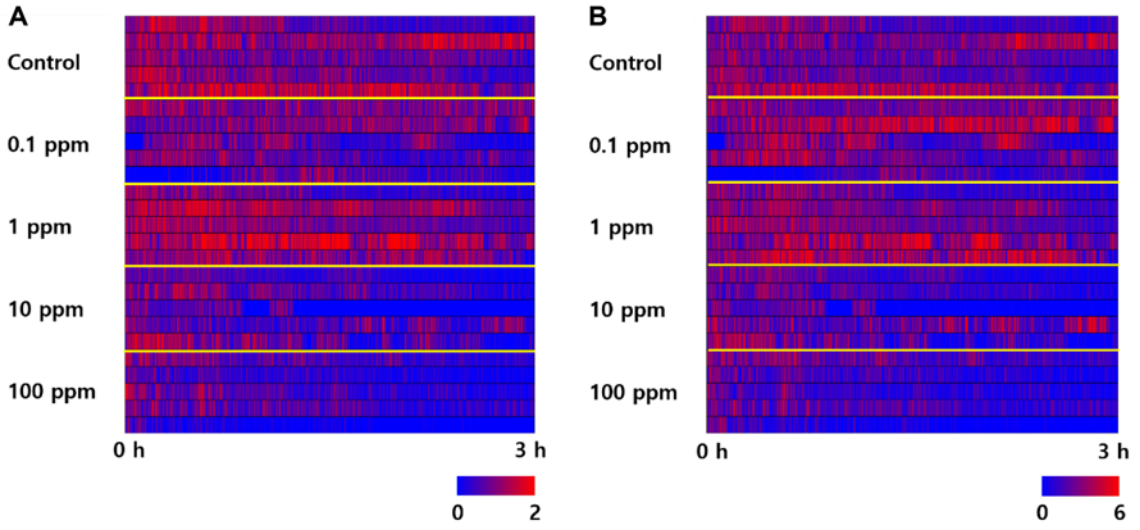


**Fig. 3.** Locomotion heatmap of individual larval zebrafish exposed to the S1 disinfectant at respective concentrations over time. Images of heatmap results from (A) individual activity and (B) swimming speed, respectively. Each row represents a different animal. Sample size n = 5 animals per condition.

swimming speed analysis of the 3 h imaging (Fig. 3). The data revealed that the fish exhibited different locomotor activity and swimming speed at the respective concentrations of the disinfectant. In general, the degrees of locomotor activity of the fish well corresponded to those of swimming speed. As the concentration increased the activity of the fish decreased, except for 1 ppm at which the fish appeared to have greatest degree of activity.

### *3.2.2. Activity of zebrafish over concentration*

Zebrafish activity over disinfectant concentrations was normalized to the control. The activity of the control was shown as 1 pixel$^2$. In the sample S1, the activity decreased appeared to decrease as the concentration increased, except for 1 ppm. The activity of zebrafish exposed to sample S2 has similar trend to the sample S1. However, it tends to decrease rapidly greater than 10 ppm concentration, and the data showed almost no activity beyond 5,000 ppm for both samples (Fig. 4A). In addition, the activity of the fish exposed to the S1 at lower than 100 ppm was greater than to the S2, however the fish appeared to have less activity at greater than 100 ppm of S1, compared with S2.

### *3.2.3. Swimming speed of zebrafish over concentration*

The swimming speed of the fish exposed to the disinfectants at different concentrations was also normalized to the control (without the disinfectants), which was calculated to be 2.72 mm/s. The speed of the fish showed a tendency similar to the activity as expected. Interestingly, the tendency of rapid decrease of the swimming speed was similar to the

