Herpetological Review, 2006, 37(3), 303–304.

Effects of Tricaine Methanesulfonate (MS-222) Concentration on Anesthetization and Recovery in Four Plethodontid Salamanders

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Tricaine methanesulfonate (also known as MS-222, Tricaine, Metacaine, or ethyl *m*-amino-benzoate methanesulfonate) is a commonly used anesthetic for fish and amphibians. Previous research has shown that the pH of MS-222 solutions has an effect on anesthetic efficacy and physiological functions (Letcher 1992; Lowe 2004; Ohr 1975a,b; Robinson and Scadding 1983). Current recommendations are to buffer solutions of MS-222 to a neutral pH (Crawshaw 1993; Robinson and Scadding 1983; Ohr 1975a). MS-222 solutions can be buffered using $NaHCO_3$ (sodium bicarbonate; Cooper 2003), aqueous buffers such as dihydrogen potassium phosphate and sodium hydroxide (Lowe 2004), or can be titrated with NaOH (Ohr 1975a). Although MS-222 is widely used and the benefits of buffering solutions are well known, few studies elaborate on the effects of its concentration on amphibian anesthetization and subsequent recovery time (see Letcher 1992; Robinson and Scadding 1983). We assessed the effects of MS-222 (Sigma, catalogue number A5040-25G) concentration in aqueous solutions on the time to anesthetization and recovery. Four species of semi-aquatic, stream-breeding salamanders (*Desmognathus monticola*, *D. ocoee*, *D. quadramaculatus*, and *Eurycea wilderae*) were tested at three different concentrations (500 mg/L, 1000 mg/L, and 2000 mg/L).

Materials and Methods.—We used 45 salamanders per species for this experiment. Animals were housed with moist paper towels in plastic Gladware™ containers (15.5 × 15.5 × 5 cm) within an environmental chamber on a 12/12 light cycle at 20°C and 75% humidity. Depending upon the species and size of the salamanders, 2–10 animals were kept together in the same container. No salamander was held in the environmental chamber for more than 72 h prior to testing. Fifteen salamanders per species were tested at each of three experimental concentrations (500 mg/L, 1000 mg/L, and 2000 mg/L) of MS-222. Salamanders were tested only once, at one concentration to ensure independence between replicates. After testing, all salamanders were returned to the environmental chamber for at least 24 h before being returned to their stream of capture. Solutions were made using 0.250 g, 0.5 g, and 1.0 g of MS-222 dissolved in 500 ml of distilled water. Solutions had an instantaneous pH of 3.9–2.8, and were neutralized with varying amounts of sodium bicarbonate (0.4 g for 500 mg/L to 0.93 g for 2000 mg/L), added in small amounts while monitoring the pH with a pH meter (Oakton Instruments, product # 35624-33). Solutions were buffered to a pH of approximately 6.5 as the pH would continue to rise throughout testing.

To test the effects of concentration, salamanders were submerged in the MS-222 solution and the time to complete anesthetization was recorded. An animal was deemed anesthetized (the induction time, Robinson and Scadding 1982) when unable to right itself and reflexive and voluntary responses to gentle pinching of the limbs and tail with forceps ceased. Salamanders were then rinsed in pure distilled water and laid in a shallow dissecting tray with distilled water (partially submerging the animal). Salamanders were monitored with gentle pinching of the limbs until a response was detected. Two to three salamanders were tested in the MS-222 solution at a time, being replaced by others after induction. Solution mixtures were used repeatedly until the pH rose above 8. There was a 45 minute cut-off for induction, after which animals were removed from the MS-222 solution and omitted from the data set. The mean body mass, mean pH, mean temperature, mean induction time, mean time to recovery, and all standard errors were calculated. Analyses of covariance (ANCOVA, SPSS v.13) were run for each MS-222 concentration, comparing species to induction and recovery time, with mass as the covariate. All factors were log transformed. Tukey-Kramer's multiple comparisons tests were run to identify significant species differences based on induction and recovery times.

