



## AEEC Guidelines Formulary in Common Laboratory Species

Objective	To provide guidelines for common Laboratory Species
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Version	1

### **Introduction**

The purpose of these guidelines is to describe appropriate analgesia, anaesthesia and chemical restraint regimes for management of pain and stress in animals used in teaching or research at the Chinese University of Hong Kong.

An exception to the Guidelines must be described and justified in the AEEC application and approved by the AEEC during review process. Veterinary advice should be sought when necessary.

## Rodents (Rats): Analgesia/Anesthesia/Chemical Restraint

Drug	Dosage	Route	Frequency	Remarks
<b>Analgesic</b>				
<b>Bupivacaine</b> <sup>1,6</sup>	< 4 mg/kg	SC, TOP, IA	4 - 6h	Local anesthetic.
<b>Buprenorphine</b> <sup>1,6</sup>	0.05 mg/kg	SC	12h	
<b>Carprofen</b> <sup>1,6</sup>	2 - 5 mg/kg	SC	12 - 24h	Anti-inflammatory.
<b>Meloxicam</b> <sup>1,6</sup>	1 - 2 mg/kg	SC	12h	Anti-inflammatory.
		PO	12 - 24h	Can be added into drinking water for dosage if the animal is singly housed. Not recommended for animal with polydipsia or adipsia. 6 ml of 1.5 mg/ml Meloxicam into 500 ml drinking water (1.8 mg/kg for 300 g rats).
<b>Anesthetic</b>				
<b>Isoflurane</b> <sup>4</sup>	4 - 5% Induction	INH		Surgical anesthesia. Rapid induction and recovery. Administer Isoflurane in 100% oxygen at 0.5 - 1.0 liter/min; Induce anesthesia in an induction box and use tailor made facemask for maintenance. Need to monitor animal and adjust % accordingly.
	1 - 2% Maintenance			
<b>Ketamine + Medetomidine</b> <sup>1,2</sup>	75 mg/kg	IP		Surgical anesthesia. 0.75ml (75mg) Ketamine + 0.5ml (0.5mg) Medetomidine + 0.75ml Water For Injection BP, administer 0.2ml/100g BW of mixture.
	0.5 mg/kg			
<b>Ketamine + Midazolam</b> <sup>1</sup>	75 mg/kg	IP		Light anesthesia. 0.75ml (75mg) Ketamine + 1ml (5mg) Midazolam + 0.25ml Water For Injection BP, administer 0.2ml/100g BW of mixture.
	5 mg/kg			
<b>Ketamine + Xylazine</b> <sup>1,2</sup>	75 - 100mg/kg	IP		Surgical anesthesia. 0.75 ml (75mg) Ketamine + 0.5ml (10mg) Xylazine + 0.75ml Water For Injection BP, administer 0.2ml/100g BW of mixture.
	10 mg/kg			
<b>Ketamine + Xylazine + Acepromazine</b> <sup>3</sup>	65 mg/kg	IP		
	13 mg/kg			
	1.5 mg/kg			

### Recommended Volume for Injections:

Route	Maximum volume <sup>7</sup>
<b>SC</b>	10 ml per site
<b>IP</b>	10 ml
<b>IM</b>	0.3 ml per site
<b>IV</b>	0.5 ml

#### References:

- Flecknell, P. (1996). Laboratory Animal Anesthesia, 2<sup>nd</sup> ed., Academic Press.
- Kohn, D. F., S. K. Wixson, W. J. White, and G. J. Benson (1997). Anesthesia and Analgesia in Laboratory Animals, ACLAM Series, Academic Press.
- JAALAS 2010 Jan; 49(1):45-51.
- Boston University Research Support, Anesthesia and Analgesia: Neonatal Mice and Rats.
- James W. Carpenter. Exotic Animal Formulary, Fifth Edition.
- Recommended Surgical Analgesic Protocols for Mice and Rats. University of Texas at San Antonio.
- Ostrow, M. E., Stark, D. M. (1992). Laboratory Animal Technician Training Manual Series (Vol. 2). American Association for Laboratory Animal Science.