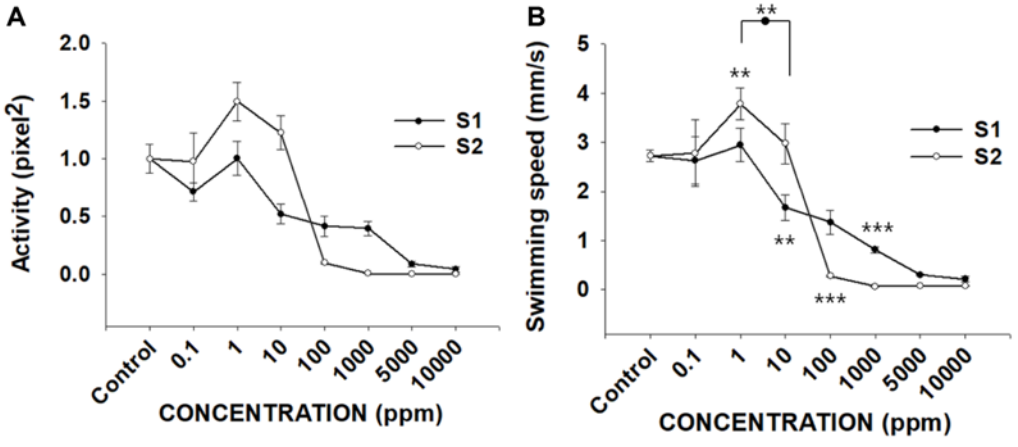


**Fig. 4.** (A) The locomotor activity and (B) the swimming speed of zebrafish exposed to the disinfectants at different concentrations. ** and *** represent $p < 0.01$ and 0.005, respectively.

activity data, indicating that there was a strong relationship between the activity and the swimming speed (Fig. 4B). A statistically significant difference occurred at concentrations above 10 ppm of the sample S1 when compared to the control. There was also a significant difference in the swimming speed between 1 ppm and 10 ppm ($p = 0.006$) of S1 sample. The sample S2 showed statistically significant differences from concentrations above 100 ppm as compared to the control. Interestingly, 1 ppm of S2 also showed a significant difference, compared to the control ($p = 0.004$).

### *3.2.4. Swimming speed of zebrafish over time*

To obtain quantitative swimming speed data over time, the average swimming speed value was calculated by dividing the total time of 15 min intervals (Fig. 5). In the case of sample S1 (Fig. 5A), the swimming speed of the control gradually decreased over time from 4.28 mm/s to 1.74 mm/s. At 0.1 ppm and 1 ppm of the concentrations, the fish have similar speed pattern to the control, whereas at 10 ppm and 100 ppm, the speed of the fish was slightly slower than that of the control. At 1,000 ppm, the fish was in motion until the first 15 min, but little movement after 30 min. At 5,000 ppm and 10,000 ppm, the swimming speed of the fish was reduced rapidly upon exposure to the sample, resulting in the swimming speed of zero after 30 min. In the case of sample S2 (Fig. 5B), the swimming speed at 0.1 ppm was similar to the control, but after 60 min the swimming speed at the concentration was suddenly increased. At 1 ppm, the swimming speed of the fish at 60 min was to 5.5 mm/s, much higher than the control. Interestingly, the swimming speed between 60 and 120 min increased, compared with earlier time points, then gradually decreased afterwards. At 100 ppm, the data showed little movement after the first 15 min, and at 1,000, 5,000 and 10,000 ppm, the swimming speed converged to zero as soon as the sample was exposed to the disinfectant.

