Reptile Wildlife Euthanasia Techniques

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"*The question is not, can they reason? Nor, can they talk? But, can they suffer?*"
-Jeremy Bentham, Philosopher, 1780

Define wildlife rehabilitation:

Wildlife rehabilitation is the act of providing temporary care to injured, sick, or orphaned wildlife with the goal of **releasing** them back into the wild.[1]

Pain?

Reptiles have all the anatomy necessary to feel pain and suffer and therefore should be treated with the same humane standards as other species, which includes providing them with proper analgesics.[2] Sladky and Mans provide a review of clinical analgesia in reptiles.[3]

Legality

- If the animal is wild, its "owner" is the State, Federal, or Tribal authority.[4]
- Some endangered or migratory species may require approval by the appropriate federal agency prior to euthanasia, however, if the animal is actively suffering, agencies will usually defer to the veterinarian's discretion.
    - Migratory birds = U.S. Fish and Wildlife Service (USFW)
    - Marine mammals = National Oceanic and Atmospheric Administration (NOAA)
    - Rest usually = State

Define euthanasia

- Derived from the Greek terms "*eu*" = well or good and "*thanatos*" = death. [5,6]
- Definition: ending the life of an animal in a way that eliminates or minimizes pain and distress.[5,6] The technique employed should result in rapid loss of consciousness followed by cardiac or respiratory arrest and, ultimately, a loss of brain function.
- Actually determining the pain/distress of a method of euthanasia can be very difficult because as humans we will never fully know/understand the subjective experience of the animal.[7] We use our best judgement; paddling, vocalizations, convulsions before apparent loss of consciousness is obviously suffering. We also use our knowledge of physiology and assume suffering in the absence of behaviors if a physiological process theoretically leads to suffering.
- Pain, distress and suffering are subjective affective experiences that are perceived in the brain. Therefore, one must be conscious and alive to suffer.

- General rule: a gentle death that takes longer is preferable to a rapid but more distressing death and conversely, if all other methods are equally humane, the quickest method should be chosen.[8]
- Taking a life is abhorrent to many. We often forget this and need to keep it in mind.[9]
- What's the difference between euthanasia and humane killing? [6,7,9]
    - Most veterinary/animal fields define euthanasia simply as "good death", however, the human field does not. Consider the death penalty—we don't consider these humans "euthanized" or eligible for "euthanasia". Applying this to animals, we do not consider healthy cattle going to slaughter to be going for euthanasia. That's because the decision whether or not to end a life, is central to the euthanasia definition. The decision of whether or not to end a life must be based on the animal's welfare, that is, to anticipate what the animal would want & and what is in the animal's best interest. So even though we won't be discussing today the decision on whether or not to euthanize, we should keep this in mind. And finally, even though humane slaughter and humane depopulation (i.e. with animal disease outbreaks) may not be considered true euthanasia, we can consider these techniques when we are looking for the most humane way to euthanize our patients in wildlife rehabilitation.

Evaluating euthanasia methods:[6]

- Animal factors:
    - Ability to induce loss of consciousness and death with a minimum of pain and distress [10]; compatibility with species, age, and health status
    - Minimize exposure to human presence and other animals that could be perceived as predators, loud noises, stress vocalizations that can serve as sources of anxiety.[10,11]
    - Time required to induce loss of consciousness[5]
    - Reliability/irreversibility

- Human factors:
    - Safety of personnel
    - Documented emotional effect on observers or operators
        - Emotional impact : The potential emotional and psychological effects on the people performing the euthanasia, and on observers must be acknowledged [5]
        - "*No matter what the situation, the act of performing euthanasia on a wild animal involves emotions. While we can't remove the emotions, we can develop guidelines which will help make the actual decision a little easier and hopefully remove some of the doubt*." [12]
    - Drug availability, human abuse potential, legal requirements
        - Lay rehabilitators are often left to perform euthanasia without a veterinarian (emergency situations, evenings, weekends, etc.); alternatives need to be available for these situations in which controlled drugs, advanced training or equipment is not available.[13]
    - Ability to maintain equipment in proper working order

- Remains (carcass) factors: Compatibility with intended animal use and purpose
    - Intracardiac injections can potentially damage heart tissue both mechanically and chemically
    - Commercial euthanasia solutions are NOT sterile
    - Barbiturates can precipitate in tissues.[14]

