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Contents: Transcript

Monitoring for Humane Endpoints: Developing an Appropriate Strategy

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Slide 1 (OLAW Online Seminar)

>>*Silk:* Hello. Today is Thursday September 20, 2018. I'm Susan Silk, the Director of the Division of Policy and Education at OLAW, and today it is my pleasure to welcome our speaker, Debra Hickman, to the [OLAW Online Seminars](#) to present **Monitoring for Humane Endpoints: Developing an Appropriate Strategy**.

Slide 2 (Monitoring for Humane Endpoints)

Dr. Hickman is the Director of the Laboratory Animal Resources Center at Indiana University in Indianapolis, Indiana. In addition to her clinical and administrative responsibilities, Dr. Hickman directs an active laboratory that explores how the biotechnology, handling, and housing selections that are used in research affect the well-being of animals used in research. She has a special interest in the biotechnology, including euthanasia, of small rodents.

It's my pleasure to welcome you to the OLAW Online Seminar and now to hand the microphone over to Dr. Hickman.

Slide 3 (Objectives)

>>*Hickman:* Thank you, Susan. Good afternoon everyone. These are the objectives for today's talk, as we discuss the creation of appropriate humane endpoints. Because sometimes there can be confusion over the definition of endpoints, I'd like to start us off with a review of what endpoints are, so that we are clear. From there, we're going to move on to an overview of the assessment of pain and chronic distress and some of the tools that we have available to help us with this assessment. And then we are going to close by talking about some best practices to develop humane endpoints using case studies as examples to help stimulate this discussion.

Slide 4 (Definition of Endpoints)

We'll start by talking about study endpoints.

Slide 5 (Study Endpoints)

When a scientist is designing their study, they begin by formulating their hypothesis, then determine the steps that they will take to test their hypothesis. This includes determining what data will be collected and when it will be collected. In their grants and protocols, they outline how and when data collection for the project will occur, if everything goes as projected. Study endpoints are established by the scientist, at the beginning of the study, as part of the experimental design. The study endpoint is when the scientist is done with data collection.

Slide 6 (Study Endpoints: Toxicity Testing)

In this first example of a study endpoint, the research team is proposing to give a test compound to the animals on day zero; and apparently this test compound is predicted to have an effect on the kidney. The scientist is proposing that they do weekly measurements of BUN and creatinine between days 7 and 90 to monitor changes in kidney function. And they propose that at day 90 that they will euthanize the animals for tissue collection. This would be an example of a study endpoint.

Slide 7 (Study Endpoints: Tumor Study)

In this second example of a study endpoint, we have a scientist who is injecting tumor cells into the animal to see if their interventional strategy will slow or stop growth. They are proposing to measure the tumor size on a weekly basis. The study will end 3 months, 90 days, after the initial injection of the tumor cells to determine if their intervention slowed the growth of the tumor.

Slide 8 (Study Endpoints: Behavioral Testing)

In this third example of a study endpoint, the animal is being trained to perform a specific behavioral task, for example, a spatial discrimination task or an operant box task. The animal will be fasted, to provide incentive to work for their food reward in the behavioral apparatus. The project is projected to take approximately 2 weeks.

Slide 9 (Study Endpoints: Multiple Sclerosis)

In this example, the scientist is proposing to test the effectiveness of a new therapeutic agent in the treatment of multiple sclerosis, the animal model of which is experimental autoimmune encephalomyelitis or EAE. They are proposing to induce the multiple sclerosis syndrome, then treat for 1 month to see if their treatment slowed the progression of the condition.

Slide 10 (Humane Endpoints)

Part of the review process for studies using animal models includes the harm-benefit analysis. The IACUC is charged to ensure that any potential harm to the animal is outweighed by the potential benefits associated with the proposed activities. While study endpoint is the termination of data collection, a humane endpoint describes the criteria that are used to determine when it is appropriate to terminate the study for an individual animal, or a cohort of animals, before data collection is complete. This decision is made for humane reasons because of concerns that the potential harm to the animal is now outweighing the potential benefits to be obtained from the proposed research project.

Slide 11 (Humane Endpoints, continued)

It is worth realizing that a humane endpoint does not always mean euthanasia. It can also mean terminating a painful procedure or giving a treatment that can help alleviate pain or

distress for the animal. For example, if a fasted animal is losing weight, this could trigger a need to adjust its feed intake or remove it from the study.

Slide 12 (Humane Endpoints: 3Rs)

The premise behind humane endpoints was formally defined through the implementation of Russell and Burch's 3Rs: refine, replace, reduce. Specifically, refinement challenges us to ensure that we are taking steps to minimize the pain and/or distress to be experienced by the animals. We can do this through things like providing analgesia or training an animal to accept an intervention, such as an injection – basically, evaluating how we perform the study to see if we can find ways to minimize the potential pain or distress. And, it includes recognition that if pain or distress cannot be minimized and the potential benefit does not outweigh the potential harm, then we have an ethical responsibility to stop the study.

