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*Note: Text has been edited for clarity.*

## **Update on the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition**

*Speakers:*

- Axel Wolff, MS, DVM, NIH Office of Laboratory Animal Welfare
- Samuel Cartner, DVM, PhD, DAACLAM, University of Alabama at Birmingham

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View Recording: <https://youtu.be/eRFQlOJ6DAI> (YouTube)

### **Slide 1: Update on the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition**

>> *Nicolette:* Good Afternoon. Today is Thursday September 10<sup>th</sup>, 2020. I'm Nicolette Petervary, part of the Division of Policy and Education at OLAW, and today it is my pleasure to welcome our speakers, Drs. Samuel Cartner and Axel Wolff, to the OLAW Online Seminars to present the seminar titled "Update on the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition."

Samuel C. Cartner, DVM, PhD, is the Director of the Animal Resources Program at the University of Alabama at Birmingham (UAB), in Birmingham, Alabama. He received his DVM from Auburn College of Veterinary Medicine and PhD from UAB. He is a Diplomat of the American College of Laboratory Animal Medicine (ACLAM).

His research interest includes the refinement and application of animal models to improve human and animal health. Dr. Cartner is the chairperson of the laboratory animal working groups on both of the American Veterinary Medical Association (AVMA) Panels on Animal Euthanasia and Depopulation.

Dr. Axel Wolff currently serves as Deputy Director, in the Office of Laboratory Animal Welfare (OLAW). At OLAW he has also served as the Director of the Division of Compliance Oversight and as a Senior Assurance Officer. Prior to joining OLAW, Dr. Wolff was the director of the Veterinary Resources Program, in NIH's intramural biomedical research support program. Dr. Wolff's interest in unique research animals has involved him in work with armadillos, chimpanzees, and fruit bats as well as the more common species.

During this presentation, Drs. Wolff and Cartner will address comments that OLAW received from a request for information regarding the implementation of the 2020 AVMA Guidelines. These comments, and the AVMA's response, can be found in their entirety (apart from personally identifiable information that was removed) on the webinar webpage under Resources.

It is my pleasure to welcome you to the OLAW Online Seminar and now to hand the microphone over to Axel.

**Slide 2: PHS Policy References the AVMA Euthanasia Guidelines**

>> Axel: Thank you, Nicolette. Let me start by stating that since its inception, the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals ([Policy](#)) has incorporated by reference the American Veterinary Medical Association's (AVMA) Guidelines on the Euthanasia of Animals. It 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 reason in writing by the investigator.

**Slide 3: OLAW is Committed to Using Sound Professional Guidance**

The Office of Laboratory Animal Welfare (OLAW) continues to support the collective assessment of subject matter experts assembled on various AVMA panels addressing various methods of humane killing, such as euthanasia and depopulation, of a wide variety of animal species.

**Slide 4: The AVMA Guidelines are Based on Peer-Reviewed Scientific Literature**

Updates to guidelines are made when a preponderance of new peer-reviewed literature points to a need for revisions to ensure that the most humane methods are being used. Panel decisions are science- and evidence-based rather than relying on anecdotal or opinion pieces.

**Slide 5: CO<sub>2</sub> Flow Rate Recommendations Revised Based on New Studies**

The previously recommended CO<sub>2</sub> flow rates of 10% - 30% were heavily criticized by users based on observational assessments rather than physiological findings. The current recommended flow rates of 30% - 70% were established based on new published evidence.

**Slide 6: Scientific Debate Leads to Improvement**

Debate among professionals in all scientific fields is the norm and helps stimulate ongoing efforts for continuing improvement.

**Slide 7: Decision Should Not be Based on:**

Neither AVMA nor OLAW supports decisions on humane killing of animals based on cost, convenience, tradition, unsupported anecdotes, or any other reasons other than a science- and evidence-based assessment with the ultimate goal being maximum animal welfare.

**Slide 8: Public Comments**

During this webinar we will include discussion of the public comments that were submitted to OLAW in response to the [Request for Information \[RFI\] on the Implementation of the Updated AVMA Guidelines for the Euthanasia of Animals: 2020 Edition](#). The responses to this RFI will be posted on the AVMA landing page at a future date. I will also address two topics that raised questions and provide the reasoning behind the decisions made by the AVMA panel.