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## Rodents (Mice): Analgesia/Anesthesia/Chemical Restraint

Drug	Dosage	Route	Frequency	Remarks
<b>Analgesic</b>				
<b>Bupivacaine</b> <sup>1,6</sup>	< 4 mg/kg	SC, TOP, IA	4 - 6h	Local anesthetic.
<b>Buprenorphine</b> <sup>1,6</sup>	0.05 mg/kg	SC, IP	12h	
<b>Carprofen</b> <sup>1,6</sup>	2 - 5 mg/kg	SC	12 - 24h	Anti-inflammatory.
<b>Meloxicam</b> <sup>1,6</sup>	1 - 2 mg/kg	SC	12h	Anti-inflammatory.
	1 – 5 mg/kg	PO	24h	Can be added into drinking water for dosage if the animal is singly housed. Not recommended for animal with polydipsia or adipisia. 3 ml of 1.5mg/ml Meloxicam into 200 ml drinking water (3.75 mg/kg for 30 g mice).
<b>Anesthetic</b>				
<b>Isoflurane</b> <sup>4</sup>	4 - 5% Induction	INH		Surgical anesthesia. Rapid induction and recovery. Administer Isoflurane in 100% oxygen at 0.5 - 1.0 liter/min. Induce anesthesia in an induction box and use tailor made facemask for maintenance. Need to monitor animal and adjust % accordingly.
	1 - 2% Maintenance			
<b>Ketamine + Medetomidine</b> <sup>1,2</sup>	75 mg/kg	IP		Surgical anesthesia. 0.38ml (38mg) Ketamine + 0.5ml (0.5mg) Medetomidine + 4.12ml Water For Injection BP, administer 0.1ml/10g BW of mixture.
	1 mg/kg			
<b>Ketamine + Midazolam</b> <sup>1</sup>	100 mg/kg	IP		Light anesthesia. 0.5ml (50mg) Ketamine + 0.5ml (2.5mg) Midazolam + 4.0ml Water For Injection BP, administer 0.1ml/10g BW of mixture.
	5 mg/kg			
<b>Ketamine + Xylazine</b> <sup>1,2</sup>	100 mg/kg	IP		Surgical anesthesia. 0.5 ml (50mg) Ketamine + 0.25ml (5mg) Xylazine + 4.25ml Water For Injection BP, administer 0.1ml/10g BW of mixture.
	10 mg/kg			
<b>Ketamine + Xylazine + Acepromazine</b> <sup>3</sup>	65 mg/kg	IP		
	13 mg/kg			
	1.5 mg/kg			

### Recommended volume for injections:

Route	Maximum volume <sup>7</sup>
<b>SC</b>	3 ml per site
<b>IP</b>	3 ml
<b>IM</b>	0.05 ml per site
<b>IV</b>	0.2 ml

#### References:

- Flecknell, P. (1996). Laboratory Animal Anesthesia, 2<sup>nd</sup> ed., Academic Press.
- Kohn, D. F., S. K. Wixson, W. J. White, and G. J. Benson (1997). Anesthesia and Analgesia in Laboratory Animals, ACLAM Series, Academic Press.
- JAALAS 2010 Jan; 49(1):45-51.
- Boston University Research Support, Anesthesia and Analgesia: Neonatal Mice and Rats.
- James W. Carpenter. Exotic Animal Formulary, Fifth Edition.
- Recommended Surgical Analgesic Protocols for Mice and Rats. University of Texas at San Antonio.
- Ostrow, M. E., Stark, D. M. (1992). Laboratory Animal Technician Training Manual Series (Vol. 2). American Association for Laboratory Animal Science.

## Rodents (Hamsters): Analgesia/Anesthesia/Chemical Restraint

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Drug	Dosage	Route	Frequency	Remarks
<b>Analgesic</b>				
<b>Buprenorphine</b> <sup>5</sup>	0.01 - 0.05 mg/kg	SC	6 - 12h	
<b>Carprofen</b> <sup>5</sup>	2 - 5 mg/kg	SC	12 - 24h	Anti-inflammatory.
<b>Meloxicam</b> <sup>5</sup>	0.5 mg/kg	SC	12 - 24h	Anti-inflammatory.
		PO		Can be added into drinking water for dosage if the animal is singly housed. Not recommended for animal with polydipsia or adipsia. 1 ml of 0.5 mg/ml Meloxicam into 100 ml drinking water (0.5 mg/kg for 100 g hamsters).
<b>Anesthetic</b>				
<b>Isoflurane</b> <sup>5</sup>	2 - 5% Induction	INH		Surgical anesthesia. Rapid induction and recovery. Administer Isoflurane in 100% oxygen at 0.5 - 1.0 liter/min; Induce anesthesia in an induction box or using face mask.
	0.25 - 4% Maintenance			Face mask or via endotracheal tube (preferred) for maintenance. Need to monitor animal and adjust % accordingly.
<b>Ketamine + Medetomidine</b> <sup>5</sup>	100 - 200 mg/kg	IP, SC		Surgical anesthesia.
	0.25 mg/kg			
<b>Ketamine + Xylazine</b> <sup>2</sup>	200 mg/kg	IP		Surgical anesthesia.
	10 mg/kg			
<b>Sedative</b>				
<b>Medetomidine</b> <sup>5</sup>	0.1 mg/kg	SC		Moderate sedation.

