Effects of 2-Phenoxyethanol on cuttlefish Sepia officinalis L. (Cephalopoda: Sepiiidae)

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Abstract: Effects of 2-Phenoxyethanol (2-PhOH) on cuttlefish Sepia officinalis (L.) were investigated. The five concentrations (0.10, 0.15, 0.20 and 0.30 ml/L) of 2-PhOH were dissolved directly into 15 L transparency glass aquarium containing of 10 L continuously aerated seawater (pH 7.68; O₂ 6.8 mg/L, salinity 37‰ at 19.7°C). After 2-PhOH treatments, the cuttlefishes were transferred immediately to a polyester recovery tank with 450 L of well aerated seawater and ranged from 0.2-0.6 ml/L (Summerfelt and Smith, 1990; Guiderhus and Marking, 1987; Mattson and Riple, 1989; Josa et al., 1992; Hseu et al., 1996, 1997, 1998; Kaminski et al., 2001; Ortuño et al., 2002; Marsić et al., 2005; Tsantilas et al., 2006). However, scarce data are available about the effects of 2-PhOH in terms of appropriate anesthetic and its doses or its toxicity for cephalopods, especially the cuttlefish Sepia officinalis. The cuttlefish, S. officinalis is one of the most easily cultured cephalopods (Richard, 1971; Pascual, 1978; Boletzky and Hanlon, 1983; Forsythe and., 1994; Lee et al., 1998; Domingues et al., 2001a, 2001b, 2002, 2003a), and is a commercially important species throughout the world (Roper et al., 1984). Furthermore, it is highly adaptable to life in captive conditions (Forsythe et al., 1994; Domingues et al., 2001a, 2001b, 2002, 2003a, 2003b, 2005, 2006; Sykes et al., 2006; Şen, 2009). The animals are particularly difficult to handle as they are not only quick but also have a very sensitive skin. Even, due to its habit to grab and hold things, the animal is not easy to handle all along treatment. Records

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are needed on the toxicity or safety exposure times and concentrations of 2-Phenoxyethanol in aquaculture applications such as transportation, measuring or weighing and sorting of *S. officinalis*. Therefore, this study was, first, designed to evaluate effects of 2-PhOH on adult *S. officinalis*.

**MATERIALS AND METHOD**

A total of 52 specimens of *S. officinalis* (L., 1758), were captured off the Izmir Bay by trammel nets on April 2, 2012. The individuals were acclimatized in an open flow-through filtered seawater (pH 8.2, salinity 37 ± 0.2‰, dissolved O₂ 7 ± 0.5 mg/L, temperature 15.5 ± 0.5°C by Extech® DO700 Multiparameter instrument) system and cylindrical polyester tanks (450 L volume) were placed in the PhD. H. Okan KAMACI Aquaculture Investigation and Application Unit of the Fisheries Faculty of Ege University (Urla, Izmir, TURKEY) one month before the application. The specimens were fed ad libitum with low market price pieces of fish species (i.e. *Sardina pilchardus*, *Engraulis encrasicolus*) by hand. The following day, uneaten part or remains were removed by siphoning. Photoperiod was adjusted naturally.

The mean body weight of 30 cuttlefishes was 224.46 ± 56.20 g (n= 30; ANOVA, P > 0.05) were used in the experiment. The five concentrations (0.1, 0.15, 0.2, 0.25, and 0.3 ml/L of 2-PhOH) were selected, and also maximum exposure time was applied as 15 minutes. In order to determine the effects of 2-PhOH, six cuttlefishes were used individually in each dose of the agent. The 2-PhOH concentrations were dissolved directly into 15 L transparency glass aquarium containing 10 L of continuously aerated seawater (pH 7.68, O₂ 6.8 mg/L, salinity 37% at 19.7°C). After the treatments, cuttlefishes were instantly transferred to a polyester recovery tank with 450 L of well-aerated seawater, where they were observed in 48-h due to any mortality.