OLAW will not be changing any of our guidance in response to the comments we received or the AVMA's response to the comments. No further clarifications will be provided at this time, and our expectations remain the same. Institutions should use the [2020 Edition of the AVMA Guidelines for the Euthanasia of Animals](#) unless scientifically justified and reviewed and approved by the IACUC. OLAW expects full implementation after October 1, 2020.

**Slide 9: Manual Blunt Force as a Method of Euthanasia**

When performed correctly, manually applied blunt force trauma is an acceptable method of euthanasia for suckling pigs. However, the Guidelines also state that "individuals should actively seek alternatives," which may appear contradictory. This method is acceptable with conditions, but user fatigue may result

in the conditions not being met, which could negatively impact the technique. Also, the procedure is aesthetically displeasing and potentially distressing to individuals performing it as well as to the public. Anyone performing the technique must be trained and standard operating procedures should be in place which include information about other methods to help mitigate operator error or safety concerns.

#### **Slide 10: Cardiac Compression in Birds**

Another common concern was the use of thoracic compression as a method of euthanasia for wild avian and mammalian species. This procedure, also known as cardiac or cardiopulmonary compression, is usually used in field studies but there are limited data on the degree of distress induced and time to unconsciousness or death. It is possible that pain and distress does occur before the animals become unconscious. Some birds die from aortic rupture, causing exsanguination, and AVMA states that exsanguination is to be performed only on unconscious animals. Therefore, thoracic compression is considered to be unacceptable for conscious animals but may be used in deeply anesthetized or insentient ones. Anesthetics can be administered in the field by open drop or injectable methods, using noncontrolled agents. Also, practical portable CO<sub>2</sub> chambers have been demonstrated at workshops and supported by OLAW for euthanasia purposes in the field for small birds and mammals.

A good resource for field euthanasia methods is the [March 28<sup>th</sup> \[correction 29<sup>th</sup>\], 2018 OLAW Online Seminar](#). A link to this resource is provided on the slide.

#### **Slide 11: Scientific Justifications Allows Flexibility**

Deviations from the Guidelines have always been allowed with appropriate scientific justification and IACUC review and approval.

Thank you and I will now turn this over to Dr. Samuel Cartner.

#### **Slide 12: AVMA Guidelines for the Euthanasia of Animals: 2020 Edition**

>> *Sam*: Thank you, Axel, for the invitation to participate in this webinar, and thanks to all the participants for their interest.

I think your attendance confirms your recognition that euthanasia of animals is one of the most sensitive and important tasks that people working with animals can perform.

Reviewing and updating the AVMA Guidelines improves the techniques employed and ensures animals are treated respectfully and humanely even in challenging situations.

I hope that this webinar will be informative and allow continued feedback that result in improved Guidelines.

#### **Slide 13: History of the AVMA's Panel on Euthanasia**

I'd first like to start with a little bit of history about the Panel. The 2020 panel was comprised of 14 members. Eleven of which chaired working groups. There were three techniques groups, and eight species groups. There was also a trained ethicist that authored the introduction. The working groups each had four to six additional members that were considered experts in their species or field of study. The panel generated the final product of a 121-page report, which is 20 pages longer than the 2013 Edition.

As in 2013, the full AVMA membership was solicited with an open comment period, and the Panel continues to exist as a virtual entity to respond to inquiries.

Since the 2013 Edition, the AVMA has developed separate guidelines for humane slaughter, and depopulation. The AVMA also hosted the second symposium on Humane Endings in 2018. This is an excellent venue for sharing critical thinking on these important and sensitive subjects and shows the AVMA's continued commitment to data-based improvements that meet societal needs.

**Slide 14: History of the AVMA's Panel on Euthanasia**

I would like to review some of the major changes in the 2013 Edition that in many instances contributed to revisions in the 2020 Edition.

The 2013 Edition included a significantly expanded introduction that emphasized the importance of minimizing distress as well as pain. The introduction discussed the activities to reduce distress prior to euthanasia and post euthanasia activities such as carcass disposal. The introduction discussed how animal welfare considerations are balanced against the merits of the experimental design and merits of the research and state that respectful and humane treatment of research animals will be largely up to IACUCs. It also includes a compelling, thoughtful, and thorough discussion of ethical issues such as the intentional killing of healthy animals.