### Recommended volume for injections:

Route	Maximum volume <sup>7</sup>
<b>SC</b>	4 ml per site
<b>IP</b>	4 ml
<b>IM</b>	0.1 ml per site
<b>IV</b>	0.3 ml

#### References:

1. Flecknell, P. (1996). Laboratory Animal Anesthesia, 2<sup>nd</sup> ed., Academic Press.
2. Kohn, D. F., S. K. Wixson, W. J. White, and G. J. Benson (1997). Anesthesia and Analgesia in Laboratory Animals, ACLAM Series, Academic Press.
3. JAALAS 2010 Jan; 49(1):45-51.
4. Boston University Research Support, Anesthesia and Analgesia: Neonatal Mice and Rats.
5. James W. Carpenter. Exotic Animal Formulary, Fifth Edition.
6. Recommended Surgical Analgesic Protocols for Mice and Rats. University of Texas at San Antonio.
7. Ostrow, M. E., Stark, D. M. (1992). Laboratory Animal Technician Training Manual Series (Vol. 2). American Association for Laboratory Animal Science.

## Rabbits: Analgesia/Anesthesia/Chemical Restraint

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Drug	Dosage	Route	Frequency	Remarks
<b>Analgesic</b>				
<b>Buprenorphine</b> <sup>1</sup>	0.01 - 0.05 mg/kg	IV, SC, IM	q12h	
<b>Carprofen</b> <sup>2</sup>	1.5 mg/kg+	PO	q12h	Anti-inflammatory.
	1 - 2 mg/kg	SC, IV	q12h	
<b>Ketoprofen</b> <sup>1,2</sup>	3 mg/kg	IM	q12h	Anti-inflammatory.
	1 - 3 mg/kg	IM	q12h	
<b>Lidocaine Hydrochloride</b> <sup>3</sup>	2 - 4 mg/kg	SC		Local anesthetic.
<b>Lidocaine 2.5% + Prilocaine 2.5% (Emla cream)</b> <sup>1</sup>		TOP to Skin		Facilitates IV catheter placement.
<b>Meloxicam</b> <sup>2</sup>	0.2 - 0.3 mg/kg	PO	q12h	Anti-inflammatory.
	0.2 mg/kg	SC	q12h	
<b>Anesthetic</b>				
<b>Acepromazine</b> <sup>1</sup>	0.25 - 1 mg/kg	IM		Pre-anesthetic; sedative; tranquilizer.
	1 - 5 mg/kg	SC		Pre-anesthetic.
<b>Isoflurane</b> <sup>1</sup>	3 - 5% Induction	INH		Surgical anesthesia. Rapid induction and recovery. Face mask for induction.
	1.5 - 1.75% Maintenance		Face mask or via endotracheal tube (preferred) for maintenance. Need to monitor animal and adjust % accordingly.	
<b>Ketamine + Acepromazine</b> <sup>2</sup>	50 mg/kg	IM		Surgical anesthesia.
	1 mg/kg			
<b>Ketamine + Medetomidine</b> <sup>2</sup>	15 mg/kg	SC		Surgical anesthesia.
	0.25 mg/kg			
<b>Ketamine + Midazolam</b> <sup>5</sup>	25 mg/kg	IM		Surgical anesthesia.
	1 mg/kg			
<b>Ketamine + Xylazine</b> <sup>1</sup>	K 10 mg/kg	IV		Surgical anesthesia.
	X 3 mg/kg			
	K 35 mg/kg	IM		
	X 5 mg/kg			
<b>Ketamine + Xylazine + Acepromazine</b> <sup>2</sup>	35 mg/kg	IM		Surgical anesthesia.
	5 mg/kg			
	1 mg/kg			
<b>Sedative</b>				
<b>Acepromazine</b> <sup>2,5</sup>	0.75 - 1.0 mg/kg	IM		Moderate sedation.
	0.1 - 0.5 mg/kg		Calming effect.	
<b>Diazepam</b> <sup>3,4,5</sup>	1 - 2 mg/kg	IV		Moderate to deep sedation.
	5 - 10 mg/kg	IM, IP		Deep sedation and muscle relaxation.
<b>Ketamine</b> <sup>2</sup>	25 - 50 mg/kg	IM		Moderate to heavy sedation.
	15 - 20 mg/kg	IV		
<b>Medetomidine</b> <sup>2</sup>	0.1 - 0.5 mg/kg	IM, SC		Mild to profound sedation.
<b>Midazolam</b> <sup>3</sup>	2 mg/kg	IV, IP		Moderate to deep sedation.
<b>Xylazine</b> <sup>2,5</sup>	1 - 5 mg/kg	SC, IM		Pre-anesthetic.

