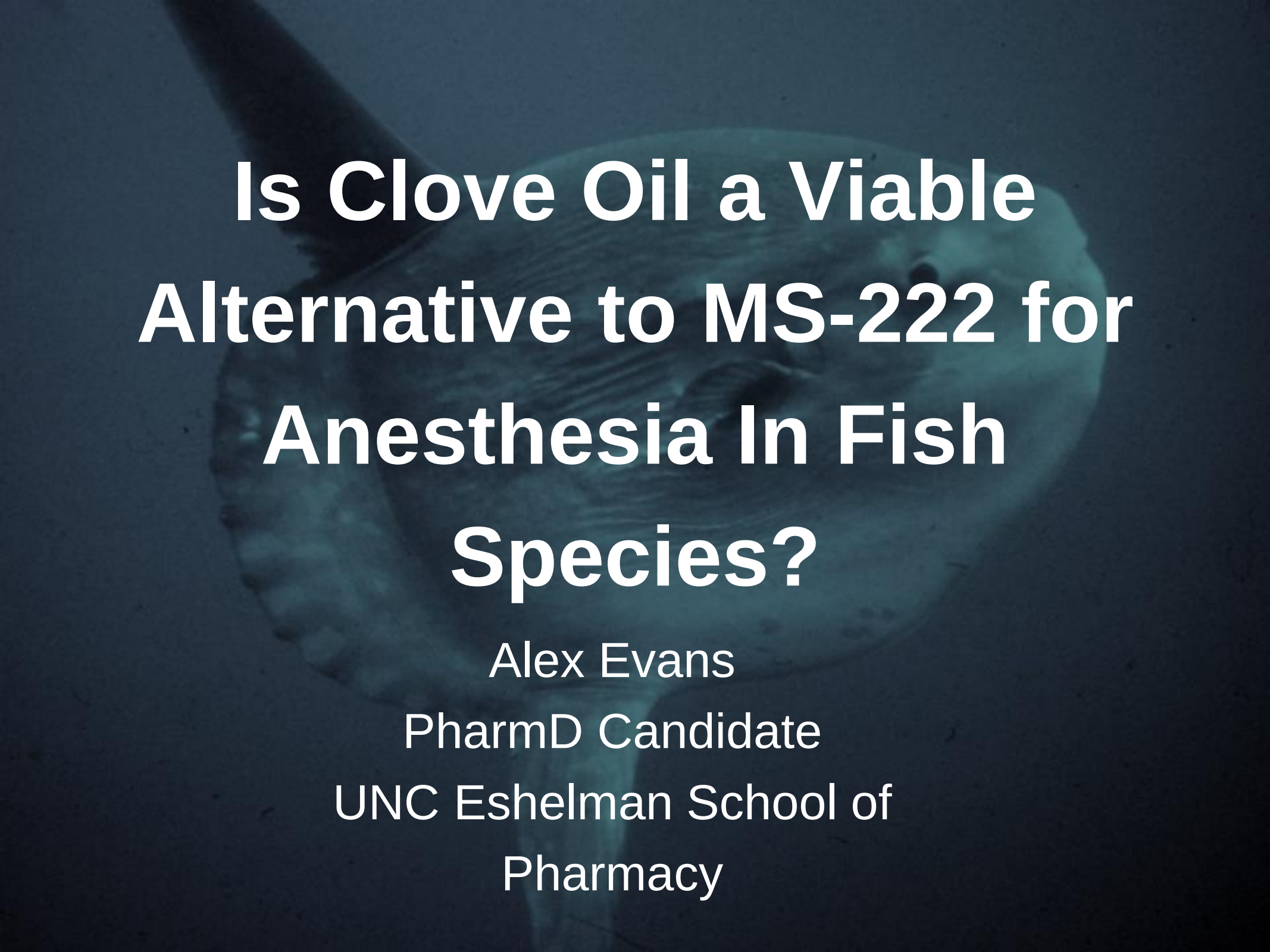


Is Clove Oil a Viable Alternative to MS-222 for Anesthesia In Fish Species?

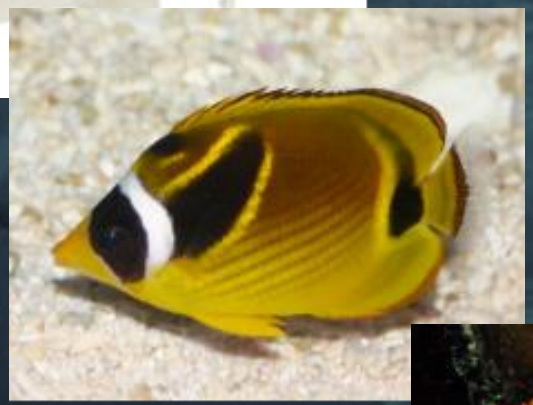
Alex Evans
PharmD Candidate
UNC Eshelman School of Pharmacy

Objectives

- Discuss the principles of surgical anesthesia in fish
- Provide an overview of the phamacotherapy of MS-222 and clove oil
- Discuss advantages and limitations of both agents
- Evaluate the literature regarding the use of clove oil in comparison to MS-222 in fish species
- Provide recommendations regarding the current dilemma

What is a “fish”?

Teleosts: true bony fish



Elasmobranchs: sometimes called “fish”

Not fish - invertebrates!!

Why anesthesia in fish?

- Fisheries science/research
- Pet fish
- Aquaculture
- Zoo and aquarium medicine

Tufts University, 2010

Gas Exchange Strategies

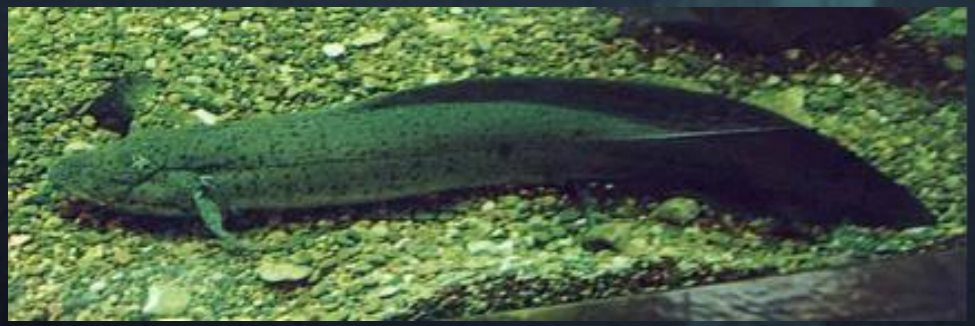
- *Ram ventilation* - fish must continually move water over its gills for oxygen exchange (tuna)
- *Buccal Pumper* - fish is capable of forcing water into its mouth and over gills for oxygen exchange (nurse shark)
- *Aquatic surface respiration* - skims air-water surface for additional oxygen (*mullet)*
- *Accessory respiratory organs* - allows the use of atmospheric air (Dipnoi, or “lungfish”)