- Environmental impacts of the method or remains, including safety for predators or scavengers should the animal's remains be consumed; Barbiturates should not be used where carcasses can potentially be consumed.[15]

We must view the above within a practical systems view/process flow:

- Handling/restraint
- Euthanasia methods/agents: 2+ phases [16]; multi-stage process recommended.[2,17,18,19]
  - Loss of consciousness (unaware of surroundings, cannot feel pain, fear, distress)
    - Sedation = animal may be aroused to a conscious state with sufficient stimulation[6]
    - Anesthesia = unconsciousness and cannot be aroused[6]
  - Causing death
- Confirmation of death
- Disposal of remains, necropsy needed (and why)

CASE #1

COMMON SNAPPING TURTLE, MORIBUND, BARELY ALIVE, HIT BY CAR, NEEDS TO BE EUTHANIZED: @ veterinary clinic, ask what euthanasia method would others use for this? Sodium pentobarbital

SODIUM PENTOBARBITAL:

- Is a barbiturate developed in 1930s as an anesthetic[20] that has fallen out of use as an anesthetic. Currently, it is overdosed to cause death/euthansia[7].
- **"Sodium pentobarbitone** is an effective and humane method of euthanasia in reptiles"[19]
- They are poikilotherms (survive at a WIDE temperature range), and ectotherms (get their body heat from the outside world).
  - Vs humans—we only survive at a narrow temperature range (90-114 deg F?) homeotherms and are endotherms, make our own heat

- Come in two general formulations, both of which are ~390 mg/ml.
  - Sodium pentobartibal alone; dose ~1 ml/4.5 kg (10 lbs.); DEA schedule II
    - Fatal-Plus® (Vortech Pharmaceuticals): thin (not viscous) pH=9.6-11
    - Pentasol® (Virbac): pentobarbital sodium powder, reconstitute in 250 ml=392 mg/ml Virbac pH 11.1
  - Sodium Pentobarbital plus phenytoin sodium (50 mg/ml) →hastens cardiac arrest, so you can't get high…you just die…humans are less likely to abuse (Phenytoin sodium = pH 12)
    - Euthasol® (Virbac): pH=12-13 Thick, viscous
    - Beuthanasia-D®: Merck Animal Health: pH= 12-13 to release, 10-13 during shelf life

But how to give the sodium pentobarbital?

ROUTES of sodium pentobarbital administration:

INTRAVENOUS

- "The intra-venous route can be used by well-trained personnel and result in quicker death. May be difficult and likely stressful to try to hit a vein in an awake animal as most reptile phlebotomy is done blindly."[19]
- Ideally, pentobarbital is given intravenous (100mg/kg) [17]. But painful?

(sidebar)

What causes pain on injection?

needle features, injection site, volume injected, injection speed, osmolality, viscosity and pH of formulation, as well as the kind of excipients employed, including buffers and preservatives.[21]

What pH is painful?

- pH human blood 7.35[22]
- Ph between 4.5-8 doesn't hurt??
- pH 5-9 safe for injection per some nursing place
- *In vitro* experiments have demonstrated that solution pH values of 2.3 and 11 kill venous endothelium cells on contact.[22]
- IC/intra lungs painful[23]

Sodium pentobarbital solutions are caustic and it can hurt if given in an inappropriate spot. Pentobarbital and phenytoin are considered weak acids and must be formulated at a high pH to ensure solubility [22]. The alkalinity is assumed to be painful [19]. Some veterinarians recognize this and have will address this problem by diluting pentobarbital in isotonic saline, Anecdotally seeing reduced pain upon injection[19]. In theory, this does not influence the pH enough to make a difference because the pH scale is logarithmic. An alternative practice to attenuate the pain associated with this injection is to mix the pentobarbitone with lignocaine which has been shown to decrease (but not eliminate) the pain associated with intraperitoneal injections of pentobarbitone in mice.[19]

(back to sodium pentobarbital routes)

INTROCOELOMIC

- Intracoelomic administration is decribed[24], however, all formulations of sodium pentobarbital are caustic, and some advise they not be given extravascularly as it is perceived to be painful[17], [23].
- the intraperitoneal route may be used but it is slower acting[25]

ORAL

- PO, tastes bitter, prohibited in certain states [26] Has been used by some veterinarians [19] (typically via gavage tubing). Barbiturates have high oral bioavailability and can reach the brain quite quickly.[19] A disadvantage is this method will be slower than IV so in a moribund animal may be ineffective.

