PROPOFOL AS AN IMMERSION ANESTHETIC AND IN A MINIMUM ANESTHETIC CONCENTRATION (MAC) REDUCTION MODEL IN GOLDFISH (*CARASSIUS AURATUS*)

Author(s): Julie A. Balko, V.M.D., Sarah K. Wilson, B.A., Gregory A. Lewbart, M.S., V.M.D., Dipl. A.C.Z.M., Brian R. Gaines, B.A., M.S., and Lysa P. Posner, D.V.M., Dipl. A.C.V.A.A.

Source: Journal of Zoo and Wildlife Medicine, 48(1):48-54.
Published By: American Association of Zoo Veterinarians
https://doi.org/10.1638/2016-0079.1
URL: http://www.bioone.org/doi/full/10.1638/2016-0079.1

Journal of Zoo and Wildlife Medicine 48(1): 48–54, 2017

PROPOFOL AS AN IMMERSION ANESTHETIC AND IN A MINIMUM ANESTHETIC CONCENTRATION (MAC) REDUCTION MODEL IN GOLDFISH (*CARASSIUS AURATUS*)

Julie A. Balko, V.M.D., Sarah K. Wilson, B.A., Gregory A. Lewbart, M.S., V.M.D., Dipl. A.C.Z.M., Brian R. Gaines, B.A., M.S., and Lysa P. Posner, D.V.M., Dipl. A.C.V.A.A.

Abstract: Propofol is a novel immersion anesthetic in goldfish (*Carassius auratus*). Objectives were to characterize propofol as an anesthetic and assess its suitability in a minimum anesthetic concentration (MAC) reduction model. Using a crossover design, eight goldfish were submerged in 1, 5, or 10 mg/L propofol. Data included induction time, recovery time, heart rate, opercular rate, and response to supramaximal stimulation. Baseline MAC (Dixon's up-and-down method) was determined, and 15 fish were anesthetized with propofol on 4 consecutive days with MAC determination on the fifth day, weekly, for 1 mo. Using a crossover design, MAC of propofol ($n = 15$) was determined 1 hr following administration of i.m. butorphanol 0.05, 0.5, and 1 mg/kg, dexmedetomidine 0.01, 0.02, and 0.04 mg/kg, ketoprofen 0.5, 1, and 2 mg/kg, morphine 5, 10, and 15 mg/kg, or saline 1 ml/kg. Comparisons were performed with Wilcoxon signed-rank tests ($P < 0.05$) and Tango's score confidence interval. Propofol at 1 mg/L did not produce anesthesia. Induction time with 10 mg/L (112, 84–166 s) was faster than 5 mg/L (233, 150–289 s; $P = 0.0078$). Heart and opercular rates for 5 and 10 mg/L were 36 (24–72) beats/min, 58 (44–68) operculations/min and 39 (20–48) beats/min, 57 (48–80) operculations/min, respectively. Recovery time was 249 (143–396) s and 299 (117–886) s with 5 and 10 mg/L, respectively. Response to supramaximal stimulation was not significantly different with 5 mg/L (1/8) compared with 10 mg/L (0/8). Baseline and weekly MAC following daily exposure was 8.4 and 9.0, 8.1, 8.1, and 8.7 mg/L, respectively. MAC reduction was no more than 8% following any drug or dosage. Propofol at 5 and 10 mg/L produced anesthesia, and anesthetic needs were similar following repeated exposure. Propofol was not suitable to test MAC reduction in goldfish in this study.

Key words: Analgesia, Anesthesia, *Carassius auratus*, Goldfish, Propofol.

INTRODUCTION

There is increasing evidence to support pain perception in fish, including a complete nervous system, anatomically diffuse and various types of nociceptors, descending modulatory pathways, and both learned and protective responses to noxious stimuli.[18] With heightened recognition and treatment of pain in these species, objective, reliable, and repeatable measures of analgesic efficacy are warranted. In higher vertebrates, a minimum alveolar concentration (MAC) reduction model is widely used for this purpose, with MAC defined as the alveolar concentration of an anesthetic agent at which 50% of a population will fail to respond to a supramaximal stimulus with gross, purposeful movement.[5] Comparison of anesthetic needs before and after administration of a proposed analgesic, as evidenced by MAC, allows for confirmation and comparison of analgesic potency.[5] A MAC reduction model was recently validated in goldfish (*Carassius auratus*) using the common fish anesthetic tricaine methanesulfonate (MS-222).[22] Although the model proved successful in the short term, with repeated exposure to MS-222, baseline anesthetic needs increased over time.[22] Because a consistent baseline is crucial in a MAC reduction model, identification of an alternative anesthetic for use in fish is needed.

