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Note: Text has been edited for clarity.

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Field Euthanasia Methods for Wildlife

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Broadcast Date: March 29, 2018

View Recording: <https://youtu.be/ZXhWcYPWmpc> (YouTube)

Slide 1 (Field Euthanasia Methods for Wildlife)

>> *Swapna:* Today is March 29, 2018. I am Dr. Swapna Mohan and it is my pleasure to welcome our speakers for today Dr. Tracy Thompson and Dr. Brent Morse to the OLAW Online Seminars to present **Field Euthanasia Methods for Wildlife**.

Dr. Thompson serves as the Chair and Attending Veterinarian for the National Park Service IACUC which oversees research activities involving vertebrate wildlife in National Park Service units. Dr. Thompson holds a veterinary degree from Washington State University's College of Veterinary Medicine. She has had a rich and varied career, involving companion animal practice, veterinarian for captive zoo and wild animal facilities, Attending Veterinarian (AV) and research associate for the Venom Lab at the Western Institute for Biomedical Research, an instructor and AV for a Veterinary Technology program, and as a Veterinary Medical Officer with the USDA, APHIS, Animal Care.

Dr. Morse is the Acting Director of the Division of Compliance Oversight at OLAW. He earned his veterinary degree from Washington State University in 1987. Prior to joining OLAW in 2006, he served in the Army Veterinary Corps as a Veterinary Officer, and in the Public Health Service Commissioned Corps at the NIH.

And now it's now my pleasure to hand over the microphone to Dr. Tracy Thompson.

Slide 2 (Objectives)

>> *Tracy:* Thank you, Swapna. And thank you to OLAW for inviting me to speak to all of you about field euthanasia methods. The goal of this talk is to get folks thinking about what provisions are required when reviewing protocols for wildlife work in the field, and

ensuring that methods are in place to relieve pain and distress for the animals involved in that work.

The objectives for this session are:

- to review euthanasia and humane killing methods,
- to choose methods based on compliance with regulatory requirements and also consider professional society guidance regarding these methods,
- to discuss challenges and conditions for performing euthanasia in the field, and
- to examine the IACUC's role and responsibility for ensuring appropriate methods and training for field euthanasia.

Slide 3 (Euthanasia)

The Greek word euthanasia or *eu thanatos* means good death. According to the *Guide*: "*Euthanasia* is the act of humanely killing animals by methods that induce rapid unconsciousness and death without pain or distress. Unless a deviation is justified for scientific or medical reasons, methods should be consistent with the *AVMA Guidelines on Euthanasia* (AVMA 2007 or later editions)." Current PHS Policy refers to the AVMA 2013 Guidelines or later editions.

Slide 4 (American Veterinary Medical Association (AVMA))

The American Veterinary Medical Association (AVMA) convenes a committee of experts periodically to put together a series of guidelines for the appropriate euthanasia methods for different species in different environments. Under the AVMA Guidelines for the Euthanasia of Animals, it designates that Euthanasia is: "...ending the life of an individual animal in a way that minimizes or eliminates pain and distress." The Guidelines also state that: "A good death is tantamount to the humane termination of an animal's life."

The most recent version, the 2013 Guidelines for the Euthanasia of Animals, added a section within 7.6 that specifically addresses the challenges that are inherent in work on free-ranging wildlife. Essentially, this paragraph sums up the fact that for free-ranging animals, the efficacy of the method chosen for euthanasia may be limited by the circumstances under which the euthanasia is being performed. And so, the optimal method for terminating an animal's life under specific circumstances may not strictly conform to the definition of euthanasia provided in the *Guide* or the AVMA Guidelines.

Slide 5 (Humane Killing)

The AVMA refers to humane killing, as we saw in the previous slide, as methods of killing other than those deemed "acceptable methods of euthanasia", and may be justified in situations with free-ranging wild animals. And it also indicates that the quickest and most humane means of terminating the life of free-ranging wildlife in a given situation may not always meet all criteria established for euthanasia. For example, a gunshot according to the AVMA cannot be considered euthanasia unless the bullet placement is to the head. And we'll address this particular situation a little bit later as we move forward.

Slide 6 (Regulations and Guidelines)

So where do the regulations and guidelines stand with regard to an IACUC's role or researcher's role in choosing appropriate methods of euthanasia or humane killing? The regulations and guidance for researchers and IACUCs from both the regulatory and the funding and oversight agencies deal with minimizing pain and distress as well as euthanasia.

OLAW has guidance on humane methods of euthanasia, as does the USDA Animal Care program. With regard to OLAW, as we already alluded to, it expects institutions to comply with the PHS Policy and adhere to the *Guide for the Care and Use of Laboratory Animals* which includes reference to the AVMA Guidelines.

The USDA updated their Animal Care Policy #3 in October of 2017 to include a statement "Licensees and registrants, in consultation with their attending veterinarians, can use methods of euthanasia that meet the definition of euthanasia in the Animal Welfare regulations... Appropriate methods [of euthanasia] may include, but are not limited to, those described in the "AVMA Guidelines for the Euthanasia of Animals"."

Slide 7 (Taxa and Professional Societies)

Additionally, there are professional organizations, some listed here, such as the [American Society of Mammalogists](#) [PDF], the [Ornithological Council](#), the [American Fisheries Society](#) [PDF], the [American Society of Ichthyologists and Herpetologists](#) [PDF], that publish specific guidelines based on field conditions that researchers may encounter as well as the variations among the taxa or the species. These are informed by subject matter experts and are updated periodically to reflect current changes or refinements in field procedures and standards. Links to these organizations are provided on the [OLAW webpage](#) as well as here on the slide and taxa specific guidelines can be used in a supplementary role as long as they do not conflict with the primary standards that OLAW sets forth regarding your Assurance if you're an Assured institution.

There are additional resources available for humane euthanasia of wildlife from other sources, such as the [Canadian Council on Animal Care](#) (CCAC) [PDF] and the NC3Rs from the UK, the [National Center for the 3R's](#). These provide useful information on the conditions and criteria to be considered when choosing a method of euthanasia for wildlife. [*Attendee Comment: Canadian Council on Animal Care guidelines on the care and use of wildlife is currently under review.*]

And now, Brent Morse will talk about OLAW guidance on euthanasia methods.

