CHARACTERIZATION OF TOTAL DISSOLVED SOLIDS (TDS) TOXICITY TO Ceriodaphnia dubia ASSOCIATED WITH EFFLUENT DISCHARGES FROM A MEAT PACKAGING INDUSTRY

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ABSTRACT

Characterization of total dissolved solids (TDS) toxicity of effluent discharges from a meat packaging company was determined with Ceriodaphnia dubia 3-brood toxicity tests. Results showed that chloride was the primary toxic element of TDS in the effluent. However, other ions such as sulfate and sodium contributed to TDS toxicity, especially when they were in an excessive amount. C. dubia was able to tolerate TDS as high as 2943.55 and 1314.76 mg/l based on acute and chronic effects, respectively. These findings indicate that TDS is not a good regulatory predictor for toxicity.

Keywords: TDS, chloride, major ion toxicity, chronic toxicity test, Ceriodaphnia dubia

1. INTRODUCTION

Total dissolved solids (TDS) are common components in meat packaging industries due to the abundant use of salts (i.e., NaCl) during processing. Toxicity related to TDS is mainly due to specific combinations and concentrations of contributing ions such as sodium, potassium, magnesium, chloride, sulfate, nitrate, and bicarbonate. The correlation between increasing TDS concentration and toxicity may vary with different ionic combinations. Therefore, TDS concentration is not a good predictor for toxicity. However, some regulatory authorities have set TDS limits for many effluents.

Because cations or anions are not present as individual constituents, it must be considered that TDS effects in the effluents are caused by combinations of ions. Understanding the effects of various ions is fundamental for predicting and characterizing TDS toxicity. For example, Na+ is not the major contributor to aquatic toxicity, but the associated anion Cl− is more toxic than Na+(3).

A variety of approaches can be used to characterize TDS toxicity. For example, conductivity can be used as a general screening tool. According to the United States Environmental Protection Agency (U.S. EPA), when conductivity exceeded 1,000 μmhos/cm, TDS may become a concern for Ceridaphnia dubia chronic toxicity. Furthermore, U.S. EPA Phase I Toxicity Identification Evaluation (TIE) can provide useful information. If the results from Phase I TIE of an effluent with high conductivity do not significantly reduce or eliminate toxicity, the concentrations of ions in the effluent may be responsible for the toxicity and should be further evaluated. Using synthetic water, which mimics the major ions in the effluent under evaluation, have also been useful to assess TDS toxicity.

The choice of the test organism used for the study is of considerable importance when measuring TDS toxicity. Several studies have indicated that C. dubia and Daphnia pulex are the most sensitive test species to sodium chloride. Furthermore, C. dubia was also sensitive to many types of pollutants that made them the best candidate for toxicity testing and as recommended species in standard methods. As a result, C. dubia was chosen as the test species for this study.

The objective of this study was to characterize TDS toxicity of effluent discharges from a meat packaging industry to C. dubia survival and reproduction. To achieve this objective, several effluents and artificial test waters were tested using standard toxicity test procedures. The results of these tests were used to calculate correlations between contributing ions and toxicity. Results of the calculations were then used to predict primary toxicants or ions in the effluent that were responsible for acute and chronic toxicity to C. dubia.

2. MATERIALS AND METHODS

2.1. Test organisms

The cladocerans, Ceridaphnia dubia, were obtained from Aquatic Biosystems, Fort Collins, CO, and were cultured according to U.S. EPA methods.
2.2. Effluent sampling, preparation of synthetic water, and acute and chronic toxicity tests