Neiffer et Al. 2009

Domenici et Al. 2007

Species differences in anesthesia strategies

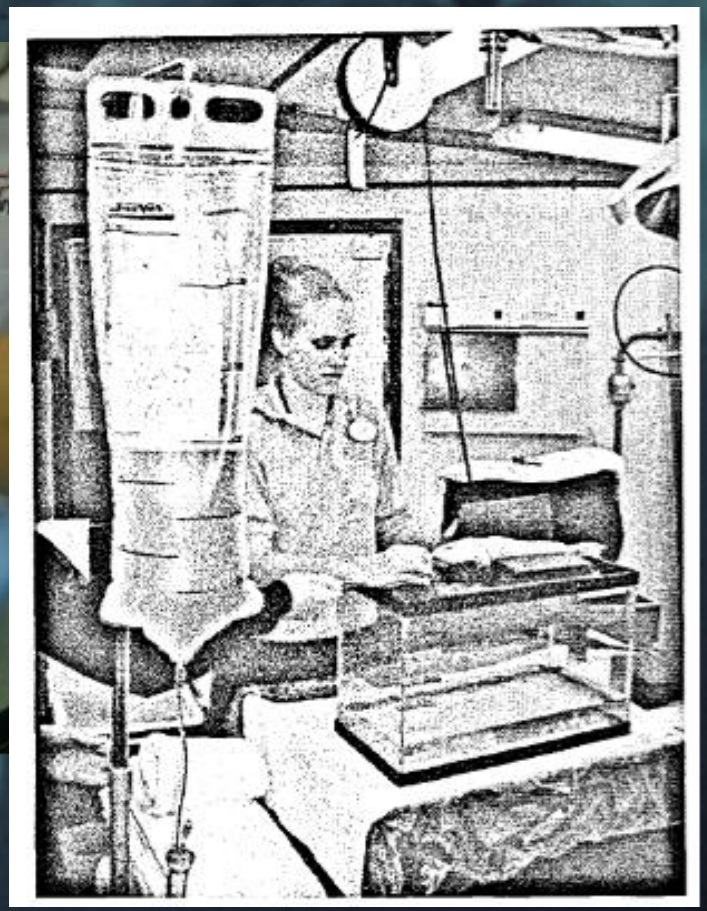
- Species that rely entirely on dissolved oxygen will have faster induction rates
- Species that rely heavily on atmospheric oxygen might suffocate if given the traditional setup
  - Need to be on a moist substrate during surgery and recovery



Neiffer et Al. 2009

Procedure

- Non-recirculating and recirculating systems



Neiffer et Al. 2009

Stages of Anesthesia and Recovery

Stages of Anesthesia:

I - Onset of erratic opercular movement

II - Partial loss of equilibrium; continued efforts to right itself

III - Total loss of equilibrium; no efforts to right itself

IV - Induction; total loss of voluntary movement and reactivity

V - Medullary collapse; total cessation of opercular movement

Stages of Recovery:

I -Reappearance of opercular movement

II - Partial recovery of equilibrium; efforts to right itself

III - Full recovery of e equilibrium; successful righting

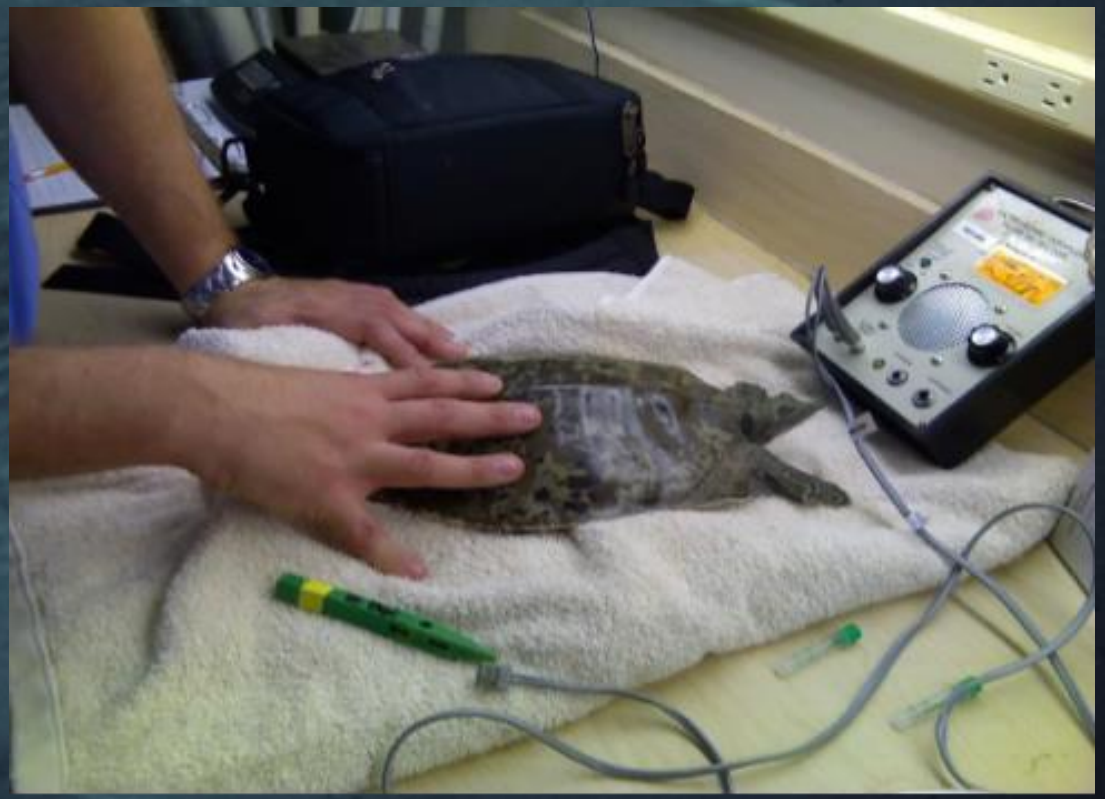
IV - Response to external stimuli

V - Behavioral recovery; normal swimming activity

Sladky et Al. 2001

Monitoring

- Respiratory rate
  - Dependent on ventilation strategy
- Heart rate
- Temperature, DO, pH
- Ammonia levels