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Reversal Agents				
Yohimbine <sup>1</sup>	0.2 - 1.0 mg/kg	IM, IV		Xylazine reversal.

**Recommended volume for injections:**

Route	Maximum volume <sup>6</sup>
SC	30 ml per site
IP	50 ml
IM	1 ml per site
IV	5 ml

References:

1. James W. Carpenter. Exotic Animal Formulary, Fifth Edition.
2. Paul Flecknell (2003). Manual of Rabbit Medicine and Surgery.
3. Kohn, D.F., S.K. Wixson, W. J. White, and G.J. Benson (1997). Anesthesia and Analgesia in Laboratory Animals, ACLAM Series, Academic Press.
4. C.J. Green, (1982). Animal Anaesthesia.
5. Mark A. Suckow, Fred A. Douglas. The Laboratory Rabbit.
6. Ostrow, M. E., Stark, D. M. (1992). Laboratory Animal Technician Training Manual Series (Vol. 2). American Association for Laboratory Animal Science.

## Pigs: Analgesia/Anesthesia/Chemical Restraint

Drug	Dose	Route	Frequency	Remarks
<b>Analgesic</b>				
<b>Aspirin</b> <sup>1</sup>	10 - 20 mg/kg	PO	12h	Anti-inflammatory.
<b>Buprenorphine</b> <sup>2</sup>	0.05 - 0.1 mg/kg	IM, IV	8-12h	
<b>Carprofen</b> <sup>2</sup>	2 - 4 mg/kg	SC, PO	24h	Anti-inflammatory.
<b>Meloxicam</b> <sup>2</sup>	0.2 - 0.4 mg/kg	SC, PO, IM	24h	Anti-inflammatory.
<b>Morphine</b> <sup>1</sup>	0.05 - 0.1 mg/kg	IM		Analgesia; < 20 mg in total. Can cause vomiting. Recommended to give post-operatively.
<b>Anesthetic</b>				
<b>Atropine</b> <sup>1</sup>	0.02 - 0.04 mg/kg	SC, IM, IV (for emergen cies)		Pre-anesthetic.
<b>Isoflurane</b> <sup>1</sup>	4 - 5% Induction	INH		Anesthesia. Rapid induction and recovery. Face mask for induction.
	1 - 2% Maintenance			Face mask or via endotracheal tube (preferred) for maintenance. Need to monitor animal and adjust % accordingly.
<b>Ketamine + Acepromazine</b> <sup>3</sup>	22 - 33 mg/kg	IM		Light anesthesia.
	1.1 mg/kg			
<b>Ketamine + Diazepam</b> <sup>3</sup>	10 - 15 mg/kg	IM		Light anesthesia.
	0.5 - 2 mg/kg			
<b>Ketamine + Medetomidine</b> <sup>3</sup>	10 mg/kg	IM		Light anesthesia.
	0.08 mg/kg			
<b>Ketamine + Xylazine</b> <sup>1</sup>	10 - 15 mg/kg (10 mins after given xylazine)	IM		Short-term anesthesia; anesthesia induction; follow by Isoflurane for maintenance.
	2 - 6 mg/kg			
<b>Ketamine + Xylazine + Butorphanol</b> <sup>1</sup>	11.0 mg/kg	IM		Short-term anesthesia.
	2.2 mg/kg			
	0.2 mg/kg			
<b>Propofol</b> <sup>3</sup>	2.5 - 3.5 mg/kg	IV		Surgical anesthesia.
	6 - 8 mg/kg (if no pre-med given)			
	14 - 20 mg/kg/hr (for CRI maintenance)			
<b>Sedative</b>				
<b>Acepromazine</b> <sup>1</sup>	0.1 - 0.5 mg/kg	IM, IV		Tranquilization.
<b>Butorphanol</b> <sup>2</sup>	0.1 - 0.3 mg/kg	IM, IV		Mild Analgesia effect.
<b>Diazepam</b> <sup>2</sup>	0.5 - 10 mg/kg	IM		Minimal sedation; muscle relaxants.
	0.44 - 2.0 mg/kg	IV		
<b>Ketamine + Medetomidine</b> <sup>3</sup>	5 mg/kg	IM		
	0.03 - 0.08 mg/kg			
<b>Medetomidine</b> <sup>2</sup>	0.02 - 0.04 mg/kg	IM, IV, SC		

