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The AVMA Guidelines for the Euthanasia of Animals: 2013 Edition

Speaker: Samuel Cartner, DVM, PhD, DACLAM, The University of Alabama at Birmingham
Moderator: George Babcock, PhD, University of Cincinnati
Broadcast Date: September 19, 2013. View recording at http://www.youtube.com/watch?v=c8ivjAysVBI (YouTube).

Slide 1 (OLAW Online Seminar)
>> *Silk:* Hello everyone. I am Susan Silk, the Director of the Division of Policy and Education at the NIH Office of Laboratory Animal Welfare and the producer of this webinar series. Today I'm pleased to introduce a new moderator of OLAW's online webinars, but first I want to thank Dr. Jerry Collins for his service as moderator since the initiation of this series. All of us at OLAW thoroughly enjoyed working with Jerry and we wish him the very best as he transitions into a lighter professional workload with less time at OLAW and more time with two adorable grandchildren. And now I have the pleasure of introducing our new moderator, Dr. George Babcock. Dr. Babcock is Professor of Surgery and Cancer and Cell Biology at the University of Cincinnati and Shriners Hospital for Children, where he has been Chair of the IACUC for 14 years. Dr. Babcock is an immunologist. Specifically he studies inducible heat shock proteins and alteration of the apoptotic response which eventually leads to sepsis in individuals following severe thermal injuries or trauma. A second area of study is the effect of the immune system on bacterial translocation across the intestinal barrier following severe injury. And finally, his laboratory investigates the mechanism by which certain dietary nutrients alter the immune response. Dr. Babcock's laboratory conducts these studies using murine models. Dr. Babcock.

>> *Babcock:* Hello, today is September 19, 2013. Again, welcome to the OLAW Online Webinar: **The AVMA Guidelines for the Euthanasia of Animals: 2013 Edition**. I am pleased to welcome a large group of distinguished speakers who were panelists on the AVMA [American Veterinary Medical Association] Panel on Euthanasia that developed the 2013 update that we will be discussing today.

Dr. [Samuel] Cartner will be the primary speaker. Dr. Cartner is Associate Vice President for Animal Research Services and Director of the Animal Resources Program at the University of Alabama at Birmingham [UAB]. He received his DVM degree from Auburn University and his PhD from UAB. He completed the laboratory animal training program at UAB and is a

Diplomate in the American College of Laboratory Animal Medicine. His research interests include genetic susceptibility to infectious diseases and the development of animal models of human and animal diseases. Recently Dr. Cartner has focused on investigations that lead to improvements of laboratory animal care and use. His publication on the euthanasia of laboratory mice led to his participation on the American Veterinary Medical Association 2011 Panel on Euthanasia.

As the Director of the UAB Animal Resources Program, Dr. Cartner is dedicated to providing the highest quality laboratory animal care for the biomedical research community at UAB and participating with professional organizations that promote animal care and use. Dr. Cartner has served in multiple roles with ACLAM [American College of Laboratory Animal Medicine], AVMA, the American Society for Laboratory Animal Practitioners and the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). One current activity is to coordinate the first International Animal Welfare Forum on [Animal] Euthanasia sponsored by the AVMA. Sam has assembled a group of experts from the AVMA Panel to provide subject matter expertise.

Joining him today will be Dr. Steven Leary, Assistant Vice Chancellor for Veterinary Affairs and Director of the Division of Comparative Medicine at Washington University. Dr. Leary was the Chair of the AVMA Panel. Dr. Cheryl Greenacre will be joining us from the University of Tennessee where she is Associate Professor of Avian and Zoological Medicine. Dr. Robert Meyer will be signing in from Mississippi State University, where he is Professor in the Department of Clinical Sciences with certification and primary interest in veterinary anesthesiology. Dr. David Miller will contribute expertise in wildlife biology.
Also we have joining us from the AVMA, Dr. Emily Patterson-Kane. And I'd like to thank all the Panel members for generously volunteering to participate in the webinar.

We are also pleased to have representatives from the oversight agencies participating in the webinar. Dr. Pat [Patricia] Brown is the Director of the Office of Laboratory Animal Welfare at the National Institutes of Health. With her today is Axel Wolff. Dr. Wolff is Director of the OLAW Division of Compliance Oversight. Carol Clarke is also with us today. Dr. Clarke is a Research Staff Specialist with Animal Care, Animal and Plant [Health] Inspection Service of the United States Department of Agriculture. And finally, I am pleased to welcome Dr. John Bradfield, Senior Director of AAALAC International. First we will hear from the oversight agencies regarding their positions on the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. Dr. Brown.

Slide 2 (AVMA Guidelines Adoption Status)
>> *Brown:* Good morning, George or good afternoon, I take that back. The PHS Policy, section IV.C.1.g., as you are probably all aware, requires that Institutional Animal Care and Use Committees (IACUCs) reviewing PHS-conducted or supported research projects determine that methods of euthanasia used will be consistent with the recommendations of the AVMA Guidelines for the Euthanasia of Animals, unless a deviation is justified for

scientific reasons in writing by the investigator. As of September 1, 2013, all IACUC reviews must use the 2013 Edition of the AVMA Guidelines [Download the AVMA Guidelines – PDF].

>> *Babcock:* Okay, Dr. Clarke?
>> *Clarke:* Good morning or good afternoon. The U.S. Department of Agriculture endorses the AVMA Guidelines for the Euthanasia of Animals, the 2013 Edition. The AVMA Guidelines are in accordance with the definition of euthanasia as found in the Animal Welfare Act Regulations, Section 1.1 Definition.

Slide 3 (AVMA Guidelines Adoption Status)
>> *Babcock:* Okay, Dr. Bradfield?
>> *Bradfield:* Hello, everyone. It's John Bradfield, Senior Director at AAALAC International, it's a pleasure to be part of this webinar today and this important topic of discussion. As it turns out as we hold the webinar today, the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition is currently under review by the AAALAC Council on Accreditation. They're considering this document as in previous versions for potential adoption as an AAALAC reference resource. An AAALAC reference resource is a document or guidance material to aid institutions on topics relevant to accreditation. And reference resources are also used by site visit teams when evaluating programs and also by the Council when discussing issues identified during the site visits.

Previous editions of the AVMA Guidelines have been adopted as reference resources by the Council and have been widely used in the accreditation program around the world. Because the Guidelines are so important to the AAALAC process, the Council is very careful and detailed and deliberate when reviewing them. The information provided in them has significant and direct impact on expectations regarding euthanasia procedures and we expect a final decision, likely at the next Council meeting held this weekend [September 21 and 22, 2013] in Bethesda, Maryland. So stay tuned for more information from AAALAC on the Guidelines themselves.

Because the Council has not yet finalized its consideration of the Guidelines, I'll be unable to provide detailed information on the 2013 revision, but as discussion warrants I can certainly comment on AAALAC's interpretation of the topic in general. Thanks again for the opportunity to participate and look forward to the webinar.

Slide 4 (AVMA Guidelines for the Euthanasia of Animals: 2013 Edition)
>> *Babcock:* Okay, Dr. Cartner?
>> *Cartner:* Thank you, George. I want to thank you and OLAW for the opportunity to participate in the webinar this afternoon and also thank our distinguished panel of speakers.