Doppler sonography to measure HR in *Apalone spinifera*

Neiffer et Al.
2009

Therapeutic Options: MS-222

- Only FDA-approved anesthetic
- Only available agent in food animals
  - 21-day withdrawal time
- **Chemical name**: Tricaine methanesulfate
  - Parent compound: benzocaine
- **MOA**: Sodium channel blocker
- **Dosage range**: 10-250 mg/L, depending on species
- **Adverse effects**: respiratory depression, hyperglycemia, hyperkalemia, polycythemia

Sladky et Al. 2001
www.ivis.org
Coyle et Al. 2004

Therapeutic Options: Clove Oil

- NOT FDA approved
- **Active ingredients**: isoeugenol, eugenol, methyleugenol
  - *Aqui-S*: standardized product approved in Australia, Chile, New Zealand, Korea, Costa Rica and Honduras with zero withdrawal time
- Mix clove oil 1:10 with 95% ethanol to yield a 100mg/mL solution
- **MOA**: Unknown
- **Dosage range**: 2-200mg/L, depending on species
- **Adverse effects**: respiratory depression, hyperglycemia, hyperkalemia, polycythemia

www.aqui-s.com

Coyle et Al. 2004

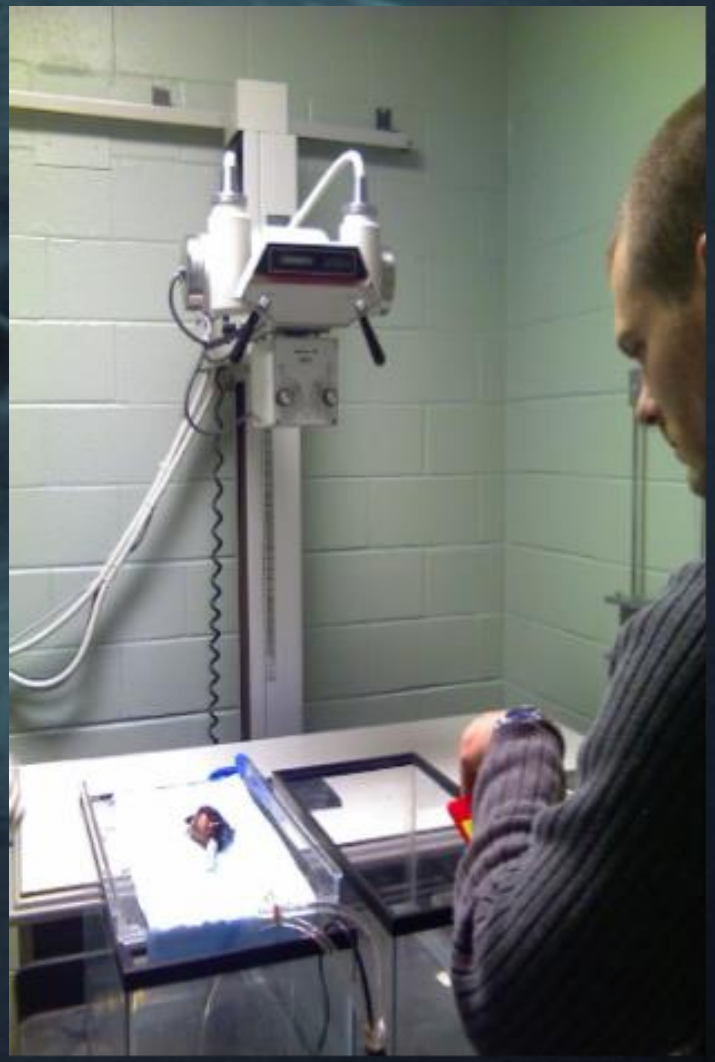
FDA CVM Comments

- Clove oil is not standardized
- Neither clove oil or any of its components are being studied under a NADA
- Carcinogenicity unknown
- Concerns over food supply
- Ecosystem concerns