Nine composite and grab samples of effluents from the meat packaging company were collected at the end of pipe zone from December 2004 until March 2006. The preliminary samples were used as a screening test for acute and chronic toxicity, whereas the remaining effluent samples and synthetic waters were used for regular chronic toxicity tests. For the screening tests, two acute and two chronic tests were performed. In addition, limited Phase I TIE studies were employed in order to evaluate metal and chlorine contributions to the effluent toxicity. Ethylenediamine tetraacetic acid (EDTA) was used during Phase I TIE to evaluate metal toxicity, while sodium thiosulfate was used to evaluate chloride toxicity. Three different concentrations of EDTA (2, 4, and 6 mg/l) were used for the test based on EPA recommendations. Sodium thiosulfate (Na2S2O3·5H2O) was added at 0.5, 1, and 2 times the theoretical equivalency concentration (TEC) required for chlorine removal. The TEC was calculated to be 2 moles of sodium thiosulfate for 1 mole of chloride, or 7.0 mg/l of sodium thiosulfate hydrate per 1 mg/l chlorine. The amount of sodium thiosulfate added to the effluent was based on chloride concentrations in the effluent.

The standard method 2002.0 was used for acute toxicity testing with *C. dubia*. The method measured the acute toxicity of effluents by exposing *C. dubia* to five different concentrations of effluents (6.25%, 12.5%, 25%, 50%, and 100%) and one standard control in a static non-renewal system for 48 hours. Moderately-hard reconstituted water known as American Society for Testing and Materials (ASTM) water with hardness of 100 mg CaCO3/l and alkalinity of 60 mg CaCO3/l was used for standard control and dilution water. Preparation and chemical composition of the ASTM water is listed in Table 1. Nanopure water, produced from filtration of distilled water through a Barnstead Nanopure Water Purification System, was used to make solutions. These tests were conducted in a controlled environmental room maintained at water temperature of 25±1 °C, and a 16-h light/8-h dark photoperiod. During the light period (16-h), the light was maintained at ambient levels. Neonates less than 24-h of age were used to initiate tests. Six neonates were placed in each chamber. Five replicates (i.e., total 30 organisms) were employed for each test concentration. Test organisms (*C. dubia*) were housed in 30-ml polystyrene containers (Plastic Inc., St. Paul, MN, USA). Food consisted of algae suspension (*P. subcapitata*) and yeast-trout chow-cherophyll leaves (YTC) given daily. Survival and reproduction were measured each day for seven days (i.e., through three-broods). Tests were deemed acceptable if control survival was at or above 80% and the average reproductive output within control groups was ≥15 neonates.

In addition to the ASTM water, city tap water and receiving waters of a small stream system were also used for the study as comparative controls. Both city tap water and receiving waters were collected as grab samples. The effluent was mimicked using synthetic waters and was then used for characterizing the TDS toxicity. The synthetic waters were prepared to have TDS and chlorine concentrations similar to the effluent, which were around 2500 and 500 mg/l, respectively. Preparation for synthetic waters is presented in Table 2.

2.3. Toxicity confirmation with MgCl2

Because chloride concentrations in the effluent were constantly higher than other ionic TDS constituents, toxicity confirmation with MgCl2 was performed to test chloride toxicity at low TDS concentrations. Chloride concentrations were set from 62.50 mg/l to 1000 mg/l. Standard *C. dubia* chronic toxicity test was performed according to U.S. EPA method mentioned above. ASTM water was used as control and dilution water for the test.

2.4. General water quality

Water samples for each test were monitored for temperature, pH, ammonia, dissolved oxygen (DO), alkalinity, hardness, conductivity, bicarbonate, chlorine, major ions, metals, and total dissolved solids (TDS). Measurements of the first three parameters were conducted with an expandable ion-analyzer (Orion Research Model EA920). DO was measured with an oxygen meter (YSI model 51A). Water hardness and alkalinity were analyzed with the EDTA and the bromocresol green-methyl red titrimetric procedures, respectively. Conductivity was measured with a conductivity meter (Amber Science Model 604), while total chloride concentrations were measured with a DPD colorimeter (Hach Model DR100).
Table 1. Preparation of American Society for Test and Materials (ASTM) water

<table>
<thead>
<tr>
<th>Water Type</th>
<th>NaHCO₃</th>
<th>CaSO₄·2H₂O</th>
<th>MgSO₄</th>
<th>KCl</th>
<th>pH</th>
<th>Hardness</th>
<th>Alkalinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mod Hard</td>
<td>96.0</td>
<td>60.0</td>
<td>60.0</td>
<td>4.0</td>
<td>7.4 - 7.</td>
<td>80 - 100</td>
<td>60 - 70</td>
</tr>
</tbody>
</table>

1) U.S. EPA, 2002 (5)
2) Expressed as mg CaCO₃/l

Nanopure water was used as solvent, all chemicals were reagent grade.