Slide 5 (Presentation Goals)
I want to talk about our goals this afternoon, which include reviewing the history of the Report on Euthanasia and review some of the major changes in the AVMA Guidelines that occurred in the 2013 Edition. And emphasize particularly the changes to the lab animal

methods of euthanasia. And then we have a great ending session where our panelists will be responding to questions and issues of interest from the audience.

Slide 6 (1963 Panel on Euthanasia)
So if we take a quick look at the history, the first Panel on Euthanasia was convened in 1963. And they were directed by the AVMA to review methods of euthanasia for unwanted animals, review the literature, observe the field activities, consult with others and give the recommendation. The Report was eight pages long.

Slide 7 (History)
Note that 1963 was also the same year that the first *Guide for the Care and Use of Laboratory Animals* was published. There have been seven additional revisions since 1963, approximately every seven to 10 years, including our last revision in 2013.

Slide 8 (1972 and 1978 Reports)
The first revision occurred in 1972, next slide please, which added laboratory animals to the first Report. And in that Report it included carbon dioxide and decapitation as a recommended method of euthanasia for laboratory rodents. The next revision in 1978 added cervical dislocation for mice and poultry and it also added a few statements about food animals. Interestingly, following the publication of the Guidelines in July of 1978, Dr. Warren from Kent, England, submitted a letter to the editor of the AVMA that drew attention to a publication, a 1975 publication, by Mikeska and Klemm that described persisting EEGs for 13.6 seconds after decapitation. So this started a decade or two of debate about humaneness of decapitation.

Slide 9 (1986 Report)
In 1996 [he meant to say 1986] the Report was revised, next slide please, and it included CO_2 as a method of euthanasia with [the] recommendation of a minimal flow rate of 20% displacement. And this was [based on] a publication by Hornett in 1984. Because of the attention drawn to Mikeska and Klemm's paper, the Panel recommended that decapitation should be used only after the animal has been sedated or lightly anesthetized unless the head will be immediately frozen in liquid nitrogen subsequent to severing. The recommendation for cervical dislocation in the 1986 Report included the weight limit specifications for rodents less than 200 grams and rabbits less than a kilogram, with the preference for them to be lightly anesthetized.

Slide 10 (1993 Report)
The Report was again revised in 1993, and because the IACUC had formally been introduced into the Amendments of the Animal Welfare Act in 1985 and the Public Health [Service] Policy [on Humane Care and Use of Laboratory Animals] in 1986, the IACUC was formally named in the AVMA Guidelines – Report on Euthanasia. And in 1993 the Report stated that there was no change in the recommendation for carbon dioxide, CO_2, but the recommendations for cervical dislocation were that it should be scientifically justified and approved by the IACUC and the same for decapitation. They also added special

considerations for equine, food animal, zoo, wildlife and aquatics. [The 1993 Report was also the first consistent use of the terms “acceptable” and “conditionally acceptable.”]

Slide 11 (2000 Report)
The next revision was in 2000. CO_2 was an acceptable method. Dry ice [was eliminated as a] source [of CO_2]. The recommendations for cervical dislocation stayed the same – it should be scientifically justified and approved by the IACUC. There was a significant change for decapitation. The recommendation was that it was a “conditionally acceptable” method when its use is required by the experimental design and approved by the IACUC. So note that they did not use the terms “acceptable” or “conditionally acceptable” with [cervical dislocation as they did with decapitation].

Slide 12 (2007 Guidelines)
In 2007 [the name changed from the Report to the Guidelines]. The [2007 Report] also included maceration as [an] acceptable [method] for newly hatched chicks. And they added the caution statement that we've all come to notice on the front of the Guidelines.

Slide 13 (Caution Statement)
This caution statement came about because of the statement in the Guidelines that a combination of pentobarbital with a neuromuscular blocking agent is not an acceptable method of euthanasia. The AVMA quickly responded, saying that the 2000 Report did not have any intention to be used for human lethal injection and that the applications of barbiturates and paralyzing agent and potassium chloride were not cited in the Report and the Report never mentioned pancuronium bromide or Pavulon in the Report.

Slide 14 (Panel on Euthanasia 2013)
So that brings us up to the 2013 Edition. The Panel was convened in 2011 and there were 14 Panel members [with 11 members leading] working groups. [The working groups] included three methods groups: inhalant, non-inhalant and physical methods, and there were eight species or environmental groups. Each working group had five or six members, [so] there were more than 70 people that participated in the review, and they included veterinarians and non-veterinarians with an expertise across a wide range of expertise, species and specialties. We ended up with a document that was about 102 pages long, quite a bit different from the first Edition in 1963.

Slide 15 (Changes)
So now I wanted to focus on some of the major changes in the 2013 Edition. The introduction emphasized processes prior to and after euthanasia, so the introduction was greatly expanded. There is discussion about end of life decisions, there's a decision tree, there's a discussion about life worth living. There are diagrams and specific guidance on some of the techniques. And we have also included a glossary, which includes definitions of some terms that are used [inconsistently in previous editions] such as unconsciousness, which we defined as loss of righting reflex, the accepted definition by the College of Veterinary Anesthesiologists.

Slide 16 (Separate Guidelines)
We also separated out depopulation and slaughter. They will be discussed in separate documents. As you know, the draft document for humane slaughter of animals is available now for comment with a deadline of September 30th [2013] for comments. And in our 2013 [Edition], we defined euthanasia as ending of life in an individual animal in a way that minimizes or eliminates pain and distress.

Slide 17 (“Acceptable with Conditions”)
Another major change is the use of the terms “acceptable with conditions”. In the 2000 edition, it was used as “conditionally acceptable”, and we have changed this to “acceptable with conditions” because these [methods] are considered to be equivalent to acceptable methods [when] the criteria for – when a criteria for application of the method can be met. All the methods are dependent upon IACUC approval and there's no reference to scientific justification in the 2013 Edition.

Slide 18 (“Acceptable with Conditions” (continued))
“Acceptable with conditions” methods are based on conditions that must be met to consistently produce humane death. And they may have a greater potential for operator error or a safety hazard. And they may not be quite as well documented in the literature. Some may require a secondary method to ensure death.

Slide 19 (Changes (continued))
Other changes in the 2013 Edition include cervical dislocation of poultry, which is an “acceptable with conditions” method. The discussion talks about appropriate size and training and that cervical dislocation must occur with luxation of the cervical vertebra without crushing the vertebra. We have changed the recommendation for thoracic compression to unacceptable and there is a section on captive invertebrates that include a discussion of euthanasia of spiders and insects.

Slide 20 (Changes to Laboratory Animals Guidelines)
So now if we focus on the changes now to the lab animals section. As mentioned before, there's a separate section for laboratory animals. We focus primarily on rodents, rabbits, and aquatics. The other species are referred to other sections in the Guidelines.

Slide 21 (Rodents)
The acceptable methods [for euthanasia of rodents] are IP or IV barbiturates or a lethal dose of dissociative [agents] such as ketamine. “Acceptable with condition” methods include all the inhalant agents such as isoflurane, CO_2. We also include under “acceptable with conditions” cervical dislocation, decapitation, and microwave irradiation. The main condition to be met for CO_2, which is an issue to be discussed more completely today, is that the displacement rate should be 10 to 30 percent [of the euthanasia chamber volume per minute]. Again based on previous publications. We also have [recommended]

tribromoethanol as an “acceptable with conditions” method for euthanasia with the conditions being it must be prepared and stored and administered correctly.