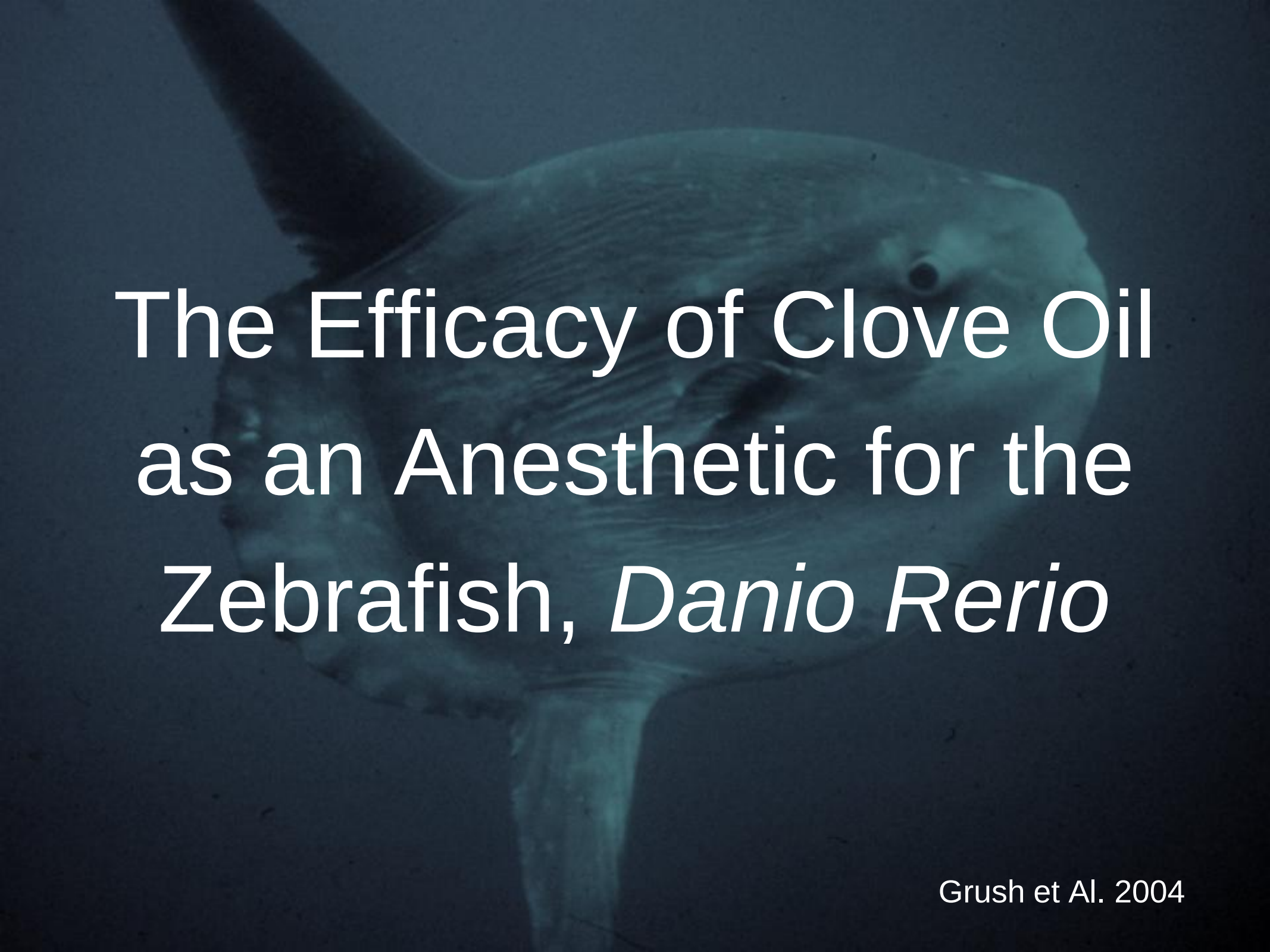
www.fda.gov

Other Therapeutic Options

- Benzocaine
- Medotomidate
- 2-Phenoxyethanol
- Quinaldine/Quinaldine Sulfate
- Azaporene
- Isoflurane/Halothane
- Oxygen (Elasmobranchs)



Neiffer et Al. 2009



The Efficacy of Clove Oil as an Anesthetic for the Zebrafish, *Danio Rerio*

Grush et Al. 2004

Overview

- 1200 zebrafish
- 1-month old, all sexually immature
- Housed in ten 50L aquaria
  - 24+/-2° C
  - 13 L:11 D
- Study arms:
  - **MS-222**: 100,120, 140, 160, 180, 200ppm
  - **Eugenol**: 60, 80, 100, 120, 140ppm

Grush et Al. 2004

Study Design

Ten zebrafish randomly selected and placed into 20L experimental aquarium

Time to stage 4 and 5 anesthesia recorded. Fish removed and placed in recovery tank once stage 5 anesthesia reached.

Time to stage 3 or 4 anesthesia recovery measured

Process repeated 3x for all concentrations of MS-222 and eugenol

Grush et Al. 2004

Study Design

Fish used in experiment were left in recovery tank for one day, then transferred back to the recovery holding tank for a 14-day period.

Abnormal behaviors and mortality during this 14-day period were recorded

Other studies:

- 96h LD_{50} for eugenol
- Ethanol exposure effects over 96 hours

Grush et Al. 2004

Results

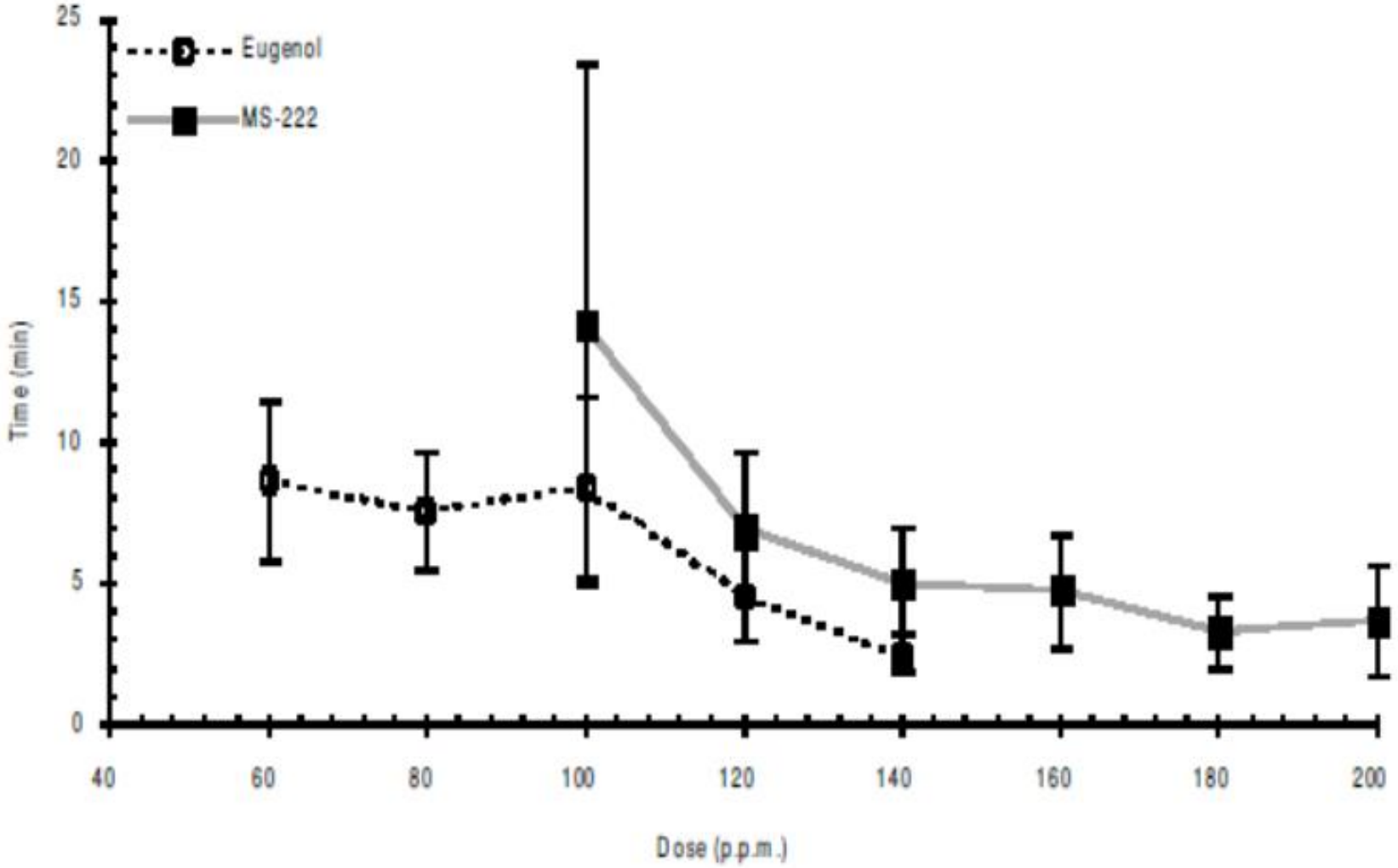


FIG. 3. Time required for zebrafish to achieve stage 5-anesthesia at various concentrations of clove oil and MS-222. Data points represent the mean ± SEM, $n = 10$.