INTRACARDIAC

Intracardiac injection may only be used on a fully anaesthetized animal as this is very painful [25]

Intramuscular or subcutaneous should not be used as they are not effective and may cause pain.[25]

PHYSIOLOGY RELAVENT TO SODIUM PENTOBARBITAL (and other methods of euth) (and why it doesn't work)

"Significant differences in anoxia tolerance exist among chelonian species that can be attributed, at least in part, to the magnitude of metabolic depression, the effectiveness of lactic acid buffering, and the size of glycogen stores. "[27]

Northern fresh water turtle brains are ANOXIA TOLERANT (especially WPT/RES)

- "Although a number of common species have been studied, there is still the possibility that there is actually a relatively continuous spectrum of anoxia tolerance, and that by chance studies have only dealt with those towards the ends of the spectrum. With this caveat in mind, the following accounts are organized according to the species' degree of anoxia tolerance."[28]
- Electrical and biochemical brain activity is believed to persist in the anoxic chelonian brain for a considerable period of time [29]
- Some species of Freshwater turtles (*Trachemys* and *Chrysemys* genera) can survive many weeks without oxygen (commonly used as model animals for vertebrate anoxia tolerance).[30]
- Some freshwater turtle brains can survive anoxia for months (i.e. under the water during winter)[31], [32]
- *Trachemys scripta* which can withstand complete anoxia for days at room temperature to weeks in winter hibernation[27], [28], [33] (34 calls them a "true facultative anaerobe)
- even at room temperature, 24 h of anoxia and re-oxygenation results in no evident loss of neurons.[34]

These turtles may appear comatose d/t brain and body →DOWN REGULATION

- Can downregulate brain to become virtually comatose[31]
- one mechanism to extend anoxic survival is entrance into a state of deep reversible hypo-metabolism[35];
- energy demand is reduced to meet the energy supplied by anaerobic glycolysis[35].
- Energy demanding processes are greatly suppressed[35]; [27]
- Ion channels, and neurotransmitters are downregulated by multiple intertwined mechanisms, suppression of action potentials, protein regulation, reviewed here [35]

NEUROPROTECTION/OTHER TISSUE PROTECTION (why they don't die of reperfusion)

- A variety of protective mechanisms are activated at the molecular level in anoxic turtle brain, many of which may not only protect against damage under anoxic conditions, but also ameliorate oxidative stress when oxygen is restored. [35]
    - include increases in heat shock proteins, anti-apoptotic factors, the MAP kinases, antioxidants
    - modulation of the p53 pathway
- Protection upon reoxygenation: Unlike the mammalian brain, which shows an overproduction of reactive oxygen species (ROS) following hypoxia or ischemia/reperfusion (Hashimoto et al., 2003 Hashimoto, T., Yonetani, M. and Nakamura, H. (2003). Selective brain hypothermia protects against hypoxic-ischemic injury in newborn rats by reducing hydroxyl radical production. *Kobe J. Med. Sci.* 49, 83-91.), the turtle brain appears to suppress ROS production upon re-oxygenation (Milton et al., 2007 Milton, S. L., Nayak, G., Kesaraju, S., Kara, L. and Prentice, H. M. (2007). Suppression of reactive oxygen species production enhances neuronal survival in vitro and in vivo in the anoxia-tolerant turtle *Trachemys scripta*. *J. Neurochem.* 101, 993-1001.; Pamenter et al., 2007 Pamenter, M. E., Richards, M. D. and Buck, L. T. (2007). Anoxia-induced changes in reactive oxygen species and cyclic nucleotides in the painted turtle. *J. Comp.*
- *Physiol. B* 177, 473-481.).