The 2013 Guidelines for the first time included diagrams for the proper placement of physical methods in livestock.

It also included a glossary that was developed to define important terms in a specific manner.

Previous versions of the Guidelines used the term "conditionally acceptable" for those methods that required specific conditions. This recommendation was often considered a substandard, less than ideal method. We changed the recommendation from conditionally acceptable to acceptable with conditions. The 2013 Guidelines emphasize that acceptable and acceptable with conditions are an equal standard.

There is no reference nor requirement for scientific justification in the 2013 Guidelines. This is because there was no clear definition of scientific justification which led to its inconsistent application by IACUC committees.

**Slide 15: History of the AVMA's Panel on Euthanasia**

The 2013 Edition states that manual or mechanical cervical dislocation may be used for poultry of appropriate size and species when performed by competent personnel who correctly apply the technique.

The 2013 Panel [on Euthanasia] also determined that thoracic compression is an unapproved method based on the lack of data demonstrating the cause of death and length of consciousness.

This Edition for the first time discussed the euthanasia of captive invertebrates which comprises 95% of the animal kingdom's species.

Inhalant anesthetics and CO<sub>2</sub> are acceptable with conditions. One of the conditions for the use of CO<sub>2</sub> is to limit the CO<sub>2</sub> flow rate to 10 - 30% displacement of the euthanasia chamber volume per minute.

### **Slide 16: Guidelines for the Euthanasia of Animals**

As mentioned before, our Laboratory Animal Working Group was comprised of experts in the field, and most of these participated in the 2013 Edition. In 2020, we added Dr. Debra Hickman who is one of the primary researchers of CO<sub>2</sub> use in rodents. The process for updating the 2013 Edition started as an interim update. However, the AVMA Board believed the proposed changes to be significant enough to require a full review process with an appropriate period for stakeholder comment.

The current update was initiated at the beginning of 2018 with a meeting of the panel to identify areas where there have been research development or feedback has been provided about potential inconsistencies or difficulties in application. And the timeline was set for changes to be implemented.

A second meeting of the Panel occurred at the culmination of the Humane Endings Symposium in November of 2018.

While there is not time in this presentation to cover all the revisions to the Guidelines in detail, I will cover the more significant updates with an emphasis on the Laboratory Animals Section.

### **Slide 17: Introduction**

The introduction of the 2020 Edition has added text to distinguish between unconsciousness and other states of sedation or tranquilization. Sedated or tranquilized animals can be aroused with sufficient stimulation. Thus, animals may still be consciously aware of, and connected to, their environment. Therefore, an effective dose of a general anesthetic should be used when performing euthanasia with methods causing distress or noxious stimulation prior to loss of consciousness.

### **Slide 18: Laboratory Animals**

There has been considerable discussion and much new research about the use of CO<sub>2</sub> in the euthanasia of laboratory rodents. The recommendation for the slow fill method with a 10 - 30% fill rate appeared in the 1986 Guidelines but was emphasized in the 2013 Edition. As a result, much research has been undertaken.

This research has supported the use of higher flow rates. The 2020 Edition recommends a 30 - 70% volume displacement rate per minute.

### **Slide 19: Physiological, Behavioral, and Histological Response of Male C57BL/6N Mice to Different CO<sub>2</sub> Chamber Replacement Rates**

Here's one of the several citations in the 2020 Guidelines that supported higher flow rates [Boivin et al. 2016]. In this study, the activity levels, the behavioral responses, physiological, and histopathological parameters were evaluated in mice exposed to 15, 30, 50, or 100% volume displacement rate. They found no difference between the groups and concluded that the higher flow rates would be acceptable for euthanizing adult mice.

### **Slide 20: Boivin – Full Recumbency**

While there are many charts and tables of data in this publication, I wanted to show this one as it addresses one of the main concerns of the panel, which was "Would higher flow rates result in animals experiencing pain prior to becoming unconscious?"