Grush et Al. 2004

Results

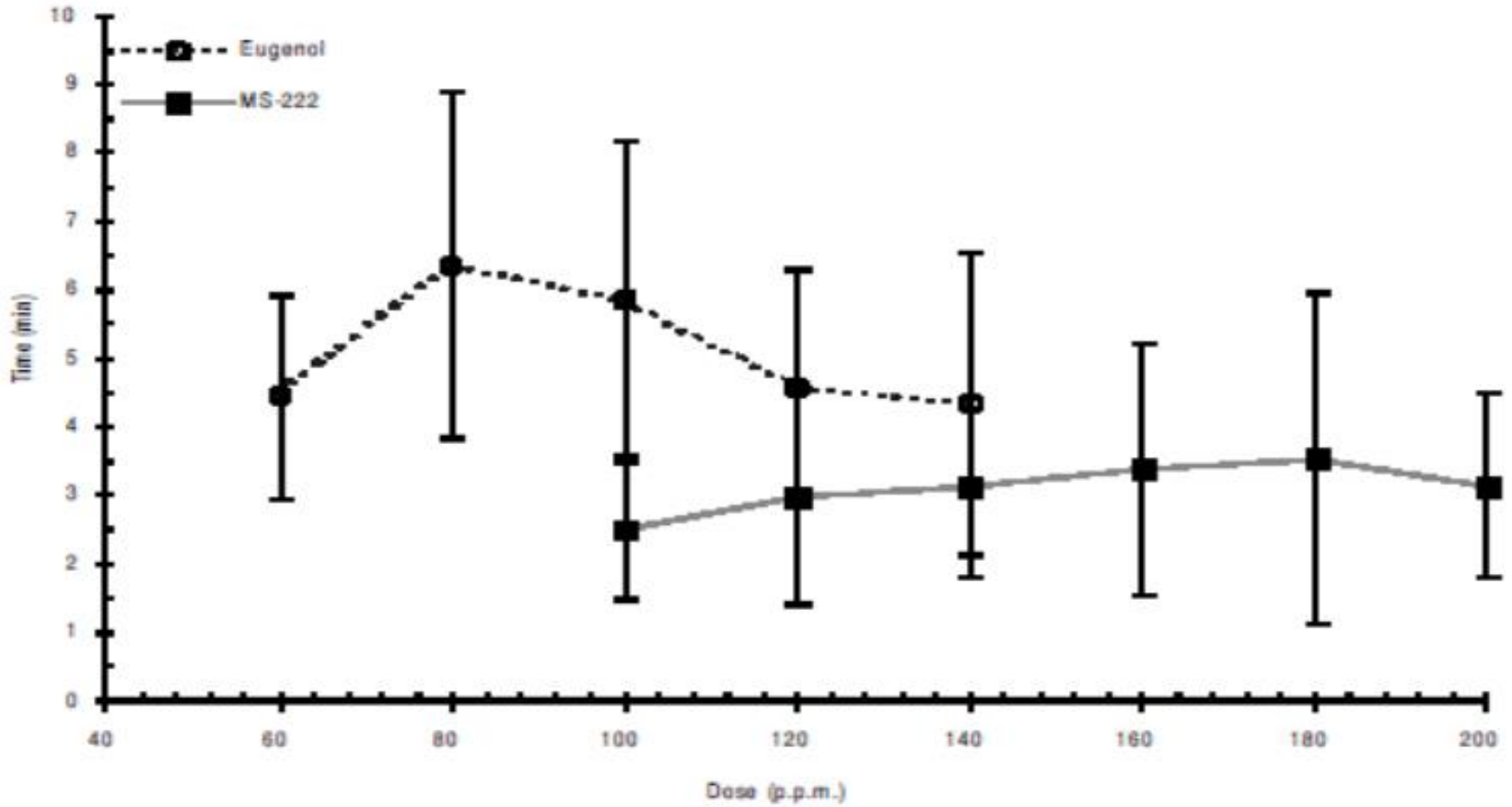


FIG. 4. Time required for zebrafish to achieve stage 3-recovery from anesthesia at various concentrations of clove oil and MS-222. Data points represent the mean ± SEM, $n = 10$.

- **Ethanol exposure effects**: All fish exposed to 1400ppm ethanol over 96 hours survived

Grush et Al. 2004

Conclusions

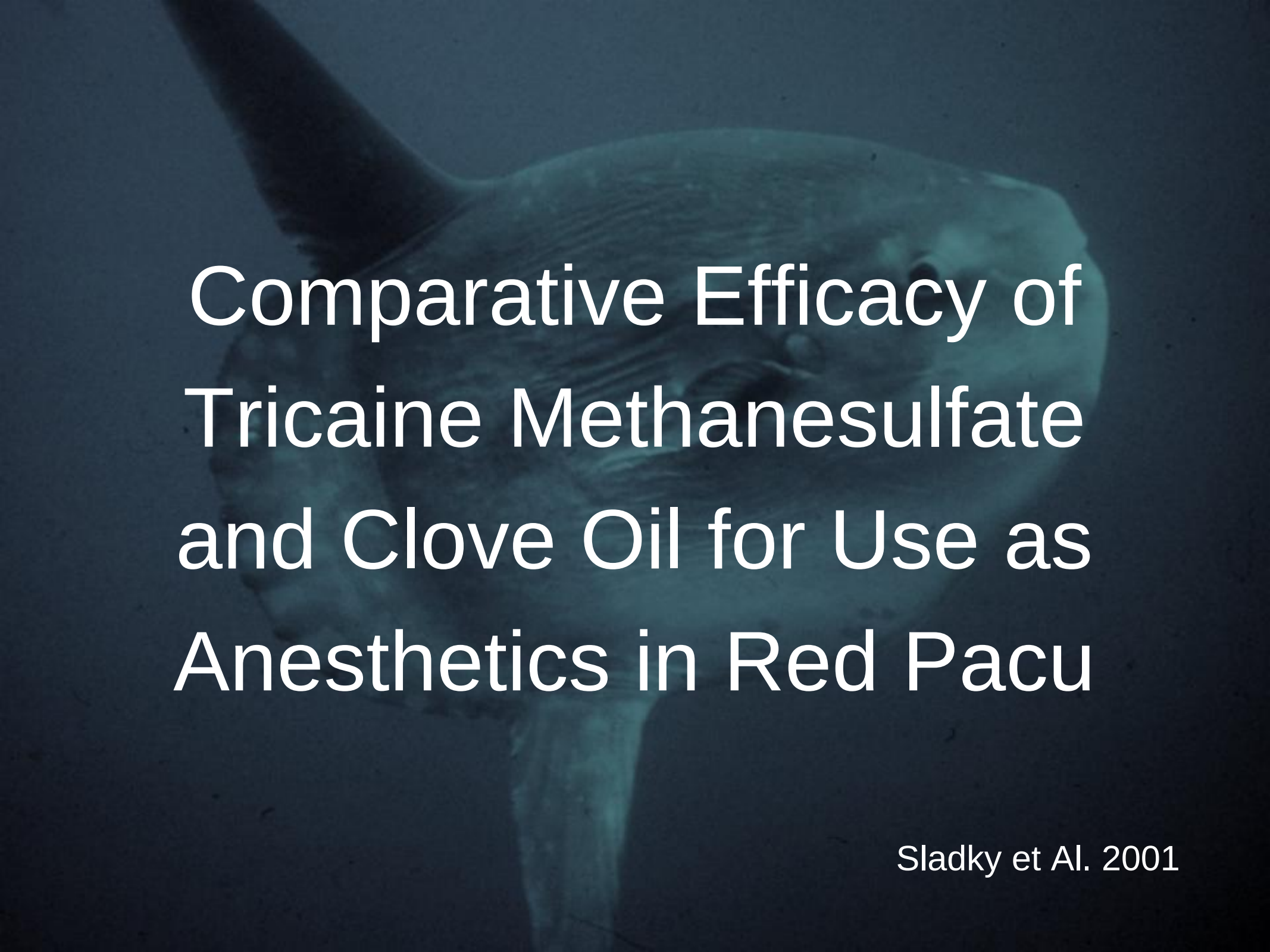
- Induction times were faster and at lower concentrations with eugenol than MS-222
- Recovery times were prolonged with eugenol
- Times to induction were concentration-dependent