Slide 22 (Neonatal Rodents)
Another change is a discussion of euthanasia in neonatal rodents which [was not included] in the previous Edition. And when we talk about rodents we have to think about those that are altricial versus the precocial young. [The Guidelines recognize the difference between rodents – those with altricial and those with precocial young.] Precocial young need to be treated, such as guinea pigs, as adults. All the neonatal rodents can be euthanized using IP barbiturates, and “acceptable with conditions” methods include the same gaseous anesthetics or CO_2, but if you use CO_2 or anesthetics you may have [need] a long period of exposure required. You need to confirm euthanasia as required by PHS Policy, which can be some physical method following the administration of the gas. [The PHS Policy requires that Assured institutions base their programs of animal care and use on the *Guide*. The *Guide*, on page 124, suggests use of a secondary method of euthanasia to confirm death.] The altricial young can also be euthanized using rapid freezing when they are five days of age or less or hypothermia, seven days of age or less.

Slide 23 (Rabbits)
We [the laboratory animal section] also have discussed euthanasia of rabbits. Small numbers of rabbits may be best euthanized using the same techniques as recommended in the private practice or companion animals section, with sedation and IV barbiturates. “Acceptable with conditions” methods include inhalants and carbon dioxide, which in rabbits may be best done with sedation. In production settings, captive bolt designed for rabbits may be best for large numbers. Cervical dislocation is also an “acceptable with conditions” method, which requires a demonstration of proficiency.

Slide 24 (Zebrafish)
Since zebrafish are one of the most frequently used animal models in biomedical research, we [the laboratory animal working group] felt like it was important to include them in the laboratory animal section. The acceptable methods include MS222 [tricaine methanesulfonate], which can be followed by a physical method such as immersion in sodium or calcium hypochlorite. Also an acceptable method is rapid chilling, in which you have to expose [fish] to a 2 to 4-degree ice bath, not in contact with the ice, until loss of the orientation and operculum movements and followed by the appropriate holding times, which [is] 10 minutes for adults and 20 minutes for fry. Once they've lost their orientation, they [can] be euthanized by exposure to bleach. It's also acceptable for other tropical or subtropical fish that are less than 3.8 centimeters, which is the size of zebrafish, to be euthanized in this method. It [Rapid chilling] is not appropriate for temperate, cool or cold water-tolerant fin fish.

Slide 25 (Rapid Chilling, Maceration, Clorox)
This slide depicts some of the common methods euthanasia of zebrafish, which include the ice water bath, using a spawning tank, exposure to Clorox for embryos less than three days

and the maceration machine, which is appropriate for euthanasia of adult zebrafish, but the younger stages should be followed by a secondary method.

Slide 26 (Frogs)
So to conclude my section – I do have one final section on frogs, which are a common model. They can be euthanized by immersion in MS222. They may require prolonged exposure, and so people may choose to confirm death by one of the physical methods such as decapitation. Benzocaine hydrochloride can also be used and is available as a gel.

Slide 27 (Living Document)
Finally, the last part of my overview is that the AVMA intends for the Guidelines to be a living document. And the Panel will be maintained so that we can review new information and address needed changes as they come about. As mentioned earlier, the AVMA has agreed to sponsor an animal welfare forum in 2014 on animal euthanasia, slaughter and depopulation.

Slide 28 (Questions and Issues)
So now I would like to recognize other members of the Panel. Dr. Cheryl Greenacre, who is the leader of the avian working group; Dr. Bob Meyer, who is the leader of the inhalant group; David Miller, who is the leader of the reptiles, zoo and wildlife working group; and also I think Dr. Emily Patterson-Kane will participate who was the animal welfare scientist representing the Animal Welfare Division of the AVMA. George, I'm going to turn it back to you.

Slide 29 (Question 1)
>> *Babcock:* Okay, now we're going to cover some of the questions that have been submitted. The first question is: **[Question 1] Why do the AVMA Guidelines recommend low flow CO_2 euthanasia? Low flow CO_2 euthanasia takes longer. Would it be more humane for the animals to die more quickly?** Dr. Meyer is going to discuss this.

Slide 30 (Pain)
>> *Meyer:* Thanks. This is something that the Panel has really struggled a bit with because there's a lot of information out there in the literature; and, again, this is extensively reviewed in the beginning of the Report in Part 2 under the Methods of Euthanasia. But basically, all inhaled gases or vapors basically require a critical concentration within the alveoli of the blood to work. So basically all inhaled methods have the potential to adversely affect animal welfare simply because of the onset of unconsciousness is not immediate. That's largely for this reason that the inhaled methods became conditionally accepted – or acceptable.

Distress can be created by the properties of the agent itself. You can have – in the case of CO_2 that would be things like pungency or the hypocarbia itself. But also by conditions under which the agent is being administered, home cage or dedicated chamber, gradual displacement versus prefilling, things like that. These – the distress can be – can be kind of – manifest itself behaviorally. For example, overt escape behaviors or by things like aversion,

which would be essentially approach avoidance type behaviors. You could also see physiological changes. All of these things have been reported with the inhaled agents and with CO_2 in particular. And while some studies have reported overt behavioral signs of distress, other studies really haven't consistently found these effects. But the majority of the literature seems to be that the slower flow rates where you seem to have less of these particular issues.

So let's kind of start with maybe just defining what pain is. The International Association for the Study of Pain [IASP] defines it as a conscious experience. It's basically an unpleasant sensory or emotional experience that's associated with actual or potential tissue damage. Importantly, activity induced in either nociceptor or nociceptor pathways by a noxious stimulus is not pain, *per se*, because pain is always a psychological state. What this means is that we can have nociceptor stimulation in an unconscious patient or an unconscious animal, which would not necessarily be pain. From a welfare standpoint, we can have nociceptor activation in animals that might be unconscious. But if the animals are conscious, and we have nociceptor activation, then we are going to have pain.

Slide 31 (Unconsciousness)
So unconsciousness is defined as the loss of individual awareness. This occurs basically when the brain's ability to integrate information is either blocked or disrupted. In the case of anesthetics or gases, that's going to be a blocking of the integration and ability to integrate if we are using physical methods that would be a physical disruption of the brain's ability. It turns out with anesthetics inhaled or injectable, this loss of individual awareness basically is a non-linear process. But largely, again because loss of consciousness is not instantaneous, all inhaled methods have the potential to cause distress.

Now, in animals, the loss of consciousness is defined as the loss of righting reflex, also called the loss of position. So basically if you were exposing an animal to an inhaled anesthetic, at the point where they essentially become recumbent and are unable to right themselves from external recumbency, at that point we would make the call that that animal has become unconscious. In humans the analogous point would be essentially the point where they lose the ability to respond to a spoken command. And again that's – you know, since the – essentially the introduction of anesthesia as a discipline in 1847, we really haven't gotten much beyond that as a good, functional, whole animal indicator or whole person indicator of loss of consciousness. So in humans, it's loss of response to spoken command. In animals, it's the loss of ability to spontaneously right themselves. Those are fairly well accepted definitions for loss of consciousness.

We also know that both in humans and animals, we know that memory and memory formation and awareness are suppressed with anesthetic concentrations less than 50% of those needed to abolish movement. It turns out that movement can be a lot of movement that we see. Especially some of the movement that we use like pedal [withdrawal] reflexes, things like that are actually spinally mediated not cerebral cortical mediated. But we do know that memory and awareness are lost very early in the anesthesia process.