The red horizontal arrow shows the 40% CO<sub>2</sub> concentration in the chamber which is accepted as the concentration that causes pain. The purple, green, red, and blue lines represent the CO<sub>2</sub> concentration

in the chamber at the different flow rates and the yellow arrows represent the mean point of full recumbency of the mice in that group. As you can see, that even at the higher flow rates of 50 and 100%, the mice are unconscious before the CO<sub>2</sub> concentration in the chamber reaches 40%.

**Slide 21: Evaluation of Low versus High Volume per Minute Displacement CO<sub>2</sub> Methods of Euthanasia in the Induction and Duration of Panic-Associated Behavior and Physiology**

In another study published by Dr. Hickman and her group [Hickman et al. 2016] they looked at parameters indicative of distress such as gasping that is considered to be a sign of air hunger distress and determined that at the lower flow rate of 10% volume displacement rate, that gasping occurs more frequently and longer. They also reported behavioral signs of agitation at the higher flow rate of 100% volume displacement rate.

**Slide 22: Hickman – Prolonged Gasping**

This is the same graph shown by Dr. Wolff that is from the Hickman publication that demonstrates prolonged gasping of the rats euthanized with 10% volume displacement rate, represented by the red line. The rats demonstrated gasping for 2.5 mins longer than the other displacement rates.

**Slide 23: Hickman – Running Behavior**

In this same study, only the rats exposed to the higher volume displacement rate of 100% (represented by the blue line), demonstrates increased running behavior. This was interpreted as a panic response. Increased running was not observed in the other groups.

**Slide 24: Laboratory Animals**

The 2020 update includes a number of techniques that have been added for rabbits. These appear in the laboratory animals section even though the new techniques primarily apply to euthanasia of rabbits in a production setting.

In a controlled setting such as a biomedical research facility where researchers have access to restraint devices and controlled drugs, intravenous injection of barbiturates is the primary method.

Blunt force trauma should only be used in emergency situations under extenuating circumstances such as an injured rabbit too large for the operator to effectively perform cervical dislocation and an operator without any supporting resources.

**Slide 25: Efficacy of Blunt Force Trauma, a Novel Mechanical Cervical Dislocation Device, and a Non-Penetrating Captive Bolt Device for On-Farm Euthanasia of Pre-Weaned Kits, Growers, and Adult Commercial Meat Rabbits**

This is the major publication [Walsh et al. 2017] that supported the use of non-penetrating captive bolt and cervical dislocation using a novel device. This study was especially important as blunt force trauma has historically been the most frequently used on the farm method of euthanasia of rabbits. Abattoirs have discontinued the practice due to inefficiency and operator fatigue. The study evaluated the induction of insensibility and degree of brain destruction by the three methods on three sizes of rabbits.

**Slide 26: [Picture of Captive Bolt Device]**

This is a picture of the non-penetrating captive bolt device used. It is a modified pneumatic nail gun weighing approximately 750 g with a mushroom-shaped nylon bolt head that extends 2 cm from the end of the barrel. The device attaches to a standard air compressor. While a single discharge appeared to be sufficient for inducing immediate insensibility to all rabbits, the device was discharged twice in rapid

succession in the same location. The needed operating air pressure was determined to be 90 psi for adult rabbits (> 12 wks), 70 psi for grower rabbits (6 - 12 wks), and 55 psi for pre-weaned kits [150 g and larger, < 5 wks]. A minimum pressure of 50 psi is needed to discharge the device. This is the device that is used in the abattoirs in Canada.

**Slide 27: [Picture of Positioning of Captive Bolt Device]**

This is a picture showing proper restraint and positioning of the device. Note the animal is gently restrained in a plastic container with non-slip flooring and the rear of the rabbit against the wall of the container. The operator's hand resting on the shoulder blades with thumb and forefinger placed gently around the cervical area pressing down. There is a cloth on the floor to prevent slippage and the device is positioned between the ears and eyes on the frontal and parietal bones in the center of the forehead.

The advantage to this device includes that it doesn't penetrate skin, reducing biosecurity and esthetic concerns. It has also been validated for euthanasia of turkey and piglets.

