
ALFAXOLONE USE IN *Xenopus laevis*: COMPARISON OF IV, IM, IP AND WATER IMMERSION OF ALFAXOLONE WITH DOSES OF 18 MG/KG AND 18 MG/L

Eva Hadzima, DVM, MVDr,^{1*} Mark A. Mitchell, DVM, MS, PhD Dipl ECZM (Herpetology),² Zdenek Knotek, MVDr, PhD Dipl ECZM (Herpetology),³ Drury Reavill DVM, Dipl ABVP (Avian and Reptile & Amphibian),⁴ Dipl ACVP, Angelica Filip AHT,¹ Stacey Parker,¹ and Katherine Weston¹

¹Dewinton Pet Hospital, #450, 412 Pine Creek Rd, Dewinton, T0L0X0, AB, Canada; ²University of Illinois, 1008 W. Hazelwood Drive, Small Animal Clinic M/C 004, Urbana, IL 61802 USA; ³University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1/3, 612 42 Brno, Czech Republic; ⁴Zoo/Exotic Pathology Service, 2825 KOVR Drive, West Sacramento, CA 95605 USA

ABSTRACT

The purpose of this study was to evaluate the clinical and cardiopulmonary effects of Alfaxolone, a neurosteroid, as an anesthetic in *Xenopus laevis*. Forty male *X. laevis*, weighing between 26-76 g, were anesthetized at a dose of 18 mg/kg via IV, IM, IP injections and water immersion of 18 mg/L, with a 2-wk washout time between trials. Several vitals and reflexes were monitored at 10-min intervals over 180 min for each trial. Heart rate was evaluated by placing a Doppler probe on the ventral pectoral girdle or auxiliary region of *X. laevis* (Table 1). Respiratory rate was calculated by visually counting thoracic excursions and gular movements (Tables 2, 3). Escape reflex was evaluated by the attempt to move off of an open palm (Table 4). Righting reflex was monitored by placing the animal on its dorsum and counting the time required to correct its position (Table 5). Corneal reflexes were tested using a wet q-tip (Table 6). Superficial pain was examined by pinching the skin of the dorsal or ventral surface of the rear feet with a mosquito hemostat (Table 7) and deep pain was evaluated by pinching the third and fourth digits of the rear feet (Table 8). As per data analysis of each *X. laevis*, IV and IM injections were found to provide the best response to Alfaxolone, resulting in deep sedation for 1-3 hr. For most IP individuals, sedation lasted between 10-60 min. Alfaxolone immersion at 18 mg/L did not provide proper immobilization or anesthesia.

LITERATURE CITED

1. Chinnadurai S. 2014. Advances in amphibian clinical therapeutics. J Exot Pet Med, 23:51-54.
2. Gilron I, Coderre TJ. 1996. Preemprive analgesic effects of steroid anesthesia with alfaxolone in the rat formalin test. Anesthesiol, 84:572-579.
3. Green S. 2010. The Laboratory Animal Pocket Reference Series: The Laboratory *Xenopus* sp. Book, 1-156
4. Guenette SA, Beaudry F, Vachon P. 2008. Anesthetic properties of propofol in African clawed frogs (*Xenopus laevis*). J Am Assoc Lab Anim Sci, 47(5):35-38.



