Human Health and Ecological Risk Assessment
For the Use of Wildlife Damage Management Methods
by APHIS-Wildlife Services

Chapter XXVII

USE OF IMMOBILIZATION AND EUTHANASIA DRUGS IN WILDLIFE DAMAGE MANAGEMENT

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EXECUTIVE SUMMARY

The U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services (WS) uses immobilization and euthanasia (I&E) drugs to capture, restrain, and euthanize animals in wildlife damage management and other wildlife management activities and research. This risk assessment is a qualitative evaluation of the risks and hazards to human health, target, and nontarget wildlife, fish, and the environment from the use of I&E drugs by WS for mission work. This risk assessment evaluates the anesthetics (ketamine hydrochloride, tiletamine hydrochloride), sedatives (azaperone, butorphanol tartrate, dexmedetomidine, medetomidine hydrochloride, midazolam, nalbuphine, xylazine and combinations thereof), and tranquilizers (acepromazine, zolazepam) collectively referred to immobilization drugs. It also evaluates accessory drugs (atipamezole hydrochloride, atropine, doxapram, naltrexone, tolazoline, yohimbine hydrochloride) and the euthanasia agents potassium chloride and sodium pentobarbital.

WS use patterns, protocols, and label requirements on the use and safety when handling and administering I&E drugs minimize the direct and indirect risks to the public and WS applicators. WS requires applicators to be trained and certified in the use of I&E drugs before use. WS applicators typically use hand injection (syringes and syringe poles) and remote darting (dart guns and blowpipes) for immobilization and accessory drugs. In contrast, only hand injection is used to deliver euthanasia agents.

The risks to the public from WS I&E drug use are negligible due to the low potential for exposure. The public does not have access to WS I&E drugs and is not directly exposed to the drugs during or following WS use. Applicators may be at risk of exposure. However, most of the I&E drugs are stored in bottles with a septum, which reduces accidental oral, dermal, or inhalation exposure. Additionally, a well-trained applicator may miss their target when darting an animal due to extenuating circumstances such as an animal moving, but they make every attempt to recover darts that missed their target. WS has no records of accidental I&E drug exposure to the public or WS applicators. WS follows withdrawal periods and tags animals, per Animal Medical Drug Use Clarification Act, and WS Immobilization and Euthanasia Manual guidelines, with ear tags, neck collars, or another identifiable mark, indicating they were treated to prevent consumption of animals treated with an immobilization or accessory drug; animals may also be disposed of with deep burial or another appropriate disposal of euthanized wildlife.

Cumulative impacts on human health are not expected because of the lack of exposure during WS applications. The exposure and cumulative risks to ecological resources are negligible for all I&E drugs covered in this risk assessment.

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1 INTRODUCTION

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Wildlife Services (WS) administer immobilization and euthanasia (I&E) drugs to animals as a necessary component of wildlife damage management (WDM) and associated research. During the capture and relocation of animals, chemical immobilization can be a valuable method for restraining wildlife, decreasing stress, and increasing animal and human safety. Chemical immobilization enhances opportunities for personnel to participate in cooperative wildlife research and management programs. Chemical immobilization also provides humane options for euthanasia. Euthanasia is a necessary tool in WDM, particularly in urban environments where human safety is a concern, where zoonotic diseases such as rabies occur, or when an animal is injured. WS considers the safety of the animal, personnel, and the public in its decision to use and select I&E drugs for mission work.

The Food and Drug Administration (FDA) has regulatory oversite of pharmaceuticals used in animals under the Federal Food, Drug, and Cosmetic Act and the Animal Medicinal Drug Use Clarification Act (AMDUCA) (21 CFR part 530). Some of these drugs are controlled substances; the Drug Enforcement Administration (DEA) has regulatory oversite of their acquisition and possession. The Controlled Substances Act, as amended, requires a DEA registration to purchase controlled substances and requires complete recordkeeping, proper storage, inventorying, and disposal, throughout the lifecycle of use. WS personnel are authorized to use approved I&E drugs following completion of the WS training and certification program or other approved training. Policies of the WS I&E program comply with DEA and FDA regulations and comply with requirements of the states and territories in which personnel administer these drugs. To ensure appropriate use, storage, and control of I&E drugs, WS established an Immobilization & Euthanasia sub-committee under the WS Safety & Health Committee to 1) review and approve I&E and accessory (I&E) drugs; and 2) establish training and certification requirements. The subcommittee approves I&E drugs for WS personnel per WS Directive 2.430[1]. WS State Directors and Field Station Leaders are responsible for obtaining a state-level controlled substance permit (when required and permissible by state law) to obtain DEA registration for procuring and storing controlled substances needed for mission work. If state law allows only licensed medical professionals, including veterinarians, to obtain state-level controlled substance permits, WS State Directors and Field Station Leaders can operate under a consulting veterinarian's registration.

State veterinary authorities (on staff or advisory) are involved in the oversight of I&E activities. WS establishes procedures in each state for administering drugs used in wildlife capture and restraint that are approved by these authorities. Veterinary authorities in each state have discretion under AMDUCA to establish withdrawal times (i.e., a period after a drug is administered that must lapse before an animal may be used for food) for specific drugs. Animals humans could consume within the withdrawal period should be marked with ear tags, neck collars, or another identifiable mark.

[1] WS Directives referenced in this document are found @ http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/wildlifedamage under Wildlife Damage – Program Directives

This human health and ecological risk assessment (HHERA) qualitatively evaluates potential risks and hazards to human health and the environment to exposure from WS use of I&E drugs in WDM. The methods used in this HHERA to assess potential human health effects follow standard regulatory guidance and methodologies from the National Research Council (1983) and the U.S. Environmental Protection Agency (USEPA 2020b) and generally conform to additional Federal agency standards. The methods used in the HHERA to assess potential ecological risk to nontarget fish and wildlife and the environment generally follow the USEPA (2020b) methodologies. This assessment starts with identifying the hazard (problem formulation) and evaluates toxicity (the dose-response assessment) and exposure (identifying exposed populations and exposure pathways for these populations). Lastly, combining toxicity and exposure information provides a determination of adverse human health or ecological risks (risk characterization).

I&E drugs and their delivery methods are the primary concerns of this risk assessment. An overview of approved drugs, delivery devices, and WS use patterns of substances is provided. Delivery methods assessed in this document should not be considered an exhaustive evaluation of all the delivery methods employed by WS. Risks associated with some delivery methods are discussed in greater detail in other WS methods risk assessments, such as the use of firearms (dart guns), hand capture, and biological sampling (e.g., use of “sharps” - syringe needles, scalpels, and other sharp objects), and carcass disposal (disposition of carcasses euthanized with I&E drugs)[2].

1.1 I&E Drugs and Other Substances Available for Use

The I&E drugs used by WS represent different categories of pharmaceuticals: 1) anesthetics (dissociative substances that induce insensitivity to pain), 2) sedatives (substances that induce sleep or calmness), 3) tranquilizers (substances that reduce anxiety or tension), 4) accessory substances (reversals, antibiotics, or enhancers for other drugs), and 5) euthanasia agents (substances that induce a humane death). The following are the primary I&E drugs used by WS for WDM and associated research:

1. Anesthetics
    a. Ketamine hydrochloride (HCl) (e.g., Ketaset®, Vetalar®)
    b. Tiletamine HCl + Zolazepam (e.g., Telazol®)
2. Sedatives
    a. Azaperone
    b. Butorphanol tartrate
    c. Dexmedetomidine
    d. Medetomidine HCl (e.g., Domitor®)
    e. Midazolam
    f. Nalbuphine
    g. BAM (Butorphanol tartrate, Azaperone, and Medetomidine HCl)

[2] Risk assessments on the use of firearms, hand capture and biological sampling, and carcass disposal in wildlife damage management are found at https://www.aphis.usda.gov/aphis/ourfocus/wildlifedamage/programs/nepa/ct-ws-risk_assessments and discuss those concerns.

    h. NAM (Nalbuphine, Azaperone, and Medetomidine HCl; NalMed-A)
    i. Xylazine (e.g., Rompun®, Cervizine™, AnaSed®)
3. Tranquilizers
    a. Acepromazine
4. Accessory Drugs/ Substances
    a. Atipamezole HCl (e.g., Antisedan)
    b. Atropine
    c. Doxapram (e.g., Dopram-V®, Dopram®)
    d. Naltrexone
    e. Tolazoline HCl
    f. Yohimbine HCl
5. Euthanasia Agents
    a. Potassium chloride
    b. Sodium pentobarbital (e.g., Beuthanasia®-D, Sleepaway®, Fatal-Plus® Solution)

Appendix 1 provides information on some over-the-counter topical antibiotics that WS may use during I&E drug applications.

1.2 Delivery Methods

WS personnel may administer I&E drugs by hand or with a remote delivery system. Hand delivery involves a syringe or syringe pole (reusable or disposable syringe attached to the end of a long handle). Hand syringes are usually used for animals completely restrained chemically (already immobilized with a drug) or manually with methods such as a catch pole or sequestration in a cage trap. Syringe poles are used for animals restrained loosely, such as in a cage, chute, or foothold trap. Remote delivery systems include blowpipes and dart guns (USDA APHIS 2019b). Although uncommon, some immobilization (e.g., alpha-chloralose) or sedation drugs (e.g., midazolam) may have approved dosage forms for oral administration and could be administered to animals in hand baits.

WS personnel choose the most appropriate delivery method for the situation. Risks associated with using a firearm, sharps, and carcass disposal are discussed in other risk assessments and not discussed further here, as noted above.

- **Syringes** are the standard method for WS to inject I&E drugs into animals. A syringe is a simple reciprocating pump consisting of a plunger that fits tightly within a tube called a barrel. The plunger can be linearly pulled and pushed along the inside of the tube, allowing the syringe to take in and expel solutions through a discharge opening at the front end of the tube. The open end of the syringe may be fitted with a hypodermic needle to direct the flow into and out of the barrel.

- **Syringe Poles** are poles 1.5–5 ft long (445–150 cm) with a syringe at one end. Syringe poles, sometimes called jab sticks, may have a thumb button or spring-loaded plunger on one end to inject the drug. The syringe pole automatically delivers the drug on contact via a plunger or other mechanism but is manually forced. Poles and needles are typically made of aluminum and steel, with the syringe made of nylon. Some syringe poles can have regular syringes inserted into the tube and used. Syringes can be reusable or disposable. Syringe poles are generally used only for immobilization.

- **Dart Guns** (Projectors) generally use compressed air or cartridge blanks to propel a dart containing an immobilizing drug. Darts generally consist of a needle and a syringe or syringe-like device inside the dart that injects the immobilizing drug into the target animal upon impact. WS personnel could use dart guns on the ground or during aerial operations. Dart guns generally have an effective range of up to 40 meters, and careful planning and extensive practice must be used to properly administer the dose without injuring the animal. Dart projectors are generally most effective for immobilizing larger animals such as white-tailed deer and bears[3].

- **Blowpipes (blowguns or blow tubes)** also propel a dart containing an immobilizing drug, but unlike dart guns, blowpipes generally consist of just a long tube about 1–3 meters in length. When using a blowpipe, the user propels the dart by placing their mouth over one end of the tube and blowing a burst of air into the tube instead of using compressed air or cartridge blanks to propel the dart to the target. These generally involve a lot of practice to generate enough force to propel a dart with accuracy. WS generally uses blowpipes to immobilize smaller animals at close range, as their effective range is generally less than 20 meters.

- **Hand Baits** may be used to deliver some immobilizing and sedative agents in the future. Alpha-chloralose, an immobilization agent that FDA has implemented enforcement discretion to allow its use for sandhill cranes under certain conditions and potential for additional bird species in the future, is administered via baiting. Due to the limited allowed use of alpha-chloralose, it is not covered in this risk assessment. Typically, alpha-chloralose can be made into a paste that can be put on bread, corn, or another substrate the target species will eat. Midazolam has approved human drug dosage forms for oral administration that veterinarians could prescribe for extra-label use in animals under certain circumstances.

1.3 Use Pattern

WS conducts WDM with a variety of I&E drugs to protect agriculture, property, natural resources, and human and animal health and safety throughout the United States. Activities associated with management actions are recorded in the WS Management Information System (MIS)[4]. Data from FY11[5] to FY15 and FY16 to FY20 show the use of I&E drugs (Table 1), primarily ketamine, xylazine, tiletamine-zolazepam, tolazoline, yohimbine, potassium chloride, and sodium pentobarbital in various formulations. New drugs that were used starting in FY17 were nalbuphine-azaperone-medetomidine (NAM) and atipamezole. Yohimbine was not used in FY20. Tables 1a-d give five-year averages and shows similar usage between the two five-year averages, other than the newly added drugs.

WS used ketamine to immobilize 6,091 animals and tiletamine-zolazepam to immobilize 52

[3] See the Introduction to Risk Assessments – Chapter I for scientific names. These are only given in this document if not used in that Chapter.

[4] The MIS (Computer-based Management Information System) is used for tracking WDM activities. Throughout the text, data for a year (i.e. FY11 to FY15) will be given and is from the MIS. MIS reports are not be referenced in the text or Literature Cited Section because MIS reports are not kept on file. A database is kept that allows queries to be made to retrieve the information needed.

[5] FY11 equals the federal Fiscal Year 2011, which is October 1, 2010–September 30, 2011 (the year is denoted by FY11, FY12, and so on).

animals from FY11 to FY15 (Tables 2a-b). MIS only reports the primary capture method, so animals reported as euthanized could have been euthanized using any American Veterinary Medical Association (AVMA) approved method. However, most of the 298 animals euthanized (Tables 2a-b) were likely from euthanasia following immobilization (Tables 3a-b have animals euthanized, and several are double counted between Tables 2a-b and 3a-b because animals were immobilized followed by euthanasia). WS euthanizes immobilized animals primarily to obtain biological samples (e.g., brainstems to test for rabies) because the animal is suffering due to injury or removal as part of management action. It should be noted that FY16–FY20 drug use data are included in Tables 1a-d, use of the different drugs by species and state can be found in Appendix 2.

Table 1a. The annual average volume of anesthetic I&E drugs WS used in WDM activities from FY11 to FY15 and FY16 to FY20.

Drug (cc unless noted)	Unit of Measure	Avg. Amount FY11–FY15	Avg. Amount FY16–FY20
Ketamine (100 mg/mL)	cc	90.3	102.6
Ketamine (200 mg/mL)	cc	-	15.3
Ketamine/Xylazine 2:1	cc	2.0	-
Ketamine/Xylazine 3:1	cc	1.3	-
Ketamine/Xylazine 5:1	cc	4,405.2	3,333.9
Tiletamine-Zolazepam	mg	20,114.4	21,449.3

Table 1b The annual average volume of sedative I&E drugs WS used in WDM activities from FY11 to FY15 and FY16 to FY20.

Drug (cc unless noted)	Unit of Measure	Avg. Amount FY11–FY15	Avg. Amount FY16–FY20
Nalbuphine-Azaperone-Medetomidine (NAM)	cc	-	16.6
Xylazine (100 mg/mL)	cc	45.5	43.8
Xylazine (300 mg/mL)	cc	-	4.2

Table 1c. The annual average volume of reversal (accessory drugs) I&E drugs WS used in WDM activities from FY11 to FY15 and FY16 to FY20.

Drug (cc unless noted)	Unit of Measure	Avg. Amount FY11–FY15	Avg. Amount FY16–FY20
Atipamezole (5 mg/mL)	cc	-	19.4
Atipamezole (25 mg/mL)	cc	-	0.02
Tolazoline (100 mg/mL)	cc	17.7	26.5
Yohimbine (2 mg/mL)	cc	45.4	22.1

Table 1d. The annual average volume of I&E drugs WS used in WDM activities from FY11 to FY15 and FY16 to FY20.

Drug (cc unless noted)	Unit of Measure	Avg. Amount FY11–FY15	Avg. Amount FY16–FY20
Potassium Chloride	cc	88.5	133.6

Drug (cc unless noted)	Unit of Measure	Avg. Amount FY11–FY15	Avg. Amount FY16–FY20
Sodium Pentobarbital (Fatal-Plus® Solution)	cc	394.4	113.4
Sodium Pentobarbital (390 mg/mL)	cc	8,191.2	3,777.9

Table 2a. The annual average number of animals immobilized with ketamine with or without xylazine, by WS in WDM activities from FY11 to FY15 throughout the United States and the final disposition of the animal (euthanized or freed). To standardize the data for the table, drugs tracked in cubic centimeters were converted to mg of drug used.

Species[1]	Euthanized	Freed	Ketamine (mg)	Xylazine (mg)
Gray Wolf	0	23	8,118	1,504
Gray Fox	1	9	505	104
Black Bear[2]	0.4	27	11,802	3,198
Raccoon	167	5,523	331,321	65,950
Striped Skunk	103	200	17,907	3,560
Virginia Opossum**	7	8	769	155
Other Predators (12 spp.)[3]	3	18	2,406	311
Rodents/Rabbit (4 spp.)	0.6	0.8	24	4
White-tailed Deer (captive)*	0.4	0	173	87
Total (22 spp.)[3]	**282**	**5,809**	**373,025**	**74,873**

* Introduced Species ** - Introduced populations Other predator = badger, bobcat, feral cat*, coyote, dog*, fisher, red fox, mountain lion, mink, river otter, eastern spotted skunk, and hooded skunk; Rodents/rabbit = eastern fox squirrel, eastern gray squirrel, woodchuck, and eastern cottontail.

[1] Individual accounts of species are given only for those species that had an annual average of more than 10 taken except federally listed threatened, endangered, or candidate (T&E) species and eagles are included no matter the number.

[2] One black bear was treated with ketamine, tiletamine-zolazepam (avg. 200 mg), and xylazine.

[3] Other predator species include domestic dogs, a subspecies of the wolf, and thus, not included in the total species count.

Table 2b. The annual average number of animals immobilized with tiletamine-zolazepam, with or without xylazine, by WS in WDM activities from FY11 to FY15 throughout the United States and the final disposition of the animal (euthanized or freed). To standardize the data for the table, drugs tracked in cubic centimeters were converted to mg of drug used.

Species[1]	Euthanized	Freed	Tiletamine-Zolazepam (mg)	Xylazine (mg)
Gray Wolf	0.8	12	6,414	0
Black Bear[2]	0	5	2,805	0
- Louisiana Black Bear[T&E 3]	0	2	1,095	0
Grizzly Bear[T&E]	0	2	1,600	0
Other Predators (8 spp.)[4]	8	4	1,883	0
Feral Swine	6	8	3,470	1,410
Other Ungulates (3 spp.)	0	3	804	804
Eastern Fox Squirrel	1	0	25	0
Total (15 spp.)	**16**	**36**	**17,966**	**2,214**

* Introduced Species Other predator = badger, coyote, dog*, gray fox, red fox, mountain lion, raccoon, and striped skunk; Other ungulate = mule deer, red deer*, pronghorn.

[1] Individual accounts of species are given only for those species that had an annual average of more than 10 taken except federally listed threatened, endangered, or candidate (T&E) species and eagles are included no matter the number.
[2] One black bear was treated with ketamine, tiletamine-zolazepam (ave. 200 mg), and xylazine.
[3] T&E = Federally listed threatened and endangered species – the Louisiana black bear is no longer listed but included.
[4] Other predator species include domestic dogs, a subspecies of the wolf, and thus, not included in the total species count.

Tiletamine-zolazepam was used less frequently than ketamine (Tables 2a-b) by WS personnel to immobilize animals. It was primarily used for wolves, feral swine, and bears. Xylazine was used with tiletamine-zolazepam for ungulates.

Raccoons (91%) and striped skunks (5%) were the most frequently immobilized species with the anesthetic ketamine (Tables 2a-b) and were the most frequent species euthanized with a sodium pentobarbital solution (97%) (Tables 3a-b). Raccoons and striped skunks are targets of the National Rabies Management Program, where animal use activities support enhanced surveillance to track the prevalence of rabies in wildlife and monitoring to test animal serum for rabies antibodies; brain stems are required for standard rabies diagnosis, which requires the animal to be euthanized for postmortem collection of tissue. In summary, 22 species were immobilized with ketamine and often accompanied by xylazine, a sedative.

Tables 3a-b list the animals WS euthanized between FY11 and FY15. The actual number of animals euthanized likely is higher, but the take was recorded under another I&E drug in the database. For example, WS immobilized and euthanized raccoons to collect brainstem tissue samples for rabies diagnosis; in the database, only the drug used to immobilize the raccoon was recorded with take because only one method can be associated with take, and the drug used for euthanasia is listed but not associated with the take of the raccoon.

Table 3a. The annual average number of animals euthanized with potassium chloride euthanasia drugs by WS in WDM activities from FY11 to FY15 throughout the United States.

Species	Euthanized	Amount Used (cc)
Raccoon	29	76.9
Striped Skunk	5	10.8
Woodchuck	0.2	0.2
Total KCl	**34**	**87.9**

Table 3b. The annual average number of animals euthanized with sodium pentobarbital (sodium pentobarbital (390 mg/mL) and Fatal Plus solution) euthanasia drugs by WS in WDM activities from FY11 to FY15 throughout the United States.

Species[1]	Euthanized	Amount Used (cc)
Virginia Opossum**	226	377.3
Feral/Free-roaming Cat*	55	28.6
Common Gray Fox	15	35.1
Raccoon	462	1,323.9
Striped Skunk	2,980	6,160.6
Other Predator (8 spp.)	12	39.7
California Ground Squirrel	44	24.1
Other Rodent (7 spp.)**	8	7.3

Species[1]	Euthanized	Amount Used (cc)
Desert Cottontail	1	0.9
Black-tailed Mule Deer	0.2	1.2
Birds (4 spp.)	2	1.6
Total Sodium Pentobarbital	**3,805**	**8,002.5**

* Introduced Species ** - Introduced populations
Other predator – bobcat, coyote**, dog*, red fox**, black bear, hooded skunk, western spotted skunk, long-tailed weasel
Other rodent – beaver**, muskrat, woodchuck, western gray squirrel, eastern fox squirrel**, brown rat*, black rat*
Birds – rock dove*, common barn owl, American kestrel, American crow
[1] Individual accounts of species are given only for those species that had an annual average of more than 10 taken.

Between FY11 and FY15, WS used tolazoline or yohimbine on 23 animals to reverse the effects of sedatives (Tables 4a-b). All the animals dosed with the antagonist drugs successfully recovered. These drugs are rarely used, but they are necessary for certain situations to ensure animals recover quickly. The two drugs were used on six species, mostly wolves (65%) and feral swine (13%). It should be noted that the animals given reversals in Tables 4a-b were also immobilized and counted in Tables 2a-b.

Table 4a. The annual average number of animals recovered with Tolazoline (100mg/mL) alpha-2 antagonist drugs by WS in WDM activities from FY11 to FY15 throughout the United States.

Species	Freed	Drug Used (cc)
Feral Swine*	3	76.9
Mule Deer	0.8	7.1
Total Tolazoline	**3.8**	**83.0**

* Introduced Species

Table 4b. The annual average number of animals recovered with Yohimbine alpha-2 antagonist drugs by WS in WDM activities from FY11 to FY15 throughout the United States.

Species	Freed	Drug Used (cc)
Gray Wolf	15	33.4
Other Predator (3 spp.)	4	4.3
Total Yohimbine	**19**	**37.7**

* Introduced Species Other predator = black bear, bobcat, and raccoon
[1] Individual accounts of species are given only for those species that had an annual average of more than 10 taken, target and nontarget numbers combined. All federally listed threatened, endangered, or candidate species and eagles are included.

Table 5 summarizes the WS I&E drugs used by state programs. In all, 38 WS state programs used immobilization drugs. Most immobilizing drugs are used for the collection of biological samples, where animals are captured in cage traps, immobilized, sampled, and released; however, depending on the sample necessary, e.g., blood or brainstem, the animal may or may not be euthanized prior to sample collection. It should be noted that rabies was diagnosed in 2% of the animals tested by enhanced surveillance, and rabies is a lethal disease with a case-fatality rate approaching 100%. Animals are also immobilized to apply identification tags or tracking collars for monitoring.

Table 5. States where WS used wildlife drugs for FY11–FY15.

State	Ketamine (mg)	Tiletamine-Zolazepam (mg)	Xylazine (mg)	Alpha$_2$ Antag. (cc)	Euthanasia (cc)
AK	-	48	-	-	10.7
AL	12,873	-	2,585	-	-
AZ	771	600	165	-	-
CA	30	-	10	11.3	7,024.4
CO	-	2,379	1,254	-	-
FL	7,893	-	1,573	0.7	39.7
GA	9,279	-	1,799	-	-
ID	-	1,800	-	-	-
KS	-	1,100	960	-	56.1
KY	37	-	7	-	0.4
LA	-	1,195	-	-	-
MA	8,685	-	1,679	-	-
MD	9,103	-	1,782	-	-
ME	13,466	112	3,710	-	-
MI	2,345	-	415	7.3	-
MN	4,126	-	492	0.2	-
MO	-	1,370	-	-	-
MS	-	-	-	-	-
MT	-	8,300	-	-	-
NC	5,750	-	1,141	-	-
ND	-	-	-	-	4.8
NE	-	-	-	-	388.0
NH	2,947	-	591	-	-
NJ	-	-	-	-	-
NV	-	-	-	-	38.2
NY	68,169	-	13,671	-	0.8
OH	69,091	-	13,738	-	10.6
OR	-	605	-	-	31.9
PA	7,973	-	1,585	1.4	14.6
TN	25,405	-	4,936	-	13.4
TX	1,694	100	316	-	22.9
UT	-	-	-	-	434.0
VA	27,379	-	5,523	-	-
VT	34,304	-	6,872	-	8.8
WA	-	-	-	-	1.2
WI	11,279	155	2,165	28.1	-
WV	45,745	-	9,129	-	3
WY	5,051	1,044	1,002	-	10.4
Total	**373,395**	**18,808**	**77,100**	**49.0**	**8,113.9**
States	**23**	**13**	**25**	**6**	**19**

2 ANESTHETICS

WS uses several anesthetics during its WDM activities and associated research. Anesthetics produce unconsciousness and loss of pain perception making the target animal unresponsive to stimuli (USDA APHIS 2019b). WS uses the anesthetics ketamine and tiletamine (tiletamine is used as a combination product of tiletamine and zolazepam) more than the other anesthetics available because of their efficacy, cost, and availability. Research indicates the environmental fate of residues of injectable anesthetics has not been determined (AVMA 2020).

The toxicity of anesthetic drugs to the target species, humans, and nontarget species is a primary concern. The anesthetics discussed are ketamine, tiletamine/zolazepam, and associated products found in these solutions. Table 6 provides toxicity values for these drugs. These values will be discussed in the following sections on each drug.

Table 6. Acute toxicity values for anesthetic drugs and their components.

Chemical	Test Species	Test Type	LD_{50} Test Result	Reference
Ketamine HCl	Brown Rat*	Acute oral	447 mg/kg-bw	(Zoetis 2014b)
Ketamine HCl	House Mouse*	Acute oral	617 mg/kg-bw	(Zoetis 2014b)
Ketamine HCl	Brown Rat*	Intravenous	58.9 mg/kg-bw	(Zoetis 2014b)
Ketamine HCl	House Mouse*	Intravenous	55.9 mg/kg-bw	(Zoetis 2014b)
Benzethonium chloride[1]	Brown Rat*	Acute oral	368 mg/kg-bw	(Zoetis 2014b)
Benzethonium chloride	Brown Rat*	Intravenous	19 mg/kg-bw	(Zoetis 2014b)
Benzethonium chloride	Brown Rat*	Subcutaneous	119 mg/kg-bw	(Zoetis 2014b)
Benzethonium chloride	House Mouse*	Acute oral	338 mg/kg-bw	(NCBI 2023k)
Tiletamine[2]	Brown Rat*	Acute oral	>5,000 mg/kg-bw (ATE)	(Zoetis 2017a)
Mannitol[3]	House Mouse*	Acute oral	22 g/kg-bw	(Zoetis 2017a)
Mannitol[3]	Brown Rat*	Acute oral	13,500 mg/kg-bw	(Zoetis 2017a)
Zolazepam[2]	Brown Rat* (adult)	Acute oral	398 mg/kg-bw	(Zoetis 2017a)

* Domestic laboratory strains
[1] Ingredient in ketamine
[2] Active ingredient in Telazol
[3] Ingredient in Telazol

2.1 Ketamine Hydrochloride (e.g., Ketaset®, Vetalar®)

2.1.1 Chemical Description and Product Use

Ketamine (CAS No. 6740-88-1; $C_{13}H_{16}ClNO$; chemical structure in Appendix 3) is a dissociative anesthetic (anesthesia that does not necessarily involve a loss of consciousness) used to immobilize wildlife, primarily mammals and birds. It can be administered intramuscularly, intravenously, and subcutaneously (EMEA 1997, NCBI 2023f). While its mechanism of action is not well understood, this cyclohexanone derivative is thought to block *N*-methyl-D-aspartate

(NMDA) receptors[6] and may also interact with sigma receptors (NCBI 2023f). The drug is used to eliminate pain and minimize fear and anxiety. Dissociative anesthetics generally produce muscle tension and increased heart rate and can cause excessive salivation and seizures (USDA APHIS 2019b). As a result, ketamine is usually combined with other drugs such as xylazine (see 3. Sedatives below), atropine to reduce salivation or a tranquilizer. The combination of drugs is used to anesthetize the animal, maximize reduction of pain and stress, and increase human and animal safety. Ketamine can be purchased in 100 mg/mL concentrations under various brand names. Ketaset®, Vetalar®, and Ketalar® are the more commonly used brands (WHO 2014). The commercially available pharmaceutical form of injectable ketamine consists of 10% ketamine HCl (CAS No. 1867-66-9), 0.01% benzethonium chloride (CAS No. 121-54-0), and sterile water for injection (Zoetis 2014b). Concentrated ketamine can be purchased with a veterinary prescription through a compounding pharmacy. Ketamine is a DEA Schedule III controlled substance (Kreeger and Arnemo 2012, USDA APHIS 2019b). WS uses ketamine, often combined with other drugs, for capturing wildlife with dart guns, blowguns, and syringe poles (e.g., for animals incapacitated from disease or injuries) or for anesthetizing wildlife with blowguns (typically skunks), syringe poles, and hand syringes when already captured using another method, such as cage traps. In some cases, oral delivery of ketamine by squirting it into the mouth of an excited animal can calm the animal enough to allow injection of an additional drug via the hand or syringe pole (USDA APHIS 2019b), which has been used by WS when working with raccoons, skunks, and other species caught in box traps (USDA APHIS 2019b). If the animal is removed instead of released, ketamine may be used as part of a two-step method prior to administering euthanasia drugs. Remote delivery does have the possibility of missing the target and ejecting ketamine on the ground.

2.1.2 Physical and Chemical Properties

Two enantiomers exist for ketamine: S(+)-ketamine and R(-)-ketamine. The S(+) enantiomer has a 3- to 4-fold greater affinity for the NMDA receptor than the R(-) form (WHO 2014). Ketamine has a molar mass of 237.727 g/mol and is a white crystalline powder with minimal odor (NCBI 2023f). Ketamine HCl injection is acidic, with a pH of 3.5 to 5.5. It has a specific gravity of 1.008–1.028; its solubility in water is 200 g/L (NCBI 2023f, Zoetis 2014b). Its melting point is 92–93°C (NCBI 2023f, WHO 2014). Its estimated vapor pressure is 5.15×10^{-5} mm Hg at 25°C, and the estimated Henry's Law Constant is 1.38×10^{-8} atm-m^3/mol at 25°C (NCBI 2023f). Ketamine has a Log K_{ow}[7] of 2.2, indicating it is lipophilic (NCBI 2023f). Ketamine HCl is stable under normal storage conditions (Zoetis 2014b).

2.1.3 Environmental Fate

In China and Taiwan, ketamine is detected widely in surface waters, with major sources from hospital and municipal wastewater (Wang et al. 2018). In the United Kingdom, ketamine was

[6] The NMDA receptor is a glutamate receptor and ion channel protein found in nerve cells that is very important for controlling synaptic plasticity and memory function.

[7] Log K_{ow} is the ratio of the concentration of a solute between water and octanol that is commonly used as a measure of hydrophobicity. Substances with high Log K_{ow} values tend to adsorb more readily to organic matter in soils because of their low affinity for water. Chemicals with very high Log K_{ow} values (e.g., 4.5) may have the potential to bioaccumulate in an organism.

detected in wastewater influent and effluent (Baker and Kasprzyk-Hordern 2013). If ketamine or its metabolites enter waterways, the only elimination pathway is photolysis, which generates toxic byproducts. One study demonstrated that at higher concentrations, ketamine irradiated by sunlight rapidly induces acute biological toxicity in aquatic organisms (Li et al. 2017). Phototransformation half-lives for ketamine and norketamine in river waters were 12.4 and 10.1 hours, respectively (Lin et al. 2014). Lin et al. (2014) found that even though photolysis reduced the concentration of ketamine and norketamine (a metabolite of ketamine) in aquatic systems, both exhibited higher Microtox® acute toxicity after 14–20 hours of sunlight exposure. In a study on the adsorption and degradation of ketamine in four soils, ketamine was reported as likely to be mobile in soil (Xega et al. 2019). WS takes precautions to avoid the release of ketamine to aquatic environments, such as proper disposal of unused products and carcasses.

2.1.4 Metabolism

Ketamine is not completely metabolized in any species. Ketamine has rapid absorption and excretion. Ketamine has a high lipid solubility and low plasma protein binding and, therefore, is rapidly absorbed in humans when administered through the intramuscular (T_{max}[8] 5–15 min), nasal (T_{max} 20 min), or oral routes (as a solution) (T_{max} 30 min). Ketamine is metabolized in the liver and intestines, making its bioavailability low when given orally (17%) or rectally (25%) (WHO 2014). The liver metabolizes ketamine, and its primary metabolite, norketamine, with enzymes from the cytochrome P450 family[9]. Norketamine is 1/3 to 1/2 (or 0.3–0.5) as potent as ketamine (Kim et al. 2007, Lin et al. 2014, Wang et al. 2005), and other metabolites include hydroxyl-norketamine and dehydronorketamine (Lin et al. 2014). Approximately 90% of a ketamine dose is excreted in the urine within 72 hours (WHO 2014).

Tissue residues of ketamine were studied in horses (*Equus ferus caballus*), cows, and pigs following intravenous or intramuscular injection; animals were slaughtered 24 hours after treatment. Ketamine residues could be detected in the liver, kidneys, and in one muscle sample of horses (intravenous (IV) treatment). In contrast, one cow (intramuscular treatment) had ketamine residues detected in the liver and kidneys. One pig had ketamine residues detected in a fat sample. No residues were detected at the injection site or in milk (EMEA 1997). Johnson et al. (2023b) measured ketamine residues in raccoons at 2, 4, and 6 days post-immobilization with 0.096 mL/kg ketamine/xylazine (5:1 mixture of ketamine and xylazine), with one raccoon receiving an additional dosage for a final drug dosage of 0.101 +/- 0.011 mL/kg. Ketamine was detected in a tissue sample on days 2 and 6.

2.1.5 Hazard Identification

Ketamine is different from most anesthetics because it stimulates the cardiovascular system. These effects are not usually problematic but could be fatal in individuals with significant ischemic heart disease or high blood pressure (Haas and Harper 1992). In addition, ketamine is a mild

[8] T_{max} concentration is the time a drug is at maximum in serum.

[9] Cytochromes P450 (CYPs) are a superfamily of enzymes that, in mammals, oxidize steroids, fatty acids, and xenobiotics, and are important for the clearance of various compounds and hormone synthesis and breakdown.

respiratory depressant, similar to opioids but dissimilar from most anesthetics (WHO 2014). Ketamine does not appear to affect reproduction (EMEA 1997).

FDA previously approved ketamine for human use as an analgesic and sedative; however, the FDA recently granted breakthrough status to esketamine, a form of ketamine, which could be used as an antidepressant (Feller 2016). Ketamine overdoses can cause unconsciousness and slowed breathing (DEA 2020). Other adverse effects include hallucinations, blood pressure changes, amnesia, lack of coordination, nausea, and respiratory depression. Ketamine produces muscle tension that results in staring, exposing the eyes to light and dirt, and requiring the application of ophthalmic ointments by WS personnel to protect the eyes of anesthetized animals (USDA APHIS 2019a).

2.1.5.1 Acute Toxicity

For acute oral toxicity or LD_{50} [10], ketamine is a toxicity category II for rats with an oral LD_{50} of 447 mg/kilograms of body weight (kg-bw) and toxicity category III for house mice with an LD_{50} of 617 mg/kg-bw (Table 6). Ketamine is more acutely toxic when given intravenously, with an LD_{50} of 58.9 mg/kg-bw in rats and 55.9 mg/kg-bw in mice (Table 6). The ketamine HCl injection formulation is an eye and skin irritant in rabbits (Zoetis 2014b).

2.1.5.2 Sublethal and Chronic Toxicity

Rats receiving 10 mg/kg/day ketamine HCl intravenously for 6 weeks had a no observed adverse effect level (NOAEL) at the maximum dose (Acosta-Jamett et al. 2010); however, there was a slight but not significant decrease in food intake and moderate weight gain depression (EMEA 1997). Similarly, dogs receiving 40 mg/kg-bw/day ketamine HCl intramuscularly for 6 weeks also resulted in a NOAEL (Pfizer 2008), but they did have slight weight loss or anorexia (EMEA 1997). Ketamine HCl was not found to be teratogenic for rats (NOAEL of 120 mg/kg/day, intramuscular), rabbits (NOAEL of 24 mg/kg-bw/day, intramuscular), and mice (NOAEL of 300 mg/kg-bw/day, intravenous) (Zoetis 2014b). In another study, histopathological changes in rat fetuses were observed at doses 10 times those used in humans for anesthesia (WHO 2014), while a study in female dogs injected with 25 mg/kg-bw intramuscularly 6 times during one trimester of pregnancy did not show adverse effects (EMEA 1997). The WS use of ketamine in the anesthesia of wildlife would not subject individual animals to consecutive, multi-day dosages.

2.1.6 Dose-Response Assessment

2.1.6.1 Human Health Dose Response

Ketamine has very strong analgesic and neuroleptic effects in humans. Ketamine is typically administered intravenously at an induction dose of 0.5–1.5 mg/kg-bw, but it can also be administered intramuscularly (4–6 mg/kg-bw) or rectally (8–10 mg/kg-bw)(Brunton and Knollmann 2022). At induction doses, blood pressure, and heart rate increase initially, while respiration rate

[10] LD_{50} is the Lethal Dose of 50% (one half) of a group of test animals. The LD_{50} is one way to measure the short-term poisoning potential, the acute toxicity, of a material.

stays the same. Side effects include hallucinations and impeded psychomotor functions for several hours after a single injection (EMEA 1997). Evidence suggests that the margin of safety with ketamine overdose is wide. Adverse outcomes were not observed in nine healthy children treated in the emergency room that accidentally received 5 to 100 times the intended dose of ketamine (Green et al. 1999). Liver damage could occur if prolonged or repeated use of ketamine occurs. In one study, six patients were scheduled to receive two continuous intravenous 100-hour S(+)-ketamine infusions (infusion rate 10–20 mg/hour) 16 days apart. Three individuals developed hepatotoxicity (liver failure) after the second infusion began (WHO 2014). The WHO (2014) found a dose above 11.3 mg/kg-bw intravenously may be lethal to humans; for a 60 kg person, this is equivalent to an intravenous dose above 680 mg.

Data on human exposure to ketamine during pregnancy is not available, but clinical evidence suggests that ketamine may depress fetal functions when 2 mg/kg-bw (intravenous) is administered (WHO 2014).

2.1.6.2 Ecological Dose Response

Intravascular injection of 30 mg/kg-bw ketamine into adult Coho salmon (*Oncorhynchus kisutch*) and juvenile rainbow trout provided anesthesia. Still, the length of anesthesia differed between the species, likely based on fish size (Graham and Iwama 1990). Anesthesia lasted for up to 4 hours for Coho salmon and 1 to 2 hours for trout. After injection, the fish experienced an interruption in ventilation, which created hypoxia and acidosis; however, the fish normalized as ventilation was restored naturally. Under anesthesia, the fish did not show swimming behavior, and some fish lost balance. In the initial seconds after injection, the fish exhibited struggling, with researchers suspecting observed elevated CO_2 and depressed O_2 as the cause. In another study, rainbow trout injected intramuscularly with 130 mg/kg-bw and 150 mg/kg-bw ketamine were anesthetized for 20 minutes and 50 to 80 minutes, respectively. However, the recovery period was long, lasting up to 90 minutes, and the fish displayed ataxia and excitement (Oswald 1978).

In aquatic invertebrates, a 20 mg/kg-bw dose of ketamine was injected into the hemolymph of blue crab (*Callinectes sapidus*) gave short-term (under 10 minutes) light anesthesia (Quesada et al. 2011). The criteria for light anesthesia were a loss of righting reflex and defensive response, slow and occasional limb movement, and slow limb withdrawal when pressed with forceps. Bradycardia (slower than normal heart rate) was an observed side effect, but the crab's heart rate approached baseline within 10 minutes. An intramuscular dose of 90 µg/g-bw of ketamine provided deep anesthesia in virile crayfish (*Faxonius* (*Orconectes) virilis*) for one hour or longer (Brown et al. 1996). Crayfish fully recovered from anesthesia, and no adverse effects were noted.

In black bears, an estimated 6.9 +/- 0.4 mg/kg-bw ketamine, along with 3.5 +/- 0.2 mg/kg-bw xylazine was administered using a syringe pole (n=9) or dart (n=1) to immobilize the animals (Williamson et al. 2018). Bears were approachable at a mean time of 7.6 minutes. The bears had a higher heart rate but did not exhibit hyperthermia. During recovery and following the reversal with yohimbine, the bears exhibited incoordination and ataxia while trying to walk. The bears were then monitored for over six months, and no mortality or unusual movements were noted during this time (Williamson et al. 2018).

Cardiovascular and behavioral responses were evaluated in adult gray wolves administered 6.6 mg/kg-bw ketamine and 2.2 mg/kg-bw xylazine intramuscularly by hand or syringe pole (Kreeger et al. 1987). Induction time averaged 4.6 minutes. The animals experienced bradycardia and hypertension.

Telesco and Sovada (2002) evaluated doses of ketamine and xylazine to determine a dosage appropriate to immobilize swift foxes. In three trials, they evaluated dosage ratios of 2.27:1.2, 5.68:1.2, and 11.4:1.2 mg/kg-bw ketamine and xylazine, respectively. In a fourth trial, they evaluated a higher dosage of 11.4:2.4 mg/kg-bw ketamine and xylazine. Induction and recovery were smooth, and the induction times and recovery periods were similar for the dosages in the first three trials. However, the immobilization time increased with the increase in dose. Foxes in trials 1 and 2 had increased salivation, compulsive licking, vomiting, and muscle twitching. Heart rate and respiratory rate were depressed but were within the range established for domestic canids. The authors suggest a dosage of 10 mg/kg-bw ketamine and 1 mg/kg xylazine for a handling time of 40 minutes.