Slide 8 (PHS Policy and OLAW Guidance)

>>*Brent*: Thank you, Tracy. The PHS Policy states that "methods of euthanasia used will be consistent with the recommendations of the AVMA Panel on Euthanasia, unless a deviation is justified for scientific reasons in writing by the investigator."

The IACUC is responsible for oversight of live vertebrate animal activities including field euthanasia of wildlife. The IACUC must ensure that proposed studies are in accord with the *Guide* where applicable and that methods of humane killing approved by the IACUC are case specific and the exception to using methods in the AVMA Guidelines noted as acceptable or acceptable with conditions.

While PHS funding of wildlife studies is not common, it does occur. However, with the establishment of a formal MOU between the National Science Foundation [NSF] and OLAW in 2015, OLAW is now responsible for ensuring compliance with the PHS Policy for all NSF grants and cooperative agreements involving research with live vertebrate animals. This includes NSF-funded field studies utilizing wild animals. And now, back to Tracy.

Slide 9 (Need for Euthanasia or Humane Killing)

>>Tracy: Thank you, Brent. So what would indicate the need for euthanasia with regard to wildlife research activities? Unlike laboratory animal facilities, sometimes the goal for wildlife studies is to collect specimens, such as voucher specimen collections for museums or to collect tissues or samples for diagnostic procedures. For such studies, euthanasia is expected as part of the [planned activities] and should be prepared for in advance of going out and conducting the work.

However, when and if something goes wrong while conducting field research, are you as a researcher and is your IACUC aware of what methods are available to use in order to relieve pain and suffering that animals in research activities might experience? Choosing a method in such a case would depend upon the type, extent, and severity of injury that might occur during capture and handling and other procedures [handling].

Other factors to consider would be the physical condition of the animal and its ability to survive. For example, some veterinarians might have seen wild animals that appear severely injured in the field and would consider euthanasia as the best option for relief of the animal's suffering. [This is a part of wild nature if the injury is not human-induced. These animals may die, but] often the animals may go on to survive successfully. When animals are found in conditions like that in the field, it is as a result of natural conditions other than those of the research activities involved, and often (as documented through a camera trap, or collaring and marking data that's been collected) these animals have been shown to go on to survive. So, it may be best to allow that animal to remain in the population instead of choosing to add in a method of euthanasia for a naturally occurring condition. [*Dr. Thompson clarifies: Various options must be considered if an animal has a pre-existing condition when it is captured. It may be best to allow the animal to remain in the population or it may be best to relieve its suffering. The decision is often determined by the parameters stated in the permit to capture the animal which may restrict the researcher from performing euthanasia.*]

Again, permission in permitting for doing the work in the field may also dictate what is and is not acceptable for wildlife that's captured or found in the field to be injured. In the

case of threatened and endangered species, there may be restrictions in the permits that do not allow euthanasia to be performed on those species. And in such cases, what kind of questions can the IACUC ask and what kind of provisions might be put in place in advance to consider minimization of pain and distress, knowing that there's limitations due to the permitting process?

If an animal injury is human caused, particularly in relation to the research activity, euthanasia should always be a contingency. So, if animals are to be captured and handled for research activities, are the personnel trained and prepared to humanely kill an animal that becomes injured or moribund as a result of those research activities?

Slide 10 (Factors to Consider When Choosing a Method)

Numerous factors may go into the selection of a method of euthanasia. Some of the things for the IACUC and investigator to consider while selecting a method are listed on this slide.

Safety, of course, is a primary consideration for both the safety of the personnel involved, and animals. Can the animal be restrained effectively? Are you able to get close enough to physically administer a method of euthanasia that's appropriate? Are the personnel administering the method sufficiently trained in that procedure? Are the necessary drugs and/or equipment available to have out in the field in order to perform those methods safely and effectively? Will the method be aesthetically acceptable to bystanders, observers, or other personnel involved?

Disposition of the carcasses following euthanasia and consideration of potential impact on the environment are also very important factors to be considered when selecting a method of euthanasia. How will and can the carcass be disposed of out in the field? Could there be secondary impact on other animals, such as scavengers? Would a different method be chosen if the animal is acting strange, such as if there are concerns about rabies or other neurological conditions? Is the method suitable to collect the appropriate diagnostic samples depending on what presentation – clinical signs presented – by the animal in the field?

Slide 11 (Euthanasia Methods)

The methods themselves are usually broken down into two categories: chemical and physical methods. The chemical methods usually involve the administration of an agent, such as an anesthetic, an analgesic, or other agent that might produce rapid unconsciousness as was referred to in the OLAW definition of euthanasia.

Sometimes more than one agent might need to be administered, as in the case of animals that are immobilized in the field, such as with a potent opioid, and then the animal would require euthanasia as it is being handled or worked on. It might be given an additional dose of opioid or anesthetic agent or a potassium/magnesium salt or gunshot – so a two-

step method of euthanasia is fairly common with when dealing with wildlife procedures in the field.

Physical methods engage in the physical disruption of the central nervous system or the cardiopulmonary system of the animal. Many of these methods also should follow a two-step procedure, whether administering a chemical agent first followed by the secondary physical method, or a two-step physical method as outlined in the AVMA and other taxa guidelines, such as decapitation followed by pithing in reptiles and fish.

Slide 12 (Field Limitations)

There are many field limitations. I'm only going to cover a few of these in a general sense. But when you go from a very controlled environment such as in a laboratory setting to the field and free-ranging wildlife, there are conditions that are very different and often unpredictable and variable.

Safety is certainly at the top of the list for any method chosen for any procedure that is being conducted in the field. When remote delivery systems are used to deliver chemical immobilization agents, certainly there are safety concerns with working in dangerous terrain, or the animals themselves may pose a risk from infectious diseases, such as zoonotic diseases, or because of aggressive behavior. The safety of the drugs themselves is certainly a concern. Many of the drugs that we use in wildlife are a lot more potent or very different from what veterinarians use in clinical practice, such as the potent opioids. These require very specific safety handling procedures to protect the research staff working with those agents or working with the carcasses after administration of those agents.

Again for chemical methods, additional limitations might be whether or not the research staff are experienced and qualified to administer chemical methods in an appropriate manner, licensure issues, such as transporting certain drugs, like controlled substances, or requirements by state and federal agencies especially those regarding dispensing and administering drugs in the field. Those all come into play when we're talking about using chemical methods of euthanasia and anesthesia out in the field with wild animals. So these should be considerations that the PI has addressed and can provide to the IACUC in advance of going out to conduct their work.