Table 2. Preparation of synthetic test waters. Type A, B, and G used dechlorinated city tap water as the solvent, others used nanopure water. (mg/l reagent added to the solvent)

<table>
<thead>
<tr>
<th>Water Type</th>
<th>NaCl</th>
<th>MgSO₄·7H₂O</th>
<th>CaSO₄</th>
<th>MgCl₂·6H₂O</th>
<th>KCl</th>
<th>KNO₃</th>
<th>Na₂SO₄</th>
<th>NaNO₃</th>
<th>CaCO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type-A</td>
<td>82.43</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>909.12</td>
</tr>
<tr>
<td>Type-B</td>
<td>247.30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>681.84</td>
</tr>
<tr>
<td>Type-C</td>
<td>800</td>
<td>100</td>
<td>500</td>
<td>50</td>
<td>10</td>
<td>200</td>
<td>500</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Type-D</td>
<td>700</td>
<td>500</td>
<td>1,000</td>
<td>50</td>
<td>10</td>
<td>200</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Type-E</td>
<td>750</td>
<td>200</td>
<td>1,000</td>
<td>150</td>
<td>50</td>
<td>100</td>
<td>200</td>
<td>1,500</td>
<td>-</td>
</tr>
<tr>
<td>Type-F</td>
<td>700</td>
<td>100</td>
<td>1,000</td>
<td>150</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Type-G</td>
<td>-</td>
<td>1,000</td>
<td>1,000</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Type-H</td>
<td>800</td>
<td>1,000</td>
<td>1,500</td>
<td>-</td>
<td>50</td>
<td>200</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Bicarbonate was calculated from alkalinity according to the equation in the Standard Methods (11). Major cations and metal concentrations were measured using inductively coupled plasma-optical emission spectroscopy (Varian VISTA-MPX CCD Simultaneously ICP-OES), major anions were measured using ion chromatography (Dionex Model ICS 2500 IonPac AS18 with AG18 guard column). TDS were measured and calculated based on methods published in the Standard Methods (11). A total of 15 ml from a well-mixed sample was filtered through glass fiber filter (PALL, Ann Arbor, MI), and washed with three successive nanopure water rinses. Filtrates were collected in pre-weighed evaporating dishes and then oven dried at 180°C for at least one hour, cooled in a desiccator, and weighed. The cycle of drying, cooling, desiccating, and weighing was repeated until a constant weight was obtained or until weight loss was less than 0.5 mg of previous weight. The TDS (mg/l) then calculated based on the Equation 1.

\[(A-B) \times 1000 = \frac{\text{sample volume, ml}}{B} \times 1000\]  

where \(A = \text{weight of dried residue + dish (mg)}\)  
\(B = \text{weight of dish (mg)}\)

2.5. Data analysis

Concentrations of cations and anions resulting in 10% (IC10), 25% (IC25), and 50% (IC50) inhibition of reproduction and resulting in 50% mortality (LC50) were calculated using interpolation p-percent inhibition concentration (ICp) software version 2.0, 1993 (U.S. EPA Environmental Research Laboratory, Duluth, MN). Significant differences in survival between controls and treatment groups were analyzed with Fisher’s exact tests (5), while significant differences in cation and anion concentrations between controls and treatment groups were tested with analysis of variance (ANOVA), followed by the Tukey honest significant difference (HSD) test using Statistica® software (StatSoft, Tulsa, OK, USA). Correlation between cation and anion concentrations versus survival and reproduction was calculated using Sigmaplot® Version 8.0 (Systat Software, Point Richmond, CA).
3. RESULTS AND DISCUSSION

