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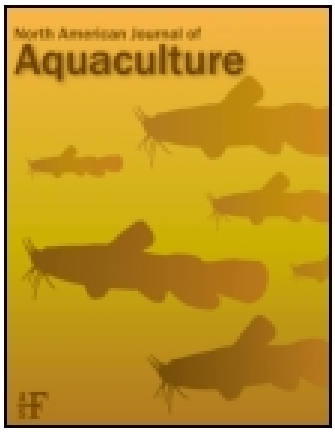

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ARTICLE

Comparison of the Toxicity of Wofasteril Peracetic Acid Formulations E400, E250, and Lspez to *Daphnia magna,* with Emphasis on the Effect of Hydrogen Peroxide

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Abstract

Commercial peracetic acid (PAA) formulations are acidic mixtures of PAA, hydrogen peroxide (H_2O_2), acetic acid, H_2O, and stabilizers to maintain the equilibrium of the concentrations. Different PAA formulations show diverse PAA : H_2O_2 ratios, potentially leading to different toxicities at the same PAA concentration due to the different concentrations of H_2O_2 and stabilizers used. To confirm any potential differences in toxicity, we performed 24-h toxicity tests using *Daphnia magna* with three commercial PAA formulations (Wofasteril): E400, E250, and Lspez. The experiments were carried out in standard dilution water and with increased water hardness, salinity, or dissolved organic carbon to reflect various natural conditions. Results showed that the toxicity to *Daphnia* was greatest for Lspez, intermediate for E250, and lowest for E400. An E400 + H_2O_2 mixture, which possessed a composition theoretically identical to the E250 formulation, had toxic effects and 24-h LC50 values similar to those of E250. This indicates an additive effect of H_2O_2 on the toxicity of PAA formulations. Moreover, a significant positive correlation was found between *Daphnia* mortality and the 3-h concentration of total peroxide (PAA and H_2O_2), with an *r*-value higher than that of PAA alone. A significant negative correlation between the total peroxide : PAA molar ratio and the 24-h LC50 value was observed, indicating that the toxicity of PAA formulations to *Daphnia* is due to the combined effect of both PAA and H_2O_2.

Peracetic acid (PAA) formulations—mixtures of PAA, hydrogen peroxide (H_2O_2), acetic acid, H_2O, and stabilizers—are increasingly used for water treatment and pathogen control in aquaculture (Madsen et al. 2000; Pedersen et al. 2009). The PAA formulations are effective disinfectants and have limited (if any) potential impacts on fish, fish consumers, or the environment (Wagner et al. 2002; Crebelli et al. 2005; Dell'Erba et al. 2007). Investigations have revealed the effectiveness of PAA

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against various fish pathogens (Smail et al. 2004; Meinelt et al. 2007, 2009; Straus and Meinelt 2009; Sudová et al. 2010; Jussila et al. 2011; Marchand et al. 2012; Picon-Camacho et al. 2012; Straus et al. 2012b; Farmer et al. 2013; Jussila et al. 2014) and the toxicity of PAA to several fish species and crayfish (Kouba et al. 2012; Straus et al. 2012a; Marchand et al. 2013).

In previous studies, commercial PAA formulations were chosen as test materials, but few studies have included comparisons of different PAA formulations. Straus and Meinelt (2009) investigated the acute toxicity of two PAA formulations—Minnfinn (4.5% PAA, 22% H_2O_2) and Wofasteril E400 (40% PAA, 12% H_2O_2)—against *Ichthyophthirius multifiliis* isolated from Golden Shiner *Notemigonus crysoleucas* and Green Swordtails *Xiphophorus hellerii*. The results showed that Minnfinn had significantly higher toxicity against both *I. multifiliis* isolates compared with Wofasteril E400 at the same PAA concentration. Despite these findings, Straus and Meinelt (2009) did not discuss the differences; their results indicated that the PAA formulation with a higher ratio of H_2O_2 to PAA was more toxic. Marchand et al. (2012) reported different inhibitory effects of PAA formulations on the *in vitro* growth of *Flavobacterium columnare* and *Saprolegnia parasitica*. They tested the effects of H_2O_2 concentration, PAA : H_2O_2 ratio, and PAA concentration and found a positive correlation between the growth reduction rate and the H_2O_2 concentration, indicating an additive effect of H_2O_2 on the reduction of pathogens *in vitro* by PAA. Marchand et al. (2012) relied on the theoretical data given in the material safety data sheets for the PAA formulations and did not include measurements of the actual PAA or H_2O_2 concentration. Marchand et al. (2013) demonstrated variation in acute toxicity of different PAA formulations to Zebrafish *Danio rerio* embryos, and they hypothesized that the variation was likely due to the impact of PAA formulations on reducing the pH, potentially leading to different levels of acidosis in Zebrafish embryos. Moreover, in the Marchand et al. (2013) study, the PAA formulations with a higher H_2O_2 : PAA ratio did not demonstrate greater toxicity.