Anesthetics are frequently used in fish for both diagnostic and therapeutic purposes and are an integral component of a MAC reduction model. Propofol (2,6-diisopropylphenol), a γ-aminobutyric acid (GABA) receptor agonist, is a short-acting central nervous system depressant widely used as an intravenous anesthetic in mammalian species.[19] Previous research has confirmed the presence of GABA receptors in the brain and spinal cord of teleost fish;[2,20] however, few studies have been conducted regarding the use of propofol as a fish anesthetic. As the gills provide a

From the Departments of Clinical Sciences (Lewbart) and Molecular and Biomedical Sciences (Balko, Posner, Wilson), College of Veterinary Medicine, North Carolina State University, 1060 William Moore Drive, Raleigh, North Carolina, 27607, USA; and Department of Statistics, College of Sciences, North Carolina State University, 2311 Stinson Drive, Raleigh, North Carolina, 27607, USA (Gaines). Correspondence should be directed to Dr. Balko (jbalkovmd@gmail.com).

unique route of absorption, particularly for lipophilic drugs, the high lipid solubility of propofol[19] makes it a candidate for administration via immersion, and several studies have reported efficacy via this route in species including silver catfish, nile tilapia, koi, and goldfish.[6,8,15,21] The current study aimed to clarify the use of propofol as an immersion anesthetic in goldfish and assess if anesthetic needs for propofol, as evidenced by MAC, changed following repeated exposure. Additionally, this study evaluated the suitability of propofol for use in a MAC reduction model by assessment of MAC following administration of several analgesic drugs. As prior studies have demonstrated opioid receptors (μ, κ)[4] and a cyclooxygenase pathway,[9,11] as well as clinical response to α2 adrenergic agonists in fish,[1] drugs that bind these receptors (morphine, butorphanol, dexmedetomidine) or interrupt the inflammatory cascade (ketoprofen) were used in the current study.

We hypothesize that 1) propofol via immersion will produce anesthesia in goldfish, 2) daily administration of propofol via immersion in goldfish will not result in a change in MAC over time, and 3) administration of intramuscular butorphanol, dexmedetomidine, ketoprofen, or morphine will all decrease the MAC of propofol via immersion in goldfish.

MATERIALS AND METHODS

Animals and husbandry

This study was conducted May–November 2014 at North Carolina State University, College of Veterinary Medicine, and was approved by the Institutional Laboratory Animal Care and Use Committee. Thirty-nine adult, red comet goldfish, approximately 4–5 cm long and 20–30 g, of unspecified sex were obtained from a commercial fishing hatchery (Blue Ridge Fish Hatchery, Kernersville, North Carolina 27284, USA). Thirty-eight fish were enrolled in this study, divided into three populations (15 fish, 15 fish, and 8 fish) and housed in three separate tanks: two 284-L (75 gallon) tanks and one 151-L (40 gallon) tank, respectively. Each tank was equipped with a mesh cover, sponge filter, air stone, drain, and calibrated digital thermometer (Coralife 00232, Coralife, Franklin, Wisconsin 53132, USA). Tank water temperature was allowed to equilibrate with the climate-controlled room temperature (23.1–23.3°C), and both were recorded daily. The photoperiod of the room was automatically maintained on a 12-hour light/dark cycle. Water quality was assessed daily (Tetra Easy Strips 6-in-1 and Ammonia Aquarium Test Strips, Spectrum Brands, Blacksburg, Virginia 24060, USA), and 50% water changes were completed as needed to maintain water quality within the following parameters: pH 6.9–7.4, nitrite 0.00–0.04 ppm, and total ammonia nitrogen 0.0–0.5 ppm. Commercial aquarium salt (Instant Ocean Sea Salt, Spectrum Brands) was used to maintain water salinity at two to three parts per thousand. City tap water, treated with sodium thiosulfate and allowed to incubate for a minimum of 3 weeks, was used as the fresh water source. Fish were fed a commercial pelleted diet (Koi and Goldfish Food, Blue Ridge Fish Hatchery) every Monday, Wednesday, and Friday. In addition to water temperature and pH, dissolved oxygen content (Dissolved Oxygen Meter, Sper Scientific Limited, Scottsdale, Arizona 85260, USA) was measured on all test days. The study was divided and conducted in four phases.

