Chronic Toxicity of Dispersed Orange H-4RL to *Daphnia Magna*

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**Keywords:** Disperse orange S-4RL; Chronic Toxicity; Aquatic organism, Risk assessment.

**Abstract.** Chronic toxicity of disperse orange S-4RL (DO S-4RL), an azo dye, was assessed on *Daphnia magna* (*D. magna*) under standardized conditions of testing for 14 days. The results showed that parameters in terms of number of offspring per adult, number of broods per adult and longevity were decreased with the exposure concentration of DO S-4RL increasing, as well as body length, whereas days to first brood were affected not so apparently by DO S-4RL. Additionally, the intrinsic natural increase rate (*r*) was significant reduced when the concentration of DO S-4RL was 6 µg/L.

**Introduction**

Dye compounds have been increasingly used due to consumer demand for color diversity and resistance to light exposure, washing and perspiration. It is estimated that over $7 \times 10^5$ tons of synthetic dyes are produced worldwide per year [1]. Azo dyes exhibit a great variety of colors and represent more than 50% of all dyes annually produced [2,3]. Approximately 10-15% of the dyes are released into the environment. Many researches have found that azo dyes could induce several toxic effects, such as genotoxicity, mutagenicity, cytotoxicity and carcinogenicity. Nevertheless, due to different chemical structure, each dye must be studied individually. In addition, a large number of dyes, containing azo dyes, are discharged into water bodies via industrial effluents. Therefore, azo dyes are assumed to be toxic to aquatic organisms. For example, disperse orange S-4RL (DO S-4RL) is representative for azo dyes and there are more than 30 commercial products on the market. The dye has previously been shown to have a high acute toxicity to *D.magna* after 48h exposure [4].

In order to gain a better understanding of the potential effects of DO S-4RL, we evaluate the chronic toxicity of DO S-4RL using model organism *D.magna*. In present study, survival, growth and reproduction of *D.magna* during a 14-d exposure period under semistatic test condition would be detected. The results would be display the most sensitive endpoint(s) which could be used for aquatic environment risk assessment.

**Material and Method**

**Chemicals and Reagents**

An analytical standard of disperse orange S-4RL (CAS:12223-23-3, purity 100%) was obtained from JiHua Group (Hangzhou, China). The chemical structure is shown in Figure 1. Stock solutions were directly prepared in Milli-Q water without carrier solvent. Each test solution was diluted with the culture medium of *D.magna*.

![Figure 1 The structural formula of disperse orange H-4RL.](image)

**Test Organisms**

*D.magna* were originally obtained from the institute of hydrobiology, Chinese Academy of Sciences (Wuhan, China). The test organisms were maintained at 22±1 °C in M4 culture medium.
with photoperiod of 12 h/day and a density of < 50 animals per liter. The medium was renewed three times a week, and *D.magna* were fed daily with the alga *Scenedesmus obliquus*, which were cultured in the laboratory using a nutrient medium.

**Chronic Toxicity Assays**

The chronic effects of DO S–4RL on the reproductive output and length were assessed based on the OECD method [5]. Prior to testing, a sensitive test for *D.magna* to potassium dichromate (K$_2$Cr$_2$O$_7$) was performed as a positive control, and the LD$_{50}$ (24h) value was in the range of 0.6-1.7 mg/L[6].

Chronic testing was proceeded as follows. Neonates (6-24h) were raised individually in 100-mL beaker containing 40mL test solution for 14 days. Exposure concentrations were 1, 2, 6, 10, 14, 18 mg/L. Ten replicates for each treatment were performed. This was a static renewal test, and medium which comprised culture medium with *Scenedesmus obliquus* and pesticide was renewed everyday. Endpoints were length of body and reproduction in terms of the number of neonates per surviving organism, the days to first brood.

The intrinsic rate of natural increase ($r$) could be determined by the following equations:

\[
R_0 = \sum l_x m_x
\]

(1)

\[
T = \frac{\sum l_x m_x}{\sum l_x m_x}
\]

(2)

\[
r = \ln R_0 / T
\]

(3)

Where $l_x$ is the proportion of individuals surviving to age $x$, $m_x$ is the age-specific fecundity (number of neonates produced per surviving female at age $x$). Values of $r$ in this study were calculated based on 14-day test. It has been recommended as a superior laboratory toxicological endpoint compared to the acute mortality as it combines lethal and sublethal effects into one comprehensive parameter.

**Results and Discussion**

The results of sensitive test for *D.magna* were shown in table 1. Lethally rates of different concentration of K$_2$Cr$_2$O$_7$ on *D.magna* after 24 h were 15.0, 20.0, 45.0, 55.0 and 95.0%. Through probit analysis, LD$_{50}$-24h of K$_2$Cr$_2$O$_7$ on *D.magna* was calculated and the value was 1.16 mg/L. Therefore, the LD$_{50}$-24h was in accordance with the requirements of sensitivity of LD$_{50}$-24h *D.magna*, and the organism can be used for the toxicity of assessment.