In male and female pigeons, 30 mg/kg-bw ketamine was administered intramuscularly to evaluate the drug's clinical effects (Azizpour and Hassani 2012). The induction time averaged 4.50 minutes, and the anesthesia duration averaged 8.13 minutes. The birds displayed poor muscle relaxation. Recovery was reported as "stormy"; birds displayed severe convulsions and wing flapping.

In laboratory snakes (unspecified), 50 mg/kg-bw ketamine given intramuscularly provided sedation/analgesia within 10 minutes, peaking at 30 minutes, lasting 60 minutes, and taking less than 72 hours to reach complete recovery (Green et al. 1981). Snakes maintained muscular tone and exhibited slow writhing movement when handled. In raptors, 15 mg/kg-bw ketamine was given intramuscularly into the pectoral muscles, with an onset of sedation/analgesia in 3 minutes, reaching peak effect in 7 minutes and attaining full recovery within 1–5 hours (Green et al. 1981). A ketamine dose of 20–50 mg/kg-bw intramuscularly or intravenously or 15‑20 mg/kg-bw provides chemical restraint in rabbits (Plumb 2018). In horses, a dose of 2 mg/kg-bw of ketamine given intravenously after administering a sedative (xylazine or xylazine and butorphanol) provides anesthesia in the field (Plumb 2018). In swine, ketamine doses range from 11 mg/kg-bw to 22 mg/kg-bw, given intramuscularly (Plumb 2018).

2.2 Tiletamine Hydrochloride and Zolazepam (Telazol®)

2.2.1 Chemical Description and Product Use

Telazol® is a mixture of tiletamine (an anesthetic) and zolazepam (a tranquilizer), which selectively interrupts association pathways to the brain. Tiletamine (CAS No. 14176-50-2; $C_{12}H_{17}NOS$; chemical structure in Appendix 3) is a dissociative anesthetic like ketamine but is 2.5–5 times more potent. It also generally works faster and lasts longer (Fowler and Miller 1999, USDA APHIS 2019b). Tiletamine can only be purchased as Telazol (NCBI 2023b). Telazol consists of 50 mg/mL zolazepam HCl (CAS No. 33754-49-3; $C_{15}H_{16}ClFN_4O$; Appendix 3), 50 mg/mL tiletamine HCl,

less than 6% mannitol (CAS No. 69-65-8), and sterile water for injection (Zoetis 2019). Zolazepam is a benzodiazepine derivative tranquilizer that minimizes the muscle tension and seizures associated with tiletamine (Cording et al. 1999). Muscle tension from Telazol is variable in different animal species; it produces extensive muscle tension in dogs but has a more relaxed response in coyotes, wolves, and bears (Fowler and Miller 1999, USDA APHIS 2019b). The small volume required for dosing, ease of administration, wide safety margin, and dose-related effects, as well as it being a DEA Class III controlled substance, make Telazol a popular anesthetic drug for WS WDM activities. WS uses Telazol in wildlife capture and anesthesia with all types of delivery methods, including remote delivery with dart guns to direct hand-injection via syringe where possible. Remote delivery does have the possibility of missing the target and ejecting tiletamine and zolazepam on the ground.

2.2.2 Physical and Chemical Properties

Telazol, a 1:1 mixture of tiletamine and zolazepam, is sold as an off-white powder and must be reconstituted with sterile water before use. After it is reconstituted, the shelf life is seven days at room temperature and 56 days if refrigerated (Zoetis 2017a). Telazol is slightly soluble in water (Zoetis 2017a).

Tiletamine has a water solubility of 0.0965 mg/ml (DrugBank 2021e). Its estimated vapor pressure is $1.86x10^{3}$ mm Hg at 25ºC, and the estimated Henry's Law constant is $1.34x10^{-8}$ atm-cu m/mol at 25°C (NCBI 2023b). The estimated log Kow for tiletamine is 2.79, indicating that it is a lipophilic drug (DrugBank 2021e). It is considered acidic, with a pH ranging from 3.5 to 5.5. Its boiling point is 100°C, and its density is 1.52. It is stable under normal use conditions. Tiletamine's estimated bioconcentration factor is 15.8 (USEPA 2023b).

Zolazepam is a crystalline structure. Its estimated pKa is 4.69 (USEPA 2023a). Its predicted water solubility is 269 mg/L (DrugBank 2021d). Its predicted vapor pressure is $1.05x10^{-8}$ mmHg, log Kow is 1.56, Henry's Law Constant $1.33x10^{-9}$ atm-m^3/mol, and melting point is 181°C (USEPA 2023a). Zolazepam's estimated bioconcentration factor is 18 (USEPA 2023a).

2.2.3 Environmental Fate

Little information and research are available on tiletamine and zolazepam's environmental fate or transport. This is because the direct environmental release is unlikely except for a missed dart; the drug is administered to animals through injection. The USEPA predicted that the biodegradation half-life (DT_{50}) in soil for tiletamine is 4.07 days and for zolazepam is 3.36 days (USEPA 2023a); however, no experimental data are currently available. Tiletamine is soluble in water and based on its estimated vapor pressure and Henry's law constant, it is not considered volatile. Due to the pharmaceutical drug approval process, environmental fate studies are limited. Telazol, equal parts tiletamine and zolazepam, is not classified as environmentally hazardous. Both tiletamine and zolazepam have bioconcentration factors that indicate very low bioconcentration potential in aquatic environments. As with many compounds, large and frequent spills may have harmful or damaging effects on the environment. Releases into the environment

of Telazol, tiletamine, or zolazepam should be avoided. Data are not available concerning the environmental degradability, nor are data available concerning the bioaccumulative potential or soil mobility of Telazol. Adverse environmental effects such as ozone depletion, photochemical ozone creation potential, endocrine disruption, and global warming potential are not expected from this component (Zoetis 2017a).

2.2.4 Metabolism

Telazol is a non-narcotic, non-barbiturate, injectable anesthetic. The onset of anesthesia generally occurs within 5 to 12 minutes (Zoetis 2019). Muscle relaxation is optimum for the first 20 to 25 minutes and then diminishes (USDA APHIS 2013, Zoetis 2019). If administered intravenously, anesthesia lasts 15–20 minutes, whereas if administered intramuscularly, anesthesia lasts 30–45 minutes (West et al. 2007). Tiletamine, one of the two drugs in Telazol, has a high lipid solubility and is rapidly absorbed through the intramuscular route (Riviere and Papich 2009). Little information is available regarding the enzymatic pathways involved in the metabolism of tiletamine and zolazepam (Kumar et al. 2006). Species metabolize Telazol differently and subsequently have differing recoveries. In pigs administered 10 mg/kg-bw Telazol (1:1 ratio of tiletamine and zolazepam) intramuscularly, the elimination half-life was 3.7 and 8.4 hours for tiletamine and zolazepam, respectively (Kumar et al. 2006). Zolazepam takes longer to metabolize in cats, while tiletamine takes longer to metabolize in dogs. According to the Telazol product label, the elimination half-life for tiletamine and zolazepam administered intravenously in dogs is 0.87 hours and 0.41 hours, respectively (Zoetis 2019). In black bear, concentrations of tiletamine and zolazepam (Telazol) in the serum, liver, muscle, and urine were measured 3, 7, 14, and 21 days after administration of 500 mg Telazol, except for two bears that required 1,000 mg and 1,250 mg Telazol (Ryan et al. 2009). Tiletamine was not detected on day 14, and zolazepam was not detected on day 21; on day 14, only two bears had detectable levels of zolazepam in the urine (Ryan et al. 2009). The authors found black bears quickly metabolize and eliminate Telazol to undetectable levels within 7–14 days. Animals differ in their recovery response. Pigs awaken slowly and calmly, while horses can have an agitated recovery if they are not provided with additional sedation (Riviere and Papich 2009).

2.2.5 Hazard Identification

FDA-approved Telazol for use in dogs and cats; however, the drug is widely used as an anesthetic agent for wildlife. Tiletamine is labeled as harmful if swallowed or inhaled (NCBI 2023b). Zolazepam is labeled as a skin, eye, and respiratory irritant (NCBI 2023s). Telazol can cause staring, which can expose the eyes to light and dirt and cause the eyes to dry out, requiring the application of ophthalmic ointments by WS personnel to protect the eyes of anesthetized animals (USDA APHIS 2019b). The Telazol product label indicates there may be pain upon intramuscular injection, particularly in cats (Zoetis 2019).

Telazol is not known to be carcinogenic (Zoetis 2017a). The SDS classifies Telazol as reproductive toxicity (i.e., to an unborn child) category 2, although no data are accessible to support this classification (Zoetis 2017c). However, zolazepam, an active ingredient in Telazol,

did not have reproductive effects in adult rats exposed to doses up to eight times the therapeutic dose (Lin et al. 1993).

2.2.5.1 Acute Toxicity

Telazol has an oral acute LD_{50} >5,000 mg/kg-bw in the rat (Zoetis 2017c). Mannitol, an ingredient in Telazol, has an oral LD_{50} of 22 g/kg-bw and 13,500 mg/kg-bw in the mouse and rat, respectively (Zoetis 2017c). Tiletamine is a category IV for acute oral toxicity in adult rats (LD_{50} >5,000 mg/kg-bw), whereas zolazepam is a category II (Oral LD_{50} of 398 mg/kg-bw; Table 6).

2.2.5.2 Sublethal and Chronic Toxicity

Information is limited on the sublethal and chronic toxicity of Telazol. Zolazepam has a lowest observed adverse effect level ((LOAEL); 3-month study) of 10 mg/kg-bw/day in the dog and monkey and a no observed adverse effect level (NOAEL) of 10 mg/kg-bw/day in the rat (Zoetis 2017c). In a study in rabbits, 32 or 64 mg/kg-bw Telazol was administered intramuscularly, and the anesthesia effects were monitored (Brammer et al. 1991). Seven days post-injection, renal tubular necrosis was present in all high-dose rabbits and three rabbits in the low-dose group, indicating that Telazol is nephrotoxic at both doses and did not produce analgesia.

2.2.6 Dose-Response Assessment

2.2.6.1 Human Health Dose Response

Human toxicity data for Telazol is limited due to its FDA-approved use exclusively in veterinary medicine. Telazol causes rapid anesthetic effects, but sudden alertness is not uncommon as the effects of the drug subside (Quail et al. 2001). In one case report, a female survived injecting herself with an estimated 200 mg of Telazol, or 100 mg of each ingredient, based on the 2 mL missing from a Telazol vial (Quail et al. 2001). The authors describe this dose as similar to the 3.6 mg/kg-bw given to chimpanzees for anesthesia. The subject of the case report also used diazepam, making it difficult to attribute subsequent symptoms to Telazol exposure. She was obtunded and hypotensive but had a gag reflex, which is consistent with Telazol effects but could also be effects from diazepam. There are two reports of death caused by self-injected drug intoxication of Telazol (Chung et al. 2000, Cording et al. 1999). In one case, a male self-injected Telazol and ketamine (Cording et al. 1999). This male had postmortem concentrations of tiletamine in the blood, urine, and liver of 295 ng/mL, 682 ng/mL, and 196 ng/g, respectively, and zolazepam concentrations in the same tissue of 1.71 ng/mL, 1.33 ng/mL, and 15.5 ug, respectively (Cording et al. 1999). The concentration of ketamine was 37 ng/mL and 381 ng/mL in the blood and urine, respectively. The authors did not estimate the amount of Telazol self-administered. In another case, a male had concentrations of 0.85 mg/L tiletamine and 3.3 mg/L zolazepam in the blood (Chung et al. 2000). Injection-site tissue has 25.1 mg/kg tiletamine and 23.3 mg/kg zolazepam (Chung et al. 2000). The authors did not estimate the amount of Telazol self-administered.

Similarly, data on the human toxicity of tiletamine and zolazepam independently is lacking. However, since tiletamine belongs to the same class of drugs as ketamine, it can be assumed that the pharmacokinetics between these drugs is similar (Cording et al. 1999). Tiletamine has the potential to affect the central nervous system in humans. Adverse effects include dizziness, light-headedness, headache, nausea, and blurred vision (NCBI 2023b).

2.2.6.2 Ecological Dose Response

Animal studies have shown that the acute induction intramuscular dose for Telazol (1:1 tiletamine:zolazepam) in dogs is 6.6 to 9.9 mg/kg-bw at a maximum dose of 13.6 mg/kg-bw, and in cats, 9.7 to 11.9 mg/kg-bw at a maximum intramuscular dose of 32 mg/kg-bw (Dechra Veterinary Products LLC 2020). In monkeys, 50 mg/kg-bw intravenously (intravenous) causes brief and mild clonic seizures (Lin et al. 1993).

The lowest observed adverse effect level (LOAEL) for zolazepam in dogs and monkeys was a dosage of 10 mg/kg/day for three months (route of administration not provided) (Zoetis 2017c). Central nervous system toxicity was associated with treatment for monkeys, and both central nervous and gastrointestinal system toxicity was associated with dogs (Zoetis 2017a). A study involving four cats anesthetized with 20 mg/kg of tiletamine (route of administration not provided) between days 10 and 50 of gestation produced normal offspring (Zoetis 2013).

In aquatic invertebrates, a 30 mg/kg-bw dose of tiletamine-zolazepam injected into the hemolymph of blue crab (*Callinectes sapidus*) gave short-term (under 10 minutes) deep anesthesia (Quesada et al. 2011). Bradycardia was an observed side effect, but the crab's heart rate approached baseline within 10 minutes.

In iguanas, an intramuscular injection of 10.53 ± 4.2 mg/kg-bw tiletamine-zolazepam caused immediate but not excessive excitement, followed by muscle relaxation a few minutes later (Mauthe von Degerfeld 2004). The mean induction time was 6.5 minutes, with a recovery time of around 45 minutes. Respiratory rates decreased, and cardiac rates increased slightly (Mauthe von Degerfeld 2004).

In yellowtail amberjack (*Seriola lalandi*), an oral dose of 8–9 mg/kg-bw tiletamine-zolazepam caused sedation that lasted a maximum of 12 hours. These fish were responsive to the touch and recovered between 12 and 48 hours after dosing. Four of the 14 fish died due to the unplanned consumption of additional oral doses (as cited in (West et al. 2007)). Goldfish, given 50 mg/kg-bw ketamine by gavage, reached full sedation after 30 minutes (Raines and Clancy 2009). Both river stingray (*Potamotrygon motoro*) and wipers (hybrid striped x white bass (*Morone saxatilis* x *M. chrysops*)) reached light sedation (loss of equilibrium, very slow swimming, loss of muscle tone) at 20 mg/kg-bw and 300 mg/kg-bw by oral route, respectively (Raines and Clancy 2009).

3 SEDATIVES

WS uses sedatives in conjunction with anesthetics such as ketamine and Telazol to calm animals prior to euthanasia, relocation, placing a radio collar on the animal for tracking, collection of biological samples, and other WDM activities, especially where the safety of people and animals is a concern. Sedatives cause drowsiness, muscle relaxation, and central nervous system depression more than tranquilizers while reducing anxiety and producing calm with the animal awake. WS may use the sedatives azaperone, butorphanol, dexmedetomidine, medetomidine HCl, nalbuphine, xylazine, or a combination of these.

The sedatives WS administers to animals have central and/or peripheral nervous system effects. Primary effects include sedation, analgesia, bradycardia, respiratory depression, mild hypotension, and a reduction in antidiuretic hormone. Peripheral effects include vasoconstriction, which can lead to hypertension and reduced gastrointestinal movements (Clarke and England 1989).

The toxicity of sedative drugs to the target species, people, and nontarget species is a primary concern. Table 7 gives toxicity values for sedative drugs. These are discussed in the following sections on each drug or combination of drugs. Toxicity data were available for the sedatives azaperone, butorphanol, dexmedetomidine HCl, medetomidine HCl, nalbuphine HCl, and xylazine.

Table 7. Acute toxicity values for sedative drugs and their components.

Chemical	Test Species	Test Type	LD_{50}	Reference
Azaperone	Guinea Pig*	Acute oral	202 mg/kg-bw	(NCBI 2023c)
Azaperone	House Mouse*	Acute oral	385 mg/kg-bw	(NCBI 2023c)
Azaperone	Brown Rat*	Acute oral	245 mg/kg-bw	(NCBI 2023c)
Azaperone	House Mouse*	Intravenous	42 mg/kg-bw	(NCBI 2023c)
Azaperone	Brown Rat*	Intravenous	25 mg/kg-bw	(NCBI 2023c)
Azaperone	Brown Rat*	Intravenous	28.1 mg/kg-bw	(Pitman-Moore 1982)
Azaperone	House Mouse*	Subcutaneous	179 mg/kg-bw	(NCBI 2023c)
Azaperone	Brown Rat*	Subcutaneous	450 mg/kg-bw	(NCBI 2023c)
Azaperone	House Mouse*	Intraperitoneal	63 mg/kg-bw	(NCBI 2023c)
Butorphanol tartrate	Dog	Acute oral	>50 mg/kg-bw	(NCBI 2023w)
Butorphanol tartrate	Monkey^	Acute oral	>50 mg/kg-bw	(NCBI 2023w)
Butorphanol tartrate	House Mouse*	Acute oral	395 mg/kg-bw	(NCBI 2023w)
Butorphanol tartrate	Brown Rat*	Acute oral	315 mg/kg-bw	(NCBI 2023w)
Butorphanol tartrate	Dog	Intravenous	10 mg/kg-bw	(NCBI 2023w)
Butorphanol tartrate	House Mouse*	Intravenous	36 mg/kg-bw	(NCBI 2023w)
Butorphanol tartrate	Brown Rat*	Intravenous	17 mg/kg-bw	(NCBI 2023w)
Butorphanol tartrate	House Mouse*	Subcutaneous	299 mg/kg-bw	(NCBI 2023w)
Butorphanol tartrate	Brown Rat*	Subcutaneous	425 mg/kg-bw	(NCBI 2023w)
Butorphanol tartrate	Dog	Intramuscular	17 mg/kg-bw	(NCBI 2023w)
Butorphanol tartrate	House Mouse*	Intramuscular	208 mg/kg-bw	(NCBI 2023w)
Butorphanol tartrate	Brown Rat*	Intramuscular	255 mg/kg-bw	(NCBI 2023w)
Butorphanol tartrate	House Mouse*	Intraperitoneal	192 mg/kg-bw	(NCBI 2023w)
Butorphanol tartrate	Brown Rat*	Intraperitoneal	127 mg/kg-bw	(NCBI 2023w)
Dexmedetomidine HCl	Dog	Intravenous	2 mg/kg-bw	(Zoetis 2017b)

Chemical	Test Species	Test Type	LD_{50}	Reference
Midazolam	Brown Rat*	Oral	1600 mg/kg-bw	(NCBI 2023u)
Midazolam	Brown Rat*	Intravenous	75 mg/kg-bw	(NCBI 2023u)
Midazolam	Brown Rat*	Intramuscular	>50 mg/kg-bw	(NCBI 2023u)
Midazolam	House Mouse*	Oral	1600 mg/kg-bw	(NCBI 2023u)
Midazolam	House Mouse*	Intramuscular	>50 mg/kg-bw	(NCBI 2023u)
Midazolam	House Mouse*	Intravenous	50 mg/kg-bw	(NCBI 2023u)
Medetomidine HCl	Brown Rat*, Adult	Acute oral	31 mg/kg-bw	(UK Competent Authority 2014)
Medetomidine HCl	Water Flea (*Daphnia magna*)	48 Hr EC_{50} acute immobilization	4.5 mg/L	Bätscher, 2007b in (UK Competent Authority, 2014)
Medetomidine HCl	Pacific Oyster (*Crassostrea gigas*)	48 Hr EC_{50} embryo-larval development	2.5 mg/L	Fox and Sharpe, 2012 in (UK Competent Authority, 2014)
Nalbuphine HCl	Dog	Acute oral	1100 mg/kg-bw	(NCBI 2023m)
Nalbuphine HCl	Dog	Intravenous	140 mg/kg-bw	(Hospira 2019)
Xylazine	Brown Rat*, Adult	Acute oral	130 mg/kg-bw	*As cited in* (IPCS 2015)
Xylazine	Water Flea	48 Hr EC_{50}	>18 mg/L	(University of Hertfordshire 2018a)

* Domestic laboratory strains ^ Species not known – not in source document

3.1 Azaperone

3.1.1 Chemical Description and Product Use

Azaperone (CAS No. 1649-18-9; $C_{19}H_{22}FN_3O$; chemical structure in Appendix 3) is a sedative and works by depressing nerve functions. It belongs to the class of butyrophenones, with most drugs in this class being antipsychotic drugs. Azaperone blocks dopamine receptors in the brain and, at low doses, is an alpha-receptor blocker (Mestorino et al. 2013). This effect decreases the animal's response to external signals and makes it indifferent to its surroundings (Mestorino et al. 2013). Azaperone is a restricted-use drug and may be used by or on the order of a licensed veterinarian. Azaperone is used in veterinary medicine to reduce stress in pigs during handling and transport and aggression when pigs are comingled. Azaperone intramuscular doses for sedation range from 0.5 to 2.0 mg/kg-bw, depending on the species (IPCS 1991). WS uses all delivery methods to inject target animals with sedatives during WDM activities. WS uses azaperone combined with butorphanol tartrate and medetomidine HCl (BAM™) and nalbuphine and medetomidine HCl (NAM); WS does not use azaperone independently. It should be noted that NAM does not use drugs regulated by the U.S. Drug Enforcement Agency, which is beneficial in many areas and has been shown to be effective in immobilizing black bears (Wolfe et al. 2016a). However, another recent study reported effectiveness, but with some concerns for the safety of personnel and animals, when using BAM or NAM compared to ketamine-xylazine for the immobilization of raccoons (Johnson et al. 2023a).

3.1.2 Physical and Chemical Properties

Azaperone is an odorless, white powder (University of Hertfordshire 2018c). Its molecular weight is 327.40 g/mol (University of Hertfordshire 2018c), with a melting point of 92°C (University of Hertfordshire 2018c). It is moderately water-soluble (50 mg/L at 20°C) (University of Hertfordshire 2018c). Its log K_{ow} is 3.3 (University of Hertfordshire 2018c), indicating it is more lipophilic than hydrophilic.

3.1.3 Environmental Fate

Azaperone is not expected to escape into the atmosphere, as it is nonvolatile. Azaperone is moderately soluble in water, and its log K_{ow} indicates it will bind to the organic matter in soil and aquatic environments and be less available in solution. It strongly adsorbs to soil (K_{oc} 304,656) and is unlikely to leach from soil (Choi et al. 2014). The DT_{50} values range from 115.5–277.3 days for amended (added powdered blood meal as a biological fertilizer) and untreated soils (Choi et al. 2014). Azaperone was found in rivers on the Iberian Peninsula during an experiment that screened for 205 chemicals (Fàbrega et al. 2013). Bioaccumulation is unlikely because studies indicate azaperone excretion and metabolism is quick; tissue residues were less than 1 percent in male Wistar rats after 96 hours (FAO 1991).

3.1.4 Metabolism

The distribution of azaperone in rats treated with a subcutaneous injection of 1 mg/kg peaked within 30 minutes in blood, liver, and brain (*as cited in* (IPCS 1991)). Elimination of azaperone from the brain and blood was rapid; within 8 hours, concentration was down to 1 percent. Elimination from the liver was slower, down 25 percent within 8 hours. Within 24 hours, rats excreted 20–25% in urine, and within 48 hours excreted 60–80% in feces. No azaperone was detected in tissues four days after treatment (*as cited in* (IPCS 1991)). Azaperol is a metabolite of azaperone (Rauws et al. 1976). The half-life of azaperol in rats after intravenous injection was 45 minutes for the liver and 15 minutes for the kidney and brain (Rauws et al. 1976).

3.1.5 Hazard Identification

Azaperone is moderately toxic (category III for oral toxicity). In rodents, toxic signs were ptosis (drooping of the upper eyelid), sedation, tremors, and clonic seizures (loss of consciousness with violent muscle contractions) (IPCS 1991). In one study, male rats were given doses of 5, 20, or 80 mg/kg bw daily by gavage for 74 days (*as cited in* (IPCS 1991)). At the high dose, rats showed signs of severe sedation, ptosis, and decreased food consumption and body weight throughout the experiment. At the 20 mg/kg bw dose, moderate sedation and ptosis occurred.

3.1.5.1 Acute Toxicity

Azaperone is moderately toxic to mammals. The acute oral LD_{50} for guinea pigs (*Cavia porcellus*), mice, and rats is 202 mg/kg-bw, 385 mg/kg-bw, and 245 mg/kg-bw, respectively (NCBI 2023c) (Table 7). In a study using dogs, azaperone did not cause mortality after subcutaneous injection

of 2.5 to 40 mg/kg-bw and up to 20 mg/kg-bw given orally by gavage (Pitman-Moore 1982). After oral administration, some dogs vomited. In another study using adult male dogs, intramuscular injections of 20 and 40 mg/kg-bw and intravenous injections of 10 and 20 mg/kg-bw did not cause mortality (Pitman-Moore 1982). Both intramuscular and intravenous injections at the low dosage caused muscular tremors and tranquilization. All dogs had decreased muscle reaction to painful stimuli, extreme muscle incoordination, and increased sweating. At the high dosages, dogs developed tachycardia and had increased respiratory rate and muscle spasms, causing backward arching of the spine, neck, and head. All symptoms subsided in all dogs 2.5 to 3 hours after administration. Intravenous injections of 30 and 60 mg/kg-bw and intramuscular injections of 60 mg/kg-bw in female cats did not cause mortality (Pitman-Moore 1982). Both intravenous doses caused an increase in the respiratory rate, tremors/convulsions, impaired locomotion, and temporary bradycardia (Pitman-Moore 1982). Female cats that received the low intravenous dose recovered in 5-10 minutes. The 60 mg/kg-bw intramuscular dose had less effect than the intravenous 60 mg/kg-bw; no convulsions were observed in the cats. Azaperone administered intravenously had an LD_{50} of 42 mg/kg-bw and 25 mg/kg-bw in mice and rats, respectively (Table 7). Azaperone is considered a skin sensitizer (NCBI 2023c).

3.1.5.2 Sublethal and Chronic Toxicity

In a subacute study, male and female rats were given azaperone orally through food over 15 weeks (Pitman-Moore 1982). Azaperone concentrations were 10, 40, and 160 mg/100 g food, which provided an approximate daily oral dose of 10, 40, and 160 mg/kg-bw. Food consumption decreased in males fed 40 and 160 mg/100 g food, and males fed a 160 mg/100 g dose had a decrease in body weight. Females given the high dose also had a reduction in food consumption, and there was evidence of some changes in the female genital organs and hypophysis (pituitary). At the high dose, both males and females had a decrease in cholesterol, and males had an increase in urobilinogen. In another subacute study, male and female rats were given azaperone subcutaneously six days a week for 13 weeks (Pitman-Moore 1982). Doses were 2.5, 10, and 40 mg/kg-bw. At the highest dose, males had a significant decrease in average body weight. The weight of the thymus in animals, given the high dose, was significantly decreased. In a subacute study on dogs, azaperone was given orally in gelatin capsules six days a week for three months. Doses were 1.25, 5, and 20 mg/kg-bw (Pitman-Moore 1982). No mortality occurred. Body weight, blood pressure, and heart rate were within normal range. Gross pathology and histological evaluation did not reveal any dose or drug-related changes. In a chronic toxicity study, male and female rats received diets containing 0, 10, 40, and 160 mg azaperone/100 g food, which was approximately 0, 10, 40, and 160 mg/kg-bw (Pitman-Moore 1982). Study durations were six, twelve, and eighteen months in length. Gross pathology examinations of the animals that died during the studies did not find evidence of drug or dose-related causes. No adverse effects were observed through hematology, serum analysis, urinalysis, gross pathology, and organ weights. At the highest dose, male and female rats had reduced food consumption and body weight gain. At the highest dose, females tended to develop some changes in the genital organs and pituitary. In a 24-month study on dogs, azaperone was orally administered in gel capsules at doses of 1.25, 5, and 20 mg/kg-bw daily for six days per week (Pitman-Moore 1982). One dog died from causes unrelated to the dosing study. No chronic toxicity was detected during the dog study, which

measured heart rate, blood pressure, electrocardiogram, and hematological and biochemical parameters. A NOAEL of 630 µg/kg-bw was established based on neurobehavioral effects in dogs following oral administration (IPCS 1998). In one study, male rats were given doses of 5, 20, or 80 mg/kg-bw daily by gavage for 74 days (*as cited in* (IPCS 1998)). No reproductive effects were observed for the three doses (IPCS 1998).

Azaperone tested negative in mutagenicity tests (NCBI 2023q) . A review of the evidence indicates azaperone is unlikely to be genotoxic, carcinogenic (IPCS 1998), teratogenic or embryotoxic (Pitman-Moore 1982).

3.1.6 Dose-Response Assessment

3.1.6.1 Human Health Dose Response

Azaperone is primarily used as a veterinary drug and uncommonly as an antipsychotic drug in humans. Information is limited on the effects of azaperone on humans. In a study, ten human males received 0.5 mg azaperone three times a day (3x/day; administration method not provided), increasing to 20 mg 3x/day (estimated 1 mg/kg-bw/day) over a 17-day period (IPCS 1991). After this increase, the maximum dose was given 3x/day for two months. No symptoms were observed up to 2 mg given 3x/day (estimated about 0.1 mg/kg bw/day). At the higher doses, sedation was observed. At 20 mg given 3x/day, the patient developed dizziness. No changes in hematology or blood chemistry were observed prior to or at the end of the 2-month treatment period.

A pig breeder exposed to azaperone developed contact dermatitis (Brasch et al. 1991). The breeder regularly handled the drug for three years giving intramuscular injections of azaperone (0.5 mL/20 kg) behind the ear of his piglets. He did not wear gloves when handling azaperone, and spillage on the hands regularly occurred. The photoallergy to azaperone was confirmed through photo patch test results and the breeder's observation that symptoms worsened after sunlight exposure.

The FDA has not established a residue tolerance limit for azaperone in food products for human consumption. The Food and Agriculture Organization (FAO 2018) reports maximum residue limits in pigs of 60 µg/kg in muscle and fat and 100 µg/kg in liver and kidney. The FDA limits the use of azaperone to control aggressiveness when mixing or regrouping weanling or feeder pigs weighing up to 80 pounds to 2.2 mg/kg-bw by deep intramuscular injection (21 Code of Federal Regulations (CFR) 522.150). The FDA has not established an acceptable daily intake of azaperone in humans; the FAO (2018) reports an acceptable daily intake of 0–6 µg/kg-bw.

3.1.6.2 Ecological Dose Response

Azaperone is moderately toxic to mammals, and although it is more lipophilic, it is unlikely to bioaccumulate due to its short half-life. The disadvantages of azaperone are that it can cause a drop in blood pressure and excitation at low doses in horses (Kreeger and Arnemo 2012). Studies on azaperone, including acute, chronic, and subchronic toxicity studies on aquatic vertebrates

and invertebrates, are limited. Male and female spiny dogfish (*Squalus acanthias*) were given 4 mg/kg azaperone through the gill slit (Latas 1987). Visual observations of behavior showed that treated animals swam normally and did not show escape behavior immediately after treatment and release into a holding tank. In contrast, untreated animals had excited and agitated swimming behavior and showed signs of escape behavior (hitting the sides of the tank) after release. Treated animals accepted food after treatment whereas untreated animals did not feed for several days. All but one of the dogfish were alive three months after the experiment; the one animal died during capture and sampling.

3.2 Butorphanol

3.2.1 Chemical Description and Product Use

Butorphanol (CAS No. 42408-82-2; $C_{21}H_{29}NO_2$; chemical structure in Appendix 3) and butorphanol tartrate (CAS No. 58786-99-5; $C_{25}H_{35}NO_8$; chemical structure in Appendix 3) are used in sedation and pain management in animals (NCBI 2023t). Butorphanol is a DEA Class IV controlled substance (DEA, 2018), and Federal law restricts its use by or on the order of a licensed veterinarian (21 CFR § 522.246). Butorphanol is an analgesic (pain relief without total loss of feeling or muscle movement) that acts on the central nervous system, having an affinity for opioid receptors. The combination of butorphanol tartrate with azaperone tartrate and medetomidine HCl (BAM™) improves the quality of the anesthesia (blockage of all feeling) (see section 3.7 below). The APHIS WS Feral Swine Program uses butorphanol in combination with medetomidine and midazolam. For feral dogs, WDM or WS may combine butorphanol with dexmedetomidine and midazolam (these other drugs are covered in this RA). In equids, the intravenous dose for butorphanol is 0.1 mg/kg-bw (21 CFR § 522.246 2023). Sedation doses in small mammals range from 0.1 to 0.4 mg/kg-bw for intramuscular injection (West et al. 2007). WS administers butorphanol through intravenous or intramuscular routes, whereas remote (dart) delivery is by intramuscular route. WS research staff have also begun using drug combinations such as Butorphanol, Azaperone, Medetomidine (BAM), and Medetomidine, midazolam, and butorphanol (MMB) for immobilizing wildlife such as feral swine.

3.2.2 Physical and Chemical Properties

Butorphanol has a melting point of 216°C and a log K_{ow} of 3.3, indicating it is lipophilic (DrugBank 2021b). It is moderately soluble in water (0.16 g/L) (HMDB 2019d). Its molecular weight is 327.468 g/mol (NCBI 2023t). Butorphanol tartrate's predicted chemical properties are a melting point of 214°C, a logP of 4.19, and a Henry's law of 8.37×10^{-10} atm-m^3/mole at 25°C (USEPA 2019b).

3.2.3 Environmental Fate

No information is currently available on the environmental fate processes and impact of butorphanol. It has a short half-life in animals and is excreted through urine and feces. It is moderately soluble in water, but its mobility in soil is unknown (the soil organic carbon-water partition coefficient is unavailable).

3.2.4 Metabolism

The onset of analgesia is a few minutes for intravenous injection of butorphanol, 10 to 15 minutes for intramuscular injection (Hospira 2023). The analgesic effects last about four hours (Riggs et al. 2008, West et al. 2007). Butorphanol is mostly metabolized in the liver (DrugBank 2021b). Hydroxybutorphanol is a major metabolite of butorphanol, and norbutorphanol is produced in smaller amounts (EMEA 1998). Butorphanol has an elimination half-life of about 18 hours, with elimination occurring through urine and feces (DrugBank 2021b). The half-life of butorphanol was approximately 71 minutes in Holstein calves given 0.025 mg/kg bw butorphanol tartrate administered intramuscularly (Baldridge et al. 2011). The mean serum half-life was 1.62 hours in adult male and female dogs administered intramuscular or subcutaneous butorphanol tartrate at 0.25 mg/kg-bw (Pfeffer et al. 1980). One study evaluated butorphanol tartrate in two raptor species and found significant differences in plasma concentration-time profiles; throughout the study, concentrations were consistently higher in great horned owls than in red-tailed hawks (Riggs et al. 2008). In two of six red-tailed hawks, butorphanol concentrations in plasma after intravenous injection of 0.5 mg/kg-bw butorphanol tartrate into the jugular vein were undetectable 8 to 12 hours after administration; the half-life of the elimination phase was 0.997 hours (Riggs et al. 2008). In great horned owls, the plasma concentration of butorphanol after intramuscular or intravenous injection of 0.5 mg/kg-bw butorphanol tartrate were undetectable in two of six birds 12 to 24 hours after administration; the half-life of the elimination phase was 4.155 hours for intravenous injection into the jugular and 1.859 hours for intramuscular injection (Riggs et al. 2008).

3.2.5 Hazard Identification

The effects of a toxic dose of butorphanol are similar to those of opioid drugs . Symptoms include cardiovascular effects, hypoventilation, coma, and death (DrugBank 2021b).

3.2.5.1 Acute Toxicity

Butorphanol tartrate has an oral LD_{50} in mice of 395 mg/kg-bw, indicating it is moderately toxic (Table 7). In humans, adverse effects felt from butorphanol toxicity include sedation, dizziness, nausea, clamminess, sweating, headache, vertigo, weakness, anxiety, nervousness, and confusion (WHO 2006).

3.2.5.2 Sublethal and Chronic Toxicity

EMEA (1998) summarizes a reproductive study where rats received 10, 40, and 160 mg/kg-bw/day of butorphanol tartrate for 63 days prior to mating for males and for 14 days prior to mating and through the gestation period and lactation period for females. A reduction in the number of conceptions occurred at the 160 mg/kg-bw/day dose. From this study, butorphanol tartrate had a NOEL of 40 mg/kg-bw/day for embryotoxicity (EMEA 1998).

Butorphanol did not show evidence of carcinogenicity based on a two-year study where mice and rats were given butorphanol tartrate orally in the diet up to 60 mg/kg-bw/day (FDA 1998). Butorphanol was not genotoxic in bacteria assays (FDA 1998).

3.2.6 Dose-Response Assessment

3.2.6.1 Human Health Dose Response

Butorphanol is an analgesic for the relief of moderate to severe pain. In people, the recommended dose varies depending on the patient's age, body weight, and underlying physical conditions. The usual recommended dose for pain treatment is 1 mg administered intravenously, intramuscularly, or as a nasal spray (FDA 1998). The duration of analgesia varies depending on the pain level and the route of administration. Usually, it lasts 3 to 4 hours with intramuscular and intravenous doses, with additional doses given as necessary (FDA 1998).

After receiving 20 µg/kg butorphanol intravenously, patients reported feeling dizziness, drowsiness, or nausea (Wetchler et al. 1989). Other toxicity symptoms observed are sweatiness, headache, anxiety, confusion, and lightheadedness (*as cited in* (WHO 2006)). In many overdose cases, people were not tolerant to the effects of opiates, and the initial dose was more than 2 mg butorphanol tartrate injection (FDA 1998).

3.2.6.2 Ecological Dose Response

Butorphanol (0.4 mg/kg-bw) injected intramuscularly into harbor seals (*Phoca vitulina*) decreased anxiety and relaxed the animal prior to collecting biopsy samples (Tuomi 2000). The seals were lethargic for 1-4 hours after injection but responded to commands and ate and swam normally within one hour. Butorphanol caused behavioral changes in sheep in the form of restlessness, continual vocalization, and chewing at 0.1 and 0.2 mg/kg-bw intravenously. At 0.4 mg/kg-bw intravenously, sheep lost control of their body movements (ataxia) (Waterman et al. 1991). In this study, the 0.5 mg/kg-bw dose and the 0.1 mg/kg-bw dose provided effective anesthesia to thermal treatments for 60 and 80 minutes, respectively. In dogs, butorphanol had an LD_{50} of 50 mg/kg-bw (category II for acute toxicity) (Plumb 2018). In green iguana, butorphanol at 1.5 mg/kg-bw or 8.0 mg/kg-bw intramuscularly gave analgesic effects in response to electrostimulation of the tail with no adverse side effects at the evaluated doses (Greenacre et al. 2006). Respiratory depression and extreme sedation in some reptile species occur at subcutaneous and intramuscular doses greater than 10 mg/kg-bw (Greenacre et al., 2008 *in* (West et al. 2007)). In avian species, 1.0-5.0 mg/kg-bw of butorphanol given intramuscularly provides an analgesic effect (*as cited in* (West et al. 2007)). Doses of butorphanol between 25-33 mg/kg-bw subcutaneously and 0.25-0.5 mg/kg-bw intramuscularly had analgesic effects on amphibians and fish, respectively (as cited in (West et al. 2007)). Butorphanol (0.5 mg/L) added to tank water (72-h bath) shortened the time to consume food and normal behavior after surgery in the eastern red-spotted newt (*Notophthalmus viridescens*) (Koeller 2009).

3.3 Dexmedetomidine

3.3.1 Chemical Description and Product Use

Dexmedetomidine (CAS No. 113775-47-6; $C_{13}H_{16}N_2$; chemical structure in Appendix 3), an imidazole compound, is an agonist of adrenergic alpha-2 receptors. Dexmedetomidine causes sedation and analgesia (Plumb 2018). Dexmedetomidine is also the active component within medetomidine, a racemic[11] form of dexmedetomidine; however, it is approximately twice as potent as medetomidine (Ko et al. 2009). Dexmedetomidine and medetomidine are reviewed in separate sections because contrasting evidence has raised questions about the common assumption of equipotency. The levomedetomidine in medetomidine may still play a role in drug interactions that would not be found in dexmedetomidine alone (Siegenthaler et al. 2020).

Alpha-2 adrenoceptor agonist drugs have effects on both the central and peripheral nervous systems. Central nervous system effects include sedation, analgesia, bradycardia, respiratory depression, mild hypotension, and a reduction in antidiuretic hormone. Peripheral nervous system effects include vasoconstriction, which can lead to hypertension and a reduction in gastrointestinal movements (Clarke and England, 1989).

FDA approved the use of dexmedetomidine for short-term sedation and analgesia (<24 hours) of humans in 1999 (Kaur and Singh 2011) under the trade name Precedex® (Ko et al. 2009). Dexmedetomidine became commercially available for use in dogs in 2006 and cats in 2007 (FDA 2010) under the trade name Dexdomitor® (Ko et al. 2009, Neto 2009). It can be used to provide chemical restraint of hyperexcitable animals (Neto 2009) during WDM activities. Dexdomitor is a 0.5 mg/mL solution (Ko et al. 2009) available in 10 mL multi-dose vials (FDA 2010).

WS uses dexmedetomidine to chemically restrain wildlife, including for research purposes. WS uses the injectable formulation with all delivery devices, including remote darting or hand-syringe.

3.3.2 Physical and Chemical Properties

Dexmedetomidine is a solid with a molecular weight of 200.285 g/mol (NCBI 2023g). Dexmedetomidine is freely soluble in water with a predicted solubility of 0.174 mg/mL and has a Log K_{ow} of 2.8 (NCBI 2023g), indicating it is more lipophilic than hydrophilic. Dexmedetomidine HCl is the HCl salt form of dexmedetomidine and is used as a sedative or analgesic in animals (NCBI 2023g). This drug is sold under the trade name Dexdomitor, which is a white or off-white, crystalline, water-soluble substance with a molecular weight of 236.7 g/mol (Zoetis 2017b).

3.3.3 Environmental Fate

The use pattern for dexmedetomidine indicates its release into the environment is unlikely. No data is available on this product's degradability, bioaccumulation potential, or mobility in soil (Zoetis 2017b). As a lipophilic compound, dexmedetomidine is rapidly distributed in the body and readily penetrates the blood-brain barrier (EMA 2021). The protein binding of dexmedetomidine

[11] Composed of dextrorotatory and levorotatory forms of a compound in equal proportion.

is also high (84–95%) in mice, rats, dogs, monkeys, and humans (EMEA 2002a). In rats and dogs, biotransformation of dexmedetomidine occurs in the liver; metabolites are pharmacodynamically inactive (EMEA 2002a).

