Acute toxicity and risk evaluation of the CSO disinfectants performic acid, peracetic acid, chlorine dioxide and their by-products hydrogen peroxide and chlorite

Chhetri, Ravi Kumar; Baun, Anders; Andersen, Henrik Rasmus

Published in:
Science of the Total Environment

Link to article, DOI:
10.1016/j.scitotenv.2019.04.350

Publication date:
2019

Document Version
Peer reviewed version

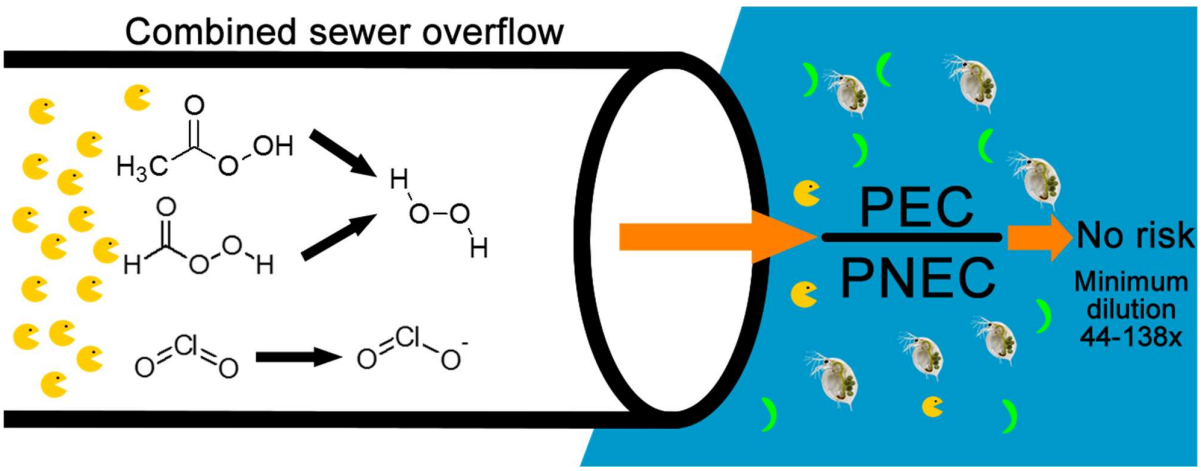
This is a post print of the article accepted for publication 24th April 2019 in the Science of Total Environment.
DOI: 10.1016/j.scitotenv.2019.04.350

Acute toxicity and risk evaluation of the CSO disinfectants performic acid, peracetic acid, chlorine dioxide and their by-products hydrogen peroxide and chlorite

Ravi Kumar Chhetri[a], Anders Baun[a], Henrik Rasmus Andersen[a1]

[a]Department of Environmental Engineering, Technical University of Denmark, Bygningstorvet, Building 115, 2800 Kgs. Lyngby, Denmark.

[1]Corresponding author: Henrik@ndersen.net



Highlights

- PAA, PFA and ClO_2 were more toxic than hydrogen peroxide and chlorite
- ClO_2 was most toxic to *D. magna* and PFA was most toxic to *V. fischeri*
- Environmental risk assessment of disinfectants was done for CSO disinfection
- Water quality limits and required dilutions of disinfected CSO found for recipients
- 70 and 138-fold dilution of CSO in receiving water needed for PFA or PAA

Abstract

The ecotoxicological evaluation of combined sewer overflow (CSO) disinfectants, with their degradation products, is important for ensuring safe use. For this form of toxicity, data for organisms representing different trophic levels are needed. We studied the toxicity of the alternative disinfectants peracetic acid (PAA), performic acid (PFA) and chlorine dioxide (ClO_2) and their degradation products hydrogen peroxide (H_2O_2) and chlorite (ClO_2^-) on *Vibrio fischeri* and *Daphnia magna*. ClO_2 was more toxic to *D. magna* (EC_{50} <0.09 mg/L) and PFA was most toxic to *V. fischeri* (EC_{50} 0.24 mg/L). EC_{50} of PFA, PAA, ClO_2, H_2O_2 and ClO_2^- on *D. magna* were 0.85, 0.78, <0.09, 3.46 and 0.36 mg/L, respectively. Similarly, EC_{50} of PFA, PAA, ClO_2, H_2O_2 and ClO_2^- on *V. fischeri* were 0.24, 0.42, 1.10, 5.67 and 30.93 mg/L, respectively. For both PFA and ClO_2, the degradation in water was faster than for PAA, H_2O_2 and chlorite. Using these data together with literature values, we derived environmental quality standards. By combining these with typical concentrations of disinfectants used for CSOs, we estimated the dilution required for discharging CSOs after disinfection, which can be used for quick assessment of the environmental feasibility of disinfection systems at specific CSO sites. Minimal dilutions in the receiving water, in the orders of 44, 70 or 138-fold, are needed for ClO_2, PFA and PAA, respectively. This highlights PFA as the most widely applicable disinfectant, taking into account both its efficiency and the lower risk of unwanted environmental effects.

Keywords: Combined Sewer overflows, Disinfection, Ecotoxicity, *Vibrio fischeri*, *Daphnia magna*

1 Introduction

Combined sewer systems are common in many cities where wastewater is mixed with rainwater and transported to a wastewater treatment plant for processing. During significant rainfall events, the design capacity of combined sewer systems can be exceeded, resulting in the discharge of untreated combined sewer overflows (CSOs) to nearby surface waters. Discharge of untreated CSOs worsens the quality of receiving waters, and thus it cannot be used for recreational purposes, due to infection risk. Disinfection of inflowing

CSO water was studied recently with a view to reducing the microbiological load in the receiving surface water and minimize the impact of discharging untreated CSO (Chhetri et al., 2016; FRODO, 2014).

Among various disinfectants, chlorine is the most well-known disinfectant used in the water industry (White, 2010). However, the toxic by-products of chlorination are of environmental concern (Bayo et al., 2009; Boczek et al., 2010; Emmanuel et al., 2004; Hrudey and Charrois, 2012; Nurizzo et al., 2005; Watson et al., 2012; White, 2010), and other chlorine-based disinfectants are therefore often used. Chlorine dioxide (ClO_2) is one example of such an alternative disinfectant; it does not react with organic and inorganic compounds present in different water types to generate toxic by-products (Hofmann et al., 1999). One of the methods employed to synthesize ClO_2 is by reacting chlorite with strong acid:

$$5ClO_2^- + 4H^+ \rightarrow 4ClO_2 + Cl^- + 2H_2O \qquad \text{Equation 1A}$$

During disinfection, ClO_2 is reduced to chlorite as a degradation product, as shown in equation 1B (Korn et al., 2002; Lee et al., 2004):

$$ClO_2 + e^- \rightarrow ClO_2^- \qquad \text{Equation 1B}$$

Performic acid (PFA) and peracetic acid (PAA) have been used as alternatives to chlorine-based disinfectants, and like ClO_2 they do not generate toxic by-products (Chhetri et al., 2014; Liberti and Notarnicola, 1999). PFA has been used to disinfect primary and secondary WWTP effluents (Gehr et al., 2009; Ragazzo et al., 2013) as well as combined sewer overflows (Chhetri et al., 2015). However, it is unstable and needs to be generated on-site, when needed, as a quaternary equilibrium mixture of performic acid (PFA), formic acid, hydrogen peroxide and water:

$$CHO - OH + H_2O_2 \rightleftharpoons CHO - OOH + H_2O \qquad \text{Equation 2A}$$

$$CHO - OOH + 2e^- \rightarrow CHO - O^- + HO^- \qquad \text{Equation 2B}$$

PFA degrades into formic acid, hydrogen peroxide and water, the former of which is not toxic to aquatic fauna and is readily biodegradable (Gehr et al., 2009; USEPA, 2001).