Slide 13 (Humane Endpoints: Five Freedoms)

The welfare sciences have a corollary in the Five Freedoms. These are freedoms that we are challenged to meet when working and living with animals in all capacities, including animals used in research.

- We are expected to ensure that animals have freedom from hunger or thirst by ready access to fresh water and a diet to maintain full health and vigor.
- They should have freedom from discomfort by providing an appropriate environment including shelter and a comfortable resting area.
- They should have freedom from pain, injury, or disease achieved by prevention or rapid diagnosis and treatment.
- They should have freedom to express most, if not all, normal behavior by providing sufficient space, proper facilities, and company of the animal's own kind.
- And, they should have freedom from fear and distress by ensuring conditions and treatment which avoid mental suffering.

When we design humane endpoints, we can use these 5 freedoms as a guide to our decision making. [Reference: Brambell Report, 1965]

Slide 14 (Generic Humane Endpoints)

Over my career, I've had the opportunity to see a lot of very generic endpoints. For the vast majority of studies, endpoints such as those on this slide, can be appropriate – weight loss, inability to ambulate, inability to access food or water. But, one of the big problems with these generic endpoints is that they are very subjective. And I suspect that many in the audience have had a situation where you're dealing with these kinds of endpoints, but they are so nonspecific that the scientist can have one opinion how to interpret these endpoints and the veterinarian can have a different interpretation.

For example, to me, this mouse is clearly hunched with its hair coat exhibiting piloerection. There is a suggestion of ocular discharge. If you touched him, you would probably note that he is nonresponsive and thin. But, would everyone be in agreement that this animal needs to be euthanized today? Could supportive care be implemented to help the animal survive for a few more days to reach the study endpoint? There are high stakes for all parties involved, so it can be difficult to reach consensus, leading to a delay or a lack of intervention for the animal when endpoints are unclear. For that reason, it is better to define objective endpoints that can be agreed upon in advance.

Slide 15 (What tools do we have to be more objective?)

So, the first question is, what tools do we have to be more objective?

Slide 16 (How do we measure well-being?)

And a great place to start is by rephrasing the question into, how do we measure well-being?

Slide 17 (Venn diagram by David Fraser)

So, this is a Venn diagram created by David Fraser which helps us to define the different parameters that we would like to have in balance to create good animal well-being. It helps us define what we should be looking at – basic health and functioning, natural living, and affective states – when we are assessing the well-being of an animal. It serves as a guide to our evaluation. Ideally, we would like the overlap of all three of these components to be in balance at the center of this diagram for an animal who is in a good state of well-being. But, we need to understand what is meant for each of these categories to be able to understand how to use them in our assessment of animal well-being. So, let's start by looking at basic health and functioning.

Slide 18 (Basic Health & Functioning: Growth)

Assessment of basic health and functioning can include things like reproductive success and appropriate growth as compared to known growth charts. As reproduction is generally costly to an animal, if the animal is successfully reproducing, then this can suggest that the well-being of the animal is good. However, this can be an insensitive measure, because you may not be accurately capturing a reduction in the overall success of reproduction for the animal. For example, the litter size may be smaller than expected for the strain of the mouse. Likewise, if an animal is growing slower than would be projected based on known growth charts, this can be a basic health assessment that there is potentially a concern about the health and well-being of the animal.

Slide 19 (Basic Health & Functioning: Physiology)

In addition to reproductive state, assessment of the overall animal can also be of use. An animal that is acutely stressed will show clinical signs consistent with activation of the fight or flight response, examples of which are demonstrated here – increased heart rate, increased respiratory rate, dilated pupils, and so on. However, chronic stimulation of the fight or flight response results in additional changes, such as alterations in immune and gastrointestinal function, in addition to alterations in neurochemical parameters such as corticosterone.

Slide 20 (Clinical Exam)

The clinical exam is an important tool when assessing an animal, and review of the things we typically check on a clinical exam can assist in the development of appropriate humane endpoints. As I noted in the previous slide, increases or decreases in body temperature, pulse, and respiratory rate, affectionately referred to in clinical medicine as the "TPR", can provide information about the physiologic status of the animal. In some studies, increases may be a sign of distress, but in other studies, decreases could signal morbidity. It's important to understand the model when interpreting the clinical findings. Body weight assessment and bloodwork, also parts of a clinical exam, may provide additional information.

Slide 21 (Body Temperature)

Body temperature has been proposed as a method of assessing animal well-being and determining a humane endpoint time – specifically looking at a drop in temperature as an indicator of morbidity and impending mortality in infectious disease studies. The rectal

thermometer works well for most of the larger species, but taking the temperature of a small rodent can be more challenging. I've put a few examples of alternative methods of measuring temperature on the slide here. All of them have plusses and minuses. Probably the most reliable is the telemetry transmitter because it provides information about the core body temperature, but it does require surgery to implant into the animals. Infrared thermometers on the tail and from a distance work in rodents and other species, but there is conflicting literature out there on how effective they are. Attempting to measure temperature in haired areas can lead to unreliable results. In the absence of being able to use the infrared thermometer or telemetry implant, then probably a rectal thermometer is going to be the best to measure body temperature.