3.3.4 Metabolism

WS typically administers dexmedetomidine intramuscularly. Dexmedetomidine is lipophilic and subsequently well-absorbed after intramuscular administration (EMA 2021). In dogs given an intramuscular dose of 50 µg/kg-bw, peak plasma levels were reached after 0.6 hours. The elimination half-life ($T_{1/2}$) is 40–50 minutes (EMA 2021). Metabolism of dexmedetomidine occurs in the liver in rats, dogs (EMEA 2002a), and cats (EMA 2021); metabolites lack pharmacological activity (EMEA 2002a); (EMA 2021). In dogs, major biotransformation pathways include hydroxylation, glucuronic acid conjugation, and N-methylation in the liver (EMEA 2002a); (EMA 2021). Dexmedetomidine elimination in cats and dogs depends on hepatic (liver) blood flow and is excreted mainly in the urine (EMA 2021).

3.3.5 Hazard Identification

Dexmedetomidine is FDA-approved for humans and animals. As with all alpha-2 agonists, the potential exists for an animal administered dexmedetomidine to experience prolonged sedation, bradycardia, cyanosis, vomiting, apnea, or death (Drugs.com 2015). Dexmedetomidine HCl is considered minimally toxic for acute oral exposure (category IV), moderately toxic for single-exposure target organs (category III), and hazardous to the skin and eyes (category III) (Cayman Chemical 2023b). The toxicity of dexmedetomidine is high in all studied species, with sedation, piloerection, bulging of the eyes, salivation (rats), and convulsions (rats and dogs) are the most common dose-related clinical signs in mice, rats, and dogs (EMEA 2002a). In some dogs and cats, a decrease in respiratory rate, vomiting, or corneal opacities may also occur (EMA 2021). Rare instances of pulmonary edema have been reported. This drug should not be used in animals with cardiovascular disorders or severe systemic disease (EMA 2021). In cases of overdose in dogs and cats, atipamezole can be used as an antagonist (EMA 2021). Other adverse effects include hypotension, bradycardia, dry mouth, sinus arrest, and transient hypertension.

3.3.5.1 Acute Toxicity

In dogs, the intravenous LD_{50} is 2 mg/kg-bw (Table 7).

3.3.5.2 Sublethal and Chronic Toxicity

Dose-related sedation and piloerection were observed in rats and dogs during repeated dose studies using dexmedetomidine (EMA 2011a). Dose ranges for these studies were 20–500 µg/kg-bw/day (rats, subcutaneous and intramuscular), 10–1,250 µg/kg-bw/day (rats, intravenous), and 10-250 µg/kg-bw/day (dogs, intramuscular and intravenous). Rats also exhibited exophthalmos (bulging of the eye), and dogs exhibited sporadic muscle twitches, irregular respiration, and atrioventricular block; corneal keratitis and opacity were observed in both species (EMA 2011a). In another 28-day repeated dose chronic toxicity study, dogs receiving daily intravenous injections

of dexmedetomidine HCl had treatment-related effects, including impacts to the liver and central nervous system. A LOAEL of 0.01 mg/kg-bw was derived from this study (Zoetis 2017b). In another study, rats receiving intramuscular injections of dexmedetomidine HCl for 28 days had treatment-related effects associated with the eyes, adrenal gland, lungs, and the male reproductive system. The NOAEL in this study was 0.02 mg/kg-bw (Zoetis 2017b).

Dexmedetomidine HCl (Dexdomitor) was administered intramuscularly at ten times the recommended dose of 40 µg/kg-bw to 3 female and three male 7-month-old cats. Sedation occurred within 15 minutes of dosing and lasted a minimum of 4 hours, with full recovery occurring in 8−24 hours. During sedation, some cats experienced corneal dehydration and opacity, miosis, pale skin and gingiva, salivation, and watery ocular discharge. Vomiting occurred 7−11 hours after dosing in all but one animal. No mortality was observed (NLM 2014).

3.3.6 Dose-Response Assessment

3.3.6.1 Human Health Dose Response

Dexmedetomidine (dose range of 0.2 to 1.4 µg/kg-bw/hour intravenously) is used most frequently in humans for the sedation of adult patients in intensive care units for sedation when necessary (EMA 2011a). Elimination of the drug in humans is rapid, with an elimination half-life of 2.6 hours, and occurs primarily by metabolism (EMA 2011a). However, the mean elimination half-life was prolonged to 3.9, 5.4, and 7.4 hours for individuals with mild, moderate, and severe hepatic impairment, respectively (EMA 2011a).

Researchers in one study determined most of the radiolabeled dose of dexmedetomidine was recovered in urine (~94%). Unchanged parent drug was not observed in the urine, and only trace amounts were noted in feces (EMA 2011a). Radioactivity declined over a period of 9 days, with trace amounts present up to 24 days (EMA 2011a).

One study evaluating the effect of age after subjects received a 10-minute intravenous infusion of 0.6 µg/kg-bw dexmedetomidine indicated a higher sensitivity in young individuals. It increased sensitivity in females more so than males both during and immediately after infusion (EMA 2011a). While dexmedetomidine had no effect on male or female fertility, and no teratogenic effects were observed in animals, the drug should not be used during pregnancy due to the potential effect on fetal heart rate (EMA 2011a).

Effects of dexmedetomidine on healthy volunteers include bradycardia, sinus pauses, hypertension, hypotension, respiratory depression, decreased heart rate, hypothermia, increased plasma human growth hormone, and hyperglycemia starting 0.2 to 0.7 µg/kg-bw/hour following intravenous administration (Keating 2015, Venn et al. 2003). Exposure may be associated with the accidental splashing of the drug on the skin or mucus membranes, which could result in sedation or hemodynamic changes (EMEA 2002a). One estimate of a worst-case scenario involving accidental injection of dexmedetomidine meant for an 80 kg dog suggests that profound hemodynamic changes could occur as a result (EMEA 2002a).

3.3.6.2 Ecological Dose Response

Aquatic and terrestrial ecotoxicity data is limited or unavailable for dexmedetomidine. Dexmedetomidine may cause harm to aquatic life (Santa Cruz Biotechnology 2018); however, under normal use conditions, dexmedetomidine is not expected to pose a risk to the environment. The predicted environmental concentration surface water value for dexmedetomidine is 0.00013 µg/L, which is below the action limit of >0.01 µg/L (EMA 2011a).

3.4 Medetomidine Hydrochloride

3.4.1 Chemical Description and Product Use

Medetomidine HCl (CAS No. 86347-15-1; $C_{13}H_{17}ClN_2$; chemical structure in Appendix 3) is a high alpha-2 agonist receptor affinity that produces dose-dependent sedation and analgesia (NCBI 2023i, Sinclair 2003). Alpha-2 adrenoceptor agonist drugs have central and peripheral effects. Medetomidine is frequently used in combination with ketamine to produce a satisfactory level of analgesia and muscle relaxation and can be reversed by yohimbine and atipamezole (Sinclair 2003). WS prefers to use atipamezole as a reversal agent for medetomidine or dexmedetomidine (USDA APHIS 2017). This combination of drugs is frequently used in WDM for work with cervids, large felids, mustelids, and bears and is useful with remote dart delivery (West et al. 2007). The FDA approved medetomidine HCl in a 1 mg/mL concentration in the United States in 1995 under the trade name of Domitor® (FDA 2020). The drug is available in a concentrated form (i.e., 10, 20, and 40 mg/mL) with a prescription from a compounding pharmacy. WS uses medetomidine to sedate wildlife, often for research purposes, administering the drug intramuscularly and using hand or remote drug delivery methods except for oral administration.

3.4.2 Physical and Chemical Properties

Medetomidine HCl is a white or off-white, odorless, water-soluble substance with a molecular weight of 236.7 g/mol. Its melting point is 176–179°C (Santa Cruz Biotechnology 2019), and it is stable in abiotic hydrolysis and photolysis (UK Competent Authority 2014). Medetomidine is a lipophilic drug with a log K_{ow} of 3.1 (DrugBank 2021f).

3.4.3 Environmental Fate

Medetomidine HCl is considered stable in abiotic hydrolysis and photolysis studies and does not readily biodegrade. It also is not considered bioaccumulative (UK Competent Authority 2014).

3.4.4 Metabolism

Medetomidine can be administered intramuscularly, intravenously, or subcutaneously. When given intramuscularly, medetomidine, due to its high lipid solubility, is rapidly absorbed, and peak plasma levels are reached within 30 minutes (*as cited in* (Kaartinen 2009)), with plasma protein binding of medetomidine at 92–95% (*as cited in* (Kaartinen 2009)). In cats given an intramuscular dose of 40 µg/kg-bw medetomidine, the peak plasma concentration is reached at approximately 0.24 hours, and the half-life is one hour. The elimination half-life ($T_{1/2}$) of 80 µg/kg-bw of

medetomidine intramuscularly administered is 1.28 hours in dogs (*as cited in* (Kaartinen 2009)). Elimination occurs as a result of biotransformation in the liver (*as cited in* (Kaartinen 2009)). The primary metabolite is the N-glucuronide conjugate (UK Competent Authority 2014). When given intravenously, the onset of action is rapid, and the peripheral effects on the cardiovascular system are greater than when the drug is administered intramuscularly. The elimination half-life of 80 µg/kg-bw of medetomidine administered intravenously is 0.97 hours (*as cited in* (Kaartinen 2009)). Subcutaneous administration results in slower and highly variable absorption times (Kaartinen 2009).

3.4.5 Hazard Identification

As with all alpha-2 agonists, the potential exists for an animal administered medetomidine to experience prolonged sedation, bradycardia, cyanosis, vomiting, apnea, or death (Sinclair 2003). Spontaneous muscle contractions may be observed in some target animals (Sinclair 2003, Zoetis 2021). After drug administration, blood pressure is initially increased due to peripheral vasoconstriction, then decreases to normal or slightly below normal levels. Respiratory responses include an initial slowing of respiration that returns to normal within two hours (Zoetis 2021). It has been noted that medetomidine should not be used in dogs with cardiac disease, respiratory disorders, or liver or kidney diseases. It also should not be used when dogs are stressed due to extreme heat, cold, or fatigue (Zoetis 2021), suggesting wildlife with these conditions could be at risk (these conditions would be generally unknown when tranquilizing wild animals but anticipated to be minimal since wild animals rarely would get supplemental veterinary care).

3.4.5.1 Acute Toxicity

Many studies involving medetomidine were performed with medetomidine versus medetomidine HCl. While this risk assessment focuses primarily on studies specific to medetomidine HCl, it is important to note that medetomidine and medetomidine HCl can be regarded as equivalent from a toxicological perspective. This is due to the dissociation of HCl from medetomidine in an aqueous environment (UK Competent Authority 2014). The LD_{50} in rats for medetomidine HCl is 31 mg/kg-bw (Table 7) (UK Competent Authority 2014). In animal safety studies, dogs tolerated up to five times the recommended intravenous dosage of medetomidine and up to 10 times the recommended intramuscular dose. A single intravenous administration of 10 times the recommended dose in dogs caused a prolonged anesthesia-like condition with increased spontaneous muscle contractions (Zoetis 2021).

3.4.5.2 Sublethal and Chronic Toxicity

In a 28-day repeated dose subcutaneous toxicity study, rats received 0, 0.1, 0.4, or 1.6 mg/kg-bw/day of medetomidine HCl in saline. No deaths occurred; however, treatment-related effects included reduced body weight gain and corneal opacity of the eye. A subcutaneous LOAEL of 0.1 mg/kg-bw/day was derived from this study (Hirsimaki, 1986a *in* (UK Competent Authority 2014)). Similarly, in a 28-day study of beagles receiving daily intramuscular injections of medetomidine HCl in saline of 0, 0.8, 0.24, or 0.4 mg/kg-bw/day, no deaths occurred at any dose level. No

treatment-related effects were noted aside from diarrhea and corneal opacity in the mid-and high-dose groups. An intramuscular NOAEL of < 0.8 mg/kg-bw/day was derived from this study (Hirsimaki, 1986b *in* (UK Competent Authority 2014)). The animal safety studies reported by Pfizer (Zoetis 2021) indicate that repeated doses of 3 or 5 times the recommended dose of medetomidine can cause profound sedation, bradycardia, reduced respiratory rates, and spontaneous twitching. In vitro and in vivo studies of medetomidine indicate that it is not genotoxic (UK Competent Authority 2014). Information on the reproductive effects of medetomidine is limited (Zoetis 2021).

3.4.6 Dose-Response Assessment

3.4.6.1 Human Health Dose Response

Medetomidine is extensively metabolized in people and rapidly excreted. The main metabolite is the N-glucuronide conjugate. Medetomidine and its metabolites are distributed throughout the body and can cross the placenta and be excreted in breast milk (UK Competent Authority 2014). The drug is suspected of damaging fertility or the fetus (Cayman Chemical 2023a). Following the intravenous injection of radioactive-labeled medetomidine in people, the maximum blood concentration of labeled medetomidine was reached in 10 minutes. Elimination of labeled medetomidine from blood was rapid, with an elimination half-life of approximately 3 hours. Medetomidine metabolites are excreted mainly via urine. In humans, an average of 95% of medetomidine metabolites are excreted in the urine after 9 days, with approximately 85% excreted within 24 hours of drug administration. Effects of medetomidine on humans include hypotension, hypertension, bradycardia, reduced salivation, decreased blood pressure, decreased heart rate, and decreased cardiac output. Medetomidine does not appear to accumulate in tissues and organs (UK Competent Authority 2014). Central nervous system effects include sedation, analgesia, bradycardia, respiratory depression, mild hypotension, and a reduction in antidiuretic hormone. Peripheral nervous system effects include vasoconstriction, which can lead to hypertension and a reduction in gastrointestinal movements (Clarke and England 1989). The NOAEL for intravenous administration of medetomidine in humans is 0.4 µg/kg bw (Abbott, 1988 *in* (UK Competent Authority 2014)).

3.4.6.2 Ecological Dose Response

Zebrafish (*Danio rerio*) have an acute 96-hour LC_{50} of 30 mg/L medetomidine hydrochloride, which is considered slightly toxic (Bätscher, 2007a in (UK Competent Authority 2014)). Medetomidine is considered very toxic to fish in chronic exposures (UK Competent Authority 2014). Medetomidine exposures from 0.5 to 50 nM via intraperitoneal injection or by water for a maximum of 54 days caused body paleness within 30 days in Atlantic salmon (*Salmo salar*), rainbow trout, Atlantic cod (*Gadus morhua*), and turbot (*Scophthalmus maximus*) (Lennquist et al. 2010). Exposure of fry from lumpfish (*Cyclopterus lumpus*) and turbot to medetomidine concentrations from 5–10 nM caused decreased oxygen consumption, but the effect was reversible (Lennquist et al. 2010). In a study involving the diatom *Skeletonema costatum*, the 72-hour no observable effect concentration (NOEC) for growth rate after exposure to medetomidine was 0.253 mg/L, and the lowest observable effect concentration (LOEC) was 0.447 mg/L

(Maunder and Vaughan, 2011 *in* (UK Competent Authority 2014)). This study's examination and other studies indicate that medetomidine is very toxic to algae (UK Competent Authority 2014).

Medetomidine, given intramuscularly at a dose of 0.01 mg/kg has analgesic and mild sedative effects in Pacific walrus (*Odobensus rosmarus*) (Moore et al. 2010). Table 7 summarizes aquatic toxicity values for fish and aquatic invertebrates.

3.5 Midazolam

3.5.1 Chemical Description and Product Use

Midazolam (CAS No. 59467-70-8; $C_{18}H_{13}ClFN_3$; chemical structure in Appendix 3) is a benzodiazepine and is commonly used in humans to sedate patients prior to procedures and treat generalized seizures (NCBI 2023u) and in outpatient procedures such as endoscopy and dentistry (Nordt and Clark 1997). Similarly, in veterinary medicine, midazolam is used to sedate animals prior to medical procedures (Plumb 2018).

Midazolam is used as an anticonvulsant and muscle relaxant in wildlife immobilization (Kreeger and Arnemo 2012). Benzodiazepines work by inhibiting the effects of the gamma-aminobutyric acid (GABA) neurotransmitter (Kreeger and Arnemo 2012), which produces a calming effect, relaxes skeletal muscles, and induces sleep (NCBI 2023u). In animal immobilization, midazolam is often used in combination with dissociative agents (e.g., ketamine) during anesthesia to prevent convulsions that may occur from dissociative agents (West et al. 2007). Midazolam has minimal cardiovascular and respiratory effects (Kreeger and Arnemo 2012). It is a schedule IV-controlled substance. Midazolam is aqueous-based and is administered intramuscularly, intravenously, subcutaneously, and orally (Kreeger and Arnemo 2012). In the U.S., midazolam comes in liquid solutions and syrup formulations. Midazolam is revisable with flumazenil (West et al. 2007).

WS uses midazolam to sedate animals during physical or mechanical restraint and to aid the induction of anesthesia with other drugs. WS uses midazolam in combination with medetomidine and butorphanol (MMB) in feral swine immobilization. Research has shown that MMB is preferable for immobilizing feral pigs due to the adequate level of immobilization and no post-recovery morbidity when compared to other drug combinations used for feral swine (Ellis et al. 2019). The optimized ratio of a combination of these drugs is 0.06 mg/kg-bw medetomidine, 0.3 mg/kg-bw midazolam, and 0.3 mg/kg-bw butorphanol. Medetomidine and butorphanol are covered elsewhere in this document. WS administers midazolam using intramuscular injection and remotely administers the drug when immobilizing large animals such as feral swine.

3.5.2 Physical and Chemical Properties

Midazolam is a solid, white, to light yellow color, with a molecular weight of 325.8 (NCBI 2023u). It has a boiling point of 497°C and a melting point of 161–164°C (NCBI 2023u). It is moderately soluble in water with a solubility of 54 mg/L; the hydrochloride salt of midazolam, which is formed in situ, is soluble in aqueous solutions (DrugBank 2023a, NCBI 2023u). Its estimated vapor pressure is 5.12×10^{-9} mm Hg at 25°C and estimated Henry's Law Constant is 2.80×10^{-11} atm-

m^3/mol at 25 °C (NCBI 2023u). It has a low Log K_{ow} of 3.30 at pH 7 (EMA 2011b). It has a bitter taste (Nordt and Clark 1997).

3.5.3 Environmental Fate

The use pattern for midazolam indicates its release into the environment is unlikely. Information on its environmental fate properties is limited. Its chemical properties indicate it is moderately soluble in water and is non-volatile. Its Log K_{ow} indicates it does not have the potential to bioaccumulate in living organisms (EMA 2011b) and would not readily bind to organic matter.

3.5.4 Metabolism

Midazolam is metabolized in the liver (Plumb 2018) and excreted in the urine (Nordt and Clark 1997). The duration of action is 1–2 hours (Nordt and Clark 1997), and the elimination half-life is about 1.5–3 hours (Nordt and Clark 1997, Plumb 2018). The metabolite alpha-hydroxy-midazolam has a 1-hour elimination half-life (Nordt and Clark 1997). The elimination half-life for midazolam is short. The intravenous dose (dose not given) elimination half-life in adults is approximately 3 hours (mean) (DrugBank 2023a). The intramuscular administration of 10 mg midazolam had an elimination half-life of 4.2 hours (DrugBank 2023a). Patient recovery time following intravenous injection averaged 27 minutes (Nordt and Clark 1997). In people with hepatic or renal problems, sedation effects may last longer due to the decreased metabolism and clearance of midazolam (Nordt and Clark 1997).

In dogs, the midazolam half-life averages 77 minutes (Plumb 2018). In horses, the median half-life is 216 minutes after 0.05 mg/kg-bw and 408 minutes after 0.1 mg/kg-bw intravenous dose (Plumb 2018).

3.5.5 Hazard Identification

Midazolam is FDA-approved for human use. Midazolam causes sleep, sedation, and amnesia in humans and reduces anxiety (Nordt and Clark 1997). Overdose exposure of people to benzodiazepines may decrease respiratory rates and cause ataxia, lethargy, slurred speech, sleepiness, and coma (Kreeger and Arnemo 2012). Adverse effects from midazolam are rare in humans; less than 5% of patients experience pain on injection, local irritation, headache, nausea, vomiting, and hiccups (Plumb 2018).

In animals, residual sedation is the most common side effect post immobilization (West et al. 2007) and potential respiratory depression (Plumb 2018).

3.5.5.1 Acute Toxicity

In rats, midazolam has an oral, intravenous, and intramuscular LD_{50} of 1,600 mg/kg-bw, 75 mg/kg-bw, and >50 mg/kg-bw (NCBI 2023u). In mice, midazolam has an oral, intramuscular, and intravenous LD_{50} of 1,600 mg/kg-bw, >50 mg/kg-bw, and 50 mg/kg-bw, respectively (NCBI 2023u) (Table 7).

3.5.5.2 Sublethal and Chronic Toxicity

Midazolam is not mutagenic, genotoxic, or carcinogenic (NCBI 2023u). Oral doses of 1, 4, or 16 mg/kg-bw midazolam had no adverse effects on male or female fertility in rats when dosed before and during mating (males and females) and throughout gestation and lactation in females (NCBI 2023u). In 13-week subchronic studies, dogs given up to 45 mg/kg-bw/day and rats given up to 200 mg/kg-bw/day demonstrated minimum toxicity for midazolam with increased liver weight in rats at high doses (NCBI 2023u).

3.5.6 Dose-Response Assessment

3.5.6.1 Human Health Dose Response

The intravenous dose of midazolam in adults ranges from 0.02–0.03 mg/kg-bw and may be repeated at intervals to achieve sedation (Nordt and Clark 1997). In healthy adults, 1–3 mg midazolam given intravenously usually achieves adequate sedation for most medical procedures, and more than 5 mg is usually not necessary (Nordt and Clark 1997). A muscular dose of 0.07–0.08 mg/kg-bw is given for conscious sedation (Nordt and Clark 1997). In humans, the onset of sedation following intramuscular and intravenous injection has been reported as early as 5 minutes, with peak effects seen within 15–20 minutes and 3 minutes, respectively (Nordt and Clark 1997).

3.5.6.2 Ecological Dose Response

In horses, 0.05 mg/kg-bw and 0.1 mg/kg-bw midazolam did not produce sedation; rather, it produced agitation and ataxia (Plumb 2018). Midazolam doses of 0.1–0.5 mg/kg-bw intramuscularly have been used on raptors (West et al. 2007). In raptors, 1 mg/kg-bw intramuscularly causes mild sedation and relaxation (Plumb 2018).

Canids caught in traps have been sedated with 3–4 mg/kg-bw midazolam given as tablets in food (West et al. 2007). Syrup formulations intended for oral administration are approved human drugs in the U.S. and could be prescribed for extra-label use in animals under certain circumstances. In wild felines, typical intramuscular and oral dosages for a tiger (*Panthera tigris*) are 0.08–0.14 mg/kg-bw (15 mg/adult tiger or lion (*P. leo*)) (West et al. 2007). In rabbits, a dose of 0.5–2.0 mg/kg-bw midazolam is given intramuscularly, subcutaneously, or intravenously for premedication and sedation (West et al. 2007).

Eight 1-to-3-year-old donkeys were given 0.1 mg/kg-bw midazolam intravenously to study the pharmacokinetics and pharmacodynamics of midazolam from the baseline time (zero) through 9-time intervals up to 48 hours post-administration (Odette et al. 2022). There was no change in heart rate, respiratory rate, or head height, and no differences in tactile and auditory stimulation and agitation-sedation throughout the study. Rectal temperature significantly increased between 90 and 720 minutes after administration; however, the authors think this was not a drug-related effect but a normal diurnal variation. Ataxia and recumbency without sedation significantly increased between 3 and 15 minutes after administration. Midazolam was detectable in the

plasma between 3- and 30-minutes post-administration. After 45 minutes, it was detectable in the plasma of 5 donkeys at 45 minutes and 3 donkeys at 60 minutes. After 60 minutes, midazolam was not detectable in any of the donkeys.

Midazolam is commonly used in combination with other sedatives (e.g., butorphanol, medetomidine) and anesthetics (e.g., ketamine). In red foxes (*Vulpes vulpes*), the induction time for the combination of ketamine (30.8 mg/kg-bw)-midazolam (0.6 mg/kg-bw) was 4–6 minutes, with immobilization effects lasting 14–23 minutes after intramuscular injection (Kreeger et al. 1990, West et al. 2007). The combination of midazolam (0.5 or 1 mg/kg-bw) with medetomidine (0.06 mg/kg-bw) has been successful in red foxes by intramuscular injection, with an induction time of 5–8 minutes, immobilization time of 20–25 minutes, and a recovery time of 10 minutes after atipamezole administration (Shilo et al. 2010). Side effects from that combination were mild hypertension in the foxes. Similarly, the combination of medetomidine (0.04 mg/kg-bw), midazolam (0.3 mg/kg-bw), and butorphanol (0.1 mg/kg-bw) was effective in sedating red fox (Bertelsen and Villadsen 2009). In feral swine, intramuscular injection medetomidine (0.06 mg/kg-bw)-midazolam (0.30 mg/kg-bw)-butorphanol (0.30 mg/kg-bw) (MMB) provided immobilization within a favorable induction time (3.8 minutes for ataxia and 12.7 minutes for lateral recumbency) (Ellis et al. 2019). Swine did not display behavior post-immobilization that would contribute to morbidity. Midazolam was not detected in muscle, fat, liver, or kidney tissue in the test animals sampled 3-, 5-, and 7-days post-immobilization.

3.6 Nalbuphine

3.6.1 Chemical Description and Product Use

Nalbuphine (CAS No. 20594-83-6; $C_{21}H_{27}NO_4$; chemical structure in Appendix 3) is a synthetic opioid used as pain medication (NCBI 2023p). Nalbuphine HCl is commonly sold as a prescription under the brand name Nubain®, which is available in two concentrations, 10 mg/mL and 20 mg/mL (FDA 2016). Nubain's analgesic potency is similar to that of morphine (FDA 2016). It appears to function as an agonist-antagonist analgesic in the central nervous system; however, the exact mechanism of action is unknown (NCBI 2023p). Nalbuphine HCl is administered via a sterile solution injected into the veins, fat, or muscles (FDA 2016). Nalbuphine has pharmacological properties similar to butorphanol and produces mild analgesia and sedation with few adverse effects (KuKanich and Wiese, 2015 *in* (Wolfe et al. 2016a)). The abuse potential for nalbuphine is low; subsequently, this opioid is not a controlled substance under the Controlled Substances Act (DEA 2023). WS would use nalbuphine for wildlife immobilization, frequently in combination with azaperone tartrate and medetomidine HCl. WS administers the drug through hand injection and syringe pole as a component of NAM. WS uses a dart gun to administer the drug when conducting bear relocations.

3.6.2 Physical and Chemical Properties

Nalbuphine is a solid with a molecular weight of 357.45 g/mol and a melting point of 230°C as a hydrochloride salt (NCBI 2023p). When combined with hydrochloric acid, it forms nalbuphine HCl, a clear solution that can dissolve in water (35.5 mg/mL at 25°C) (DrugBank 2023c). Nalbuphine

HCl has a molecular weight of 393.91 g/mol and pKa values of 8.71 and 9.96. Its log K_{ow} is 1.4, indicating the drug is slightly more lipophilic than hydrophilic (HMDB 2019a). Nalbuphine HCl is commonly sold under the brand names Nubain and Nalpain®. Nubain is stable when stored at 25°C and away from excessive light (Drugs.com 2023c).

3.6.3 Environmental Fate

Little information and research are available on nalbuphine's environmental fate and transport. The EPA predicted that the biodegradation half-life is 116 days since no experimental data is currently available (USEPA 2020). The environmental properties of nalbuphine HCl have not been thoroughly investigated; therefore, the drug's release into the environment should be avoided (Hospira 2019).

3.6.4 Metabolism

Nalbuphine is administered subcutaneously, intravenously, intramuscularly, or orally. Nalbuphine has very poor oral (enteral) bioavailability in adults and is quickly metabolized. Parenteral doses of 75 to 300 mg/kg-bw are used in infants and children (NIH 2018). Due to the high degree of conjugation in the liver at first pass, oral forms are not typically used in clinical practice (Logash et al. 2017). The plasma half-life of nalbuphine is 5 hours, with the duration of analgesic activity reported to range from 3 to 6 hours in clinical studies (DEA 2023). The maximum concentration is attained in 15–30 min, and it exits the body mostly through bile, although metabolites and the unchanged drug can be found in urine (Logash et al. 2017). Nalbuphine is expected to be concentrated in the blood, urine, and breast milk (HMDB 2019a, NIH 2018). However, blood and urine concentrations have never been quantified (HMDB 2019a).

In a study concerning the pharmacokinetics and excretion of nalbuphine via breastmilk, seven nursing women received a single 20 mg dose of nalbuphine intramuscularly. Milk samples were collected several times, ranging from 1 to 24 hours after the initial dose. The elimination half-life from milk was determined to be roughly 8 hours. It was determined that an exclusively breastfed infant in this study would receive a dosage equivalent to 1.1% of the maternal weight-adjusted dosage. Because nalbuphine has poor oral absorption, it is unlikely to adversely affect breastfed infants (NIH 2018). According to NIH, the mean absolute bioavailability of nalbuphine is 81% and 83% for the 10 and 20 mg intramuscular doses, respectively, and 79% and 76% following 10 and 20 mg of subcutaneous nalbuphine (NCBI 2023p).

3.6.5 Hazard Identification

Nalbuphine is FDA-approved for human use. Nalbuphine HCl has low acute toxicity. It is ranked as category IV for acute oral and acute inhalation toxicity (Letco 2019). According to aggregated Globally Harmonized System of Classification and Labelling of Chemicals (GHS) information from five companies, The National Institutes of Health (NIH) has determined that nalbuphine is H302 (100%, Harmful if swallowed), H332 (40%, Harmful if inhaled), and H336 (20%, may cause drowsiness or dizziness). Symptoms of nalbuphine HCl induced toxicity include irregular

heartbeat and blood pressure, a variety of disorders of the central nervous system, nausea and vomiting, respiratory depression, and symptoms of dependence and withdrawal (Hospira 2019). Other adverse effects include sedation, sweaty and clammy conditions, dizziness, vertigo, dry mouth, and headache. Less than 1% of patients experience adverse effects in the central nervous, cardiovascular, gastrointestinal, respiratory, or dermatological systems or have other miscellaneous effects (speech, urinary urgency, blurred vision, rash, pruritus, allergic reactions).

3.6.5.1 Acute Toxicity

In dogs, the acute oral LD_{50} for nalbuphine is 1,100 mg/kg-bw, and intravenous LD_{50} is 140 mg/kg-bw (Table 7). Dust or powder may irritate eye tissue (Letco 2019).

3.6.5.2 Sublethal and Chronic Toxicity

In a reproductive and fertility study, Nalbuphine HCl administered to rats subcutaneously had a NOAEL of 56 mg/kg-bw/day, with no effects at the maximum dose (Hospira 2019). In embryo and fetal development studies, nalbuphine HCl given subcutaneously had a NOAEL of 100 mg/kg-bw/day in rats and 32 mg/kg-bw/day in rabbits (Hospira 2019). Fertility in male and female rats was unaffected by doses of Nubain up to 56 mg/kg-bw/day or 330 mg/m^2/day (Drugs.com 2023c). In a 24-month oral toxicity study in rats and a 19-month oral toxicity study in mice, nalbuphine HCl had a NOAEL of 200 mg/kg-bw/day (Hospira 2019). Nalbuphine HCl is not considered carcinogenic (Hospira 2019). In people, chronic use of opioids can cause a deficiency in androgen, resulting in symptoms of infertility, impotence, amenorrhea, and erectile dysfunction (Hospira 2019).

3.6.6 Dose-Response Assessment

3.6.6.1 Human Health Dose Response

The maximum recommended human dose for Nubain (nalbuphine HCl) given intramuscularly, subcutaneously, or intravenously is 160 mg/day, or 100 mg/m^2/day for a person weighing 60 kg (Drugs.com 2023c). Respiratory depression occurs at the usual adult Nubian dose of 10 mg for a 70 kg person (Drugs.com 2023c). In non-tolerant adults, the maximum single dose should be 20 mg, with a maximum daily dose of 160 mg (Drugs.com 2023c). For inducing anesthesia, the doses range from 0.3 mg/kg-bw to 3 mg/kg-bw intravenous over a 10 to 15-minute period (Drugs.com 2023c). Nubain is an opioid drug that can impair people's mental or physical abilities while under its influence (Drugs.com 2023c). A single subcutaneous dose of 72 mg of Nubain caused symptoms of sleepiness and mild dysphoria (a state of unease) in a study population of eight healthy adult humans (Drugs.com 2023c).

3.6.6.2 Ecological Dose Response

There is limited information on nalbuphine’s effects on wildlife. Rocky Mountain elk (*Cervus canadensis nelsoni*) responded similarly at three doses of nalbuphine (0.3, 1.6, and 3.0 mg/kg-bw) given by hand intramuscularly (Wolfe et al. 2014b). The elk showed transient sedation, and some of the animals had a diminished response to human approach. Intramuscular injection with

12.5 mg/kg-bw nalbuphine in Hispaniolan parrots (*Amazona ventralis*), increased pedal thermal withdrawal thresholds up to 3 hours (analgesic effect); the higher doses of 25 mg/kg-bw and 50 mg/kg-bw did not increase thermal thresholds (Sanchez-Migallon Guzman et al. 2011). The birds displayed normal behavior, and no adverse effects, including sedation, were observed. No nalbuphine toxicity data appears to be available for aquatic nontarget organisms.

3.7 Butorphanol Tartrate, Azaperone, and Medetomidine Hydrochloride (BAM™)

3.7.1 Chemical Description and Product Use

The patented sedative drug mixture BAM™ [US Pat. 7795263] consists of butorphanol tartrate, azaperone, and medetomidine HCl. Each drug was evaluated separately in Sections 3.2, 3.1, and 3.4, respectively. BAM provides short (5–10 minutes) induction times and quick reversal times when using the indicated reversal agent. BAM is potent in small quantities and used for a broad range of species, making it practical to remotely immobilize wildlife with a dart or any hypodermic injection delivery method. The drug is designed so that most North American cervids can be darted with 2 cc's or less. In most cases, BAM is reversed in five to ten minutes using naltrexone HCl and atipamezole when given intramuscularly (Harms et al. 2018, McDermott et al. 2020). WS uses BAM to immobilize wildlife for a variety of WDM activities. BAM was not used from FY11–FY15 but has been used since that data was analyzed.

3.7.2 Physical and Chemical Properties

BAM is commonly used as a premixed 6:4:3 ratio (volume/volume/volume) of butorphanol, azaperone, and medetomidine from respective source solutions (Wolfe et al. 2014a). The mixed solution is predominantly clear and contains a combination of the chemical properties of each of its components. BAM is sold as a a premixed solution containing 27.3 mg of butorphanol, 9.1 mg of azaperone, and 10.9 mg of medetomidine (Wedgewood Pharmacy 2024).

3.7.3 Environmental Fate

Limited or no information is available on the environmental fate processes and impact of BAM or the individual drug components. WS does not use I&E drugs frequently and at the same site, so the risk of environmental contamination is low.

3.7.4 Metabolism

The components of BAM are metabolized with a default withdrawal period of 30 days. The withdrawal period is likely shorter for white-tailed deer because laboratory analyses did not detect butorphanol, azaperone, medetomidine, or atipamezole in the liver or muscle of white-tailed deer after 11 days (W.R. Lance, unpublished data *in* (Wolfe et al. 2016a). In black bear, no tissue residues of butorphanol, azaperone, and medetomidine were detected 8 days post-injection (Wolfe et al. 2020). In raccoons immobilized with 0.016 mL/kg BAM (two raccoons were given an additional half-dose of BAM for a final dosage of 0.020 +/- 0.001 mL/kg), both butorphanol and

azaperone residues were detected in homogenized muscle, fat, liver, and kidney tissue 2, 4, and 6 days post-immobilization; medetomidine was not detected in the tissue (Johnson et al. 2023b).

3.7.5 Hazard Identification

BAM's toxicity arises as a result of its parts. As previously described, butorphanol tartrate, azaperone, and medetomidine HCl are moderately toxic. Common toxic signs associated with high doses of the mixture include sedation, tremors, cardiovascular effects, hypoventilation, coma, and death. In a recent study, 27% (4/15) of the raccoons immobilized with 0.016 mL/kg BAM (two raccoons were given an additional half-dose of BAM for a final dosage of 0.020 +/- 0.001 mL/kg) experienced hypoxemia below 70%. Seventy-two percent (13/18) of raccoons were given supplemental oxygen (Johnson et al. 2023a).

3.7.5.1 Acute Toxicity

See the acute toxicity of the individual drugs in this mixture (Sections 3.1.5.2, 3.2.5.2, and 3.4.5.2). Table 7 summarizes acute toxicity data.

3.7.5.2 Sublethal and Chronic Toxicity

See this compound's three drugs for sublethal and chronic toxicity (Sections 3.1.5.3, 3.2.5.3, and 3.4.5.3).

3.7.6 Dose-Response Assessment

3.7.6.1 Human Health Dose Response

The medetomidine and butorphanol components of BAM may severely affect human respiratory and cardiorespiratory function (see the hazard identification section for these drugs). The adverse health effects from exposure to opioids such as butorphanol can be reversed with naloxone in humans (Kreeger and Arnemo 2012). Atipamezole will reverse the effects of medetomidine in animals, but it is not approved for use in humans. However, human trials have been conducted with atipamezole reversal of dexmedetomidine without severe side effects (Pertovaara et al. 2005). Greenberg et al. (2018) recommend an intramuscular dose of 100 mg atipamezole for significant medetomidine exposure where medical aid is not available.

3.7.6.2 Ecological Dose Response

The ecological dose response is summarized under the individual drug components (Sections 3.1.5.2, 3.2.5.2, and 3.4.5.2). Table 7 presents information on medetomidine's toxicity to fish and aquatic invertebrates. No ecological dose response data is available for butorphanol and atipamezole.

3.8 Nalbuphine, Azaperone, and Medetomidine (NAM)

3.8.1 Chemical Description and Product Use

The patented sedative drug combination NAM (or NalMed-A; US Pat. 9339498B2) consists of nalbuphine, azaperone, and medetomidine (Wolfe et al. 2016b). These drugs were evaluated separately in Sections 3.5, 3.1, and 3.4, respectively. Governmental drug enforcement agencies do not regulate the drugs in NAM as controlled substances, but a veterinarian prescription is required.

The NAM combination uses a relatively small volumetric quantity suitable for accurate and safe delivery by remote dart or syringe pole (jab stick) (Wolfe et al. 2016a). The preferred relative weight ratio relationship of the pharmaceutically effective ingredients is 40 mg/mL of nalbuphine HCl, 10 mg/mL of azaperone tartrate, and 10 mg/mL of medetomidine HCl. This ratio was used in a study evaluating the use of NAM for raccoon immobilization (Johnson et al. 2023a). WS may use this combination of NAM to immobilize and sedate wildlife.

3.8.2 Physical and Chemical Properties

NAM is a premixed solution in a single vial to be used in the field. See the physical and chemical properties above for each drug in NAM.

3.8.3 Environmental Fate

Limited or no information on the environmental fate processes and impact of BAM and NAM or the individual drug components is currently available. WS does not frequently use I&E drugs; therefore, the risk of adverse environmental impacts is low.

3.8.4 Metabolism

The components of NAM are likely metabolized rapidly after recovery with a similar withdrawal time to BAM (Wolfe et al. 2016a). Nalbuphine HCl has a reportedly short elimination half-life in dogs (Pao et al. 2000). Azaperone is metabolized by the liver and rapidly eliminated (Plumb 2018). Mestorino et al. (2013) reported that azaperone was not detected in pigs even when euthanized 6 hours post-injection. The Food Animal Residue Avoidance Databank recommends 8 days post-injection as a withdrawal time for azaperone (*as cited in* (Wolfe et al. 2018)). Cook et al. (2016) reported that tissue residues for components of a similar immobilization combination (butorphanol, azaperone, and medetomidine) and the antagonists atipamezole and naltrexone were not detected 11 or 21 days post-injection in white-tailed deer (*O. virginianus*). Wolfe et al. (2018) reported that NAM tissue residues (0.01 mg/kg) were detected in liver and muscle tissue samples from elk euthanized within 40 min post-injection and one animal that died 12–24 hours post-injection but not in tissues from any of the animals euthanized at 3, 6, 14, 21, or 28 days post-injection. Wolfe et al. (2018) also reported that tissue residues for the antagonists naltrexone, atipamezole, and tolazoline were detected in the liver and muscle of the animal that died 12–24 hours post-injection. Only naltrexone was detected in the liver from the two elk euthanized on day 3 without antagonist residues detected thereafter. In raccoons immobilized with 0.018 mL/kg NAM (one raccoon was given an additional half-dose of NAM for a final dosage of 0.020 +/-0.0003 mL/kg), both nalbuphine and azaperone residues were detected in homogenized muscle, fat,

liver, and kidney tissue 2, 4, and 6 days post-immobilization; medetomidine was not detected in the tissue (Johnson et al. 2023b).

3.8.5 Hazard Identification

Nalbuphine HCl is a synthetic opioid agonist-antagonist widely used for wildlife capture as an analgesic and supplement to balance anesthesia. Medetomidine is a potent alpha-2 adrenoreceptor agonist with good sedative and analgesic properties. Azaperone is a neuroleptic in the butyrophenone class of tranquilizers (Wolfe et al. 2018). The synergistic NAM combination of opioid and alpha-2 agonist drug immobilization relies more heavily on a potent alpha-2 agonist rather than on a potent opioid. NAM combinations have low volume and reversible effects as well as the benefit of greatly diminished regulatory control and greater accessibility by nonveterinary field personnel (Wolfe et al. 2014b).

NAM is used for reversible chemical immobilization under field conditions in a variety of species, such as Rocky Mountain elk (Wolfe et al. 2014b) and black bears (*Ursus americanus*) (Wolfe et al. 2014b, Wolfe et al. 2016a). NAM has good quality sedation in American bison (*Bison bison*) with a relatively low delivery volume (0.8 mL/100 kg containing the mean dose of 0.4 mg/kg-bw nalbuphine, 0.08 mg/kg-bw medetomidine, and 0.08 mg/kg-bw azaperone) and rapid and smooth antagonism (Wolfe et al. 2017). Side effects include hypoxemia (low oxygen levels in the blood) in American bison and raccoons (Johnson et al. 2023a, Wolfe et al. 2017) and respiratory rate depression in American black bears (Wolfe et al. 2016a).

Nalbuphine HCl is related to the more-potent opioids (such as etorphine HCl, carfentanil citrate, and thiafentanil oxalate). However, nalbuphine HCl is not regulated by the US Drug Enforcement Administration (DEA 2023) because the human abuse potential with nalbuphine appears to be quite low (Wolfe et al. 2014b).

3.8.5.1 Acute Toxicity

Table 7 gives the acute toxicity data for nalbuphine, azaperone, and medetomidine HCl and the sections summarizing this data under the individual drugs.

3.8.5.2 Sublethal and Chronic Toxicity

See the sublethal and chronic toxicity sections under the individual drugs.

3.8.6 Dose-Response Assessment

3.8.6.1 Human Health Dose Response

The human health dose response for nalbuphine, azaperone, and medetomidine are discussed individually in this risk assessment.