- Because the downregulation of energy pathways and protection against cell death and oxidative stress are hallmarks of anoxia tolerance, it is not surprising to find evidence of p53 activation in the turtle (Zhang et al., 2013).
- Decreased ROS production, robust repair mechanisms, protection of structure and function, upregulation of protective pathways, increased antioxidants, NEUROGENESIS [33]

NORTH VS SOUTH Same species, but different latitudes do these things to different degrees:

- In the northern parts of their ranges in North America, turtles may spend more than half of their lives in an overwintering state.[28]
- The three northern subspecies are very anoxia-tolerant, and can remain responsive for more than 150 days in anoxic water, but the southern subspecies can survive only about half that time, having a more rapid fall in pH (Fig. 5; Ultsch et al., 1985; Reese et al., 2004a). [28]
- Northern turtles are better able to buffer accumulated lactate vs southern turtles, and thus survive longer in anoxic water[36]
- aquatic turtles with far northern ranges are more anoxia tolerant than southern animals even within a single species (probably because of the need to survive potentially long periods under ice or in hypoxic mud) (Ultsch, 2006 Ultsch, G. R. (2006). The ecology of overwintering among turtles: where turtles overwinter and its consequences. *Biol. Rev. Camb. Philos. Soc.* 81, 339-367.).

LACTIC ACID BUFFERING[27]

- Buffer lactate with bone and sequesters it there as well, helps that there is so much well vascularized bone in the shell [28]

BRAIN CELL REGENERATION

- "When heavily damaged by global ischemia, *T. scripta* showed evidence of neuronal reproduction within 3 weeks[37] "
- Can regenerate nerve cells[33]

Freezing/cold/anoxia tolerance adaptations

- Depends on location, Ontario turtles tolerate anoxia much better vs Nebraska turtles[38]
- Painted and snapping turtles have been seen brumating simply in the water (not in mud) but also in the mud, muskrat burrows/lodges, solo, together, seeps/springs, cattail stands, under banks, under logs, out in open water, etc. so many different environments [28]

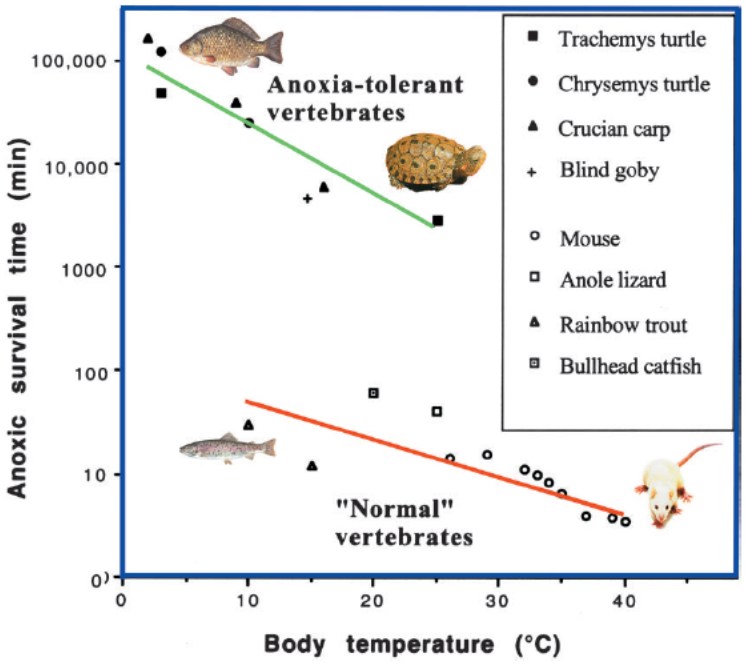

FIG. 1. Anoxic survival time in "normal" vertebrates and in anoxia tolerant vertebrates. Note that cold blooded vertebrates in general are as sensitive to anoxia as mammals, if temperature is taken into account, and that the anoxia-tolerant vertebrates survive anoxia about 1000 times longer than other vertebrates. In general, metabolic rate falls sharply with body temperature. A main reason why anoxia tolerant vertebrates survive anoxia longer at cold temperatures is probably that their glycogen stores last longer. For anoxia-intolerant vertebrates, a main benefit of a reduced temperature is that it slows down the loss of ATP and ion homeostasis, and the subsequent onset of degenerative processes. Redrawn from Lutz et al. (2003).