As I mentioned, environmental factors – you're out who knows where doing the field work – the variability and challenges that may occur in the field can definitely impact the methods that are selected. There are days when it's incredibly windy and you cannot accurately shoot a dart from a remote delivery system in order to immobilize an animal and then provide appropriate euthanasia. Terrain that is dangerous that could potentially isolate you from the animal and therefore limit your access to that animal to safely administer an appropriate method of euthanasia.

So there are many potential issues with regard to the environmental impact, and as I mentioned already disposal questions – disposition of the carcass – definitely needs to be a consideration for the safety of the environment and the other animals that live there, as well as humans that are involved.

Appropriate restraint of animals is essential to conducting field procedures, particularly euthanasia. Capture methods of wildlife should be well-established for the species to minimize chase and handling times in order to avoid any stress that humans are causing those animals from becoming distressed. Distress can lead to unanticipated injury or illness and then may necessitate an unplanned euthanasia or may lead to suffering and death of those animals that would not be able to be relieved appropriately in time.

With regard to physical methods, appropriate methods of restraint to properly and safely administer that method is essential. For example, when considering cervical dislocation of bats, rabies is a potential risk to any handler if they are working around bats, so physically restraining a bat poses a potential risk for human health and therefore other methods or certainly the qualifications of those individuals and personal protective equipment should be well thought out before a physical method of euthanasia would be used in those situations.

Certain physical methods such as gunshot or decapitation may have an unacceptable aesthetic impact on personnel, students, or members of the public and may impact their perception of how humane those methods actually are. So again, aesthetic components of some of these methods needs to be considered and precautions for situations addressed at the time that euthanasia would need to be administered.

Again, environmental impact, more commonly we think of that with the chemical methods and secondary toxicities potentially due to carcasses left in the environment. But the use of lead ammunition is also an issue with regard to disposal of animals that would have gunshot killing performed in the field. So I would encourage those researchers that have gunshot as a method of euthanasia to consider using non-lead ammunition or be able to ensure that that carcass can be removed or deeply buried in the environment to protect other animals from coming upon lead fragments or lead in the body.

Slide 13 (Special Field Considerations)

One of the issues with free-ranging wildlife is we have an enormous variety of size and species and conditions in which those animals live. Marine species are a particularly challenging group for any researcher or any wildlife personnel that work around these animals in terms of providing a humane method of euthanasia either due to stranding situations or if you're working with these animals in a free-range situation in research.

Again, chemical methods and physical methods are available and public and personnel safety and impact need to be considered. But again the size of these animals and the visibility, especially with regard to stranding, can pose some very challenging situations.

This picture in particular shows you the sheer size of an animal that's been stranded and this was provided by a researcher who I would definitely keep on your list if you're ever going to have folks doing marine species work as a source for good information on appropriate methods of euthanasia for these animals. Obviously there's a lot of adaptations and we'll talk more about that as we go through other methods and other species. This is using a large trocar attached to a pressurized dispensing system, sprayer system, to administer a chemical euthanasia method intracardiac for this large whale. Again, if any of our researchers are involved with stranding networks or marine mammal research in the field, please bring in additional expertise as well as work with that researcher in order to determine what the most appropriate methods might be should euthanasia be needed in the field.

Slide 14 (Special Field Considerations)

Other considerations for selection of euthanasia methods should include the unique species or physiologic characteristics that may influence efficacy and safety. Venomous reptiles can be handled safely in the field by trained and knowledgeable personnel but the methods of euthanasia will depend on how safely that can be administered in the field. And so again, things like that should be thought-out and provide a good justification prior to those folks going out and conducting any work.

Species that commonly exist in low oxygen or in torpid states may not effectively be euthanized by inhalant or even injectable agents so often two-step methods, or killing methods, that often are unacceptable under the AVMA Guidelines, may be your best choice. Scientific justification should definitely be supplied by the researcher to the IACUC for these circumstances and these species. Again, risks for consumption by humans or other animals if chemical agents might be used in the field should be considered before carcasses are left in the field in a manner that would allow predation or potential contact by humans or other animals.

Slide 15 (Injectable Agents)

Let's talk about the methods themselves. Just briefly, here is a picture of some of the injectable agents that are commonly used in wildlife immobilization, alpha-2 agonists in combination with dissociatives and sedatives. An overdose of a chemical immobilization agent that I mentioned earlier may be used fairly commonly out in the field if that's part of the handling and capture method. But also barbiturates such as Euthasol or Sleepaway, can be carried into the field and be administered via intravenous, intracardiac, or intraperitoneal methods. Again disposition, I cannot emphasize enough, we need to make sure that those the animals can be removed or deeply buried, taken to a landfill, or incinerated.

Slide 16 (Inhalants)

Other methods that are fairly accessible under certain conditions in the field include inhalant agents, particularly for small animals that can be put into chambers that are

easily adaptable to carry in the field. Gas anesthetics such as isoflurane can be used very safely following a “drop method” technique, and I’ll show a set up for another one commonly used in the field. This particular image on the slide is one that is being recommended by the USGS, US Geological Service. Dr. Anne Ballman is teaching courses on euthanasia in the field for bats for submission for White-nose [syndrome] testing. That is a 50 ml conical centrifuge tube with a cotton ball soaked with isoflurane in the bottom with the bat inside that conical tube. And again, the benefit of this is you can minimize the amount handling by the personnel with the bat and can submit the bat in this container to the laboratory for analysis. So this is a very convenient and easy way to take this to the field. You can pre-prepare these tubes to go into the field and seal them tightly for probably up to 24 hours from what we’ve had reports back from folks doing this work.

Slide 17 (Inhalant Field Euthanasia Set-up)

Here is a much more common set up for small mammals in the field. Again using the drop technique and a tightly sealable plastic container to hold animals securely. In the picture you’ll see a tea strainer but any type of mesh device that can allow the isoflurane to be vaporized from the soaked cotton balls, but not come in contact with directly with the animal that would be in this chamber, is the best method in order to minimize irritation to the mucous membranes from the isoflurane. The equipment is very affordable and transportable, and the IACUC should ensure that the researchers are trained to use this and have access to isoflurane to carry into the field with them. Thus regarding, they would need to have a veterinary relationship to get that particular prescription to carry it into the field. PPE – or proper personal protective equipment should be available in order to protect the personnel involved in handling these animals for euthanasia. And then, I will walk you through just briefly the technique using this equipment.