3.8.6.2 Ecological Dose Response

In bison (*Bison bison*), hand injection of a mean dosage of 0.4 mg/kg-bw nalbuphine, 0.08 mg/kg-bw medetomidine, and 0.08 mg/kg-bw azaperone safely immobilized the animals (Wolfe et al. 2017). Bison had respiratory rates within normal limits, but researchers observed hypoxemia. Based on a study in American black bears, a recommended dose for immobilization is 0.44–0.88 mg nalbuphine/kg-bw, 0.11-0.22 mg azaperone/kg-bw, and 0.11-0.22 mg medetomidine/kg-bw (Wolfe et al. 2016a). As observed in other wildlife dosed with NAM, the bears had a depressed respiratory rate. In Rocky Mountain elk, successful immobilization was achieved at a concentration of 40 mg/mL nalbuphine, 10 mg/mL medetomidine, and 10 mg/mL azaperone dosed at 1.8–2.0 mL for darting elk (Wolfe et al. 2014b). There was a tendency towards hypoxemia in the elk. In raccoons immobilized with 0.018 mL/kg NAM (one raccoon was given an additional half-dose of NAM for a final dosage of 0.020 +/-0.0003 mL/kg), hypoxemia below 70% oxygen saturation was observed in 6% of study animals (Johnson et al. 2023b). See Table 7 for information on medetomidine's toxicity to fish and aquatic invertebrates; information is lacking for nalbuphine and azaperone toxicity in aquatic species.

3.9 Xylazine

3.9.1 Chemical Description and Product Use

Xylazine (CAS No. 7361-61-7; $C_{12}H_{17}ClN_2S$; chemical structure in Appendix 3) is an alpha-2 adrenergic agonist (the sedative works primarily on the alpha-2 adrenergic receptors in the brain and body) inhibiting the release of norepinephrine in the central and peripheral nervous system (Kreeger and Arnemo 2012). Alpha-2 adrenoceptor agonist drugs have central and peripheral nervous system effects. Central effects include sedation, analgesia, bradycardia, respiratory depression, mild hypotension, and a reduction in antidiuretic hormone. Peripheral effects include vasoconstriction, which can lead to hypertension and reduced gastrointestinal movements (Clarke and England 1989). Xylazine can be purchased as Rompun®, Cervizine™, and AnaSed®. The drug is supplied as a sterile solution. Each mL of solution contains 20 mg xylazine, 0.9 mg methylparaben, 0.1 mg propylparaben, and water for injection (AnaSed® 2022). Some brands are available in concentrations of either 20 mg/mL (for small animals) or 100 mg/mL (for large animals). The drug is used as a sedative that calms nervousness, irritability, and excitement, usually by depressing the central nervous system. Xylazine is commonly used with ketamine to produce relaxed anesthesia but can also be used alone to facilitate physical restraint. The two most commonly used ratios are a 2:1 and a 5:1 ketamine: xylazine mixture. WS often uses a ketamine and xylazine combination as an alternative to Telazol (USDA APHIS 2019b).

When combined with ketamine, xylazine helps minimize muscle tension, reducing heat production but can lower body temperatures when working in cold conditions (Fowler and Miller 1999). Animals sedated with xylazine are usually responsive to stimuli; therefore, individuals handling these animals should minimize sight, sound, and touch (USDA APHIS 2019b). WS uses xylazine alone to chemically restrain wildlife, such as white-tailed deer, or in combination with other approved program drugs. WS administers the drug using hand injection with a hand syringe or syringe pole and remote darting.

3.9.2 Physical and Chemical Properties

Xylazine is a white or almost white crystalline substance (FAO 1997). It has a molar mass of 220.334 g/mol (NCBI 2023d) and a melting point of 165–168°C (USDA AMS 2002). It is soluble in methanol (50 mg/mL) but practically insoluble in water (FAO 1997, Sigma-Aldrich Corporation 2002b, USDA AMS 2002). Xylazine is stable under normal storage conditions but is incompatible with strong oxidizing agents (Cayman Chemical 2022b). Xylazine is a highly lipid-soluble organic base (log K_{ow} 2.8) (Le Vet BV 2017, NCBI 2023d).

3.9.3 Environmental Fate

Information on the environmental fate of xylazine is limited in the scientific literature. In laboratory studies, Choi et al. (2014) found xylazine to be mobile in soils with the potential to leach. The rate of degradation and dissipation within artificial soil environments was slow, which may indicate an ability to accumulate (Choi et al. 2014, Pugajeva et al. 2017). The DT_{50} values ranged from 92.4–138.6 in amended and control soils (Choi et al. 2014). Xylazine was found in rivers on the Iberian Peninsula (Fàbrega et al. 2013). Its detection in waters on the Peninsula may be due to the colocation of the manufacturer (Pugajeva et al. 2017).

3.9.4 Metabolism

Due to xylazine's lipid-soluble organic base, it can be found in a high concentration in the kidneys, liver, central nervous system, and diaphragm within minutes of intravenous injection. Xylazine is metabolized rapidly, with approximately 70% of the metabolites eliminated in the urine and 30% in enteric elimination (Le Vet BV 2017). The effects of xylazine diminish within 15-90 minutes after administration but are not completely reversed until it is fully metabolized (2–36 hours) (EMEA 2002b) *in* (USDA AMS 2002).

In one study, when horses, cattle, sheep, and dogs were administered the drug intravenously, xylazine decreased to undetectable levels within a few hours. Metabolism of xylazine was fastest in sheep and dogs and slowest in horses (FAO 1997). Half-lives were the same when horses, cattle, sheep, and dogs received xylazine intramuscularly, and the maximum values were reached within 15 minutes for horses, sheep, and dogs. Pharmacokinetic parameters could not be determined in cattle due to low concentrations of the drug in bovine plasma (FAO 1997). The biological half-live for xylazine ranges from 1–58 days (*as cited in* (USDA AMS 2019).

3.9.5 Hazard Identification

Cattle, horses, dogs, and cats are the target species for xylazine; however, this drug is also used for carrying out WDM activities. Xylazine is frequently used with drugs such as ketamine to enhance sedative and analgesic qualities. Adverse effects from xylazine include respiratory depression, bradycardia, hypotension, reversible arrhythmia, dry mouth, and hyperglycemia, with considerable variation of effects among species. Thermoregulation also can be impacted (Le Vet BV 2017). Xylazine has caused premature parturition in cats and cattle (Le Vet BV 2017).

Because xylazine affects the central nervous system, it can produce complications such as bloat. Bloat occurs most frequently in ruminants and can be fatal (USDA APHIS 2019b).

Strongly sedated animals are drowsy and will walk with an uncoordinated gait, which can lead to injury or death if physical hazards such as steep ledges or water are nearby. Sedated animals are also more susceptible to predators. Xylazine usually makes an animal's breathing slower and shallower, which can be a concern if an animal has increased oxygen demands from being captured (USDA APHIS 2019b).

Cardiac arrhythmia, hypotension, seizures, central nervous system, and respiratory depression may occur in the event of an accidental overdose of xylazine (Le Vet BV 2017). If breathing is too shallow or slow, xylazine can be antagonized by alpha-2 adrenergic antagonists such as yohimbine or tolazoline (Le Vet BV 2017, USDA APHIS 2019b).

The SDS indicates xylazine hydrochloride is a skin and eye irritant and can cause serious eye damage (Sigma-Aldrich Corporation 2023); however, xylazine is not a skin or eye irritant (Sigma-Aldrich Corporation 2022).

3.9.5.1 Acute Toxicity

Acute oral toxicity of xylazine in rats (Table 7) was determined to be moderately toxic (IPCS 2015). In one study involving adult dogs and cats receiving intramuscular or intravenous administration of xylazine at 10 times the recommended therapeutic dose, one cat out of three receiving the intravenous dose died, and two dogs out of four receiving the intramuscular dose died. The remaining animals experienced convulsions, unconsciousness, and respiratory depression but recovered with no apparent long-term effects. The authors of this study concluded that xylazine was slightly toxic (*as cited in* (IPCS 2015)).

3.9.5.2 Sublethal and Chronic Toxicity

One study concluded that beagles receiving oral administration dosages of 0, 10, 30, or 100 mg xylazine (0, 0.3, 0.9, or 3 mg/kg-bw) for 13 weeks had no treatment-related adverse effects (*as cited in* (IPCS 2015)). However, xylazine concentration in the feed was not analyzed for confirmation of content, homogeneity, and stability. The Joint FAO/WHO Expert Committee on Food Additives has retained a NOEL of 3 mg/kg-bw from this study (EMEA 2002b).

In a study on embryotoxicity/teratogenicity that does meet scientific standards, pregnant rats receiving xylazine (0, 1, 4, or 16 mg/kg-bw per day) showed treatment-related maternal effects, including underactivity, ataxia, and slightly reduced body weight gain in the high dose (16 mg/kg bw) group only. Subsequently, the NOAEL in this study was 4 mg/kg-bw. No teratogenic effects were observed (*as cited in* (IPCS 2015)). Xylidine, a metabolite of xylazine, may be carcinogenic to humans as a result of multiple-dose studies in animals (IARC 1993).

Studies on neurotoxic effects were not identified in the literature. Endocrine effects have been observed in livestock (Greene and Thurmon 1988); however, xylazine is not on the USEPA endocrine disruptor list for humans.

3.9.6 Dose-Response Assessment

3.9.6.1 Human Health Dose Response

Xylazine is not approved for use in humans but has recently come under DEA concern regarding an increasingly recognized potential for abuse. Accidental or intentional ingestion or injection of xylazine by humans is hazardous (Hoffmann et al. 2001). It includes symptoms such as central nervous system depression, respiratory depression, hypo- and hypertension, bradycardia, tachycardia, ventricular arrhythmias, and transient hyperglycemia. Inhalation of xylazine can cause similar symptoms (Yong-Fu et al. 2013). The lowest published toxic dose of xylazine in humans is 14 mg/kg-bw (intramuscular) (NCBI 2023d). Oral doses of 170 µg/kg-bw have been reported to produce initial pharmacological effects, while acute toxic effects start at 700 µg/kg-bw (EMEA 2002b). Fatal doses recorded have been as low as 40 mg, while some individuals have survived exposures above 2,400 mg (Ruiz-Colón et al. 2014). A case report described one nearly fatal human suicidal overdose through drinking 4 mL of xylazine (Gallanosa 1981). Accidental fatal overdoses associated with xylazine have occurred (Reyes et al. 2012). The use of xylazine in illicit street drugs, including fentanyl, cocaine, and heroin, has increased in the United States in recent years leading to an increased prevalence of xylazine in fatal opioid overdoses (Alexander et al. 2022).

3.9.6.2 Ecological Dose Response

Dose and effect information for vertebrate terrestrial nontarget organisms is limited to studies on domestic animals (see Hazard Identification section). Xylazine's effects are of short duration, which is preferred particularly in field anesthesia or sedation when rapid recovery is needed (West et al. 2007).

Information on aquatic effects from xylazine exposure is limited. In one study using the freshwater cladoceran, *Daphnia magna,* the acute 48-hour median effective concentration (EC_{50}) was >18 mg/L (University of Hertfordshire 2018a). A 20 mg/kg dose of xylazine injected into the hemolymph of blue crabs (*Callinectes sapidus*) gave short-term (under 10 minutes) light anesthesia (Quesada et al. 2011). The criteria for light anesthesia were a loss of righting reflex and defensive response, slow and occasional limb movement, and slow limb withdrawal when pressed with forceps. Bradycardia was an observed significant side effect, but the crab's heart rate approached baseline within 10 minutes. Xylazine is not recommended for use in salmonids based on a study where the lowest effective dose (100 mg/kg-bw) produced apnea during induction and recovery and caused convulsion activity in brown and rainbow trout (Oswald 1978).

4 TRANQUILIZERS

WS uses tranquilizers to calm an animal and reduce fear or sensitivity to stress. They are used with anesthetic, narcotic, or sedative drugs to offset side effects and facilitate the transport and holding of wild animals (Kock et al. 2012).

Animals that need to be relocated or released may need to be chemically immobilized for safe handling. Immobilizing drugs such as ketamine, xylazine, and Telazol have been used safely on animals tranquilized with acepromazine and propionylpromazine hydrochloride (PPZH). APHIS no longer uses PPZH; thus, PPZH is not covered in this assessment. The use of tranquilizer drugs by WS employees is restricted to individuals who have completed specific training requirements (USDA APHIS 2019a).

4.1 Acepromazine

4.1.1 Chemical Description and Product Use

Acepromazine (CAS No. 61-00-7; $C_{19}H_{22}N_2OS$; chemical structure in Appendix 3), a phenothiazine derivative, is a tranquilizer that acts as a dopamine receptor antagonist in the central nervous system, causing sedation, muscle relaxation, and reduction in spontaneous activity. This drug was first used as an antipsychotic agent in humans but is now most frequently used as a sedative to calm anxious animals (NCBI 2023n). Acepromazine maleate is used in veterinary medicine to tranquilize dogs, cats, and horses (Forney 2013). The drug is also occasionally used to calm trapped canids (West et al. 2007). By itself, acepromazine is not an effective immobilizing agent (West et al. 2007), but the combination of acepromazine maleate and medetomidine HCl is safe and effective for use on wildlife species (Wolfe et al. 2014a). WS combines acepromazine with other drugs, such as BAM and NAM, to immobilize wildlife species. Field applications are through hand injection or with a syringe pole.

4.1.2 Physical and Chemical Properties

Acepromazine is used in combination with benzyl alcohol to create an injectable solution (Bayer Healthcare 2014). Its molecular weight is 326.46 g/mol (NCBI 2023n). The solution is yellow and odorless with a density of 0.99 g/cm^3 at 20°C and is water soluble with a predicted solubility of 0.0098 mg/mL (Bayer Healthcare 2014, DrugBank 2021c). Its flash point is >93.0°C, and its boiling point is 110°C. The predicted log K_{ow} for acepromazine is 4.32 and 3.49, indicating the drug is lipophilic (HMDB 2019e). This solution is stable under normal conditions (Boehringer Ingelheim 2019).

4.1.3 Environmental Fate

Information on acepromazine's environmental fate is limited in the scientific literature. In laboratory studies, acepromazine strongly adsorbs to soil (K_{oc} 52,138) but quickly dissipates or degrades, with DT_{50} values of 4.1–7.0 days in untreated and amended soils (Choi et al. 2014).

The preferential binding of acepromazine to soil suggests it is unlikely to leach or runoff and impact surface or groundwater (Choi et al. 2014).

4.1.4 Metabolism

Acepromazine may be administered subcutaneously, intravenously, orally (Bayer Healthcare 2015), or intramuscularly (Forney 2013). The effects of this drug will last from 1 to 4 hours and depend upon the dosage and species. Acepromazine is metabolized by the liver and excreted in the urine (Forney 2013). A major metabolite observed in humans is 2-(1-hydroxyethyl)promazine (Elliott and Hale 1999), and a major metabolite observed in the urine and plasma of horses is hydroxyethylpromazine sulphoxide (Wieder et al. 2012). In horses, the metabolism rates appear to depend upon the route of administration. In a recent study assessing intravenous administration of acepromazine, the drug was detectable in plasma for 3 hours but barely detectable in urine at any point, indicating rapid metabolism. The greatest conversion of acepromazine occurred in the first 10 minutes following intravenous administration (Schneiders et al. 2012). In contrast, Chou et al. (1998) reported that acepromazine could be detected for up to 48 hours in serum after intramuscular administration. The elimination half-life of 30 mg acepromazine intravenously administered to horses was 2.0 ± 0.5 hours (Schneiders et al. 2012). In another study, horses intravenously injected with acepromazine demonstrated an elimination half-life of approximately 3 hours (Ballard et al. 1982).

4.1.5 Hazard Identification

Acepromazine, combined with other drugs, has been used in various wildlife species but is routinely used in dogs, cats, and horses. It acts rapidly in dosed animals with a low order of toxicity (Bayer Healthcare 2015). Side effects include hypotension due to decreased vasomotor tone, respiratory rates, thermoregulatory abilities, and bradycardia. Acepromazine also causes a dose-dependent decrease in hematocrit in dogs and horses (Forney 2013). In horses, acepromazine can cause penile prolapse at doses of 0.01 mg/kg-bw and higher (Ballard et al. 1982). Phenothiazines such as acepromazine may increase the toxicity of organophosphates (Bayer Healthcare 2015). Acepromazine should not be used in animals that are pregnant, dehydrated, anemic, or in shock, or have liver disease, heart disease, epilepsy, injury, or debilitation. An overdose of acepromazine results in excessive sedation, slowed respiration and heart rate, pale gums, unsteady gait, poor coordination, or an inability to stand. In some cases, unconsciousness, seizures, and death may occur (Forney 2013).

Acepromazine decreases an animal's ability to thermoregulate; thus, temperature extremes and inclement weather conditions are hazardous to tranquilized animals. Acepromazine should not be used in high ambient temperatures (West et al. 2007).

4.1.5.1 Acute Toxicity

In animal toxicity studies, acepromazine has a low-order acute toxicity with an oral LD_{50} of 256.8 mg/kg-bw in laboratory house mice and 400 mg/kg-bw in laboratory rats (Bayer Healthcare 2015). The intravenous LD_{50} in mice and rats was 65 mg/kg-bw and 95 mg/kg-bw (Table 8).

Table 8. Acute toxicity values for acepromazine and its components.

Chemical	Test Species	Test Type	LD_{50}	Reference
Acepromazine maleate	House Mouse*	Acute oral	256.8 mg/kg-bw	(Bayer Healthcare 2015)
Acepromazine maleate	Brown Rat*	Acute oral	400 mg/kg-bw	(Santa Cruz Biotechnology 2007)
Acepromazine maleate	Brown Rat*	Intravenous	95 mg/kg-bw	(Santa Cruz Biotechnology 2007)
Acepromazine maleate	House Mouse*	Intravenous	65 mg/kg-bw	(Santa Cruz Biotechnology 2007)
Benzyl Alcohol (component)	Brown Rat*	Acute oral	1230–3100 mg/kg-bw	(Bayer Healthcare 2014)

* Domestic laboratory strains.

4.1.5.2 Sublethal and Chronic Toxicity

In animal studies, acepromazine shows low-order chronic toxicity (Bayer Healthcare 2015). Chronic toxicity tests of acepromazine maleate in rats indicated no deleterious effects on renal or hepatic function or hemopoietic activity (Bayer Healthcare 2015). No adverse effects were observed in a study where beagles were treated for six months with daily doses of 20 to 40 mg/kg-bw (Bayer Healthcare 2015). In another study, with increasing doses of acepromazine maleate up to a level of 220 mg/kg-bw daily, beagles showed some signs of pulmonary edema and hyperemia of internal organs, but no animals died (Bayer Healthcare 2015).

4.1.6 Dose-Response Assessment

4.1.6.1 Human Health Dose Response

Acepromazine was halted for human use in the 1950s due to side effects and a lack of efficacy (Collard and Maggs 1958). As a result, acepromazine is now used exclusively in veterinary medicine, and human toxicokinetic data is limited. Bayer (2014) reports that acute oral toxicity of acepromazine maleate in humans has a lowest dose that will cause death (LD_{Lo}) of 496 mg/kg, while Algren and Ashworth (2015) discuss in more detail the toxicokinetic data associated with an intentional acepromazine overdose. Following the ingestion of 950 mg of acepromazine, an adult woman experienced increased drowsiness, tachycardia, and hypotension. Her 1-hour post-ingestion plasma acepromazine level was 63 ng/mL, which decreased to 8.9 ng/mL 8 hours post-ingestion. The elimination half-life was 2.95 hours. Central nervous system and respiratory depression resolved within 8 hours of exposure, which is similar to previous case studies (Algren and Ashworth 2015).

4.1.6.2 Ecological Dose Response

Ecological dose response data for acepromazine and terrestrial nontarget vertebrates is limited to the previously discussed studies using laboratory and domestic animals.

No effects data appear to be available testing the toxicity of acepromazine to fish and aquatic invertebrates. During a 72-hour test, the benzyl alcohol component of acepromazine maleate resulted in a median inhibition concentration (IC_{50}) greater than 100 mg/L for algae (Bayer Healthcare 2014). During a 0.5-hour test, exposure to the benzyl alcohol component of acepromazine maleate on *Photobacterium phosphoreum,* a bioluminescent bacterium, resulted in an EC_{50} of 71.4 mg/L (Bayer Healthcare 2014).

5 ACCESSORY DRUGS

All of the accessory drugs, except over-the-counter antibiotics (Appendix 1), that WS uses are to reduce side effects from sedatives or reverse sedation. Alpha-2 antagonist drugs displace alpha-2 agonist sedatives from receptor sites in the brain, reversing sedation effects (Kock et al. 2012). These drugs are used to speed recovery and are most effective if administered intravenously. They can be injected into the muscle but metabolize more slowly (USDA APHIS 2019b).

The toxicity of accessory drugs to the target species, people, and nontarget species is a primary concern. Table 9 gives toxicity values for most accessory drugs and substances (for atropine, see Table 12). These are discussed in the following sections for each drug. Toxicity data were available for the atipamezole, doxapram HCl, tolazoline HCl, and yohimbine.

Table 9. Acute toxicity values for immobilization recovery drugs.

Chemical[1]	Test Species	Test Type	LD_{50} Test Result	Reference
Atipamezole	Brown Rat*	Subcutaneous	44 mg/kg-bw	(Bahri 2008, Zoetis 2017d)
Doxapram HCl	Brown Rat*	Acute oral	261 mg/kg-bw	(Boehringer Ingelheim 2018)
Doxapram HCl	House Mouse*	Acute oral	270 mg/kg-bw	(EMEA 1999)
Doxapram HCl	Dog	Acute oral	150 mg/kg-bw	(EMEA 1999)
Doxapram HCl	House Mouse*	Intravenous	75 mg/kg-bw	(Ward et al. 1968)
Tolazoline HCl	Brown Rat*	Acute oral	1200 mg/kg-bw	(Akorn Animal Health 2015;2018a)
Tolazoline HCl	House Mouse*	Acute oral	400 mg/kg-bw	(Akorn Animal Health 2018a)
Tolazoline	House Mouse*	Intraperitoneal	160 mg/kg-bw	(NCBI 2023e)
Tolazoline	House Mouse*	Intravenous	40 mg/kg-bw	(NCBI 2023e)
Naltrexone	House Mouse*	Acute oral	1100-1500 mg/kg-bw	(NCBI 2023y)
Naltrexone	Brown Rat*	Acute oral	1450 mg/kg-bw	(NCBI 2023y)
Naltrexone	Guinea Pig	Acute oral	1490 mg/kg-bw	(NCBI 2023y)
Naltrexone	House Mouse*	Subcutaneous	551 mg/kg-bw	(American Society of Health-System Pharmacists 2017)
Naltrexone	House Mouse*	Intravenous	180 mg/kg-bw	(American Society of Health-System Pharmacists 2017)
Yohimbine HCl	House Mouse*	Acute oral	43 mg/kg-bw	*As cited in* (IPCS 1992)
Yohimbine HCl	House Mouse*	Subcutaneous	20 mg/kg-bw	(IPCS 1992)
Yohimbine HCl	Brown Rat*	Intraperitoneal	55 mg/kg-bw	(Cayman Chemical 2020)
Yohimbine HCl	House Mouse*	Intraperitoneal	45 mg/kg-bw	(Cayman Chemical 2020)

* Domestic laboratory strains.
[1]Atropine acute toxicity values can be found in Table 12.

5.1 Atipamezole

5.1.1 Chemical Description and Product Use

Atipamezole HCl (CAS No. 104075-48-1; $C_{14}H_{17}ClN_2$; chemical structure in Appendix 3) has a high affinity for alpha-2 receptors in humans and rodents (Bahri 2008). Atipamezole is approved by the FDA to reverse the sedative and analgesic effects of medetomidine HCl and dexmedetomidine HCl in dogs (Bahri 2008) but is also commonly used in other species (West et al. 2007). It is 200−300 times more selective for the alpha-2 receptor than yohimbine, which provides a more rapid reversal of anesthesia. Atipamezole was developed in conjunction with medetomidine (alpha-2 agonist) and, more recently, dexmedetomidine, but it is also administered to reverse the effects of xylazine (University of Colorado 2012). In the United States, atipamezole is sold under the trade name Antisedan®. Each mL of the drug contains 5.0 mg atipamezole HCl, 1.0 mg methylparaben, 8.5 mg sodium chloride, and water for injection (Antisedan® 2012). WS intends to administer atipamezole HCl intravenously or intramuscularly by hand with a syringe needle to reverse the effect of sedation by medetomidine, dexmedetomidine, and xylazine in wildlife (USDA APHIS 2019b). Atipamezole works more efficiently through intravenous injection but can be injected intramuscularly, although it will take longer to take effect (USDA APHIS 2019b).

5.1.2 Physical and Chemical Properties

Atipamezole is a crystalline solid with a 248.75 g/mol molecular weight. Atipamezole is soluble in organic solvents such as ethanol, dimethyl sulfoxide, and dimethyl formamide. It is stable under normal storage conditions (Cayman Chemical 2022a). Its predicted log K_{ow} is 3.32 and 2.95 (HMDB 2019b).

5.1.3 Environmental Fate

Information concerning the environmental fate of atipamezole in the scientific literature is limited. The environmental fate of atipamezole under WS use is undetermined.

5.1.4 Metabolism

Atipamezole is rapidly absorbed following intramuscular injection. The onset of arousal usually occurs within 5 to 10 minutes after injection in most species, depending on the depth and duration of sedation (Jalanka and Roeken 1990, Vähä-Vahe 1990, Zoetis 2020). The elimination half-life is 1.3 hours in rats (Bahri 2008) and approximately 3 hours in dogs (Bahri 2008, Zoetis 2020). In raccoons, residues of atipamezole were detected in homogenized muscle, fat, liver, and kidney tissue 2, 4, and 6 days post-administration of a concentration of 5.0 mg atipamezole per 1.0 mg medetomidine. Atipamezole undergoes extensive hepatic biotransformation, and metabolites are excreted primarily in the urine (Bahri 2008, Zoetis 2020).

5.1.5 Hazard Identification

Atipamezole is a weak lipophilic base. When intramuscular administration occurs, absorption is rapid (Bahri 2008). Atipamezole displaces alpha-2 adrenergic agonist drugs and rapidly blocks or reverses sedative and analgesic drug effects (Bahri 2008). In dexmedetomidine-treated dogs, 57% could stand after 5 minutes, and 96% stood after 15 minutes. Dogs treated with medetomidine had similar responses (Zoetis 2020). In medetomidine-treated cats, the median arousal time was 5 minutes, and the walking time was 10 minutes (Vähä-Vahe 1990). Atipamezole is highly selective, minimizing undesirable effects (Bahri 2008). Atipamezole is well-tolerated in healthy dogs receiving doses 10 times the recommended dose or repeated doses at one, three, and five times the recommended dose (Zoetis 2020). Atipamezole is not considered a carcinogen (Zoetis 2017d).

5.1.5.1 Acute Toxicity

Acute toxicity data is limited for atipamezole. The subcutaneous LD_{50} in rats is 44 mg/kg-bw (Table 9). Atipamezole is well-tolerated in healthy dogs receiving 10 times the recommended dose or repeated doses at one, three, and five times the recommended dose (Zoetis 2020).

5.1.5.2 Sublethal and Chronic Toxicity

Information on the sublethal and chronic effects of atipamezole is limited. The finding that dogs tolerated receiving 10 times the recommended dose of atipamezole suggests minimal chronic toxicity potential (Pfizer 2016).

5.1.6 Dose-Response Assessment

5.1.6.1 Human Health Dose Response

Atipamezole has a wide safety margin in humans, with doses up to 100 mg administered intravenously without adverse effects (Jalanka and Roeken 1990, Pertovaara et al. 2005). The elimination half-life in humans receiving atipamezole intravenously is 1.7−2.0 hours. Cardiovascular or subjective side effects were not reported at doses up to 30 mg (Pertovaara et al. 2005). Motor restlessness, increased blood pressure and salivation, sweating, shivering, and cold hands were reported after a dose of 100 mg, but not with smaller doses. The highest atipamezole dose (100 mg) also increased systolic and diastolic blood pressure and plasma norepinephrine concentration, while lower doses had no significant effects (Karhuvaara et al. 1990).

5.1.6.2 Ecological Dose Response

We were unable to find information to evaluate the ecological dose response. Atipamezole is not classified as environmentally hazardous (Zoetis 2017d).

5.2 Atropine

5.2.1 Chemical Description and Product Use

Atropine (CAS No. 51-55-8; $C_{17}H_{23}NO_3$; chemical structure in Appendix 3) is an alkaloid produced by several plant families, including plants in the Solanaceae family. Atropine is manufactured usually by extraction from solanaceous plants, deadly nightshade (*Atropa belladonna*), Jimsonweed (*Datura stramonium*), and corkwood (*Duboisia myoporoides*) or through synthetic processes (IPCS 2002).

Atropine is an anticholinergic agent that blocks the neurotransmitter acetylcholine in the nervous system. In veterinary medicine, atropine sulfate (anhydrous, CAS No. 55-48-1; $C_{34}H_{48}N_2O_{10}S$; chemical structure in Appendix 3) is given to reduce salivary and airway secretions during general anesthesia (NCBI 2023r). For example, ketamine HCl is an anesthetic agent commonly used on wild carnivores (Acosta-Jamett et al. 2010), but side effects include excessive salivation, among other effects (Riviere and Papich 2017). In animals, dosages of atropine sulfate are mostly within the range of 0.01 mg/kg to 0.05 mg/kg (West et al. 2007). In several avian species, 0.01–0.02 mg/kg-bw atropine sulfate may be given as a pre-anesthetic intramuscularly or intravenously. In pigs and rabbits, 0.04 mg/kg-bw is used, and 0.05 mg/kg-bw is used in laboratory rats, mice, gerbils (*Meriones unguiculatus*) and hamsters (*Mesocricetus auratus*) (West et al. 2007). Table 10 summarizes recommended atropine sulfate dosages in several animals. WS would use atropine sulfate as an intramuscular injection by hand syringe and needle to reduce salivation and airway secretions and increase the heart rate in xylazine-immobilized animals.

Table 10. Recommended atropine sulfate dosages for animals.

Animal	Dose (mg/kg-bw)	Injection Type	Notes
Crocodiles, alligators, most avian spp.	0.01-0.02	Intramuscular	
Most avian species	0.01-0.02	Intravenous	
Bear	0.02	Intramuscular	
Otter	0.02	Intramuscular	
Domestic ferret	0.02-0.05		may be given as a premedication to maintain the heart rate and decrease secretions
Common Ostrich (*Struthio camelus*)	0.035		
Pig	0.04	Intramuscular	
Rabbit, viverrids, procyonids	0.04	Intramuscular	
Avian species	0.04-0.1	Intramuscular, intravenous	bradycardia
Rat, mouse, gerbil, hamster	0.05	Intramuscular	In rabbits, 0.2-2.0 mg/kg briefly caused moderate tachycardia

Reference: (West et al. 2007)

5.2.2 Physical and Chemical Properties

Atropine is a colorless or white crystalline powder and odorless with a molecular weight of 289.4 g/mol (NCBI 2023r). It has a melting point of 118.5°C (NCBI 2023r). Atropine is not volatile, with

a low vapor pressure of $2.59x10^{-3}$ mPa at 20°C (University of Hertfordshire 2018b) and a Henry's Law Constant of $2.73x10^{-13}$ atm-m^3/mole at 25°C (NCBI 2023r). The pH of atropine is alkaline (NCBI 2023r). Atropine has a log K_{ow} of 1.83 at 25°C (relatively low lipophilicity) (NCBI 2023r). Atropine is available in 0.5 and 2.0 mg/mL concentrations (USDA APHIS 2019b).

Atropine sulfate anhydrous is a colorless crystal or white crystalline powder that is odorless and very bitter (IPCS 2002) with a molecular weight of 676.83 g/mol. It has a melting point of 190 to 194°C (IPCS 2002). Atropine sulfate has the same vapor pressure, Henry's Law Constant, and log K_{ow} as atropine (NCBI 2023r).

5.2.3 Environmental Fate

Literature on the fate of atropine and atropine sulfate in the air, soil, and water is limited. Atropine is nonvolatile, indicating movement into the air during administration or through respiration from a treated animal is not a pathway. Atropine is soluble in water. Although atropine is photosensitive, atropine sulfate is slowly degraded by light (NCBI 2023r). The log K_{ow} and pK_a values for atropine suggest it would be mobile in plant xylem (Jandrić et al. 2013). Some evidence of atropine uptake in plants and binding to soil has been found. In a greenhouse study, researchers measured the uptake of atropine and its transformation products in soil by wheat (Jandrić et al. 2013). They applied 0.1 mg/kg ^{14}C-atropine or 0.2 mg/kg ^{14}C-atropine to soil. Fifteen days after planting, roots and leaves had measurable amounts of atropine or its transformation products. Atropine (labeled and unlabeled) was detected in the leaves, stems, roots, and seeds, with the highest percentage detected in the leaves and the lowest in the seeds, likely due to the amounts of water moving into the leaves through transpiration (Jandrić et al. 2013). In the same paper, researchers reported atropine in soil leachate 30 days after planting, but the amount decreased after 60 days, likely due to atropine binding to soil (Jandrić et al. 2013). They further measured the amount of ^{14}C-atropine and transformation products bound to soil 30 days after planting and determined 60 percent was bound to soil (Jandrić et al. 2013). Bacteria, including strains of *Corynebacterium* and *Pseudomonas* commonly found in nature, can break down atropine in as little as 48 hours of incubation at 30°C (Kedzia et al. 1961). Several bacterial species contain esterase, which breaks down atropine into tropinol and tropic acid (Jandrić et al. 2013).

5.2.4 Metabolism

In the human body, hepatic metabolism eliminates approximately half of a dose of atropine, with the remainder of atropine and atropine sulfate excreted unchanged in the urine (Kalser 1971, Van Der Meer et al. 1986). After an intramuscular injection of 2 mg of 2,4-^{14}C-atropine into one male, 77–94% of the dose was excreted in urine within 24 hours, and at least one-third remained unchanged (Kalser 1971). In another paper, the percent of atropine excreted in parent form was relatively high (the range of 44–69%) (Jjemba 2006). One human male (weight and age not given), when given a dose of 2 mg of atropine sulfate and 100 uCi [^{3}H]-atropine sulfate, excreted in his urine noratropine, atropine-N-oxide, tropine, and tropic acid, all metabolites of atropine, with 50% of the dose excreted unchanged (Van Der Meer et al. 1986). Atropine half-life is approximately 4 hours (NCBI 2023r).

Intraperitoneal injection of ^{3}H-atropine into rats at doses ranging between 1.5 mg/kg-bw and 10 mg/kg-bw resulted in half-lives of 40 to 46 minutes in plasma, 52 to 56 minutes in the heart, 50 to 58 minutes in the kidney, 71 to 82 minutes in the brain, and 97 to 106 minutes in fat tissue (Harrison et al. 1974).

5.2.5 Hazard Identification

Atropine and atropine sulfate interacts with the nervous system, competing with acetylcholine for binding sites on the muscarinic receptors. Atropine and atropine sulfate is used to resolve neurotoxicity from organophosphate insecticides and other toxic nerve agents. However, atropine toxicity can cause nervous system effects such as hyperactivity, hallucinations, and hypersensitivity (IPCS 2002). Toxic amounts of atropine can cause drowsiness, stupor, convulsions, and coma in humans (NCBI 2023r). Effects at 0.5 mg atropine were dry mouth and sweat inhibition (NCBI 2023r). Additional effects at 1 mg and 2 mg included increased heart rate and pupil dilation. At 5 mg, all the above symptoms were observed, with difficulty in speaking, swallowing, urinating, restlessness, headache, and fatigue. At 10 mg or greater doses, symptoms include blurred vision, flushed, hot, dry skin, hallucinations, delirium, and coma (NCBI 2023r). The eyes of treated animals may be sensitive to light from pupil dilation.

Toxic effects in humans exposed to atropine and atropine sulfate have been seen following oral, intramuscular, intravenous, and ocular routes (Table 11). In humans, pupil dilation and hallucinations or distorted perception were observed at intramuscular doses of 0.001 mg/kg-bw and 0.175 mg/kg-bw atropine, respectively (Table 11).

An occupational exposure incident was illustrated by a 28-year-old respiratory therapist who administered atropine sulfate ten times over a 24-hour period (NCBI 2023r). She experienced anxiety, palpitations, dry mouth, dizziness, and blurred vision, and she was hot and flushed. She recovered in 48 hours. Of 240 clinical evaluations of 268 children exposed to doses up to 17-fold higher than standard doses of atropine for their age from accidental misuse of automatic atropine injectors, none experienced mortality or life-threatening complications (Amitai et al. 1992). Serum atropine levels were 6.2 to 61 nanograms (ng)/mL, much higher than that observed after therapeutic doses (Amitai et al. 1992). Deaths followed ocular applications of 1.6 and 2 mg (Heath 1950, Morton 1939) and oral doses of 100 mg (Legroux, 1962 (in French) (IPCS 2002)) in three-year-old children. A 6-year-old boy ingested a 200 mg atropine sulfate solution and developed symptoms, including agitation, vomiting, and fever. The child received treatment and was released from the hospital after four days but continued to experience bilateral mydriasis for a week (Aliyev et al. 2015). Atropine sulfate levels in the blood 11 and 13 hours after admission were 1.93 µg/mL and below the limit of quantification, respectively. Tables 11a-b provide toxicity doses and effects of atropine and atropine sulfate in humans.

Table 11a. Toxicity doses and effects of atropine in humans: reported lowest dose causing a toxic effect (TDLo) and reported lowest dose causing lethality (LDLo).

Species	Test type	Dose	Effect	Reference
Human	TDLo IM	0.001 mg/kg-bw	Pupil dilation	(NCBI 2023r)
Human	TDLo Oral	0.033 mg/kg-bw	Visual field changes, muscle weakness	(NCBI 2023r)

Man	TDLo IM	0.175 mg/kg-bw	Hallucinations, distorted perceptions	(NCBI 2023r)
Man	TDLo IV	0.014 mg/kg-bw	Headache, blood pressure elevation	(NCBI 2023r)
Man	LDLo (unreported)	0.143 mg/kg-bw		(NCBI 2023r)

TDLo - Reported lowest dose causing toxic effect; LDLo - Reported lowest dose causing lethality; IM - Intramuscular; IV - Intravenous

Table 11b. Toxicity doses and effects of atropine sulfate in humans: reported lowest dose causing a toxic effect (TDLo) and reported lowest dose causing lethality (LDLo).

Species	Test type	Dose	Effect	Reference
Child	TDLo Oral	0.02 mg/kg-bw	Decreased urine volume, behavioral excitement, change in heart rate	(NCBI 2023h)
Adolescent male	TDLo IV	0.028 mg/kg-bw	Not provided	(Lacouture et al. 1983)
Adult, male, and female	TDLo IM	0.028 mg/kg-bw	Ataxia, increase in pulse rate	(NCBI 2023h, White et al. 1956)
Adult male	TDLo (unreported)	0.014 mg/kg	Behavioral effects (hallucination, distorted perceptions); headache	(Herschman et al. 1991, NCBI 2023h)
Adult female	TDLo (multiple)	0.044 mg/kg-bw	Arrhythmia; behavioral effects (hallucinations, distorted perceptions)	(Elkins-Sinn 1995)
Adult female	TDLo Ocular	0.02 mg/kg-bw	Arrhythmia	(Elkins-Sinn 1995)
Adult male	LDLo Oral	357 mg/kg-bw	Acute pulmonary edema	(NCBI 2023h)

TDLo - Reported lowest dose causing toxic effect; LDLo - Reported lowest dose causing lethality; IM - Intramuscular; IV - Intravenous

5.2.5.1 Acute Toxicity

Atropine and atropine sulfate can be toxic to mammals, depending on the dose and route of exposure. Atropine's oral LD_{50} for rats, guinea pigs, and rabbits falls within USEPA's acute toxicity category III, as does the intramuscular LD_{50} for rats (Table 12), indicating atropine is slightly toxic. Atropine's oral LD_{50} for mice is in acute toxicity category II (Table 12), indicating atropine is moderately toxic. LD_{50} values for dermal and inhalation exposures and eye and skin irritation studies and skin sensitization studies are unavailable.

Table 12. Mammalian toxicity of atropine and atropine sulfate.

Species	Route	Atropine LD_{50}	Atropine Sulfate LD_{50}	Reference
Brown Rat*	Oral	500 mg/kg-bw	600 mg/kg-bw	Atropine: (Lewis 1996) atropine sulfate: (NCBI 2023h)
Brown Rat*	Subcutaneous	250 mg/kg-bw	540 mg/kg-bw	(NCBI 2023h;r)
Brown Rat*	Intramuscular	920 mg/kg-bw	--	(Lewis 1996)

Species	Route	Atropine LD_{50}	Atropine Sulfate LD_{50}	Reference
Brown Rat*	Intravenous	73 mg/kg-bw	37 mg/kg-bw	Atropine: (Lewis 1996) atropine sulfate: (NCBI 2023h)
Brown Rat*	Intraperitoneal	280 mg/kg-bw	215 mg/kg-bw	(Lewis 1996)
House Mouse*	Oral	75 mg/kg-bw	468 mg/kg-bw	(Lewis 1996)
House Mouse*	Intradermal	550 mg/kg-bw	--	(NCBI 2023r)
House Mouse*	Subcutaneous	--	400 mg/kg-bw	(NCBI 2023h)
House Mouse*	Intravenous	30 mg/kg-bw	31 mg/kg-bw	(Lewis 1996)
House Mouse*	Intraperitoneal	30 mg/kg-bw	150 mg/kg-bw	(NCBI 2023h;r)
Guinea pig	Oral	1,100 mg/kg-bw	--	(NCBI 2023r)
Guinea pig	Subcutaneous	--	480 mg/kg-bw	(NCBI 2023h)
Guinea pig	Intraperitoneal	400 mg/kg-bw	--	(NCBI 2023r)
Domestic Rabbit*	Oral	600 mg/kg-bw	--	(NCBI 2023r)
Domestic Rabbit*	Intradermal	500 mg/kg-bw	--	(NCBI 2023r)
Domestic Rabbit*	Intramuscular	--	414 mg/kg-bw	(NCBI 2023h)
Domestic Rabbit*	Intravenous	50 mg/kg-bw	70 mg/kg-bw	(NCBI 2023h;r)
Domestic Rabbit*	Intraperitoneal	--	200 mg/kg-bw	(NCBI 2023h)
Cat	Intravenous	--	36 mg/kg-bw	(NCBI 2023h)
Dog	Intravenous	--	60 mg/kg-bw	(NCBI 2023h)

* Domestic laboratory strains.

5.2.5.2 Sublethal and Chronic Toxicity

Standardized toxicity studies on the effects of subchronic and chronic exposure to atropine and atropine sulfate are unavailable. School children did not develop adverse effects on intraocular pressure or other injury effects after receiving atropine eyedrops daily for nearly five years (NCBI 2023r). However, a chronic reference dose for atropine and atropine sulfate has not been established.