Slide 22 (Body Weight)

Although it is used extensively to assess animal well-being, there are some significant pitfalls associated with body weight. Body weight can change significantly, as much as 10-15%, over the course of the day. Body weight is also very labor intensive and requires specialized equipment. Like the rat in this picture, you have to have a scale and a schedule of weighing so that you can identify changes. What you are looking for is how has the animal's body weight changed from baseline over time. And, depending on the study, you can have a number of things that can confound that change. For example, if you are working with very young animals and they should be growing over the course of the study but they are not gaining weight; then those animals are actually declining in weight over time, even though their weight appears stable. So, when working with growing animals you should be comparing those weights to an age-matched control to tell whether the animal is actually losing weight or not.

Tumor growth can also mask the cachexia and muscle loss associated with it. So you can have an animal whose weight stays stable over time, but the body condition of that animal – the muscle mass of that animal – deteriorates and disappears over time. But, because the tumor is growing, the tumor bulk counterbalances the loss of muscle mass. So if you were relying fully on body weight, you'll miss the fact that the animal has lost muscle condition.

Slide 23 (Body Condition Score: Mice)

This takes us to what I believe is the most valuable tool in the humane endpoints tool chest that we have and that is the use of the body condition score. The body condition score for mouse was first reported in 1999 and you can see the chart here. One of the great advantages of the body condition score is it does not require a baseline. You're not comparing to a past time point, you're assessing the animal in the here-and-now and determining what its body score is. Is it obese, a score of 5, where you cannot palpate the hips or spine; is it well-conditioned, a score of 3, where you can palpate the hips and the spine with slight pressure; or is it emaciated, a score of 1, where the hips and the spine are prominent? The technique doesn't require specialized equipment. If you have a hand, you can do this because it is performed by running your thumb over the base of the lumbar spine and seeing how well you can palpate the spine and hip bones. It is age independent. You can tell the condition of the mouse regardless of whether it should be growing or not.

Body condition score can also be very appropriate for many tumor studies with the caveat that often depends on where the scientist has injected the tumor cells. If the scientist is injecting the tumor cells over the flank, that can interfere with your ability to do body

condition scoring; but you can work with your scientist on the best place for tumor inoculation to help protect against that potential problem.

The most wonderful thing for us in the biomedical research field, is we started with the mouse in 1999 and these body condition scores have been characterized for a multitude of species since then. [Reference: Ullman-Cullere & Foltz, 1999]

Slide 24 (Body Condition Score: Rat)

For example, in 2010, there was a body condition score published by my lab that characterizes the body condition score for rats. We had originally hypothesized that the mouse body condition score would be appropriate for rats, but there are some species specific differences in fat deposition. The ability to palpate the hip bones is more critical in rats and the spine is less informative. An obese rat, with a score of 5, will still have a palpable vertebral column, while a mouse with the same score will not. So, it is important to use a body condition score chart that has been characterized for the species.

As we look at this slide, I am also going to make a point about body condition scores. The original mouse body condition score allowed the use of plusses and minuses, but for our rats, we suggested limiting the score to just 1, 2, 3, 4 or 5. No plusses or minuses. This is intentional because lowering the number of options helps to minimize inter-observer variability when you are assigning scores to animals results in more consistent scores. It is a recommendation of mine that you do not get into the trap of plusses or minuses, but that you stay with the 1, 2, 3, 4 or 5. [Reference: Hickman & Swan, 2010]

Slide 25 (Body Condition Score: Rabbit)

Here is an example of a rabbit body condition score. Again, we are palpating the hips and spinal area, but with a larger species, it's possible to also notice visual differences in how the fat is deposited over the body. The ideal conditioned rabbit, score 3, does not have an abdominal bulge, while the over-conditioned rabbit, score 5, has fat rolls that are in contact with the ground when the rabbit is at rest. [Reference: <https://rabbitsrequirerights.com/health/>]

Slide 26 (Body Condition Score: Companion Animals)

There are multiple body condition score charts for cats and dogs that have been published by pet food companies. These score charts generally suggest palpation of the rib cage and observation of fat deposition on the abdomen. The emaciated dog or cat, score of 1, will have ribs that are potentially visually obvious especially on a short haired animal, while the desired body condition score, a score of 3, will require gentle palpation to identify the ribs.

Slide 27 (Body Condition Score: Macaque)

For those who are using nonhuman primates, there is a body condition score chart for macaques. However, my recommendation would be that you focus on the whole numbers in this scoring chart and not attempt to use the descriptions of the half scores. By including the half scores, 5 options increases to 9 options, with very subtle differences which can lead to more significant inter-observer variability. [Reference: Summers, L., 2012]

Slide 28 (Bloodwork)

A complete cell count, measuring white and red blood cells, can provide additional information in the assessment of the well-being of animals. For multiple species, ranging

from rodents to humans, if the neutrophil:lymphocyte ratio is elevated, generally above 0.4, but the total white blood cell count is within normal limits, this has been shown to be a strong indicator of chronic stress. Alterations in the white blood cell count can suggest infections or other problems. Increases in the hematocrit, also known as packed cell volume, can help to diagnose dehydration and other problems. Although blood samples are not always practical, depending on the species and study, and one should always consider the effect of the collection on the well-being of the animal, these tests can provide additional information for decision making.