Slide 18 (Step 1: Charge the Chamber)

So again, courtesy of Dr. Michelle Verant for these photos from the National Park Service. Step 1 is you charge the chamber with the isoflurane soaked cotton ball within that tea strainer.

Slide 19 (Step 2: Add Animal)

The animal is placed in the chamber with the tea strainer.

Slide 20 (Step 3: Monitor)

Having a clear chamber is very important. It allows you to monitor the animal visually for signs of euthanasia – so loss of righting reflex, lack of movement, slow respirations or no respirations – before opening the chamber. Of course this should be done in a very well ventilated area to prevent any side effects to the humans involved.

Slide 21 (Step 4: Verify Death)

And then the last step once the animal appears to have succumbed to the overdose of isoflurane that you would verify death appropriately through checking for heart rate and respiration, and then if needed a secondary physical

method could then be more safely applied since the animal would be deeply anesthetized or already euthanized.

Slide 22 (Inhalants)

Additional methods of euthanasia by inhalant method, again this is a classic laboratory set-up for CO₂ euthanasia for mice or rats. It is acceptable with conditions for some wildlife by the American Veterinary Medical Association. Taking this particular apparatus is obviously not going to happen in the field, but modifications can be made. And I will show you an example of those modifications. Again, this is a controversial method of euthanasia – there are a lot of feelings one way or the other about utilizing it – but it is another option for field researchers to have in their arsenal of methods knowing that different scenarios may require different choices.

Slide 23 (Field CO₂ Chamber)

Here is a modification that can be taken into the field very effectively and easily. The reference for this modification is on the slide [Development of a compact system for field euthanasia of small mammals; Murray V. Ellis, *Journal of Mammalogy*, 98(4):1211–1214, 2017], Murray Ellis in Australia published this in the *Journal of Mammalogy* last year and we here in the National Park Service have rebuilt this based on his information and guidance. He's very excited for field researchers to have access to something like this when it's an appropriate method for field euthanasia.

It is a system that can be calibrated. We have a mini regulator that is very commonly acquired through home beer brewing industries and very affordable. And they vary in how precise the calibration can be. So you would want to make sure that you looked at what would be best for the situation which your researchers would need for calibration. We were able to calibrate our system for the 20% volume displacement per minute and the system definitely needs to be calibrated in order to ensure that it will work effectively every time. The source of the CO₂ is from prefilled canisters that are purchased either through sporting goods supply stores for bicycle inflation of tires or through paint ball guns, but also there's an adapter for some of these regulators that will fit the CO₂ chambers or canisters that we use for some of our remote delivery systems that are CO₂ powered such as the Dan-Inject rifles. So a lot of wildlife researchers or biologists would have access to those and it would be very adaptable for them to use this in the field for, again, certain species that would be more amenable to CO₂ as a euthanasia method. There is a [ball valve] that helps provide some gas flow restriction and calibration, but it's again very affordable, very convenient and lightweight system that is in the AVMA guidance for acceptable methods of euthanasia that can be adequately adjusted to field work.

Slide 24 (Components for Field CO₂ Unit)

Just to give you a little close-up on those components, since they're hard to see in that previous photo. On the bottom of the left-hand side of the photo with the cartridges is the large canisters that go into the Dan-Inject rifles and then on the top are the ones used for

bicycle tires. So these are available commercially very easily and affordably. And on the right is the regulator that we purchased that has the adapter that's separate there for the larger canisters.

Slide 25 (Immersion and Topical)

There are some unique species that you deal with in the lab as well as those of us that deal with them in the field. Fish and amphibians don't necessarily respond to euthanasia methods as effectively as some of our mammals and birds do. So these methods that are listed on the slide are very effective, but there are some limitations that need to be considered. For reptiles some of these can also be used, for instance, the MS-222 in injectable form. Again there may be other methods that are more appropriate depending on the species.

AQUI-S is an Investigational New Animal Drug (or INAD) through the Fish and Wildlife Service and it is something that I would highly recommend if you have someone doing a lot of fish work for them to pursue whether or not getting a permit to use that agent would be worth their while. Mostly because the MS-222, while very effective and proven, cannot be put back into the environment, either dumped in the environment or the carcasses being put back in the environment, whether it be for anesthesia or euthanasia. Whereas AQUI-S does not have a withdrawal time for food fish. It is something to consider. Our amphibians are unique in that their skin acts as part of their respiration for a lot of species and so topical application of benzocaine, which is related to MS-222, is a very effective method for anesthesia and euthanasia in these species. Often these would be recommended to have again a two-step method, maybe a physical method to ensure death for these species.

Slide 26 (Physical Methods)

Speaking of physical methods, you've heard me talk about gunshot and cervical dislocation. Again, these are methods that disrupt physically the central nervous signal to the body or induce cardiopulmonary failure. Gunshot is probably the most commonly accepted method for humane killing of wildlife and certainly is the one that many of our wildlife researchers are most qualified and trained in performing in the field.

Cervical dislocation, and this is very familiar to those of you working in laboratories, is another form of physical euthanasia that is common in small mammals, birds, and some reptiles. There are penetrating captive bolts that are now available for use in the field, both in small and large versions. Again, it requires a certain level of training and comfort to use these for the species involved, but when you do have folks that are appropriately trained, these can be used in a variety of species and so it may be a good secondary method to avoid some of the issues that we talked about with chemical or gunshot euthanasia methods in the field.

Decapitation is more common in smaller animals or in fish, but again often requires a secondary method to be performed to ensure death is humane. So there are some

controversial methods listed here that are still fairly familiar to a lot of our wildlife researchers and often used in the field that may not meet the AVMA requirements for a humane euthanasia method [e.g., thoracic or cardiac compression, pit fall traps, kill traps]. And so in some cases these animals are required for the conditions to be already deeply anesthetized or used as a final secondary method to confirm death.

Some species, though, [these controversial methods] may be the best choice for capturing and/or euthanizing these species in the field. So relying on the expertise of the researcher and having them provide the scientific justification, taxa-specific references, et cetera, to the IACUC to consider in order to make sure that it is hopefully never going to be needed, but if it is, it is the best choice for those conditions in those species.

Choosing an optimal physical method of euthanasia in free-ranging wildlife requires consideration for the qualifications and expertise of the personnel, the reliability of the method for that particular species, the research objectives, and overall the ability to perform that safely in the field.