Study design

Phase I (n = 8): The objective of phase I was to evaluate the effects of three doses of propofol via immersion and evaluate induction time, recovery time, opercular rate, heart rate, and response to supramaximal stimulation in goldfish. In a dose-escalating, crossover design with a 1-wk washout period between each dose, eight fish were immersed in each of three propofol concentrations: 1, 5, and 10 mg/L (PropoFlo, Abbott Laboratories, North Chicago, Illinois 60064, USA). These doses were extrapolated from a previous study of propofol immersion in koi.[15] Each fish was moved via fishnet from its main housing tank to a 1,000-ml glass beaker (Fisher Scientific, Pittsburgh, Pennsylvania 15275, USA) containing one of the aforementioned propofol concentrations in 500 ml fresh water. Fresh propofol concentrations were created for each fish using new 1-ml syringes (Monoject, Medtronic, Minneapolis, Minnesota 55432, USA). Immediately after transfer, an opercular rate (OR_1) was recorded, and fish were observed for signs of anesthesia, with a sedation rubric used to assess response (Appendix 1). Trials were terminated for any fish that did not reach a sedation score of 3 by 30 min; these fish were transferred to a glass beaker of fresh water and observed until full recovery with no additional testing performed. When a sedation score of 3 was reached, the induction time was recorded, and fish were immediately removed from their beaker. Fish were weighed using a portable, digital gram scale (CS5000, Ohaus, Parsippany, New

Jersey 07054, USA), length was measured, and heart rate was determined using a Doppler crystal (Doppler Flow Detector, Model 811-B, Parks Medical Electronics, Inc., Aloha, Oregon 97078, USA) primed with ultrasound transmission gel (Aquasonic 100, Parker Laboratories, Inc., Fairfield, New Jersey 07004, USA) and placed over the cranial ventrum. Response to supramaximal stimulation was assessed using a 25-gauge needle (Monoject, Medtronic) inserted into the caudal epaxial musculature. Gross, purposeful movements including flicking of the tail or fins, wriggling of the body, and lifting of the head were deemed a positive response, whereas no movement, muscle fasciculations, shuddering, or any nonpurposeful movements characterized a negative response. The response was recorded, and fish were transferred to a 1,000-ml glass beaker containing 500 ml fresh water. Immediately after transfer, an opercular rate (OR_2) was recorded, and fish were observed for complete recovery, characterized by an intact righting reflex when pushed into a lateral orientation. When noted, a recovery time was recorded and an opercular rate (OR_3) was obtained. Following the final experiment of the day, all fish were returned to the main housing tank and monitored routinely. All glassware was cleaned daily with Fisherbrand Versa-Clean (Fisher Scientific).