Results.—Induction time generally decreased and time to recovery increased with increasing MS-222 concentration (Table 1). The smaller salamanders, *D. ocoee* and *E. wilderae*, closely followed this pattern with little variation. The larger two species, *D. quadramaculatus* and *D. monticola*, exhibited the most variation in both induction and recovery times. Most notably, *D. quadramaculatus* had a substantially longer recovery time at 1000 mg/L than either the 500 or 2000 mg/L concentrations. It was found that species differences were highly significant ($P<0.001$) at all concentrations for both induction and recovery times. Tukey-Kramer's multiple comparisons showed extreme variability in species differences both between concentrations and between induction and recovery times. The covariate mass was significant for both the 500 and 2000 mg/L concentrations ($F_{1,57} = 6.39$, $P = 0.014$; $F_{1,54} = 7.84$, $P = 0.007$ respectively). Contrary to the low and high concentrations, mass was not significant in the 1000 mg/L concentration ($F_{1,59} = 0.095$, $P = 0.760$). Mass was also not significant in relation to recovery time for any MS-222 concentration, though was nearly significant in the anomalous 1000 mg/L concentration ($F_{1,59} = 3.62$, $P = 0.062$). We had 100% survival of all tested salamanders at all concentrations and no abnormal behavior or visible external impairments were incurred as a result of testing. The temperature of aqueous MS-222 solutions was held constant (mean temperature = 26.9°C, SE = 0.061, N = 173) and the pH was kept neutral (mean pH = 7.15, SE = 0.020, N = 173).

Discussion.—Increasing the concentration of MS-222 in a neutral solution led to shorter induction times and longer recovery times. These results correspond to those found by Robinson and Scadding (1983) and Letcher (1992). As concentration increased, variation in induction time within species generally decreased, while variation in recovery time generally increased. The larger salamander species, *D. quadramaculatus* and *D. monticola*, generally took longer to reach complete induction than the smaller *D. ocoee* and *E. wilderae* (Table 1). *Desmognathus monticola* was the most anomalous species tested, showing no consistent pattern between concentrations, and many individuals did not become inducted within the 45 minute cut-off (note sample sizes in Table 1). For its size, *D. ocoee* reached induction more slowly and re-

TABLE 1. Mean and standard error (±SE) estimates of body mass (g), induction time (min), time to recovery (min), pH, and temperature (°C) for each species in each MS-222 concentration (mg/L).

	Mass	Induction	Recovery	pH	Temperature
D. quadramaculatus					
500 (N = 14)	9.35 (±1.13)	14.04 (±2.19)	12.64 (±2.14)	7.29 (±0.10)	26.43 (±0.14)
1000 (N = 15)	9.71 (±1.09)	6.03 (±0.37)	21.99 (±1.02)	7.30 (±0.09)	27.00 (±0.00)
2000 (N = 15)	11.06 (±1.41)	3.10 (±0.18)	18.65 (±2.38)	6.88 (±0.02)	28.00 (±0.00)
D. monticola					
500 (N = 14)	3.46 (±0.50)	7.82 (±0.76)	6.95 (±0.74)	7.00 (±0.06)	26.43 (±0.23)
1000 (N = 15)	3.77 (±0.57)	5.13 (±0.82)	8.33 (±0.91)	7.11 (±0.06)	26.93 (±0.27)
2000 (N = 10)	4.61 (±0.57)	8.29 (±1.77)	7.43 (±0.95)	7.13 (±0.08)	27.00 (±0.33)
D. ocoee					
500 (N = 15)	0.82 (±0.99)	12.73 (±1.86)	3.67 (±0.24)	7.00 (±0.00)	26.00 (±0.00)
1000 (N = 15)	0.83 (±0.12)	4.60 (±0.27)	6.96 (±0.35)	7.30 (±0.00)	27.00 (±0.00)
2000 (N = 15)	0.67 (±0.09)	2.35 (±0.08)	9.72 (±0.78)	7.35 (±0.00)	28.00 (±0.00)
E. wilderae					
500 (N = 15)	0.61 (±0.10)	4.70 (±0.31)	8.50 (±0.36)	7.14 (±0.02)	26.60 (±0.13)
1000 (N = 15)	0.59 (±0.10)	2.36 (±0.12)	14.12 (±0.56)	7.35 (±0.09)	27.00 (±0.00)
2000 (N = 15)	0.71 (±0.07)	1.90 (±0.13)	21.54 (±1.15)	6.91 (±0.00)	26.00 (±0.00)

covered faster than expected in 500 mg/L. *Desmognathus quadramaculatus* also exhibited unexpected variation in its recovery time in the 1000 mg/L concentration.