Slide 29 (Venn Diagram)

Next, let's look at natural living and basic behavior.

Slide 30 (Exploration of Natural Living: Strategies)

Using the mouse as an example, we can look at how they spend their time in the wild and then how we apply enrichment strategies to their cage, to make their lives better. Because mice like to burrow and build nests, we can give them deep bedding and nesting material to improve their lives. They like to forage and chew, so we can give them foods to search for and toys to chew on. Most mice prefer social groups, so we can house them socially. Understanding of the mouse ethogram can help us identify ways to measure their behavior to identify if they are exhibiting poor well-being.

Slide 31 (Nesting Material Integration)

For example, the nest building behavior gives us an intriguing test that we can use to see if a mouse is interested in engaging in its normal behaviors. We can measure how long it takes a mouse to retrieve a piece of nesting material and integrate it into its nest as a reflection of how the mouse is feeling. If the mouse is in pain or stressed, the time to integrate will be much longer. If the mouse is feeling fine, the time to integrate it will be much more rapid. This technique has been described for use in monitoring health in a number of different recent publications and there is a JOVE video describing how to perform the assessment. [References: Rock et al., 2014, Yuan et al., 2018, Corder et al., 2018, Oliver et al., 2018, <https://www.jove.com/video/51012/nest-building-as-an-indicator-of-health-and-welfare-in-laboratory-mice>]

Slide 32 (Quantifiable Grooming)

We also know that mice and rats groom extensively, so this is an interesting idea that has been published recently that I found fascinating when I was putting together this talk. This proposed scoring system is a way of assessing how well the animal is grooming itself. Although we frequently look for things like poor hair coat or piloerection, by the time the animal is expressing those physiologic and clinical changes, the animal is probably feeling pretty sick and not doing very well. What this grooming assay allows you to do is, by putting nontoxic fluorescent powder at the base of the skull, score 1 on the sheet, over time you can check and see if the animal is taking the time to groom itself and how much grooming it's doing by assessing when the fluorescence is ending up on the feet, in scores 2 and 3, and when it finally disappears from the back of the animal, score 5. This is another intriguing measure of well-being that I think we are going to see used more frequently in humane endpoint development, but it does need some additional characterization. [Reference: Oliver et al., 2018]

Slide 33 (Zebrafish Behavior)

Looking to a non-mammalian model, zebrafish are continuing to grow in popularity as a research model and their small size can make physiologic assessment more challenging.

However, there is an extensive body of literature regarding behavioral characteristics of zebrafish that can assist in the development of deviations from the zebrafish ethogram. For example, the top pictures demonstrate changes in swim pattern associated with a predator threat and the bottom picture show how you can use tracking software to assess behavioral interactions between fish. [Reference: Stewart, A.M., 2014]

Slide 34 (Grimace Scale: Mice)

A discussion about endpoints and potential pain and distress is not complete without touching on the grimace scale. This is one of those objective assessment tools that has been primarily described for determining whether analgesics are operating in the way they are expected to. But when you are looking at end of life in an ill animal, the grimace scale will also give you information about whether the animal is experiencing pain or distress. One of the caveats though is that there is this effect of cage side analgesia where when you are staring at the animal to try and determine what its grimace scale score is, you could inadvertently make the animal not express those behaviors. This is a side effect of a prey species hiding evidence of pain or distress when it is being observed by a predator species. So to do this correctly, you really have to set up a video, leave the room, and then come back and look at still images to see the actual score. This means that one of the downsides to this assessment tool is that it's retrospective and requires specialized equipment to be at its maximum effectiveness. [Reference: <https://www.nc3rs.org.uk/grimacescales>]

Slide 35 (Grimace Scale: Rat, Rabbit)

However, we do have the ability to use this in a multitude of other species that we commonly see in the laboratory animal research community. So in addition to the mice, we have grimace scales characterized for rats and rabbits and a number of other species. Unlike the body condition score, the grimace score for rats is very similar to the grimace score for mice. For rabbits, nose shape and ear flattening are going to be more significant measures. You can obtain very nice posters of these grimace scales and more at the NC3Rs website on this slide. [Reference: <https://www.nc3rs.org.uk/grimacescales>]

Slide 36 (Venn Diagram)

The last component to consider when assessing animal well-being is the affective state.

Slide 37 (Affective State)

Measurement of the affective state currently requires the use of sophisticated behavioral tests, such as operant box training or spatial discrimination tests like the one pictured here, to assess judgement bias, or the use of mazes to assess learning and memory. Because these tests are very involved, they are not always practical for use in endpoint development, but I have included them here to complete the discussion of assessment of well-being.

Slide 38 (Thou shalt never perform...)