The use of PAA as a disinfectant in wastewater treatment was introduced about 30 years ago (Antonelli et al., 2006; Baldry, 1983; Falsanisi et al., 2006; Kitis, 2004; Koivunen and Heinonen-Tanski, 2005; Luukkonen et al., 2015), and recently it has been used to disinfect combined sewer overflows (Chhetri et al., 2016, 2014). Commercial PAA is available as an acidic quaternary equilibrium mixture of PAA, hydrogen peroxide, acetic acid and water:

$$CH_3COOH + H_2O_2 \rightleftharpoons CH_3CO - OOH + H_2O \qquad \text{Equation 3A}$$

$$CH_3CO - OOH + 2e^- \rightarrow CH_3CO - O^- + HO^- \qquad \text{Equation 3B}$$

Residues left after PAA use are acetic acid, hydrogen peroxide and water. Hydrogen peroxide tends to degrade slower than PAA (Wagner et al., 2002) and it has a stringent discharge limit in relation to surface water.

To assess the potential impact of discharged disinfected effluents in receiving waters and related aquatic ecosystems, it is important to evaluate the ecotoxic effect of residual disinfectants. Generic aquatic risk assessments rely on laboratory-based tests carried out on organisms from different trophic levels in the ecosystem (ECHA, 2008). Some of the most commonly used ecotoxicity tests for this purpose are: the bacterial luminescence inhibition test with *Vibrio fischeri*, the crustacean immobilization test with *Daphnia magna* and the algal growth rate inhibition test with the freshwater green algae *Pseudokirchneriella subcapitata*. This test array covers degraders, primary producers and zooplankton, but it does not include the predator level, for which tests with fish are usually included. However, for ethical reasons the use of vertebrate for chemicals testing should be minimized (ECHA, 2008), and there are already several studies on the correlation between *V. fischeri* and acute toxicity tests with fish (Kaiser, 1998; Wang et al., 2016).

At present, only a few studies have reported data on the ecotoxicity of PFA, PAA, hydrogen peroxide, ClO_2 and chlorite, and there is no consistent information regarding the toxic effect of disinfected effluents. We have

recently studied the ecotoxicity of alternative disinfectants on microalgae, *P. subcapitata* (Chhetri et al., 2017a), but for aquatic environmental risk assessment, it is important to obtain data for organisms representing different trophic levels in the ecosystem. Thus, in order to ensure the environmental safety of disinfectants, ecotoxicity data from other organism groups is needed urgently. Furthermore, ecotoxic data on disinfectants are important for obtaining permission from the authorities to use them in full-scale applications, to ensure that disinfected effluents do not have toxic effects on the aquatic ecosystem. Moreover, the degradation kinetics of disinfectants depends on the water matrix in which they are applied, and hence the effect concentration (EC) may be influenced. In general, nominal concentrations of chemical compounds are used for interpreting toxicity results. However, it is known that PFA, PAA, ClO_2 and hydrogen peroxide degrade fast when employed for disinfection (Chhetri et al., 2015, 2014; DesiCSO, 2014; Hey et al., 2012). To our knowledge, most of the toxicity test results currently available for these disinfectants are based on nominal concentrations, and the degradation kinetics of disinfectant in the test medium is not available for interpreting the test results.

With the aim of providing quantitative environmental risk estimates for the use of chemical disinfectants for treating combined sewer overflows, the objective of this study was to evaluate comprehensively the ecotoxicity of the disinfectants PFA, PAA, ClO_2 and their degradation products hydrogen peroxide and chlorite. This was done by complementing already existing datasets with toxicity data for microbial and crustacean toxicity tests. Moreover, degradation kinetics of disinfectants and degradation products on the test medium was measured, to account for the fact that disinfectant concentration decreases over time.

2 Materials and methods

2.1 Chemicals

ABTS (2,200-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt), sodium chlorite and hydrogen peroxide (35% w/w) were all purchased from Sigma-Aldrich (Brøndby, Denmark). All chemicals were

of reagent grade. PAA solution containing 30–40% (w/w) of technical grade disinfectant was supplied by Sigma-Aldrich (Brøndby, Denmark). Chlorine dioxide was synthesized as described by Hey et al. (2012). In short, 400mL of demineralized water was mixed with 25 mL of 9% HCl and 7.5% $NaClO_2$. The reaction mixture was allowed to react overnight and was then diluted to 1000 mL with demineralized water. This resulted in an approximately 1 g/L chlorine dioxide solution, which was quantified using the method described below. PFA was prepared in two steps as described by Chhetri et al. (2014), before each experiment.

2.2 Chemical analysis

PFA and PAA concentrations were analyzed using the colorimetric method described by Chhetri et al. (2014), based on the selective oxidation of ABTS by PFA or PAA, without interference from hydrogen peroxide. Hydrogen peroxide was analyzed using the titanium oxide-oxalate colorimetric assay (Antoniou and Andersen, 2015), chlorine dioxide was measured using a Hach Lange test kit LCK 310 and chlorite concentration was measured using Ion Chromatography coupled with an IonPac AS14 analytical column (4 mm × 250 mm, Dionex) and an IonPac G14 guard column (4 mm × 50 mm, Dionex). The eluent consisted of 8 mM Na_2CO_3 and 1 mM $NaHCO_3$. Chlorite was quantified by a Jasco 870-UV (Japan) UV-detector at λ = 340 nm.

2.3 Bioassays

For each inhibition tests, five concentrations of PFA, PAA, ClO_2, H_2O_2 and chlorite were tested in two types of experiments: range finding test and a final test.

2.3.1 Microbial toxicity

The toxicity in relation to the photobacterium *V. fischeri* was measured with the commercial BioTox™ (AboatoxOy, Finland) assay kit. The tests were carried out in accordance with the ISO 11348-3 (2007) test method. Each test consisted of five concentrations with two replicates and two control replicates without adding any test chemical. Prior to the assay, the pH of all samples was adjusted to 7.0 ± 0.2 with 1M NaOH or 1M H_2SO_4 solutions. NaCl was added to obtain a final chloride concentration of 20 g/L (2% w/v) in the samples.