Contrary to Lowe (2004), mass played a significant role in time to induction in two of the three concentrations (500 and 2000 mg/L), although mass was not significant in time to recovery in any concentration. But in accordance to Lowe (2004), species differences were most significant ($P<0.001$) and present in all concentrations for both induction and recovery times, but the inconsistency of significant differences between species attests to the variability within this system. The latter may be indicative of a phylogenetic variation (Lowe 2004), but other factors influencing the induction and recovery times of salamanders may include the time since last feeding, age, sex, and reproductive status.

It was our goal to provide information for practical and efficient use of MS-222. Researchers should choose the most appropriate concentration for their needs of handling or operating upon salamanders. At 1000 and 2000 mg/L induction times for all species were 6 minutes or less, making recovery time the most important consideration when selecting a concentration in which to anesthetize salamanders. If longer recovery times are required, animals can be left in MS-222 solutions for an extended period of time beyond the time of last response with no fatal effects (Robinson and Scadding 1983; pers. obs.), although this has not been explicitly tested. Higher concentrations of MS-222 may cause some physiological stress (Robinson and Scadding 1983) and should be avoided if possible.

Although all of these salamander species share similar life history characteristics and three are congeners, extensive variation was present. Preliminary testing of species' responses to MS-222 is recommended. For more control over the pH of a solution, we also recommend following buffering procedures described by Lowe (2004) or Robinson and Scadding (1983). Further, for extended anesthetization, intraceolomic injection may be preferred (Letcher 1992).

Acknowledgments.—All procedures were approved by the University of Missouri Animal Care and Use Committee (protocol #3951). Salamanders were collected under USFS permit number HIG 6493. We thank Highlands Biological Station for laboratory facilities, and Tim Altnether and John Crawford for assistance in collection of salamanders. Valued comments were provided by John Crawford, Andrew Cox, Thomas Pauley, Jason Lowe, and Deanna Olson.

LITERATURE CITED

COOPER, J. E. 2003. Urodela (Caudata, Urodela): Salamanders, Sirens. *In* Murray E. Fowler and R. Eric Miller (eds.), Zoo and Wild Animal Medicine, 5th ed., pp. 33–40. W. B. Saunders Company, Philadelphia, Pennsylvania.

CRAWSHAW, G. J. 1993. Amphibian Medicine. *In* Murray E. Fowler and R. Eric Miller (eds.), Zoo and Wild Animal Medicine, Current Therapy 3, pp. 131–139. W. B. Saunders Company, Philadelphia, Pennsylvania.

LETCHER, J. 1992. Intracelomic use of tricaine methanesulfonate for anesthesia of bullfrogs (*Rana catesbeiana*) and leopard frogs (*Rana pipiens*). Zoo Biol. 11:243–251.

LOWE, J. 2004. Rates of tricaine methanesulfonate (MS-222) anesthetization in relation to pH and concentration in five terrestrial salamanders. Herpetol. Rev. 35:352–354.

OHR, E. A. 1975a. Tricaine methanesulfonate – I. pH and its effects on anesthetic potency. Comp. Biochem. Physiol. C. 54:13–17.

———. 1975b. Tricaine methanesulfonate – II. Effects on transport of NaCl and H_2O. Comp. Biochem. Physiol. C. 54:19–22.

ROBINSON, M. E., AND S. R. SCADDING. 1983. The effect of pH on tricaine methanesulfonate induced anaesthesia of the newt *Notophthalmus viridescens*. Can. J. Zool. 61:531–533.