Phase II (n = 15): The objective of phase II was to determine the MAC of propofol delivered via immersion. Using a previously validated, modified Dixon's up-and-down method,[3,22] a population of 15 fish was sequentially anesthetized by exposure to a propofol test solution for exactly 4 min and then exposed to a supramaximal stimulus. With this technique, if the first fish has a positive response to stimulation, the subsequent fish is exposed to 0.3 mg/L more propofol. If the response is negative, the subsequent fish is exposed to 0.3 mg/L less propofol. This incremental dose change (~10%) was extrapolated from a previous study.[22] The algorithm is then continued for all study individuals, and the median value for the population—the median concentration above which 50% of study subjects responded to the stimulus and below which 50% of study subjects did not—is established as the population's MAC. To determine an appropriate propofol concentration for use in the first fish, a pilot MAC study was undertaken starting with 4.5 mg/L extrapolated from phase I data. However, the stepwise increases proved insufficient to prevent response to supramaximal stimuli following 4 min of exposure; thus, the pilot study was repeated the following day, testing the first fish on day 2 using the last propofol concentration used on day 1 (7.5 mg/L). A more even distribution of positive and negative responses was generated on day 2; thus, the resultant MAC value (8.1 mg/L) was used as the starting propofol concentration for the MAC determination trial, conducted on day 3. For this trial, the first fish was transferred via fishnet from its main housing tank to a 1,000-ml glass beaker containing an 8.1 mg/L propofol dilution in 500 ml fresh water. Fresh propofol dilutions were made for each fish using new U-100 insulin syringes (BD, Franklin Lakes, New Jersey 07417, USA). After 4 min, fish were removed from the beaker, and a supramaximal stimulus was administered via a 25-gauge needle inserted into the caudal epaxial musculature. Gross, purposeful movements including flicking of the tail or fins, wriggling of the body, and lifting of the head were deemed a positive response, whereas no movement, muscle fasciculations, shuddering, or any nonpurposeful movements characterized a negative response. The response was recorded, and the fish was moved to a 1,000-ml glass beaker containing 500 ml fresh water. Based on the response—positive or negative—a propofol concentration 0.3 mg/L higher or lower was created using 500 ml fresh water and was then used for immersion of the subsequent fish. This protocol was continued for all 15 fish, and a MAC value was established. Following the final anesthesia for the day, the fish were observed for complete recovery, returned to the main housing tank, and monitored routinely. To avoid excessive muscle trauma, supramaximal stimuli were altered between the right and left caudal epaxial musculature on subsequent days. Fish were not individually marked; thus, daily testing order was random.

Phase III (n = 15): The objective of phase III was to determine whether MAC would change following repeated exposure to propofol over time. Fifteen goldfish (phase II population) were anesthetized 5 days per week—consisting of daily exposure on Monday through Thursday and MAC determination on Friday—for a total of 4 consecutive weeks. Baseline MAC was used as the exposure concentration for week 1, with each Friday's redetermined MAC value then used as the exposure concentration for the following week (weeks 2–4). For daily exposure, each fish was transferred via fishnet from the main housing tank to a 1,000-ml glass beaker with a propofol dilution in 500 ml fresh water. Fresh propofol concentrations were made for each fish using new U-100

insulin syringes. After 4 min in solution, each fish was moved to a 1,000-ml glass beaker containing 500 ml fresh water. Following the final anesthesia for the day, all fish were returned to the main housing tank and monitored routinely. On Friday of each week, MAC was redetermined following the phase II protocol, using each week's exposure concentration as the starting propofol dose for the first fish. The location of supramaximal stimuli application (right or left caudal epaxial musculature) was recorded and alternated each week to avoid excessive muscle trauma.

Phase IV (n = 15): The objective of phase IV was to assess whether administration of various analgesics at several doses would decrease the MAC of propofol via immersion. In a randomized, crossover design, a population of 15 naïve goldfish were administered each of four analgesics at three separate doses and a saline control with a 1-wk washout period between trials. Drug doses were chosen based on current recommendations in fish medicine, as well as previous studies in goldfish and included butorphanol 0.05, 0.5, and 1 mg/kg (Torbugesic 2 mg/ml, Zoetis, Inc., Kalamazoo, Michigan 49007, USA), dexmedetomidine 0.01, 0.02, and 0.04 mg/kg (Dexdomitor 0.1 mg/ml, Zoetis, Inc.), ketoprofen 0.5, 1, and 2 mg/kg (Ketofen 100 mg/ml, Fort Dodge Animal Health, Fort Dodge, Iowa 50501, USA), and morphine 5, 10, and 15 mg/kg (morphine sulfate 15 mg/ml, Hospira, Inc., Lake Forest, Illinois 60045, USA). A saline control volume of 1 ml/kg (0.9% sodium chloride injection USP, Hospira, Inc.) was chosen to produce a comparable volume per fish weight to the tested drugs. All drugs, except morphine, were diluted with 0.9% sodium chloride to secondary concentrations and created new on each test day to produce reasonable drug delivery volumes: 1 and 0.1 mg/ml (butorphanol), 0.01 mg/ml, (dexmedetomidine), and 1 mg/ml (ketoprofen). Baseline MAC was determined for this population (MAC_i) as described in phase II 1 wk prior to any drug administration. On experiment days, each fish was individually anesthetized with propofol via immersion. Once anesthetized, each fish was weighed, the drug volume was calculated, and that week's drug administered intramuscularly via a new U-100 insulin syringe into the caudal epaxial musculature. Site of injection (right or left) was alternated between experiments to minimize muscle trauma. After injection, fish were moved to an individual glass beaker of fresh water until full recovery and then moved to one of two shared recovery tanks, with the first eight injected fish in the first tank and the remaining seven fish in the second tank. One hour after the last fish was injected, MAC was determined for the population following the protocol described in phase II and starting with all eight fish from the first recovery tank. A pharmacokinetic study of morphine in teleost fish was used as the basis for the 1-hr interval.[14] As fish were not individually identified, the exact time interval from drug administration to MAC determination ranged from 1 to 3 hr. One week following the last drug tested, MAC was redetermined for the population (MAC_f).