After mixing 100 µL of test solutions with 100 µL luminescent bacterial suspensions, light emission was measured after 5, 15 and 30 min contact time at a temperature of 15°C. Relative inhibition at 5, 15 and 30 min was calculated on the basis of controls to which no test compound was added.

2.3.2 Crustaceans immobilization test

The immobilization tests with the crustacean *D. magna* were performed using the method and testing conditions prescribed by ISO 6341 (2012). The strain of *D. magna* used in this study originated from specimens captured in Langedammen, Birkerød, Denmark, in 1978 and has been cultured in the laboratory since that time. Tests with *D. magna* neonates (less than 24 h old) were carried out at 20±2°C in the dark. Each test consisted of five concentrations with four replicates and four control beakers, without adding any testing chemical. In each replicate, 25 mL of testing solution was placed in 100 mL glass beakers and five neonates were added. The number of immobile *D. magna* neonates was counted after 2 h, 6 h, 18 h, 24 h and 48 h of incubation with the test solutions. Animals were counted as dead, if they remained settled at the bottom of the test container and did not swim within 15 s of observation. Mortality values were given as the percentage of dead *D. magna* neonates compared to the initial number of animals added. The control group was used to ensure that no mortality occurred in beakers without the addition of the test compounds.

2.3.3 Statistical analyses of bioassays

Effect concentrations (EC) with 95% confidence intervals for the inhibition of *V. fischeri* were estimated using the statistical program LOG457. A concentration-response curves were fitted by non-linear regression assuming a logarithmic-normal distribution of data. The ToxCalc™ v5.0 program was used to calculate the acute toxicity of *D. magna*. Effect concentrations with 95% confidential intervals were calculated using the probit model along with linear regression by maximum-likelihood estimation (Tidepool Scientific).

3 Results and discussion

3.1 Toxicity values

Effect concentrations (EC_{10} and EC_{50}) of the disinfectants and degradation products obtained from *V. fischeri* and *D. magna* toxicity are presented in Table 1. Concentration response curves corresponding to these EC values at different exposure times are presented in the supporting information (Figure S1 and S2). Among the tested compounds, ClO_2 was the most toxic to *D. magna*, followed by chlorite and PAA (Table 1). However, with respect to microbial toxicity after 30 min of incubation, PFA showed the highest toxicity level followed by PAA. Chlorite is the degradation product of ClO_2, and it is worth noting that it is significantly less toxic to the photobacterium *V. fischeri* than ClO_2, i.e. 28 times less toxic when comparing EC_{50} values (Table 1). However, chlorite was found to be 86 times more toxic to *D. magna* than to *V. fischeri* when comparing EC_{50} values (Table 1).

Table 1: Effect concentration (EC_{10} and EC_{50}) for V. fischeri at 30 min contact time and effect concentration (EC_{10} and EC_{50}) for D. magna at 48 h contact time of disinfectants (PFA, PAA and ClO_2) and their degradation products (H_2O_2 and ClO_2^-). EC_{10} and EC_{50} of P. subcapitata at 72 h contact time of PFA, PAA, ClO_2, H_2O_2 and ClO_2^- was derived from Chhetri et al.*(2017b). *All concentrations are in mg/L and are based on nominal concentrations. 95% confidence intervals are shown in parenthesis.*

Organism	Endpoint	Disinfectant chemicals			Degradation products	
		PFA	PAA	ClO_2	H_2O_2	ClO_2^-
Vibrio fischeri	EC_{10}	0.18 (0.13-0.23)	0.27 (0.26-0.27)	0.09 (0.04-0.24)	1.06 (0.52-2.16)	5.80 (3.63-9.25)
	EC_{50}	0.24 (0.21-0.27)	0.42 (0.41-0.44)	1.10 (0.66-1.84)	5.67 (4.20-7.65)	30.93 (24.96-38.33)
Daphnia magna	EC_{10}	0.59 (0.35-0.73)	0.53 (0.28-0.66)	<0.02	2.59 (1.79-3.01)	0.07 (0.01-0.18)
	EC_{50}	0.85 (0.67-0.98)	0.78 (0.59-0.95)	<0.09	3.46 (2.97-3.96)	0.36 (0.11-0.72)
*Pseudokirchneriella subcapitata**	EC_{10}	0.19 (0.12-0.39)	0.23 (0.10-0.53)	0.06 (0.05-0.07)	1.78 (1.63-1.94)	0.59 (0.59-0.60)
	EC_{50}	0.34 (0.29-0.39)	1.38 (0.96-1.99)	0.16 (0.15-0.17)	2.90 (2.87-2.92)	1.10 (1.10-1.11)

The influence of a longer incubation time on the effect concentrations (EC_{50}) of disinfectants PFA, PAA and ClO_2, and their degradation products H_2O_2 and chlorite on *V. fischeri*, is presented in Figure 1. Longer incubation

times would be expected to lead to higher toxicity, and this is also the pattern found for PFA, ClO_2 and chlorite. For PAA and H_2O_2, it was found that toxicity decreased (i.e. the EC_{50} values increased) in line with increasing exposure time. This might be due to the reaction of the catalase enzyme present in *V. fischeri* cells that decomposes hydrogen peroxide into water and oxygen.