- [31]

AGE MATTERS

- Baby painteds hatch in fall, stay in ground, and live off yolk and come out in the spirng[38]
- Interestingly, although hatchlings of many turtle species survive in the nest in their first winter through freeze tolerance (Storey, 2006 Storey, K. B. (2006). Reptile freeze tolerance: metabolism and gene expression. *Cryobiology* 52, 1-16.) or super-cooling (Packard and Packard, 2003), in general hatchlings are far less able to tolerate anoxic submergence than adults (Reese et al., 2004). It has been suggested that this is because of their incomplete shell development, as the shell is important for lactate buffering (Ultsch, 2006 Ultsch, G. R. (2006). The ecology of overwintering among turtles: where turtles overwinter and its consequences. *Biol. Rev. Camb. Philos. Soc.* 81, 339-367).
- Snappers emerge the same year they were laid. They cannot overwinter in the nest (Packard & Packard, 1990; Packard, Ruble & Packard, 1993; Costanzo et al., 2001) [28]
-

THUS: HYPOTHERMIA (i.e. placing animal in freezer to cause death or dropping into liquid nitrogen) **is an unacceptable method** of euthanasia for two reasons, [17],[23],

1. they may not die (b/c of above physiology)
2. This methods as a standalone method is not acceptable for euthanasia of reptiles[25] as Ice crystals formed in the body are presumed to be painful [23]

i. Neonatal rodents can be rapidly frozen in liquid nitrogen (-196 degrees C) if < 4 g in body weight[23] as causes instantaneous freezing of the body. The AVMA 2020 is considered appropriate under these conditions[39]
ii. Freezing of deeply anesthetized animals in a situation where human safety cannot be compromised *may* be justifiable.[39]

(BACK TO CASE) LUCKILY, YOU HIT A TAIL VEIN, how does sodium pentobarbital work?

How sodium pentobarbital works in mammals:[26]

Stage 1: voluntary excitement
- Bloodstream → heart → cerebral cortex
- Begins to lose consciousness/coordination, hyperesthesia, loses voluntary motor, loss of superficial pain → disorientation, possible movement

Stage 2: involuntary excitement
- Bloodstream → heart → cerebral cortex → cerebrum
- Loss of consciousness → possible uncontrolled motor activity (paddling, vocalizations)

Stage 3: surgical anesthesia
- Bloodstream → heart → cerebral cortex → cerebrum → cerebellum (~5 sec)
- Cannot feel pain, doesn't respond to visual/auditory, reflexes begin to disappear

Stage 4: medullary paralysis
- Bloodstream → heart → cerebral cortex → cerebrum → cerebellum
- Depresses breathing, heartbeat, blood pressure→anoxia→brain cell death* (~40sec) → possible heart fibrillation, agonal breaths, muscle spasms (unconscious, reflex)
    - *20% of the oxygen consumed by the body is used by the human brain[35] (making ATP to maintain membrane potentials). Thus, the brain is one of the first organs to fail in anoxia, interruption of the oxygen supply to the brain for more than a few minutes leads to irreversible neuronal death [35]
        - Notes (won't go into this) →"Without oxidative phosphorylation, ATP-dependent neuronal processes including ion transport and neurotransmitter reuptake decline sharply. Without pumping, ion gradients fail and neurons depolarize, releasing excessive levels of excitotoxic neurotransmitters such as glutamate and dopamine. The overstimulation of glutamate [*N*-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)] receptors increases intracellular calcium levels and triggers multiple internal cascades that result in cell damage and death, including activation of lipases, endonucleases and proteases, and mitochondrial-dependent apoptosis" (Lipton, 1999 Lipton, P. (1999). Ischemic cell death in brain neurons. *Physiol. Rev.* 79, 1431- 1568.).

HOW DO WE KNOW IF A TURTLE IS DEAD? WHAT HAVE YOU DONE IN THE PAST?
- Walk through physiology of following scenarios:
    - Doppler to check heart (problem: can have REALLY SLOW heart beats, may not place probe correct)
    - Leave on floor for 48 hours, check for smell/rigor (problem: just means body tissues are rotting, doesn't mean the brain is rotting)
- All these methods look for the stop of the heart➔ stop of blood flow➔ how does this "kill" mammals? By causing irreversible brain cell death d/t anoxia….what happens to turtle brain cells in anoxia/ they survive!
- So we need a new way to confirm death in turtles (and really we can apply to all reptiles because we don't know how tolerant most species brains are to anoxia)