The most important take away from this rapid review of tools available for assessment of animal well-being is that one should never make decisions based on a single criterion. Ideally, the endpoint assessment, if it is a true well-being assessment, will include multiple components. If you have worked in my laboratory or heard me speak previously, you will recognize this as Dr. Hickman's fundamental rule of assessing animal well-being: Thou shalt never perform a well-being study that only evaluates one component. Why is this rule so important for humane endpoints?

Slide 39 (“elephant”)

There is an old story of blind people touching an elephant and guessing what they are touching. Depending on what part of the elephant they are touching, each person has a very different idea of what they are touching. One person thinks they’re touching a spear, one thinks it is a snake. Another thinks the elephant is a wall, while a fourth is convinced they are feeling a rope. And no one is getting the answer right because they are so focused on a single component of the whole, which is the elephant.

Slide 40 (“elephant with enrichment”)

As an example, in an enrichment case, we can have the same problem by only using a single test. If one study documents that the rats preferentially spend a significant amount of time in the enrichment housing tube, the conclusion drawn will be that the tube is a beneficial enrichment that improves animal well-being. But, what happens if another study evaluates the behavior of the animal in an open field maze? If that study shows that rats with the enrichment tube spend significantly less time in the center of the open field maze, suggesting that they are much more anxious, compared to rats without the enrichment tube, does that suggest that the enrichment tube is creating anxiety? Since we cannot talk directly to the rat, or at least I don’t claim to speak rat, we have to rely on the indirect tests to determine the actual feelings of the rat. But, if we rely on only one test, we will miss being able to understand the whole.

Slide 41 (“elephant with mouse in nest”)

The same principle applies with humane endpoint selection. Are his eyes a little squinty because you just woke him up to assess him or is he exhibiting an increase in his grimace scale? Is his grimace scale accurate or is he concealing pain because you are staring at him? Is he not integrating the nesting material in a timely manner because he is in pain or distress or because it is the light period of the cycle and sleeping is a priority for this nocturnal animal? You need more context to determine the state of well-being for this mouse.

Slide 42 (Development of Humane Endpoints)

So, after all of this, how should one go about developing humane endpoints?

Slide 43 (Theory of Developing Humane Endpoints)

The approach to developing appropriate humane endpoints is very study dependent. Unfortunately, there is no “magic bullet” perfect solution that fits all cases, though there are tools that can have more universal application. The researcher, the IACUC, and the veterinary staff need to work to understand what is happening to the animal. What is the expected response and what kinds of complications can be anticipated? If those complications arise, how will they be identified and what will happen? Will they be a trigger for supportive care? Will they indicate that it’s time to remove the animal from the study? Will they indicate that it is time to euthanize the animal? A key point is that the criteria should be specific and objective, with clear agreement of what score will trigger what action. Again, we should be sure that we are looking at the basic health and functioning and the natural living, or behavior, at a minimum when developing these endpoints.

Slide 44 (Study Endpoints: Toxicity Testing)

To discuss how we can apply these tools that we’ve talked about, I’m going to walk us through a number of case studies and some theoretical approaches that can be used to determine what would be an appropriate humane endpoint. We’ll start with the toxicity

testing example from the beginning of the presentation. As a reminder, the study endpoint, the one the researcher is hoping for, is that the mice will be euthanized 90 days after administration of the test compound.

Slide 45 (Toxicity Testing: Humane Endpoints)

Because the compound in this example is anticipated to cause kidney problems, we have some good potential endpoints to work with. Assessment of body weight, or more preferentially, body condition score, would be good. Checking hydration status, either by performing the skin tent test, where you pinch the skin and assess how long a delay for the skin to return to normal, or by looking at the packed cell volume of a blood sample would also be good. The scientist is already planning to assess renal function with weekly blood collections and this will be good information for everyone. Depending on the animal model, imaging of the kidney to assess gross damage, may also be valuable. As a general indicator of overall animal health, assessing grooming or nest-building behavior may also be of use.

Slide 46 (Toxicity Testing: Objective Endpoints)

But, for the endpoints to be of use, they need to be objective. So, if we are developing endpoints for the toxicity study in a mouse, these ones may be appropriate. Euthanasia will be performed, regardless of completion of data collection, when the mouse has a body condition score of 1, a BUN level greater than 45 mg/dL, a creatinine greater than 1.2 mg/dL, or a time to integrate nesting material greater than 15 minutes. What should be clearly decided in advance is if euthanasia is indicated if only one of these criteria are present or if some combination of these criteria are present. Between the scientist, who is familiar with their model, and the veterinary team, they should be able to reach agreement regarding what combination of these items will trigger the decision to euthanize the mouse.