3.1. Preliminary study

There was no toxicity observed for the acute test, since all animals survived at all effluent concentrations. However, test results showed that there was some toxicity observed for reproduction (Fig. 1). The IC10, IC25, and median inhibition concentration (IC50) were calculated at 1.88, 5.09, and 69.57% effluent, respectively (Table 3). Significant ($p<0.05$) reduction of C. dubia reproduction started to appear at 6.25% effluent concentration (Fig. 1). Toxicity identification evaluation (TIE) Phase I study with EDTA and sodium thiosulfate revealed no significant differences between treated and untreated effluent. Based on these preliminary results, the toxicity test for toxicity characterization and comparison among water types would be performed using chronic toxicity procedures.

### Table 3. Total dissolved solids (TDS) and major ion concentrations in controls, effluent, and synthetic waters

<table>
<thead>
<tr>
<th>Water Type</th>
<th>Conductivity (mhos/cm)</th>
<th>TDS (mg/l)</th>
<th>Cl (mg/l)</th>
<th>SO$_4^{2-}$ (mg/l)</th>
<th>PO$_4^{3-}$ (mg/l)</th>
<th>NO$_3^{-}$ (mg/l)</th>
<th>Ca$^+$ (mg/l)</th>
<th>Na$^+$ (mg/l)</th>
<th>Mg$^{2+}$ (mg/l)</th>
<th>K$^+$ (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASTM$^1$</td>
<td>322</td>
<td>213.87</td>
<td>1.75</td>
<td>87.96</td>
<td>U</td>
<td>U</td>
<td>11.40</td>
<td>18.67</td>
<td>11.44</td>
<td>2.03</td>
</tr>
<tr>
<td>DTW$^2$</td>
<td>1960</td>
<td>1314.76</td>
<td>172.12</td>
<td>602.58</td>
<td>U</td>
<td>U</td>
<td>13.57</td>
<td>373.31</td>
<td>7.64</td>
<td>5.52</td>
</tr>
<tr>
<td>SW$^3$</td>
<td>1713</td>
<td>1259.54</td>
<td>249.47</td>
<td>176.87</td>
<td>U</td>
<td>U</td>
<td>9.72</td>
<td>63.78</td>
<td>114.98</td>
<td>30.27</td>
</tr>
<tr>
<td>Effluent$^4$</td>
<td>3281</td>
<td>2458.85</td>
<td>552.06</td>
<td>377.63</td>
<td>143.70</td>
<td>149.75</td>
<td>29.58</td>
<td>404.28</td>
<td>15.82</td>
<td>58.19</td>
</tr>
<tr>
<td>Type-A</td>
<td>2336</td>
<td>1394.67</td>
<td>333.62</td>
<td>575.60</td>
<td>U</td>
<td>U</td>
<td>73.64</td>
<td>500.38</td>
<td>40.82</td>
<td>16.05</td>
</tr>
<tr>
<td>Type-B</td>
<td>2666</td>
<td>1628.45</td>
<td>509.11</td>
<td>568.63</td>
<td>U</td>
<td>U</td>
<td>90.52</td>
<td>591.62</td>
<td>58.09</td>
<td>17.17</td>
</tr>
<tr>
<td>Type-C</td>
<td>3132</td>
<td>2039.78</td>
<td>600.13</td>
<td>739.86</td>
<td>U</td>
<td>U</td>
<td>33.33</td>
<td>267.37</td>
<td>15.27</td>
<td>70.24</td>
</tr>
<tr>
<td>Type-D</td>
<td>3235</td>
<td>2233.11</td>
<td>549.13</td>
<td>797.88</td>
<td>U</td>
<td>U</td>
<td>32.42</td>
<td>164.83</td>
<td>172.77</td>
<td>54.97</td>
</tr>
<tr>
<td>Type-E</td>
<td>3448</td>
<td>2315.56</td>
<td>574.93</td>
<td>701.71</td>
<td>U</td>
<td>U</td>
<td>95.52</td>
<td>370.83</td>
<td>405.32</td>
<td>14.97</td>
</tr>
<tr>
<td>Type-F</td>
<td>4249</td>
<td>2834.00</td>
<td>575.69</td>
<td>1237.55</td>
<td>U</td>
<td>U</td>
<td>29.01</td>
<td>316.87</td>
<td>12.98</td>
<td>163.19</td>
</tr>
<tr>
<td>Type-G</td>
<td>3449</td>
<td>2849.34</td>
<td>172.89</td>
<td>1633.49</td>
<td>U</td>
<td>U</td>
<td>1.06</td>
<td>242.90</td>
<td>104.68</td>
<td>22.14</td>
</tr>
<tr>
<td>Type-H</td>
<td>3965</td>
<td>2934.55</td>
<td>546.03</td>
<td>1280.22</td>
<td>U</td>
<td>U</td>
<td>27.88</td>
<td>225.88</td>
<td>199.09</td>
<td>27.48</td>
</tr>
</tbody>
</table>