The criteria for anesthetic effects were evaluated to Seol et al. (2007) and where loss of sucking intensity under anesthesia (Stage A3) and recovery of regular breathing (R4). Anesthetizing the cuttlefish involved several stages, beginning with a change in body color (Stage A1) to the loss of sucking intensity (Stage A3), at which stages the specimen was immediately transferred to a recovery tank were considered. Recovery time was estimated from the point at which the cuttlefish recovered normal activity (Stage R3) and regular breathing (Stage R4).

One-way analysis of variance and Duncan’s multiple range tests were applied to determine the statistical significance of the differences among the induction time means and among the recovery means for the species, using the SPSS 15.0 package program. Furthermore, transformation to √x + 0.1 was applied when non-parametric statistical conditions occurring. Additionally, the survival rates of the groups were statistically tested by chi-square test. Level of significance was taken at P < 0.05.

**RESULTS**

The major point of this study was that 2-PhOH acted like toxic affects and did not anesthesia on the cuttlefish in these experimental conditions. At among 0.15 and 0.30 ml/L of 2-PhOH concentrations, more than 50% mortality occurred within 3-5 minutes. Toxic affect of 2-PhOH was increased by dosages and observed shorter than 5 minutes at 0.15 and 0.20 ml/L of 2-PhOH, and 3 minutes at 0.25 and 0.30 ml/L of 2-PhOH. On the other hand, at 0.1 ml/L of 2-PhOH concentration neither anesthetic affects nor mortality occurred on the cuttlefishes within the 15-minute treatment period. The percentages of survival rates were estimated at 100% for 0.10 ml/L, 33.3% for 0.15 and 0.20 ml/L or 16.7% for 0.25 and 0.30 ml/L of 2PhOH concentrations. There were significant differences among the survival rates of the treatments (P<0.05). Additionally, the recovery stages of *S. officinalis* were, first described, and the survivor cuttlefish recovered within 5 minutes and survived over 48 hours. The induction time and the recovery time among the trials were not significantly different (P>0.05). The 2-PhOH doses caused hyperactivity and trauma such as violently inking, sudden swimming movements, hit to the aquarium walls of itselfs, and trying to jumping out of the aquarium, etc, in the cuttlefishes. Affects of 2-PhOH treatments depend on exposure time and concentrations on *S. officinalis* were shown in the Table 1. Its noted that A3 criteria could not be shown at the Table 1, because the anaesthetic effects of 2-PhOH was not observed.

**DISCUSSION**

It is well known that 2-PhOH is a safely and effectively anesthetic at lower doses (0.2-0.6 ml/L) for fish (Guilderhus and Marking, 1987; Maylonas et al., 2005; Weyl et al., 1996; Josa et al., 1992; Hseu et al., 1996, 1997, 1998; Kaminski et al., 2001; Ortunõ et al., 2002; Maršic et al., 2005; Tsantilas et al., 2006), but it works for the cephalopod (e.g. *Eledone moschata*) at higher concentrations (1.2-1.6 ml/L) (Şen and Tanrıkul, 2009). On the other hand, it is recorded that anesthesia with 2-PhOH under controlled conditions, and its fatality or toxicity is mainly depends on the exposure time and the concentration (Şen and Tanrıkul, 2009). Additionally, Basaran et al. (2007) showed that the toxicity of 2-PhOH was clearly depended on the dose and exposure time on European sea bass, *Dicentrarchus labrax*, juvenile. Furthermore, in the current study, 2-PhO H did not run as an anesthetic even in the minimum concentrations (0.15 - 0.30 ml/L), and acted toxic on the adult *S. officinalis*.

Messenger et al. (1985) pointed out that magnesium chloride is an effective anesthetic and narcotizing agent for several cephalopods (e.g. *Sepia officinalis*, *Locolo forbesi*, *Alloleuthis subulata*, *Octopus vulgaris*, *Eledone cirrhosa*) at temperatures ranging from 13 to 22°C. Although the authors reported that achieving to the anesthesia of *S. officinalis* without any mortality and trauma, they could not determine to recovery stages for *S. officinalis*. 