Grush et Al. 2004

Limitations

- Only studied in the zebrafish
- No physiologic parameters measured
- Stages of anesthesia and recovery are not defined

Grush et Al. 2004



Comparative Efficacy of Tricaine Methanesulfate and Clove Oil for Use as Anesthetics in Red Pacu

Sladky et Al. 2001

Overview

- 15 captive-raised red pacu
- All approximately 4 years old
- All housed in the same aquarium for > 3 years prior to the study
- Study arms:
  - MS-222 (50, 100, 200mg/mL)
  - Eugenol (50, 100, 200mg/L)
- Within-subjects complete crossover design

Sladky et Al. 2001

Study Design

- Each fish housed separately in a 60L aquarium during the study period.
- Baseline respiratory rate observed in its home aquarium
- Fish removed from aquarium, blood sample #1 taken

Fish placed in 4L water in 8L plastic bag, fish allowed to acclimate for 2-3 minutes. Second baseline RR recorded.

Anesthetic added. Fish removed from tank and blood sample #2 taken when: a) Stage IV anesthesia reached b) 600 seconds had passed. Fish returned to home aquarium.

Resuscitation initiated if no opercular movement seen within 5 minutes of being returned to home aquarium

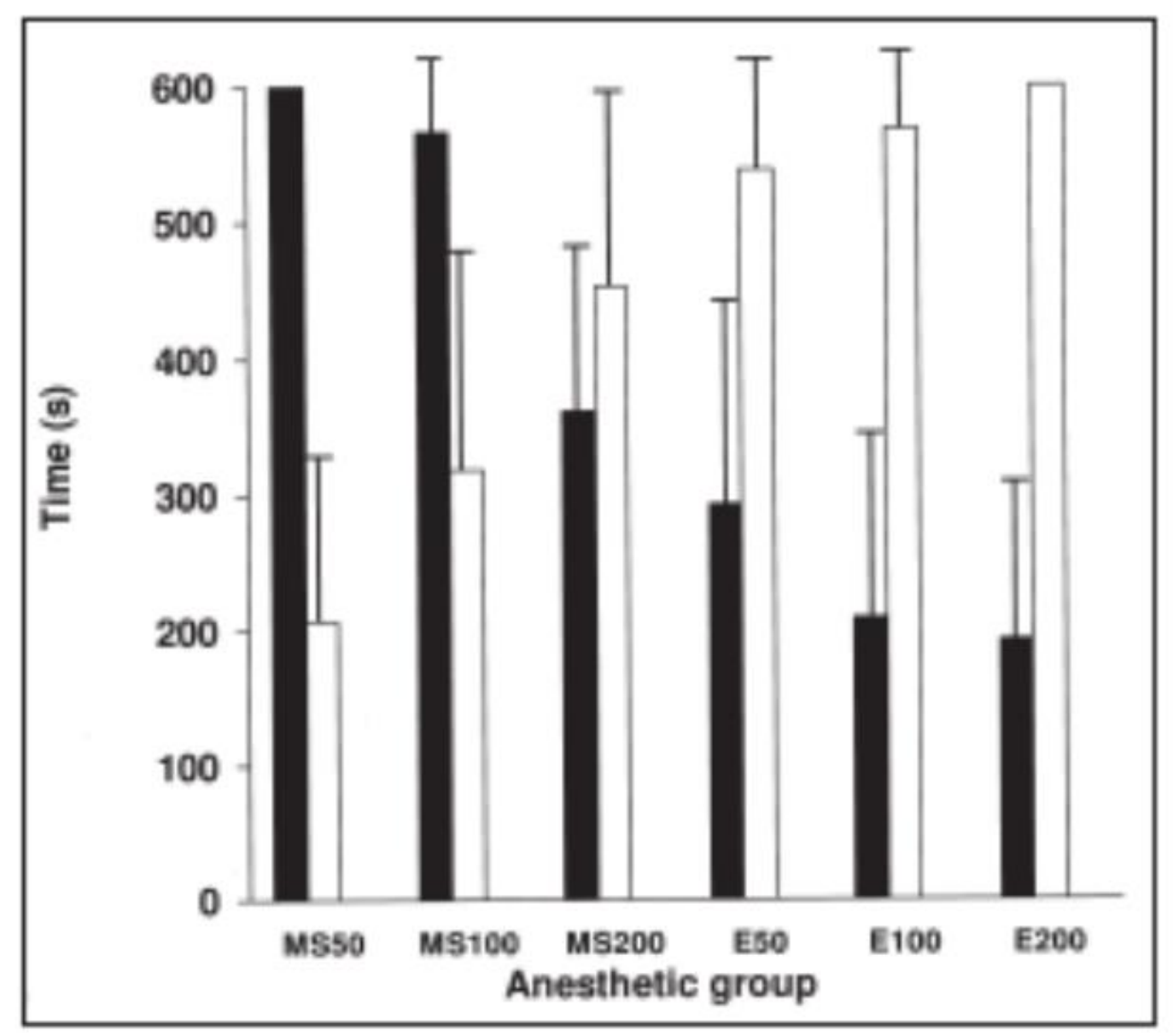
Sladky et Al. 2001

Study Design

- Behavioral reaction to needle measured as 0 (no observable reaction) and 1 (observable reaction
- Blood sample was collected from caudal artery or vein
- *Labs obtained:* Glucose, sodium, potassium, pH, PO_2, PCO_2, hematocrit, hemoglobin