### *3.2.5. Relationship between behavior responses and survival rate of the fish*

To find out the relationship between locomotive activity and

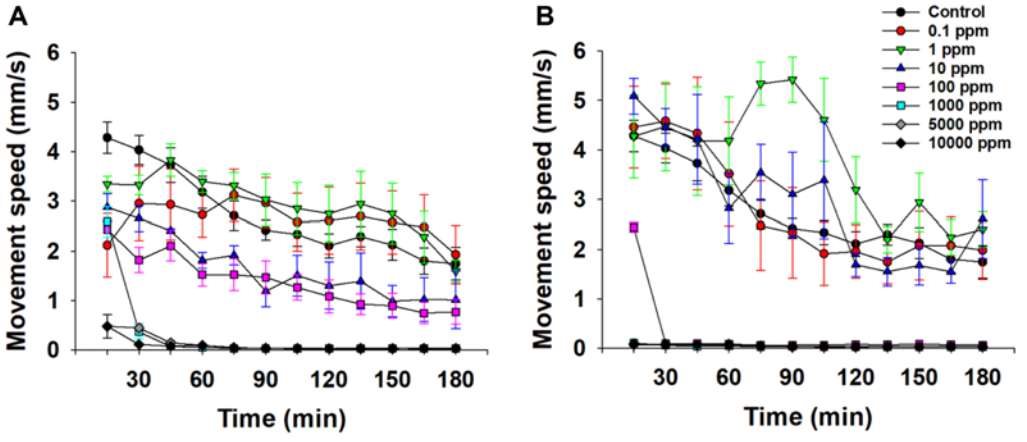


**Fig. 5.** The swimming speed changes of zebrafish exposed to (A) S1 and (B) S2 at different concentrations over time, respectively ($n \geq 5$).

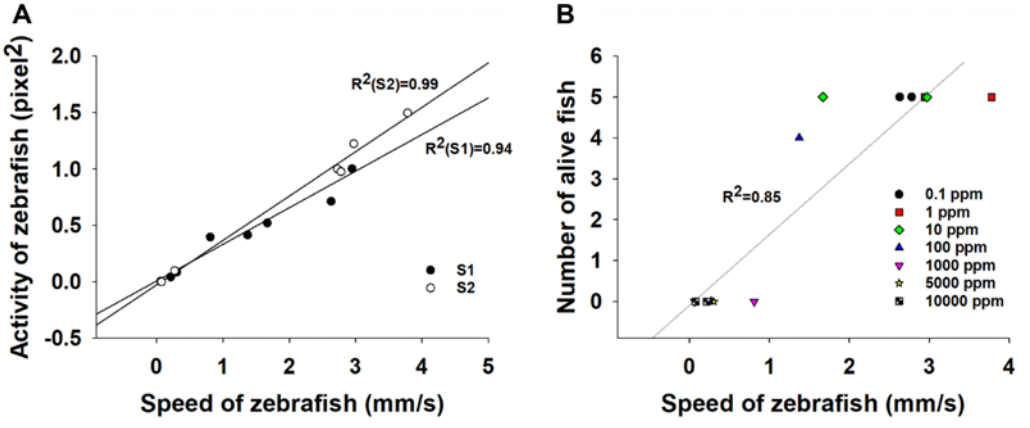


**Fig. 6.** (A) Correlation between locomotive activity and swimming speed of zebrafish exposed to the samples. (B) Correlation between swimming speed of the fish and the number of alive fish exposed to the disinfectants (S1, S2) at different sample concentrations.

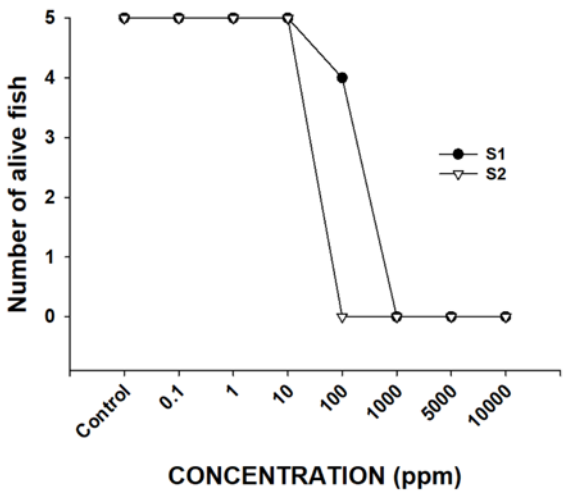


**Fig. 7.** The number of alive zebrafish after 3 h of exposure to the disinfectant samples S1 and S2 at different concentrations.

swimming speed of the fish, the linear regression method was used, and the result showed the strong relationships between the two behavior responses of the fish, regardless of the type of disinfectants tested (Fig. 6A). Furthermore, there appeared to have a relationship between swimming speed of the fish and the survival rate of the fish exposed to the disinfectants (S1, S2) at different concentrations ($R^2 = 0.85$) (Fig. 6B).