Slide 27 (Gunshot Considerations)

Again, just briefly, a little bit more about gunshots. This is something that ideally should be done away from the public for safety concerns, but also for the aesthetic concerns I mentioned earlier. Gunshots to the head may not always be possible or even the best option. This could be due to distance that the researcher can get to the animal in order to perform a different method or a gunshot method directly to the head. It also has a potential to interfere in diagnostic or other sample collection that needs to be done because it will destroy those anatomical features in the head if gunshot is applied there.

It also poses the risk to spread any infectious material that might exist, so for instance, in chronic wasting disease or in rabies, using gunshot to the head would not be the most appropriate method for preserving the specimens for testing, but also to minimize the risk for spread of disease. Again, neurological signs such as animals with chronic wasting disease or rabies do not indicate gunshot to the head as the best method. So other than [gunshot] to the head as I mentioned earlier, the AVMA does not consider that to be euthanasia, but in those situations that do not allow a gunshot to the head to be the most safe or appropriate method, it still may be the most humane and efficacious method to kill or euthanize that animal to place a gunshot elsewhere. It will definitely need to be a two-step method and again, the purpose of this is to minimize pain and suffering so picking the best method first is always the goal, but recognizing that there may be situations where that second choice may be the most humane even if it would not be considered humane under more normal circumstances.

Slide 28 (Disposition)

I've talked a lot about disposition, and repetition is the key to learning, so carcasses that are not fit for human consumption should not be left in the field. So animals with chemicals on board that could cause harm to humans or other animals or the environment

and lead ammunition when used in these animals, there needs to be a way to safely dispose, hopefully remove those carcasses from the environment or deeply bury to minimize the risk. But what would the value of that animal be if it's left on the landscape? So we're talking about choosing the most appropriate method of euthanasia, maybe there are opportunities that you would look at the best method to be something to allow that animal to be left in the landscape environment. Other animals depend on these remains as a food source and so therefore leaving that animal out there may be appropriate.

The other way to consider using dead animals and again maybe selecting the best, most appropriate method of euthanasia, would be can information be gleaned from these animals in spite of having to euthanize them. Mostly we are talking contingency euthanasia here when it's not part of the plan for research. Are there museums that would value those specimens to be given to their collections? And therefore maybe certain methods of euthanasia may not be appropriate in order to preserve that specimen for a museum collection.

The same thing with the diagnostics. If laboratories can benefit from tissues or other samples from those carcasses, are there methods of euthanasia that might need to be considered to allow those kind of data resources to be collected.

Slide 29 (Ethical Considerations)

So I wanted to talk more about the ethical considerations. According to the AVMA Guidelines, they state that research objectives may limit the use of some euthanasia agents or methods for wildlife species. While Section 14 of the current AVMA Guidelines under Euthanasia and Veterinary Medical Ethics addresses mostly animals used in research laboratories, I believe some of this should apply to wildlife that are used in research activities particularly that the IACUC must apply the principles of refinement, replacement, and reduction, and ensure a respectful death for research animals. So refinement should be one of those things that are considered at the drafting of the research protocol by the wildlife researcher, but also during IACUC deliberations.

Slide 30 (IACUC Role and Responsibility)

So with regard to the IACUC's role and responsibility, we've talked quite a bit about those methods and how to select some of those methods for euthanasia, particularly those that would be consider acceptable or humane when conducting field research using a wide variety of species. So the IACUC obviously will need to be evaluating and considering those methods as to whether they're acceptable, acceptable with conditions in accordance with the AVMA guidance. Are they unacceptable or are adjunct methods being presented that need to have scientific justification or other supporting evidence through literature searches and professional guidance for the IACUC to have appropriate information to approve a condition or a method that would be less acceptable?

Preparedness for getting out to the field and being prepared for the worst is so important in the selection for euthanasia methods. I can honestly say it's one of those areas that we

struggle with most within our IACUC when we review projects at the National Park Service. And that often researchers leave that contingency for euthanasia out of the picture. These researchers and their students and/or staff that are conducting field work should be prepared to perform euthanasia whether it's part of the research activities or as an emergency measure for any injured or ill animals that are affected by the research activities themselves.

The IACUC absolutely plays a big role in assuring preparedness through assessing the training of the personnel and ensuring that all of the individuals that might be involved are going to be proficient in the methods that are listed in the research project for a contingency or planned euthanasia. We hope that methods are never needed in the field if it's not part of the planned activity, unless it's an emergency, but they must be done appropriately and quickly to meet those veterinary medical ethics that we just talked about and ensure that pain and distress is minimized.

Additional assessments that the IACUC can make when evaluating personnel training are the individual's knowledge of the species, the knowledge of the conditions under which euthanasia would be required, that the personnel understand appropriate handling and restraint and how the effects of restraint affect the behavior of that animal. How to minimize pain and distress when utilizing those methods of capture and restraint, and understanding the mechanisms of producing a humane death while being aware of what's necessary to ensure an efficient and purposeful euthanasia.

I mentioned earlier that will equipment and supplies be readily available on site in a variety of circumstances, and that should help dictate what methods be proposed or selected? Is refresher training available when field seasons pass and there hasn't been a need to perform euthanasia in the field? This is where the attending veterinarian or the veterinary staff at an institution can be valuable in establishing initial as well as refresher training for personnel conducting field work in euthanasia methods that are appropriate.

IACUCs can suggest or consider refinements in wildlife research protocols each year at either the annual reviews or during de novo reviews. This should be particularly true for those methods that might not strictly conform with the definition of euthanasia provided by the AVMA Panel on Euthanasia, but have been scientifically justified for the purposes of the study. Or if during the course of the field work procedures are found to produce greater risk for injury or distress which might lead to the need euthanasia, this is a perfect time for the PI and the IACUC to discuss what other options might be available and to ensure that training and proficiency is in place before going back out the next season.

Slide 31 (Summary)

So we've covered all of our objectives, to review euthanasia methods that might be commonly used in wildlife research activities. We've considered the regulatory requirements and also the other professional society guidance and using those to guide the IACUC and the researcher in deciding which methods are most appropriate. We've

discussed the challenges and conditions under which euthanasia may be performed in the field and thereby how to select the most appropriate methods. And then finally, we examined the IACUC's role and responsibility for ensuring that those methods are appropriate and that the field personnel are qualified in performing those.

Thank you very much, and now I will take questions from the audience if we have time.