As described above, H_2O_2 may play an important role in the toxicity of PAA formulations. To understand this relationship, the results of acute toxicity tests must be correlated with the actual PAA : H_2O_2 composition in different PAA formulations. Such information has been lacking in previous studies; therefore, our objective was to fill this gap.

METHODS

Chemicals.—Three Wofasteril PAA formulations were obtained from Kesla Pharma Wolfen GmbH (Greppin, Germany): E400 (40% mass/volume [m/v] PAA, 12% m/v H_2O_2), E250 (25% m/v PAA, 30% m/v H_2O_2), and Lspez (3% m/v PAA, 40% m/v H_2O_2). The standard dilution water was prepared according to the German standard DIN EN ISO 7346-3:1997 and was composed of 294 mg of $CaCl_2 \cdot 2H_2O$, 123.3 mg of $MgSO_4 \cdot 7H_2O$, 63.0 mg of $NaHCO_3$, and 5.5 mg of KCl in 1,000 mL of H_2O. Sea salt was Tropic Marin brand (Dr. Biener GmbH, Watenberg, Germany). The main components of sea salt are NaCl and KCl and account for approximately 85% of the total salt mass; the rest is composed of SO_4^{2-}, Mg^{2+}, and Ca^{2+} ions. A preparation of HuminFeed (Humintech GmbH, Düsseldorf, Germany) containing about 40% organic carbon was used as a source of dissolved organic carbon (DOC). All chemicals were reagent grade.

Daphnia magna toxicity tests.—*Daphnia magna* were cultured in media composed of 2.4 g of $CaCl_2$, 60 mg of KCl, 1.23 g of $MgSO_4 \cdot 7H_2O$, and 550 mg of $NaHCO_3$ in 10 L of distilled water; cultures were maintained at 26°C, with a 12-h light : 12-h dark photoperiod and continuous aeration. An algae suspension (*Scenedesmus* sp.) was used as feed for *Daphnia*. The toxicity tests were performed according to the German standard DIN 38412-11:1982-10. *Daphnia* that were 6–24 h old were obtained from the culture by double sieving: first with a 650-μm sieve to remove the oversized *Daphnia* and then with a 200-μm sieve. These *Daphnia* were then transferred into crystal dishes; 20 *Daphnia* and 40 mL of test solution were placed into each dish.

Test solutions were (1) standard dilution water, (2) standard dilution water with extra hardness (2.5-fold standard dilution water), (3) standard dilution water with extra NaCl (0.3% NaCl), (4) standard dilution water with extra sea salt (0.3% sea salt), and (5) standard dilution water with extra DOC (8 mg of DOC/L [20 mg of HuminFeed/L]). Treatment ranges were 0.5–1.5 mg/L for Wofasteril E400; 0.5–1.5 mg/L for E250; and 0.1–0.5 mg/L for Lspez. There was also a positive control containing potassium dichromate ($K_2Cr_2O_7$) at 1.9 mg/L. The crystal dishes ($n = 3$ per treatment) were incubated (without light or feed) at 20°C for 24 h in a KBW 720 Incubator (Binder GmbH, Tuttlingen, Germany); the *Daphnia* were then observed under a dissecting microscope (Olympus SZH-ILLB). *Daphnia* that were unable to swim were categorized as dead.

To investigate the effect of H_2O_2 on the toxicity of PAA formulations, a separate sample was prepared by adding H_2O_2 to the E400 samples to simulate the PAA : H_2O_2 ratio of the E250 formulation.