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<b>Midazolam</b> <sup>2</sup>	0.1 - 0.5 mg/kg	IM, IV		
<b>Xylazine</b> <sup>1</sup>	1 - 2 mg/kg	IM, SC		
<b>Reversal Agents</b>				
<b>Atipamezole</b> <sup>2</sup>	0.1 - 0.2 mg/kg	IV, IM		Medetomidine reversal.
<b>Flumazenil</b> <sup>1</sup>	0.01 - 0.1 mg/kg	IV		Midazolam reversal.
<b>Yohimbine</b> <sup>2</sup>	0.05 - 0.1 mg/kg	IV		Xylazine reversal.
<b>Miscellaneous</b>				
<b>Dantrolene</b> <sup>1</sup>	20 mg/kg	PO	12h	Treat hyperthermia in stress-susceptible animals.
	2 - 6 mg/kg	IV	12h	

### Recommended volume for injections:

Route	Maximum volume
SC	3 ml per site
IM	10 ml per site
IV	30 ml

#### References:

1. Muir. W.W and Hubbel J.A.E, (2013) Handbook of Veterinary Anaesthesia (5<sup>th</sup> Edn.).
2. AALAS Learning Library, 2022.
3. Flecknell, P, (2015), Laboratory Animal Anaesthesia (4<sup>th</sup> Edn.).

## Ferrets: Analgesia/Anesthesia/Chemical Restraint

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Drug	Dosage	Route	Frequency	Remarks
<b>Analgesic</b>				
<b>Buprenorphine</b> <sup>1,2</sup>	0.01 - 0.05 mg/kg	IM, SC	8 - 12h	May cause respiratory depression.
<b>Carprofen</b> <sup>1,2</sup>	2 - 5 mg/kg	PO, SC	12 - 24 h	Anti-inflammatory.
<b>Meloxicam</b> <sup>1,2</sup>	0.2 mg/kg loading dose, then 0.1 mg/kg	SC, IM, PO	24 h	Anti-inflammatory.
<b>Anesthetic</b>				
<b>Isoflurane</b> <sup>1,2</sup>	3 - 5% Induction	INH		Face mask for induction.
	1 - 3% Maintenance			Face mask or via endotracheal tube (preferred) for maintenance. Need to monitor animal and adjust % accordingly.
<b>Ketamine + Diazepam</b> <sup>1,2</sup>	16 mg/kg	IM		Surgical anesthesia (induction). Partially reversible with Yohimbine.
	3 mg/kg			
<b>Ketamine + Xylazine</b> <sup>1,2</sup>	12.5 mg/kg	IM		Surgical anesthesia (induction). Reversible with Atipamezole or Yohimbine.
	0.75 mg/kg			
<b>Sedative</b>				
<b>Diazepam</b> <sup>1,2</sup>	0.5 - 3 mg/kg	IM		Good muscle relaxant.
<b>Midazolam</b> <sup>1,2</sup>	0.5 - 3 mg/kg	IM, SC		Good muscle relaxant.
<b>Reversal Agents</b>				
<b>Atipamezole</b> <sup>1,2</sup>	0.4 - 1.0 mg/kg	IM, SC, IV, IP		Xylazine reversal. Administer as equal volume of Xylazine.
<b>Yohimbine</b> <sup>1,2</sup>	0.2 - 1.0 mg/kg	IV, IM		Xylazine reversal.