Slide 32 (Questions)

>>*Swapna*: Thank you, Tracy. There are some questions that we received before the broadcast started and I'll read some of those out and we can address them. We also welcome live questions from the audience. So if you have any questions, please type them into the questions pane in your control panel. And if you think of a question later after the broadcast is over, you can send it to OLAW at the email address provided on our website. [olawdpe@mail.nih.gov]

Slide 33 (Question 1)

The first question we received was: **When euthanasia is required for animals that are not the target species (accidental trapping, injury), does the IACUC need to review the methods for euthanasia for these cases as well?**

>>*Tracy*: So that is definitely a best practice. I think we need to consider that when we review wildlife research activities it's always a good idea to address the possibility of non-target species and thereby if the non-target species are as likely as the target or even more likely to sustain injuries requiring euthanasia in the field, it would be good to consider not only which non-target species might be captured, but what method of euthanasia might be appropriate. We cannot say that they are required to do this since these animals are not the subject for the research activity, but I would recommend it as a best practice to at least have that consideration in place for being prepared to perform appropriate euthanasia.

Slide 34 (Question 2)

>>*Swapna*: Thank you, the next question was: **What are some of the common euthanasia methods employed for stranded marine mammals?**

>>*Tracy*: Thanks. I addressed this a little bit. And again, it really does depend on the size and condition of the animal, where they're found, and who is available to respond. Chemical euthanasia is usually the preferred choice, especially if the carcass can be removed from the site. Again, most common or similar to other species in overdose of barbiturates or anesthesia agent or a two-step method such as anesthesia followed by potassium chloride administration. There are some methods that can be employed that involve physical methods and all of those are really something that should be evaluated on a case-by-case basis by those folks that are expert in the field, particularly with those species. And again, depending on what types of diagnostic samples or other use might be needed from those animals.

Slide 35 (Question 3)

>>*Swapna*: **Do wild rodent species react the same way to carbon dioxide as laboratory rodents? There might be some species such as moles that can survive hypoxia for long periods of time.**

>>*Tracy*: Yes, depending on the physiology of the species as I mentioned on that one slide about special considerations, there are definitely some very similar responses to some of our rodent species and other small mammals. But animals that experience torpor, such as ground squirrels or marmots or some of our fossorial species like moles, again, physiological conditions are common with our traditional rodent species too for neonates and others that might be resistant to inhalant methods of euthanasia like CO₂. But yes, for the more common rodent species it is very much the same, but this is where that researcher who should be very knowledgeable about their species and should be able to provide scientific justifications as well as taxa-specific information, if there's guidance – if there's deviations that need to occur from the AVMA Guidelines, that should be available to the IACUC from the researcher.

Slide 36 (Question 4)

>>*Swapna*: And the next question is: **Can the field CO₂ chamber maintain a 10-30% flow rate as required by the AVMA guidelines?**

>>*Tracy*: Yes. The regulator that I showed you was adequate for us to be able to calibrate the device to a 20% displacement rate. And we were able to employ a comparison with a standard research CO₂ unit chamber that's used for laboratory mice to show side-by-side that they were equivalent to maintain a 20% flow rate and have the same effect on both sets of mice. So it was very effective. We just used the 25-gram CO₂ cartridge, which was not shown in the picture. It's a little bit larger than that 16-gram bicycle inflation cartridge and it was able to be calibrated using that and maintain a 20% flow rate for euthanasia of several laboratory mice

Slide 37 (Question 5)

>>*Swapna*: The next question is: **Can you address how field study investigators should plan for having veterinary oversight for dealing with clinical illnesses, adverse events, etc. – when the studies are far from the institution and institutional veterinarians?**

>>*Tracy*: That's an excellent question. A lot of our biologists and field researchers have a lot of training, especially in the species that they work with. But for instance, here in the National Park Service, I'm the attending vet, but I'm here in Fort Collins, Colorado and can't be out in Yellowstone or up in Alaska or down in the Gulf islands to help out and I certainly don't have the expertise for all the species that our researchers are dealing with out there.

What we've done and what I would recommend to the PI, that their institution is to establish ahead of time, again the relationship with their attending vet, as well as have that attending vet help them make connections with veterinarians that are in the area of the research activities for a lot of purposes, for those adverse events, but also for

securing access to certain drugs and other equipment and maybe also to have an additional person that might have expertise to come and provide guidance, such as I mentioned Dr. Harms earlier when it came to marine mammals. So I highly encourage the researchers to make those relationships with a local veterinarian, but also have that institutional relationship with their attending veterinarian.

When it comes to clinical illnesses, it's probably something we don't have time to get into today with regard to wildlife. I mentioned it a little bit with regard to stumbling upon injured or ill wildlife that's not part of the research activity. That's something that I would really recommend that you have a plan in place and know what the limitations and liabilities would be to intervene in a natural state for an animal.

Slide 38 (Questions?)

>>*Swapna*: Thank you. We have lots of questions coming in live from the audience so I'll start reading those out.

[Question 6] **In the case of reptiles and amphibians, is it safe to generalize and recommend decapitation and pithing as a method, specifically for venomous snakes. Wouldn't blunt force trauma such as crushing the skull or brain be more appropriate for snakes who can sustain brain activity after decapitation? How would blunt force trauma be used safely on venomous species?**

>>*Tracy*: As I mentioned, venomous snakes definitely, venomous animals in general would need certain provisions in place and expertise by the researcher going out in the field with regard to handling and what appropriate methods of euthanasia would be. I did not recommend blunt force trauma nor decapitation and pithing for those species, specifically because the American Society of Ichthyologists and Herpetologists guidance would recommend what the most appropriate method would be based on species or taxa.

Again, it's something that should be considered on the species, the situations that the researcher and the animals will be found in, and what is most appropriate for that particular animal or species or taxa. So I would not certainly generalize that those methods of physical euthanasia are always best for snakes or for reptiles in general. There are definitely lots of methods out there and I mentioned captive bolt as well as inhalants and other things that might be a better option, but it would depend on what the circumstances and training would be.

>>*Swapna*: The next is not a question, it's a comment that the Canadian Council On Animal Care guidelines on the care and use of wildlife is currently under review and revision so we should wait for the latest version to come out, hopefully soon.