$^1$Standard control using American Society for Testing and Materials (ASTM) water with hardness of 100 mg CaCO$_3$/l
$^2$Dechlorinated tap water (DTW) used for comparative control 1 using dechlorinated city tap water
$^3$Stream water (SW) used for comparative control 2
$^4$Effluent from a meat packaging company

U = Undetected (under detection limit)

Type A – H = Artificial (synthetic) waters

**Figure 1.** Correlations between meat packaging effluent concentrations and C. dubia reproduction determined in whole effluent toxicity (WET) tests. Results are given as means. Asterisks denote statistically significant different from control ($p<0.05$). The arrow shows the reproduction of control animal.
3.2. Conductivity, total dissolved solid (TDS) and major ion concentrations

Conductivity, total dissolved solids (TDS) and major ion concentrations of the effluent, control waters, and mimicked effluent (synthetic waters) are listed in Table 3. High conductivity (3251 \( \mu \)mhos/cm) and high TDS concentration in the effluent (2583.85 mg/l) were mainly due to high chloride, sodium, and sulfate ions. These ions were very low in the standard control (ASTM water), but relatively high in comparative controls 1 and 2. Sulfate was the dominant anion in comparative control 1, while chloride was the dominant anion in comparative control 2 (Table 3). Both of them had similarly high concentration of sodium. All the synthetic waters had considerably high concentrations of chloride, sodium, and sulfate, similar to the effluent (Table 3).

3.3. Toxicity characterization and comparison among water types

All artificial water and effluent affected C. dubia reproduction significantly (p<0.50). However, none of them affected survival (Fig. 2). Total dissolved solids (TDS) strongly correlated with C. dubia reproduction (Fig. 3). Significant reduction of C. dubia reproduction began at a TDS concentration of 1394.67 mg/l (Fig. 3). The IC10, IC25, and median-inhibition concentration (IC50) were calculated at 1213.46, 1328.32, and 1435.81 mg/l TDS, respectively (Table 3). Chloride strongly correlated with C. dubia reproduction (Fig. 4). The IC10, IC25, and median-inhibition concentration (IC50) were calculated at 95.35, 171.37 and 455.07 mg Cl/l, respectively (Table 4). Sulfate and sodium were unlikely causes of reduction in C. dubia reproduction due to weak correlations between the two ions and C. dubia reproductive output (Fig. 5 and 6).

3.4. Toxicity confirmation with MgCl\(_2\)

Toxicity confirmation with MgCl\(_2\) having low TDS values showed that chloride level at 500 mg/l and above significantly reduced C. dubia reproduction (Fig. 7). The chloride non-observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) to C. dubia were 250 and 500 mg/l, respectively, while its chronic value (CV) was 353.55 mg/l (Fig. 7). The IC10, IC25, and median-inhibition concentration (IC50) were calculated at 226.87, 328.30 and 444.24 mg Cl/l, respectively (Table 4).