### Recommended volume for injections:

Route	Maximum volume
SC	10 ml per site
IP	10 ml
IM	0.3 ml per site
IV	0.5 ml

#### References:

1. Plumb, DC. Plumb's Veterinary Drug Handbook. Stockholm, Wis.: Ames, Iowa: PharmaVet; Distributed by Blackwell Pub., 2005.
2. M. Johnston. Clinical Approaches to Analgesia in Ferrets and rabbits. Seminars in Avian and exotic pet medicine Vol 14, Issue 4, 2005.

## Amphibians: Analgesia/Anesthesia/Chemical Restraint

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Drug	Dosage	Route	Frequency	Remarks
<b>Analgesic</b>				
<b>Aspirin</b> <sup>2</sup>	2.5 mg/L	Immersion	30 mins duration	
<b>Lidocaine hydrochloride</b> <sup>1,2</sup>	5 mg/L	Immersion	30 mins duration	
<b>Morphine</b> <sup>2</sup>	48 mg/L	Immersion		Larvae.
<b>Anesthetic</b>				
<b>Benzocaine (Orajel)</b> <sup>5</sup>	0.1 ml/10 g	Cutaneous		Anesthesia 15 - 60 mins.
<b>Isoflurane</b> <sup>1-4</sup>	Mix 3ml with 3ml KY jelly + 1.5 ml water. Cover 2/3 of a 1 x 1 cotton gauze square with impermeable backing. Place on frogs back, remove when anaesthetized.	Cutaneous		Short procedures. Must use chemical hood to mix and deliver.
<b>Tricaine methanesulfonate (MS222)</b> <sup>1-4</sup>	500 mg – 2g/L (For Frog)	Immersion		Surgical anesthesia; Must be buffered with Sodium Bicarbonate (or similar) to pH 7.0 – 7.5.
	200 – 500 mg/L (For Tadpole)			Embryo: 1 – 14 days post fertilization. Mortality if > 10 mins.

References:

1. Anaesthesia and Analgesia in Laboratory Animals, Fish et al. 2<sup>nd</sup> Edition.
2. Formulary for Laboratory Animals, Hawk et al, 3<sup>rd</sup> Edition.
3. Laboratory Animal Medicine, ACLAM, 3<sup>rd</sup> Edition.
4. Veterinary Drug Handbook, Plumb. 3<sup>rd</sup> Edition.

## Fish: Analgesia/Anesthesia/Chemical Restraint

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Drug	Dosage	Route	Frequency	Remarks
<b>Analgesic</b>				
<b>Aspirin</b> <sup>2</sup>	2.5 mg/L	Immersion	30 mins duration	
<b>Buprenorphine</b> <sup>1,2</sup>	0.01 – 0.3 mg/kg	Immersion		
<b>Lidocaine hydrochloride</b> <sup>1,2</sup>	1 - 5 mg/L	Immersion	30 mins duration	
<b>Morphine</b> <sup>2</sup>	48 mg/L	Immersion		
	2.5 – 50 mg/kg	IM		
<b>Anesthetic</b>				
<b>Ice (Hypothermia)</b>		Contact	< 10 mins duration	Embryo: 1 – 14 -Day post fertilization. Mortality if > 10 mins.
<b>Lidocaine hydrochloride</b> <sup>1,2</sup>	325 mg/L	Immersion		Surgical anesthesia.
<b>Tricaine methanesulfonate (MS222)</b> <sup>1,2</sup>	50 – 100 mg/L	Immersion		Light anesthesia; Must be buffered with Sodium Bicarbonate (or similar) to pH 7.0 – 7.5.
	100 – 200 mg/L			Surgical anesthesia; Must be buffered with Sodium Bicarbonate (or similar) to pH 7.0 – 7.5.
<b>Sedative</b>				
<b>Lidocaine hydrochloride</b> <sup>1,2</sup>	300 mg/L	Immersion		Sedation.
<b>Tricaine methanesulfonate (MS222)</b>	50 mg/L	Immersion		Sedation; Must be buffered with Sodium Bicarbonate (or similar) to pH 7.0 – 7.5.

References:

1. Anaesthesia and analgesia in laboratory adult zebrafish: a question of refinement. T Martins, A Valentin, N Pereira and L Antunes. *Laboratory Animals* 2016, Vol 50 (6).
2. Anaesthesia, Analgesia and Euthanasia of the Laboratory Zebrafish. C. Collymore. *Zebrafish in Biomedical research* 2020. Elsevier.