#### *3.2.6. Zebrafish survival and LC50 data*

In the three-hour experiment, both the control group and the S1 group, the fish treated at concentrations of 0.1, 1, and 10 ppm survived (Fig. 7). At 100 ppm, one died, and at concentrations of 1,000, 5,000, and 10,000 ppm, all fish died. In the case of S2 sample, the control and the groups the fish treated at 0.1, 1, and 10 ppm survived. all tested fish died at concentrations above 100 ppm. The LC50 for S1 and S2 were calculated at 135.76 ppm and 31.42 ppm, respectively.

## 4. Discussion

In this study, the aquatic toxicity of the disinfectant formulation containing SPC and TAED by a newly developed quantitative analysis using aquatic organisms such as zebrafish as well as by assessing a traditional LC50 and survival rate using the medaka. The LC50 of the disinfectant formulation (S1) we measured with the medaka after 96 h was 88.39 mg/L (88.39 ppm), whereas our data also revealed that the LC50 of the disinfectant with zebrafish was 135.76 ppm after 3 h (Fig. 7). While the LC50 of the same formulation as the tested disinfectant (S1) and the species has not been measured previously, the reported LC50 of SPC (active ingredient of the tested disinfectant) with similar fish, fathead minnow (*Pimephales promelas*) was 71 mg/L (71 ppm) [31]. A previous study reported that both fish had similar LC50 when exposed to a number of toxic chemical compounds [32]. Although it is very hard to directly compare the data from different laboratories due to the differences in experimental conditions (*e.g.*, the species, the tested chemical compounds, time, *etc.*), we may be able to compare the lethal concentration range of the tested chemical compounds exposed to similar species [33].

Regarding the effect of the disinfectant on the DO of the water exposed to the disinfectant, there appeared to have a low or no relationship between the two, regardless of tested concentrations as confirmed by a previous study found no relationship between toxicity of the seawater and biochemical oxygen demand using the same species [34]. However, there was a relationship between the pH of the water and the survival of the fish from our data (Fig. 2B). Interestingly, when the tested disinfectant (S1) was dissolved in water at 1.0 wt% (10,000 ppm), which can actually be used as a concentration with disinfecting effects and also much greater than tested concentrations, the pH was ~10.6, which was similar to the pH at 250 ppm. Previous studies indicate that most fish species survive from acute exposure for a few days to pH as acidic as 4.5 or as alkaline as 9.0, but not above pH 10 [35].

Alkaline water has also caused side effects of inhibiting ammonia excretion in fish and increasing $CO_2$ excretion, therefore, the increase of ammonia levels in the fish can be expected as observed in rainbow trout [36,37]. Therefore, it is possible to assume that the LC50 of the disinfectant can be affected by the pH of the water due to alkaline nature of the disinfectant dissolved in the water. On the other hand, the LC50 of the commercially available disinfectant (S2 in this study), which is highly acidic (pH 2.2-2.7) when it is dissolved in water (1.0 wt%, or 10,000 ppm), was much lower than the tested disinfectant (S1). Thus, it is possible to assume that the pH of the water may have adverse effects on survival rate of the tested organisms. It would be interesting to investigate the main cause for the acute toxicity of the disinfectants whether it is the pH of the water or chemical compound itself. However, it would be beyond the scope of the current study.