[Question 7] The next question, Tracy, asks: **Are isoflurane euthanized animals unsafe to be left in the field or consumed by other animals?**

>>*Tracy*: My understanding is they would be safe to be left in the field. Most of the folks that I'm aware of that are doing this would be bringing those animals out because they're

going to probably submit them for diagnostic testing or for voucher purposes to have in a museum for future testing or other needs for using that specimen. So I would say that isoflurane being a volatile agent would be safe and would not be something that would cause a secondary toxicity.

>>Swapna: And I suppose it's metabolized by the animals.

>>Tracy: I'm not aware of a study that's looked at it, to be honest, Swapna.

>>Swapna: The next question is for Brent. [Question 8] **Does the carbon dioxide used in the field method need to be medical grade?**

>>Brent: No. Just an example, welding grade CO₂ is often more pure than medical grade. The PI and the IACUC should consider using the best grade that's available for the circumstances expected in the field. And for example, the CO₂ canisters that Tracy showed on the slide for the field CO₂ system would be acceptable and could be considered by the IACUC as a refinement to previously used methods.

>>Swapna: Thank you. Tracy, the next question is: [Question 9] **Are there any restrictions on taking controlled substances into the field?**

>>Tracy: Maybe. It definitely depends on the State Veterinary Practice Act as well as the Veterinary [Medicine] Mobility Act. That's another reason to have a really good relationship with your attending vet as well as the veterinarian in the area that you might be practicing. So for instance not even controlled substances, I was told, and whether this is accurate or not because I'm not practicing in Washington State, but that the state of Washington restricts researchers from taking isoflurane out into the field. So therefore their researchers are not currently using those methods for small mammal or other field euthanasia methods. So I do advise you all to make sure that your researcher is working very closely with a licensed veterinarian that's familiar with the regulations or that they have their DEA license and are covered appropriately that way for using and transporting control substances.

>>Swapna: And next question: [Question 10] **Are there any proven downsides to using Eugenol for euthanizing fish? And can you clarify that it is okay to dispose of the carcass in the field after this method?**

>>Tracy: Yes. And for full disclosure, I have not personally used the agent, but the Fish and Wildlife Service had lifted the band on withdrawal times and disposing of carcasses that had Eugenol administered or AQUI-S 20E. They have published or at least announced that it is safe to release specimens back into the wild that have been anesthetized and/or euthanized with AQUI-S.

>>Swapna: [Question 11] **When using gunshots with lead bullets, can the head be removed and the body of the carcass left in the wild?**

>>Tracy: That's a really great question. It's probably going to depend on the type of shot that's used and the animal involved. Some lead shot is going to shatter and be left in the environment. So still deeply burying and/or trying to minimize the amount of lead

remnants that might be around would be really important. Because obviously, if you are leaving part of a carcass around, there may be contamination within that carcass from the lead shot remnants as well as from adjacent to the animal – and then any scavengers could come in contact with those remnants.

>>*Swapna*: [Question 12] **You have mentioned several physical methods, most commonly decapitation or cervical dislocation. So in traditional laboratory animal medicine, the use of adjunctive methods of euthanasia is acceptable when the animal is fully anesthetized. Can you comment on the use of bilateral thoracotomy or exsanguination methods for use in wildlife species?** I suppose the person who is asking this means if the animals are fully anesthetized.

>>*Tracy*: Yes, I would definitely consider for a confirmatory purpose. So the overdose of an anesthetic or euthanasia solution with then following it by bilateral thoracotomy or exsanguination would be very acceptable method of confirming death, especially if you could use the exsanguination samples for further testing. I don't see any reason why that shouldn't be considered. There are not very many wildlife researchers out there that are probably as comfortable to do exsanguination that I've experienced. So it can be species dependent and conditions dependent. Certainly bilateral or a single thoracotomy following an overdose or an appropriate physical method should be an acceptable way to euthanize an animal in the field.

>>*Swapna*: I know that it is over time, but we are getting a lot of good questions coming in, so we would like to go ahead. To the audience – if you must disconnect you can, we will have the answers ready for you to read when we post our transcript. Our captioner has left because we have gone over the hour, but these answers will be provided in the transcript when we post it online.

The next question is for Brent. [Question 13] **There has been much discussion about the use of penetrating bolt gun as a stand-alone method of euthanasia. Some contend that this must be only the first method followed by exsanguination. And we have found that bolt gun on wild pigs is highly effective when used alone. So what do you advise?**

>>*Brent*: This is a performance standard. If it works, then you need to consider which species it works on. So if it works on wild pigs, would it be effective on domestic pigs, or would it be effective on other species? If this is approved by the IACUC then it would be an appropriate method.

>>*Swapna*: The next question is also for you, Brent. [Question 14] **Our IACUC reviews wildlife management protocols that focus on control of invasive rats, mongoose, and cats in Hawaii. Investigators are requesting use of kill traps, such as Conibear and Timms, for rapid euthanasia because of remote locations of field sites. This is quite controversial within the committee, but we have approved the use of these traps with sufficient justification. Can you give us any guidance on this issue?**

>>*Brent*: It's good to hear that it is controversial within the committee. The committee needs to deliberate over these types of methods of euthanasia or humane killing. The IACUC may approve a method not listed in the AVMA Guidelines if it is scientifically justified, but they really should consider the most humane method.

>>*Swapna*: Thank you. The next question is for both of you. [Question 15] **How can IACUCs verify a researcher's proficiency at using a physical method of euthanasia in the field, such as gunshot or captive bolt?**

>>*Brent*: This is Brent. I'll hand it over to Tracy in a minute. We have to rely partly on the investigator's experience. The IACUC may request that the investigator get tested at a shooting range, or perhaps practice on [targets or carcasses] for the captive bolt. Everything else that Tracy mentioned for proficiency in the method that is to be used applies here as well. Tracy, do you have other things to add?

>>*Tracy*: It is definitely – you are depending on what the researchers are going to provide to you and we need to have some trust that they are providing the correct information and current information. We have had our IACUC struggle with that question as well – how do we determine that they are proficient. We have reached out to attending veterinarians at other institutions that work with these researchers, to confirm that these individuals have been properly trained or refreshed on how to perform such techniques. Especially when we get responses back from researchers saying "I have never had to euthanize an animal in the field. And if I did, I'd probably do decapitation or cervical dislocation". Well, okay, "But when is the last time you did that?", and "Do you have the equipment with you to do it correctly?"