In one study, male rats treated with 125 mg/kg-bw/day atropine for 10–17 days prior to mating with untreated females resulted in a lower pregnancy rate due to reduced male fertility (Sato et al. 2005). Female rats in gestation give atropine at days 7 to 19 led to adverse effects on the behavioral development of pups, namely deficiency in avoidance learning (Watanabe et al. 1985). Chick eggs injected with 0.6 to 1.5 mg atropine during the interval of 4 to 12 days incubation did not show signs of developmental effects (IPCS 2002). Atropine usage is short-term, and human fertility impairments have not been investigated.

Atropine and atropine sulfate are not mutagenic (IPCS 2002), and atropine is not carcinogenic (Schmähl and M. Habs 1976). Atropine and atropine sulfate studies were negative in mutagenicity testing, and studies were summarized in IPCS (2002). In a long-term study on rats given 6 mg/kg-bw/week atropine, atropine was not found to be carcinogenic (Schmähl and M. Habs 1976). Atropine is not listed as an endocrine disruptor (PAN 2018). In a laboratory study, atropine

treatment suppressed T-cell responses in spleen cells of rats exposed to approximately 2 mg/kg-bw atropine/day subcutaneously for 3 weeks (Razani-Boroujerdi et al. 2008).

5.2.6 Dose-Response Assessment

5.2.6.1 Human Health Dose Response

The typical adult oral and parenteral (intramuscular, intravenous, or subcutaneous) dosage of atropine sulfate is 0.4–0.6 mg (range 0.1–1.2 mg) every 4–6 hours; for children, the oral and parenteral dose is 0.01 mg/kg-bw or 0.3 mg/m^2 but not to exceed 0.4 mg every 4–6 hours (NCBI 2023r). “The estimated lethal doses of atropine in adult humans are greater than or equal to 10 mg” (*as cited in* (Jandrić et al. 2013)).

Intravenous doses of atropine sulfate at doses of 0.75 and 1.5 mg caused tachycardia in adult males and females ranging in age from 21 to 41 years, with a peak effect at 2 to 4 minutes followed by a slow decrease (Lönnerholm and Widerlöv 1975). Changes in blood pressure (increase in diastolic pressure) occurred at the highest dose (1.5 mg). At lower doses of 0.25 and 0.40 mg, subjects experienced bradycardia (slower than normal heart rate). At all doses, saliva secretion was inhibited with a clear dose-response relationship. In another study, seven adult males were given 0, 1.5, 3.0, and 6.0 mg/70 kg[12] atropine sulfate dissolved in saline, and physiological and behavioral performance was measured over 24 hours (Higgins et al. 1989). Heart rate and pupil diameter increased as the atropine dose increased. Tests of behavioral performance (response rate and latency and correctly completing the task) showed a decrease as the atropine dose increased, with diminished performance across all behavioral tasks at the 6.0 mg dose and often at the 3.0 mg dose (Higgins et al. 1989). In general, at these two dose levels, the changes in behavioral performance peaked around 1.5 hours post-application and lasted for 7 to 9 hours, with no effects observed at 24 hours (Higgins et al. 1989). Atropine’s percent bioavailability in the body during use is 50 (Jjemba 2006), and the percentage of the parent compound excreted in clinical settings is 50, which is considered relatively high (Ali-Melkkilä et al. 1993).

5.2.6.2 Ecological Dose Response

Mammals

The acute toxicity of atropine and atropine sulfate to mammals is summarized in Section 5.2.4. In general, atropine and atropine sulfate have slight to moderate acute oral, inhalation, and dermal toxicity to mammals based on available data.

Intravenous infusion of 0.1 mg/kg atropine sulfate alone or five minutes prior to the intravenous infusion of 80 µg/kg-bw of xylazine in goats caused an increase in arterial blood pressure and heart rate (xylazine causes a decrease in blood pressure and heart rate) (Kokkonen and Eriksson 1987). Atropine reduced salivation but did not inhibit saliva dripping (Kokkonen and Eriksson 1987). Atropine did not suppress sedation in xylazine-treated goats, nor did it prevent respiratory

[12] The dose was normalized based on the mean body weight of the test subjects.

suppression. Some goats became restless and shook their heads for two minutes after the injection of atropine (Kokkonen and Eriksson 1987).

Intravenous injection of 0.2 mg/kg-bw atropine in twelve male and female dogs (mean weight of 12.5 +/- 0.5 kg) caused an increase in heart rate from around 87 beats/minute to around 244 beats/minute within two minutes and remained at this rate for six minutes before decreasing (Chassaing et al. 1979). Atropine disappearance in the blood was rapid within the first 20 minutes, and the heart rate decreased during this time (Chassaing et al. 1979). In another study, dogs (10 to 20 kg in weight) given 0.5 mg/kg-bw atropine sulfate intravenously experienced salivation inhibition, decreased gastric secretion, and tachycardia around 160 beats/min greater than normal within 15 seconds of administration (Rush et al. 1970). There were no other observed changes in behavior other than restlessness (Rush et al. 1970).

Atropine had different behavioral disturbances in dogs at five different ages (newborn, 3 weeks, 6 weeks, 3 months, and adult) due to the development of two enzymes (choline acetyltransferase and cholinesterase) as the animal matures (Winbladh 1973). Behavioral disturbances in 6-week old, 3-month old, and adult dogs given 0.5 mg/kg-bw atropine included ataxia, non-responsiveness, non-retreating behavior, and increased motor activity (Winbladh 1973). In newborn dogs, atropine had no effect at doses from 0.5 to 5 mg/kg-bw (Winbladh 1973). In 3-week old dogs, those that were more mature had symptoms similar to adult dogs, while less mature dogs did not display symptoms even at the 5 mg/kg-bw dose (Winbladh 1973). Table 10 summarizes recommended atropine sulfate dosages in several animals.

Birds

Information on the acute toxicity of atropine and atropine sulfate in wild birds is limited. Atropine sulfate was mildly toxic to 14–20 day old chickens dosed intramuscularly into pectoral muscles with 100 mg/kg-bw atropine sulfate (Shlosberg et al. 1997). The researchers did not see adverse short-term or long-term effects at doses below 100 mg/kg-bw (0.5, 25, 50 mg/kg-bw). Atropine treatment in ratites (large flightless birds such as ostrich) to manage bradycardia is variably effective (West et al. 2007); xylazine may cause bradycardia in ratites. In a study evaluating drugs that dilate pupils in wild birds, double-crested cormorants around two to three months old were given 1% (4 drops) of atropine in the eye at four 15-minute intervals (Loerzel et al. 2002). No effect on pupil diameter or other side effects was observed.

Reptiles and Terrestrial Phase Amphibians

Information on the acute and subchronic toxicity of atropine and atropine sulfate in reptiles and terrestrial-phase amphibians is lacking.

Invertebrates

Atropine sulfate fed (3mg/1 g diet) or applied daily for 2–5 days to the mesothorax in 1 µl ($1x10^{-2}$ M aqueous acetone solution) doses to sixth-instar western spruce budworms (*Choristoneura occidentalis*) did not cause delays in maturity, developmental abnormality, or mortality (Smith 1971).

Aquatic Vertebrates and Invertebrates

Available data suggest that atropine and atropine sulfate are practically non-toxic to fish and aquatic invertebrates. Based on the effects on heart rate during a 30-minute exposure, rainbow trout at embryonic stages 22 and 29 and larval stage 30 have a NOEC of 28.94 mg/L atropine sulfate (USEPA 2019a). In the same study, a LOEC of 28.94 mg/L atropine sulfate was based on increased heart rate at embryonic stage 26 and larval stage 32. Atropine is practically non-toxic to zebrafish with an EC_{50} value of 559.1 mg/L and a no observable adverse effect concentration (NOAEC) of 400 mg/L atropine (Ali et al. 2014). USEPA classifies atropine as teratogenic based on the zebrafish assay.

An LC_{50} was not calculated for brine shrimp (*Artemia salina*) because toxicity was not observed at the highest dose of atropine sulfate tested (>1,000 mmol/L) (Barahona and Sánchez-Fortún 1999). In another study, the LC_{50} for *A. salina* was 22,700 µmol/L atropine sulfate (Calleja et al. 1994). Atropine sulfate is practically non-toxic to the freshwater cladoceran, *D. magna,* with reported LC_{50} values of 354 mg/L and 104 mg/L at 24 and 48 hours, respectively (Carvalho et al. 2003). In another study using *D. magna,* the 24-hour EC_{50} value was 0.372 +/-0.038 mM atropine sulfate with recovery (i.e., mobility) observed 3 hours after exposure (Lilius 1994). In another study, the EC_{50} for *D. magna* was 513 µmol/L (Calleja et al. 1994). In laboratory studies, the marine bacterium *Photobacterium phosphoreum* had LC_{50} values of 8,010 µmol/L atropine sulfate (Calleja et al. 1994). For freshwater species, the LC_{50} for freshwater rotifers (*Brachionus calyciflorus*) was 481 µmol/L and for fairy shrimp (*Streptocephalus proboscideus*), 951 µmol/L atropine sulfate (Calleja et al. 1994).

5.3 Doxapram

5.3.1 Chemical Description and Product Use

Doxapram (CAS No. 309-29-5; $C_{24}H_{30}N_2O_2$; chemical structure in Appendix 3) is a central respiratory stimulant that affects the carotid and aortic chemoreceptors and the medullary respiratory centers (Kreeger and Arnemo 2012). WS uses doxapram to stimulate breathing to speed the recovery of the animal after xylazine sedation and Telazol® (tiletamine + zolazepam) anesthesia. However, it is not a pure antagonist because it does not operate on the same receptors as the agonists. Doxapram is also used to reverse hypoventilation or apnea caused by barbiturate anesthesia in dogs and anesthesia from ketamine, ketamine-xylazine, ketamine-promazine, and opioids such as carfentanil (Kreeger and Arnemo 2012). The duration of respiratory stimulation is brief (5-10 minutes); doses may be repeated every 15 minutes (Kreeger and Arnemo 2012). Doxapram is sold as Dopram-V® (veterinary formulation) and Dopram® (human formulation). Each 1 mL of doxapram injection contains 20 mg doxapram HCl, 0.9% benzyl alcohol (preservative), and water for injection (Drugs.com 2023a, FDA 2005). WS administers doxapram intramuscularly by hand using a syringe to already immobilized animals.

5.3.2 Physical and Chemical Properties

Doxapram is an oral or injectable colorless liquid (APHA 2015). It has a pH between 3.5 and 5 (Drugs.com 2023a) and a melting point of 217–219ºC (NCBI 2023o). Its molecular weight is 378.5 g/mol (NCBI 2023o). Doxapram is sparingly soluble[13] (NCBI 2023o) and stable and non-reactive under normal use, storage, and transport (Boehringer Ingelheim 2018). It is incompatible with strong oxidizing agents (Boehringer Ingelheim 2018). Doxapram (e.g., Dopram-V®, Dopram®) is sold in 20 mg/mL concentrations (APHA 2015, USDA APHIS 2019b), and its preservative is benzyl alcohol (DOPRAM-V 2013). The solution contains doxapram HCl (CAS No. 7081-53-0; (APHA 2015).

5.3.3 Environmental Fate

We were unable to find information in the scientific literature on the environmental fate of doxapram in soil, water, and air. A literature search revealed that doxapram is not readily biodegradable (DrugBank 2023b).

5.3.4 Metabolism

Doxapram stimulates breathing but can also be used to speed up the recovery of an animal by increasing the metabolism of the immobilizing drug. While Telazol has no antagonist, doxapram (not a true antagonist) can be used for this purpose (USDA APHIS 2019b). In dogs given 10 mg/kg and 20 mg/kg doxapram intravenously, blood concentrations of doxapram and its metabolites were at peak levels immediately after injection and declined rapidly in the first hour. The metabolism of doxapram decreased after the first hour allowing for the presence of doxapram after 24 hours. A dog given doxapram with radioactive carbon had feces with 29% of the administered radioactivity after 48 hours and an additional 9% the following 24 hours (DOPRAM-V 2013).

5.3.5 Hazard Identification

Doxapram is FDA approved for humans. Doxapram stimulates respiration by primarily affecting the brain stem and is used to speed awakening and return reflexes after anesthesia or to stimulate respiration during and after general anesthesia (DOPRAM-V 2013). Dogs and horses have dramatic responses to doxapram, while respiratory stimulation is moderate in cats and slight in rats (DOPRAM-V 2013). Respiratory stimulation was observed in anesthetized dogs after intravenous, intramuscular, intraperitoneal, oral, sublingual, and subcutaneous administration (DOPRAM-V 2013). Adverse effects include seizures, hypertension, tachycardia, and arrhythmia.

5.3.5.1 Acute Toxicity

Doxapram is in category III for acute oral toxicity (Boehringer Ingelheim 2018). The acute oral LD_{50} is 261 mg/kg-bw in rats, 270 mg/kg-bw in house mice, and 150 mg/kg-bw in dogs (Table 9).

[13] Sparingly soluble materials are those that require 30 to 100 ml solution to dissolve 1 g of material.

5.3.5.2 Sublethal and Chronic Toxicity

Reproduction studies in rats receiving oral or intramuscular doses of doxapram up to 1.6 times the human dose have revealed no evidence of impaired fertility or harm to the fetus (FDA 2005).

5.3.6 Dose-Response Assessment

5.3.6.1 Human Health Dose Response

Following a single intravenous injection of doxapram HCl at 0.5–1.0 mg/kg, respiratory stimulation generally occurs within 20–40 seconds with peak effect at 1–2 minutes. The duration of the effect varies from 5–12 minutes (FDA 2005). Side effects from the use of doxapram that are indicative of subconvulsive central nervous system stimulation include hypertension, tachycardia, arrhythmias, coughing, sneezing, vomiting, itching, tremors, muscle rigidity, sweating, flushing, and hyperpyrexia (NCBI 2023o). Doxapram overdose symptoms include tachycardia, skeletal muscle hyperactivity, and enhanced deep tendon reflexes (NCBI 2023o).

5.3.6.2 Ecological Dose Response

Information is lacking on the effects of doxapram on aquatic species and wildlife.

5.4 Naltrexone

5.4.1 Chemical Description and Product Use

Naltrexone (CAS No. 16590-41-3; $C_{20}H_{23}NO_4$; chemical structure in Appendix 3) is designated an orphan drug by the US Food and Drug Administration (FDA) in the maintenance of alcohol and opiate cessation (NCBI 2023y). Naltrexone is a competitive inhibitor of the opioid receptors and displaces opioid drugs from these receptors, thus reversing their effects (NCBI 2023y).

Naltrexone can antagonize all opiate receptors. There are FDA-approved products for oral and intramuscular administration that contain naltrexone, but the only approved veterinary drug that contains naltrexone is Trexonil® (intramuscular). The human drug oral dosage forms (tablets) could be prescribed for extra-label use in animals by a veterinarian under certain circumstances. Subcutaneous treatments are not approved by the FDA and can result in serious side effects (Alkermes Inc. 2016). WS uses naltrexone to reverse the effects of sedative immobilization drugs such as butorphanol tartrate and nalbuphine and those drugs in the combinations BAM and NAM. Naltrexone is long-lasting compared to other opioid antagonists. It is preferred in wildlife when potent opioids are used because it minimizes the potential re-narcotization from long-lasting opioids and minimizes the need to re-dose animals, particularly when personnel safety concerns exist (Plumb 2018). Naltrexone is also used to antagonize butorphanol in swine (West et al. 2007).

5.4.2 Physical and Chemical Properties

Naltrexone is an insoluble solid at room temperature, with a melting point of 168–170°C (O'Neil 2013). As hydrochloride salt, the solubility of naltrexone is 100 mg/mL (McEvoy 1990). The molecular weight of naltrexone a.i. is 341.4 g/mol, with a vapor pressure of $3.11x10^{-11}$ and a Henry's Law constant of $4.22x10^{-19}$ atm-m^3/mol at 25°C (NCBI 2023v;y).

5.4.3 Environmental Fate

Naltrexone's use in the maintenance of animal populations may result in its release to the environment through various waste streams (NCBI 2023y). If released into water, naltrexone is expected to adsorb to suspended solids and sediment based on the estimated organic carbon octanol-water partition coefficient (K_{oc}) (NCBI 2023v). Hydrolysis is not expected to be a significant environmental fate process since this compound lacks functional groups that hydrolyze under environmental conditions (pH 5 to 9) (NCBI 2023v). Volatilization from water surfaces is also not expected based upon this compound's acid dissociation constant value, and if released to air, an estimated vapor pressure of $3.1x10^{-11}$ mm Hg at 25°C indicates naltrexone will exist primarily in the particulate phase (NCBI 2023v). Particulate-phase naltrexone will be removed from the atmosphere by wet or dry deposition. Naltrexone is not expected to be susceptible to direct photolysis by sunlight or undergo hydrolysis in the environment (NCBI 2023v), and if released to soil, naltrexone is expected to have low mobility based upon an estimated (Koc) of 1,040 (USEPA 2012). The acid disassociation constant (pKa) values of naltrexone are 8.38 and 9.93 (Kaufman et al. 1975), indicating that this compound will exist almost entirely in the cation form in the environment and cations generally adsorb more strongly to soils containing organic carbon and clay than their neutral counterparts (NCBI 2023v). Volatilization from moist soil is not expected because the compound exists as a cation, and cations have a propensity not to volatilize (NCBI 2023v). An estimated bioconcentration factor (BCF) of 9 suggests a low potential for bioconcentration in aquatic organisms (NCBI 2023v). Occupational exposure to naltrexone may occur through inhalation of dust during production, storage, and transport. Risks of accidental exposure to naltrexone primarily involve those who prescribe, prepare or administer the drug (NCBI 2023v). Biodegradation data in soil or water are not available.

5.4.4 Metabolism

In humans, naltrexone is metabolized in the liver principally by reduction of the 6-keto group of naltrexone to 6-beta-naltrexol (6-beta-hydroxynaltrexone) (American Society of Health-System Pharmacists 2017). Naltrexone metabolites undergo conjugation with glucuronic acid. The major fraction of total drug and metabolites in both plasma and urine consists of conjugated metabolites (American Society of Health-System Pharmacists 2017, NCBI 2023y). The primary route of elimination of the parent drug and its metabolites is the kidney (53% to 79% of the dose) (T3DB 2023). However, urinary excretion of unchanged naltrexone accounts for less than 2% of an oral dose, and fecal excretion is a minor elimination pathway (T3DB 2023). The renal clearance for naltrexone ranges from 30 to 127 mL/min. It suggests that renal elimination is primarily by glomerular filtration with a half-life of 4 hours for naltrexone and 13 hours for the active metabolite 6 beta-naltrexol (T3DB 2023).

Naltrexone may also undergo partial reabsorption by the renal tubules. Most urinary excretion of naltrexone occurs within the first 4 hours after oral administration. Following the oral administration of 50 mg of radiolabeled naltrexone in one patient, approximately 93% of the radiolabeled dose was excreted within 133 hours; about 79 and 14% were excreted in urine and feces, respectively (NCBI 2023v). In raccoons, residues of naltrexone were detected in homogenized muscle, fat, liver, and kidney tissue 2, 4, and 6 days post-administration of 0.233 mg/kg naltrexone to reverse butorphanol or nalbuphine (Johnson et al. 2023b).

Cases of hepatitis and clinically significant liver dysfunction have been observed in association with naltrexone hydrochloride exposure. When patients exposed to naltrexone presented with elevated transaminases, other potential coexisting causative or contributory conditions were often present, including pre-existing alcoholic liver disease, hepatitis B and/or C infection, or concomitant usage of other potentially hepatotoxic drugs (NLM 2017a).

Symptoms of opioid withdrawal appear within five minutes of naltrexone hydrochloride exposure and last up to 48 hours. Confusion, somnolence, and visual hallucinations have occurred. Significant fluid losses from vomiting and diarrhea have required intravenous fluid administration. (NLM 2017a). Other adverse effects of naltrexone potentially include abdominal cramping, nausea, vomiting, nervousness, insomnia, joint or muscle pain, skin rashes, and itchy skin (Plumb 2018).

5.4.5 Hazard Identification

5.4.5.1 Acute Toxicity

Acute naltrexone toxicity in mice, rats, and dogs resulted in death secondary to tonic-clonic seizures and/or respiratory failure (NCBI 2023v). The oral LD_{50} in mice is 1.1 g/kg-bw, with death occurring from respiratory depression or tonic-clonic seizure (Plumb 2018). The subcutaneous injection LD_{50} in dogs is 200 mg/kg-bw (Plumb 2018). Weight loss was observed in monkeys following the subcutaneous administration of 100 mg/kg-bw of naltrexone. Prostration, seizures, and death were observed in monkeys after 300 mg/kg-bw subcutaneous doses of naltrexone (NCBI 2023y). Hypoactivity, salivation, and emesis occurred in monkeys following oral administration of 1 g/kg-bw doses. Seizures and death occurred following oral administration of 3 g/kg-bw (American Society of Health-System Pharmacists 2017).

Bradycardia has been observed following intravenous (iv) naltrexone hydrochloride doses of 5-80 µg/kg-bw in unanesthetized dogs. In naltrexone hydrochloride-treated cats (1 mg/kg-bw), total brain oxygen consumption was reduced by approximately 48%, and blood flow to the entire brain region decreased by about 40% (American Society of Health-System Pharmacists 2017).

Increases in mean arterial pressure, cardiac output, stroke volume, and left ventricular contractility were observed in dogs with hypovolemic shock following administration of naltrexone hydrochloride doses ranging from 2.5–10 mg/kg-bw as a rapid intravenous injection or 2 mg/kg-

bw rapid intravenous injection followed by an intravenous infusion of 2 mg/kg-bw per hour for 4 hours (American Society of Health-System Pharmacists 2017, NCBI 2023v).

5.4.5.2 Subacute and Chronic Toxicity

In a 2-year study of the carcinogenic potential of naltrexone, increases in the frequency of mesotheliomas in male rats and tumors of vascular origin in both male and female rats were observed. No evidence of carcinogenicity was observed in several other 2-year studies in mice or rats receiving naltrexone dosages of 30 or 100 mg/kg-bw daily (47 or 150 times greater than the usual dosage in humans), respectively (NCBI 2023v).

5.4.6 Dose-Response Assessment

5.4.6.1 Human Health Dose Response

Oral naltrexone treatment has been observed to result in hormonal changes in women (50 mg), and intramuscular naltrexone (intramuscular) has also been observed to potentially be hepatotoxic, resulting in a hepatotoxicity severity grade of 3 (NCBI 2023v). Patients receiving 800 mg of naltrexone hydrochloride daily for up to 1 week in one study showed no evidence of toxicity. However, lower dosages reportedly have been hepatotoxic in some patients. No serious adverse effects were observed following the administration of single naltrexone doses of up to 784 mg (as the extended-release intramuscular injection) in several healthy individuals (American Society of Health-System Pharmacists 2017).

High doses of naltrexone greater than 100 mg/kg-bw have been observed to cause excess salivation, depression/reduced activity, tremors, and convulsions (T3DB 2023).

In a study of 250 male opioid-dependent patients, non-statistically significant changes in liver enzyme levels were most often observed in hepatitis-C-positive patients. The majority of the elevations greater than three times the upper limit of normal occurred in patients with chronic HCV infection (Mitchell Jr et al. 2012).

Abrupt opioid withdrawal may lead to systemic sequelae, including acute liver injury. It is advised to seek medical attention if people exposed to naltrexone experience symptoms of acute hepatitis (NCBI 2023v). Additionally, subcutaneous injection of naltrexone can cause severe site-related reactions. Only oral and intramuscular treatments are approved by the FDA (Prescriber's Desk Reference 2023).

Tolerance to naltrexone can lead to possible accidents or injury due to side effects such as loss of coordination, slowed reaction time, sleepiness, impaired judgment, overdose, and death (T3DB 2023).

5.4.6.2 Ecological Dose Response

In veterinary medicine, naltrexone (2–5 mg/kg-bw oral) has been used to treat behavioral disorders (Plumb 2018).

Anesthesia of wild ass with a single 3-mL dart containing 4.4 mg etorphine, 10 mg detomidine, and 10 mg butorphanol was reversed with an i.v. combination of 200 mg naltrexone and 20 mg atipamezole. The reversal was rapid and smooth, with animals standing and alert in approximately 2 minutes following the administration of the antagonists (West et al. 2007).

Naltrexone has been observed to increase the CNS effects of yohimbine, e.g., anxiety, tremors, nausea, palpitations, and cortisol levels (NCBI 2023v). In a Sokolowska-Mikolajczyk 2002 study to determine the role naltrexone has on endogenous opioid peptides in luteinizing hormone (LH) secretion in fish, sexually mature male Eurasian (common) carp were intravenously injected with naltrexone-opioid receptor antagonist (5 or 50 µg/kg-bw) in the period of natural spawning (June) or gonad recrudescence (December). Blood samples were taken every minute, up to 10 min after the naltrexone injection. It was observed that in June, naltrexone significantly lowered LH levels in comparison to saline-injected males. In December, there were no differences between saline and naltrexone-injected carps. These results detailed that, in male carp, LH secretion under the influence of naltrexone depends on the stage of gonad maturity and also suggests that the feedback of gonadal steroids on LH release could be mediated by the endogenous opioids (Sokolowska-Mikolajczyk et al. 2002).

5.5 Tolazoline

5.5.1 Chemical Description and Product Use

Tolazoline HCl (CAS 59-97-2; $C_{10}H_{13}ClN_2$; chemical structure in Appendix 3) is a combination alpha-1 and alpha-2 antagonists that reverse the sedative effects of xylazine HCl. It belongs to the imidazole derivative group and competitively inhibits alpha-adrenoreceptors. Tolazoline HCl is used in human medicine (Prisoline™, 25 mg/mL and for domestic animals such as horses and cattle (Tolazine™). Tolazoline was approved by the FDA in 1996 in a 100 mg/mL concentration, but it is routinely compounded and available in a 200 mg/mL concentration in 30 mL multiple-use vials. In wildlife applications, tolazoline has been shown to be effective for use in equids and cervids (Monteith et al. 2012, Plumb 2018). To reverse the sedative effects of xylazine HCl in horses, 4.0 mg/kg-bw of tolazoline HCl is used (Plumb 2018). In cervids, 4 mg/kg-bw of tolazoline reversed xylazine in deer, reducing time to standing (Monteith et al. 2012). WS would administer tolazoline through intramuscular or intravenous hand injection with a syringe and needle to reverse the sedative effects of xylazine in wildlife, including ungulates and carnivores, when needed and for the appropriate species (USDA APHIS 2019b).

5.5.2 Physical and Chemical Properties

Tolazoline is a crystalline solid. A stock solution may be made by using an organic solvent such as ethanol (Cayman Chemical 2022c). WS uses tolazoline as an injectable solution. It has a molecular weight of 196.7 g/mol (Cayman Chemical 2022c). The commercially available human injection has a pH between 3 and 4 (Mikota and Plumb 2017). Tolazoline HCl has a melting point

of 174°C. Its experimental log K_{ow} is 2.65, indicating that the drug is more lipophilic than hydrophilic (DrugBank 2021a). Tolazoline is soluble in water, with solubility ranging from 0.373–1.36 g/L, and the pKa is 10.3 (NCBI 2023e). The estimated vapor pressure is $2.4x10^{-6}$ mmHg, and the estimated Henry's Law constant is $5.18x10^{-8}$ atm-m^3/mol, both at 25°C (USEPA 2023d). The drug should be stored at a controlled room temperature of -20°C for long-term storage (Cayman Chemical 2022c).

5.5.3 Environmental Fate

Information is lacking in the scientific literature on the fate and persistence in soil, water, and air of tolazoline. Tolazoline is not volatile based on its vapor pressure. The estimated biodegradation half-life for tolazoline is 3.36 days (USEPA 2023d). The estimated soil adsorption coefficient (K_{oc}) is 141 L/kg, indicating it is moderately mobile in soil, and the estimated bioconcentration factor is 5.07 (ComTox), indicating tolazoline has a low risk of bioaccumulation.

5.5.4 Metabolism

The biological half-life for tolazoline is 313 hours and is mostly excreted in urine within 2–4 weeks following administration (***as cited in*** (USDA AMS 2019)). In horses given a 4 mg/kg-bw dose of tolazoline intravenously, the elimination half-life was 2.08 hours (Plumb 2018).

5.5.5 Hazard Identification

Information on the acute toxicity and sublethal and chronic toxicity of tolazoline in the scientific literature was limited. The biological half-life for tolazoline is 313 hours and is mostly excreted in urine within 2–4 weeks following administration (***as cited in*** (USDA AMS 2019)). Tolazoline is in toxicity category II for acute oral toxicity study (Cayman Chemical 2022c). The oral LD_{50} in rats is 1,200 mg/kg-bw, and in house mice, 400 mg/kg-bw (Table 9) (Cayman Chemical 2022c). It is listed as category II for skin corrosion and irritation (Cayman Chemical 2022c). We did not find published information on the carcinogenicity of tolazoline.

5.5.6 Dose-Response Assessment

5.5.6.1 Human Health Dose Response

In humans, tolazoline causes vasodilation and is used to treat hypo- and hypertension, respiratory distress in newborns, and congenital heart disease (***as cited in*** (USDA AMS 2019)). Studies on the toxicity, lethal dosages, and carcinogenicity of tolazoline in humans are unavailable in the scientific literature.

5.5.6.2 Ecological Dose Response

In veterinary medicine, tolazoline is used to reverse the effects of xylazine. Tolazoline can result in tachycardia, defecation, vomiting, salivation, and edema (Kreeger and Arnemo 2012). Studies on the toxicity and lethal dosages in animals are limited (Table 9). Tolazoline is practically nontoxic to fish, with a 96-h LC_{50} of 354 mg/L in fathead minnows (*Pimephales promelas*) (Sigma-Aldrich

Corporation 2019). Side effects of tolazoline include increases in blood pressure, peripheral vasodilation, and tachycardia.

Horses given 1, 3, and 5 times the recommended dose of 4 mg/kg-bw tolazoline (without prior xylazine dosing) every 6 hours for 3 doses had gastrointestinal disturbances (Akorn Animal Health). No hematologic, serum, or urinalysis measurements were affected by tolazoline at doses equal to or less than the 5 times dose or 20 mg/kg-bw (Akorn Animal Health). Overdoses at 5 times the recommended rate for tolazoline have been linked to death in horses (Akorn Animal Health). After an intravenous tolazoline dose of 4 mg/kg-bw in horses, heart rate decreased beginning 2 minutes post-injection, reaching its lowest point 45 minutes post-injection (Plumb 2018). Serum glucose concentrations increased through 1.5 hours post-injection (Plumb 2018). The withdrawal period is 8 days after administering to livestock intended for slaughter, and a milk discard period of four days in dairy animals (Plumb 2018, USDA AMS 2019).

5.6 Yohimbine Hydrochloride

5.6.1 Chemical Description and Product Use

Yohimbine HCl (CAS No. 65-19-0; $C_{21}H_{26}N_2O_3 \cdot HCl$; chemical structure in Appendix 3), a plant alkaloid (NCBI 2023a), easily penetrates the blood-brain barrier and causes reversal of the sedative effects of xylazine usually within 2–10 minutes (IPCS 1992). Yohimbine also reverses cardiac side effects, such as arrhythmia and bradycardia, which can occur as a result of xylazine (Akorn Animal Health 2018a). Yohimbine is not a specific alpha-adrenergic antagonist; therefore, it can cause cholinergic, serotonergic, and dopaminergic receptor activity. Because yohimbine and other adrenergic antagonists do not fully antagonize ketamine, they should not be administered to animals until ketamine has had adequate time to metabolize in the animal (Kreeger and Arnemo 2012). Yohimbine HCl was approved by the FDA in 2 mg/mL and 5 mg/mL strengths and is compounded in 30 mL multiple-use vials. In cervids, yohimbine HCl is administered intravenously at a dose of 0.2–0.3 mg/kg-bw (FDA 2024). Veterinary formulations of yohimbine include Yobine® (Akorn Animal Health 2018b) and Antagonil® (NCBI 2023a). The recommended dosage for dogs to reverse xylazine is 0.11 mg/kg (NADA 140-866 Yobine Injectable Solution) (Akorn Animal Health 2018b).

Yohimbine was first used successfully to reverse the sedative effects of xylazine in cervids. The drug produces consistent results in non-domestic members of the dog and cat families (Canidae and Felidae), but it produces inconsistent results in bovids (Bovidae). WS administers yohimbine HCl intravenously to immobilized animals to reverse the sedative effects of xylazine in wolves and other predators and other appropriate wildlife species, but not necessarily ungulates or hoofed mammals. Yohimbine can be injected intramuscularly, if necessary, but it will be slower to act (USDA APHIS 2019b).

5.6.2 Physical and Chemical Properties

Yohimbine HCl is a white to off-white, water-soluble powder with a molecular weight of 390.90 g/mol. Its melting point is 288–290°C. Aqueous solutions have a neutral pH (Sigma-Aldrich

Corporation 2002a). Its predicted solubility in water is 0.35 g/L (HMDB 2019c). The experimental log K_{ow} for yohimbine is 2.73 indicating the drug is more lipophilic than hydrophilic (HMDB 2019c). Yohimbine HCl solutions are light-sensitive (Sigma-Aldrich Corporation 2002a).

5.6.3 Environmental Fate

We were unable to find information in the scientific literature on the degradability, bioaccumulative potential, or mobility in air and soil for yohimbine HCl. Yohimbine is slightly soluble in water (IPCS 1992). The predicted median biodegradation half-life is 144 days, the estimated soil adsorption coefficient (Koc) $1.37x10^4$ L/kg, indicating it is hardly mobile in soil, and the estimated bioconcentration factor 14.2 (USEPA 2023c).

5.6.4 Metabolism

Owen et al. (1987) researched the impact that oral administration of a single dose of yohimbine HCl had on eight young males, and results showed the drug was rapidly absorbed (absorption half-life 11 min) into systemic circulation and rapidly eliminated from plasma (elimination half-life 36 min). Less than 1% of the parent compound in the dose was recovered in the urine in a 24-hour period, suggesting that yohimbine is primarily eliminated through hepatic metabolism (Hedner et al. 1992, Owen et al. 1987). Yohimbine is metabolized into 11-hydroxy-yohimbine and 10-hydroxy-yohimbine. The 11-hydroxy-yohimbine metabolite is the only one detected in plasma and has alpha-2 adrenergic antagonist properties (Berlan et al. 1993, Federal Institute for Risk Assessment 2016, Le Corre et al. 1999).

The pharmacokinetics of yohimbine HCl varies between individuals. Routine administration of yohimbine HCl three times daily for 6 days had no effect on pharmacokinetics (Sturgill et al., 1997 *in* (Federal Institute for Risk Assessment 2016)). Oral bioavailability data show significant variation, ranging from 7% to 87% (EFSA 2013). The consumption of fat-rich food has the potential to reduce the absorption of yohimbine HCl (Grasing et al. 1996).

The mean half-life of yohimbine HCl injection is 46.7 ± 24.4 min in steers, 76.1 ± 23.1 min for a small dose, and 52.8 ± 27.8 min for a large dose in horses, and 104.1 ± 32.1 min for dogs (Jernigan et al. 1988). Yohimbine HCl produced no behavioral changes in steers and horses. However, 1 to 2 minutes after dogs were injected, numerous dogs had increased respiratory rates, muscle tremors, hyperemic mucous membranes, salivation, emesis, and mildly agitated behavior. These symptoms lessened after 15 to 20 minutes, and normal behavior returned after 90 to 150 minutes (Jernigan et al. 1988).

5.6.5 Hazard Identification

Yohimbine has been used medicinally by people for centuries at low doses, but side effects could be seen at higher doses. Yohimbine readily stimulates the central nervous system (EFSA 2013), but can cause side effects such as muscle tremors, increased blood pressure, dizziness, vomiting, anxiety, and nausea (Federal Institute for Risk Assessment 2016). Other adverse effects include excitation, insomnia, hypertension, and tachycardia. FDA, though, approved the use of yohimbine HCl for humans. Doctors may prescribe yohimbine to humans for the treatment of sexual

dysfunction, as an aphrodisiac, or for fatigue and exhaustion (Kommission, 1987, 1990 *in* (Federal Institute for Risk Assessment 2016)). In humans, yohimbine is not recommended for use with antidepressants and other mood-altering drugs.

Yohimbine is effective for reversing xylazine in horses, cats, and dogs but is less effective in ruminants. Yohimbine is not as effective at reversing newer drugs such as medetomidine (West et al. 2007). Research indicates that dogs receiving five times the recommended dose of yohimbine (0.55 mg/kg) experienced transient seizures and muscle tremors.

5.6.5.1 Acute Toxicity

Yohimbine's oral LD_{50} value (43 mg/kg-bw) and subcutaneous LD_{50} value (20 mg/kg-bw) in house mice indicate it is highly toxic (Table 9). The reported lowest dose causing lethality (LD_{Lo}) of yohimbine given to mice and rabbits intravenously was reported to be 16 mg/kg-bw and 11 mg/kg-bw, respectively (Table 9). The LD_{Lo} for subcutaneous administration of yohimbine in rabbits was 50 mg/kg-bw (IPCS 1992).

5.6.5.2 Sublethal and Chronic Toxicity

Dogs tolerated yohimbine at 3 and 5 times the intended dose, but at the 5x dose, dogs displayed brief seizures and muscle tremors, though no lasting effect (Akorn Animal Health 2018b). Data is unavailable for yohimbine's carcinogenicity, teratogenicity, and mutagenicity (IPCS 1992).

5.6.6 Dose-Response Assessment

5.6.6.1 Human Health Dose Response

A study evaluating the effectiveness and safety of a high dose of yohimbine HCl (36 mg/day for 25 days) in 29 men resulted in drug-related adverse effects in 2 patients (7%). One man experienced a hypertensive crisis, while the other experienced severe palpitation (Kunelius et al. 1997). Another study of healthy volunteers receiving oral yohimbine of 20 or 40 mg caused dose-dependent increases in blood pressure, heart rate, and plasma noradrenaline (Murburg et al., 1991 *in* (EFSA 2013)). In another situation, a 37-year-old bodybuilder was admitted to the hospital with acute neurotoxicity after ingesting 5 g of yohimbine. The patient experienced malaise, vomiting, loss of consciousness, and repeated seizures but recovered (Giampreti et al., 2009 *in* (EFSA 2013)).

5.6.6.2 Ecological Dose Response

In zebrafish larvae, yohimbine HCl had an EC_{50} value of 14.5 mg/L and a NOAEC of 10 mg/L (Ali et al. 2014). Phenotypic abnormalities included pericardial edema (fluid retention), yolk sac edema, dispersed pigment cells, and an uninflated swim bladder, and in this zebrafish assay, yohimbine HCl was teratogenic (Ali et al. 2014).

6 EUTHANASIA AGENTS

WS uses chemical agents to euthanize animals for postmortem disease diagnosis or investigations or when an animal is injured. WS personnel refer to WS Directive 2.505 Lethal Control of Animals for the current policy on euthanasia. WS follows the AVMA Guidelines on Euthanasia as required by WS policy.

Table 13. Acute toxicity values for euthanasia drugs.

Chemical	Test Species	Test Type	LD_{50} (LC_{50})	Reference
Potassium Chloride	Brown Rat*	Acute oral	3,020 mg/kg-bw	OECD, 2001
	Brown Rat*	Intravenous	142 mg/kg-bw	OECD, 2001
	House Mouse*	Intravenous	117 mg/kg-bw	OECD, 2001
	Brown Rat*	Intraperitoneal	660 mg/kg-bw	OECD, 2001
	Water Flea	48-hr LC_{50}	177 mg/L	OECD, 2001
Sodium Pentobarbital	Brown Rat*	Acute oral	118 mg/kg-bw	(Merck Animal Health 2014, Vortech Pharmaceuticals 2015, Zoetis 2014a)
	Dog	Acute oral	65 mg/kg-bw	(Merck Animal Health 2014, Vortech Pharmaceuticals 2015)
	House Mouse*	Acute oral	239 mg/kg-bw	(Zoetis 2014a)

* Domestic laboratory strains.

6.1 Potassium Chloride

6.1.1 Chemical Description and Product Use

Potassium chloride (KCl; CAS No. 7447-40-7; chemical structure in Appendix 3) is a salt that can cause cardiac arrest when administered intravenously as a saturated solution (340 grams in 1 liter of water). Approximately 120 mL KCl is injected into the target animal (Shearer and Ramirez 2013). WS administers KCl through intravenous or intracardiac injection after the animal is immobilized with an anesthetic or anesthetic sedative combination. Death usually occurs within a couple of minutes after injection. WS typically uses KCl to euthanize small mammals such as mongooses, striped skunks, raccoons, and woodchucks (Table 3a).

6.1.2 Physical and Chemical Properties

KCl, a white or colorless crystal, is odorless and has a strong saline taste. Its molecular weight is 74.55 g/mol (NCBI 2023j). It has a boiling point of 1,500°C and a melting point of 771°C (NCBI 2023j). It has a pH of 7 and is soluble in water (35.5 g/100 g water at 25°C) (NCBI 2023j). It has a low vapor pressure and is not volatile at ambient temperatures (NCBI 2023j). KCl has a predicted log K_{ow} of 0.2, indicating the drug is equally partitioned between the lipid and aqueous phases.

6.1.3 Environmental Fate

KCl is naturally occurring in the environment, in soil, and in water (OECD 2001). KCl is soluble in water. In soil, KCl is fairly mobile; the type and amount of clay minerals, pH, and organic matter affect transport and leaching (OECD 2001). Potassium is less mobile in soil than chloride, but chloride readily bonds with potassium and leaches from the soil (OECD 2001). KCl has a low vapor pressure (5.73 hPa at 906°C) and is unlikely to disperse into the atmosphere due to low volatility (OECD 2001). The high-water solubility and low K_{ow} suggest that KCl does not bioconcentrate or bioaccumulate.

6.1.4 Metabolism

Ingested potassium is mostly absorbed by passive diffusion in the membrane of the upper intestine and distributed to all tissues where it plays a role in biological functions (OECD 2001). Potassium mediates the osmotic balance of body fluids (OECD 2001). The excretion of potassium is mostly in urine, with some in feces (OECD 2001).

6.1.5 Hazard Identification

KCl naturally occurs in the environment and is essential to cellular function in the body. It occurs in food and is used in various medical treatments. Oral toxicity is rare because large doses induce vomiting, and KCl is rapidly eliminated from the body (NCBI 2023j). In humans, oral overdoses of KCl can cause hyperkalemia (excess potassium in the blood), muscular weakness, vertigo, mental confusion, hypotension, and cardiovascular changes (NCBI 2023j). In one report, a subcutaneous injection caused skin burning and lesions, but the dose was not given (NCBI 2023j). Clinical signs of toxicity in animals include convulsions, exhaustion, and respiratory failure (OECD 2001). Other signs include gastrointestinal issues, increased thirst and urine excretion, and fever (OECD 2001). Saturated doses of KCl impact cardiac function and can be lethal.

6.1.5.1 Acute Toxicity

KCl has low acute oral toxicity in mammals (Table 13), with an LD_{50} in rats of 3,020 mg/kg-bw (OECD 2001). The physical properties of KCl make the potential for inhalation and dermal exposure low (OECD 2001).