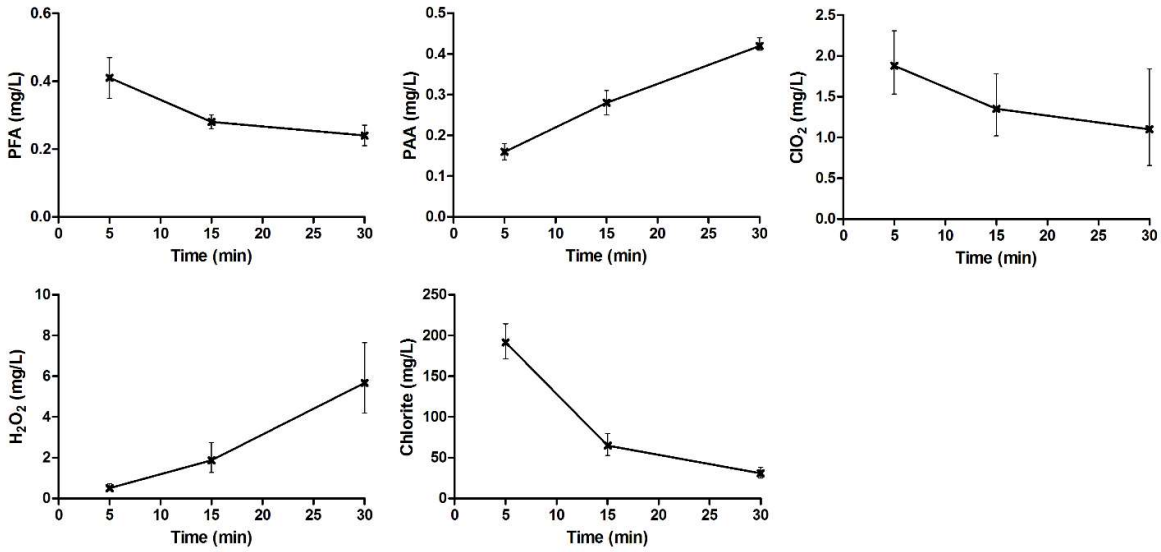


Figure 1: Median effect concentration (EC_{50}) for V. fischeri measured at different time intervals for the disinfectants performic acid (PFA), peracetic acid (PAA) and chlorine dioxide (ClO_2), and their degradation products hydrogen peroxide (H_2O_2) and chlorite (ClO_2^-). Error bars indicate 95% confidence intervals of results at each tested concentration. Note: Different scales on the primary axes.

Effect concentrations (EC_{50}) of PFA, PAA, ClO_2, H_2O_2 and chlorite to *D. magna* at different exposure times are presented in Figure 2. Full concentration-response curves were obtained at all observation time points (i.e. 2, 6, 24, 48 hours), and although the EC_{50} values for PFA, PAA and H_2O_2 were at their highest after 2 hours of incubation, the observed toxicity was almost stable over the exposure time. Instant responses of *D. magna* were observed when the animals were exposed to PFA, PAA and H_2O_2; however, low toxicity was observed after 2 h of contact time in the toxicity test. For ClO_2 and chlorite, toxicity in relation to *D. magna* increased in line with increasing exposure time. Mortality in the ClO_2 test increased by a factor of three during the first 24 h. Similarly, the toxicity of chlorite increased 16 times in the observation period from 2 h to 48 h.

In general, literature data on the aquatic toxicity of the tested disinfectants and their degradation products are limited and somewhat contradictory. A study conducted by Liu et al. (2015), for instance, found lethal concentration (LC_{50}) values for *D. magna* exposed to PAA formulations with different PAA:H_2O_2 ratios ranging from 0.18 -0.77 mg/L. The lowest LC_{50} values were observed for the highest PAA:H_2O_2 ratios (i.e. when less H_2O_2 was present in the PAA formulation). The *D. magna* $LC_{50,\ 48\ h}$ values of PAA, reported by ECETOC (2001), range from 0.35-1.1 mg/L, whereas Antonelli et al. (2009) found an LC_{50} value of 0.15 mg/L for PAA. For H_2O_2, ECETOC (2001) reported an $LC_{50,\ 24\ h}$ value of 7.7 mg/L for *D. magna*, which is two times higher than what was found in the present study. Mattei et al. (2006) reported a chlorine dioxide 24 h LC_{50} value of 0.02 mg/L for *D. magna*, which is 4.5 times lower than the LC_{50} (0.09 mg-ClO_2/L) observed in our study. Furthermore, the 24 h LC_{50} value for PAA of 0.03 mg/L observed by the same authors was 22 times lower than found in this study. For PAA toxicity on *V. fischeri*, Antonelli et al. (2009) reported an EC_{50} value of 0.13 mg/L, which is three times lower than we obtained.

Toxicity values (EC/LC_{50}) less than 1 mg/L provide the classification “Acute toxic 1”, i.e. very toxic to aquatic organisms according to CLP regulations (EU Commission, 2011). This means that PFA, PAA, ClO_2 and chlorite are considered as very toxic for aquatic organisms, whereas this is not the case for H_2O_2.

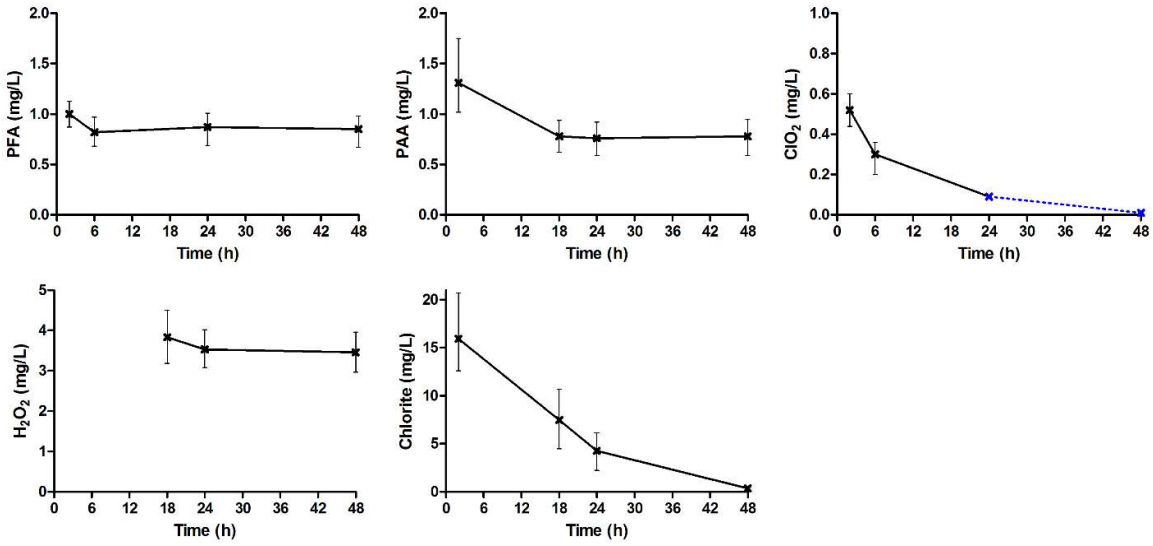


Figure 2: Effect concentration (EC_{50}) of D. magna at different time intervals from disinfectants performic acid (PFA), peracetic acid (PAA) and chlorine dioxide (ClO_2), and their degradation products hydrogen peroxide (H_2O_2) and chlorite (ClO_2^-). Error bars indicate 95% confidence interval (n=4) of results at each tested concentration. Note: different scales on the primary axes.

3.2 Concentration profiles in test media

Concentration profiles were obtained by measuring concentrations of disinfectants and degradation products over time in the media used for testing the toxicity of *D. magna* (Figure 3). Figure 3 shows these profiles measured at 0, 2, 6, 18, 24 and 48 h of incubation in the media, i.e. the same time points for which the mortality of *D. magna* was recorded. However, the lowest concentrations (0.16 and 0.3 mg/L) of PFA, PAA and chlorine dioxide in test media could not be quantified, since these were below the limit of quantification of the colorimetric assay. Hence, the concentration profiles are not shown in Figure 3.