-
-
5. Guenette SA, Helie P, Beaudry F, et al. 2007. Eugenol for anesthesia of African clawed frogs (*Xenopus laevis*). *Vet Anaesth Analg*, 34(3):164-170.
 6. Hollis DM, Goetz FW, Roberts SB, et al. 2004. Acute neurosteroid modulation and subunit isolation of the γ -aminobutyric acidA receptor in the bullfrogs, *Rana catesbeiana*. *J Mol Endocrinol*, 32:921-934.
 7. Jones, K. 2012. Therapeutic review: alfaxalone. *J Exot Pet Med*, 21:347-353
 8. Jurox Pty Ltd. 2010. Alfaxan anaesthetic injection - technical notes. Rutherford, Australia, Jurox Pty Ltd.
 9. Kischinovsky M, Duse A, Wang T, Bertelsen MF. 2012. Intramuscular administration of Alfaxalone in red-eared sliders (*Trachemys scripta elegans*) – effects of dose and body temperature. *Vet Anaesth Analg*, 13-20.
 10. Lafortune M, Mitchell MA, Smith J. 2001. Evaluation of medetomidine, clove oil, and propofol for anesthesia of leopard frogs, *Rana pipiens*. *J Herpetol Med Surg*, 11(4):13-18.
 11. McMillan MW, Leece EA. 2011. Immersion and brachial/transcutaneous irrigation anaesthesia with alfaxalone in Mexican axolotl. *Vet Anaesth Analg*, 38: 619-623.
 12. Mitchell MA. 2009. Anesthetic considerations for amphibians. *J Exot Pet Med*, 18(1):40-49.
 13. Mitchell MA, Riggs S, Singleton B, et al. 2009. Evaluating the clinical and cardiopulmonary effects of clove oil and propofol in tiger salamanders (*Ambystoma tigrinum*). *J Exot Pet Med*, 18:50-56.
 14. Mitchell MA, Riggs SM, Singleton CB, Diaz-Figueroa O, Hale LK. 2009. Evaluating the clinical and cardiopulmonary effects of clove oil and propofol in tiger salamanders (*Ambystoma tigrinum*). *J Exot Pet Med*, 18(1):50-56.
 15. Orchinik M, Murray TF, Moore FL. 1994. Steroid modulation of GABAA receptors in amphibian brain. *Brain Res*, 646: 258-66.
 16. Posner LP, Bailey KM, Richardson EY, Harms CA. 2013. Alfaxalone anesthesia in bullfrogs (*Rana catesbeiana*) by injection or immersion. *J Zoo Wildl Med*, 44(4):965-971.
 17. Posner LP, Burns P. 2009. *Veterinary Pharmacology and Therapeutics Ninth Edition: Injectable anesthetic agents*. Ames, IA: Wiley-Blackwell, 265-300.
 18. Rasmussenn NJ, Rosendal T, Overgaard J. 1978. Althesin in neurosurgical patients: effects on cerebral hemodynamics and metabolism. *Acta Anaesthesiol Scan*, 22:257-269.
 19. Ross A, Guenette SA, Helie P, et al. 2006. Case of cutaneous necrosis in African clawed frogs, *Xenopus laevis*, after the topical application of eugenol. *Can Vet J*, 47(11):1115-1117.
 20. Smith JM, Stump KC. 2000. Isoflurane anesthesia in the African clawed frogs (*Xenopus laevis*). *Contemp Top Lab Anim Sci*, 39(6):39-42.
 21. Wright KM. 2001. *Amphibian Medicine and Captive Husbandry: Restraint techniques and euthanasia*. Malabar, FL: Krieger Publishing, 1:114-120.

Table 1. Heart rate of *X. laevis* when treated with 18mg/kg of Alfaxalone.

Time (min)	Median Heart Rate (BPM)	10-90%	Min-Max
0	36.0	24 – 55	6.0 – 60.0
10	41.0	24 – 60	12.0 – 72.0
20	38.0	26 – 48	12.0 – 65.0
30	36.0	24 – 50	6.0 – 60.0
40	36.0	28 – 48	12.0 – 60.0
50	40.0	28 – 56	12.0 – 72.0
60	38.0	28 – 48	6.0 – 60.0
70	36.0	26 – 48	6.0 – 60.0
80	40.0	28 – 54	6.0 – 60.0
90	40.0	32 – 54	12.0 – 60.0
100	42.0	32 – 54	18.0 – 60.0
110	40.0	31 – 54	6.0 – 60.0
120	40.0	30 – 48	10.0 – 60.0
130	40.0	30 – 52	16.0 – 64.0
140	40.0	28 – 52	18.0 – 62.0
150	40.0	29 – 53	12.0 – 60.0
160	40.0	31 – 50	12.0 – 60.0
170	41.0	31 – 51	6.0 – 66.0
180	40.0	31 – 57	12.0 – 60.0

Table 2. Respiratory rate *X. laevis* when treated with 18mg/kg of Alfaxalone.