Sladky et Al. 2001

Induction and Recovery Times



Sladky et Al. 2001

Physiologic Changes

Group	Glucose (mg/dl)	Sodium (mg/dl)	Potassium (mg/dl)	pH	Po_2* (mm Hg)	Pco_2* (mm Hg)	Hct (%)	Hemoglobin (g/dl)
MS 50								
Before	56.9 ± 12.0	140.2 ± 5.6	2.2 ± 0.6	7.75 ± 0.05	26.1 ± 20.7	4.4 ± 0.6	18.5 ± 2.5	6.1 ± 0.9
During	76.1 ± 16.4	143.5 ± 2.6	3.2 ± 0.4	7.49 ± 0.13	7.7 ± 4.5	7.7 ± 1.9	20.5 ± 2.6	6.9 ± 1.0
Change (%)	+24.6 ± 8.6	+1.9 ± 3.0	+28.3 ± 21.8	−3.3 ± 1.4	−55.4 ± 33.9	+39.2 ± 17.8	+9.7 ± 10.1	+10.0 ± 12.6
MS 100								
Before	58.7 ± 7.4	140.9 ± 4.3	2.6 ± 0.5	7.74 ± 0.11	24.1 ± 26.2	4.3 ± 0.9	18.9 ± 3.1	6.3 ± 1.1
During	74.9 ± 14.9	142.1 ± 3.3	3.1 ± 0.3	7.44 ± 0.11	3.9 ± 0.7	9.1 ± 3.0	20.9 ± 2.9	7.3 ± 1.2
Change (%)	+19.4 ± 15.7	+0.9 ± 2.4	+15.8 ± 14.7	−3.9 ± 1.2	−65.5 ± 26.8	+48.1 ± 18.0	+9.1 ± 11.3	+10.3 ± 12.9
MS 200								
Before	58.7 ± 11.9	140.9 ± 3.5	2.5 ± 0.5	7.72 ± 0.06	26.8 ± 22.5	4.6 ± 1.1	20.3 ± 3.8	6.9 ± 1.4
During	73.4 ± 15.5	141.8 ± 4.3	3.1 ± 0.4	7.45 ± 0.10	3.93 ± 0.7	8.6 ± 1.3	22.7 ± 3.0	7.7 ± 1.0
Change (%)	+19.1 ± 9.8	+0.6 ± 1.5	+18.2 ± 11.5	−3.5 ± 0.8	−76.3 ± 14.5	+46.1 ± 7.9	+10.8 ± 10.1	+10.8 ± 11.8
E 50								
Before	56.0 ± 7.7	141.4 ± 3.0	2.4 ± 0.4	7.73 ± 0.06	25.2 ± 16.6	4.4 ± 0.8	18.9 ± 3.1	6.3 ± 1.1
During	76.8 ± 15.0	143.1 ± 4.9	3.3 ± 0.3	7.41 ± 0.05	3.5 ± 0.5	8.9 ± 1.0	21.4 ± 2.8	7.2 ± 1.1
Change (%)	+25.7 ± 10.0	+1.2 ± 1.7	+27.2 ± 12.0	−4.2 ± 0.6	−77.8 ± 15.8	+50.4 ± 9.3	+16.0 ± 11.7	+15.9 ± 13.9
E 100								
Before	58.7 ± 12.7	140.1 ± 5.4	2.2 ± 0.4	7.75 ± 0.06	33.1 ± 36.8	4.1 ± 0.4	19.0 ± 3.2	6.3 ± 1.1
During	72.3 ± 20.3	143.3 ± 3.3	3.5 ± 0.3	7.44 ± 0.09	3.93 ± 1.0	8.4 ± 1.7	20.6 ± 3.1	7.1 ± 1.1
Change (%)	+17.2 ± 9.9	+2.2 ± 4.0	+35.2 ± 13.1	−4.0 ± 1.1	−73.1 ± 28.5	+49.4 ± 10.7	+7.0 ± 14.8	+9.9 ± 13.5
E 200								
Before	55.2 ± 5.7	141.8 ± 1.9	2.5 ± 0.5	7.81 ± 0.30	20.8 ± 23.97	4.6 ± 0.9	20.1 ± 3.2	6.7 ± 1.0
During	66.0 ± 10.7	141.5 ± 3.7	3.5 ± 0.4	7.50 ± 0.08	5.2 ± 2.1	7.4 ± 1.4	19.3 ± 2.1	6.3 ± 0.6
Change (%)	+15.2 ± 10.3	−0.3 ± 2.5	+28.4 ± 13.0	−3.8 ± 3.2	−59.4 ± 26.9	+37.7 ± 13.2	−4.8 ± 17.8	−7.0 ± 17.7

Percentage change is indicated as an increase (+) or decrease (−) of values obtained before anesthesia, compared with values obtained during anesthesia. *The Po_2 and Pco_2 values were determined on mixed venous-arterial blood samples.

Response to Needle Puncture

Eugenol:

- 50mg/L: 10/15
- 100mg/L: 14/15 [6/15 resuscitated]
- 200mg/L: 14/15 [11/15 resuscitated]

MS-222:

- 50mg/L: 8/15
- 100mg/L: 3/15
- 200mg/L: 1/15

Sladky et Al. 2001

Conclusions

- Margin of safety for eugenol was narrower
- Eugenol is less effective as an anesthetic
- Induction times are more rapid and recovery times are more prolonged with eugenol
- Physiologic changes were similar between groups

Sladky et Al. 2001

Limitations

- Small trial
- Only studied in red pacu
- Incomplete data on resuscitation rates

Conclusions

- Thousands of species
- Efficacy of an agent in one species is not reliably extrapolated to another (ex. MS-222 in sturgeon)
- Must use MS-222 in food animals
- Consider any evidence available for that species or similar species
- Clove oil seems to be associated with prolonged recovery times and faster induction rates

Veterinary Pharmacy Pearls

Veterinary Pharmacy Pearls

- **Where do I learn how to give OTC advice to veterinary patients?**
  - You can't! AMDUCA, 1994 [www.fda.gov/cvm/s340.htm]
  - A valid veterinarian-client-patient relationship must be established
- **Vetsulin:** Porcine in origin
  - U40 Insulin
  - Separate syringes