6.1.5.2 Sublethal and Chronic Toxicity

A NOAEL for KCl is 1,820 mg/kg-bw/day based on a 2-year feeding study in rats. KCl was fed to a group of 50 male rats at doses of 110, 450, or 1,820 mg/kg-bw/day (OECD 2001). Gastritis was the only treatment-related effect. In this study, KCl had no carcinogenic effects. Developmental effects and survival rates were not observed in offspring from pregnant mice or pregnant rats given up to 235 mg/kg-bw and 310 mg/kg-bw, respectively (OECD 2001).

6.1.6 Dose-Response Assessment

6.1.6.1 Human Health Dose Response

KCl is necessary for several biological functions. In humans, the daily dietary intake for potassium is 50–100 mmol (2–4 g), and for chloride is 100–250 mmol (3.5–9 g) (OECD 2001). Therapeutic oral doses of potassium for adults are 3.0–7.5 g/day for replacement (OECD 2001). However, KCl at high dosages can be lethal; the oral lethal dose is estimated at 500–5,000 mg/kg-bw (OECD 2001). A serum potassium level of 40 mg/100 mL in humans is fatal (OECD 2001).

6.1.6.2 Ecological Dose Response

Toxicity studies have indicated that KCl is practically non-toxic to freshwater organisms. In acute toxicity tests, the 48-hour LC_{50} values for channel catfish and *Daphnia magna* were 720 mg/L, and 177 mg/L, respectively, and the 120-hour EC_{50} for *Nitzschia linearis* (algae species) was 1,337 mg/L (OECD 2001). Table 13 summarize KCl toxicity to fish and aquatic invertebrates. KCl is an important nutrient and constituent for terrestrial species, including mammals and birds as well as plants. KCl has low acute oral toxicity in mammals. Potassium is one of the three major nutrients for plants.

6.2 Sodium Pentobarbital

6.2.1 Chemical Description and Product Use

Sodium pentobarbital (CAS No. 57-33-0; $C_{11}H_{17}N_2NaO_3$; chemical structure in Appendix 3) is a barbiturate that rapidly depresses the central nervous system to the point of respiratory arrest (USDA APHIS 2019b). Intravenous injection is the most rapid and reliable technique for administering sodium pentobarbital. An intraperitoneal injection may be used when it would cause less distress than an intravenous injection (AVMA 2020). The use of chemical immobilization to minimize distress may precede the administration of sodium pentobarbital (USDA APHIS 2019b). WS typically uses sodium pentobarbital administered by intracardiac route, following anesthesia, as part of a 2-step method to euthanize small mammals such as striped skunks, raccoons, and Virginia opossums (Table 3).

Numerous euthanasia products are on the market with varying compositions of active and other ingredients; these products are DEA Schedule II, III, or IV controlled products. Sleepaway® contains 26% pentobarbital sodium, 20.7% propylene glycol, and 7.8% isopropyl alcohol (Zoetis 2014a), while Beuthanasia®-D Special contains 39% pentobarbital sodium, 5% phenytoin sodium (anticonvulsant and antiarrhythmic agent and central nervous system depressant), 10–20% propylene glycol, and less than 10% of ethyl alcohol and benzyl alcohol (Merck Animal Health 2014). Fatal-Plus solution contains 390 mg/mL sodium pentobarbital, 0.01 mg/mL propylene glycol, 0.29 mg/mL ethyl alcohol, and 0.2 mg/mL benzyl alcohol (Vortech Pharmaceuticals 2015).

6.2.2 Physical and Chemical Properties

Sodium pentobarbital is a clear liquid mixture with color added to some formulations. For example, Beuthanasia-D solution is pink, and Fatal Plus is blue (Merck Animal Health 2014, Vortech

Pharmaceuticals 2015). Many sodium pentobarbital formulations are soluble in water (Vortech Pharmaceuticals 2015, Zoetis 2014a). Fatal Plus solution has a pH ranging from 9.6−11.0 and a specific gravity of 1.105 (Vortech Pharmaceuticals 2015). Sleepaway euthanasia solution smells like alcohol, has a specific gravity of 1.062, and has a flash point of 39.4°C (Zoetis 2014a). Sodium pentobarbital is stable under normal conditions (Merck Animal Health 2014, Vortech Pharmaceuticals 2015, Zoetis 2014a). Hazardous decomposition products include carbon oxides (Merck Animal Health 2014, Vortech Pharmaceuticals 2015).

6.2.3 Environmental Fate

Research involving euthanized animals suggests sodium pentobarbital can leach from the deceased animal tissue into the surrounding soil (Payne et al. 2015). The persistence of a pharmaceutical in soil or sediment depends upon its photostability, binding, adsorption capabilities, and degradation rate (Díaz-Cruz et al. 2003). Pentobarbital is not susceptible to direct photolysis by sunlight. Pentobarbital has an estimated soil organic carbon-water partition coefficient (Koc) of 28 and pKa of 7.8 (NCBI 2023l). Based on the Koc value for pentobarbital, the drug is expected to have very high soil mobility (NCBI 2023l). The pKa value suggests pentobarbital will exist partially in anion form in the environment. As a result, pentobarbital is less likely to be absorbed by soil, which makes it more susceptible to uptake by groundwater (Saha 2016). If pentobarbital enters surface or groundwater sources, pentobarbital is not expected to adsorb to suspended solids and sediment. The Henry's Law constant for pentobarbital is $8.4x10^{-13}$ atm-m^3/mole (NCBI 2023l). Thus, volatilization is not expected to be an important fate process (NCBI 2023l). One recent study examined the rate of degradation of pentobarbital in sand, topsoil, and potting soil over 17 weeks. At the end of the study, approximately 17% of the pentobarbital remained in the sand, 19% remained in the topsoil, and 10% remained in the potting soil. In sand and topsoil, bacterial degradation appears to be the primary mechanism for the degradation of pentobarbital (Bagsby et al. 2018).

Pentobarbital was detected in groundwater samples taken from a water well located 300 meters from a landfill in Florida approximately 15 years after the landfill received waste. Resampling 7 years later also revealed the presence of pentobarbital (NCBI 2023l). The pKa of pentobarbital indicates that the drug will exist partially in the anion form if released into the environment. Pentobarbital is not expected to volatilize from dry soil surfaces based upon an estimated vapor pressure of $3.0x10^{-10}$ Hg at 25°C. It is not expected to be susceptible to direct photolysis. Biodegradation data is not available (NCBI 2023l). An estimated bioconcentration factor of 11 suggests the potential for bioconcentration of pentobarbital in aquatic organisms is low (NCBI 2023l).

6.2.4 Metabolism

When pentobarbital is administered orally or rectally, the onset of action is 1-minute following intravenous administration, 10–25 minutes intramuscularly, and 15–60 minutes orally or rectally (NCBI 2023l). Pentobarbital is metabolized by the liver, which oxidizes the drug into more polar compounds such as carboxylic acid (NCBI 2023l). Between 40% and 50% of an oral dose of

pentobarbital is excreted in urine as hydroxypentobarbital (NCBI 2023l). Plasma concentrations of pentobarbital decline in two phases, with a half-life of approximately 4 hours for the first phase and 35–50 hours for the second phase (NCBI 2023l). The plasma half-life for pentobarbital in adults is 15 to 50 hours and is dose-dependent (NLM 2017b).

6.2.5 Hazard Identification

In humans, relatively low doses of barbiturates depress the sensory cortex, decrease motor activity, and cause drowsiness. Larger doses distort judgment, suppress motor activity, reduce respiration, and produce sleep. Increasing the dosage further causes anesthesia. Acute overdose with barbiturates results in areflexia (muscles do not respond to stimuli), constriction of the pupils, oliguria (low urine output), tachycardia, hypotension, lowered body temperature, and coma. Circulatory collapse, respiratory arrest, and death could also occur in overdose situations (NCBI 2023l). Sodium pentobarbital, in certain formulations, is regularly used as an illicit drug. The formulations of sodium pentobarbital that WS uses are denatured and have no street value.

6.2.5.1 Acute Toxicity

Sodium pentobarbital has acute oral LD_{50} toxicity values of 65 mg/kg-bw in dogs, 118 mg/kg-bw in rats, and 239 mg/kg-bw in house mice (Table 13), placing it in toxicity category II (moderately toxic). It is considered an eye irritant (Zoetis 2014a). Sodium pentobarbital may cause skin irritation after prolonged contact (time not given) and may cause temporary irritation and redness of the eyes on contact (Oak 2015).

6.2.5.2 Sublethal and Chronic Toxicity

Exposure of 5 hours or longer (dose not provided) caused a loss of neuronal cells and was associated with cognitive learning loss and memory loss in primates (NLM 2017b). Dogs were dosed orally with either 50, 150, or 500 mg pentobarbital/day for eight weeks (FDA 2002). Dogs that received the 150 and 500 mg/day dose had statistically higher liver weights. Based on the study, a NOEL for pentobarbital was 50 mg/day (FDA 2002).

In general, barbiturates are pregnancy category D drugs that have been shown to cause fetal damage. Barbiturates can cross the placental barrier and move throughout the fetal tissue, with the highest concentrations in the liver, brain, and placenta (Johnson and Sadiq 2022).

6.2.6 Dose-Response Assessment

6.2.6.1 Human Health Dose Response

Barbiturates can be habit-forming. Daily administration of 400 mg or greater of pentobarbital for 90 days has the potential to produce some degree of physical dependence on the drug. Symptoms of acute intoxication with barbiturates include unsteady gait, slurred speech, and involuntary eye movements. Chronic intoxication includes confusion, poor judgment, irritability, and insomnia. Barbiturate withdrawal can be severe and may cause death (Drugs.com 2022).

Potentially lethal doses of pentobarbital are approximately 30 µg/mL (NLM 2017b). Barbiturates can cross the placental barrier following oral or parenteral administration. Barbiturates are also distributed to milk but are found at lower concentrations than those in plasma (NLM 2017b).

6.2.6.2 Ecological Dose Response

Researchers in one study injected red swamp crayfish (*Procambarus clarkii*) with 90.0 mg/kg-bw sodium pentobarbital and observed behavioral changes (Winegar et al. 1988). In fathead minnows, sodium pentobarbital has a 96-hour LC_{50} of 49.5 mg/L, indicating it is slightly toxic (Table 13).

Animals euthanized with sodium pentobarbital are toxic to scavengers. Following several secondary poisoning incidents, the FDA updated its euthanasia solution regulations to state "*euthanized animals must be properly disposed of by deep burial, incineration, or other method in compliance with the state and local laws to prevent consumption of carcass material by scavenging wildlife*" in 2003 (21 CFR § 522.900 2017). Well-vascularized organs such as the liver have the highest concentrations of pentobarbital; subsequently, the degree of intoxication will depend on the amount and type of tissue ingested. Bald and golden eagles tend to move quickly to fresh carcasses, prefer viscera, and have a narrow tolerance for barbiturates making them more susceptible to secondary poisoning from pentobarbital than other species (Krueger and Krueger 2000). Eagle and other species deaths have been reported in at least 16 states and Canada as a result of scavenging on carcasses of euthanized farm animals or pet horses left in the field or small animal carcasses that were unburied or exposed at landfills (Krueger and Krueger 2000). Animals that ingest tissue contaminated with pentobarbital may become disoriented or comatose, and death may occur (Krueger and Krueger 2000).

7 EXPOSURE ASSESSMENT AND RISK CHARACTERIZATION

Determining the potential human and environmental impact of accidental or unintentional exposure to an I&E drug involves characterizing the risk of accidental exposure to the WS personnel administering the drugs, the risk of an environmental release of I&E drugs, and the fate of these compounds when released in the environment. Risks associated with accidental exposure to people, nontarget animals, and the environment depend on the chemical attributes and environmental and biological fate of the individual compound and its various formulations summarized in this document. Acute and chronic toxicity data as well as environmental fate information, are summarized earlier in this document, when available, and are integrated with the exposure analysis to characterize the risk of I&E drugs to the public and WS personnel and to nontarget wildlife and domestic animals.

Environmental fate describes the processes by which I&E drugs move and are transformed in the environment. The environmental fate processes include 1) mobility and migration potential to groundwater and surface water, 2) persistence and degradation, and 3) plant uptake. Aquatic and terrestrial effects toxicity data for many of the I&E drugs and environmental fate data are

unavailable or limited. A literature search indicates that mobility in soil data does not exist for most I&E drugs discussed in this HHERA. Overall, there is low potential for the environmental release of most I&E drugs from the proposed use pattern in this risk assessment.

7.1 I&E Drug Administration

WS uses I&E drugs in low volume. Tables 1a-d summarized the drug formulations and quantity used, while Tables 2a-b, 3a-b, and 4a-b summarized WS animal take with different types of drugs. Human and nontarget species exposures to I&E drugs, and resulting risks, are mostly via accidental exposures that can occur during the administration of the I&E drugs.

Table 14. WS Method of I&E drug administration.

Drug Type	Drug	Method of Administration
Anesthetic	Ketamine HCl	• Hand injection using a hand syringe or syringe pole • Remote injection using darts propelled with rifles, pistols, or blow guns • Oral delivery (wearing gloves) by squirting into the mouth to calm an excited animal enough to allow injection of an additional drug (USDA APHIS 2019b)
Anesthetic	Tiletamine HCl + Zolazepam	• Hand injection using a hand syringe or syringe pole • Remote injection using darts propelled with rifles, pistols, or blow guns
Sedative	Azaperone, Butorphanol, Dexmedetomidine, Medetomidine HCL	• Hand injection using a hand syringe or syringe pole • Remote injection using darts propelled with rifles, pistols, or blow guns
Sedative	Midazolam	• Hand injection using a syringe pole • Remote injection using propelled darts • Oral (for seizures; extra-label use only) • Nasal (for seizures) • Rectal (for seizures)
Sedative	Nalbuphine	• Hand injection using a hand syringe or syringe pole • Remote injection using darts as part of NalMedA
Sedative	Butorphanol tartrate + Azaperone + Medetomidine (BAM)	• Hand injection using a hand syringe or syringe pole • Remote injection using darts propelled with rifles, pistols, or blow guns
Sedative	Nalbuphone + Azaperone + Medetomidine (NAM)	• Hand injection using a hand syringe or syringe pole • Remote injection using darts propelled with rifles, pistols, or blow guns
Sedative	Xylazine	
Tranquilizer	Acepromazine	• Hand injection using a hand syringe or syringe pole • Remote injection using darts propelled with rifles, pistols, or blow guns

Accessory Drug	Atropine	• Hand injection using a hand syringe and needle; For the treatment of bradycardia (low heart rate), intravenous injection is considered the most appropriate route of administration due to poor circulation but atropine can be given intramuscularly when appropriate.
Accessory Drug	Doxapram	• Hand intramuscular injection using a needle/syringe
Accessory Drug	Naltrexone	• Hand injection using a hand syringe and needle; antagonists work most rapidly when given intravenously but they are often given intramuscularly for a smoother recovery and for human safety concerns.(USDA APHIS 2019b) • Oral (extra-label use only)
Accessory Drug	Atipamezole, Tolazoline, Yohimbine HCL	• Hand injection using a hand syringe and needle; antagonists work most rapidly when given intravenously but they are often given intramuscularly for a smoother recovery and for human safety concerns.(USDA APHIS 2019b)
Accessory Substance	Over-the-Counter Antibiotics	• Applied topically by hand directly wearing latex or nitrile gloves
Euthanasia Agent	Potassium Chloride	• Only by hand using a syringe and needle, administered by intravenous or intracardiac injection (USDA APHIS 2019b)
Euthanasia Agent	Sodium Pentobarbital	• By hand using a syringe or syringe pole, administered by intravenous or intracardiac injection; intraperitoneal for small rodents (USDA APHIS 2019b)

7.2 Human Health Exposure and Risk Assessment

The exposure pathways for I&E drugs used by WS include dermal, oral, injection, and inhalation. This assessment focuses primarily on the environmental exposure to I&E drugs, not human protection and safety in the use of sharp instruments or cartridge-fired projectors. Personnel are advised to wear hearing and eye protection when using cartridge-fired projectors (USDA APHIS 2019b).

The potential for human exposure to I&E drugs is greatest for WS personnel, cooperators, or veterinarians who administer drugs for the WDM program. WS published the USDA-APHIS WS I&E Manual (USDA APHIS 2019b) to provide field personnel with information on legal responsibilities and policies, I&E drugs, delivery systems, administration of drugs, dosages, handling of immobilized animals, and human safety. WS restricts the use of I&E drugs to individuals who complete 16 hours of training and pass an administered exam (USDA APHIS 2019a). Certification is valid for 5 years, and continuing education is required to maintain certification (USDA APHIS 2019b). WS also stores I&E drugs properly and securely in an approved cabinet or safe or approved container and maintains accurate records for drug distribution and use, including proper disposal. WS avoids using remote delivery (darts) in populated areas (USDA APHIS 2019b). These restrictions minimize the risk of unintentionally exposing personnel and the public to these drugs. WS applicators have not reported any accidents of I&E drugs that could result in exposure to workers and the public.

7.2.1 Dermal Exposure

Personnel using these drugs could accidentally spray themselves with contents from drug vials or spill drugs while loading darts, syringes, and syringe poles. Dermal exposure may also occur during injection due to the animal's sudden movements (West et al. 2007). Splashed drugs can be absorbed through the eyes, mucous membranes of the nose and mouth, skin, or through abrasions and cuts. To minimize exposure to I&E drugs, WS employees wear nitrile/vinyl/latex gloves and goggles or a face shield when loading and handling a syringe or dart (USDA APHIS 2019b) and avoid the practice of recapping needles wherever possible. Accidental dermal exposure could still occur should personnel touch their face, skin, or other body parts with contaminated gloves. WS employees must also wear long-sleeved shirts or sleeve protectors and appropriate eye protection (USDA APHIS 2019b) during I&E activities.

7.2.2 Injection Exposure

Personnel may accidentally have a needlestick injury while handling darts, syringes, or syringe poles or by careless disposal of used syringes and needles. To minimize these risks, WS employees carry darts or loaded syringes in labeled, rigid, enclosed containers and do not reuse needles. All used syringes and needles are placed in a biohazard sharps container for disposal, and empty drug vials are returned to the State Director's office or WS National Wildlife Research Center (USDA APHIS 2019b). WS personnel avoid using their hand to recap a needle; instead, they place the cap on a wood block or piece of putty and press the needle into the cap (USDA APHIS 2019b). Several innovations to protect personnel are becoming more common, including syringes with retractable needles that "cap" the needle without recapping by hand. Although unlikely to occur, there is a risk of injection exposure from darts that ricochet and strike personnel.

7.2.3 Inhalation Exposure

Inhalation exposure is negligible for I&E drugs that are stored in bottles with a septum in which WS personnel inserts a needle to withdraw the proper dose. During mixing and loading, exposure through inhalation is unlikely for nonvolatile drugs. For many I&E drugs, the volatility is unknown. Inhalation during administration also is unlikely because the drug is contained in a syringe, and personnel inject the drug directly into the animal or use remote darting. Inhalation could occur during hand injection if the syringe breaks because of the animal's sudden movement and releases an aerosol of the drug (West et al. 2007). Using a syringe pole or remote darting reduces the risk of aerosol exposure.

7.2.4 Oral Exposure

Oral exposure through drinking water is negligible due to the WS use pattern and lack of exposure to water resources. Oral exposure from dietary consumption of treated animals as food sources is negligible for I&E drugs to the public. WS follows protocols and regulations regarding the secure storage, use, and disposal of I&E drugs, removing the chance the public would obtain the drugs. Dietary exposure of the public to I&E drugs is minimized by following recommended drug doses

and complying with withdrawal periods in association with hunting seasons for game species. The withdrawal interval recommendation for atropine given intramuscularly, intravenously, or subcutaneously in cattle is 28 days for meat and 6 days for milk (FARAD 2019). In black bears, research indicates it is safe to use Telazol (tiletamine and zolazepam) up to 15 days before hunting season based on 7–14-day metabolization and elimination to undetectable levels (Ryan et al. 2009). Table 15 presents suggested withdrawal times following intramuscular injection of several I&E drugs.

Most of the animal species WS treats with I&E drugs are not classified as food animals by the FDA (Tables 3, 4, and 5). Potential food animals released back into the environment are marked with an ear tag indicating they should not be used for food (USDA APHIS 2019b). The immobilization and accessory drugs that WS administers are in doses that are generally not toxic to the animal. The length of time immobilization and accessory drugs persist in a treated animal depends on the drug and the animal treated. Most immobilization and accessory drugs have short half-lives (around one to six hours) (e.g., sedatives, anesthetics, tranquilizers, most of the accessory drugs except for tolazoline) and rapidly metabolize in the body. Negative impacts from the consumption of animals treated with an immobilization and/or accessory drug by WS are unlikely because of the safeguards WS has in place to reduce exposure (adhering to withdrawal periods, labeling animals that were treated) and the metabolism and excretion rate of the drug in the treated animal (see Table 15 for withdrawal periods). The public is unlikely to consume animals treated with immobilization and accessory drugs before the drugs are metabolized.

WS personnel attempt to recover every dart that missed its intended target and spent darts that fall to the ground (USDA APHIS 2019b). A complete exposure pathway is not identified for surface water and groundwater. Small amounts of I&E drugs may be released into the soil during administration/application of the drug or through excretion in urine/feces; however, soil concentrations would be extremely low due to the low quantities of I&E drugs used by WS, the small amount of waste excreted and the metabolism and degradation of I&E drugs.

WS follows strict protocols when euthanizing an animal to prevent exposure to the public and nontarget species. WS administers a lethal potassium chloride or sodium pentobarbital injection into the target animal through a needle and syringe, not a dart. WS personnel monitor the animal post-injection, remove euthanized animals from the environment, and follow the appropriate regulations regarding disposal. The public would not have access to these animals.

As with the public, oral exposure of WS personnel to I&E drugs is negligible. Should personnel eat, drink, smoke, or touch their mouths with contaminated gloves, oral exposure could occur. WS personnel are trained in the proper storage, handling, and disposal of I&E drugs as well as the post-treatment periods for target species.

Table 15. Suggested withdrawal times of several immobilization and accessory drugs used in free-ranging wildlife and domestic livestock in North America.

Drug	Withdrawal Period (Days) Free-ranging Wildlife	Withdrawal Period (Days) Domestic Livestock	Reference
Acepromazine (intramuscular)	14	7	(Cattet 2003)
Atipamezole	-	14	(WAFWA 2010)
Butorphanol	-	2	(*as cited in* (Baldridge et al. 2011))
Ketamine (intramuscular)	3	3	(Cattet 2003)
Medetomidine (intramuscular)	14	n/a	(Cattet 2003)
Tolazoline HCl (intramuscular)	30	n/a	(Cattet 2003)
Yohimbine (intramuscular)	30	7	(Cattet 2003)
Zolazepam and Tiletamine (intramuscular)	14	365	(Cattet 2003)

7.2.5 Risk Characterization of Human Health Exposures

Although the I&E drugs characterized in this risk assessment have the potential to pose a hazard to human health (see hazard identification and dose-response assessment sections), the potential for exposure of I&E drugs from WS use pattern as well as safety policy and procedures is expected to be low resulting in minimal risks to human health. The greatest risk to human health is to workers who administer these drugs to immobilize or euthanize animals. The risk to these workers from oral, inhalation, injection, and dermal exposure is minimized by adherence to the proper use of personal protective equipment. In remote delivery, a small volume of the drug is delivered, and for some drugs, the concentration of the drug is high (West et al. 2007). Although some I&E drugs could be hazardous to humans at high doses, the low potential for exposure to these drugs suggests that adverse effects to well-trained workers will be minimal. Accidental exposure may occur if unprotected body parts are splashed with a drug while preparing, loading, or administering the drug to an animal. The exposure frequency would be low when following the label safety precautions. In addition, it is unlikely during an accidental exposure that an applicator would receive an entire dose of the drug. The WS Immobilization and Euthanasia training manual (WS 2019) includes instructions on human first aid in the event that an accidental exposure does occur.

The risk to the general public from exposure to I&E drugs from WS use is expected to be negligible. The lack of direct exposure and safeguards to prevent secondary exposure minimize the risks to the public.

During hand injection, workers are at risk of bite wounds, kicks, and crushing injuries (West et al. 2007). After administering immobilizing drugs, WS personnel use caution approaching the animal; the animal may not be fully immobilized and may respond to loud noises depending on the drug

used. Personnel work quickly with the sedated animal to minimize the need to give the animal additional drugs to prolong sedation.

7.3 Ecological Exposure and Risk Assessment

WS administers drugs through hand injection with a hand or syringe pole or remote darting (Table 14). WS has no records of unintentionally injecting or darting nontarget species.

7.3.1 Aquatic Exposure Assessment

The potential for aquatic exposure to I&E drugs is minimal. The primary pathways for aquatic exposure during drug administration would be from darts that miss the target animal and land in water, spillage during the administration of a drug and subsequent runoff, or an animal that is drugged entering an aquatic water body and excreting that drug. These activities have a low probability of occurrence and suggest that exposure to potential residues would be infrequent and localized—WS personnel attempt to recover any darts that may miss the target animal. Aquatic exposure to euthanasia drugs would not occur since the administration is through direct hand injection (not remote darting), and euthanized animals are removed and disposed of according to Federal, State, and local regulations. Euthanasia drugs come with a warning reminding the user that euthanized animals must be properly disposed of by deep burial (where allowed), incineration, or other authorized methods to prevent the consumption of carcasses by scavenging wildlife (Drugs.com 2023b). Animals that received an immobilization drug may enter water bodies after treatment; however, the metabolism and excretion of immobilization drugs and the low frequency of this type of exposure suggest a very low probability of drug exposure to aquatic habitats.

7.3.2 Terrestrial Exposure Assessment

WS use pattern for I&E drugs is unlikely to result in exposure to the terrestrial environment and nontarget terrestrial species. WS personnel administer the drugs through direct hand injection or remote injection.

Exposure of nontarget terrestrial species to water or aquatic food sources contaminated with I&E drugs is unlikely. As discussed in the aquatic exposure section above, the exposure potential of water resources to I&E drugs is negligible. Therefore, dietary exposure through drinking water or consuming aquatic species is negligible. Secondary exposure of predators and scavengers to immobilizing/accessory drugs is unlikely because most drugs are used to restrain an animal temporarily. In the unlikely event that a predator or scavenger consumes an animal with detectable amounts of an immobilizing/accessory drug, the dose they would ingest is unlikely to be lethal. This is due to the metabolism of immobilizing/accessory drugs as well as the dose administered, which is a nonlethal dose in the target animal.

WS follows Directive 2.505, Lethal Control of Animals, and Directive 2.515, Disposal of Carcasses, when determining the disposition of carcasses. WS personnel follow Federal, State,

and local regulations regarding the disposal of animals treated with euthanasia agents. The immediate disposal of euthanized animals removes the risk of toxicity to scavengers. WS follows drug label instructions. If guidance is lacking, carcasses must be disposed of via deep burial (where allowed), incineration, or at a landfill approved for such disposal. Subsequently, the fate and distribution of euthanasia drugs in the environment do not indicate a risk of secondary exposure.

Exposure of invertebrates and plants to I&E drugs is unlikely, given the use pattern of the drugs.

7.3.3 Risk Characterization of Ecological Exposures

The number of animals listed as euthanized by immobilizing drugs in the WS Program can be misleading. Overdosing an animal with an immobilizing drug and/or accessory drug is rare as the doses WS administers to target animals are below the toxicity level. WS personnel likely immobilized the animal with the reported immobilization drug but euthanized it by different methods. After immobilizing an animal, WS may euthanize the animal if it is injured, for disease sampling, research, or if the animal was trapped at a site causing damage. For example, during FY11-15 (Table 2a) and FY16-20 (Appendix 2) WS reports 167 and 43 raccoons with a final disposition of euthanized that were immobilized under ketamine, respectively. The method of euthanasia was likely a method other than ketamine.

Tissue damage and irritation will occur at the site of injection. Animals immobilized through darts can experience localized tissue bruising or trauma caused by the energy of dart impact (West et al. 2007). Inaccurate placement of the dart can cause injury if it penetrates a sensitive area (West et al. 2007) or can administer the drug into the wrong tissue, e.g., fat tissue instead of muscular tissue.

Residues of human pharmaceuticals have been widely detected in the environment, which leads to concerns about their potential to cause effects in nontarget species (Gunnarsson et al. 2008). Some of these pharmaceuticals are drugs WS administers; however, these detections were not from WS uses. Many aquatic species have similar target molecules to those in humans (Kostich and Lazorchak 2014). While large or frequent spills could have a negative impact on nontarget species, WS infrequently uses I&E drugs, and when it does, it only uses limited quantities.

Direct and indirect risk to nontarget mammals, birds, and reptiles is expected to be negligible based on WS use patterns for I&E drugs and lack of exposure. Similarly, the risk to aquatic organisms is expected to be negligible due to the lack of significant exposure. I&E drugs are not expected to be transported to aquatic habitats in sufficient amounts that could result in a risk to aquatic species.

8 UNCERTAINTIES AND CUMULATIVE EFFECTS

The uncertainties associated with this risk evaluation arise primarily from a lack of information about the effects of I&E drugs, their formulations, metabolites, and potential mixtures to nontarget organisms in the environment and the lack of environmental fate for some drugs. These uncertainties are not unique to this assessment but are consistent with uncertainties in human health and ecological risk assessments with any environmental stressor. In addition, there is uncertainty regarding the frequency of use and the locations where WS will use these drugs.

Another area of uncertainty is the potential for cumulative impacts on human health and the environment from the proposed use of I&E drugs. Areas, where cumulative impacts could occur include co-exposure to other chemicals with a similar mode of action and exposure to other chemicals that could affect the toxicity of I&E drugs.

The impacts on wildlife species are expected to be negligible when put in context with other stressors since the drugs are used infrequently and in limited areas. Cumulative impacts on aquatic organisms will also be negligible because there is an extremely low probability of exposure to aquatic habitats from the proposed use of I&E drugs.

Cumulative impacts on human health from the use of I&E drugs are not anticipated. Human exposure and risk are negligible for the general public. DEA scheduled drugs require safeguards and security to prevent theft by the public. The probability of exposure is greatest for workers involved with administering these drugs. However, the risk to this group will be negligible based on the low risk of exposure to I&E drugs when following applicable safety protocols, including wearing the appropriate personal protective equipment. There is potential for exposure to other chemicals used during wildlife management; however, the use of personal protective equipment reduces the potential for cumulative impacts related to exposure to numerous chemicals. WS does not anticipate cumulative risk to the public from exposure to multiple chemicals because of the methods of application and program controls, which include marking animals that people may want to harvest to indicate the animal was treated with an immobilization/accessory drug.

9 SUMMARY

WS use patterns, protocols, and label instructions for I&E drugs minimize the direct and indirect risks to the public and WS applicators. Exposure and risk to terrestrial and aquatic nontarget organisms are also negligible for all I&E drugs WS uses.

10 PREPARERS: WRITERS, EDITORS, AND REVIEWERS

10.1 Writers for "Use of Immobilization and Euthanasia Drugs in Wildlife Damage Management Risk Assessment"

Primary Writer: Andrea Lemay
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10.2 Internal Reviewers

USDA APHIS Wildlife Services

Reviewer: Are R. Berentsen
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Experience: Biologist/wildlife biologist at NWRC for 17 years. Prior experience, 10 years wildlife research at Utah State University, University of California

Reviewer: Anthony G. Duffiney
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Education: BS Fisheries and Wildlife Biology, Michigan State University
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European starlings, common and boat-tailed grackles, rock pigeons, American crows, common ravens, and ring-billed gulls.

Reviewer: John Forbes
Position: State Director, West Virginia
Education: BS Wildlife Resources, West Virginia University; MS Wildlife Ecology, Mississippi State University
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Reviewer: (John) Tyler Genders
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Reviewer: Amy T. Gilbert
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Reviewer: Steve Greiner
Position: USDA-APHIS-Wildlife Services, Safety and Occupational Health Manager, Fort Collins, CO
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Reviewer: Chad M Heuser
Position: USDA-APHIS-WS, State Director, UT
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Reviewer: Daniel Hirchert
Position: USDA-APHIS-WS, State Director, Sun Prairie, WI
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Reviewer: Shylo R. Johnson
Position: USDA APHIS WS NWRC, Biologist
Education: BS Biology – College of Idaho, MS Animal Behaviour and Welfare – University of Edinburgh
Experience: Seventeen years of service with NWRC using immobilization and euthanasia drugs

Reviewer: Michael McBride
Position: Attending Veterinarian, APHIS, Wildlife Services
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Experience: I was a zoo veterinarian for 16 years prior to joining APHIS. During this time, I anesthetized and immobilized thousands of animals and dozens of different species. I am very familiar with all the drugs spoken about in the document. Since joining APHIS, I have become an I&E instructor to provide anesthesia instruction to WS staff. I also serve on the WS I&E Sub-committee.

Reviewer: Daniel Morgan
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Reviewer: Dave Ruid
Position: USDA-APHIS-WS, District Supervisor, Rhinelander, WI (Member of the WS I/E Committee)
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Reviewer: Caleb Wellman
Position: USDA-APHIS-WS, Staff Wildlife Biologist, Sandusky, OH
Education: BS in Fish and Wildlife Management, The Ohio State University
Experience: 19 years of service in wildlife damage management with Wildlife Services, including 10 years with a focus on the immobilization of mesocarnivores.

10.3 Peer Review

The Office of Management and Budget requires agencies to have peer review guidelines for scientific documents. The APHIS guidelines were followed to have "Use of Immobilization and Euthanasia Drugs in Wildlife Damage Management" peer reviewed. WS worked with the Association of Fish and Wildlife Agencies to have experts review the documents.

10.3.1 Peer Reviewers Selected by the Association of Fish and Wildlife Agencies

Colorado Parks and Wildlife
Arizona Game and Fish Department
Wisconsin Department of Natural Resources

10.3.2 Comments

1. All game species should be tagged/marked whenever drugs are used. Add to Executive summary.
   Response: WS follows the guidance in our Immobilization and Euthanasia manual based upon the Animal Medical Drug Use Clarification Act (AMDUCA). The manual states that personnel will not chemically immobilize food animals, as defined by the State, within a specified withdrawal period before or during a harvest season unless the animal is visually tagged, held in quarantine for the specified period, or euthanized. Therefore, all game species must be marked within the specified period if they are not held or euthanized, but not whenever drugs are used. We have added language to the Executive Summary to clarify this procedure.

2. Section 1.2: Some syringe poles have a spring-loaded or CO2-charged trigger, not all.
   Response: We have changed the wording of the paragraph on syringe poles to clarify that not all syringe poles have a spring-loaded or CO2-charged trigger.

3. KCL should only be used in anesthetized animals. According to the text which indicates that only one method of take can be provided and if another IE drug was used it should have prevented KCl being indicated. KCl should never be used without a general anesthetic.
   Response: WS only uses potassium chloride in animals that have been anesthetized. We have verified this by checking individual work tasks associated with potassium chloride. Every animal euthanized with potassium chloride was anesthetized first.

4. Section 2.1.4 Johnson et al. (2023b) study discussing ketamine residues in raccoons post-immobilization. What was the result of this study?
   Response: We have included the Johnson et al. (2023b) study results, which detected ketamine in tissue samples on days 2 and 6 post-immobilization.

5. Section 3.1.5.1 Do the authors mean panting instead of sweating in reference to a reaction to intramuscular and intravenous injections of Azaperone?

Response: The study's authors referenced in this section (Pitman-Moore 1982) specifically indicate increased sweating. We are repeating the original authors' direct statement in this section.

6. Reviewer suggests including the phrase “with an anesthetic or anesthetic sedative combination.” After this sentence in Section 6.1.1 “WS administers KCl through intravenous or intracardiac injection after the animal is immobilized.”
   Response: We have edited this section of text to include the phrase “with an anesthetic or anesthetic sedative combination.

7. Executive summary “It is rare for a well-trained applicator” to miss their target during remote darting” Reviewer states: This seems inaccurate; however, I may be unfamiliar with WS darting practices. Remote darting of free-ranging wildlife is inherently challenging. In my experience, missing a free-ranging animal target is actually not uncommon for experienced individuals conducting remote darting and extremely common during helicopter darting operations. Animals may move at just the wrong moment or a wind gust might catch your dart. However, if WS personnel are primarily darting in highly controlled situations, perhaps this is not the case.
   Response: Remote darting of free-ranging wildlife can be challenging. However, WS does not often dart wildlife from helicopters and more regularly darts free-ranging wildlife in more controlled situations.

8. Section 2.1.1 “Concentrated ketamine can be purchased with a veterinary prescription.” All forms of ketamine used in wildlife require veterinary prescription/oversight - so this seems like an odd statement. Perhaps what would be more appropriate would be to say concentrated ketamine can be purchased through a compounding pharmacy?
   Response: We have edited this statement for clarity. All forms of ketamine do require veterinary prescription oversight. We have included in this statement that concentrated ketamine can be purchased with a veterinary prescription through a compounding pharmacy.

9. Section 2.2.2 It might be worth also adding that Telazol is sold in a vial containing a 1:1 ratio of tiletamine to zolazepam. This will help to make the later discussions on mg/kg dosing more clear since both drugs are at the same concentration in this mixture.
   Response: We have included the 1:1 ratio information in this statement for clarity.

10. Section 2.2.6.2 This is where it would be helpful to make sure it is understood that Tiletamine and zolazepam are at the same concentration in the vial. Otherwise, it might be unclear whether you are referring to just tiletamine or just zolazepam with this dosing information.
    Response: We agree that adding the ratio of tiletamine to zolazepam in Section 2.2.2 makes it more clear that the drugs are at the same concentration in the vial.

11. Section 3.4.1: There seems to be some confusion between what requires a prescription versus what is acquired through a compounding pharmacy. Nearly all immobilization drugs administered to wildlife require a veterinary prescription. Some drugs have to be specifically compounded into formulations with high concentration to facilitate appropriate wildlife dosing in small volumes.
    Response: We have edited this sentence to include that the medetomidine HCL is a concentrated form and is being purchased through a compounding pharmacy.

12. Section 3.6.1 To my knowledge - most nalbuphine used for wildlife immobilization will actually be purchased through a compounding pharmacy and not purchased in the commercially available concentrations.
    Response: We understand that Nalbuphine HCL may be purchased from compounding pharmacies for use with Azaperone and Medetomidine HCL. This section describes Nalbuphine HCL alone, and the statement about concentrations available is accurate.

13. Section 3.8.1 “Non-veterinarians may administer NAM without encountering record- and safe-keeping requirements (Wolfe et al. 2016a).” May want to clarify this statement that non-veterinarians my administer NAM with a veterinary prescription without encountering DEA record and safe keeping requirements. A valid veterinary client-patient relationship is required under AMDUCA and there could still be record keeping requirements from the prescribing veterinarian to ensure that legally required relationship is valid.
    Response: We have deleted this statement from the section. Record-keeping requirements are covered in another section.

14. Section 3.8.1: “In a study evaluating the use of NAM raccoon immobilization, NAM came formulated with 40 mg/mL nalbuphine, 10 mg/mL azaperone, and 10 mg/mL medetomidine (Johnson et al. 2023a). This is the formulation listed in the NalMed-A patent. Seems odd to cite it under this publication when multiple publications have used this formulation and the 2016 patent describes this formulation?
    Response: We have cited Johnson et al. 2023a here because we reference the study done by our WS NWRC staff utilizing the drug combination. We have cited the 2016 patent and other studies throughout this section.

15. Table 14. Atipamezole, and Atropine: This might benefit from revision to differentiate between recommendations for atropine vs atipamezole. Uncertain of USDA WS protocols, but we have seen adverse reactions to full doses (and even 50% doses) of atipamezole given intravenously (tremors, ataxia, head-pressing) and zoopharm has reported issues with renarcotization when full dose of atipamezole is given intravenously. Recommendation should be to give most of atipamezole intramuscularly and likely no more than 33% of the dose should go IV.
    Response: We have edited Table 14 to account for the potential hazard to human safety with an intravenous injection for all alpha-antagonists. We have also changed the atropine method of administration to correctly identify its use.

16. I assume unrecovered darts, if any, would be documented? Any statistics at hand to back up success of dart recovery?
    Response: WS Immobilization and Euthanasia manual instructs employees to recover every dart/missed dart used. Therefore WS does not track dart recovery.

17. Executive summary: “WS follows withdrawl periods and often tags animals…” "often tags" could be open for interpretation or misinterpretation. Would it be more appropriate to say something like "tags according to x,y,z guidelines" etc.
    Response: WS follows the guidance in our Immobilization and Euthanasia manual based upon the Animal Medical Drug Use Clarification Act (AMDUCA). The manual states that personnel will not chemically immobilize food animals, as defined by the State, within a specified withdrawal period before or during a harvest season unless the animal is: visually tagged, held in quarantine for the specified period, or euthanized. Therefore, all game species must be marked within the specified period if they are not held or euthanized, but not whenever drugs are used. We have added language to the Executive Summary to clarify this procedure.

18. Section 7.2.5 Is it worth mentioning/enforcing that training/protocols cover accidental exposure? I.e. if it were to happen, staff are prepared to know what to do?
    Response: It is worth mentioning that training/protocols cover accidental exposure. We have edited this section to include references to the WS Immobilization and Euthanasia training manual, which covers steps to take in case of accidental exposure. These include maintaining field copies of SDS and/or labels, notifying local hospitals before conducting operations, noting the time of exposure, and transporting the exposed person to a local emergency clinic.

19. Is there consideration for updating the database so that this information can be accurately summarized? I don't know if that is important long-term or not, but would possibly prevent future misinterpretation of the data.
    Response: The database that is used to summarize WS operational data is being updated to a new system. It is possible that irregularities such as only being able to list a single method of take with an animal will be resolved in the tracking software.

20. Table 14. Tolazoline, yohimbine HCl: The one area where a risk might need to be addressed is in the statements that drug antagonists are “most effective when given intravenously. Full intravenous administration of some drug antagonists can create a hazard for human safety as animals may have rapid recoveries that do not allow sufficient time for humans to move to a safe distance from the recovering animal. They can also pose a hazard to the animals with certain drug combinations that may result in difficult recoveries or renarcotization when antagonists are given intravenously. I would suggest revising this to either specify what is meant by “most effective” to ensure that is not confused with the recommendation for route of administration or to specify the recommended route of administration or simply list available routes of administration.

Response: We have edited Table 14 to account for the potential hazard to human safety with an intravenous injection for all alpha-antagonists.

Comments received not requiring a response.

1. Overall, I found the document very thorough and detailed. From the perspective of a state wildlife manager, I found no significant questions or concerns and think that potential risks were adequately considered and addressed. Specifically, I found all portions of the assessment to be thorough and complete. I also found mitigation recommendations/actions as described to minimize adverse impacts were well-reasoned and defensible.

2. Document is complete and of good quality. Standard operating procedures described in the document will minimize or mitigate the risk to human, animal, and environmental health. Descriptions of hazards and risks are accurate and complete. Assumptions and uncertainties are clearly stated. References appear appropriate.

3. This document is thorough and describes the various immobilization and euthanasia drugs accurately and in sufficient detail without overly in-depth literature reviews for each substance.