Time (min)	Median Respiratory Rate (per min)	10-90%	Min-Max
0	20	12 – 36	6 – 42
10	12	8 – 26	2 – 45
20	12	6 – 24	2 – 36
30	12	5 – 20	2 – 32
40	12	4 – 20	2 – 30
50	12	6 – 24	2 – 48
60	12	6 – 24	2 – 48
70	12	6 – 24	2 – 28
80	12	6 – 24	2 – 48
90	12	6 – 24	2 – 36
100	16	6 – 24	2 – 48
110	16	6 – 24	4 – 36
120	12	6 – 26	1 – 54
130	12	6 – 29	4 – 40
140	12	6 – 29	2 – 48
150	16	7 – 28	2 – 42
160	16	12 – 24	2 – 36
170	16	12 – 24	6 – 36
180	16	12 – 24	6 – 36

Table 3. Differences in respiratory rate noted by anesthetic route.

Multiple Comparisons					95% Confidence Interval	
(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
IP	bath	-1.9591	1.51792	0.698	-6.1980	2.2799
	IM	1.1782	1.54008	0.940	-3.1227	5.4790
	IV	5.8772*	1.51792	0.002	1.6382	10.1162
	control	-.5029	1.69709	0.998	-5.2422	4.2364
bath	IP	1.9591	1.51792	0.698	-2.2799	6.1980
	IM	3.1373	1.54008	0.258	-1.1636	7.4381
	IV	7.8363*	1.51792	0.000	3.5973	12.0752
	control	1.4561	1.69709	0.911	-3.2832	6.1954
IM	IP	-1.1782	1.54008	0.940	-5.4790	3.1227
	bath	-3.1373	1.54008	0.258	-7.4381	1.1636
	IV	4.6990*	1.54008	0.025	0.3982	8.9999
	control	-1.6811	1.71694	0.864	-6.4759	3.1136
IV	IP	-5.8772*	1.51792	0.002	-10.1162	-1.6382
	bath	-7.8363*	1.51792	0.000	-12.0752	-3.5973
	IM	-4.6990*	1.54008	0.025	-8.9999	-.3982
	control	-6.3801*	1.69709	0.003	-11.1194	-1.6408
control	IP	.5029	1.69709	0.998	-4.2364	5.2422
	bath	-1.4561	1.69709	0.911	-6.1954	3.2832
	IM	1.6811	1.71694	0.864	-3.1136	6.4759
	IV	6.3801*	1.69709	0.003	1.6408	11.1194

Based on observed means.

The error term is mean square (error) = 20.737.

*. The mean difference is significant at the 0.05 level.



Table 4. Differences in escape reflexes noted between treatment groups.

Time (min)	10	20	30	40	50	60	70	80	90	100	110	120	130
Treatment													
IP-Control	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.018	0.038	0.196
IP-IV	0.888	0.424	0.606	0.293	0.097	0.104	0.074	0.192	0.203	0.265	0.767	0.719	0.719
IP-Bath	0.003	0.004	0.068	0.005	0.055	0.152	0.134	0.134	0.192	0.226	0.279	0.265	0.696
IP-IM	0.181	0.606	0.389	0.252	0.118	0.181	0.339	0.355	0.584	0.988	0.628	0.628	0.126
Control-IV	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.068	0.125
Control-Bath	0.196	0.000	0.000	0.000	0.003	0.018	0.008	0.196	0.196	0.196	0.213	0.441	0.441
Control-IM	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.008	0.038	0.018
IV-Bath	0.008	0.000	0.016	0.000	0.000	0.006	0.001	0.013	0.017	0.037	0.143	0.406	0.481
IV-IM	0.161	0.791	0.767	1	0.815	0.673	0.389	0.719	0.406	0.293	0.791	0.424	0.239
Bath-IM	0.000	0.001	0.004	0.000	0.000	0.007	0.014	0.029	0.055	0.279	0.152	0.192	0.097

Table 5. Differences in righting reflexes noted between treatment groups.

Time (min)	10	20	30	40	50	60	70	80	90	100	110	120	130
Treatment													
IP-Control	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.008	0.008	0.018	0.125	0.312
IP-IV	0.542	0.481	0.606	0.424	0.265	0.308	0.143	0.239	0.118	0.181	0.521	0.308	0.339
IP-Bath	0.002	0.000	0.006	0.000	0.001	0.006	0.004	0.031	0.126	0.134	0.214	0.406	0.839
IP-IM	0.079	0.424	0.389	0.252	0.226	0.214	1	0.815	0.584	1	0.791	0.406	0.126
Control-IV	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.018	0.068
Control-Bath	0.312	0.000	0.000	0.000	0.003	0.008	0.018	0.068	0.125	0.125	0.312	0.441	0.441
Control-IM	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.018	0.038	0.018
IV-Bath	0.001	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.068	0.074	0.265
IV-IM	0.323	1	0.767	0.767	1	0.791	0.097	0.265	0.239	0.181	0.767	0.913	0.628
Bath-IM	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.017	0.134	0.171	0.118	0.097

Table 6. Differences in corneal reflexes noted between treatment groups.