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However, only two anesthesia stages (the chromatophores showed quickening waves of color change, and cessation of movement (death) also closing of the funnel) were observed during the study among the criteria described by Messeneger et al. (1985). Additionally, the recovery criteria (a-Recovery of activity; start of the arm movement following to the fin movement, but breathing is labored; b-Start of the chromatophores showed quickening waves of color change and originating of two dark spots on the posteriodorsal of the mantle; c- Start swimming and sink, brownish color on the body) were, first recorded in S. officinalis in the present study.

As reported by O'Dor et al. (1977), for squid, 2-3% urethane as an anesthetic agent, is effective in seawater, providing handling ability after only a few minutes' exposure and a recovery period of 3–15 minutes. Unfortunately, urethane is now considered unsuitable material because of its carcinogenic properties (Ross and Ross, 1999). It should be noted that both these materials cause initial hyperactivity, which can be traumatic. Moreover, the present results clearly showed that 2-PhOH cause hyperactivity and trauma in the cuttlefishes.

In conclusion, according to the present results, the 0.10 ml/L of 2-PhOH concentration did not cause any mortality or toxicity on S. officinalis within 48-hour. However, at the same dose, the individuals' body color became pale and monitored partial sedation, only. By the way, toxic effect of 2-PhOH was monitored at among concentrations 0.15 and 0.30 ml/L in this species. Finally, the current study demonstrated that 2PhOH could not be recommended for S. officinalis due to its inefficiency as an anesthetic and/or its toxicity. At the same time, it needs to more detailed studies should be performed related to physiological effects of 2-PhOH on cuttlefish.

**REFERENCES**


**Table 1**. Effects of 2-PhOH treatments on S. officinalis L depends on exposure time and concentrations.

<table>
<thead>
<tr>
<th>Description</th>
<th>Remarkable behaviour</th>
<th>0.15 ml/L</th>
<th>0.20 ml/L</th>
<th>0.25 ml/L</th>
<th>0.30 ml/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (sec)</td>
<td>Time (sec)</td>
<td>Time (sec)</td>
<td>Time (sec)</td>
<td>Time (sec)</td>
<td>Time (sec)</td>
</tr>
<tr>
<td>Change in body colour and activity</td>
<td>The chromatophores showed quickening waves of colour change, and start hyperactivity</td>
<td>63±12</td>
<td>41.7±33</td>
<td>5.5±1.0</td>
<td>43.8±4.8</td>
</tr>
<tr>
<td>Change in mantle cavity shape and colour</td>
<td>Shrinkage of body and fins, and colour becomes pale and darkish brown colour</td>
<td>168.8±111.6</td>
<td>183.2±56.6</td>
<td>16.3±9.2</td>
<td>66.2±22.5</td>
</tr>
<tr>
<td>Body spasm</td>
<td>Close the eyes and violently ejecting ink</td>
<td>256.7±205.8</td>
<td>194±57.8</td>
<td>82±35.6</td>
<td>78±22.2</td>
</tr>
<tr>
<td>Cessation of movement (death)</td>
<td>Become variegated of body colour, full blossomed the arms, contraction of the whole body, and also closing of the funnel</td>
<td>256.7±205.8</td>
<td>231.7±91.2</td>
<td>139.8±79.9</td>
<td>93.5±19.0</td>
</tr>
<tr>
<td>Recovery of activity</td>
<td>Recovery of activity; start of the arm movement following to the fin movement, but breathing is labored</td>
<td>96.7±34.1</td>
<td>271.8±235.6</td>
<td>11±24</td>
<td>-</td>
</tr>
<tr>
<td>Recovery of body colour</td>
<td>The start of the chromatophores showed quickening waves of colour change and originating of two dark spots on the posteriodorsal of the mantle</td>
<td>155.3±25.8</td>
<td>237.8±990.0</td>
<td>12±70.4</td>
<td>-</td>
</tr>
<tr>
<td>Recovery of regular swimming and colorization (R3)</td>
<td>Start swimming and go down, brownish colour of the body</td>
<td>292.5±6.9</td>
<td>106.7±165.7</td>
<td>230.3±16.8</td>
<td>30±73.5</td>
</tr>
</tbody>
</table>