Slide 47 (Toxicity Testing: Zebrafish) [Correction: slide amended to indicate scores ranging from 0-3 and not 1-4]

As an example of how you can take more subjective criteria and then create an assessment tool, let's apply this zebrafish score chart to our hypothetical toxicity testing model. To use this tool, the fish are assessed for their general health, body score, presence of abnormal abdominal muscle tone, abdominal distension, behavior. A score of [0 to 3] is assessed for each of these descriptors. If the total score is 0, the fish are normal and no other action is needed. If the total score is 1-4, then there are moderate changes in well-being and monitoring should be increased to daily. If the score is 5 to 8, then there are significant changes in the well-being of the fish and it should be monitored twice daily. If the score is greater than 8, or if there is a score of 3 in any category using this score chart, this is the trigger for immediate euthanasia. By clearly listing what should be looked at and a score assigned to each assessment, the scientist, veterinarian, and IACUC can create a clear roadmap of monitoring and pre-determine when euthanasia will be performed. [Reference: <https://www.humane-endpoints.info/>]

Slide 48 (Study Endpoints: Tumor Study)

In this example study, nude mice are being used to passage tumor cell lines. In these types of models, the scientists would prefer to grow those tumors as large as they can because they are minimizing overall animal use by growing large tumors. Although overall reduction of animal numbers is good, it is very important to have a discussion between the scientist, the veterinary staff, and the IACUC upfront on what the expectations are for

the tumor growth and humane endpoints to ensure refinement of the procedure. We do have some good potential objective options in this case.

Slide 49 (Tumor Study: Humane Endpoints)

Body condition score can be very helpful because as the tumor grows, it's going to mask the weight loss associated with the tumor cachexia. What would be an appropriate maximal tumor size? Historically, a tumor size of 1 cm volume has been considered an appropriate endpoint, but when you look at the whole animal and its response to the tumor, this may not be the definitive criteria for your endpoint. Tumor ulceration may be more important than tumor size, especially when working with subcutaneous tumors. There have been studies that suggest that mice can tolerate tumor sizes of 2 cm volume, but that the body condition scores drop rather rapidly once a tumor becomes ulcerated, regardless of size, suggesting that the quality of life deteriorates very fast once the skin is broken.

And then looking at mouse behavior – the nesting behavior – and how they are interacting with their environment would also be good indicators of animal well-being in a model like this. Because these models typically use nude mice, grooming patterns may not be the most beneficial. In my experience, body condition score and nesting behavioral assessments will be more valuable in assessing animal well-being in these studies as compared to tumor burden. And, the presence of ulceration should, at a minimum, trigger more frequent monitoring, if not a decision to euthanize.

Slide 50 (Study Endpoints: Behavioral Testing)

In our behavioral testing example, the scientist is proposing to mildly fast rats, feeding them 80% of their projected feed intake based on body weight on a daily basis. Their study is expected to take approximately 2 weeks and they will be weighing the rats every day as part of the feed restriction.

Slide 51 (Behavioral Testing: Humane Endpoints)

Because our scientist is going to be weighing the rats every day as part of the study, we can use body weight with some confidence in this study. A loss of 15-20% body weight or a body condition score of 2 may not actually be a humane endpoint, but they could serve as indicators that the scientist needs to increase the feed provided to an individual animal. If the animal continues to lose weight as compared to baseline or develops a body condition score of 1, then it would be time to euthanize the animal. Assessment of time to incorporate nesting material or grooming patterns can also be used as potential endpoints in this case.

Because the scientist is using an animal in a behavioral test, they can glean some good information about the animal's overall state of being based on how they behave in the behavioral apparatus. If an animal is not motivated to perform its behavioral task, especially if it has been performing it previously, this can at least suggest that additional examination is needed. For example, I once assessed a rat that had apparently forgotten how to perform its operant box task. We could find nothing overtly wrong with the animal; but a few weeks later, it developed a head tilt and a pituitary tumor was noted at necropsy – the scientist had been able to diagnose the problem based on behavior long before there were classic clinical signs. So, failure at the active behavioral assessment can also be a screening tool for developing humane endpoints.

Slide 52 (Study Endpoints: Multiple Sclerosis)

As a refresher for our example model of multiple sclerosis, our scientist is proposing to induce the experimental autoimmune encephalomyelitis, or EAE, mouse model of multiple sclerosis. They believe they have a potential therapeutic agent and would like to track the progress of the condition for one month after induction of EAE. Now, I chose this one as an example because this is an intense animal model and can be a very challenging model to write your endpoints for.

Slide 53 (Multiple Sclerosis (EAE): Expected Outcomes)

The very first thing that the IACUC and veterinary staff need to realize is that there are multiple models of this condition just as there are multiple expressions of multiple sclerosis in people. Multiple sclerosis is a devastating disorder that involves loss of neuromuscular function in an ascending paralysis that can ebb and wane in the relapsing and remitting version or get progressively worse in the chronic progression in humans. The mouse models are similar, but the specific endpoints selected are going to be heavily dependent upon which model is being used.

If you are working with the relapsing/remitting model which is an SJL mouse model, you have the situation where the mice do get very sick. In my experience, a technician has notified me that they have an animal that is down and unable to move and I need to come and assess it immediately; and by the time the scientist or I arrive and get there, the animal has improved so much you cannot identify it from the other mice moving about in the cage. It is amazing how they will go from having lost significant amounts of weight to recovering very quickly in this model. That can make this a challenging model for developing appropriate endpoints.

