

## Effects of 2-Phenoxyethanol on cuttlefish *Sepia officinalis* L. (Cephalopoda: Sepiidae)

### 2-Fenoksietanolün sübye *Sepia officinalis* L. üzerine etkisi

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**Özet:** *Sepia officinalis* üzerine 2-fenoksietanolün (2-PhOH) etkisi incelenmiştir. 2-PhOH'ün farklı dozları (0,10; 0,15; 0,20; 0,25 ve 0,30 ml/L) 15 litrelik şeffaf cam akvaryumun içinde bulunan ve sürekli havalandırılan 10 litre deniz suyunda (pH 7,68; O<sub>2</sub> 6,8 mg/L; sıcaklık 19,7°C ve tuzluluk ‰37) çözündürülmüştür. 2-PhOH uygulamalarından sonra sübyeler hemen içinde 450 litre iyi havalandırılmış deniz suyu bulunan polyester ayılma tanklarına nakledilmiştir, burada 48 saat boyunca ölüm olup olmayacağı gözlenmiştir. Ortalama vücut ağırlığı 224,46 ± 56,20 gr (n=30) olan sübyelerden her deneme grubu için tek tek olacak şekilde 6 birey kullanılmıştır. Elde edilen sonuçlara göre, 0,10 ml/L 2-PhOH konsantrasyonunda ne anestetik etki nede ölüm gözlenmiştir, ama 0,15 ile 0,30 ml/L 2-PhOH konsantrasyonlarında %50'nin üzerinde ölüm olmuştur. Yaşama yüzdeleri 0,10 ml/L için %100, 0,15 ve 0,20 ml için %33,3 ve 0,25 ve 0,30 ml/L için %16,7 olarak hesaplanmıştır (P<0,05). Hayatta kalanlar 5 dakika içinde ayılmıştır ve 48 saatten fazla yaşamışlardır. Sonuç olarak, 2-PhOH anestetik olarak etkisiz oluşu ve toksisitesi nedeniyle *S. officinalis* için önerilmemektedir.

**Anahtar kelimeler:** 2-Phenoxyethanol, anestezi, sübye, *Sepia officinalis*, toksisite.

**Abstract:** Effects of 2-Phenoxyethanol (2-PhOH) on cuttlefish *Sepia officinalis* (L.) were investigated. The five concentrations (0.10, 0.15, 0.20, 0.25 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, O<sub>2</sub> 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, where they were observed in 48-h due to any mortality. Six cuttlefishes, the mean body weight was 224.46 ± 56.20 g (n=30) were used individually in each experiment. According to the present results, at 0.1 ml/L of 2-PhOH level neither anesthetic effects nor mortality occurred on the cuttlefish, but more than 50% mortality occurred between 0.15 and 0.30 ml/L of 2-PhOH concentrations. The percentages of survival were 100% for 0.10 ml/L, 33.3% for 0.15 and 0.20 ml/L, and 16.7% for 0.25 and 0.30 ml/L of 2-PhOH concentrations (P<0.05). The reminders recovered within 5 minutes and survived over 48 hours. Finally, the current study demonstrated that 2-PhOH could not be recommended for *S. officinalis* due to its inefficiency and toxicity.

**Keywords:** 2-Phenoxyethanol, anaesthesia, cuttlefish, *Sepia officinalis*, toxicity.

### INTRODUCTION

Anesthetic agents are widely used fisheries and aquaculture to immobilize animals for transport, vaccination, measuring or weighing, sorting and tagging, sampling for blood or gonadal biopsies, and collection of gametes, besides to comfortable handling, permit the performance of painful procedures and decrease stress (Le Bras, 1982; Summerfelt and Smith, 1990; Kreiberg and Powell, 1991; Gewick et al., 1999; Ross and Ross, 1999; Small, 2003). Several anesthetic such as MS222, 2-Phenoxyethanol, clove oil, etc. has been commonly used in fish or fisheries operations (Summerfelt and Smith, 1990; Guilderhus and Marking, 1987; Maylonas et al., 2005). Among them 2-Phenoxyethanol is considered very suitable for aquaculture practices because of its easy preparation, low price, rapid action, fast and uneventful recovery (Pucéat et al., 1989; Weyl et al., 1996) and bactericidal and fungicidal characteristics (Jolley et al., 1972). It is well recorded that the effective anesthetic concentrations of 2-PhOH in several of species of fish have been reported and ranged from 0.2-0.6 ml/L (Summerfelt and Smith, 1990;

Guilderhus and Marking, 1987; Mattson and Riple, 1989; Josa et al., 1992; Hseu et al., 1996, 1997, 1998; Kaminski et al., 2001; Ortunó et al., 2002; Maršič 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

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  $37 \pm 0.2\text{‰}$ , dissolved  $O_2$   $7 \pm 0.5$  mg/L, temperature  $15.5 \pm 0.5^\circ\text{C}$  by Extech® DO700 Multiparameter instrument) system and cylindrical polyester tanks (450 L volume) were placed in the Ph.D. 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  $224.46 \pm 56.20$  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,  $O_2$  6.8 mg/L, salinity  $37\text{‰}$  at  $19.7^\circ\text{C}$ ). 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  $\sqrt{x+0.1}$  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 Tannkul, 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 Tannkul, 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-PhOH 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*, *Loligo forbesi*, *Alloteuthis subulata*, *Octopus vulgaris*, *Eledone cirrhosa*) at temperatures ranging from 13 to  $22^\circ\text{C}$ . 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*.

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

Description	Remarkable behaviour	0.15 ml/L Time (sec)*	0.20 ml/L Time (sec)	0.25 ml/L Time (sec)	0.30 ml/L Time (sec)
<b>Change in body colour and activity</b>	The chromatophores showed quickening waves of colour change, and start hyperactivity	63±12	41.7±33	5.5±1.0	43.8±4.8
<b>Change in mantle cavity shape and colour</b>	Shrinkage of body and fins, and colour becomes pale and darkish brown colour	168.8±111.6	183.2±56.6	16.3±9.2	66.2±22.5
<b>Body spasm</b>	Close the eyes and violently ejecting ink	256.7±205.8	194±57.8	82±35.6	78±22.2
<b>Cessation of movement (death)</b>	Become variegated of body colour, full blossomed the arms, contraction of the whole body, and also closing of the funnel	256.7±205.8	231.7±91.2	138.8±79.9	93.5±19.0
<b>Recovery of activity</b>	Recovery of activity; start of the arm movement following to the fin movement, but breathing is labored	96.7±34.1	271.8±235.6	11±24	-
<b>Recovery of body colour</b>	The start of the chromatophores showed quickening waves of colour change and originating of two dark spots on the posteriodorsal of the mantle	155.3±25.8	237.8±990.6	128±70.4	-
<b>Recovery of regular swimming and colorization (R3)</b>	Start swimming and go down, brownish colour of the body	292.5±6.9	106.7±165.7	230.3±16.8	30±73.5

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 Messenger et al. (1985). Additionally, the recovery criteria (a-Recovery of activity; start of the arm movement following to the fin movement, but breathing was labored; b-Start of the chromatophores showed quickening waves of color change and originating of two dark spots on the posteriodorsal of 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.

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