Measurement of peracetic acid and hydrogen peroxide.—The method to determine PAA and H_2O_2 concentrations was the DPD (N,N-diethyl-p-phenylenediamine sulfate salt) photometric method. Without the peroxidase, the transparent, colorless (to light pinkish) DPD is oxidized by PAA into DPD^+, which is pinkish and has a maximum absorption value at 550 nm (Pedersen et al. 2009). When peroxidase is added, the DPD is oxidized by both PAA and H_2O_2 (Bader et al. 1988). To measure the PAA concentration, 1 mL of PAA sample and 500 μL of buffer solution A (30.25 g of $Na_2HPO_4 \cdot 12H_2O$; 23 g of KH_2PO_4; 0.01 g of NaCl; and 0.5 g of KI in 1,000 mL of H_2O) were mixed in a plastic cuvette. We then added 500 μL of DPD solution (1.6 g of DPD; 200 μL of 97% H_2SO_4; and 0.02 g of EDTA in 100 mL of H_2O). After 30 s, the absorption

at 550 nm (hereafter, absorption A) was measured with a DU 800 spectrophotometer (Beckman Coulter GmbH, Krefeld, Germany). For measurement of the combined concentration of PAA and H_2O_2 (i.e., total peroxide), we used the same procedure and wavelength as above except that buffer solution B was used to obtain the absorption (hereafter, absorption B). Buffer solution B was prepared by dissolving 5 mg of peroxidase (peroxidase from horseradish, Practical Grade II, A3800; Applichem GmbH, Darmstadt, Germany) in 100 mL of buffer solution A. Thus, absorption A represents the concentration of PAA, while absorption B represents the concentration of total peroxide.

Determination of total peroxide : peracetic acid molar ratios.—Samples of E400 and E250 at nominal PAA concentrations of 0.5, 1.0, and 3.0 mg/L and samples of Lspez at nominal PAA concentrations of 0.1, 0.2, and 0.4 mg/L were measured with buffer solutions A and B. The total peroxide : PAA molar ratios of the PAA formulations were determined through comparison of absorption A with absorption B at different nominal PAA concentrations by means of a linear relationship. Determination of total peroxide : PAA molar ratios was performed under the same conditions as the *Daphnia* toxicity tests.

Monitoring of peracetic acid and hydrogen peroxide concentrations during testing.—According to the German standard DIN 38412-11:1982-10, *Daphnia* toxicity tests must be performed in an incubator without light; therefore, concentrations of PAA and H_2O_2 in the samples were not measured during the toxicity tests. Additional samples of E400, E250, and Lspez were prepared according to the parameters in the toxicity tests but without adding *Daphnia*. The concentrations of PAA and H_2O_2 were measured after 3 h of static exposure.

Statistics.—The difference in toxicity among PAA formulations and the impacts of the various test solutions on PAA toxicity were determined via a two-tailed ANOVA test ($\alpha = 0.05$) and a two-tailed *post hoc* test (Tukey's test or Dunnett's test, $\alpha = 0.05$) applied to the data on *Daphnia* mortality at different PAA concentrations. The 24-h LC50 values (concentrations that were lethal to 50% of test organisms) were calculated via probit regression ($\alpha = 0.05$) and were demonstrated in the form of mean values with 95% confidence intervals. Relationships between *Daphnia* mortality and PAA concentration, mortality and total peroxide concentration, and 24-h LC50 and the total peroxide : PAA molar ratio were determined via two-tailed Pearson's product-moment correlation ($\alpha = 0.05$). All statistical analyses were performed with IBM SPSS Statistics version 21 (IBM, Chicago).

RESULTS

Toxicity of Peracetic Acid Formulations to Daphnia and Impacts of Different Test Solutions

As shown in Figure 1, the Lspez formulation led to 100% mortality of *Daphnia* at a PAA concentration of 0.4 mg/L, indicating higher 24-h toxicity. In contrast, the E400 and E250 formulations led to 100% mortality at a PAA concentration of 1.5 mg/L. A noteworthy finding was that the 24-h toxicity of E400 was significantly lower than that of E250 and the E400 + H_2O_2 mixture (Tukey's test: $P = 0.000$), while the 24-h toxicity of E400 + H_2O_2 was similar to that of E250 (Tukey's test: $P = 0.983$).