**Slide 28: [Picture of Cervical Dislocation Device]**

This is the mechanical cervical dislocation device that was used for cervical dislocation. The V-shaped, stainless steel device was wall-mounted at approximately shoulder height. The rabbit's head slides into the V-shaped wedge and the device acts to secure the rabbit's head prior to the operator applying a downward force to the hips and back legs. The device allows for alteration of the V-shaped opening according to the rabbit size by means of an adjustable neck plate.

**Slide 29: Efficacy of Blunt Force Trauma, a Novel Mechanical Cervical Dislocation Device, and a Non-Penetrating Captive Bolt Device for On-Farm Euthanasia of Pre-Weaned Kits, Growers, and Adult Commercial Meat Rabbits**

The total number of animals evaluated were 170. After each method of euthanasia was applied, an observer assessed the rabbit for insensibility by evaluating rabbit reflexes, postures, behaviors, and palpable heart rate. Reflexes assessed included pupillary, palpebral, toe and ear pinch withdrawal, and corneal reflex.

If the animal was not immediately insensible or it returned to sensibility, as determined by return of rhythmic breathing, vocalizations, or reflex responses, the euthanasia technique was deemed a failure, and the same method was immediately reapplied.

A euthanasia method was judged to have been effectively applied if the rabbit demonstrated immediate and irreversible insensibility, based on no vocalizations, no response to reflex testing, and lack of rhythmic breathing until cardiac arrest.

You can see that the non-penetrating captive bolt had a 100% efficacy, mechanical cervical dislocation device had one animal that was not immediately insensible and that was because the device was not adjusted appropriately for the rabbit's size, and that blunt force trauma was only 78% effective.

**Slide 30: Euthanasia of meat rabbits with carbon dioxide: behavioral and physiologic responses to gas chamber gradual- and fast-fill rates**

The lead investigator, Jessica Walsh, in this study also looked at the efficacy of CO<sub>2</sub> euthanasia. She evaluated two different flow rates, a 28% volume displacement rate per min and a 58 [%] volume displacement rate. The letter A represents increased respiration, the letter B represents the time when the rabbits lost righting reflex which was about 40 secs for the faster flow rate and 100 seconds for the

slower flow rate. C is the loss of corneal reflex and D is last breath. She reported that there were no clear signs of distress with either flow rate.

**Slide 31: On Farm CO<sub>2</sub> Euthanasia Chamber**

This is the picture of the euthanasia chamber using CO<sub>2</sub>. The port in the front allowed reflexes to be assessed during the loss of righting reflex.

**Slide 32: Other Changes**

I briefly want to review some other changes to the Guidelines that don't necessarily apply to laboratory animals but they could.

CO<sub>2</sub> was added as a technique for use with neonatal goats. There are details and conditions about how to use those in the Guidelines.

There have also be considerable refinements and additions in the area of captive bolt and gunshot use with livestock. These updates provide more images for the correct anatomical site selection for cattle and sheep.

Information has been added for llama, alpaca, deer, water buffalo, bison, chicken, turkeys, ducks, and geese.

Careful consideration was given using an anatomical site at the rear of the head of cattle. This poll shot can be effective but should be reserved for situations when a frontal position is inaccessible or unsafe.

Traditionally puntilla is used as a slaughter method for camelids. This method involves inserting a knife into the back of the neck to sever the spinal cord. Research in llamas and cattle indicates that the stab is often ineffective. Therefore, it cannot be recommended as an acceptable method of euthanasia.

**Slide 33: Selected Changes**

Since the publication of the 2013 Guidelines, feedback was received in relation to the killing of embryonated eggs. There were inconsistencies in recommendations between the avian and poultry sections. After extensive discussion it was determined that destruction by cooling or freezing must occur prior to 80% of incubation, which is 17 days for chickens.

Feedback was also received that some IACUCs are applying the laboratory animal requirements for CO<sub>2</sub> flow rates to poultry and so language was added to clarify that prefill methods are suitable for poultry.

Relatively recently, rendering plants and landfills have refused equine carcasses euthanized with pentobarbital. For this reason, adjunctive methods have been added. Anesthetizing horses with xylazine and ketamine can be followed with:

- lidocaine injected intrathecally, or
- saturated solution of potassium chloride injected IV or intracardiac, or
- saturated solution of magnesium sulfate injected IV

are acceptable with conditions.