A first-order degradation kinetics model described in equation 4 was used for curve fitting in Figure 3, with derived parameters presented in Table 2.

$$C_t = C_0 \cdot e^{-kt} \qquad \text{Equation 4}$$

In equation 4 C_t is the residual disinfectant concentration at time t, C_0 is the applied disinfectant dose, k is the rate constant and t is time. The disinfectants PFA and ClO_2 are known to be unstable in an aqueous solution (Chhetri et al., 2017a), and the fast degradation of these compounds has also been observed in *Daphnia* testing media (Figure 3). The complete degradation of PFA in all tested concentrations was observed after 12 h, whereas for ClO_2 complete degradation was not observed at the highest tested concentration (6 mg/L) after 48 h. No chlorite degradation occurred in the test media during the 48 h incubation, and only slow PAA and H_2O_2 degradation was observed. The half-lives of the disinfectants were 1.6 h on average for PFA, followed by 7.6 h for ClO_2 and 38 h for PAA, whilst the half-lives of the degradation product hydrogen peroxide was 38 h and chlorite was more than 100 h (Table 2). The findings are in agreement with the faster degradation of PFA compared to PAA, which was found when used to disinfect combined sewer overflows (Chhetri et al., 2015,

2014). Similarly, the fast degradation of chlorine dioxide was observed when it was used to remove pharmaceuticals in biologically treated wastewater (Hey et al., 2012).

Even though most of the PFA degraded during the first 12 h of the *Daphnia* toxicity test, the EC_{50} values observed from 6 h to 48 h did not change. This indicates that PFA was lethal for *D. magna* during the very first hours of contact, and due to the fast degradation (t½ 1.6h), no further mortality could be recorded. For PAA and H_2O_2, which did not completely degrade during the 48 h *Daphnia* toxicity test, the EC_{50} values were almost constant throughout the test period. This indicates a fast lethal action, with a sharply defined threshold, i.e. the animals can cope with some of the compounds without any mortality during 48 h (up to 0.3 mg/L for PAA and 1.0 mg/L for H_2O_2), but even a slight increase in concentration gives rise to mortality. This is shown quite clearly in the very steep concentration response curves showing similar patterns in toxicity for each compound at the different observation times (see Supporting Information). Therefore, it seems reasonable to base the data evaluation on nominal concentrations, i.e. for *D. magna* mortality tests there is no need to account for the degradation of the compounds with incubation periods up to 48 h.

For ClO_2, an increase in toxicity during the 48 h incubation was observed (as evidenced by the decreasing EC_{50} values show in Figure 3) despite almost complete degradation during the first 8 h. When testing the degradation product of ClO_2, i.e. chlorite, no degradation was found, but the trend in toxicity over time was similar to that of ClO_2 (see Figure 2). This indicates that although there is a clear effect of ClO_2 before it degrades, chlorite contributes to the toxicity observed in the experiment with ClO_2. It is therefore not straightforward to correct for the degradation of ClO_2, and it is reasonable to express toxicity results in terms of nominal ClO_2 concentrations.

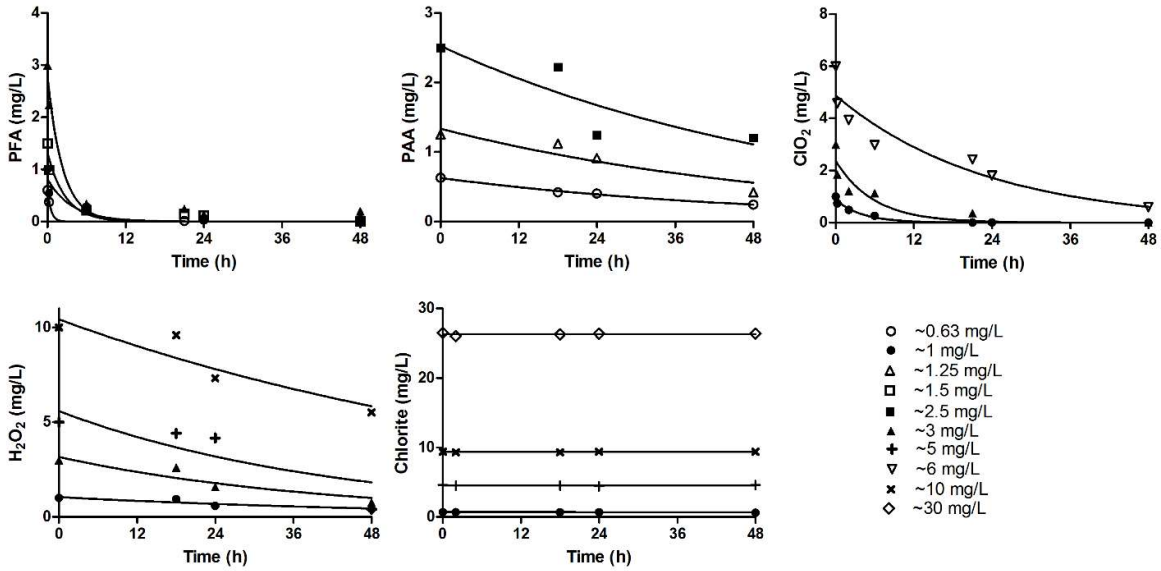


Figure 3: Concentration profiles of disinfectants (PFA, PAA and ClO_2) and their degradation products (H_2O_2 and Chlorite) in a Daphnia test medium. Symbols indicates nominal concentrations of disinfectants and their degradation products. Fitted curve is based on the first-order degradation kinetics model.

Table 2: Fitted parameters of concentration curves for concentration profiles in the Daphnia media shown in Figure 3.

Disinfectants	Nominal concentration (mg/L)	$C_{initial}$ (mg/L)	k (h^{-1})	R^2	$T_{½}$ (h)
PFA	0.6	0.6	1.9×10^{0}	0.75	0.4
	1	0.8	2.6×10^{-1}	0.86	2.6
	1.5	1.3	3.7×10^{-1}	0.94	1.9
	3	2.8	4.2×10^{-1}	0.97	1.6
PAA	0.6	0.6	2.0×10^{-2}	0.99	35.1
	1.3	1.3	1.8×10^{-2}	0.87	38.2
	2.5	2.5	1.7×10^{-2}	0.75	40.4
ClO_2	1	0.9	2.5×10^{-1}	0.97	2.8
	3	2.4	1.7×10^{-1}	0.83	4.1
	6	4.9	4.3×10^{-2}	0.88	16
H_2O_2	1	1.0	1.8×10^{-2}	0.81	38.9
	3	3.2	2.4×10^{-2}	0.86	29
	5	5.6	2.3×10^{-2}	0.71	29.7
	10	10.4	1.2×10^{-2}	0.85	57.4
Chlorite	1	0.7	2.8×10^{-3}	0.70	>100
	5	4.5	3.0×10^{-14}	N.A	>100
	10	9.4	$\sim 1.2 \times 10^{-16}$	N.A	>100
	30	26.3	$\sim 1.2 \times 10^{-16}$	N.A	>100