4. Hazards are appropriately identified and addressed in the document.

APPENDIX 1. Over-the-Counter Antibiotics

A1.1 Chemical Description and Product Use

Over-the-counter topical antibiotics such as Neosporin® are applied to skin abrasions and cuts to prevent post-capture infections. These topical antibiotics are used infrequently in small quantities. Most topical antibiotics contain one or a combination of the following anti-infective components: polymyxin B sulfate, neomycin sulfate, and bacitracin zinc (GlaxoSmithKline 2013). Polymyxin B sulfate and neomycin sulfate act against the following microorganisms: *Staphylococcus aureus*, *Escherichia coli*, *Haemophilus influenza*, *Klebsiella-Enterobacter* species, *Neisseria* species, and *Pseudomonas aeruginosa*. Bacitracin is active against most Gram-positive bacteria, *Neisseria* spp., and *Haemophilus influenza* (GlaxoSmithKline 2013). WS uses antibiotics to treat minor skin abrasions or cuts in wildlife prior to releasing the animal.

A1.2 Physical and Chemical Properties

Topical antibiotic ointments are typically a clear, pale yellow, or white gel composed of substances such as polymyxin B sulfate, neomycin sulfate, or bacitracin zinc (GlaxoSmithKline 2013, Johnson and Johnson 2009, Pfizer 2000). One common antibiotic ointment, Neosporin, is slightly soluble in water but a stable product (Pfizer 2000).

A1.3 Environmental Fate

Environmental fate of topical antibiotics is not available.

A1.4 Metabolism

Antibiotic ointments are applied topically to the skin. Systemic absorption of bacitracin, neomycin, and polymyxin B is negligible, except when applied to large areas or extended periods. Polymyxin B has a high affinity for cell membranes, so there is little systemic absorption when applied to open wounds. Bacitracin and neomycin could be absorbed systemically if applied to damaged skin. If systemic absorption does occur, bacitracin and neomycin are excreted renally (PDR 2018). Approximately 10−40% of the bacitracin is excreted within 24 hours. The elimination half-life of bacitracin is 1.5 hours (NCBI 2023x).

A1.5 Hazard Identification

Humans started using triple antibiotic ointments containing neomycin, polymyxin B, and bacitracin as prescription products in the 1950s. Triple antibiotic ointments have been available over the counter since the 1970s (Jones et al. 2006). If used in large quantities or in large areas for prolonged periods of time, neomycin sulfate can cause toxicity; neomycin sulfate, polymyxin B sulfate, and bacitracin zinc can cause nephrotoxicity; and polymyxin B sulfate can cause neurotoxicity (GlaxoSmithKline 2013). Some individuals have reported allergic sensitization after using antibiotic ointments. In one clinical study, neomycin-induced allergic skin reactions occurred

in 0.09% of individuals, while another study reported the incidence at approximately 1% (GlaxoSmithKline 2013). Increased absorption in very young children is also possible; therefore, products such as Neosporin are not recommended for use in children under two years (GlaxoSmithKline 2013).

A1.5.1 Acute Toxicity

The acute oral LD_{50} of bacitracin zinc is 2,000 mg/kg in guinea pigs and greater than 3,750 mg/kg in house mice. The acute oral LD_{50} of bacitracin zinc, neomycin sulfate, and polymyxin B sulfate in house mice is >3,750 mg/kg, 8,000 mg/kg, and 750 mg/kg, respectively (Table A3-1).

Table A1-1. Acute toxicity values for over-the-counter antibiotics.

Chemical	Test Species	Test Type	LD_{50}	Reference
Bacitracin zinc	House Mouse*	intravenous	360 mg/kg	(Akorn Animal Health 2015)
Bacitracin zinc	Brown Rat*	Intraperitoneal	190 mg/kg	(Akorn Animal Health 2015)
Bacitracin zinc	House Mouse*	Intraperitoneal	300 mg/kg	(Akorn Animal Health 2015)
Bacitracin zinc	House Mouse*	Acute oral	>3,750 mg/kg	(Akorn Animal Health 2015)
Bacitracin zinc	Guinea pig	Acute oral	2,000 mg/kg	(Akorn Animal Health 2015)
Bacitracin zinc	Japanese Quail	Acute oral	>316 mg/kg	(Akorn Animal Health 2015)
Neomycin sulfate	House Mouse*	Acute oral	8,000 mg/kg	(Akorn Animal Health 2015)
Neomycin sulfate	House Mouse*	Intravenous	17,400 mg/kg	(Akorn Animal Health 2015)
Neomycin sulfate	House Mouse*	Intraperitoneal	305 mg/kg	(Akorn Animal Health 2015)
Polymyxin B sulfate	House Mouse*	Acute oral	750-790 mg/kg	(Akorn Animal Health 2015, Pfizer 2000)
Polymyxin B sulfate	House Mouse*	Intravenous	5,400 µg/kg	(Akorn Animal Health 2015)
Polymyxin B sulfate	House Mouse*	Intraperitoneal	20,500 µg/kg	(Akorn Animal Health 2015)

* Domestic laboratory strains.

A1.5.2 Sublethal and Chronic Toxicity

The LD_{Lo} of polymyxin B sulfate given to dogs intravenously was reported to be 8 mg/kg (Akorn Animal Health 2015).

A1.6 Dose-Response Assessment

A1.6.1 Human Health Dose Response

In humans, topical antibiotic ointments may cause allergic contact dermatitis (NCBI 2023x). Polymyxin B is toxic to kidneys and the nervous system and hazardous if inhaled or ingested (Sciencelab 2013). On the other hand, Neomycin sulfate has a low absorption rate even if ingested; approximately 97% of the drug is eliminated unchanged in the feces. Prolonged consumption of oral neomycin sulfate tablets could result in systemic drug levels that are high enough to produce neurotoxicity, ototoxicity (toxic to the ear), and nephrotoxicity (NLM 2017c). Dermal absorption of bacitracin ointment is negligible. Intramuscular injection of bacitracin is widely distributed in the body (NCBI 2023x). Nephrotoxicity occurred when 200,000 units of

bacitracin were given parenterally for several days, while oral doses of up to 240,000 units caused virtually no side effects or absorption (NCBI 2023x).

A1.6.2 Ecological Dose Response

We are unable to find information on the ecological dose response for over-the-counter antibiotics.

APPENDIX 2. FY16-20 Data Tables

Table A2-1a. The annual average number of animals immobilized with ketamine, with or without xylazine, by WS in WDM activities from FY16 to FY20 throughout the United States and the final disposition of the animal (euthanized or freed). To standardize the data for the table, drugs tracked in cubic centimeters were converted to mg of drug used.

Species	Euthanized	Freed	Ketamine (mg)	Xylazine (mg)
Gray Wolf	0.2	29	10,214	2,021
Gray Fox	0	8	458	92
Black Bear	1	25	12,308	3,098
Raccoon	43	4,159	250,128	49,928
Striped Skunk	38	157	10,163	2,033
Virginia Opossum*	3	5	1,033	207
Red Fox	0.4	5	290	58
Other Predators (11 spp.)[1]	0.2	14	2,866	515
Wild Turkey	0.8	2.4	1,166	294
White-tailed Deer	0	6.6	2,382	1,016
Mule	0	0.2	0	20
Total (19 spp.)[3]	**87**	**4,405**	**291,008**	**59,282**

* - Introduced populations Other predator = badger, beaver, bobcat, coyote, domestic dog, feral/free-roaming dog, fisher, mountain lion, mink, porcupine, and river otter [1] Other predator species include domestic dogs, and feral/free-ranging dog, subspecies of the wolf, and thus, not included in the total species count.

Table A2-1b. The annual average number of animals immobilized with tiletamine-zolazepam, with or without xylazine, by WS in WDM activities from FY16 to FY20 throughout the United States and the final disposition of the animal (euthanized or freed). To standardize the data for the table, drugs tracked in cubic centimeters were converted to mg of drug used.

Species	Euthanized	Freed	Tiletamine-Zolazepam (mg)	Xylazine (mg)
Gray Wolf	0	9	5,900	0
Black Bear[2]	0.2	5	2,650	0
- Louisiana Black Bear[T&E 1]	0.2	12	6,703	0
Grizzly Bear[T&E]	0	0.6	1,300	0
Other Predators (6 spp.)[2]	2	7	1,246	0
Feral Swine	0	10	2,596	1,100
Other Ungulates (3 spp.)	0	4	1,054	710
Total (15 spp.)	**2.6**	**50.6**	**21,449**	**1,810**

Other predator = bobcat, dog*, mountain lion, raccoon, Virginia opossum, and striped skunk; Other ungulate = mule deer, white-tailed deer, moose. [1] T&E = Federally listed threatened and endangered species – the Louisiana black bear is no longer listed but included. [2] Other predator species include domestic dogs, a subspecies of the wolf, and thus, not included in the total species count.

Table A2-1c. The annual average number of animals immobilized with nalbuphine, azaperone, and medetomidine (NAM) by WS in WDM activities from FY16 to FY20 throughout the United States and the final disposition of the animal (euthanized or freed). To standardize the data for the table, drugs tracked in cubic centimeters were converted to mg of drug used.

Species	Euthanized	Freed	Nalbuphine-Azaperone-Medetomidine (cc)
Raccoon	2.6	15.6	5.7
Striped Skunk	15.4	5.8	5.5

Coyote	0.8	0.2	2.5
Feral/free-ranging Cat	0.8	1.8	1.2
Other Predator (4 spp.)	0.2	2.2	1.7
Total (8 spp.)	**19.8**	**25.6**	**16.6**

Other predator = Gray wolf, mountain lion, Virginia opossum, and porcupine.

Table A2-2a. The annual average number of animals euthanized with potassium chloride by WS in WDM activities from FY16 to FY20 throughout the United States.

Species	Euthanized	Potassium Chloride (KCl) (cc)
Raccoon	19.2	70.6
Striped Skunk	11	44.3
Feral/Free-roaming Cat	2.4	6.8
Louisiana Black Bear[T&E 2]	0.2	12
Total	**32.8**	**133.7**

Table A2-2b. The annual average number of animals euthanized with sodium pentobarbital (390 mg/ml) and Fatal Plus solution by WS in WDM activities from FY16 to FY20 throughout the United States.

Species	Euthanized	Sodium Pentobarbital (sodium pentobarbital (390mg/mL) and Fatal Plus)
Striped Skunk	1,580	3,144
Raccoon	164	55
Virginia Opossum*	87	134
Common Gray Fox	8	21.6
Other Predator (6	6.8	21.3
Fox Squirrel	2.2	2.2
Mule Deer	0.6	3.4
Total	**1848.6**	**3,381.5**

* - Introduced populations; Other predator –bobcat, coyote, red fox**, black bear, feral/free-roaming cat, mink

Table A2-3a. The annual average number of animals recovered with the alpha-2 antagonist drug Atipamezole by WS in WDM activities from FY11 to FY15 throughout the United States.

Species	Freed	Atipamezole (5 and 25 mg/mL; cc)
Gray Wolf	8.8	67.8
Coyote	1	16.6
Other Predator (4 Sp.)	14	62
White-tailed Deer	0.2	0.55
Total	24	147

* Introduced Species Other predator –black bear, feral/free-roaming cat, Mountain lion, raccoon

Table A2-3b. The annual average number of animals recovered with the alpha-2 antagonist drug Tolazoline by WS in WDM activities from FY11 to FY15 throughout the United States.

Species	Freed	Tolazoline (100mg/mL; cc)
Feral Swine*	7.2	1,912
Mountain Lion	0.8	160
Mule Deer	1.4	400

White-tailed Deer	0.4	36
Total	**9.8**	**2,508**

Table A2-3c. The annual average number of animals recovered with the alpha-2 antagonist drug Yohimbine by WS in WDM activities from FY11 to FY15 throughout the United States.

Species	Freed	Yohimbine (2 mg/mL; cc)
Gray Wolf	10.6	44
Total	**10.6**	**44**

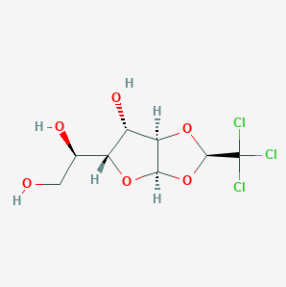
Table A2-4. States where WS used wildlife drugs for FY16–FY20.

State	Ketamine (mg)	Tiletamine -Zolazepam (mg)	Xylazine (mg)	NAM (cc)	Alpha$_2$ Antag. (cc)	Euthanasia (cc)
AL	17,073	-	3,410	-	-	-
AZ	-	74	-	-	-	-
CA	1,402	3,292	1,976	-	2,618	2,931
GA	7,026	-	1,392	-	-	-
ID	-	4,830	-	-	-	-
IL	-	174	92	-	36	-
KS	-	2.1	80	-	-	5.2
KY	675	-	135	-	-	1.8
LA	-	6,843	-	-	-	12
MA	11,257	-	2,251	-	-	-
MD	6,560	-	1,304		-	-
ME	19,839	-	3,535	-	-	15.5
MI	1,078	-	208	-	2.8	-
MN	5,628	-	866	-	0.84	-
MT	-	4,901	-	-	-	-
NC	13,604	348	2,686	-	-	-
ND	-	-	-		-	0.6
NE	-	-	-	-	-	256
NJ	3,335	-	1,211	-	.02	-
NM	180	-	420	-	-	-
NY	24,731	-	4,946	-	-	-
OH	41,517	-	8,303	-	-	1
OR	-	170	-	-	-	2.4
PA	13,428	-	2,686	-	-	0.2
TN	16,473	-	3,235	-	-	11.6
TX	917	-	183	-	-	23.4
UT	200	-	40	-	-	237
VA	27,602	-	5,520	-	-	0.4
VT	45,701	620	9,140	-	-	-
WA	-	40	-	-	-	2.4
WI	14,273	-	2,807	-	107.3	7.6
WV	18,293	-	3,659	-	-	-
WY	2,330	520	466	16.6	29.9	117
Total	**293,122**	**21,814**	**60,551**	**16.6**	**2,794.9**	**3,625.1**
States	**23**	**12**	**25**	**1**	**7**	**17**

APPENDIX 3. Chemical Structures.

Source: PubChem, accessed April 6, 2023 at https://pubchem.ncbi.nlm.nih.gov/

ANESTHETICS



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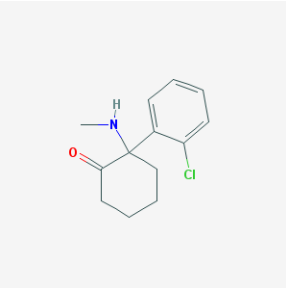

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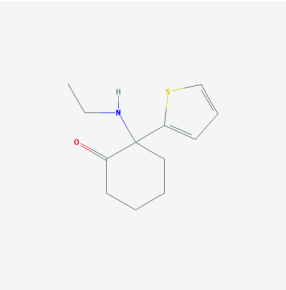

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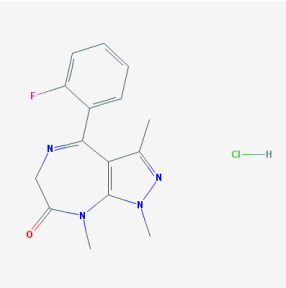

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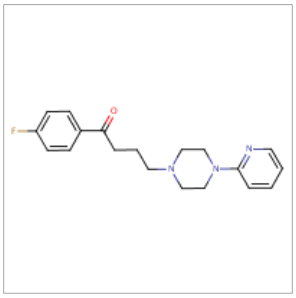

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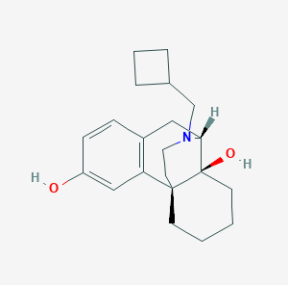

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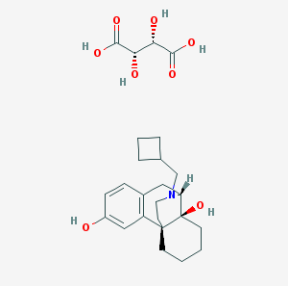

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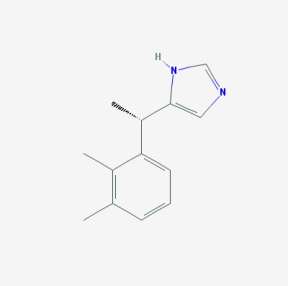

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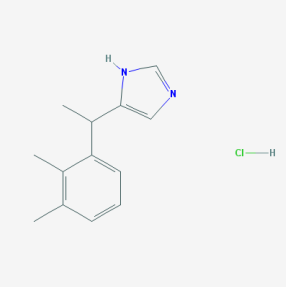

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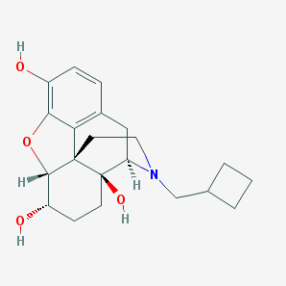

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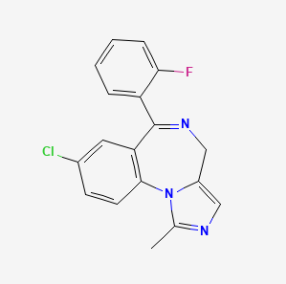

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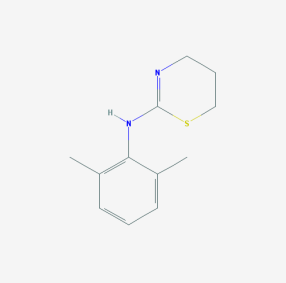

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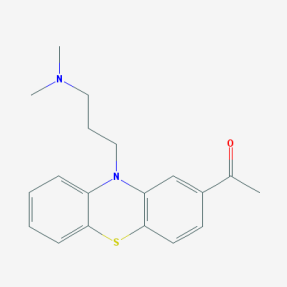

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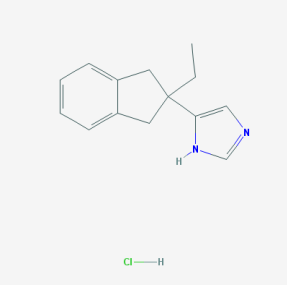

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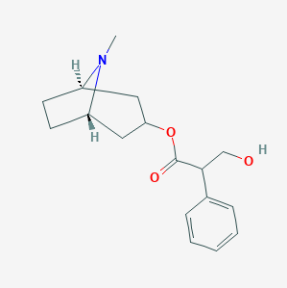

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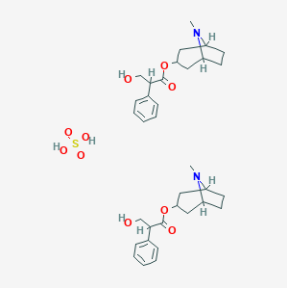

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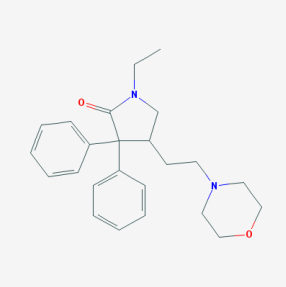

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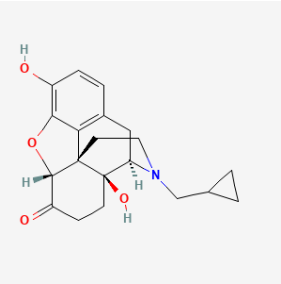

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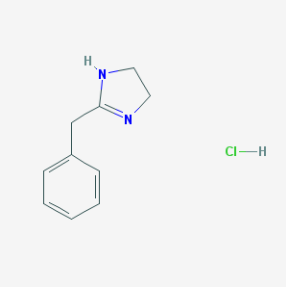

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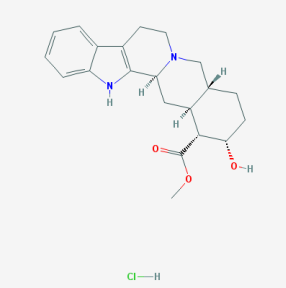
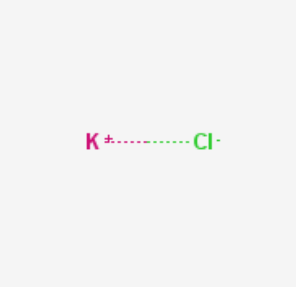


Yohimbine hydrochloride

EUTHANASIA AGENTS



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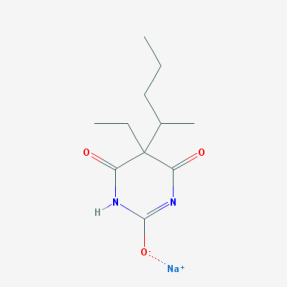

Sodium pentobarbital

REFERENCES

21 CFR § 522.246. 2023. Food and Drugs. Chapter 1. Food and Drug Administration. Department of Health and Human Services. Sub chapter E. Animal Drugs, Feeds, and Related Products, Implantation or Injectable Dosage Form New Animal Drugs. Butorphanol.

Acosta-Jamett, G., F. Astorga-Arancibia, and A. Cunningham. 2010. Comparison of chemical immobilization methods in wild foxes (*Pseudalopex griseus* and *Pseudalopex culpaeus*) in Chile. Journal of Wildlife Diseases 46:1204-1213.

Akorn Animal Health. 2015. SDS: Neomycin and Polymyxin B Sulfates and Bacitracin Zinc Ophthalmic Ointment, USP. Akorn Animal Health, Inc. Lake Forest, IL, USA.

_____. 2018a. Tolazoline Injection (tolazoline HCL, USP). Akorn Animal Health, Inc. Lake Forest, IL, USA.

_____. 2018b. Yobine® (yohimbine sterile solution) injection – veterinary use. Akorn Animal Health, Inc. Lake Forest, IL, USA.

Alexander, R.S., B.R. Canver, K.L. Sue, and K.L. Morford. 2022. Xylazine and overdoses: trends, concerns, and recommendations. American Journal of Public Health 112:1212-1216.

Algren, D.A., and A. Ashworth. 2015. Acute acepromazine overdose: clinical effects and toxicokinetic evaluation. Journal of Medical Toxicology 11:121-123.

Ali-Melkkilä, T., J. Kanto, and E. Iisalo. 1993. Pharmacokinetics and related pharmacodynamics of anticholinergic drugs. Acta Anaesthesiologica Scandinavica 37:633-642.

Ali, S., M.K. Richardson, and J. Aalders. 2014. Teratological effects of a panel of sixty water-soluble toxicants on zebrafish development. Zebrafish 11:129-141.

Aliyev, E., O. Görgülü, Z. Yalvaç, S. Biçer, E. Türköz Acar, M. Charezhaz, and A. Vitrinel. 2015. Atropine intoxication in a child after accidental ingestion of 200 mg atropine sulfate – a case report. Prensa Médica Argentina 101:1-3.

Alkermes Inc. 2016. REMA NDA 21-897 VIVITROL® (naltrexone for extended-release injectable suspension) Opioid Antagonist. Alkermes, Inc. Waltham, MA, USA.

American Society of Health-System Pharmacists. 2017. AHFS Drug Information 2017. American Society of Health Systems, Bethesda, MD, USA.

Amitai, Y., S. Almog, R. Singer, R. Hammer, Y. Bentur, and Y.L. Danon. 1992. Atropine poisoning in children during the Persian Gulf crisis: a national survey in Israel. Journal of the American Medical Association 268:630-632.

AnaSed®. 2022. Xylazine hydrochloride injection, solution [Package insert] AKORN Operating Company LLC. Gurnee, IL, USA. Rev. 06/2022.

Antisedan®. 2012. Atipamezole hydrochloride injection, solution [package insert] Orion Corporation, Espoo, Finland Rev. 10/2012.

APHA. 2015. Veterinary Compendium, search results for Dopram-V. http://www.apha.ie/AnimalHealth/VeterinaryCompendium.aspx accessed June 6, 2023.

AVMA. 2020. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. . American Veterinary Medical Association.

Azizpour, A., and Y. Hassani. 2012. Clinical evaluation of general anaesthesia in pigeons using a combination of ketamine and diazepam. Journal of the South African Veterinary Association 83:4.

Bagsby, C., A. Saha, G. Goodwin, S. Siddiqi, M. Farone, A. Farone, and P.C. Kline. 2018. Stability of pentobarbital in soil. Journal of Environmental Science and Health, Part B 53:207-213.

Bahri, L. 2008. Pharm Profile: Atipamezole. Compendium 30:256-258.

Baker, D.R., and B. Kasprzyk-Hordern. 2013. Spatial and temporal occurrence of pharmaceuticals and illicit drugs in the aqueous environment and during wastewater treatment: New developments. Science of the Total Environment 454-455:442-456.

Baldridge, S., R. Gehring, J.F. Coetzee, and J. Havel. 2011. Pharmacokinetics and physiologic effects of intramuscularly administered xylazine hydrochloride-ketamine hydrochloride-butorphanol tartrate alone or in combination with orally administered sodium salicylate on biomarkers of pain in Holstein calves following castration and dehorning. American Journal of Veterinary Research 72:1305-1317.

Ballard, S., T. Shults, A. Kownacki, J.W. Blake, and T. T. Tobin. 1982. The pharmacokinetics, pharmacological responses and behavioral effects of acepromazine in the horse. Journal of Veterinary Pharmacology and Therapeutics 5:21-31.

Barahona, M.V., and S. Sánchez-Fortún. 1999. Toxicity of carbamates to the brine shrimp *Artemia salina* and the effect of atropine, BW284c51, iso-OMPA and 2-PAM on carbaryl toxicity. Environmental Pollution 104:469-476.

Bayer Healthcare. 2014. Safety Data Sheet – Acepromazine Maleate 10mg Injectable. Version 1.0. Bayer Healthcare LLC Shawnee, KS, USA.

_____. 2015. Acepromazine maleate – acepromazine maleate injection. Archived drug label. Revised 8/2015.

Berlan, M., R. Le Verge, J. Galitzky, and P. Le Corre. 1993. Alpha-2-adrenoceptor antagonist potencies of two hydroxylated metabolites of yohimbine. British Journal of Pharmacology 108:927-932.

Bertelsen, M.F., and L. Villadsen. 2009. A comparison of the efficacy and cardiorespiratory effects of four medetomidine-based anaesthetic protocols in the red fox (*Vulpes vulpes*). Veterinary Anaesthesia and Analgesia 36:328–333.

Boehringer Ingelheim. 2018. Safety data sheet: Doxapram HCl. Boehringer Ingelheim Vetmedica, Inc. St. Joseph, MO, USA.

_____. 2019. Safety data sheet: PromAce® Injectable (acepromazine maleate). Boehringer Ingelheim Animal Health USA Inc. Duluth, GA, USA.

Brammer, D.W., B.J. Doerning, C.E. Chrisp, and H.G. Rush. 1991. Anesthetic and nephrotoxic effects of Telazol in New Zealand white rabbits (abstract only). Laboratory Animal Science 41:432-435.

Brasch, J., H. Hessler, and E. Christophers. 1991. Occupational (photo)allergic contact dermatitis from azaperone in a piglet dealer. Contact Dermatitis 25:258.

Brown, P.B., M.R. White, J. Chaille, M. Russell, and C. Oseto. 1996. Evaluation of three anesthetic agents for crayfish (*Orconectes virilis*). Journal of Shellfish Research 15:433-435.

Brunton, L., and B. Knollmann. 2022. Goodman and Gilman's the Pharmacological Basis of Therapeutics, 14th Edition. McGraw-Hill Education.

Calleja, M.C., G. Persoone, and P. Geladi. 1994. Comparative acute toxicity of the first 50 multicentre evaluation of in vitro cytotoxicity chemicals to aquatic non-vertebrates. Archives of Environmental Contamination and Toxicology 26:69-78.

Carvalho, F.D., I. Machado, M. Sánchez Martínez, A. Soares, and L. Guilhermino. 2003. Use of atropine-treated *Daphnia magna* survival for detection of environmental contamination by acetylcholinesterase inhibitors. Ecotoxicology and Environmental Safety 53:43-46.

Cattet, M. 2003. A CCWHC Technical Bulletin: Drug residues in wild meat – addressing a public health concern. Paper 46. *in* Canadian Cooperative Wildlife Health Centre: Newsletters and Publications.

Cayman Chemical. 2020. Safety Data Sheet, Yohimbine (hydrochloride). Cayman Chemical, Ann Arbor, MI, USA.

_____. 2022a. Product Information, Atipamezole (hydrochloride). Cayman Chemical, Ann Arbor, MI, USA.

_____. 2022b. Safety Data Sheet, Xylazine. Cayman Chemical, Ann Arbor, MI, USA.

_____. 2022c. Product Information, Tolazoline (hydrochloride). Cayman Chemical, Ann Arbor, MI, USA.

_____. 2023a. Safety Data Sheet, Medetomidine (hydrochloride). Cayman Chemical, Ann Arbor, MI, USA.

_____. 2023b. Safety Data Sheet, Dexmedetomidine (hydrochloride). Cayman Chemical, Ann Arbor, MI, USA.

Chassaing, C., D. Godeneche, M. Boucher, and P. Duchene-Marullaz. 1979. A comparison of changes in atropine-induced tachycardia and atropine concentration in conscious dogs. European Journal of Pharmacology 58:433-441.

Choi, J., M. Lamshöft, S. Zühlke, A.M. Abd El-Aty, M.M. Rahman, S.W. Kim, J. Shim, and M. Spiteller. 2014. Analyses and decreasing patterns of veterinary antianxiety medications in soils. Journal of Hazardous Materials 275:154-165.

Chou, C.C., C.L. Chen, A.C. Asbury, A.I. Webb, and T.W. Vickroy. 1998. Development and use of an enzyme-linked immunosorbent assay to monitor serum and urine acepromazine concentrations in thoroughbreds and possible changes associated with exercise. American Journal of Veterinary Research 59:593-597.

Chung, H., H. Choi, E. Kim, W. Jin, H. Lee, and Y. Yoo. 2000. A fatality due to injection of tiletamine and zolazepam. Journal of Analytical Toxicology 24:305-308.

Clarke, K.W., and G.C.W. England. 1989. Medetomidine, a new sedative-analgesic for use in the dog and its reversal with atipamezole. Journal of Small Animal Practice 30:343-348.

Collard, J.F., and R. Maggs. 1958. Clinical trial of acepromazine maleate in chronic schizophrenia. British Medical Journal 1:1452–1454.

Cook, W., D. Cain, T. Hensley, W. Bluntzer, W. Lance, L. Dobson, R. McDaniel, and D. Davis. 2016. Tissue residue levels of butorphanol, azaperone, medetomidine, atipamezole, and naltrexone in white-tailed deer (*Odocoileus virginianus*) at 11 and 21 days post intramuscular injection. Poultry, Fisheries and Wildlife Sciences 4:1000168.

Cording, C.J., R. DeLuca, T. Camporese, and E. Spratt. 1999. A fatality related to the veterinary anesthetic Telazol. Case Report. Journal of Analytical Toxicology 23:552-555.

DEA. 2023. Nalbuphine Hydrochloride (Trade Name: Nubain®). Drug Enforcement Administration, Diversion Control Division, Drug and Chemical Evaluation Section.

Dechra Veterinary Products LLC. 2020. TZED (TILETAMINE HYDROCHLORIDE AND ZOLAZEPAM HYDROCHLORIDE) tiletamine and zolazepam for injection. Dechra Veterinary Products LLC, Overland Park, KS, USA.

Díaz-Cruz, M.S., M.J. López de Alda, and D. Barceló. 2003. Environmental behavior and analysis of veterinary and human drugs in soils, sediments and sludge. Trends in Analytical Chemistry 22:340-351.

DOPRAM-V. 2013. doxapram hydrochloride injection, Boehringer Ingelheim Vetmedica, Inc. St. Joseph, MO USA Rev. 02/2013

https://dailymed.nlm.nih.gov/dailymed/archives/fdaDrugInfo.cfm?archiveid=101238 accessed Accessed December 20, 2023.

DrugBank. 2021a. DrugBank Online, queried Tolazoline. Drug Bank Accession number DB00797. https://go.drugbank.com/drugs/DB00797 accessed January 03, 2023.

_____. 2021b. DrugBank Online, queried Butorphanol. Drug Bank Accession number DB00611. https://go.drugbank.com/drugs/DB00611 accessed January 03, 2023.

_____. 2021c. DrugBank Online, queried Acepromazine. Drug Bank Accession number DB01614. https://go.drugbank.com/drugs/DB01614 accessed June 06, 2023.

_____. 2021d. DrugBank Online, queried Zolazepam. DrugBank Accession number DB11555. https://go.drugbank.com/drugs/DB11555 accessed June 06, 2023.

_____. 2021e. DrugBank Online, queried Tiletamine. Drug Bank Accession number DB11549. https://go.drugbank.com/drugs/DB11549 accessed January 03, 2024.

_____. 2021f. DrugBank Online, queried Medetomidine. Drug Bank Accession number DB11428. https://go.drugbank.com/drugs/DB11428 accessed January 03, 2024.

_____. 2023a. DrugBank Online, queried Midazolam. DrugBank Accession number DB00683. https://go.drugbank.com/drugs/DB00683 accessed June 06, 2023.

_____. 2023b. DrugBank Online, queried doxapram. Drug Bank Accession number DB00561. https://www.drugbank.ca/drugs/DB00561 accessed June 06, 2023.

_____. 2023c. DrugBank Online, queried Nalbuphine. DrugBank Accession number DB00844. https://go.drugbank.com/drugs/DB00844 accessed January 03, 2024.

Drugs.com. 2015. Dexmedetomidine HCl. https://www.drugs.com/vet/dexmedetomidine-hcl.html accessed January 4, 2024.

_____. 2022. Nembutal Prescribing Information. https://www.drugs.com/pro/nembutal.html accessed June 06, 2023.

_____. 2023a. Dopram-V Injectible. https://www.drugs.com/vet/dopram-v-injectable.html accessed June 06, 2023.

_____. 2023b. Fatal-Plus solution. https://www.drugs.com/vet/fatal-plus-solution.html accessed 06/06/2023.

_____. 2023c. Nubain Prescribing Information. https://www.drugs.com/pro/nubain.html accessed 06/06/2023.

EFSA. 2013. Scientific opinion on the evaluation of the safety in use of Yohimbe (*Pausinystalia yohimbe* (K.Schum.) Pierre ex Beille). EFSA Journal 11:3302.

Elkins-Sinn, I. 1995. Atropine Sulfate Injection (ESI76465) Elkins-Sinn Inc. Cherry Hill, NJ, USA. https://www.uww.edu/apps/riskmanagement/msds/atropine_sulfate_injection_elkins-sinn-_inc._x.x.xx.pdf Accessed December 20, 2023. *in.*

Elliott, S.P., and K.A. Hale. 1999. A previously unidentified acepromazine metabolite in humans: implications for the measurement of acepromazine in blood. Journal of Analytical Toxicology 23:367-371.

Ellis, C.K., M.E. Wehtje, L.L. Wolfe, P.L. Wolff, C.D. Hilton, M.C. Fisher, S. Green, M.P. Glow, J.M. Halseth, M.J. Lavelle, N.P. Snow, E.H. VanNatta, J.C. Rhyan, K.C. VerCauteren, W.R. Lance, and P. Nol. 2019. Comparison of the efficacy of four drug combinations for immobilization of wild pigs. European Journal of Wildlife Research 65: 78:12 pp.

EMA. 2011a. Assessment Report. Dexdor: dexmedetomidine. European Medicines Agency EMEA/H/C/002268.

_____. 2011b. Assessment Report. Buccolam, Midazolam. European Medicines Agency EMEA/H/C/002267.

_____. 2021. Dexdomitor: EPAR – Product Information. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/veterinary/medicines/000070/vet_med_000110.jsp&mid=WC0b01ac058001fa1c accessed 06/06/2023.

EMEA. 1997. Committee for Veterinary Medicinal Products, Ketamine Summary Report. Document EMEA/MRL/315/97-FINAL, December 1997. European Agency for the Evaluation of Medicinal Products.

_____. 1998. Committee for Veterinary Medicinal Evaluation, butorphanol tartrate summary report Document EMEA/MRL/323/97-FINAL. European Agency for the Evaluation of Medicinal Products.

_____. 1999. Committee for Veterinary Medicinal Products, Doxapram Summary Report, EMEA/MRL/533/98-Final. The European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit.

_____. 2002a. Dexmedetomidine – Scientific Discussion. European Agency for the Evaluation of Medicinal Products.

_____. 2002b. Committee for Veterinary Medicinal Products, Xylazine Hydrochloride Summary Report. Document EMEA/MRL/611/99-FINAL. European Agency for the Evaluation of Medicinal Products.

Fàbrega, F., M. Marquès, A. Ginebreda, M. Kuzmanovic, D. Barceló, M. Schuhmacher, J.L. Domingo, and M. Nadal. 2013. Integrated risk index of chemical aquatic pollution (IRICAP): case studies in Iberian rivers. Journal of Hazardous Materials 263P:187-196.

FAO. 1991. Residues of some veterinary drugs in animals and foods: Azaperone, Carazolol, Febantel, Fenbendazole, Oxfendazole, Propionylpromazine, Spiramycin, and Tylosin. Food and Nutrition Paper 41/4 Food and Agriculture Organization of the United Nations.

_____. 1997. Residues of some veterinary drugs in animals and foods: xylazine. FAO Corporate Document Repository. Agriculture and Consumer Protection. Food and Agriculture Organization of the United Nations.

_____. 2018. Codex Alimentarius. Maximum Residue Limits (MRLs) and Risk Management Recommendations (RMRs) for Residues of Veterinary Drugs in Foods. CAC/MRL 2-2018. Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO).

FARAD. 2019. Withdrawal Interval (WDI) Recommendations Atropine. http://farad.org/wdilookup/wdi_cattle.html accessed 06/06/2023.

FDA. 1998. Center for Drug Evaluation and Research, Application number 75046, butorphanol tartrate injection. U.S. Food and Drug Administration.

_____. 2002. Food and Drug Administration/Center for Veterinary Medicine Report on the Risk of Pentobarbital in Dog Food.

_____. 2005. Center for Drug Evaluation and Research Approval Package for Application Number 14-879/ S-044. Drugs@FDA: FDA Approved Drug Products, search results for Dopram. U.S. Food and Drug Administration.

_____. 2010. Freedom of Information Summary: Supplemental New Animal Drug Application, NADA 141-267, Dexdomitor. U.S. Food and Drug Administration.

_____. 2016. Nubain – (nalbuphine hydrochloride) injection, for intramuscular, subcutaneous, or intravenous use, Reference ID: 4027891. U.S. Food and Drug Administration.

_____. 2020. Approved Animal Drug Products (Green Book); Domitor. https://animaldrugsatfda.fda.gov/adafda/views/#/search accessed January 04, 2024.

_____. 2024. Approved Animal Drug Products (Green Book); Antagonil. https://animaldrugsatfda.fda.gov/adafda/views/#/home/previewsearch/140-874 accessed January 05, 2024.

Federal Institute for Risk Assessment. 2016. Scientific assessment of yohimbe (*Pausinystalia yohimbe*). Annex 5 to 5-3539-02-5543103, Germany. German Federal Institute for Risk Assessment (BfR).

Forney, B. 2013. Acepromazine maleate for veterinary use. *in* Wedgewood Pharmacy.

Fowler, M.E., and R.E. Miller. 1999. Zoo and Wild Animal Medicine. W.B. Saunders Co. Philadelphia, PA.

Gallanosa, A.G., Spyker, D.A., Shipe, J.R., and Morris, D.L. . 1981. Human xylazine overdose: a comparative review with clonidine, phenothiazines, and tricyclic antidepressants (abstract only). Clinical Toxicology 18:663-678.

GlaxoSmithKline. 2013. Prescribing information: Neosporin® Ointment. https://pdf.hres.ca/dpd_pm/00021007.PDF accessed January 2, 2024.

Graham, M.S., and G.K. Iwama. 1990. The physiologic effects of anesthetic ketamine hydrochloride on two salmonid species. Aquaculture 90:323-331.

Grasing, K., M.G. Sturgill, R.C. Rosen, J.R. Trout, T.J. Thomas, G.D. Kulkarni, P. Maines, and J.R. Seibold. 1996. Effects of yohimbine on autonomic measures are determined by individual values for area under the concentration—time curve. Journal of Clinical Pharmacology 36:814-822.

Green, C.J., J. Knight, S. Precious, and S. Simpkin. 1981. Ketamine alone and combined with diazepam or xylazine in laboratory animals: a 10 year experience. Laboratory Animals 15:163-170.

Green, S.M., R. Clark, M.A. Hostetler, M. Cohen, D. Carlson, and S.G. Rothrock. 1999. Inadvertent ketamine overdose in children: clinical manifestations and outcome. Annals of Emergency Medicine 34:492-497.

Greenacre, C.B., G. Takle, J.P. Schumacher, E.K. Klaphake, and R.C. harvey. 2006. Comparative antinociception of morphine, butorphanol, and buprenorphine versus saline in the green iguana, *Iguana iguana*, using electrostimulation. Journal of Herpetological Medicine and Surgery 16:88-92.

Greenberg, M., A. Rama, and J.R. Zuba. 2018. Atipamezole as an emergency treatment for overdose from highly concentrated alpha-2 agonists used in zoo and wildlife anesthesia. American Journal Of Emergency Medicine 36:134-137.

Greene, S.A., and J.C. Thurmon. 1988. Xylazine--a review of its pharmacology and use in veterinary medicine. Journal of Veterinary Pharmacology and Therapy 11:295-313.

Gunnarsson, L., A. Jauhiainen, E. Kristiansson, O. Nerman, and D.G. Joakim Larsson. 2008. Evolutionary conservation of human drug targets in organisms used for environmental risk assessments. Environ. Sci. Technol. 42:5807-5813.

Harms, N.J., T.S. Jung, M. Hallock, and K. Egli. 2018. Efficacy of a Butorphanol, Azaperone, and Medetomidine Combination for Helicopter-Based Immobilization of Bison (*Bison bison*). Journal of Wildlife Diseases 54:819-824.

Harrison, S.D., T.R. Bosin, and R.P. Maickel. 1974. Physiological disposition of atropine in rat. Pharmacology, Biochemistry, and Behavior 2:843-845.

Heath, W.E. 1950. Death from atropine poisoning. British Medical Journal 2:608.

Hedner, T., B. Edgar, L. Edvinsson, J. Hedner, B. Persson, and A. Pettersson. 1992. Yohimbine pharmacokinetics and interaction with the sympathetic nervous system in normal volunteers. European Journal of Clinical Pharmacology 43:651-656.

Herschman, Z.J., J. Silverstein, G. Blumberg, and A. Lehrfield. 1991. Central nervous system toxicity from nebulized atropine sulfate. Journal of Toxicology: Clinical Toxicology 29:273-277.

Higgins, S.T., B.M. Woodward, and J.E. Henningfield. 1989. Effects of atropine on the repeated acquisition and performance of response sequences in humans. Journal of the Experimental Analysis of Behavior 51:5-15.

HMDB. 2019a. Nalbuphine (HMDB0014982) The Human Metabolome Database. http://www.hmdb.ca/metabolites/HMDB0014982 accessed June 06, 2023.