Time (min)	10	20	30	40	50	60	70	80
Treatment								
IP-Control	0.068	0.003	0.038	0.068	0.008	0.068	0.068	0.068
IP-IV	0.171	0.521	0.696	0.239	0.743	0.791	0.584	0.584
IP-Bath	0.047	0.007	0.696	0.839	0.424	0.815	0.606	0.628
IP-IM	0.673	0.864	0.521	0.134	0.767	0.239	0.279	0.252
Control-IV	0.441	0.018	0.003	0.003	0.003	0.018	0.008	0.018
Control-Bath	1.0	0.441	0.008	0.003	0.018	0.038	0.125	0.196
Control-IM	0.125	0.003	0.003	0.001	0.008	0.003	0.003	0.008
IV-Bath	0.406	0.047	0.293	0.118	0.239	0.542	0.226	0.226
IV-IM	0.406	0.323	0.606	0.743	0.767	1.0	0.265	0.521
Bath-IM	0.091	0.008	0.171	0.044	0.279	0.079	0.079	0.068

Table 7. Differences in superficial pain reflexes noted between treatment groups.

Time (min)	10	20	30	40	50	60	70	80	90	100	110	120	130
Treatment													
IP-Control	0.003	0.000	0.000	0.000	0.000	0.001	0.001	0.003	0.003	0.003	0.008	0.068	0.152
IP-IV	0.864	0.424	0.406	0.279	0.192	0.064	0.004	0.001	0.308	0.606	0.791	0.815	.0791
IP-Bath	0.002	0.001	0.008	0.003	0.006	0.010	0.029	0.029	0.022	0.029	0.044	0.252	0.161
IP-IM	0.171	0.673	0.239	0.406	0.372	0.521	0.462	0.481	0.864	0.719	0.938	0.743	0.265
Control-IV	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.001	0.038	0.125
Control-Bath	0.798	0.008	0.000	0.001	0.038	0.125	0.125	0.196	0.196	0.196	0.441	0.441	0.798
Control-IM	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.018	0.018	0.068	0.038
IV-Bath	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.014	0.011	0.152	0.143
IV-IM	0.104	0.650	0.767	0.791	0.606	0.226	0.085	0.064	0.293	0.424	0.888	0.888	0.462
Bath-IM	0.000	0.000	0.000	0.000	0.000	0.001	0.005	0.003	0.079	0.111	0.064	0.192	0.037

Table 8. Differences in deep pain reflexes noted between treatment groups.

Time (min)	10	20	30	40	50	60	70	80	90	100	110	120	130
Treatment													
IP-Control	0.008	0.001	0.001	0.001	0.001	0.003	0.008	0.018	0.008	0.038	0.038	0.068	0.441
IP-IV	0.563	0.293	0.279	0.963	0.628	0.462	0.161	0.481	0.888	0.501	0.481	0.501	0.481
IP-Bath	0.004	0.001	0.001	0.001	0.001	0.051	0.037	0.009	0.009	0.044	0.047	0.085	0.406
IP-IM	0.913	0.406	0.323	0.542	0.203	0.279	0.226	0.265	0.501	0.372	0.443	0.913	0.143
Control-IV	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.008	0.196	0.196	0.622	0.798
Control-Bath	1	0.798	0.038	0.196	0.312	0.622	0.312	0.622	0.798	0.798	0.798	0.798	1
Control-IM	0.001	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.018	0.018	0.018	0.068	0.038
IV-Bath	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.009	0.252	0.252	0.791	0.791
IV-IM	0.424	0.815	0.864	0.521	0.243	0.673	0.888	0.584	0.406	0.192	0.192	0.143	0.044
Bath-IM	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.002	0.016	0.017	0.019	0.085	0.022