N.A=Not applicable

3.3 Indicative environmental risk evaluation for disinfectant application

The data generated in this study complement the datasets already existing in the literature for PFA, PAA and ClO_2. In the following section, an environmental risk evaluation of PFA, PAA , ClO_2, H_2O_2 and chlorite for CSO disinfection is performed based on the ecotoxicity data from this study and our previous study (Chhetri et al., 2017a) (Table 1), in which the concentrations of disinfectants and their degradation products were measured throughout the test period. In general, static ecotoxicity tests must be carried out under stable exposure conditions, i.e. where concentrations are maintained within 80-120% of the nominal concentration throughout the test period (OECD, 2000). Measured concentrations seem not to be reported in the remaining literature data, and so compound degradation (prior to or during testing) was not considered. For PFA and ClO_2, the data from this study and our previous study (Chhetri et al., 2017a) constitute the only data available for ecotoxicological evaluation. The starting point for the indicative environmental risk assessment of the

disinfectants and their degradation products in a CSO disinfection scenario is to estimate Predicted No Effect Concentration (PNEC). This was done in accordance with the instructions in the Technical Guidance Document for the Water Framework Directive (TGD-EQS, 2011). $PNEC_{freshwater}$ values of disinfectants and their degradation products were calculated by dividing the lowest EC_{50} value by assessment factors selected in accordance with the Technical Guidance Document (TGD-EQS, 2011). For all compounds, an assessment factor of 1000 was selected, since this is the largest permittable assessment factor (TGD-EQS, 2011) and only data from short-term toxicity tests at two different trophic levels were available. The PNEC values of PFA, PAA and ClO_2 were calculated by dividing the lowest EC_{50} values of the respective compounds in the dataset by an assessment factor (Table 3). According to the TGD-EQS (2011), PNEC values can be used as a basis for setting environmental quality standards (EQSs) with additional considerations of the potential for bioaccumulation and the persistency of the compounds. With logKow values far below 3 for PFA, PAA and hydrogen peroxide (log Kow at pH 7 of -1.63 for PFA, -0.52 for PAA and -1.57 for hydrogen peroxide), they do not have the potential for bioaccumulation, and so the risk of the secondary poisoning of predators in the aquatic ecosystem is very low. For PFA, PAA and H_2O_2, the fast degradation half-lives shown in Table 2 show furthermore that these compounds are not expected to be persistent in the aquatic environment. For ClO_2, rapid degradation was also observed in this study as well as in simulated CSO (DesiCSO, 2014); therefore, ClO_2 is also not expected to have the potential for bioaccumulation or persistence. As shown in Equation 1B, chlorine dioxide is reduced to chlorite during disinfection, and the degradation of chlorite is slower than the mother compound chlorine dioxide (Figure 3). Therefore, the predicted residual concentration of chlorite will be similar to the nominal concentration of chlorine dioxide. Like the mother compound, chlorine dioxide, chlorite is not expected to have the potential to bioaccumulate. Since none of the compounds is expected to be either bioaccumulative or persistent, PNEC values can be used directly as the overall EQS for water.

However, continuous exposure to disinfectants and their degradation products is not expected to occur in the receiving water bodies, since disinfection occurs only when a CSO event occurs. Furthermore, the data shown in Table 2 document the short half-lives of all compounds in water. The environmental quality standard of

relevance is hence the so-called "MAC-$QS_{fw,eco}$", i.e. the maximum allowable concentration for the freshwater ecosystem (TGD-EQS, 2011). The same dataset as above can be used to derive the MAC-$QS_{fw,eco}$, and since intermittent discharges are mainly believed to result in acute effects, a lower assessment factor can be used. In this case, an assessment factor of 100 was chosen, in accordance with the TGD-EQS (2011), and thus the MAC-$QS_{fw,eco}$ for all compounds was 10 times higher than the PNEC (see Table 3).

The disinfectant nominal concentration for CSO disinfection varies for different CSO structures, in line with variable CSO retention times. Moreover, disinfectant dose depends on the anticipated removal of indicator bacteria after disinfection. In disinfected effluents, residual disinfectants and their degradation products must be maintained lower than MAC-$QS_{fw,eco}$ when discharged into receiving waters, in order to avoid acute toxicity. This can be achieved either by destroying the residual disinfectants or by diluting the residual disinfectants. The dilution factors necessary to avoid toxic effects in receiving waters were calculated as the ratio between the predicted residual concentration (PRC), which is residual disinfectant concentration exposed to receiving waters after disinfection, and the MAC-$QS_{fw,eco}$ of disinfectants and their degradation products.

The PRCs illustrated in Table 2 are based on our previous studies on the full-scale disinfection of CSO in two different CSO structures. When 6 mg/L PAA was used 2 log of *Enterococcus* bacteria was inactivated leaving 0.58 mg/L of residual PAA in the effluent after 60 min contact time (Chhetri et al., 2016). Similarly, when 1-4 mg/L PFA was used, a 1-2.4 log of *Enterococcus* was inactivated, leaving 0.17-1.43 mg/L residual PFA in effluents after 20 min retention time (Chhetri et al., 2015). Similarly, ClO_2 residue was 0.04-0.63 mg/L after 20 min contact time when 5-15 mg/L ClO_2 was used to disinfect simulated CSO water (DesiCSO, 2014).

As shown in Table 3, the residual concentrations of PFA and PAA were higher than the MAC-$QS_{fw,eco}$ values for PFA and PAA. Therefore, in both cases, the dilution of disinfected effluents is required, in order to avoid the risk of toxic effects in the aquatic environment.

Table 3: Predicted No-Effect Concentrations (PNECs) for the freshwater, Environmental Quality Standards for water (EQS_{water}) and Maximum Allowable Concentration Quality Standards for the freshwater ecosystem ($MAC\text{-}QS_{fw, eco}$) for PFA, PAA, ClO_2, H_2O_2 and ClO_2^-. The individual dilution factors needed for an intermittent discharge not to be safe for a freshwater environment is the ratio between PRC and $MAC\text{-}QS_{fw, eco}$. All units are in µg/L.

	Disinfectant chemicals			Degradation products	
	PFA	**PAA**	**ClO_2**	**H_2O_2**	**ClO_2^-**
Predicted No-Effect Concentrations, PNEC	0.24	0.42	0.09	2.9	1.1
EQS_{water}	0.24	0.42	0.09	2.9	1.1
$MAC\text{-}QS_{fw, eco}$	2.4	4.2	0.9	29	11
Predicted Residual Concentration, PRC*	170-1430	880	40-630	N.A	N.A
Dilution factor (PRC/MAC-QS)	70-590	138	44-700	N.A	N.A

N.A= Not available

*The Predicted Residual Concentration (PRC) is the concentration of disinfectants after treating a combined sewer overflow event (from Chhetri et al., (2015) & DesiCSO, (2014)).