Compared with the *Daphnia* exposures conducted in standard dilution water, extra sea salt significantly reduced the 24-h toxicity of all PAA formulations (Dunnett's test: $P = 0.000$; Figure 2). Extra DOC significantly reduced the 24-h toxicity of the E400 and E250 formulations ($P = 0.000$) but did not induce a significant change in toxicity of the Lspez formulation (Dunnett's test: $P = 0.344$). Extra NaCl significantly reduced the 24-h toxicity of Lspez (Dunnett's test: $P = 0.005$) but had no significant effect on the 24-h toxicity of E400 (Dunnett's test: $P = 0.979$) or E250 (Dunnett's test: $P = 0.998$). Extra hardness significantly reduced the 24-h toxicity of E250 (Dunnett's test: $P = 0.000$) but had no significant effect on E400 (Dunnett's test: $P = 0.853$) or Lspez (Dunnett's test: $P = 0.133$) toxicity.

Twenty-Four-Hour LC50 Values

The calculated 24-h LC50 values are listed in Table 1. In standard dilution water, the Lspez formulation had the lowest 24-h LC50 value, while the E400 formulation had the highest 24-h LC50 value. The 24-h LC50 values for E250 and the E400 + H_2O_2 mixture were nearly identical and were intermediate between those of E400 and Lspez. These results corresponded to the 24-h toxicity results described above.

The addition of extra sea salt resulted in lower 24-h LC50 values for all PAA formulations relative to the LC50 values obtained with standard dilution water. Extra DOC increased the 24-h LC50 values of E400 and E250; however, the 24-h LC50 value for E400 with extra DOC was considered an

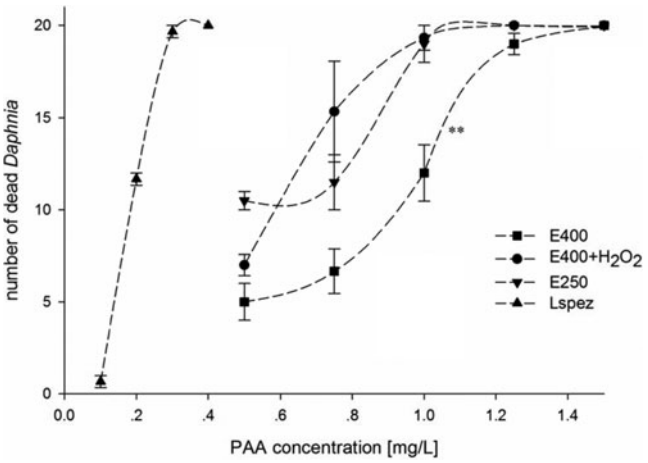

FIGURE 1. The 24-h mortality (mean ± SE) of *Daphnia magna* (20 individuals per replicate) exposed to the Wofasteril peracetic acid (PAA) formulations E400, E250, and Lspez and an E400 + hydrogen peroxide (H_2O_2) mixture at various PAA concentrations in standard dilution water. Asterisks indicate a significant difference in comparison with the result for E250 (**$P < 0.01$). Lspez was not compared with the other formulations due to its completely different data distribution.