What this all kind of translates into, I think, is that once an animal or a person has lost consciousness, once [they] have lost the ability to right them[selves] – once the animal [has] lost the ability to right themselves, then things that we may see, things we may observe, panting, paddling, vocalizations are not an animal welfare issue because the animal is not consciously aware of what's going on. Again, we know this because similar things happen in humans at similar stages of anesthesia and again most people don't have memory or awareness of what's going on.

Slide 32 (CO_2 and Distress)
So basically, the suitability of any particular inhaled agent for euthanasia is really going to depend on really whether there's distress and/or pain experienced prior to loss of consciousness. Once consciousness is lost, then, again these things may be unpleasant to watch, but they are not an animal welfare issue. Now, in the case of CO_2, CO_2 can cause distress by at least three different methods. They are listed here on the slide. One is – a big one is due to essentially pain due to formation of carbonic acid on the respiratory and ocular membranes. The CO_2 combines with water and essentially forms carbonic acid, which causes a burning sensation. I think probably everybody in the audience who has ever drunk a carbonated beverage very quickly and then burped has experienced some of that – what that potentially can feel like. Again, this is something that we know occurs.

We also know that CO_2 can cause what's called air hunger and can give us a sensation of breathlessness. Carbon dioxide is a very profound respiratory stimulant. And more recently there's been a discovery of these things called acid-sensing ion channels within the amygdala, which are associated with the fear response. These have been described in a knockout mouse. Interestingly, carbon dioxide is used in the diagnosis of panic disorder in humans. And people with panic disorder tend to be more sensitive to the inhalation of CO_2 than people who don't have panic disorder. So we know that CO_2 can cause distress and it can be caused by these various methods.

Slide 33 (Carbon Dioxide)
Now, carbon dioxide produces – essentially is an anesthetic. It's not a good anesthetic. It's not one that we use clinically that much anymore, but it is an anesthetic and it does not rely on the induction of hypoxia to produce unconsciousness and to kill. CO_2 does essentially – inhalation of increased levels of CO_2 causes the analgesia and anesthesia that is due to a direct decrease in the interstitial pH. And with the inhalation of CO_2, even at fairly low levels, between five and seven percent, we can see a reduction in both basal and evoked neural activities. You start getting up to 15 percent or so and it can produce unconsciousness. And when we start getting up around – above 15 percent it can cause death. So again it can produce – it does produce an anesthetic state and it does not rely on the induction of hypoxia.

And the chart on the right here kind of goes through and gives an example of at various percent CO_2's how much oxygen would be remaining in an air atmosphere. And you can see

that somewhere between 10 to 20%, which is where we're starting to see unconsciousness occur with CO_2, we can see the oxygen levels aren't really at a level – we were down to maybe somewhere between 14 and 18% oxygen, which are not levels that are associated with hypoxia – hypoxic death. Generally if you're using something like argon or nitrogen which relies entirely on hypoxia, you have to get oxygen levels down below two percent in order to reliably kill. So we see the CO_2 can produce unconsciousness and death with levels of oxygen that are not necessarily hypoxic.

Slide 34 (Faster CO_2 Flow Rates?)
So this brings up the issue of, you know, should we be using faster CO_2 flow rates. And we know that prefill can cause severe pain and distress prior to loss of consciousness. We know that in isolated nociceptors in rats, cats and humans, stimulation with CO_2 starts to occur somewhere between about 30 to 40% CO_2 concentration. Less than that and we don't necessarily see those nociceptors being stimulated. In humans, 30 to 40% is said to cause discomfort with 50% causing intense pain – 50% or greater causing intense pain. So prefill, I think we've pretty much accepted that that can cause an animal welfare issue because again the animals are going to be exposed to these high levels of CO_2. We know it can cause a burning on the ocular membranes and respiratory membranes at these high levels. Gradual fill, again, the literature, there's a lot of literature on this. Again, looking at various studies, looking at aversion, looking at studies where distress was reported, it turns out somewhere an inflow rate, a displacement rate somewhere between 10 to 30% per minute seemed to provide the best compromise between speed of onset and stimulation, nociception, in these species where it was reported.

Faster fills, we'll talk a little bit more about that in a minute here, but there's really limited data. Again, with the faster fills there seems to be more agitation and dyspnea. Helen Valentine's 2012 study is not in the Report, but in that particular study which came out after the Report, basically they saw more agitation and dyspnea with a 100% displacement rate in rats at slower rates.

Slide 35 (AVMA Recommends CO_2 Inflow Rate 10-30% of Chamber vol/min)
So the AVMA is recommending currently a CO_2 inflow rate of 10 to 30% of the chamber volume per minute in those species where a slower rate has been shown not to produce distress. And again, looking at the graph on the right, two axis on this. On the Y axis is percent concentration. This would be for any gas basically. And on the X axis, it would be something called a time constant. And what this graph is representing essentially is the inflow of essentially the wash-in and the buildup concentration of the gas, when you add gas to a closed container. And the solid line starting at 100% and working downward is essentially what happens to a gas that's being washed out of that container.

And as you can see, the wash-in and wash-out of gases in an enclosed space is not a linear process, it's an exponential process. It goes up very rapidly at first and then starts to flatten out as you approach 100%. On the time constant axis there you can see it goes from zero to 0.5, one, one and a half, two and so on. The time constant basically could be calculated fairly

easily. It's the volume of the container divided by the inflow rate. So for whatever the volume of the container is, you divide that by the inflow rate, the displacement rate of the gas, and that will give you the time constant for that particular container. One time constant – if you look at the X axis there, one time constant, extrapolate a line up, gets to you about 63% concentration if you're starting with the wash-in gas starting at zero. So within one time constant we're up to 63% of whatever the concentration of gas we're putting in would be. Two time constants get us to about 87%. And three time constants get us to about 95%. And it takes basically infinite time constants to get to a full 100%.

What this means then is for a 20% inflow, if you set the volume of our container to one, divide that by 0.2, that gives us a time constant of five minutes. So basically a 20% inflow would have a five-minute time constant. That means in five minutes we would be at one time constant, which would be at 63% of the wash-in gas concentration. And this relationship basically holds for any leak-free container, whether it's a cage or whether it's something larger.

Slide 36 (Fig 1, Niel and Weary, Appl An Behavioral Sci 2006)
So basically if we're using a 20% inflow rate, certain things should be happening that we should be able to avoid the issue of the nociceptor stimulation that we know occurs with higher CO_2 concentrations. We should also have the animals becoming unconscious before that point also. And also trying to minimize that period of aversion.

This is figure 1 from Niel and Weary, 2006. In this particular case they used a 20-liter box. They were using a CO_2 flow rate of 3.5-liters per minute. And for that size box that works out to a time constant essentially of 5.7 minutes. And that's going to be somewhere down the X axis down around 330 seconds approximately. And again, if you take their line, take the line from the 330, just take it straight up to where it crosses, and that's going to be around 63% or so. So in this particular situation what they saw was looking at CO_2, they were reporting essentially whether animals would stay inside of a chamber in response to a food reward or whether they would leave. And what they saw was that the animals starting around 15%, somewhere between 10 and 15% CO_2, the animals would choose to try to leave the container. That's happening somewhere down between 30 and 45 seconds, between 30 and 60 seconds approximately, where – that would be where the aversion – essentially where the animals would try to voluntarily leave the container.