Statistical analysis

Physiologic data including induction and recovery times, heart rates, and opercular rates were compared using Wilcoxon signed-rank tests, with significance set at $P < 0.05$. Response to supramaximal stimulation was compared using Tango's score confidence interval. MAC values were identified as the median propofol concentration tested in each trial.

RESULTS

*Phase I (*N = *8):* Propofol at 1 mg/L did not produce anesthesia, and all fish at this dosage were assigned a sedation score of 2; as such, no physiologic or timed data are presented for this dose. Propofol at 5 and 10 mg/L produced anesthesia in all goldfish, with a sedation score of 3 assigned to all fish. Induction and recovery times, heart rates, and opercular rates are reported in Table 1. Following anesthetic induction, yet prior to manipulation for heart rate determination, seven of eight fish at 5 mg/L and four of eight fish at 10 mg/L had an opercular rate of zero. All fish resumed operculating during the subsequent procedural step: manual removal from the water for heart rate determination. Positive response to supramaximal stimulation was observed in one of eight fish administered 5 mg/L and zero of eight fish administered 10 mg/L (95% confidence interval = –0.471, 0.240). No morbidity or mortality was noted in any fish at any dose, and physical examinations remained unchanged throughout phase I, with median body weights of 22 (range, 17–30) g and 22 (range, 18–31) g at the beginning and end of the phase, respectively.

*Phases II and III (*N = *15):* Baseline MAC and MAC following weekly repeated exposure to propofol were 8.4 and 9.0, 8.1, 8.1, and 8.7 mg/

Table 1. Physiologic and anesthetic parameters of goldfish following immersion in propofol

Parameter[a]	5 mg/L	10 mg/L	*P* value
Induction time (s)	233 (150–289)	112 (84–166)	0.0078[b]
Recovery time (s)	249 (143–396)	299 (117–886)	0.1953
Heart rate (beats/min)	36 (24–72)	39 (20–48)	0.7422
OR_1 (operculations/min)	96 (84–120)	ND[c]	
OR_2 (operculations/min)	58 (44–68)	57 (48–80)	0.2813
OR_3 (operculations/min)	48 (36–84)	54 (36–68)	0.9609

All values reported as median (range).

[a] OR_1, opercular rate immediately following placement in propofol immersion; OR_2, opercular rate immediately following placement in anesthetic-free water; OR_3, opercular rate at complete recovery.

[b] Significant difference ($P < 0.05$).

[c] ND = not detectable due to opaque nature of solution.

L, respectively (Fig. 1). No morbidity or mortality was noted in any fish in phase II or III.