So having that information to the IACUC about the training that has occurred, how recent that training has been, and whether or not that individual is appropriately prepared to be dealing with whatever that method is in the field. I see this more when we have graduate students and such who are going to go out on their own, and do some work. They may have worked with the researcher before and the researcher is proficient but they have never had to do it. So I think it's really important on both the researcher and the IACUC to ask those questions and get some kind of clarification and documentation on the fact that training has been conducted and that they are ready to do it.

Slide 39 (OLAW Online Seminars)

>>*Swapna*: Thank you, Tracy and Brent. We have a couple of announcements, so please hang on. First of all, this all we have time for today, and if you have any more questions, you can submit them via the link on the OLAW Webinars page or by email at olawdpe@mail.nih.gov. Our speakers will address them and we'll post the answers on the OLAW website. And as another reminder, the webinar materials will be posted on the [Education Resources](#) webpage.

Thank you to Dr. Tracy Thompson for a wonderful talk. And I thank all of you for participating in our webinar, with special thanks to those who sent in questions.

The next OLAW Online Seminar will be in June, where Dawn O'Connor who is the Assistant Director for Research Compliance in the Animal Care & Use Office at the University of Michigan, will be talking about facility inspections.

Slide 40 (2018 ICARE Training)

And before we go, I'd like to let you know that registration is open for the next three Interagency Collaborative Animal Research Education (ICARE) events to be held in May, June, and September of 2018, and we welcome your participation. Participants from previous events have described the ICARE workshops as interesting, challenging, and very rewarding. We encourage all those involved in U.S. animal care and use programs to register early for this exciting educational opportunity while spaces are available.

More details of these workshops can be found at the [ICARE website](#) via the url provided, or you can contact Susan Silk directly through the [email](#) or phone number [301-402-4371] provided on this slide.

Thank you, good bye.

Additional Submitted Questions Not Addressed During the Webinar

[Question 16] **What is best euthanasia technique for woodchucks in NY State that are live-caught and usually released, but what if for some reason, cannot be released back to wild?**

>> Assuming that euthanasia would be needed due to complications associated with the research activities, several questions should be addressed regarding a contingency plan for euthanasia of woodchucks: 1) What method of euthanasia is the PI/research staff trained to conduct in the field for medium to large-bodied rodents? 2) Does NY State allow researchers to utilize and transport chemical anesthesia or euthanasia drugs? 3) Would the carcass or tissues be utilized for research, museum collection, and/or diagnostic purposes? 4) Does the AV at the research institution have knowledge of the species and a recommendation for the most appropriate method?

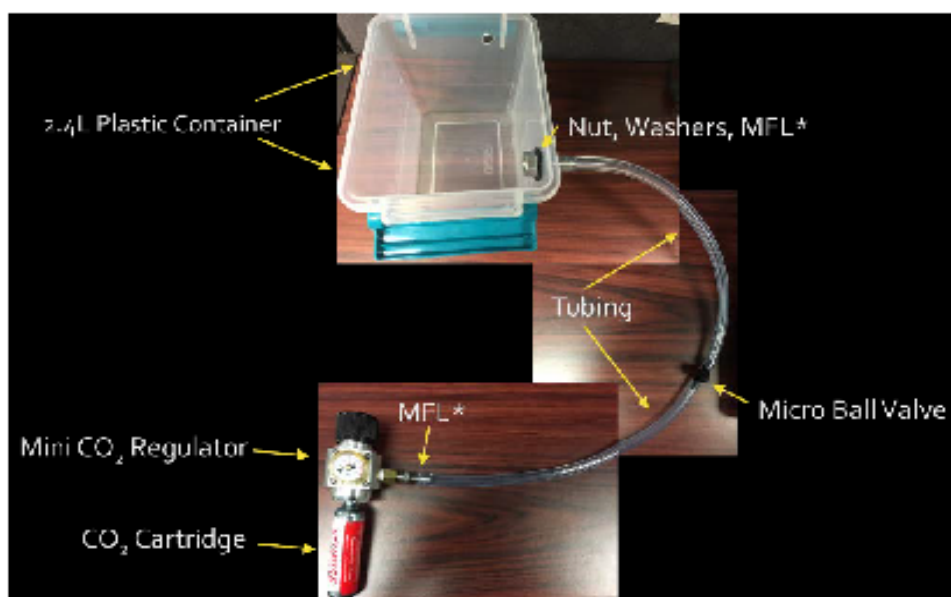
Depending on the answer to those questions, you may be able to decide what the best method would be. My thought is that a two-step method might be best (e.g., field CO₂ or isoflurane chamber followed by thoracotomy, captive bolt, or decapitation; or captive bolt followed by exsanguination or thoracotomy) but this should definitely be backed up with training of staff and approval by the AV/IACUC.

[Question 17] **Can you point us to a reference that provides instructions on how to calibrate a euthanasia chamber?**

>> Pinho, C. The positive displacement method for calibration of gas flow meters. The influence of gas compressibility. *Applied Thermal Engineering* 41 (2012) 111-115.
<https://doi.org/10.1016/j.applthermaleng.2011.12.012>

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- Insert a micro ball valve into the open end of the tubing and connect another piece of tubing to the hose barb on the other end of the micro ball valve.
- Repeat the tube softening process with the free end of the tubing and slide the tube over the hose barb portion of a second MFLCB.
- Screw in the second MFLCB into the input in the mini CO₂ regulator.



Calibration of the CO₂ Euthanasia System

The system must be calibrated before use to ensure an appropriate flow rate of CO₂ into the chamber. The flow rate recommended by AVMA for euthanasia is 20% displacement of the air volume of the chamber over 1 minute. Higher concentrations of CO₂ are irritating to mucus membranes and cause increased pain and distress to the animal.

- 20% Displacement Rate: $(\text{Volume of Container})/5 = \text{Volume Displaced in 1 Minute}$
 - Ex. For a 2.4L container $\rightarrow 2.4\text{L}/5 = 0.48\text{L/minute}$ or 480mL/minute or 240mL/30s
- Acquire a graduated container (e.g., cylinder) that is sufficient for visualizing this displacement rate, a large container to be filled with water, a timer, and the field CO₂ euthanasia system.
 - For a 2.4L container, we used a 500mL graduated cylinder and a standard recycling bin (LWH: 24"x17.5"x13.5").
- Fill the graduated container by completely submerging it horizontally in the water-filled container. Then invert it.
- Insert the end of the tubing into the opening of the graduated container (underwater).

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