It turns out that the animals become unconscious somewhere between 20 and 30% CO_2 and that's happening somewhere between 60 and 90 seconds. In other studies, again a slightly different methodologies, that might go out to about 106, 110 seconds. So basically we're talking somewhere between 30 to 60 seconds where aversion kind of starts – where the animals want to voluntarily leave the container and between 90 and 120 seconds where the animals are actually becoming anesthetized, losing posture, becoming unable to spontaneously right themselves.

And again, if this is done correctly we should be essentially be below levels of 30%, which is where we know that at least 30 to 40% in the isolated preparations, that's where the nociceptors and the ocular and nasal membranes are stimulated by CO_2. So again, this is the main reason why we're recommending the lower flow rate is because again – done at this rate the animals are becoming unconscious prior to the nociceptor stimulation and it tries to minimize the time its spent – the period where they show aversion to carbon dioxide.

Slide 37 (Quality Control with CO_2)
So with CO_2, with any inhaled method, but with CO_2 in particular, we have to look at some quality control issues probably. And the biggest ones are going to be that if you're going to try to apply this you will have to have an accurate volume on your chamber that you're using and you will have to know – have a good handle on the flow rate that you're putting into that container in order to meet these recommendations. Leaks will be problematic. They will be more problematic if they're on the sides or at the bottom of the container, than they would be at the top. But again, that's something that has to be considered.

I think if people are seeing disturbing behaviors you have to essentially, then you will have to ask whether those behaviors are occurring either before or after the loss of righting and essentially the loss of consciousness. If we're seeing those behaviors prior to the loss of consciousness, then they are animal welfare issues, but if those behaviors are occurring after the animals have lost consciousness and we're seeing things like gasping or paddling or that kind of thing, then if the animal is unconscious, then it's not a welfare issue as far as the animal is concerned, although it may be certainly unpleasant for technical staff to observe.

One thing that's been suggested is the use of inhaled anesthetics prior to carbon dioxide as a way to try to reduce distress. And the Canadian Council on Animal Care has made that recommendation in their recommendations. We didn't go quite that far. Again, all inhaled anesthetics produce – all inhaled anesthetics and gases that have been studied – produce some degree of aversion. The aversion to isoflurane, for example, is less than the aversion to carbon dioxide at least in the models that they've been studied in, but it does produce aversion. And again, we're talking probably seconds, less of a difference in terms of whether one is more aversive or less aversive than the other prior to onset of unconsciousness.

Again, it didn't make the Helen Valentine report – didn't make our guidelines as a reference, but in that particular report essentially they're using c-Fos as a marker of stress, they saw that isoflurane produced just as much distress [more actually] as carbon dioxide did when applied at the recommended flow rate. So there doesn't seem to be a lot of advantage there, plus it introduces other issues as far as human exposure to inhaled anesthetics and scavenging and that sort of thing.

Nasal bleeding has been sometimes reported, with carbon dioxide, again, if you look at Burkholder's paper 2010, they also reported – essentially – they reported pulmonary

lesions, specifically hemorrhage into the alveoli, that was consistent with terminal asphyxiation in rats euthanized with either CO_2 or argon gas. And basically they said that they observed lesions that they saw were similar to those previously described in rats euthanized with CO_2 by Danneman in 1997 and by Fawell in 1972. So this isn't anything new. Again the question is, are we seeing the bleeding prior to loss of consciousness or after loss of consciousness? Because again it appears to be a common lesion observed with asphyxiating gases. And that's, I believe, all I have to say on that.

Slide 38 (Question 2)
>> *Babcock:* All right. Thank you. The next question: **[Question 2] Why does the panel consider thoracic compression unacceptable?** Dr. Miller, would you like to address this?

Slide 39 (Thoracic Compression)
>> *Miller:* Sure, thank you. The first thing that we'll do is review what it [thoracic compression] is. [Thoracic compression] It's a method that's applied to small mammals and birds. What happens is pressure is applied to the animal's chest to prevent respiration and/or heart movement. It's used by a subset of those field biologists that work with small mammals and birds. There are several reasons why it has been used. One, it's just because it has worked and has been used for a number of years. When we interviewed the field biologists, as to their reasons for using this method, many of them indicated that what they liked about it was they didn't have to carry extra equipment, materials into the field [and] they didn't need additional training. Also, some of them also felt that for their purposes, they got better samples for what they wanted to do as part of their research.

Slide 40 (Thoracic Compression Compliance with POE Criteria for Methods)
Now, when we end up looking at what the Panel on Euthanasia used as criteria for classifying something as being euthanasia, [the] first concern – as Bob talked about a little while ago – [is that] it's reasonable to assume that these animals have similar neurologic wiring as every other animal. [Consequently] there's concern that the [thoracic] compression will cause pain. Also there's a lot of undocumented information for other criteria. One, there's no published information on time until [un]consciousness. Reliability and irreversibility are issues. That also brings up the issue of training and making sure that people are competent in using the procedure. There's also some question as to whether when people are using this method, whether they really are getting [the sample] quality [they need]. If there's a need [to use thoracic compression], there is a need to document that this is the best way, or the only way, of getting their samples, [to get the quality they need].

Slide 41 (Thoracic Compression Compliance with POE Criteria for Methods)
So in summary, when the Panel is looking at all of these criteria, there's substantial animal welfare concerns in terms of pain, distress, asphyxiation. [There is] not a lot of documentation that can support the use of this as a method of euthanasia. There [were] also concerns expressed about the performance standards so that we know when people are using it [thoracic compression] they are actually using it well and that animal welfare will be maximized under those circumstances. And also a lot of veterinarians with lots of field

experience in the United States and overseas, including myself, have also pointed out that there are a lot of practical alternatives [to thoracic compression] ranging from injectables, to the use of portable anesthetic machines, the drop method, et cetera. And what it boiled down to was that convenience – meaning not wanting to carry equipment out to the field, not wanting training [in alternative methods], et cetera – was not considered adequate justification for the use of thoracic compression. So in summary, it does not meet the criteria for euthanasia.

Slide 42 (Thoracic Compression)
However, if we go to the next slide, one of the things that the Panel realized was that there's a need for flexibility, particularly for wildlife work. And so the criteria for [the category of] humane killing was set up. Basically what humane killing says is that even if it doesn't meet the criteria for euthanasia, you still do the best that you can under the circumstances. And again the animal's welfare is what takes priority over a strict adherence to a given definition. Now, the Panel does realize that field work is hard, [and] we are not trying to make it any harder, but hopefully the criteria for [the category of] humane killing provides an alternative for where there really isn't a way of doing euthanasia in the field and again [humane killing] just reminds people to do the best they can under the circumstances. For those wanting more information, if you go to the AVMA website, there is a backgrounder that does provide additional information. And we can move on to the next slide. I think back to George.

Slide 43 (Questions 3 and 4)
>> *Babcock:* Yes, thank you, Dr. Miller. The next two questions: **[Question 3] Is it acceptable for an IACUC to decide that terminating the lives of wild animals in the field setting is humane killing rather than euthanasia?** Secondly, does the AVMA Guidelines apply to research conducted by PHS-funded investigators who have traveled to a foreign country to conduct that research? Dr. Brown, would you like to address this?