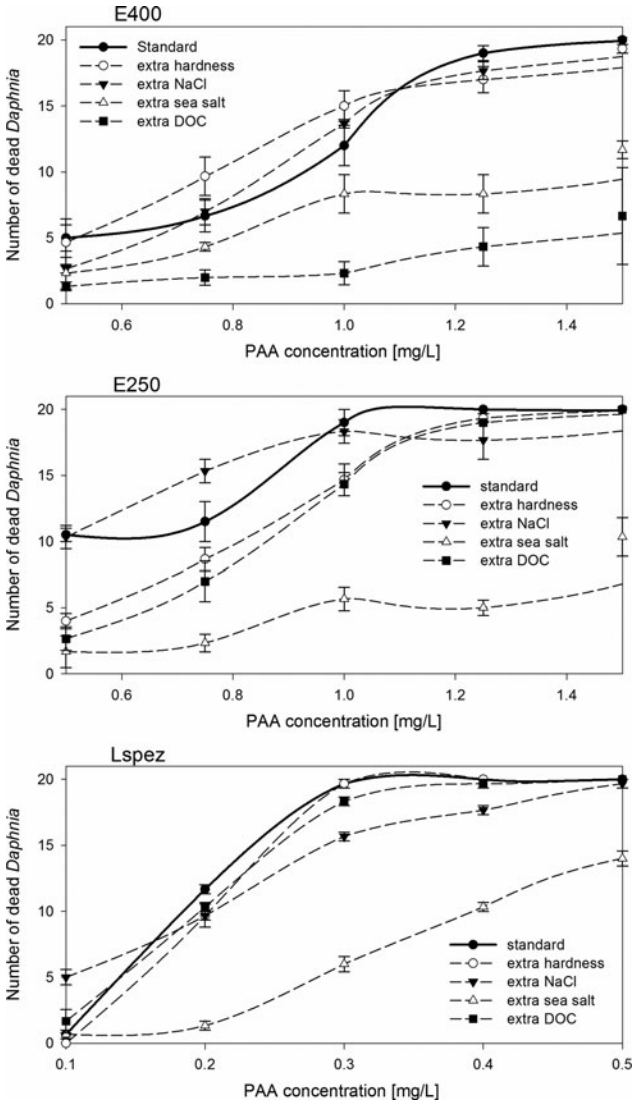

FIGURE 2. Impacts of various test solutions (standard dilution water alone or with extra hardness, NaCl, sea salt, or dissolved organic carbon [DOC]) on the 24-h mortality (mean ± SE) of *Daphnia magna* (20 individuals per replicate) exposed to the Wofasteril peracetic acid (PAA) formulations E400, E250, and Lspez at different PAA concentrations. Asterisks indicate a significant difference in comparison with the result for standard dilution water (**$P < 0.01$).

outlier. Extra hardness increased the 24-h LC50 value for E250 but had little effect on the values for E400 and Lspez. These results also corresponded to the 24-h toxicity results. For all PAA formulations, extra NaCl had little effect on the 24-h LC50.

Daphnia Mortality and the Three-Hour Concentrations of Peracetic Acid and Total Peroxide

The relationship between *Daphnia* mortality and the 3-h PAA or total peroxide concentration is demonstrated in Figure 3. For E400 and Lspez, *Daphnia* mortality showed significant positive correlations with both PAA and total peroxide ($P = 0.000$). Pearson's product-moment correlation coefficients (Pearson's r) for E400 were 0.754 (PAA) and 0.728 (total peroxide); Pearson's r-values for Lspez were 0.654 (PAA) and 0.90 (total peroxide). For E250, *Daphnia* mortality was not significantly correlated with either PAA (Pearson's $r = 0.272$, $P = 0.162$) or total peroxide (Pearson's $r = 0.286$, $P = 0.14$). When data from all PAA formulations were combined, significant positive correlations were observed between *Daphnia* mortality and both PAA and total peroxide concentration. However, Pearson's r-value for *Daphnia* mortality versus total peroxide (Pearson's $r = 0.616$, $P = 0.000$) was higher than that for *Daphnia* mortality versus PAA (Pearson's $r = 0.256$, $P = 0.011$).

Relationship between the 24-h LC50 and Total Peroxide : Peracetic Acid Molar Ratio

Figure 4 shows a significant negative correlation between 24-h LC50 values and the total peroxide : PAA molar ratio under all tested conditions (Pearson's $r = -0.692$, $P = 0.009$). The E400 and Lspez formulations with the DOC treatment were not included in the correlation calculation because the deviation in 24-h LC50 value for E400 (Table 1) and the deviation in total peroxide : PAA molar ratio for Lspez (Table 2) were too large.

DISCUSSION

Effect of Additional Hydrogen Peroxide

As depicted in Figure 1, 24-h toxicity to *Daphnia magna* differed significantly among the E400, E250, and Lspez formulations. The 24-h toxicity was lowest for E400, intermediate

TABLE 1. Mean 24-h LC50 values (with 95% confidence interval in parentheses) for *Daphnia magna* exposed to Wofasteril peracetic acid formulations E400, E250, and Lspez and an E400 + hydrogen peroxide (H_2O_2) mixture. Exposures were conducted in five test solutions: standard dilution water alone or with extra hardness, NaCl, sea salt, or dissolved organic carbon (DOC).

Test solution	E400	E250	Lspez	E400 + H_2O_2
Standard dilution water	0.774 (0.671–0.87)	0.547 (0.385–0.651)	0.181 (0.166–0.195)	0.574 (0.506–0.631)
Extra hardness	0.731 (0.665–0.792)	0.739 (0.686–0.789)	0.202 (0.188–0.205)	
Extra NaCl	0.81 (0.756–0.862)	0.487 (0.325–0.593)	0.176 (0.153–0.198)	
Extra sea salt	1.323 (1.159–1.626)	1.695 (1.368–2.835)	0.393 (0.356–0.442)	
Extra DOC	2.617 (1.641–165.163)[a]	0.79 (0.74–0.838)	0.196 (0.188–0.205)	

[a] Outlier; not considered in final statistics.