<table>
<thead>
<tr>
<th>Concentration(mg/L)</th>
<th>Lethally rate (%)</th>
<th>Dose logarithm</th>
<th>Probit</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.48</td>
<td>15.0</td>
<td>-0.319</td>
<td>3.960</td>
</tr>
<tr>
<td>0.8</td>
<td>20.0</td>
<td>-0.097</td>
<td>4.160</td>
</tr>
<tr>
<td>1.2</td>
<td>45.0</td>
<td>0.079</td>
<td>4.870</td>
</tr>
<tr>
<td>1.6</td>
<td>55.0</td>
<td>0.204</td>
<td>5.130</td>
</tr>
<tr>
<td>2.4</td>
<td>95.0</td>
<td>0.380</td>
<td>6.640</td>
</tr>
</tbody>
</table>

Acute toxicity of DO S–4RL on *D.magna* has been determined in our previous study and 48h LD$_{50}$ value was 30 µg/L[4]. Exposure concentration of chronic toxicity test based on LD$_{50}$-48h in this study. The parameters in terms of sublethal effect, such as abnormal growth or/and reproduction were investigated. Most of the monitored parameters were affected by DO S–4RL at sublethal concentrations during the chronic test. Therefore, DO S–4RL indicated a higher toxicity in the chronic toxic test since toxicity generally tends to increase with increasing exposure time.
As shown in Table 2, longevity of *D. magna* was not affected (*P > 0.05*) by DO S-4RL at low concentration when compared to control, whereas it decreased with increasing concentration at 3, 4 and 6 µg/L. Similar to our finding, longevity was also reduced by chlordane at higher concentration for *C. dubia* after 14 d exposure and for *D. magna* after 21 d exposure [7]. Yet, longevity is not so sensitive as reproductive parameters.

In addition, no significant difference was found on body length in all treatment when compared to control (*P > 0.05*) (Table 2). However, slightly decrease was observed on length which decreased from 2.7±0.4 mm at 1 µg/L to 2.5±0.1 mm at 6 µg/L. Sanchez also detect negative correlation between body size of *D. magna* and exposure concentrations[8]. Similarly, length is not the most sensitive endpoint as well as longevity.

<table>
<thead>
<tr>
<th>Concentration (µg/L)</th>
<th>control</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longevity (d)</td>
<td>13±0.9</td>
<td>14±0.0</td>
<td>13±0.3</td>
<td>14±0.0</td>
<td>12±1.1</td>
<td>11±1.3</td>
<td>10±2.1</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>2.7±0.2</td>
<td>2.7±0.4</td>
<td>2.7±0.3</td>
<td>2.6±0.3</td>
<td>2.6±0.5</td>
<td>2.5±0.4</td>
<td>2.5±0.1</td>
</tr>
</tbody>
</table>

Values are means ± S.D.

The effects of DO S-4RL on the reproduction of *D. magna* were shown in Figure 2. Adult *D. magna* in 1, 1.5, 2, 3, 4 and 6 µg/L of DO S-4RL produced an average of 23.1, 20.0, 21.4, 19.3, 14, 13 and 12 live offspring per adult, respectively. A significantly decrease in number of offspring per adult was observed when the concentration was equal to or higher than 3 µg/L (*P < 0.05*). And at 6 µg/L, the number of offspring per adult reduced 48%. Many investigators have reported that reproduction was a more sensitive index of chronic pesticide toxicity to *D. magna* [9], as we found in present study. Number of broods per adult also declined in all treatments except when the *D. magna* exposed to 2 µg/L DO S-4RL. Significant reduction in number of broods per adult was detected when the concentration was equal to or higher than 3 µg/L. Days to first brood were not affected obviously when compared to control (*P > 0.05*). We can conclude that ‘time of first reproduction’ was not a good parameter to estimate the effect of DO S-4RL on *D. magna* population. Similarly, Yao et al. also found that days to first brood were not influenced by rac-metalaxyl in terms of statistical analysis[10]. Therefore, the results indicating that reproductive parameters in terms of number of offspring and number of broods per adults were more sensitive than reproductive parameter in terms of days to first broods.
Figure 2. Reproductive parameters evaluation of DO S-4RL on *D. magna* in chronic test (14 d): (A) No. of broods per adult; (B) No. of offspring per adult; (C) days to first brood.

The result of the intrinsic natural increase rate (*r*) at concentration from 1-6 μg/L are shown in Figure 3. Compared to control, *r* value showed a significant decrease only at 6 μg/L (*P* < 0.05), but for 1-4 μg/L treatments there were no profound change for *r* value. Many authors considered that *r* is not as sensitive as reproduction[11].

Figure 3. The effect of Disperse Orange H-4RL on the intrinsic rate of natural increase(*r*) of *D. magna*.

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**References**