While the effluent was not acutely toxic to C. dubia, it affected C. dubia chronically by reducing its neonate production by 50% (IC50) at 69.57% effluent concentration (Table 4). Phase I TIE showed no significant differences between treated and untreated effluent indicating that the toxicity was more likely caused by TDS than other toxicants such as metals or chlorine. Furthermore, high conductivity values (i.e., 3280.71 \( \mu \)mhos/cm) also indicated problems due to TDS toxicity. Additional tests with synthetic waters showed that TDS concentrations of ≥1394.67 mg/l significantly affected C. dubia reproduction (Fig. 3). Strong correlations between TDS and C. dubia reproductive output (Fig. 3) gave further indication of TDS role in toxicity. Several studies have documented that TDS was the main cause of toxicity in many effluents\(^\text{1,3,12}\). However, most of them indicated that the primary contributor for TDS toxicity was the combinations and concentrations of its ionic constituents\(^\text{1,3,12}\).

The present study indicated that some major ions (i.e., chloride, sulfate, and sodium) were constantly higher in the effluent. These ions were suspected as the primary contributor of TDS toxicity of the effluent. Correlation between major ion concentrations and C. dubia reproductive output indicated that chloride was the primary contributor ion of TDS toxicity. It was shown by its stronger correlation (\( r^2 = 0.66 \)) compared to sulfate and sodium with correlation coefficient (\( r \)) of 0.23 and 0.10, respectively (Fig. 4, 5, and 6). Additional tests with synthetic waters showed that chloride concentrations above 500 mg/l significantly reduced C. dubia reproduction (Fig. 4).

Table 4. Comparison of 7-day IC10, IC25, and IC50 between effluent, total dissolved solid (TDS), and chloride on C. dubia reproduction.

<table>
<thead>
<tr>
<th>Concentration (IC)</th>
<th>% Inhibition</th>
<th>Effluent(^1)</th>
<th>TDS</th>
<th>Chloride(^2)</th>
<th>Chloride(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% (IC10)</td>
<td>1.88 ± 0.23</td>
<td>1213.46 ± 143.98</td>
<td>95.35 ± 23.35</td>
<td>226.87 ± 78.62</td>
<td></td>
</tr>
<tr>
<td>25% (IC25)</td>
<td>5.09 ± 2.45</td>
<td>1328.32 ± 9.45</td>
<td>171.37 ± 3.94</td>
<td>328.30 ± 22.07</td>
<td></td>
</tr>
<tr>
<td>50% (IC50)</td>
<td>69.57 ± 8.81</td>
<td>1435.81 ± 35.29</td>
<td>455.07 ± 29.86</td>
<td>444.24 ± 25.80</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Average TDS and chloride concentrations in a full strength effluent were 2583.85 and 552.06 mg/l, respectively.

\(^2\)Result from test with synthetic water with high total dissolved solid (TDS) concentrations.

\(^3\)Result of confirmation test with MgCl\(_2\) with low total dissolved solid (TDS) concentrations.
Figure 2. *C. dubia* reproduction (bars) and survival (line) toxicity test results for controls and synthetic waters. Results are given as means with standard deviation. Asterisks denote statistically significant different from standard control (p< 0.05). Water type-1 is the standard control using American Society for Testing and Materials (ASTM) water with hardness of 100 mg CaCO3/l; type-2 is dechlorinated tap water (DTW) used for comparative control-1; type-3 is stream water (SW) used for comparative control-2; type-4 is effluent from a meat packaging company; types A – H are artificial (synthetic) waters.

Figure 3. Correlations between total dissolved solid (TDS) and *C. dubia* reproduction. Results are given as means. Asterisks denote statistically significant different from control (p< 0.05). The arrow shows the reproduction of control animal.