### Slide 34: Selected Draft Changes

There has also been considerable research in the area of euthanasia methods for use with finfish and other aquatics:

- Exsanguination has been added as an approved secondary step.
- Recent evidence indicates that MS222 is aversive to fish including zebrafish. However, at this time the benefits of its use outweigh the distress or aversion it may cause. However, recent literature supported extending the exposure time to 30 mins.
- Metomidate is currently only listed for anesthesia and analgesia on the Index of Legally Marketed Unapproved New Animal Drugs for Minor Species by the FDA. ~~Should the index status change to include euthanasia or should the FDA approval be obtained allowing extra-label use under AMDUCA [The Animal Medicinal Drug Use Clarification Act], metomidate should be considered an acceptable agent in certain species of finfish. [Clarification: The index status will have to change to include euthanasia or FDA approval will have to be obtained allowing extra-label use under AMDUCA [The Animal Medicinal Drug Use Clarification Act], before metomidate can be considered as an acceptable agent.]~~ I know this research is progressing with a publication in development so we may have an additional acceptable euthanasia agent in the near future.
- There are also studies supporting a 2-step method for euthanasia of goldfish and some other hypoxia-tolerant species including cichlids. These revisions were added to the 2020 update.

### Slide 35: Selected Draft Changes

It is also worth mentioning some other areas where new information was considered but no changes were made at this time.

This includes techniques that will remain unacceptable including thoracic compression, and rapid freezing of reptiles. There was new literature published on thoracic compression of small birds that indicated death was due to cardiovascular rupture followed by exsanguination, as well as evidence that the time until death can be prolonged. The panel concluded that this method should remain unacceptable.

The unacceptability of drowning is mentioned here as there are reports of this method being used for the culling of kid goats, so the AVMA wishes to reiterate that this method is not acceptable for any animals.

Acceptable methods include CO<sub>2</sub>, barbiturate overdose, gunshot, and captive bolt (penetrating or nonpenetrating) with an adjunctive method.

There is also considerable research emerging for the use of ethanol. However, it was considered not sufficient yet for the technique of cranial injection for poultry to be added. However, we will assess this for the next update.

There are also mixed opinions about the use of lidocaine with the IP or intracoelomic injection of barbiturate based on one study with rats. The use of lidocaine has a range of potential benefits and problems and it was deemed that there is not enough evidence yet to recommend its routine use.

**Slide 36: Comparison of intraosseous pentobarbital administration and thoracic compression for euthanasia of anesthetized sparrows (*Passer domesticus*) and starlings (*Sturnus vulgaris*)**

This is the study [Paul-Murphy JR et al. 2017] that evaluated thoracic compression that was performed by Dr. Paul-Murphy, looking at two different species of small birds and evaluating cardiovascular parameters by DOPPLER and neurological parameters by EEG and documenting cardiovascular rupture as the cause of death following thoracic compression. The panel concluded that exsanguination on unanesthetized animals was unacceptable.

**Slide 37: AVMA Panel at Work**

This is a picture of our panel meeting in 2018. Such in person meetings have surely been rare over the last 9 months! And I hope that COVID will soon pass, and at least by 2023, the time of our next update, that we will be able to meet in person. I thank you for the opportunity to speak, and I think we're going to review our questions now that had been pre-submitted.

**Slide 38: Thank you**

Thank you Dr. Cartner!

>> *Nicolette*: Now we have some questions submitted to OLAW on this topic in advance of the webinar.

**Slide 39: Question 1:**

Question 1: The Guidelines address how to conduct the most humane method of euthanasia for animals, but what about the people who perform such procedures?

Compassion fatigue is an important issue. Is there any guidance available on mitigating the impact of end-of-life decisions and euthanasia on care staff?

**Slide 40: Question 1: Answer**

>> *Sam*: The AVMA has recognized this need and has initiated a working group under the oversight of the Steering Committee on Human Animal Interactions. They welcome a representative from OLAW to participate on this working group.