>> *Brown:* Yes, I'm going to address Question 3. So an IACUC must consider each circumstance involving wild animals involved in research in a field setting. An IACUC may determine that there are some cases where under emergency circumstances humane killing methods may be indicated. The use of humane killing methods and the circumstances where they are proposed should be fully explained by the investigator and justified to the IACUC's satisfaction. Euthanasia is the higher standard that each investigator should strive to attain. Humane killing is not to be used when euthanasia is possible. An IACUC at a PHS-Assured institution is not permitted to have a blanket approval allowing humane killing methods for all field activities where animals are killed for research purposes.

>> *Babcock:* Thank you, Dr. Brown. The second part of the question: **[Question 4] Does the AVMA Guidelines apply to field research conducted by PHS-funded investigators who have traveled to a foreign country to conduct research?** will be handled by Dr. Wolff.

>> *Wolff:* Sure. The short answer is yes. Any studies performed with live vertebrate animals by investigators from a domestic institution receiving PHS funds must have the activity reviewed and approved by the IACUC according to the same criteria as for a study performed within the United States. Therefore the field research must be conducted under the AVMA Guidelines.

Slide 44 (Question 5)
>> *Babcock:* Thank you, Dr. Wolff. The next question: **[Question 5] What was the Panel's rationale for the acceptability of cervical dislocation?** Dr. Cartner?

Slide 45 (2013: Cervical Dislocation)
>> *Cartner:* Thank you, George. This has come up in several venues. Just to review, the 2013 Guidelines recommend that cervical dislocation is an “acceptable with conditions” method. The personnel needed to be trained and demonstrate proficiency. There is no requirement for scientific justification.

Slide 46 (1978 Report: Cervical Dislocation and Decapitation)
To understand where we got to in our decision, I think we have to again review – you need to understand the history and the literature that supported the recommendations as we went along. And we mentioned some of this in the overview. In 1978, the Panel Report recommended that cervical dislocation and decapitation was appropriate for rodents. That disarticulation of the skull and cervical vertebrae is a method of producing euthanasia in mice and poultry and guillotine devices have been used for decapitating smaller laboratory animals, especially rats. It is rapid, inexpensive, and when properly done, produces instant death.

Slide 47 (1978 Warren Letter to Editor)
As I mentioned earlier, following the report in July, Dr. Warren published a letter to the editor in October where he brought the attention of the Mikeska and Klemm study to the Panel where they demonstrated there was some EEG activity, as much as up to 13 seconds after decapitation.

Slide 48 (EEG Evaluation of Humaneness of Asphyxia and Decapitation Euthanasia of the Laboratory Rat)
Just showing their publication and that the EEG activity persisted, like I said, 13.6 seconds after decapitation.

Slide 49 (1986 Report: Decapitation)
Then in '86, the Report came out that until additional information was available, the technique should be used only after the animal has been sedated or lightly anesthetized unless the head was immediately frozen in liquid nitrogen.

Slide 50 (1986 Report: Cervical Dislocation)
For cervical dislocation the recommendation was it was a humane technique to euthanize

poultry, mice and rats, less than 200 grams and rabbits less than a kilogram. And because unconsciousness may not occur immediately, it is preferable to lightly anesthetize or sedate and that IACUCs must determine that personnel have been properly trained.

Slide 51 (Decapitation Debate)
And then through those two decades there were several publications, including Vanderwolf in 1988, that did a very detailed study on the type of EEGs and it concluded that the EEG did not resemble the EEG in response to pain – the EEG that Mikeska and Klemm had documented. And Derr also published in 1991, a report that the oxygen tension in the blood was too low in the brain to support consciousness after about three seconds.

Slide 52 (1993 Report: Cervical Dislocation and Decapitation)
However, in 1993 the Report still had the same recommendation for cervical dislocation and decapitation. And that was that until additional information is available, it should only be used in research settings when scientifically justified by the user and approved by the IACUC.

Slide 53 (2000 Report: Decapitation)
I think the 2000 Panel studied the literature very closely. After their study, their recommendation was that the EEG activity that you see after decapitation does not infer the ability to perceive pain and that the loss of consciousness develops rapidly. And their recommendation was that it is a conditionally acceptable method and should be used in research settings when its use is required by scientific design. Note it doesn't say have to be scientifically justified.

Slide 54 (2000 Report: Cervical Dislocation)
Also in 2000 the Panel recommended for cervical dislocation that it was a humane technique for birds and small rodents when performed by trained personnel. In lieu of demonstrated competency that they [the animals] should be sedated or anesthetized. And in research settings this technique should be used when only scientifically justified by the user and approved by the IACUC. Note again that they did not use the term "acceptable" or "conditionally acceptable" with their recommendation and that they still required scientific justification.

Slide 55 (Loss of Cortical Function in Mice After Decapitation, Cervical Dislocation, Potassium Chloride Injection, and CO_2 Inhalation)
Following the 2000 Report, colleagues and I looked at two different measures of the loss of cortical function following the four common euthanasia methods of rodents. We looked at loss of EEG and visually evoked responses in amplitude following euthanasia. We found there was no difference between the loss of EEG and VEP following decapitation or cervical dislocation; and therefore concluded that the same parameters should apply to both applications, both methods. So that is what the [2013] Panel reviewed and came with their recommendation [that cervical dislocation is an "acceptable with conditions" method for mice and rats less than 200gm].

Slide 56 (Question 6)
>> *Babcock:* Thank you, Sam. The next question: **[Question 6] Would you review the Panel's reasoning for revising the recommendations concerning the acceptability of rapid chilling of tropical fish such as zebrafish?**
>>*Cartner:* Yes, sir. If we can go to the next slide.

Slide 57 (Evaluation of Rapid Cooling and Tricaine Methanesulfonate (MS222) as Methods of Euthanasia in Zebrafish (Danio rerio))
>> *Cartner:* There were several publications on the use of rapid chilling, some in different species. This particular one, by Wilson in 2009, used zebrafish and the figure on the right shows the time to loss of ability to swim and the loss of the opercular movements and the [time to] death [using] rapid chilling. In two to four degrees [Celsius] water the fish all lost these things between five and seven seconds. If you compare to the darker columns, which were the use of MS 222, you can see that there was a much longer time to the loss of swimming, loss of opercular movements, and death.

[While time to the loss of consciousness is very important, the amount of distress experienced by the animal is also important.] [The authors also looked] at distress activities, which were rapid opercular movements and erratic swimming. [While they recognized] that some of these behaviors may be changes that you might observe of in fish that are passing through various stages of anesthetics, they didn't observe any of these behaviors when the animals were placed in ice or water bath. So based on this paper [and] one by Blessing and one by the University of Washington, the Panel came to this conclusion that it was an acceptable method of euthanasia in zebrafish. And like I said in my earlier comments, [rapid chilling is] not [recommended] for fish that are cold water tolerant.

Slide 58 (Questions 7 and 8)
>> *Babcock:* Okay. Thank you. The next question, **[Question 7] Do you need to use low flow CO_2 euthanasia for poultry? Can you use pre-filled chambers? And how long in the chamber is required for euthanasia?** Dr. Greenacre, would you like to comment on this?

>> *Greenacre:* Yes. I would like to start out by saying there is very little literature in this area that is specific research that pertains to the questions that we still have remaining on a lot of the avian section. So do we need to use low flow CO_2 euthanasia in poultry? The application of CO_2 for chicks will be extended because they need more CO_2 or higher CO_2 levels, excuse me, but a specific exposure time is not provided for adults or chicks or any animal for that matter. And in both cases, it must be enough to cause death and death should be confirmed. So the slow flow is not a requirement for use of CO_2 in poultry, to answer that question. Can you use pre-filled chambers? And how long in the chamber is required for euthanasia of chicks? The CO_2 chamber size is not specific for poultry, but it should be designed so that – to avoid stress such as from unnatural body positions within the container.