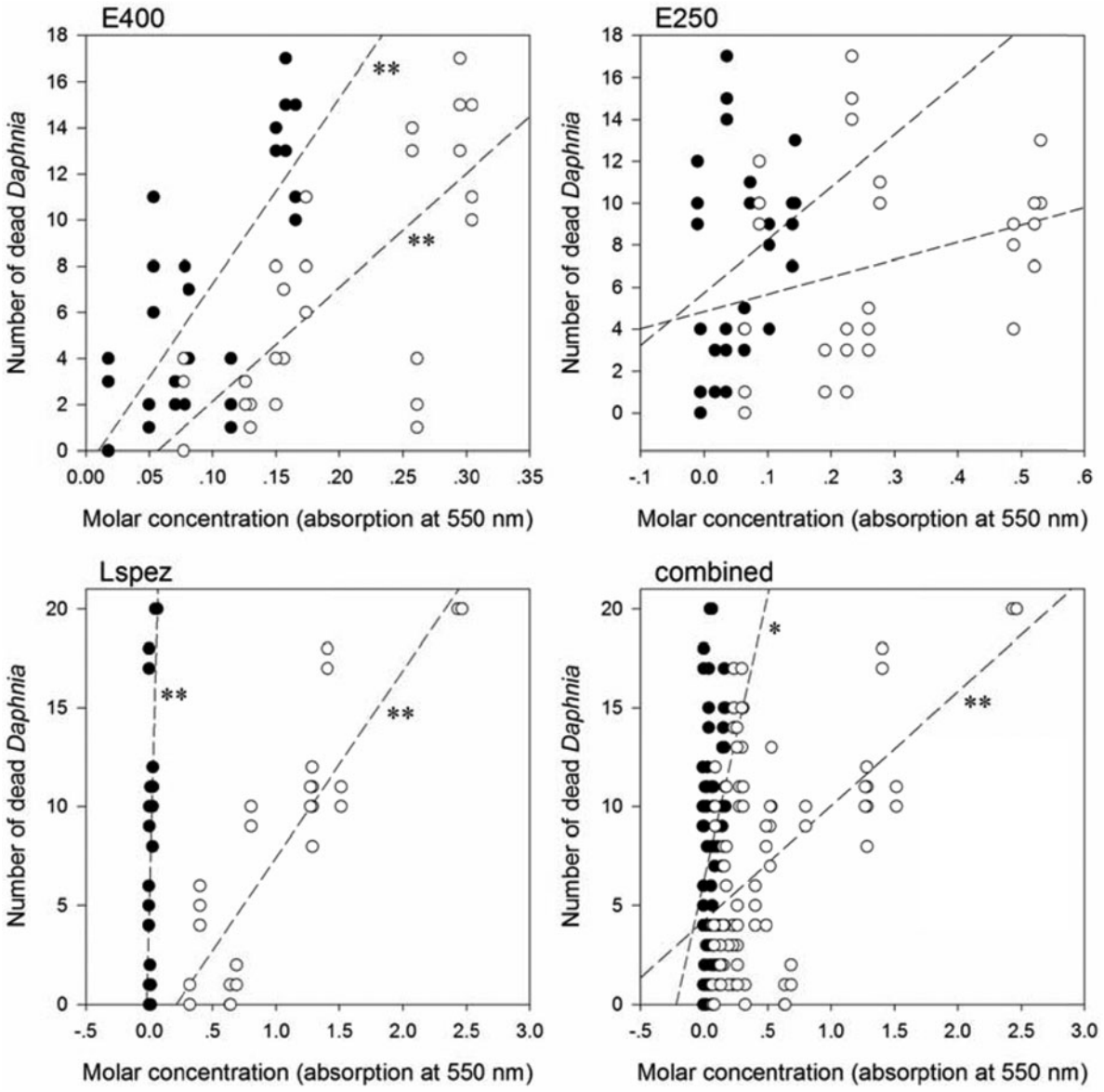

FIGURE 3. Pearson's product-moment correlations between the mortality of *Daphnia magna* (20 individuals per replicate) exposed to Wofasteril peracetic acid (PAA) formulations E400, E250, and Lspez and the molar concentration of PAA (black shaded circles) or total peroxide (open circles) in each formulation. The correlations observed for all formulations combined are also presented. Asterisks indicate significant correlations (*$P < 0.05$; **$P < 0.01$).