If your scientist is working with the chronic model, which is typically with the B6 mouse, then you get a more classic presentation of the EAE and the ascending paralysis that gets progressively worse over time with a very insignificant chance that the animals will recover. So your endpoints for the chronic EAE model are going to be different from your endpoints with the relapsing/remitting model.

Slide 54 (Multiple Sclerosis (EAE): Humane Endpoints)

With the chronic EAE model when you have an animal that isn't ambulatory, has clinical signs including a body condition score of 2 or less, a decreased body temperature, and/or dehydration, these can all be indications that it's time to euthanize the animal. However, with the relapsing/remitting model, you may be able to provide supportive care to that animal, such as supplemental fluid gels on the cage bottom, and see them recover in a relatively short period of time. You will still want to have similar endpoints in place because if the animal is not recovering after being provided with supportive care – we do want to know when to say enough is enough. Again, a decrease in body condition score, decrease in body temperature, and increase in dehydration status of the animal would all be indications that it is time to euthanize this animal even though the study may not quite be done. The presence of urine scalding (raw skin on the abdomen associated with an inability to groom urine off of the coat), especially on the abdomen, can demonstrate that the mouse is having difficulty grooming and should be euthanized, as well.

Slide 55 (Conclusion)

In conclusion, the approach to developing appropriate humane endpoints is very study dependent. Unfortunately, there is no "magic bullet" perfect solution that fits all cases, though there are tools that can have more universal application. But, if the researcher,

the IACUC, and the veterinary staff work together, they can put together a plan that anticipates potential complications and develops a plan for intervention if and when those complications arise. Reviewing literature focused on animal well-being can provide novel tools that are available to assess animal well-being in the context of humane endpoints. However, as much as possible, the criteria should be specific and objective and include behavioral and physiologic assessment. And, as was illustrated with our zebrafish example, there should be clear agreement of what score, generally a compilation of various individual assessments, or what specific criteria will trigger what action.

Slide 56 (Questions)

>>*Silk*: Thank you, Dr. Hickman. That was terrific. And I am sure the listeners do have questions. Listeners, please type your questions about humane endpoints into the chat box on your webinar screen. OLAW may edit the questions for clarity, duplication, and fidelity to today's topic. If we cannot answer all of the questions we receive in the time allotted, we will amend them to a transcript of this webinar which will be posted to the OLAW website. [See: [Webinars and Podcasts](#)] We will start with a few questions that we received before the webinar.

Slide 57 (Question 1)

Deb, where can you find guidelines and regulations on humane endpoints?

Slide 58 (Answer 1)

>>*Hickman*: Susan, that's a wonderful question and as I was putting things together, I started to put together a list of references that could go along with this talk and I discovered this website. It's one I used very extensively www.humane-endpoints.info, and they have already compiled all of that information for you. I strongly recommend that you visit this website. They have references to European, American, all sorts of international regulations, and then they have lots of literature and additional resources that can be very valuable for you. [Reference: <https://www.humane-endpoints.info/en#>]

>>*Silk*: And Lori has posted on the OLAW website a place where you can download these slides so if you're scrambling to note down that link, you can download the slides and find it there. [See: [Webinars and Podcasts](#)]

Slide 59 (Question 2)

Question 2, are there set humane endpoints like those you described in your talk or can humane endpoints be "customized" depending on the research and the animal model?

Slide 60 (Answer 2)

>>*Hickman*: That's another great question. I think part of the takeaway message is that customization is going to be very necessary. There are some things – there are some fairly generic tools that we can use, for example, that body condition score, and comparison to a normal – the animal's normal ethogram can be very, very helpful. But ultimately, it's critical for the IACUC, the researcher, and the veterinarian to look at the model that's being done, understand the model, and make sure the endpoints are appropriate for the model that is being used.

Slide 61 (Question 3)

>>*Silk*: And here's a question that came in through email before the webinar and we love when you send us those questions. This listener says, I am particularly interested in hearing thoughts on assessing endpoints for monkeys engaged in neuroscience

experiments, particularly electrophysiological and behavioral. They go on to say, there is a delicate balance between maximizing the information gleaned from any one animal, given the extensive behavioral training and preparation that goes into preparing each animal, and specific experiments. I am interested in hearing about guidelines for these determinations.

>>*Hickman*: This is an excellent question, and I honestly wish I had a great, straight answer for this person. I have been thinking about this question since I saw it last week. And there is no easy answer for this question. I think what I would say is, this goes back to the harm-benefit analysis of understanding what the benefit is that the science is hopefully getting. And then how is the animal doing, especially, yes, these long, intensive, extensive behavioral training assays are a huge investment on the part of the animal and on the part of the personnel doing the training. So I think you have to look at it on a case-by-base basis.

Again, looking at what complications you expect and having a very clear idea upfront of when are you going to say that it's time to remove the animal from the study. If you're doing a feed and water restriction study to help motivate this nonhuman primate to engage in those behavioral studies, and the behavioral experiments, you have to have a plan for that animal who just refuses to play, who doesn't want to do the behavioral test, and make sure that they're not getting dehydrated or losing weight. If whatever is being done to the animal is having physiologic or clinical complications associated with it, again, having an idea of what you're going to expect and then what complications can arise, and what clinical signs you're looking for, and clearly defining when the animal needs to be removed from the study, whether that means removed from the study and returned to the colony for use in another venue, or if it means euthanasia.