>> *Babcock:* Okay. **[Question 8] Do you have a chart for the appropriate size of poultry for cervical dislocation?**
>> *Greenacre:* For this question, the AVMA 2013 Euthanasia Guidelines did provide some guidelines, but they're not specific. And that is because the research is not specific. A key goal is to rapidly separate the vertebra without crushing the vertebra since crushing results in pain. How rapid the effects are – just how rapid cervical dislocation occurs has not been worked out and has not been in the literature specifically for poultry or any avians. And if it's found in the future that the unconsciousness is not very rapid and is not achieved very rapidly, then other secondary methods may be suggested in the future. So I do not have a chart because there is nothing that was quoted. We wanted to use literature whenever possible, not anecdotal information.

Slide 59 (Sunflower [image])
>> *Babcock:* Thank you. Since we have a little time left, Dr. Leary, Dr. Cartner, Dr. Bradfield, **would you like to make any further comments?**
>> *Cartner:* **[Comment 9 on c-Fos]** Yes, this is Sam Cartner. I wanted to go back and clarify something that Bob [said], when he was talking about the Valentine's study and the c-Fos expression in the brain of animals that have been euthanized by isoflurane or carbon dioxide. And I maybe misunderstood, but as far as c-Fos was concerned, I thought that Dr. Valentine and her group showed that there was significant difference with CO_2 showing much less c-Fos expression than isoflurane. I do know that for the behavioral changes they didn't see a difference necessarily between the groups, but they did see a significant difference with expression of c-Fos. And I may have misunderstood. I'm just confirming.

>> *Babcock:* Would any of our other Panel members like to comment on this topic or any others?

>> *Patterson-Kane:* **[Comment 10 on CO_2 euthanasia policy]** This is Emily here for the AVMA. I think there's still going to be development in how we use CO_2 alone or in combination with inhalant anesthetics. There's some areas where we don't make any particular recommendation at this point. And when that is the case, like for example, people who would like to use isoflurane and follow with CO_2, it's difficult to say with certainty whether that is or not better than with CO_2 alone.

And our advice is always to come back to the performance goal here. In particular if what you're carry out is not unacceptable and it is achieving euthanasia, so it is a death with a minimum of distress, that is really what the entire 100 pages is trying to achieve. You can use an acceptable method in an unacceptable way. The most important thing to do is to demonstrate that you've achieved euthanasia.

>> *Babcock:* Any more comments from our Panel members?

>> *Bradfield:* **[Comment 11 on CO_2 euthanasia policy]** Yes, from the AAALAC perspective we have fielded a number of questions and I think I pose them now maybe for the group to consider. From the biomedical community, in particular with regard to investigators who manage large rodent-breeding colonies, mouse colonies, in which there's a complicated breeding scheme, say back-crossing two or more genes of interest on to one background strain of mouse, it inevitably generates large numbers of genetically null mice which requires that they be appropriately euthanized at the proper time. So when you have that scenario we have heard many questions about two aspects of that. The first, which I think Dr. Meyer answered well earlier, and that is the length of time the animals spend in the chamber at the 10 to 30% displacement rate seems excessive and might there be distress and pain associated with that? I think Bob has answered that question in his earlier discussion.

But the second part of that question, I'm interested to hear from the group if there's any advice for IACUCs when they're faced with the proposition from an investigator that says look, this slower displacement rate in the chamber really takes so much longer to accomplish the task that it really impacts our ability to do research. **[Question 12]** And so for that reason perhaps alone, maybe that reason and others, **some investigators are proposing not to use the 10 to 30% displacement rate and do something faster purely for reasons of practicality. Do you have any advice for IACUCs faced with that question from investigators?**

>> *Babcock:* Would someone from the Panel like to comment on that?

>> *Cartner:* Well, this is Sam Cartner. I'll take a shot and then hopefully Bob or someone will pick up. John, I don't think that investigators need to necessarily have to use CO_2 until death is confirmed. They can use CO_2 until loss of consciousness and then they can follow that with the physical method as soon as they've lost consciousness. So even if you're using – if you wanted to use a higher displacement rate, I think the amount of time is really not that much different to the loss of consciousness. And then the other thing I wanted to mention, there are of course commercial – if they're euthanizing large numbers – in my institution when I work with breeding colonies, they're usually weaning individual cages and not a great number at one time, and they're using the home cage and – I really think that if you look at that closely they're not saving much time in doing a small number of animals. Now, if you're doing a large number of animals, then maybe we need to consider a commercial application where you could euthanize a large number of cages at the same time.

>> *Meyer:* Yeah, this is Bob. Just as a former IACUC member, I'm going to kind of go laterally here, I guess, and with the non-pharmaceutical-grade chemical question, you know, cost is not supposed to be a factor as far as whether we should use, you know, pharmaceutical versus non-pharmaceutical-grade. Am I correct in that? Anyone?

>> *Cartner:* We're talking about – Bob, we're –
>> *Meyer:* Right, I understand that, but I think, you know, you could make the same

analogy here that time shouldn't be a factor if it's affecting welfare. You know, just as we say cost isn't a factor for going with a cheaper med. I'm not sure time is necessarily a factor if it's taking them longer to do the research. And I would kind of argue it on the same – I guess the same term. Again, we're talking seconds here basically in terms of time to loss of consciousness. With a 20% inflow rate we're talking [loss of] consciousness somewhere between 90 to 110, 120 seconds. If we go with a 10% rate we're talking about 150 seconds. We're talking less than three minutes versus what's the alternative they're proposing? And yeah, it's faster, but are we creating more distress? We've said that the prefill is unacceptable because we do know that, yeah, it works really fast, but there is going to be pretty intense pain during that first 20 seconds or so. So that would be, I guess, my response would be I'm not sure that the time factor is a real issue, *per se*. The other thing is you could certainly go ahead and add the gas and walk away and go on to something else and come back.

>> *Patterson-Kane:* Yeah, the Panel considered that as a practicality but essentially the goal is to reach that quality standard and maybe to look at inventive ways to get there. And as I mentioned, there are these largely automated units now that have the large volume facility would look at investing in and you can conserve a lot of time there. But ultimately the only reason that we were really swayed by practicality is it if it would fall back and affect the animal. If we thought we were making it so impractical that a person might leave a moribund animal and choose not to euthanize them, and I think that kind of consideration would only very rarely occur in a research center.

>> *Bradfield:* I think from the AAALAC perspective, we would agree with much of what's already been described. I think the time question assumes that that question is on an equal footing with the humaneness of the procedure and I think the IACUC's focus ought to be on the humaneness of the procedure. Understanding practicality is important. But the IACUC really needs to prioritize what the issues here are and humaneness clearly seems to be the priority factor, time notwithstanding. And so if we [AAALAC] saw IACUCs approving rapid fill rates, for example, based solely on the argument that it's more convenient because it's faster for the investigative team, I think we would be concerned about such judgments.

>> *Patterson-Kane:* I would say, this is just a personal statement, not an AVMA statement, I feel that it may be possible with rapid fills done in very particular ways with particular breeds and species may develop into a method of euthanasia, but absent the data we have to treat all prefilling as the same. So if people really feel that their prefill method can be done appropriately, that would need to be documented for us to be able to accept that.