Thompson J 2009

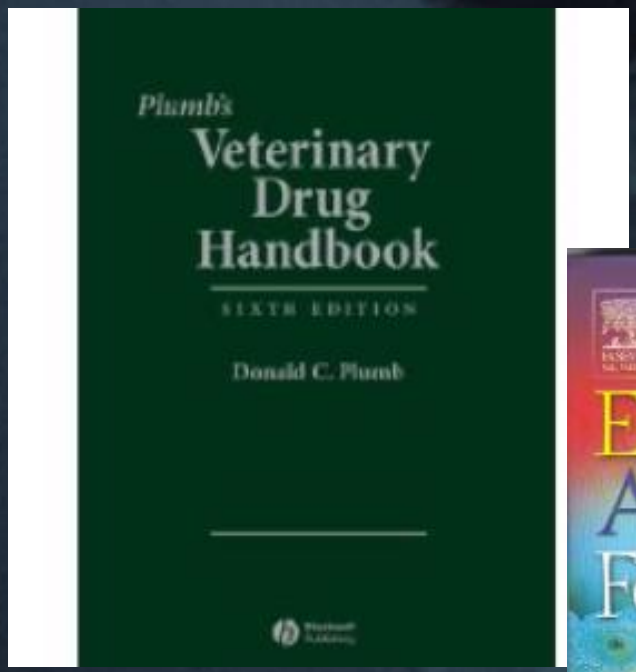
Huang J 2010

Veterinary Pharmacy Pearls

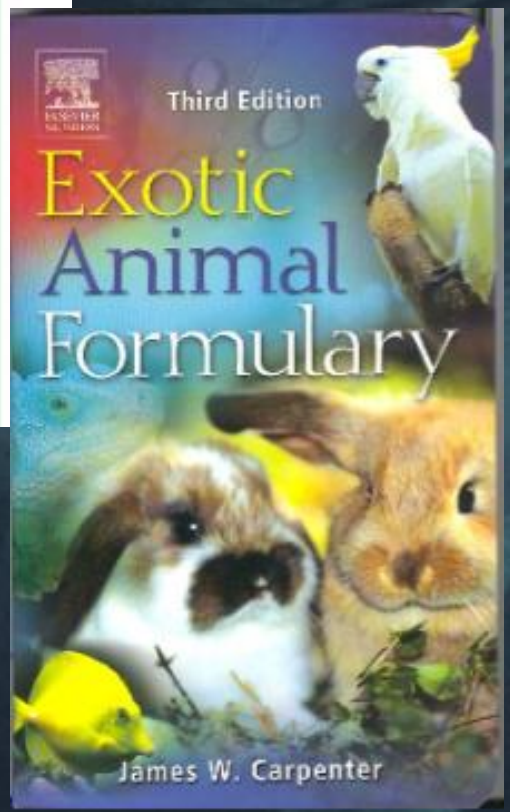
- FARAD
  - CVM's website for withdrawal times in various species [www.farad.org]
- **Some potentially inappropriate drugs in selected species:**
  - Ivermectin - turtles
  - Beta-lactams, erythromycin, clindamycin, tetracyclines - rabbits
  - Steroids - birds
  - **When in doubt - call the vet!!**

Thompson J
2009

Helpful Veterinary References



Plumb's



Exotic Animal Formulary

- Merck Veterinary Manual: www.merckvetmanual.com
- Tufts University Open Courseware: http://ocw.tufts.edu
- Walgreen's Online CE
- www.ivis.org

Some commonly encountered drugs in veterinary medicine

- **Macrocytic lactones (heartworm preventives):** Ivermectin, moxidectin, selamectin, milbemycin
- Melarsomine (Immiticide)
- **NSAIDS**: Carprofen (Rimadyl), flunixin (Banamine), meloxicam
- Otomax, Mometamax
- **Flea Treatments:** (S)-Methoprene, Fipronil
- **Antibiotics:** Enrofloxacin (Baytril), oxytetracycline, chloramphenicol, ceftiofur (Naxcel)
- **Anti-parasitics:** Pyrantel, praziquantel (Droncit), fenbendazole (Panacur)
- **Nausea:** Maropitant (Serenia)

References

- Plumb D. *Veterinary Drug Handbook.* Hoboken, NJ: Wiley-Blackwell; 2008.
- Carpenter J. *Exotic Animal Formulary.* Maryland Heights, MO: Saunders; 2004.
- Sladky K, Swanson C, Stoskopf M. Comparative efficacy of tricaine methanesulfonate and clove oil for use as anesthetics in red pacu. AJVR 2001; 62(3):337-42.
- Grush J, Noakes DLG, Moccia RD. The efficacy of clove oil as an anesthetic for the zebrafish, *Danio Rerio* (Hamilton). Zebrafish 2004; 1(1):46-53.
- Neiffer D, Stamper A. Fish sedation, analgesia, and euthanasia: considerations, methods, and types of drugs. ILAR 2009 ; 50(4): 343-360.
- Domenici P, Lefrancois C, Shingles A. Hypoxia and the antipredator behaviours of fishes. *Phil. Trans. R. Soc. B* (2007); 362: 2105–2121.
- Food and Drug Administration. Concerns related to the use of clove oil as an anesthetic for fish. Accessed at www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/ucm052520.pdf Feb 10, 2010.
- Lewbart G. Fish Anesthesia. Accessed at www.ivis.org on February 10, 2010.
- Coyle S, Durborow R, Tidwell J. Anesthetics in aquaculture. Souther Regional Aquaculture Center 2004: Publication # 3900.
- Thompson J. A practical guide to contemporary pharmacy practice. Philadelphia:Wolters-Kluwer Health; 2009.
- Huang J. Veterinary therapeutics for community pharmacists volume 1. Accessed at https://webapp.walgreens.com/cePharmacy/ Feb 14, 2010.
- Tufts university open courseware. Accessed at http://ocw.tufts.edu/Schools Feb 14, 2010.

Image References

- http://www.lrn.usace.army.mil/op/cor/rec/images/fish/striped_bass.jpg
- http://animal-world.com/encyclo/marine/butter/images/RaccoonButterflyfishWMBu_C744.jpg
- http://static.zsl.org/images/originals/3-manta-ray-2336.jpg
- http://scrapetv.com/News/News%20Pages/Everyone%20Else/images-4/Great-White-Shark-3.jpg
- http://www.richardseaman.com/Underwater/Belize/StillLifes/ReticulatedStarfish.jpg
- http://blogs.menmedia.co.uk/old/worldcup/jellyfish.jpg
- http://www.aquariumfish.net/images_01/oranda_choco_pompom_w270.jpg
- http://johneaves.files.wordpress.com/2009/06/mola_mola2.jpg
- http://www.coolscienceexperimentsforkids.com/wordpress/wp-content/uploads/2009/11/south_american_lungfish.jpg
- http://www.koi-ponds-care.co.uk/Images/Koi-PondTypes1.jpg
- http://www.aquariacentral.com/pictures/pacu4.jpg
- http://mi.eng.cam.ac.uk/research/projects/Query/img/arrow.png
- http://blogs.nature.com/nm/spoonful/zebrafish.jpg

Questions????