This result was confirmed by the MgCl₂ tests, which showed that even with low TDS concentration (i.e., 806 mg/l), chloride concentration of 500 mg/l reduced *C. dubia* production by 62.59% (Fig. 7). However, results from two test waters with chloride concentration less than 500 mg/l (Type-A and G) still reduced *C. dubia* reproduction. These waters were apparently had excessive amounts of other ions, such as sulfate and sodium (Fig. 2 and 4). Although chloride was suspected as the primary toxic element of TDS toxicity, other ions also might contribute to toxicity, especially when they were present in excessive amounts.
The results that chloride was toxic to cladocerans (i.e., *C. dubia*) supported previous studies. Birge et al. reported that the non observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) of chloride to *Daphnia pulex* were 314 and 441 mg/l, respectively, and the chronic value (CV) was 372 mg/l. These values were very similar to the NOEC, LOEC, and CV values for *C. dubia* in the present study, which were 250, 500, and 353.55 mg/l, respectively (Fig. 7). Lower chloride CV value for *C. dubia* compared to *D. pulex* could indicate that *C. dubia* was more sensitive to chloride than *D. pulex*. U.S. EPA adopted the report by Birge et al. to be used in National Criteria for chloride, which set a maximum four-day average chloride concentration of 230 mg/l for freshwater ecosystems.

Figure 4. Correlations between chloride concentrations and *C. dubia* reproduction. Chloride concentrations were measured from tests of controls, effluent, and synthetic waters. Results are given as means. Asterisks denote statistically significant different from control (p< 0.05). The arrow shows the reproduction of control animal.

Figure 5. Correlations between sulfate concentrations and *C. dubia* reproduction. Sulfate concentrations were measured from tests of controls, effluent, and synthetic waters. Results are given as means. Asterisks denote statistically significant different from control (p< 0.05). The arrow shows the reproduction of control animal.
Figure 6. Correlation between sodium concentrations and *C. dubia* reproduction. Sodium concentrations were measured from tests of controls, effluent, and synthetic waters. Results are given as means. Asterisks denote statistically significant different from control (p< 0.05). The arrow shows the reproduction of control animal.

\[
y = 16.12 - 0.01x \\
\text{r}^2 = 0.10
\]

Figure 7. Correlations between chloride on *C. dubia* reproduction. Chloride concentrations were measured from confirmation tests with MgCl$_2$. Results are given as means. Asterisks denote statistically significant different from control (p< 0.05). The arrow shows the reproduction of control animal.

\[
y = 30.14 - 0.03x \\
\text{r}^2 = 0.92
\]

**4. CONCLUSIONS**

The present study demonstrated that effluent from a meat packaging industry with high TDS concentration (i.e., averaging 2583.85 mg/l) was chronically toxic to *C. dubia*. However, no evidence was found that the effluent was acutely toxic to *C. dubia*. Additional tests with synthetic waters containing TDS up to 2943.55 mg/l observed no acute toxicity to *C. dubia* during 7-day tests. This result suggests that aquatic organisms may tolerate TDS concentrations far above the limit of 1000 mg/l set in some TDS regulatory permits\(^1\). Furthermore, as long as chloride concentrations are low, TDS concentrations as high as 1314.76 mg/l do not affect *C. dubia* reproduction. However, if TDS due to solely to potassium sulfate and potassium bicarbonate, a
concentration of 1314.76 mg/l would be acutely toxic to \textit{C. dubia}. This indicates that TDS is not a good regulatory element for effluent discharging permit. Characterization and regulation of the contributing ions in TDS is more important and relevant than TDS alone. For example, this study reported that media with chloride concentrations above 500 mg/l was chronically toxic to \textit{C. dubia} although TDS concentrations were below 1000 mg/l. Therefore, chloride may be better used for a regulatory element of effluent containing high TDS value.

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**REFERENCES**