>> *Meyer:* This is Bob again. You know, again using the wash-in/wash-out, you know, exponential equations, say you could do an inflow, a displacement rate of 100% of the chamber per minute, which would be a very high flow rate, it still gives you a one minute time constant for that chamber. It still takes one minute to get to 63%. Again, everything just becomes time compressed. So you would be you would be getting to the concentrations

a lot faster, but again there could be welfare issues associated with that, there could be distress, agitation issues. Again, we don't have good data on that to support it at this time.

>> *Babcock:* Dr. Brown, would you like to comment on OLAW's take on this?
>> *Brown:* **[Comment 13 on pharmaceutical substance policy]** I just wanted to clarify about pharmaceutical-grade substances that OLAW's position is that when they are available they should be used for euthanasia in order to avoid either toxicity or side effects that would impact the animal's welfare but also could potentially interfere with interpretation of research results. And this is something that should be – can be scientifically justified. The use of non-pharmaceutical substances can be scientifically justified and would be up to the IACUC to find that as an acceptable alternative. If there is no equivalent veterinary or human drug available for experimental use, then the highest grade equivalent chemical reagent should be used.

>> *Babcock:* Okay. Thank you. And anybody else on the Panel who would like to bring up a topic related?

Slide 60 (Upcoming OLAW Online Seminar)
If not, I would like to thank all of the panelists and speakers for joining us today. Our next webinar appears on your screen, December 12th, 2013. I would like to remind all of our people listening to please send in any questions to OLAW that they may have within a week. And we look forward to joining you for our next seminar. Thank you.

Additional Submitted Questions and Comments

[Comment 14 on revisions to the AVMA Guidelines on Euthanasia]
>> *Leary:* The AVMA Guidelines is a living document that can be amended at any time by the AVMA Animal Welfare Committee based on adequate, peer-reviewed scientific information.

[Question 15] What is the most humane method of euthanasia for newborn rodent pups? [see also slide 22]
>> *Cartner:* Decapitation, gradual cooling without contacting cold surfaces.

[Question 16] Some researchers still insist they need to do final tissue sampling for PK studies using decapitation with no drugs. What is the panels view on this?
>> *Cartner:* Decapitation is acceptable with conditions of demonstration of proficiency.

[Question 17] Has the Panel and/or AAALAC addressed the concern that introducing a heavy (i.e. heavier than air gas such as 100% CO_2 at the bottom of a tank will quickly raise the CO_2 concentration to a high level even at a low fill rate? That is, introduction of CO_2 into a container anywhere other than the top of the tank will rapidly increase CO_2 to a high level with effects not much different than a prefill.

>> *Cartner:* Gas dynamic studies have shown that CO_2 is dispersed throughout the cage at the recommend fill rate. The CO_2 does not settle to the bottom immediately while gas is inflowing.

[Question 18] Who uses microwave radiation for euthanasia?
>> *Cartner:* Neuroscientists requiring samples with immediate fixation of brain metabolites.

[Question 19] Just to recap, loss of consciousness is defined as LORR, therefore, there are no welfare concerns when you see paddling/vocalization – correct?
>> *Cartner:* Yes, see slide 31. **What about loss of consciousness associated with anesthesia? If the plane of anesthesia is light and a response is seen to surgical stimulus, is there no animal welfare impact, by the same logic?**
>> *Miller:* Correct, as long as the response is not purposeful – defined as directed movements such as lifting the head. Coughing tachypnea, pedal withdrawal, and twitching are not purposeful, directed movements.

[Question 20] Leaks were mentioned. Doesn't CO_2 displace the air in the chamber and therefore air needs to escape?
>> *Miller:* Yes. Generally any small opening or space between the top cover and the sides of the container provides sufficient space for displaced gas to escape.

[Question 21] Regarding IP administration of barbiturates and barbituric acid derivatives, please clarify if pentobarbital combination drugs (e.g., Euthasol) can be delivered IP vs. IV (with or without lidocaine) in rodents, as referred to on pages 44 and 48 of the AVMA Guidelines.
>> *Cartner:* Barbituraates and barbituric acid derivatives can be administered IP with or without lidocaine. The dose, concentration or formulation of local anesthetics have not been determined for the combination of the drugs.

[Question 22] If you can use an alternative method such as a physical method once the animal is unconscious, why can't you just turn up the CO_2 once the animal loses righting?
>> *Miller:* Turning up the CO_2 is certainly an option once consciousness is lost.

[END]

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[Comment 14 on revisions to the AVMA Guidelines on Euthanasia]
>> *Leary:* The AVMA Guidelines is a living document that can be amended at any time by the AVMA Animal Welfare Committee based on adequate, peer-reviewed scientific information.

[Question 15] What is the most humane method of euthanasia for newborn rodent pups? [see also slide 22]
>> *Cartner:* Decapitation, gradual cooling without contacting cold surfaces.

[Question 16] Some researchers still insist they need to do final tissue sampling for PK studies using decapitation with no drugs. What is the panels view on this?
>> *Cartner:* Decapitation is acceptable with conditions of demonstration of proficiency.

[Question 17] Has the Panel and/or AAALAC addressed the concern that introducing a heavy (i.e. heavier than air gas such as 100% CO_2 at the bottom of a tank will quickly raise the CO_2 concentration to a high level even at a low fill rate? That is, introduction of CO_2 into a container anywhere other than the top of the tank will rapidly increase CO_2 to a high level with effects not much different than a prefill.

>> *Cartner:* Gas dynamic studies have shown that CO_2 is dispersed throughout the cage at the recommend fill rate. The CO_2 does not settle to the bottom immediately while gas is inflowing.

[Question 18] Who uses microwave radiation for euthanasia?
>> *Cartner:* Neuroscientists requiring samples with immediate fixation of brain metabolites.

[Question 19] Just to recap, loss of consciousness is defined as LORR, therefore, there are no welfare concerns when you see paddling/vocalization – correct?
>> *Cartner:* Yes, see slide 31. **What about loss of consciousness associated with anesthesia? If the plane of anesthesia is light and a response is seen to surgical stimulus, is there no animal welfare impact, by the same logic?**
>> *Miller:* Correct, as long as the response is not purposeful – defined as directed movements such as lifting the head. Coughing tachypnea, pedal withdrawal, and twitching are not purposeful, directed movements.

[Question 20] Leaks were mentioned. Doesn't CO_2 displace the air in the chamber and therefore air needs to escape?
>> *Miller:* Yes. Generally any small opening or space between the top cover and the sides of the container provides sufficient space for displaced gas to escape.

[Question 21] Regarding IP administration of barbiturates and barbituric acid derivatives, please clarify if pentobarbital combination drugs (e.g., Euthasol) can be delivered IP vs. IV (with or without lidocaine) in rodents, as referred to on pages 44 and 48 of the AVMA Guidelines.
>> *Cartner:* Barbituraates and barbituric acid derivatives can be administered IP with or without lidocaine. The dose, concentration or formulation of local anesthetics have not been determined for the combination of the drugs.

[Question 22] If you can use an alternative method such as a physical method once the animal is unconscious, why can't you just turn up the CO_2 once the animal loses righting?
>> *Miller:* Turning up the CO_2 is certainly an option once consciousness is lost.

[END]