CONFIRMATION OF DEATH in reptiles

- "Some reptiles presented for emergency evaluation may have already experienced respiratory or cardiac arrest, unbeknownst to the owners. According to the AVMA Guidelines for the Euthanasia of Animals, it is "difficult to confirm" death in reptiles and amphibians.18 Certain reptiles, especially freshwater turtles, can tolerate prolonged periods of anoxia and corresponding severe bradycardia.19 Corneal reflexes are difficult to assess in reptiles that lack eyelids, such as snakes and some geckos. Every reptile, even if unresponsive and in respiratory arrest, should be checked for a heartbeat using the methods described above. However, the presence of a heartbeat is not synonymous with "life," as reptile and amphibian hearts can and will continue to beat for hours after brain death.18 An electroencephalogram (EEG) can determine brain activity in unconscious reptile patients that still maintain a heartbeat20; however, this test is not practical in most clinical settings. The reader is referred to Chapter 47 in this text for additional information on euthanasia.[40]"
- Can be difficult[2]
    - Reptile hearts can beat even after brain death (?) [6] and a stopped heart doesn't mean brain death…
    - death should always be confirmed by physical intervention[23]
    - Death is judged to have occurred when recovery of brain activity is not possible[29]
    - Humane methods must achieve nonreversible damage or destruction of the brain tissue to ensure the death of an animal.[18]
- Concerning euthanasia, the AVMA Guidelines state: "it is often difficult to ascertain that an amphibian or reptile is, in fact, dead."1 Although this statement has become a paradigm when dealing with euthanasia of herpetological species, the truth is there are methods to ensure reptiles and amphibians are indeed dead, and it is our responsibility as veterinarians to educate ourselves and apply those methods in a manner that respects their welfare.[2]
- Assessment of heart rate by electrocardiogram or Doppler blood flow analysis does not provide an accurate determination of death because the heart occasionally continues to beat, even hours after the apparent euthanasia. Movement of the limbs and head should also be interpreted cautiously because this is often normal even after pithing and decapitation. This is due to inherent differences in the nervous, system of reptiles and amphibians, including the presence of spinal ganglia. Nonetheless if movement is present, a thorough assessment of the patient must be performed to ensure it is deceased. [2]

- Pithing or otherwise destroying brain material great way!![2]
    - MUST be done in every "euthanized" or "dead" reptile because it's the only way to ensure they are no longer perceiving pain/suffering
- What if Heart beating upon necropsy [17] or after brain destruction [23]
    - Additionally, reflexes and other movements may remain intact b/c of increased number of spinal reflexes in these species and/or tissues being tolerant to hypoxia as well [23]
    - It's ok b/c the brain is destroyed—so they cannot suffer/feel pain

CASE #2

PAINTED TURTLE, ACTIVE BUT SPINAL TRAUMA WITH NO DEEP PAIN, DRAGGING BACK LEGS, HIT BY CAR, NEEDS TO BE EUTHANIZED: @ veterinary clinic, ask what euthanasia method would others use for this? Sodium pentobarbital, decapitation, blunt force trauma to head, shooting

What about inhalant anesthesia (i.e. used in mammals and birds, either to anesthetize or anesthetize and euthanize?

"With inhalant anesthetics, the animal can be placed in a closed receptacle containing cotton or gauze soaked with an appropriate amount of the anesthetic, or the anesthetic can be introduced from a vaporizer. The latter method may be associated with a longer induction time. Vapors are inhaled until respiration ceases and death ensues2.

- Many reptiles (especially turtles) will breath hold [17,32]
- Many reptiles are capable of holding their breath and converting to anaerobic metabolism, and can survive long periods of anoxia (up to 27 hours for some species)2,4,6. Because of this ability to tolerate anoxia, induction of anesthesia and time to loss of consciousness may be greatly prolonged when inhalants are used2,17. Death in these species may not occur even after prolonged inhalant exposure2. Therefore euthanasia by inhalation of toxic gases is not an acceptable method for Euthanasia in reptiles1,3,4,18."[25]

Isoflurane[6]:

- Smells noxious, irritates respiratory epithelium → animals may breath hold → may cause distress
- Turtles will hold their breath b/c noxious….and can for days. Not recommended for turtles

Can you hit a vein? No, turtle too small. We don't want to use intracoelomic, SC, IM, IC, etc. because of pH of solution
There is the occipital venous sinus[41] that would work well but it appears painful in awake turtles and requires a lot of uncomfortable restraint. Let's anesthetize first!