Phase IV (N = 15): Baseline MAC (MAC_i), final MAC (MAC_f), and MAC following various drug administrations are reported in Table 2. One fish was removed from the study at week 8 due to prolonged (>3.5 hr), yet complete, recovery from anesthesia following propofol immersion and administration of dexmedetomidine 0.02 mg/kg. Subjectively, this fish was clinically unthrifty and of smaller body size than the remaining fish. MAC determination for week 8 was conducted with the 14 remaining fish, but a naïve, replacement fish was used for the remainder of the drug trials without issue. In week 13, one fish failed to recover from anesthesia following propofol immersion and administration of dexmedetomidine 0.04 mg/kg; necropsy did not reveal any significant findings. Because this was the final week of drug administration, MAC determination for weeks 13 and 14 (final MAC) was conducted with the 14 remaining fish. Excluding the aforementioned two fish, no additional morbidity or mortality was noted in any other fish in phase IV, and median body weights at the beginning and end of the phase were 22 (range, 17–34) g and 26 (range, 19–37) g, respectively.

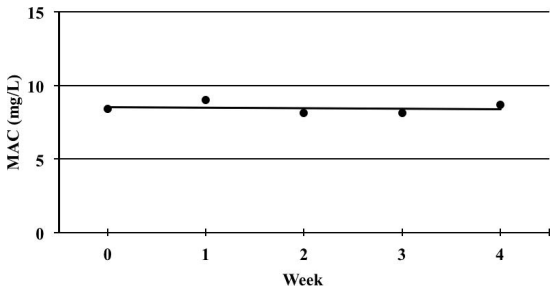

Figure 1. Baseline MAC of propofol via immersion (week 0) and MAC following weekly repeated exposure to propofol via immersion (weeks 1–4) in goldfish. The included trendline for the data points has a point–slope equation of $y = -0.03x + 8.55$.

DISCUSSION

Propofol via immersion at 5 and 10 mg/L produced anesthesia in goldfish, with only a single fish responding to supramaximal stimulation amongst both groups. Induction times were significantly faster with 10 mg/L compared with 5 mg/L, likely a result of more concentrated drug uptake over the gills. Although no standards exist for immersion anesthetic induction times in aquatic species, both 5 and 10 mg/L produced consistent and clinically relevant induction times of less than 5 min. This finding is in agreement with a recent pharmacokinetic study in rainbow trout that confirmed rapid uptake of propofol

Table 2. MAC of propofol via immersion in goldfish following drug administration

Drug	Dose (mg/kg)[a]	MAC (mg/L)	% change from MAC_i
Baseline (MAC_i)	NA	7.8	NA
Butorphanol	0.05	9.0	+15.4
Butorphanol	0.5	8.7	+11.5
Butorphanol	1	7.8	0
Dexmedetomidine	0.01	8.1	+3.8
Dexmedetomidine	0.02	8.1	+3.8
Dexmedetomidine	0.04	7.2	–7.7
Ketoprofen	0.5	8.7	+11.5
Ketoprofen	1	7.8	0
Ketoprofen	2	8.7	+11.5
Morphine	5	7.8	0
Morphine	10	9.0	+15.4
Morphine	15	7.8	0
Saline	1	8.1	+3.8
Final (MAC_f)	NA	8.4	+7.7

[a] All doses in mg/kg with the exception of saline, which is ml/kg.

within the first minute of exposure and induction times of less than 3 min following immersion in a 10 mg/L solution.[7] Recovery times were not significantly different between doses; however, a wider range of times was observed at 10 mg/L, with one fish requiring almost 15 min for full recovery. This suggests the potential for prolonged recovery times using higher doses of propofol in goldfish.

Opercular rates dropped to zero in almost 70% of fish in the 5 and 10 mg/L groups at the time of anesthetic induction. Although this resolved during physical manipulation of all fish, it is unknown how long this effect would last without manipulation. Propofol is a known dose-dependent respiratory depressant in vertebrates[16] and, supported by the current study, should also be considered as a likely complication of propofol immersion in goldfish. Physiologic parameters otherwise remained within acceptable clinical ranges with no morbidity or mortality noted in phase I.