for E250, and highest for Lspez. The E400 + H_2O_2 mixture, which had the same PAA and H_2O_2 composition as the E250 formulation, showed 24-h toxicity similar to that of E250. The 24-h LC50 values demonstrated results similar to the 24-h toxicity findings (Table 1). Our study results indicate that (1) H_2O_2 has an additive effect on the toxicity of PAA formulations; and (2) among the different PAA formulations, there are no factors other than H_2O_2 that could affect toxicity.

Effects of Various Test Solutions on Toxicity to Daphnia

The toxicity of E400, E250, and Lspez showed differing sensitivity to the evaluated test solutions in comparison with the use of standard dilution water. This result was mainly due to different PAA degradation rates. Our previous work (Liu et al. 2014) found that salinity, hardness, and DOC induced different PAA degradation rates in E400, E250, and Lspez. Different PAA degradation rates result in different PAA residues, which in turn lead to differences in toxicity.

Daphnia Mortality versus Peracetic Acid and Total Peroxide

Since the number and size of *Daphnia* used in each replicate of the toxicity tests were the same, the impact of *Daphnia* on PAA decay was presumed to be the same among replicates.

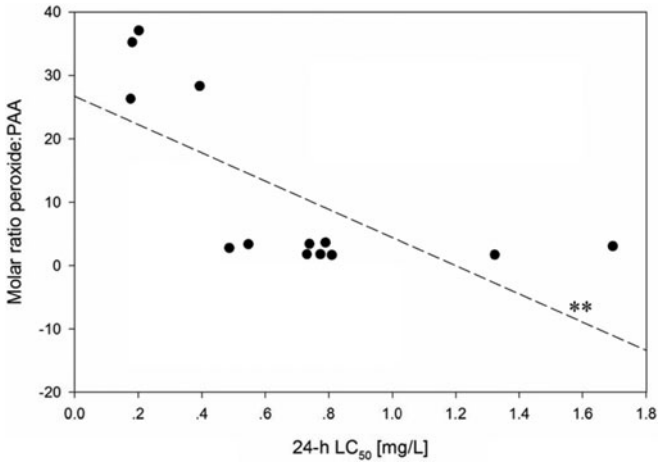

FIGURE 4. Pearson's product-moment correlations between 24-h LC50 values for *Daphnia magna* from exposure to Wofasteril peracetic acid (PAA) formulations E400, E250, and Lspez and the total peroxide : PAA molar ratio for all formulations under all test conditions (E400 and Lspez treated with extra dissolved organic carbon are excluded due to high deviation). Asterisks indicate that the correlation was significant (**$P < 0.01$).

Therefore, ignoring the impact of *Daphnia* on PAA decay, the 3-h absorptions A and B could represent intermediate concentrations of PAA and total peroxide, respectively, during the toxicity tests and thus should be directly associated with the mortality rate.

Daphnia mortality was significantly and positively correlated with both PAA and total peroxide from the E400 and Lspez formulations (Figure 3). Pearson's *r*-values for the correlations with mortality were identical between the PAA from E400 and the total peroxide from E400. However, for Lspez, total peroxide had a higher Pearson's *r* than PAA. These findings indicate that PAA and total peroxide from E400 were equally responsible for *Daphnia* mortality, whereas total peroxide from Lspez was more likely to induce *Daphnia* mortality than PAA from Lspez. As the PAA proportion of total peroxide decreased from E400 to E250 to Lspez (Table 2), PAA appeared to contribute less to toxicity in *Daphnia*.

TABLE 2. Total peroxide : peracetic acid (PAA) molar ratio for the Wofasteril E400, E250, and Lspez formulations in each of the five test solutions (DOC = dissolved organic carbon); values were calculated from the absorption values (measurements of PAA and hydrogen peroxide [H_2O_2] concentrations). Asterisks indicate coefficient of determination values (**$R^2 > 0.99$; *$R^2 > 0.95$).