If veins too small, need SC or IM anesthesia. Propofol is IV….Alfaxalone is a great choice! Not many other combos of meds work well in this species (but for other species you may have other combos that DO work well, in which case, use an overdose/double dose)
My recommendation for a feisty WPT for euthanasia: 20-30 mg/kg alfaxolone SC or IM (usually SC works well but sometimes you get it in a fat pad, in which case it isn't well absorbed. If minimal effect after 20 min, redoes. Volume of US alfaxolone doses is LARGE so the volume in a muscle belly can be painful! Anecdotally, forelimbs have better effect vs rear limbs. Consider giving half into each forelimb, or half SC and half IM.

Once anesthetized, give pentobarbital IV occipital venous sinus. (videos, diagrams, etc.)

How to confirm death? PITH

What if no controlled drugs available? We may be left with physical methods. Ideally you would have the animal anesthetized first, but all good anesthetics are controlled substances.

PHYSICAL METHODS OF EUTHANIZING TURTLES "when properly used by skilled personnel with well-maintained equipment, physical methods of euthanasia may result in less fear and anxiety and be more rapid, painless, humane and practical than other forms of euthanasia."[6]

- BLUNT FORCE TRAUMA: to the head in which the brain is destroyed [42];
    - Killing in field, for consumption or when appropriate drugs, training or outcome (i.e. eating) are not available [42]
    - Can be humane but difficult for people to do/watch[19] and can be difficult in turtles as they have thick skulls
    - This involves striking the head of the animal directly over the cranium with some hard implement or object and with sufficient force to cause immediate loss of consciousness and/or death9,14. If many animals are to be killed within a short time by the same operator it is difficult to ensure consistency in performance and therefore only a few animals should be killed by the same person using this method at any time1. Larger reptiles (crocodilians) may be rendered unconscious by this method but are less likely to be killed. The brain must be destroyed before the return of consciousness3,4,9,14, either by a further blow or by some other method such as pithing. It is considered an acceptable method for all reptiles but should only be carried out by experienced operators6 who know exactly where to strike. [25]

- DECAPITATION : This procedure involves the severing of the neck of the animal, exactly between the skull and the first cervical vertebra, using a sharp instrument (guillotine1,2,3,4,5, axe or blade1) ideally with a single very swift cut4 that leads to severance of the spinal cord.
    - alone not acceptable[17], [15]
    - Rattlesnake's heads appeared to be reacting to things they saw up to 59 min post decapitation [43]
    - "Alligators spinal cord severance not humane/appropriate, as brain activity was detected up to an hour after spinal cord severance (and "higher/more" than anesthetized alligators) [18] Analysis of results for the present study revealed that severance of the spinal cord alone was not able to totally depress EEG waves. For severance of the spinal cord alone, EEG power in the _, _, and _ frequency bands was higher after the procedure than during anesthesia. The alligators retained reflexes for an extended period and had repeated periods of electrical seizure activity that could not be expressed by motor activity because of the severed spinal cord. Because this method failed to meet our definition for a humane procedure, it cannot be recommended and should not be used in alligators. b/c of reptile brain being very tolerant of hypoxia, therefore it is assumed that the perception of pain can continue for at least several minutes after the animal's head has been removed."[19]
    - "Some reptiles may remain conscious for up to an hour after decapitation4,6,7, which makes this procedure acceptable only if the brain of the severed head is immediately destroyed by pithing2,4,6,8 or by blunt trauma."[25]
    - In particular, the assumption that decapitation results in rapid unconsciousness is disputed, for it seems that the brain of a reptile can remain viable for up to an hour after decapitation (Cooper and others, 1984; Cooper, 2003). [In mammals, electrical waves have been recorded from the brain for short periods after decapitation and there has been debate as to whether these waves were indicative of sensibility (Reilly, 1993).] Cooper, J.E. (2003). Reptiles, amphibians and fish. In: *BSAVA Manual of Wildlife Casualties* Mullineaux E, Best D, and Cooper J.E. (Editors), British Small Animal Veterinary Association, Gloucester, United Kingdom, pages 270-276.