Comparison of MAC before and after administration of a proposed analgesic allows for confirmation and comparison of analgesic potency,[5] and this is routinely used in mammalian and non-mammalian species. In species without alveoli, including birds and fish, this model is redefined as a minimum anesthetic concentration reduction model, but the same principles apply.[13] In the current study, the MAC of propofol via immersion was established in a population of goldfish and was shown to be repeatable, both within a population (phase II) and between populations (phases II and IV). The MAC of propofol did not change following repeated exposure, in contrast to MS-222, in which repeated exposure over a similar time frame demonstrated increased anesthetic needs.[22] Although statistical analysis could not be performed, inspection of the five data points (Fig. 1) shows minimal variability as the values did not deviate by more than 10%. Potential reasons for the consistency of anesthetic needs with chronic propofol exposure in goldfish include lack of upregulation of the GABA receptor or lack of detrimental effects on the mechanism of uptake (gills). Pharmacokinetics of propofol following repeated, chronic exposure in other species is unknown. The dosing consistency of propofol following repeated exposure, compared with MS-222, may make it ideal for fish requiring serial anesthetic events.

Only a single drug and dose (dexmedetomidine 0.04 mg/kg) decreased MAC, whereas the remaining drugs and doses either increased MAC or resulted in no change. This is surprising given that a previous study demonstrated MAC reduction with 8 of 12 similar drugs and doses in a goldfish MAC reduction model[22] and prior studies have established analgesic efficacy with these drugs and doses in fish.[10,17] Although interspecies variation in response to drugs should not be discounted, given prior success with equivalent drugs, inappropriate analgesic drug selection seems less likely to be a cause for the inconsistent results in the current study. A more likely hypothesis for the lack of evident MAC reduction is the small dosage changes used for MAC determination. As the doses of propofol required for anesthesia in goldfish are much smaller than equipotent doses of MS-222 (5 mg/L compared with 100 mg/L, respectively), the 10% change in Dixon's up-and-down method may have proven too small to identify changes between dosages (type II error). Other potential explanations include lack of a convention for supramaximal stimulus in goldfish, the subjective nature of the end point, and the inherent challenges in assessing small, aquatic species. These challenges were likely diminished by the use of a standardized scoring rubric and a single evaluator (JAB). Despite its potential, propofol was not suitable to test MAC reduction in goldfish in the current study.

Of 417 separate anesthetic episodes conducted in phase IV, only one mortality resulted (<0.25%). Although necropsy revealed no significant findings, its death was likely multifactorial in nature as the fish had both received the highest dose of dexmedetomidine and undergone 24 anesthetic events in the previous 12 weeks. The general health status (physical examination, weight) of the remaining fish did not negatively change throughout the study, and no external irritation secondary to propofol exposure, corroborated by its isotonic pH,[12] was noted. These considerations support propofol as a safe immersion anesthetic in goldfish. Additional advantages of propofol include its wide availability, lack of federal control, nominal cost, and ready-to-use formulation.

Limitations of this study include the lack of convention for appropriate supramaximal stimulus in fish, the small drug volumes, and the small size and aquatic nature of the subjects, which precluded more invasive monitoring.

Propofol at 5 and 10 mg/L is a safe and effective immersion anesthetic in goldfish and anesthetic needs do not change following repeated exposure over time. Propofol was not suitable

to test MAC reduction in goldfish in the current study.

LITERATURE CITED

1. Christiansen EF, Mitchell JM, Harms CA, Stoskopf MK. Sedation of red porgy (*Pagrus pagrus*) and black sea bass (*Centropristis striata*) using ketamine, dexmedetomidine and midazolam delivered via intramuscular injection. J Zoo Aquar Res. 2014;2(3):62–68.

2. Delgado L, Schmachtenberg O. Immunohistochemical localization of GABA, GAD65, and the receptor subunits $GABA_{A\alpha1}$ and $GABA_{B1}$ in the zebrafish cerebellum. Cerebellum. 2008;7(3):444–450.

3. Dixon WJ. The up–and–down method for small samples. J Am Stat Assoc. 1965;60(312):967–978.

4. Ebbesson LOE, Deviche P, Ebbesson SOE. Distribution and changes in μ– and κ–opiate receptors during the midlife neurodevelopmental period of coho salmon, *Oncorhynchus kisutch*. J Comp Neurol. 1996; 364:448–464.

5. Eger EI, Saidman LJ, Brandstater B. Minimum alveolar anesthetic concentration: a standard of anesthetic potency. Anesthesiology. 1965;26(6):756–763.