Test solution	E400	E250	Lspez
Standard dilution water	1.79**	3.36**	35.24*
Extra hardness	1.79**	3.41**	37.10*
Extra NaCl	1.66**	2.78**	26.34*
Extra sea salt	1.70**	3.06**	28.43*
Extra DOC	1.79**	3.64**	ND

When results for all formulations were combined, PAA and total peroxide exhibited significant positive correlations with *Daphnia* mortality. However, values of *P* and Pearson's *r* were much lower for PAA than for total peroxide, suggesting that for all PAA formulations, toxicity to *Daphnia* was more likely determined by the total peroxide concentration than by PAA alone.

Twenty-Four-Hour LC50 versus Total Peroxide : Peracetic Acid Molar Ratio

The 24-h LC50 results (Table 1) differed slightly from the toxicity results (Figure 2). The only difference occurred for the toxicity of Lspez in standard dilution water treated with extra NaCl, which significantly differed from that obtained with standard dilution water alone (Figure 2). The ANOVA test indicated that the mean difference in mortality between the extra NaCl treatment and standard dilution water was 0.8667—that is, less than one dead *Daphnia*. In this case, the statistical difference does not seem to be biologically significant. Therefore, we decided that the conflicting results between Table 1 and Figure 2 were inconsequential and that the 24-h LC50 values are appropriate for use in further analysis.

The total peroxide : PAA molar ratio showed a significant negative correlation with 24-h LC50 values. Therefore, the formulation with a higher total peroxide : PAA ratio has a lower 24-h LC50 value—specifically, a higher toxicity to *Daphnia*. However, in the present study as well as in previous studies, the 24-h LC50 value was defined as the LC50 of only PAA. If the LC50 of PAA is multiplied by the total peroxide : PAA molar ratio, then theoretically the LC50 of total peroxide can be obtained. Since the changes in 24-h LC50 values lead to reversed changes in total peroxide : PAA molar ratios, it is likely that the LC50 of total peroxide could be rather stable. This indicates that even different PAA formulations can have the same LC50 of total peroxide for *Daphnia*.

Mechanism of Toxicity

It has been demonstrated that chronic exposure of *Daphnia* to H_2O_2 at concentrations greater than 1.25 mg/L leads to death and/or reduced reproduction (Meinertz et al. 2008). However, *Edwardsiella tarda* is tolerant of H_2O_2 due to the presence of catalases (Srinivasa Rao et al. 2003). Many microorganisms can produce catalase to reduce the effect of H_2O_2 (Nakamura et al. 2012). For instance, Jussila et al. (2014) found no effect of H_2O_2 against spores of the infective crayfish plague *Aphanomyces astaci*. In that case, H_2O_2 may not have been able to contribute to the toxicity of PAA formulations. Peracetic acid is fat soluble and can penetrate the cell membrane (Kitis 2004). McKinney et al. (1991) described that PAA at certain concentrations can deactivate catalases, so the toxic effect of H_2O_2 within PAA formulations would be maintained. Such an effect was shown by Pedersen et al. (2009), who simultaneously measured PAA

and H_2O_2 at different concentrations. We speculate that the mode of action for toxicity of PAA formulations is primarily the action of PAA and secondarily the action of H_2O_2. Therefore, our findings should theoretically also be valid for other aquatic organisms, but further research is needed.

Strategy for Peracetic Acid Application in Freshwater Aquaculture

Pedersen et al. (2013) discussed the difficulty in maintaining a safe but effective dosage of PAA while disinfecting freshwater aquaculture systems; they emphasized that the most feasible solution is to monitor PAA consumption during the process of disinfection. In addition, we recommend monitoring the combined concentration of PAA + H_2O_2 since our study indicates that the toxicity of PAA is due to the combined effect of PAA and H_2O_2. The present findings suggest that additional, combined, or simultaneous applications of H_2O_2 could be options when applying PAA for water treatment.

Conclusions

We demonstrated that the toxicity of PAA formulations to *Daphnia* is affected by both PAA and H_2O_2. The PAA formulation with a higher H_2O_2 : PAA ratio is more toxic to *Daphnia* because of higher H_2O_2 concentrations at the same PAA concentration. We suggest that the effect of H_2O_2 cannot be ignored in toxicity studies of PAA formulations and that the LC50 values of PAA formulations should be defined as the LC50 of total peroxide rather than only PAA.

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