If dilution is the only process available to bring down the concentrations of PFA, PAA and ClO_2 in the receiving water after an intermittent discharge (i.e. a CSO event), maximum dilutions of 590 times, 138 times and 700 times, respectively, are needed. The PFA and PAA formulations have different PFA/PAA:H_2O_2 ratios with the varying toxicity values. When PFA and PAA is discharged and diluted, hydrogen peroxide is also diluted and released into the receiving waters post-disinfection. For PFA, rapid degradation (see Table 2), in practice, will mean that a lower dilution factor than 590 may also be safe for the receiving waters. Given the dilution factors shown in Table 3, it can be concluded that even though acute effects may occur at the initial point of discharge, due to the use of PFA and PAA as disinfectants, the relatively small dilution factors indicate that toxic effects will not occur after the initial dilution. ClO_2 showed the highest need for dilution, though it also has a relatively short half-life in water. This degradation leads to the formation of chlorite, but given the quite high MAC-EQ for chlorite (11 µg/L), this degradation product is not expected to lead to acute toxic effects in receiving waters after CSO disinfection with the mother compound (ClO_2).

4 Conclusion

In summary, this study presented ecotoxicity data on the alternative disinfectants PFA, PAA and ClO_2, and their degradation products hydrogen peroxide and chlorite, on three trophic levels in aquatic ecosystems.

Furthermore, a preliminary environmental risk evaluation of disinfectants and their degradation products was undertaken, and the results of the toxicity tests revealed that the disinfectants PFA, PAA and ClO_2 were more toxic than their degradation products. Among three disinfectants, ClO_2 was more toxic to *D. magna* and *P. subcapitata* than PFA and PAA. PFA showed higher toxicity to *V. fischeri* than PAA and ClO_2. PFA, PAA and hydrogen peroxide toxicity for *D. magna* was almost stable over the exposure time, whilst ClO_2 and chlorite toxicity increased with increasing exposure time. Complete degradation of PFA and ClO_2, partial degradation of PAA and hydrogen peroxide, and no degradation of chlorite were observed in the media used for *D. magna* toxicity testing.

An indicative environmental risk evaluation of disinfectants and their degradation products for disinfection of combined sewer overflows showed that of the three disinfectants, PFA needs a dilution factor of 70-590 times, PAA needs a dilution factor of 138 times and ClO_2 needs a dilution factor of 44-700 times, to avoid risk to the aquatic environment.

References

Antonelli, M., Mezzanotte, V., Panouillères, M., 2009. Assessment of peracetic acid disinfected effluents by microbiotests. Environ. Sci. Technol. 43, 6579–6584. https://doi.org/10.1021/es900913t

Antonelli, M., Rossi, S., Mezzanotte, V., Nurizzo, C., 2006. Secondary effluent disinfection: PAA long term efficiency. Environ. Sci. Technol. 40, 4771–4775. https://doi.org/10.1021/es060273f

Antoniou, M.G., Andersen, H.R., 2015. Comparison of UVC/S2O82− with UVC/H2O2 in terms of efficiency and cost for the removal of micropollutants from groundwater. Chemosphere 119, S81–S88. https://doi.org/10.1016/j.chemosphere.2014.03.029

Baldry, M.G.C., 1983. The bactericidal, fungicidal and sporicidal properties of hydrogen peroxide and peracetic acid. J. Appl. Bacteriol. 54, 417–423.

Bayo, J., Angosto, J.M., Gómez-López, M.D., 2009. Ecotoxicological screening of reclaimed disinfected wastewater by Vibrio fischeri bioassay after a chlorination-dechlorination process. J. Hazard. Mater. 172, 166–171. https://doi.org/10.1016/j.jhazmat.2009.06.157

Boczek, L. a, Johnson, C.H., Meckes, M.C., 2010. Chlorine disinfection of blended municipal wastewater effluents. Water Environ. Res. 82, 2373–2379. https://doi.org/10.2175/106143010X12681059117175

Chhetri, R.K., Baun, A., Andersen, H.R., 2017a. Algal toxicity of the alternative disinfectants performic acid (PFA), peracetic acid (PAA), chlorine dioxide (ClO2) and their by-products hydrogen peroxide (H2O2) and chlorite (ClO2−). Int. J. Hyg. Environ. Health 220. https://doi.org/10.1016/j.ijheh.2016.11.011

Chhetri, R.K., Baun, A., Andersen, H.R., 2017b. Algal toxicity of the alternative disinfectants performic acid (PFA), peracetic acid (PAA), chlorine dioxide (ClO2) and their by-products hydrogen peroxide (H2O2) and chlorite (ClO2−). Int. J. Hyg. Environ. Health 220, 570–574. https://doi.org/10.1016/j.ijheh.2016.11.011

Chhetri, R.K., Bonnerup, A., Andersen, H.R., 2016. Combined Sewer Overflow pretreatment with chemical coagulation and a particle settler for improved peracetic acid disinfection. J. Ind. Eng. Chem. 37, 372–379. https://doi.org/10.1016/j.jiec.2016.03.049

Chhetri, R.K., Flagstad, R., Munch, E.S., Hørning, C., Berner, J., Kolte-Olsen, A., Thornberg, D., Andersen, H.R., 2015. Full scale evaluation of combined sewer overflows disinfection using performic acid in a sea-outfall pipe. Chem. Eng. J. 270, 133–139. https://doi.org/10.1016/j.cej.2015.01.136

Chhetri, R.K., Thornberg, D., Berner, J., Gramstad, R., Ojstedt, U., Sharma, A.K., Andersen, H.R., 2014. Chemical disinfection of combined sewer overflow waters using performic acid or peracetic acids. Sci. Total Environ. 490, 1065–1072. https://doi.org/10.1016/j.scitotenv.2014.05.079

DesiCSO, 2014. Udredning og sammenligning af mulige aktivstoffer for kemisk desinfektion af

kloakoverløbsvand [WWW Document]. URL http://www.vandibyer.dk/31782 (accessed 5.6.15).

ECETOC, 2001. Joint Assessment of Commodity Chemicals (JACC) report No. 40. Peracetic Acid (CAS No. 79-21-0) and its Equilibrium Solutions.

ECHA, 2008. Guidance on information requirements and chemical safety assessment Chapter R.10: Characterisation of dose [concentration]-response for environment.

Emmanuel, E., Keck, G., Blanchard, J.-M., Vermande, P., Perrodin, Y., 2004. Toxicological effects of disinfections using sodium hypochlorite on aquatic organisms and its contribution to AOX formation in hospital wastewater. Environ. Int. 30, 891–900. https://doi.org/10.1016/j.envint.2004.02.004

EU Commission, 2011. Regulation (EU) No 286/2011 10.03.2011. Amending Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures.