6. GholipourKanani H, Ahadizadeh S. Use of propofol as an anesthetic and its efficacy on some hematological values of ornamental fish *Carassius auratus*. Springerplus. 2013;2(1):76.

7. Gomulka P, Fornal E, Berecka B, Szmagara A, Ziomek E. Pharmacokinetics of propofol in rainbow trout following bath exposure. Pol J Vet Sci. 2015; 18(1):147–152.

8. Gressler LT, Parodi TV, Riffel APK, DaCosta ST, Baldisserotto B. Immersion anesthesia with tricaine methanesulphonate or propofol on different sizes and strains of silver catfish *Rhamdia quelen*. J Fish Biol. 2012;81:1436–1445.

9. Grosser T, Yusuff S, Cheskis E, Pack MA, FitzGerald GA. Developmental expression of functional cyclooxygenases in zebrafish. Proc Natl Acad Sci U S A. 2002;99(12):8418–8423.

10. Harms CA, Lewbart GA, Swanson CR, Kishimori JM, Boylan SM. Behavioral and clinical pathology changes in koi carp (*Cyprinus carpio*) subjected to anesthesia and surgery with and without intra–operative analgesics. Comp Med. 2005;55(3):221–226.

11. Ishikawa T, Herschman HR. Two inducible, functional cyclooxygenase–2 genes are present in the rainbow trout genome. J Cell Biochem. 2007;102:1486–1492.

12. Henry Schein Vet. Material Safety Data Sheet: propofol [Internet]. c2007 [cited 2016 February 15]. Available from https://henryscheinvet.com/Content/SDS/041626.pdf

13. Mosley CAE, Dyson D, Smith DA. Minimum alveolar concentration of isoflurane in green iguanas and the effect of butorphanol on minimum alveolar concentration. J Am Vet Med Assoc. 2003;222(11): 1559–1564.

14. Newby NC, Robinson JW, Vachon P, Beaudry F, Stevens ED. Pharmacokinetics of morphine and its metabolites in freshwater rainbow trout (*Oncorhynchus mykiss*). J Vet Pharmacol Ther. 2008;31:117–127.

15. Oda A, Bailey KM, Lewbart GA, Griffith EH, Posner LP. Physiologic and biochemical assessments of koi (*Cyprinus carpio*) following immersion in propofol. J Am Vet Med Assoc. 2012;245(11):1286–1291.

16. Riviere JE, Papich MG (eds.). Veterinary pharmacology and therapeutics. 9th ed. Ames (IA): Wiley-Blackwell; 2009.

17. Sneddon LU. The evidence for pain in fish: the use of morphine as an analgesic. Appl Anim Behav Sci. 2003;83:153–162.

18. Sneddon LU. Pain in aquatic animals. J Exp Biol. 2015;218:967–976.

19. Stoelting RK, Hillier SC (eds.). Handbook of pharmacology and physiology in anesthetic practice. 4th ed. Philadelphia (PA): Lippincott, Williams, and Wilkins; 2006. p. 155–163.

20. Uematsu K, Shirasaki M, Storm-Mathisen J. GABA- and glycine-immunoreactive neurons in the spinal cord of the carp, *Cyprinus carpio*. J Comp Neurol. 1993;332:59–68.

21. Valenca-Silva G, Braz MG, Barreto RE, Salvadori DMF, Volpato GL. Low dose of the anesthetic propofol does not induce genotoxic or mutagenic effects in Nile tilapia. Trans Am Fisheries Soc. 2014; 143:414–419.

22. Ward JL, McCartney SP, Chinnadurai SK, Posner LP. Development of a minimum-anesthetic-concentration depression model to study the effects of various analgesics in goldfish (*Carassius auratus*). J Zoo Wildl Med. 2012;43(2):214–222.

Received for publication 4 April 2016

Appendix 1. Sedation rubric for goldfish exposed to propofol via immersion

Value	Visible characteristics
0	Normal, no visible changes
1	Slower swimming, occasional listing
2	No swimming, responds to stimulation
3	Anesthetized, lack of righting ability, no response to stimulation or removal from water