- CERVICAL DISLOCATION: This method involves separation of the skull and the brain from the spinal cord by applying pressure in a simultaneous ventral-cranial motion at the base of the skull with an appropriate tool.[25]
    - "Cervical dislocation, if carried out near to the head, causes damage to the lower brain region, resulting in rapid and painless loss of consciousness. This must always be followed immediately by destruction of the brain or section of the major blood vessels in the neck. However, research has shown that visual evoked potentials may remain up to 30 s after dislocation which may indicate lack of insensibility".[23]
    - DIFFICULT in anything larger than 200g[25]

- CAPTIVE BOLT: Captive bolt pistols are powered by gunpowder or compressed air and must provide sufficient energy to penetrate the skull2 (penetrating captive bolt) or cause fatal stunning (non-penetrating captive bolt) of the species on which they are being used.
    - Must kill/go through brain if no anesthesia[23]
    - Requires skill, good knowledge of anatomy [23]
    - "The animal must be properly restrained to ensure that only a single shot is required. Both penetrating and non-penetrating captive bolt guns must be placed directly on the skull over the brain cavity to en-sure their effectiveness. All personnel must be trained in these techniques to ensure the correct positioning of the weapon to ensure a direct hit into the brain1. An appropriate charge of the gun (air or gunpowder) must be selected to match the size of the animals. It has been shown to be very efficient for the slaughter of Pantanal caimans and American alligators, and can be used for all sizes of croco-dilian12,13 given that appropriate charge is selected. It is considered an acceptable and humane meth-od for large reptiles but should only be carried out by trained personnel who know where to position the pistol5 and thereby ensure a direct hit into the brain. In snakes the captive bolt would have to be shortened to avoid wrist injuries and damage to the equipment. However, there are ways of using the standard equipment in snakes by placing soft materials (foam, etc.) beneath the animal to soften the trajectory of the bolt after penetrating the head. Alternatively the non-penetrating bolt would not need modification although the head is quite large for some species".[25]

- SHOOTING/GUNSHOT
    - "A high level of skill is required in order to hit the brain through the two brain cases found in many rep-tiles9. In addition, with small species, and/or where the target is moving, shooting may not be effective. However, apart from this, shooting in the head to ensure immediate destruction of the brain is an effective and humane way of killing large reptiles1. It is occasionally recommended that even when this method is used the spine is severed and the brain destroyed by pithing 10,11." [25]
    - Entire section in AVMA 2020 about gunshot, energy requirements, bullet selection, etc. bullet should go through cranium and into brain…firearm safety!!

- EXSANGUINATION: By cutting the major blood vessels in the neck i.e. the carotid arteries and jugular veins.
    - not acceptable b/c tolerant to hypoxia[23]
    - This method of euthanasia is not acceptable for reptiles and other ectothermic vertebrates because of their slow metabolic rate and hypoxic tolerance.[25]

- PITHING: Carried out by inserting a sharp metal rod or probe through the foramen magnum into the base of the brain to ensure quick brain destruction3.
    - "Method acceptable for unconscious reptiles1,2,9 (e.g. stunned, anaesthetized). It may also be acceptable when performed immediately after decapitation or cervical dislocation. Pithing can be carried out in reptiles without crushing the skull4".[25]

Other random unacceptable methods:

- Hyperthermia: Raising the temperature above the critical temperature of the species4. This method is not acceptable for euthanasia of reptiles1,9,12.[25]
- Suffocation/drowning: Depriving animals of oxygen. This method is not acceptable for euthanasia of reptiles.[25]
- Drowning : This method is not acceptable for euthanasia of reptiles4.[25]

CASE #3

COMMON GARTER SNAKE, ACTIVE BUT SPINAL TRAUMA WITH NO DEEP PAIN, DRAGGING TAIL,, NEEDS TO BE EUTHANIZED: @ veterinary clinic, ask what euthanasia method would others use for this?

My recommendation: isoflurane liquid in box, once anesthetized IC pentobarbital (or leave in box for several hours. Pith after.

Recommended techniques for reptilian euthanasia (Mader 2006)

Group	Chemical	Inhalant	Physical	Deep Freezing
Lizards	YES	YES	YES	< 4 g
Snakes	YES	YES	YES	< 4 g
Chelonians	YES	NO	YES	< 4 g
Croc	YES	NO	YES	NO

Rapid freezing

a. Animals <4 g where immediate death occurs
b. Not considered to be humane for larger reptiles
c. In all other cases, animals should be rendered dead or unconscious prior to freezing

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