Falsanisi, D., Gehr, R., Santoro, D., Dell'Erba, A., Notarnicola, M., Liberti, L., Dell'Erba, A., Notarnicola, M., Liberti, L., 2006. Kinetics of PAA demand and its implications on disinfection of wastewaters. Water Qual. Res. J. Canada 41, 398–409.

FRODO, 2014. Forbedret rensning og desinfektion af overløbsvand [WWW Document]. URL http://www.udviklingssamarbejdet.dk/en/projekter/frodo

Gehr, R., Chen, D., Moreau, M., 2009. Performic acid (PFA): Tests on an advanced primary effluent show promising disinfection performance. Water Sci. Technol. 59, 89–96. https://doi.org/10.2166/wst.2009.761

Hey, G., Ledin, A., Jansen, J.L.C., Andersen, H.R., 2012. Removal of pharmaceuticals in biologically treated wastewater by chlorine dioxide or peracetic acid. Environ. Technol. 33, 1041–1047. https://doi.org/10.1080/09593330.2011.606282

Hofmann, R., Andrews, R.C., Ye, Q., 1999. Impact of Giardia Inactivation Requirements on ClO2 By-Products. Environ. Technol. 20, 147–158. https://doi.org/10.1080/09593332008616804

Hrudey, S.E., Charrois, W.J., 2012. Disinfection By-products and Human Health. IWA Publishing, London United Kingdom.

ISO, 11348-3, 2007. Water quality -- Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) -- Part 3: Method using freeze-dried bacteria [WWW Document]. URL http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=40518 (accessed 5.26.16).

ISO, 6341:2012, 2012. Water quality -- Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) -- Acute toxicity test [WWW Document]. URL http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=54614 (accessed 5.26.16).

Kaiser, K.L.E., 1998. Correlations of Vibrio fischeri Bacteria Test Data with Bioassay Data for Other Organisms. Environ. Health Perspect. 106.

Kitis, M., 2004. Disinfection of wastewater with peracetic acid: A review. Environ. Int. 30, 47–55. https://doi.org/10.1016/S0160-4120(03)00147-8

Koivunen, J., Heinonen-Tanski, H., 2005. Peracetic acid (PAA) disinfection of primary, secondary and tertiary treated municipal wastewaters. Water Res. 39, 4445–4453. https://doi.org/10.1016/j.watres.2005.08.016

Korn, C., Andrews, R.C., Escobar, M.D., 2002. Development of chlorine dioxide-related by-product models for drinking water treatment. Water Res. 36, 330–342. https://doi.org/10.1016/S0043-1354(01)00194-4

Lee, Y.-J., Kim, H.-T., Lee, U.-G., 2004. Formation of chlorite and chlorate from chlorine dioxide with Han river water. Korean J. Chem. Eng. 21, 647–653.

Liberti, L., Notarnicola, M., 1999. Advanced treatment and disinfection for municipal wastewater reuse in agriculture. Water Sci. Technol. 40, 235–245. https://doi.org/10.1016/S0273-1223(99)00505-3

Liu, D., Straus, D.L., Pedersen, L.-F., Meinelt, T., 2015. Comparison of the Toxicity of Wofasteril Peracetic Acid Formulations E400, E250, and Lspez to Daphnia magna, with Emphasis on the Effect of Hydrogen Peroxide. N. Am. J. Aquac. 77, 128–135. https://doi.org/10.1080/15222055.2014.976682

Luukkonen, T., Heyninck, T., Rämö, J., Lassi, U., Ramo, J., Lassi, U., 2015. Comparison of organic peracids in wastewater treatment: Disinfection, oxidation and corrosion. Water Res. 85, 275–285. https://doi.org/10.1016/j.watres.2015.08.037

Mattei, D., Cataudella, S., Mancini, L., Tancioni, L., Migliore, L., 2006. Tiber River Quality in the Stretch of a Sewage Treatment Plant : Effects of River Water or Disinfectants To Daphnia and Structure of Benthic Macroinvertebrates Community. Water. Air. Soil Pollut. 177, 441–455. https://doi.org/10.1007/s11270-006-9183-1

Nurizzo, C., Antonelli, M., Profaizer, M., Romele, L., 2005. By-products in surface and reclaimed water disinfected with various agents. Desalination 176, 241–253. https://doi.org/10.1016/j.desal.2004.11.012

OECD, 2000. Guidance document on aquatic toxicity testing of difficult substances and mixtures.

Ragazzo, P., Chiucchini, N., Piccolo, V., Ostoich, M., 2013. A new disinfection system for wastewater treatment: Performic acid full-scale trial evaluations. Water Sci. Technol. 67, 2476–2487. https://doi.org/10.2166/wst.2013.137

Svecevicius, G., Šyvokiene, J., Stasiunaite, P., Mickeniene, L., Syvokiene, J., Stasiŭnaite, P., 2005. Acute and

chronic toxicity of chlorine dioxide (ClO2) and chlorite (ClO2-) to rainbow trout (Oncorhynchus mykiss). Environ. Sci. Pollut. Res. 12, 302–305. https://doi.org/10.1065/espr2005.04.248

TGD-EQS, 2011. Technical Guidance for deriving Environmental quality standards, Guidance Document number 27. Common Implementation Strategy for the Water Framework Directive (2000/60/EC).

USEPA, 2001. U.S. EPA HPV Chemical Challenge Program Test Plan for the Formates Category [WWW Document]. URL http://www.epa.gov/hpv/pubs/summaries/formates/c13438tp.pdf (accessed 5.14.14).

Veschetti, E., Cittadini, B., Maresca, D., Citti, G., Ottaviani, M., 2005. Inorganic by-products in waters disinfected with chlorine dioxide. Microchem. J. 79, 165–170. https://doi.org/10.1016/j.microc.2004.10.017

Wagner, M., Brumelis, D., Gehr, R., 2002. Disinfection of Wastewater by Hydrogen Peroxide or Peracetic Acid : Development of Procedures for Measurement of Residual Disinfectant and Application to a Physicochemically Treated Municipal Effluent. Water Environ. Res. 74, 33–50.

Wang, X.H., Yu, Y., Huang, T., Qin, W.C., Su, L.M., Zhao, Y.H., 2016. Comparison of Toxicities to Vibrio fischeri and Fish Based on Discrimination of Excess Toxicity from Baseline Level. PLoS One 11, e0150028. https://doi.org/10.1371/journal.pone.0150028

Watson, K., Shaw, G., Leusch, F.D.L., Knight, N.L., 2012. Chlorine disinfection by-products in wastewater effluent: Bioassay-based assessment of toxicological impact. Water Res. 46, 6069–6083. https://doi.org/10.1016/j.watres.2012.08.026

White, G.C., 2010. Handbook of chlorination and alternative disinfectants, 5th ed. John Wiley & Sons, Inc., New York.