There was a significant difference in activity and swimming speed between S1 and S2 at respective concentrations (Fig. 4B). In the case of S1 disinfectant, the fish behavior gradually decreased as the concentration increased at concentrations below LC50, whereas the behavior values were almost steady or even greater than the control at certain concentrations (especially at 1 ppm) below LC50 for S2

disinfectant. We also found that at 1 ppm concentration, the speed increased between 60 and 120 min (Fig. 5B). We speculate that there appeared to have dynamic behavioral patterns of the fish when they are exposed to different concentrations over time. For example, a previous toxicity study using zebrafish revealed that at low concentration of 15 and 7.5 ug/L of another disinfectant (deltamethrin), the fish showed a similar tendency to have a higher swimming speed than the control during the initial exposure time up to 180 min. Thus, it may be possible to assume that in the early stages of exposure, the fish can increase their swimming speed as well as locomotor activity to avoid toxic substances in the water [38,39]. Further detailed studies may be needed to better understand these phenomena. In addition, there was a clear relationship between locomotor activity and swimming speed of the fish exposed to the disinfectants (Fig. 6A). It is noteworthy to mention that the locomotor activity and swimming speed of the fish can be observed at the concentrations well below LC50 for both tested disinfectants. Previous studies have shown the benefits of behavioral studies for assessing eco-toxicological effects of chemical compounds such as disinfectants at realistic exposure concentrations, since the concentrations of most contaminants in natural aquatic environments would be well below acute lethality such as LC50 [40,41].

The correlation between behavioral responses and the survival of the fish appeared to have a relationship between the two (Fig. 6B), therefore it is possible to assume that the swimming speed could be an additional screening indicator for the acute toxicity. Recently, research related to the methodology of the image analysis is increasingly active to evaluate the toxicity of chemical compounds or biomaterials in general and we expect that behavioral response may be useful to provide more detailed information of ecological toxicity in addition to conventional lethal concentration data [21,29]. Furthermore, in this study, a camera was used to capture the behavior of the fish in two dimensional from the top, since the actual fish's motion is in three dimensional (3D) form, future studies may be able to capture the upper and lateral data to create more detailed 3D images, which need advanced image software developments. In addition, our results demonstrated the convenience and rapidity of toxicological evaluation of chemical compounds such as disinfectants by assessing behavioral responses of aquatic organisms exposed to the environmental contaminants, as compared to the traditional lethal concentration methods. However, in order for the analysis of behavioral responses to become a favorable acute toxicity evaluation of various chemical compounds, further studies must be needed to optimize and standardize the protocols [42]. Together, our data revealed that the efficient behavioral assessment of aquatic organisms provided valuable toxicological information of disinfectants at concentrations below LC50, where conventional approaches would not produce any toxicological information. Future studies for developmental and reproductive responses of the organisms may be able to further assess ecological consequences of exposure to tested chemical compounds [43]. Cell cultures and *in vitro* techniques may be crucial to identify the mechanisms of action, leading to ultimately development of predictive models to elucidate adverse effects in human health [44].

## 5. Conclusion

In this study, the aquatic acute toxicity information of the disinfectant containing percarbonate and TAED such as LC50 and behavioral responses (*e.g.*, locomotive activity and swimming speed) of the fish were obtained by the OECD guideline and the image analysis. The results revealed that the LC50 of the medaka may be affected by the pH of the water exposed to the disinfectant. The data also demonstrated that the additional toxicity information can be generated by the quantitative image analysis by measuring locomotive activity and swimming speed of the zebrafish exposed to the disinfectant at concentrations below LC50. There was a linear relationship between the activity and the speed of the fish ($R^2$ = 0.99) and it is apparent that the behavioral responses of the fish directly related to the survival rate of the fish ($R^2$ = 0.85). Therefore, our data indicate that the behavioral responses of the fish may be useful to provide important ecotoxicological information, particularly at below lethal concentrations such as LC50.

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## Ethical Statements

The authors declare no conflict of interest.
The study protocol was approved by the Animal Care and Use Committee (IACUC) of Peachchem Co. Ltd (P-20190507-1).

## Electronic Supplementary Material (ESM)

The online version of this article (doi: 10.1007/s12257-021-0419-0) contains supplementary material, which is available to authorized users.

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