**Slide 41: Question 2:**

>> *Nicolette*: Thank you. Question 2: Why were data from other mammalian species used to conclude that rodent fetuses are unconscious in utero? Rodents differ from humans and other vertebrates in some very significant ways. What data shows that results from research in other species are applicable?

**Slide 42: Question 2: Answer**

>> *Sam*: The Guidelines reference Dr. Mellor's work from the Massey University in New Zealand. He has published many papers on mammalian development in many species. He describes that the general pattern of neurological development appears to be similar across most mammals, irrespective of when the capacities for sentience and conscious perception first appear in relation to the timing of birth. The Panel on Euthanasia agreed with Mellor and, based on work in other mammalian species, concluded that rodent fetuses are likely to be unconscious in utero and that hypoxia, therefore, would not evoke a response.

**Slide 43: Question 3:**

>> *Nicolette*: Thank you. Question 3: On page 76, section 3.3.3 of the new Guidelines, correct application of manually applied blunt force trauma is categorized as an acceptable means of euthanasia for suckling pigs. If this is the case, why does the AVMA recommend actively searching for alternatives to this method?

**Slide 44: Question 3 Answer**

>> *Sam*: I think Axel covered part of this, but the panel made this recommendation because they recognize that individuals performing blunt force trauma must be well trained and must not become physically fatigued such that performance of the technique is negatively impacted. Further, manually applied blunt force trauma is aesthetically displeasing and potentially distressing to individuals asked to perform the technique. Psychological impacts on individuals performing the euthanasia technique and general societal acceptance are a few of the many variables that must be weighed when deliberating the choice of a euthanasia method.

**Slide 45: Question 4**

>> *Nicolette*: Thank you. Question 4: Some literature strongly suggests that rapid freezing would meet the definition of euthanasia for amphibians and reptiles. In section 7.3.7 why does the AVMA limit rapid freezing to amphibians and reptiles under 4 g and require a secondary method?

**Slide 46: Question 4 Answer**

>> *Sam*: The AVMA continues to support the designation of hypothermia or freezing of amphibians and reptiles as unacceptable in animals > 4 g in weight. Rapid freezing should only be used for amphibians and reptiles < 4 g in weight and a secondary method should be used to ensure death has occurred and is irreversible. This method was based on rodent models and likely will work for ectothermic vertebrates that fall within in this weight range. However, the use of hypothermia and freezing as a euthanasia method for these species lacks the appropriate scientific literature support to document that it meets the criteria set forth in the Guidelines for the Euthanasia of Animals: 2020 [Edition].

**Slide 47: Question 5**

>> *Nicolette*: Thank you. Question 5: The 2020 Guidelines specify a temperature range for rapid chilling of zebrafish from 2 - 4°C. However, a 2018 study by Wallace et al. suggests a range of 0 - 4°C may be more appropriate. Has the Panel considered the information in this publication when revising the Guidelines?

**Slide 48: Question 5: Answer**

>> *Sam*: The Panel on Euthanasia thanks the commenter for bringing this additional publication to the Panel's attention and will consider its content during the next update of the Guidelines.

**Slide 49: Question 6**

>> *Nicolette*: Thank you. And we have one final question which is more appropriately, I think, addressed to Axel, so Axel, if you can answer first and then Dr. Cartner can weigh in. Question 6: Some research projects require specific methods of euthanasia that are not acceptable under the AVMA Guidelines but are necessary to produce valid scientific results. How can investigators and IACUCs address this issue while maintaining compliance?

**Slide 50: Question 6: Answer**

>> *Axel*: As I had mentioned earlier, methods of euthanasia that do not follow the most current version of the AVMA Guidelines for the Euthanasia of Animals may be acceptable if there is scientific justification and IACUC review and approval.

>> *Sam*: I don't have anything to add to that. The IACUCs or the oversight body at each institution should take this into consideration.

>> *Nicolette*: Thank you Axel and Sam.

**Slide 51: Thank you**

>> *Nicolette*: This concludes our online seminar. I would like to thank our speakers, Dr. Cartner and Dr. Wolff, for sharing their time and expertise today.

**Slide 52: Next OLAW Online Seminar: Topic to be Determined**

Our next online seminar will be on December 10<sup>th</sup>, 2020. Until then, thank you for listening and goodbye.

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