_____. 2019b. Atipamezole (HMDB0248699) The Human Metabolome Database. https://hmdb.ca/metabolites/HMDB0248699 accessed December 20, 2023.

_____. 2019c. Yohimbine (HMDB0015464) The Human Metabolome Database. https://hmdb.ca/metabolites/HMDB0015464 accessed January 03, 2023.

_____. 2019d. Burtorphanol (HMDB0014749) The Human Metabolome Database. https://hmdb.ca/metabolites/HMDB0014749 accessed January 02, 2023.

_____. 2019e. Acepromazine (HMDB0015552) The Human Metabolome Database. https://hmdb.ca/metabolites/HMDB0015552 accessed December 19, 2023.

Hoffmann, U., C.M. Meister, K. Golle, and M. Zschiesche. 2001. Severe intoxication with the veterinary tranquilizer xylazine in humans. Journal of Analytical Toxicology 25:245-249.

Hospira. 2019. Safety Data Sheet: Nalbuphine hydrochloride injection. https://pfe-pfizercom-prod.s3.amazonaws.com/products/material_safety_data/nalbuphine_HCl_Inj%28Hospira%29_17-June-2019.pdf accessed January 02, 2024.

_____. 2023. Safety Data Sheet: Butorphanol Tartrate - butorphanol tartrate injection , solution. https://dailymed.nlm.nih.gov/dailymed/fda/fdaDrugXsl.cfm?setid=9822ca3f-aee2-46e5-8a96-495400e65d10&type=display#:~:text=The%20analgesic%20effect%20of%20butorphanol,15%20minutes%20for%20intramuscular%20injection accessed January 05, 2024.

IARC. 1993. 2,6-Dimethylaniline (2,6-xylidine). IARC Monograph on the Evaluation of Carcinogenic Risks to Humans: Occupational exposures of hairdressers and barbers and personal use of hair colourants; some hair dyes, cosmetic colourants, industrial dyestuffs and aromatic amines. https://monographs.iarc.fr/wp-content/uploads/2018/06/mono57-22.pdf accessed January 02, 2024.

IPCS. 1991. INCHEM: Chemical Safety Information from Intergovernmental Organizations, queried azaperone, World Health Organization Food Additives Series 29. International Programme on Chemical Safety.

_____. 1992. INCHEM: Chemical Safety Information from Intergovernmental Organizations, queried yohimbine. International Programme on Chemical Safety.

_____. 1998. Toxicological Evaluation of Certain Veterinary Drug Residues in Food, queried azaperone, WHO Food Additives Series 41. World Health Organization, International Programme on Chemical Safety.

_____. 2002. INCHEM: Monograph on atropine. World Health Organization, International Programme on Chemical Safety Evaluation.

_____. 2015. INCHEM: Chemical Safety Information from Intergovernmental Organizations, queried xylazine. International Programme on Chemical Safety.

Jalanka, H.H., and B.O. Roeken. 1990. The use of medetomidine, medetomidine-ketamine combinations, and atipamezole in nondomestic mammals: a review. Journal of Zoo and Wildlife Medicine 21:259-282.

Jandrić, Z., M.N. Rathor, S. Chhem-Kieth, J. Adu-Gyamfi, L. Mayr, C. Resch, S. Bado, J. Švarc-Gajić, and A. Cannavan. 2013. Uptake of 14C-atropine and/or its transformation products from soil by wheat (*Triticum aestivum* var *Kronjet*) and their translocation to shoots. Journal of Environmental Science and Health, Part B, Pesticides, Food Contaminants, and Agricultural Wastes 48:1034-1042.

Jernigan, A.D., R.C. Wilson, N.H. Booth, R.C. Hatch, and A. Akbari. 1988. Comparative pharmacokinetics of yohimbine in steers, horses, and dogs. Canadian Journal of Veterinary Research 52:172-176.

Jjemba, P.K. 2006. Excretion and ecotoxicity of pharmaceutical and personal care products in the environment. Ecotoxicology and Environmental Safety 63:113-130.

Johnson, A.B., and N.M. Sadiq. 2022. Pentobarbital. NCBI Bookshelf ID NBK545288. National Institutes of Health, National Library of Medicine, National Center for Biotechnology Information.

Johnson and Johnson. 2009. Material Safety Data Sheet: Polysporin® Ointment. http://www.safecross.com/MSDS_Sheets/EN/05253.pdf accessed March 19, 2018.

Johnson, S., C. Ellis, C. Wickham, M. Selleck, and A. Gilbert. 2023a. Comparison of ketamine-xylazine, butorphanol-azaperone-medetomidine, and nalbuphine-medetomidine-azaperone for raccoon immobilization. In press.

Johnson, S.R., C.E. Ellis, C.K. Wickham, T. Mays, M.R. Selleck, A. Barbee, and A.T. Gilbert. 2023b. Tissue residue levels of immobilization and antagonist drugs in raccoons (*Procyon lotor*) at two, four, and six days post injection. In press.

Jones, R.N., Q. Li, B. Kohut, D.J. Biedenbach, J. Bell, and J.D. Turnidge. 2006. Contemporary antimicrobial activity of triple antibiotic ointment: a multiphased study of recent clinical isolates in the United States and Australia. Diagnostic Microbiology and Infectious Disease 54:63-71.

Kaartinen, J. 2009. Cardiovascular effects of a medetomidine constant rate infusion at different dose levels in anaesthetized dogs. Master of Science, Université de Montréal, Montreal, Quebec.

Kalser, S.C. 1971. The fate of atropine in man. Annals of the New York Academy of Sciences 179:667-683.

Karhuvaara, S., A. Kallio, M. Scheinin, M. Anttila, J.S. Salonen, and H. Scheinin. 1990. Pharmacological effects and pharmacokinetics of atipamezole, a novel α2-adrenoceptor antagonist–a randomized, double-blind cross-over study in healthy male volunteers. British Journal of Clinical Pharmacology 30:97-106.

Kaufman, J.J., N.M. Semo, and W.S. Koski. 1975. Microelectrometric titration measurement of the pKa's and partition and drug distribution coefficients of narcotics and narcotic antagonists and their pH and temperature dependence. Journal of Medicinal Chemistry 18:647-655.

Kaur, M., and P.M. Singh. 2011. Current role of dexmedetomidine in clinical anesthesia and intensive care. Anesthesia Essays and Research 5:128-133.

Keating, G.M. 2015. Dexmedetomidine: A Review of Its Use for Sedation in the Intensive Care Setting. Drugs 75:1119-1130.

Kedzia, W., J. Lewon, and T. Wisniewski. 1961. The breakdown of atropine by bacteria. Journal of Pharmacy and Pharmacology 13:614-616.

Kim, E., J. Lee, S. Choi, M. Lim, and H. Chung. 2007. Analysis of ketamine and norketamine in urine by automatic solid-phase extraction (SPE) and positive ion chemical ionization-gas chromotography-mass spectrometry (PCI-GC-MS). Forensic Science International 174:197-202.

Ko, J.C., O. Knesl, A.B. Weil, M.R. Raffe, and T. Inoue. 2009. FAQs: Analgesia, sedation, and anesthesia—Making the switch from medetomidine to dexmedetomidine. Compend. Contin. Educ. Vets. 31(suppl. 1A):1-24.

Kock, M.D., W.R. Lance, and D.A. Jessup. 2012. The art of chemical capture. Wildlife Professional 6:34-39.

Koeller, C.A. 2009. Comparison of buprenorphine and butorphanol analgesia in the eastern red-spotted newt (*Notophthalmus viridescens*). Journal of the American Association for Laboratory Animal Science 48:171-175.

Kokkonen, U.-M., and L. Eriksson. 1987. Cardiovascular and allied actions of xylazine and atropine in the unanaesthetized goad. Journal of Veterinary Pharmacology Therapy 10:11-16.

Kostich, M.S., and J.M. Lazorchak. 2014. Risks to aquatic organisms posted by human pharmaceutical use. U.S. Environmental Protection Agency, National Exposure Research Laboratory.

Kreeger, T.J., A.M. Faggella, U.S. Seal, D.L. Mech, M. Callahan, and B. Hall. 1987. Cardiovascular and behavioral responses of gray wolves to ketamine-xylazine immobilization and antagonism by yohimbine. Journal of Wildlife Diseases 23:463-470.

Kreeger, T.J., U.S. Seal, and J.R. Tester. 1990. Chemical immobilization of red foxes (*Vulpes vulpes*). Journal of Wildlife Diseases 26:95-98.

Kreeger, T.J., and J.M. Arnemo. 2012. Handbook of Wildlife Chemical Immobilization, Fourth Edition.

Krueger, B.W., and K.A. Krueger. 2000. Secondary pentobarbital poisoning of wildlife. U.S. Fish and Wildlife Service Fact Sheet. U.S. Fish and Wildlife Service.

Kumar, A., H.J. Mann, and R.P. Remmel. 2006. Pharmacokinetics of tiletamine and zolazepam (Telazol®) in anesthetized pigs. Journal of Veterinary Pharmacology and Therapeutics 29:587-589.

Kunelius, P., J. Hakkinen, and O. Lukkarinen. 1997. Is high-dose yohimbine hydrochloride effective in the treatment of mixed-type impotence? A prospective, randomized, controlled double-blind crossover study. Urology 49:441-444.

Lacouture, P.G., F.H. Lovehoy, and A.A. Mitchell. 1983. Acute hypothermia associated with atropine. American Journal of Diseases of Children 137:291-292.

Latas, P.J. The use of azaperone in the spiny dogfish (*Squalus acanthias*). O'Fallon IL: Veterinary Software Publishing, May 10-14 1987.

Le Corre, P., G. Dollo, F. Chevanne, and R. Le Verge. 1999. Biopharmaceutics and metabolism of yohimbine in humans. European Journal of Pharmaceutical Sciences 9:79-84.

Le Vet BV. 2017. Summary of product characteristics: nerfasin. Le Vet BV, Netherlands.

Lennquist, A., A. Hilvarsson, and L. Förlin. 2010. Responses in fish exposed to medetomidine, a new antifouling agent. Marine Environmental Research 69:S43-S45.

Letco. 2019. Safety Data Sheet: Nalbuphine HCl. Letco Medical, LLC, Decatur, AL, USA.

Lewis, R.J. 1996. Sax's Dangerous Properties of Industrial Materials. 9th ed. Volumes 1-3. Van Nostrand Reinhold, New York, NY.

Li, S., Y. Wang, and A.Y. Lin. 2017. Ecotoxicological effect of ketamine: Evidence of acute, chronic and photolysis toxicity to *Daphnia magna*. Ecotoxicology and Environmental Safety 143:173-179.

Lilius, H.I., B. and T. Holmström. . 1994. A comparison of the toxicity of 50 reference chemicals to freshly isolated rainbow trout hepatocytes and *Daphnia magna*. Aquatic Toxicology 30:47-60.

Lin, A.Y., W. Lee, and X. Wang. 2014. Ketamine and the metabolite norketamine: Persistence and phototransformation toxicity in hospital wastewater and surface water. Water Research 53:351-360.

Lin, H.C., J.C. Thrumon, G.J. Benson, and W.J. Tranquilli. 1993. Telazol-a review of its pharmacology and use in veterinary medicine. Journal of Veterinary Pharmacology and Therapeutics 16:383-418.

Loerzel, S.M., P.J. Smith, A. Howe, and D.A. Samuelson. 2002. Vecuronium bromide, phenylephrine and atropine combinations as mydriatics in juvenile double-crested cormorants (*Phalacrocorax auritus*). Veterinary Ophthalmology 5:149-154.

Logash, M., P. Pokotylo, and R. Stepien. 2017. Nalbuphine: some aspects of the research and applications. Medical Studies 33:146-154.

Lönnerholm, G., and E. Widerlöv. 1975. Effect of intravenous atropine and methylatropine on heart rate and secretion of saliva in man. European Journal of Clinical Pharmacology 8:233-240.

Mauthe von Degerfeld, M. 2004. Personal experiences in the use of association tiletamine/zolazepam for anaesthesia of the green iguana (*Iguana iguana*). Veterinary Research Communications 28:351-353.

McDermott, J.R., W. Leuenberger, C.A. Haymes, G.B. Clevinger, J.K. Trudeau, T.C. Carter, J.T. Hast, G.S.W. Jenkins, W.E. Bowling, and J.J. Cox. 2020. Safe Use of Butorphanol–Azaperone–Medetomidine to Immobilize Free-Ranging White-tailed Deer. Wildlife Society Bulletin 44:281-291.

McEvoy, G. 1990. AHFS drug information 90. Bethesda: MD: American Society of Hospital Pharmacists.

Merck Animal Health. 2014. Material Safety Data Sheet: Beuthanasia-D Solution (Pentobarbital Sodium / Phenytoin Formulation. Merck & Co., Inc. Rahway, NJ, 07065.

Mestorino, N., M.L. Marchetti, M. Daniele, M.A. Martínez, M.R. Martínez-Larrañaga, and A. Anadón. 2013. Tissue depletion of azaperone and its metabolite azaperol after oral administration of azaperone in food-producing pigs. Revista de Toxicologia 30:209-214.

Mikota, S.M., and D.C. Plumb. 2017. Elephant Formulary – Tolazoline HCl. http://elephantcare.org/resources/formulary/drug-index/tolazoline-hcl/ accessed 06/06/2023.

Mitchell Jr, M.C., A. Memisoglu, and B.L. Silverman. 2012. Hepatic safety of injectable extended-release naltrexone in patients with chronic hepatitis C and HIV infection. Journal of studies on alcohol and drugs 73:991-997.

Monteith, K.I., K.B. Monteith, J.A. Delger, L.E. Schmitz, T.J. Brinkman, C.S. Deperno, and J.A. Jenks. 2012. Immobilization of white-tailed deer with telazol, ketamine, and xylazine, and evaluation of antagonists. Journal of Wildlife Management 76:1412-1419.

Moore, R.P., B.L. Raphael, and P.P. Calle. 2010. Use of selective sedatives and analgesics in marine mammals in a large public aquarium. Proceedings of the International Association for Aquatic Animal Medicine Annual Conference 41:88–89.

Morton, H.G. 1939. Atropine intoxication: its manifestations in infants and children. The Journal of Pediatrics 14:755-760.

NCBI. 2023a. National Center for Biotechnology Information, PubChem Compound Summary for CID 6169, Yohimbine Hydrochloride. https://pubchem.ncbi.nlm.nih.gov/compound/yohimbine_hydrochloride accessed December 19, 2023.

_____. 2023b. National Center for Biotechnology Information, PubChem Compound Summary for CID 26533, Tiletamine. https://pubchem.ncbi.nlm.nih.gov/compound/26533 accessed December 20, 2023.

_____. 2023c. National Center for Biotechnology Information, PubChem Compound Summary for CID 15443, Azaperone. https://pubchem.ncbi.nlm.nih.gov/compound/15443 accessed December 19, 2023.

_____. 2023d. National Center for Biotechnology Information, PubChem Compound summary for CID 5707, Xylazine. https://pubchem.ncbi.nlm.nih.gov/compound/xylazine accessed December 19, 2023.

_____. 2023e. National Center for Biotechnology Information, PubChem Compound Summary for CID 5504, Tolzazoline. https://pubchem.ncbi.nlm.nih.gov/compound/Tolazoline accessed December 20, 2023.

_____. 2023f. National Center for Biotechnology Information, PubChem Compound Summary for CID 3821, Ketamine. https://pubchem.ncbi.nlm.nih.gov/compound/ketamine accessed Decemeber 19, 2023.

_____. 2023g. National Center for Biotechnology Information, PubChem Compound Summary for CID 5311068, Dexmedetomidine. https://pubchem.ncbi.nlm.nih.gov/compound/dexmedetomidine accessed December 20, 2023.

_____. 2023h. National Center for Biotechnology Information, PubChem Compound Summary for CID 60296398, Atropine sulfate. https://pubchem.ncbi.nlm.nih.gov/compound/60196398 accessed December 20, 2023.

_____. 2023i. National Center for Biotechnology Information, PubChem Compound summary for CID 68601, Medetomidine Hydrochloride. . https://pubchem.ncbi.nlm.nih.gov/compound/Medetomidine_HCl accessed December 19, 2023.

_____. 2023j. National Center for Biotechnology Information, PubChem Compound summary for CID 4873, Potassium chloride. https://pubchem.ncbi.nlm.nih.gov/compound/potassium_chloride accessed Decemeber 19, 2023.

_____. 2023k. National Center for Biotechnology Information, PubChem Compound summary for CID 8478, Benzethonium chloride. . https://pubchem.ncbi.nlm.nih.gov/compound/Benzethonium-chloride accessed December 19, 2023.

_____. 2023l. National Center for Biotechnology Information, PubChem Compound summary for CID 4737, Pentobarbital. https://pubchem.ncbi.nlm.nih.gov/compound/4737 accessed December 20, 2023.

_____. 2023m. National Center for Biotechnology Information, PubChem Compound Summary for CID 5360733, Nalbuphine Hydrochloride. https://pubchem.ncbi.nlm.nih.gov/compound/5360733 accessed December 19, 2023.

_____. 2023n. National Center for Biotechnology Information, PubChem Compound summary for CID 6077, Acepromazine. https://pubchem.ncbi.nlm.nih.gov/compound/Acetylpromazine#section=Top accessed December 19, 2023.

_____. 2023o. National Center for Biotechnology Information, PubChem Compound summary for CID 3156, Doxapram. https://pubchem.ncbi.nlm.nih.gov/compound/3156 accessed December 19, 2023.

_____. 2023p. National Center for Biotechnology Information, PubChem Compound summary for CID 5311304, Nalbuphine. https://pubchem.ncbi.nlm.nih.gov/compound/nalbuphine accessed December 19, 2023.

_____. 2023q. National Center for Biotechnology Information, PubChem Substance Record for SID 363899536, 1649-18-9, Source: Chemical Carcinogenesis Research Information System (CCRIS). https://pubchem.ncbi.nlm.nih.gov/substance/36899536 accessed December 19, 2023.

_____. 2023r. National Center for Biotechnology Information, PubChem Compound Summary for CID 174174, Atropine. https://pubchem.ncbi.nlm.nih.gov/compound/174174 accessed December 20, 2023.

_____. 2023s. National Center for Biotechnology Information, PubChem Compound summary for CID 35775, Zolazepam. . https://pubchem.ncbi.nlm.nih.gov/compound/Zolazepam#section=Safety-and-Hazards accessed December 19, 2023.

_____. 2023t. National Center for Biotechnology Information, PubChem Compound summary for CID 5361092, Butorphanol. https://pubchem.ncbi.nlm.nih.gov/compound/5361092 accessed December 19, 2023.

_____. 2023u. National Center for Biotechnology Information, PubChem Compound summary for CID 4192, Midazolam. https://pubchem.ncbi.nlm.nih.gov/compound/Midazolam accessed.

_____. 2023v. National Center for Biotechnology Information, PubChem Annotation Record for Naltrexone, Source: Hazardous Substances Data Bank (HSDB). https://pubchem.ncbi.nlm.nih.gov/source/hsdb/6750 accessed December 19, 2023.

_____. 2023w. National Center for Biotechnology Information, PubChem Compound Summary for CID 5464090, Butorphanol Tartrate. https://pubchem.ncbi.nlm.nih.gov/compound/5464090 accessed December 19, 2023.

_____. 2023x. National Center for Biotechnology Information, PubChem Compound Summary for Bacitracin. https://pubchem.ncbi.nlm.nih.gov/compound/Bacitracin accessed December 20, 2023.

_____. 2023y. National Center for Biotechnology Information, PubChem Compound Summary for CID 5360515, Naltrexone. https://pubchem.ncbi.nlm.nih.gov/compound/Naltrexone. accessed December 19, 2023.

Neto, F.J.T. 2009. Dexmedetomidine: A New Alpha-2 Agonist for Small Animal Practice. Proceedings of the World Small Animal Veterinary Association World Congress.

NIH. 2018. Drugs and Lactation Database (LactMed) [Internet]. Bethesda (MD): National Institute of Health and Human Development; 2006-. Nalbuphine. [Updated 2018 Oct 31]. https://www.ncbi.nlm.nih.gov/books/NBK501238/ accessed December 19, 2023.

NLM. 2014. Dexdomitor – dexmedetomidine hydrochloride injection, solution. https://dailymed.nlm.nih.gov/dailymed/fda/fdaDrugXsl.cfm?setid=9afc5d50-8df7-46e9-b5a0-5573627fc1bc&type=display accessed December 20, 2023.

_____. 2017a. Naltrexone hydrochloride tablet, film coated https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=49aa3d6d-2270-4615-aafa-b440859ab870 accessed December 20, 2023.

_____. 2017b. Pentobarbital sodium – pentobarbital sodium injection, solution. https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=d375b92a-baa1-4d63-9ad1-1467dcfde7d5 accessed December 20, 2023.

_____. 2017c. Neomycin sulfate – neomycin sulfate tablet. https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=3114c827-1923-437a-bc8a-a1ac20327a28 accessed December 20, 2023.

Nordt, S.P., and R.F. Clark. 1997. Midazolam: A review of therapeutic uses and toxicity. The Journal of Emergency Medicine 15:357-365.

O'Neil, M.J. 2013. The Merck index: an encyclopedia of chemicals, drugs, and biologicals. RSC Publishing.

Oak. 2015. Safety Data sheet, Nebutal Sodium Solution CII (pentobarbital sodium injection, USP). Oak Pharmaceuticals, Inc. (Subsidiary of Akorn, Inc.), Lake Forest, IL, USA.

Odette, O., B.T. Simon, L.S. Ebner, I. Lizarraga, X. Sun, and S.K. Cox. 2022. The pharmacokinetics and pharmacodynamics of midazolam after intravenous administration to donkeys (*Equus africanus asinus*). Canadian Journal of Veterinary Research 86:125-131.

OECD. 2001. Potassium chloride. Screening Information Dataset (SIDS). UNEP Publications. Organisation for Economic Co-operation and Development (OECD).

Oswald, R.L. 1978. Injection anaesthesia for experimental studies in fish. Comp. Biochem. Physiol. 60C:19-26.

Owen, J.A., S.L. Nakatsu, J. Fenemore, M. Condra, D.H.C. Surridge, and A. Morales. 1987. The pharmacokinetics of yohimbine in man. European Journal of Clinical Pharmacology 32:577-582.

PAN. 2018. Pesticide Action Network (PAN) Pesticides Database-Chemicals. Atropine. https://www.pesticideinfo.org/chemical/PRI1344 accessed June 06, 2023.

Pao, L., C. Hsiong, O.Y. Hu, and S. Ho. 2000. High-performance liquid chromatographic method for the simultaneous determination of nalbuphine and its prodrug, sebacoyl dinalbuphine ester, in dog plasma and application to pharmacokinetic studies in dogs. Journal of Chromatography B: Biomedical Sciences and Applications 746:241-247.

Payne, J., R. Farris, G. Parker, J. Bonhotal, and M. Schwartz. 2015. Quantification of sodium pentobarbital residues from equine mortality compost piles. Journal of Animal Science 93:1824-1829.

Pertovaara, A., A. Haapalinna, J. Sirviö, and R. Virtanen. 2005. Pharmacological properties, central nervous system effects, and potential therapeutic applications of atipamezole, a selective α_2-adrenoceptor antagonist. CNS Drug Reviews 11:273-288.

Pfeffer, M., R.D. Smyth, K.A. Pittman, and P.A. Nardella. 1980. Pharmacokinetics of subcutaneous and intramuscular butorphanol in dogs. Journal of Pharmaceutical Sciences 69:801-803.

Pfizer. 2000. Material Safety Data Sheet: Neosporin Ointment. Pfizer Consumer Healthcare, Morris Plains, NJ, USA.

_____. 2008. Material Safety Data Sheet, (S)-(+)-Ketamine Hydrochloride Solution. *in* Pfizer Inc, Pfizer Pharmaceuticals Group, New York, NY, USA.

Pitman-Moore. 1982. Environmental Impact Analysis Report: Azaperone. Pitman-Moore, Inc., Washington Crossing, New Jersey.

Plumb, D.C. 2018. Plumb's Veterinary Drug Handbook, 9th edition. Wiley Blackwell.

Prescriber's Desk Reference. 2023. Drug Information Vivitrol (naltrexone). https://www.pdr.net/drug-information/vivitrol?druglabelid=1199 accessed June 06, 2023.

Pugajeva, I., J. Rusko, I. Perkons, E. Lundanes, and V. Bartkevics. 2017. Determination of pharmaceutical residues in wastewater using high performance liquid chromatography coupled to quadrupole-Orbitrapmass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 133:64-74.

Quail, M.T., P. Weimersheimer, A.D. Woolf, and B. Magnani. 2001. Abuse of Telazol: An animal tranquilizer. Clinical Toxicology 39:399-402.

Quesada, R.J., C.D. Smith, and D.J. Heard. 2011. Evaluation of parenteral drugs for anesthesia in the blue crab (*Callinectes sapidus*). Journal of Zoo and Wildlife Medicine 42:295-299.

Raines, J.A., and M.M. Clancy. 2009. Sedation by orally administered ketamine in Goldfish, *Carassius auratus*, hybrid striped bass, *Morone* hybrid *saxatilis* x *M. chrysops*, and ocellated river stingray, *Potamotrygon motoro*. Journal of the World Aquaculture Society 40:788-794.

Rauws, A.G., M. Olling, J. Freudenthal, and M. Ten Ham. 1976. Azaperol, a new metabolite of the veterinary butyrophenone tranquilizer azaperone. Toxicology and Applied Pharmacology 35:333-339.

Razani-Boroujerdi, S., M. Behl, F.F. Hahn, J.C. Pena-Philippides, J. Hutt, and M.L. Sopori. 2008. Role of muscarinic receptors in the regulation of immune and inflammatory responses. Journal of Neuroimmunology 194:82-88.

Reyes, J.C., J.L. Negrón, H.M. Colón, A.M. Padilla, M.Y. Millán, T.D. Matos, and R.R. Robles. 2012. The emerging of xylazine as a new drug of abuse and its health consequences among drug users in Puerto Rico. Journal of Urban Health 89:519-526.

Riggs, S.M., G. Hawkins, A.L. Craigmill, P.H. Kass, S.D. Stanley, and I.T. Taylor. 2008. Pharmacokinetics of butorphanol tartrate in red-tailed hawks (*Buteo jamaicensis*) and great horned owls (*Bubo virginianus*). American Journal of Veterinary Research 69:596-603.

Riviere, J.E., and M.G. Papich. 2009. Veterinary Pharmacology and Therapeutics, Section 3: Anesthetics and Analgesics, Ninth Edition. Wiley Blackwell, Ames, IA.

_____. 2017. Veterinary pharmacology and therapeutics, 10th edition. John Wiley & Sons, Inc.

Ruiz-Colón, K., C. Chavez-Arias, J.E. Díaz-Alcalá, and M.A. Martínez. 2014. Xylazine intoxication in humans and its importance as an emerging adulterant in abused drugs: A comprehensive review of the literature. Forensic Science International 240:1-8.

Rush, M.L., L. Pearson, and W.J. Lang. 1970. Conditional autonomic responses induced in dogs by atropine and morphine. European Journal of Pharmacology 11:22-28.

Ryan, C.W., M.R. Vaughn, J.B. Meldrum, R.B. Duncan, and J.W. Edwards. 2009. Retention time of Telazol in black bears. The Journal of Wildlife Management 73:210-213.

Saha, A. 2016. Degradation of pentobarbital in various soil types by solid phase extraction and liquid chromatography/ mass spectrometry. Master of Science, Middle Tennessee State University.

Sanchez-Migallon Guzman, D., B. KuKanich, N.S. Keuler, J.M. Klauer, and J.R. Paul-Murphy. 2011. Antinociceptive effects of nalbuphine hydrochloride in Hispaniolan Amazon parrots (*Amazona ventralis*). American Journal of Veterinary Research 72:736-740.

Santa Cruz Biotechnology. 2007. Acepromazine Maleate, sc-207247. Safety Data Sheet. Santa Cruz Biotechnology, Inc. Santa Cruz, CA, USA.

_____. 2018. Dexmedetomidine Safety Data Sheet. Santa Cruz Biotechnology, Inc. Dallas, TX, USA.

_____. 2019. Medetomidine hydrochloride, Safety Data Sheet. Santa Cruz Biotechnology, Inc., Dallas, TX, USA.

Sato, T., Y. Ban, M. Uchida, E. Gondo, M. Yamamoto, Y. Sekiguchi, A. Sakaue, M. Kemi, and T. Nakatsuka. 2005. Atropine-induced inhibition of sperm and semen transport impairs fertility in male rats. The Journal of Toxicological Sciences 30:207-212.

Schmähl, D., and M. M. Habs. 1976. Life-span investigations for carcinogenicity of some immune-stimulating, immunodepressive and neurotropic substances in Sprague-Dawley-Rats. Z. Krebsforsch. 86:77-84.

Schneiders, F.I., G.K. Noble, R.C. Boston, A.J. Dunstan, M.N. Sillence, and A.R. McKinney. 2012. Acepromazine Pharmacokinetics: A Forensic Perspective. The Veterinary Journal 194:48-54.

Sciencelab. 2013. Material Safety Data Sheet: Polymyxin B Sulfate MSDS. *in* Sciencelab.com, Inc.

Shearer, J.K., and A. Ramirez. 2013. Procedures for the humane euthanasia of sick, injured and/or debilitated livestock. Iowa State University Extension.

Shilo, Y., R. Lapid, R. King, T. Bdolah-Abram, and A. Epstein. 2010. Immobilization of red fox (*Vulpes vulpes*) with medetomidine-ketamine or medetomidine-midazolam and antagonism with atipamezole. Journal of Zoo and Wildlife Medicine 41:28–34.

Shlosberg, A., M. Bellaiche, V. Hanji, and E. Ershov. 1997. New treatment regimens in organophosphate (diazinon) and carbamate (methomyl) insecticide-induced toxicosis in fowl. Veterinary and Human Toxicology 39:347-350.

Siegenthaler, J., T. Pleyers, M. Raillard, C. Spadavecchia, and O.L. Levionnois. 2020. Effects of medetomidine, dexmedetomidine, and their reversal with atipamezole on the nociceptive withdrawal reflex in beagles. Animals 10:1-14.

Sigma-Aldrich Corporation. 2002a. Yohimbine hydrochloride. Product Information. Sigma, St. Louis, MO, USA.

_____. 2002b. Xylazine Product Information. Sigma Saint Louis, MO, USA.

_____. 2019. Tolazoline hydrochloride safety data sheet. Sigma-Aldrich Inc., St. Louis, MO, USA.

_____. 2022. Xylazine Safety Data Sheet. Sigma-Aldrich Inc., St. Louis, MO, USA.

_____. 2023. Xylazine hydrochloride Safety Data Sheet. SIgma-Aldrich, Inc., St. Louis, MO, USA.

Sinclair, M.D. 2003. A review of the physiological effects of α2-agonists related to the clinical use of medetomidine in small animal practice. Canadian Veterinary Journal 44:885-897.

Smith, R.F. 1971. Effects of neurotropic drugs on lannate and atropine toxicity in western spruce budworm, *Choristoneura occidentalis* (Lepidoptera: Tortricidae). Journal of Invertebrate Pathology 18:139-145.

Sokolowska-Mikolajczyk, M., M. Socha, T. Mikolajczyk, J. Chyb, and P. Epler. 2002. Seasonal short-term effects of naltrexone on LH secretion in male carp (*Cyprinus carpio* L.). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 131:379-385.

T3DB. 2023. Naltrexone (T3D2864). http://www.t3db.ca/toxins/T3D2864 accessed June 06, 2023.

Telesco, R.L., and M.A. Sovada. 2002. Immobilization of swift foxes with ketamine hydrochloride-xylazine hydrochloride. Journal of Wildlife Diseases 38:764-768.

Tuomi, P.A. 2000. Butorphanol and butorphanol/diazepam administration for analgesia and sedation of harbor seals (*Phoca vitulina*) Proceedings of the International Association for Aquatic Animal Medicine Conference.

UK Competent Authority. 2014. CLH Report: Proposal for Harmonized Classification and Labelling. Substance Name: Medetomidine. Chemicals Regulation Directorate, Health and Safety Executive, United Kingdom.

University of Colorado. 2012. Veterinary Anesthetic and Analgesic Formulary, 3rd Edition, Version G.

University of Hertfordshire. 2018a. Veterinary Substances Database (VSDB): xylazine hydrochloride. https://sitem.herts.ac.uk/aeru/vsdb/Reports/1794.htm accessed June 06, 2023.

_____. 2018b. Veterinary Substances Database (VSDB): Atropine. http://sitem.herts.ac.uk/aeru/vsdb/Reports/1820.htm accessed January 03, 2024.

_____. 2018c. Veterinary Substances Database (VSDB): Azaperone. http://sitem.herts.ac.uk/aeru/vsdb/Reports/1871.htm accessed June 06, 2023.

USDA AMS. 2002. Xylazine/Tolazoline: Livestock. United States Department of Agriculture, Agricultural Marketing Service.

_____. 2019. Technical Evaluation Report: Xylazine/Tolazoline Livestock. United States Department of Agriculture, Agricultural Marketing Service.

USDA APHIS. 2013. Environmental Assessment: Mammal Damage Management in Florida. United States Department of Agriculture, Animal and Plant Health Inspection Service.

_____. 2017. Field Operations Manual for the Use of Immobilization and Euthanasia Drugs. United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services.

_____. 2019a. Wildlife Services Directive: Acquisition, storage, and use of controlled chemical immobilization and euthanasia substances, WS 2.430, dated 3/12/2019. United States Department of Agriculture, Animal and Plant Health Inspection Service.

_____. 2019b. Immobilization and Euthanasia Manual, version 20190408. United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services.

USEPA. 2012. Estimate Program Interface (EPI) Suite Ver.4.1. US Environmental Protection Agency.

_____. 2019a. Ecotox Knowledgebase: queried atropine. United States Environmental Protection Agency.

_____. 2019b. Butorphanol tartrate. https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID50904569#properties accessed June 06, 2023.

_____. 2020. CompTox Chemicals Dashboard. Nalbuphine hydrochloride. https://comptox.epa.gov/dashboard/DTXSID20177844 accessed June 06, 2023.

_____. 2023a. CompTox Chemicals Dashboard: Zolazepam. https://comptox.epa.gov/dashboard/chemical/properties/DTXSID30185278 accessed June 06, 2023.

_____. 2023b. CompTox Chemicals Dashboard: Tiletamine. https://comptox.epa.gov/dashboard/chemical/executive-summary/DTXSID5048552 accessed March 14, 2023.

_____. 2023c. CompTox Chemicals Dashboard: Yohimbine. https://comptox.epa.gov/dashboard/chemical/executive-summary/DTXSID5048552 accessed March 21, 2023.

_____. 2023d. CompTox Chemical Dashboard: Tolazoline. https://comptox.epa.gov/dashboard/chemical/details/DTXSID3023683 accessed June 06, 2023.

Vähä-Vahe, A.T. 1990. Clinical effectiveness of atipamezole as a medetomidine antagonist in cats. Journal of Small Animal Practice 31:193-197.

Van Der Meer, M.J., H.K.L. Hundt, and F.O. Muller. 1986. The metabolism of atropine in man. Journal of Pharmacy and Pharmacology 38:781-784.

Venn, M., J. Newman, and M. Grounds. 2003. A phase II study to evaluate the efficacy of dexmedetomidine for sedation in the medical intensive care unit. Intensive Care Med 29:201-207.

Vortech Pharmaceuticals. 2015. Material Safety Data Sheet: Fatal-Plus Solution. Product Code: 9373. Vortech Pharmaceuticals, Ltd. Dearborn, MI, USA.

WAFWA. 2010. A Model Protocol for Purchase, Distribution and Use of Pharmaceuticals in Wildlife. Western Association of Fish and Wildlife Agencies, Wildlife Health Committee.

Wang, K., Shih, T., and S. Cheng. 2005. Use of SPE and LC/TIS/MS/MS for rapid detection and quantification of ketamine and its metabolite, norketamine, in urine. Forensic Science International 147:81-88.

Wang, Z., Z. Xu, and X. Li. 2018. Biodegradation of methamphetamine and ketamine in aquatic ecosystem and associated shift in bacterial community. Journal of Hazardous Materials 359:356-364.

Ward, J.W., D.L. Gilbert, B.V. Franko, G. Woddard, and G.T. Mann. 1968. Toxicologic studies of doxapram hydrochloride. Toxicology and Applied Pharmacology 13:242-250.

Watanabe, T., K. Matsuhashi, and S. Takayama. 1985. Study on the postnatal neuro-behavioral development in rats treated prenatally with drugs acting on the autonomic nervous system (abstract only). Nihon Yakurigaku Zasshi 85:79-90.

Waterman, A.E., A. Livingston, and A. Amin. 1991. Analgesic activity and respiratory effects of butorphanol in sheep. Research in Veterinary Science 51:19-23.

Wedgewood Pharmacy. 2024. BAM kit. https://www.wedgewoodpharmacy.com/items/bam-kit.html accessed January 04, 2024.

West, G., D. Heard, and N. Caulkett. 2007. Zoo Animal and Wildlife, Immobilization and Anesthesia. Blackwell Publishing, Ames, Iowa.

Wetchler, B.V., C.D. Alexander, M.S. Shariff, and G.M. Gaudzels. 1989. A comparison of recovery in outpatients receiving fentanyl versus those receiving butorphanol. Journal of Clinical Anesthesia 1:339-343.

White, R.P., F. Rinaldi, and H.E. Himwich. 1956. Central and peripheral nervous effects of atropine sulfate and mepiperphenidol bromide (Darstine) on human subjects. Journal of Applied Physiology 8:635-642.

WHO. 2006. Critical review of butorphanol. 34th ECDD 2006/4.1. World Health Organization.

_____. 2014. Ketamine, Update Review Report, Agenda item 6.2. Expert Committee on Drug Dependence Thirty-sixth Meeting, Geneva, 16-20 June 2014. World Health Organization.

Wieder, M.E., B.P. Gray, P.R. Brown, S. Hudson, C.M. Pearce, S.W. Paine, and L. Hillyer. 2012. Identification of acepromazine and its metabolites in horse plasma and urine by LC-MS/MS and accurate mass measurement. Chromatographia 75:635-643.

Williamson, R.H., L.I. Muller, and C.D. Blair. 2018. The use of ketamine-xylazine or butorphanol-azaperone-medetomidine to immobilize American black bear (*Ursus americanus*). Journal of Wildlife Diseases 54:503-510.

Winbladh, B. 1973. Postnatal development of central effects of atropine and oxotremorine in dogs in relation to brain development [abstract only]. Acta Pharmacologica et Toxicologica 32:65-82.

Winegar, B.D., G.D. Bittner, and S.W. Leslie. 1988. Effects of pentobarbital on behavioral and synaptic plasticities in crayfish (abstract only). Brain Research 475:21-27.

Wolfe, L.L., H.E. Johnson, M.C. Fisher, M.A. Sirochman, B. Kraft, and M.W. Miller. 2014a. Use of acepromazine and medetomidine in combination for sedation and handling of rocky

mountain elk (*Cervus elaphus nelsoni*) and black bears (*Ursus americanus*). Journal of Wildlife Diseases 50:979-981.

Wolfe, L.L., W.R. Lance, D.K. Smith, and M.W. Miller. 2014b. Novel combinations of nalbuphine and medetomidine for wildlife immobilization. Journal of Wildlife Diseases 50:951-956.

Wolfe, L.L., H.E. Johnson, M.C. Fisher, W.R. Lance, D.K. Smith, and M.W. Miller. 2016a. Chemical immobilization in American black bears using a combination of nalbuphine, medetomidine, and azaperone. Ursus 27:1-4.

Wolfe, L.L., M.W. Miller, W. R., W.R. Lance, and D.K. Smith. 2016b. United States Patent Wolfe et al. Patent No.: US 9,339,498 B2, Date of Patent: May 17, 2016, 10 pp.

Wolfe, L.L., M.E. Wood, P. Nol, M.P. McCollum, M.C. Fisher, and W.R. Lance. 2017. The efficacy of nalbuphine, medetomidine, and azaperone in immobilizing American bison (*Bison bison*). Journal of Wildlife Diseases 53:304-310.

Wolfe, L.L., P. Nol, M.P. McCollum, T. Mays, M.E. Wehtje, W.R. Lance, M.C. Fisher, and M.W. Miller. 2018. Tissue residue levels after immobilization of Rocky Mountain elk (*Cervus elaphus nelsoni*) using a combination of nalbuphine, medetomidine, and azaperone antagonized with naltrexone, atipamezole, and tolazoline. Journal of Wildlife Diseases 54:362-365.

Wolfe, L.L., T. Mays, M.C. Fisher, and M.W. Miller. 2020. Tissue residue levels of the tranquilizer combination of butorphanol, azaperone, and medetomidine, and the antagonists, naltrexone, atipamezole, and tolazoline, in black bears (*Ursus americanus*) postimmobilization. Journal of Wildlife Diseases 56:933–936.

Xega, R., B.E. King, and K.B. Sumpter. 2019. Environmental fate of ketamine in soil and relevant waters. Report No. CCDC CBC-TR-1588. US Army Combat Capabilities Development Command Chemical Biological Center. Defense Threat Reduction Agency.

Yong-Fu, X., B. Wang, X. Wang, F. Du, M. Benzinou, and Y.J. Wang. 2013. Xylazine-induced reduction of tissue sensitivity to insulin leads to acute hyperglycemia in diabetic and normoglycemic monkeys. BMC Anesthesiology 13:33.

Zoetis. 2013. Telazol, Material Safety Data Sheet, Version 1.1. Zoetis Inc., Florham Park, NJ, USA.

_____. 2014a. Sleepaway Euthanasia Solution, Safety Data Sheet, Version 4.1. Zoetis Inc., Florham Park, NJ, USA.

_____. 2014b. Ketamine Hydrochloride Injection, Safety Data Sheet, Version 3.1. Zoetis Inc., Florham Park, NJ, USA.

_____. 2017a. Telazol®, Safety Data Sheet, Version 2. Zoetis Inc., Parsippany, NJ, USA.

_____. 2017b. Dexdomitor®, Safety Data Sheet, Version 1. Zoetis Inc., Parsippany, NJ, USA.

_____. 2017c. Telazol Safety Data Sheet Zoetis Inc., Parsippany, NJ, USA.

_____. 2017d. Atipamezole hydrochloride sterile injectable solution, Safety Data Sheet, Version 6. Zoetis Inc., Parsippany, NJ, USA.

_____. 2019. Telazol label: tiletamine and zolazepam for injection. Zoetis Inc., Kalamazoo, MI, USA.

_____. 2020. Antisedan: Product Information. Vet Label: Veterinary Product and Label Information. https://vetlabel.com/lib/vet/meds/antisedan-2/ accessed January 05, 2024.

_____. 2021. Domitor® (Medetomidine hydrochloride) Sedative and analgesic for dogs and cats Safety Data Sheet. Zotetis Australia Pty Ltd Rhodes, New South Wales, Australia.