The biggest thing is to identify that upfront. So these are tough, especially when you've got that significant – when you've got instrumentation or a lot of time and effort invested in the animal. What I usually try and remind my scientists is that humane endpoints are in place so that we can help make sure that we maximize the data we're getting from these animals. An animal who gets very, very ill or even one that unfortunately dies before we euthanize it can end up meaning that the scientist loses some of the data that they're looking for. So it's actually in the scientist's best interest to be keeping a very close eye on those animals and making sure that they can get the data before it's time the euthanize the animal before the animal dies on their own.

Slide 62 (Question 4)

>>*Silk*: What are the principal considerations in developing humane endpoints in any study?

Slide 63 (Answer 4)

>>*Hickman*: Good question, again. So the biggest thing is, ultimately, really understanding what's happening to the animal. I think one of the biggest pitfalls I see with my IACUC is that sometimes our scientists will just throw the generic endpoints in, and those generic endpoints of weight loss or unthriftiness may be appropriate, but they may not be appropriate for the model. And so protocol review time is a great time to have that dialogue between the IACUC and the scientist about what's happening, what's expected, what complications are expected, and then how are you going to be monitoring and how frequently, and what will you be monitoring for, so you can identify when those complications arise.

And then, again, clearly defining up front what's going to be the definition, what will be used to say that this is time to remove the animal from the study.

Slide 64 (Question 5)

>>*Silk*: Who should be involved in the establishment of species-specific and study-appropriate humane endpoints?

Slide 65 (Answer 5)

>>*Hickman*: Another great question. I've stressed during the talk today the scientist, veterinarian, the IACUC. One thing I wanted to add in response to this question was that it is entirely possible that the scientist, the veterinarian, and the IACUC, this may be a new model for them, this may be a new species for them, there may be contention around the decision of what the appropriate humane endpoint should look like. So I would encourage people to not forget that it may be appropriate to bring in outside subject-matter experts to help with additional information, especially if you're dealing with a model that's fairly complicated, involved, potentially has a lot of pain or distress for the animal, and get some help on how to make sure that you're minimizing that pain and distress as you develop the intervention plan and the humane endpoints.

>>*Silk*: And we do have questions coming in from our listeners now. We're reaching the end of our time together. Deb, are you able to stay on the line for more minutes?

>>*Hickman*: Yes, I am.

>>*Silk*: Terrific. And we're glad that we have our captioner. And she will stay on as long as she is able. She has assured me. I think in the future we're going to expand these and call them 75-minute rather than 60-minute seminars, but let's keep going.

Slide 66 (Question 6)

Question 6, at what phase of the study should humane endpoints be clearly defined?

Slide 67 (Answer 6)

>>*Hickman*: They should be clearly defined before any animal work starts. The last thing that we want is a situation where an animal is in distress or needs attention, and we're not clear on what the path is. And the scientist and the veterinarian and the IACUC are arguing over whether this animal has reached the agreed humane endpoints or not. That potentially can result in significant pain or distress for the animal. And so having all this in place before the study even starts is the best practice for making sure that we're doing the best by the animals.

Slide 68 (Question 7)

>>*Silk*: What are your thoughts about death as an endpoint?

>>*Hickman*: Yeah, that's a "fun" one. So, death as an endpoint, for those of you who are not familiar with those models, those are ones where to complete the study, the animal cannot be euthanized prior to death. So if you have a significant infectious disease study or if you're looking, a nice example would be an LD50 where you're looking at toxicity, if you intervene and euthanize the animal before death, you may end up skewing results. And this is a difficult one. In my experience, we ask a lot of questions of scientists who'd like to do death as an endpoint studies.

We [search] the literature quite a bit to look to see if there's been anybody who has found markers in a similar model that might be predictive of impending death. I alluded to one of those in the talk when I was talking about the temperature monitoring, because there is some evidence that in some infectious disease models for example, as you see a decline in temperature, that correlates very strongly to death is coming. And so you can reasonably predict that when the animal's temperature drops and they have achieved that level of morbidity, that they will be dying soon and you can euthanize them so that you alleviate any potential suffering that happens in that window between morbidity and mortality.

But it's not always possible. Sometimes the work, the science there that shows that that is a predictive parameter is not defined yet. So this is an area where we do need to have some additional work. And this is – our IACUC tends to like having our scientists partner with our veterinarians to assess the monitoring on any studies that are proposing to do this so that we can see whether more frequent monitoring or identify a parameter that can help us do that euthanasia early. And I think that falls from my philosophy as the attending vet that these should be rare, that as much as possible we should try to ensure that we are euthanizing prior to the animal suffering and then dying without that alleviation of that pain and suffering. It's not always possible, but again, my feeling is it should be rare, and saved for extraordinary circumstances.

Slide 69 (Questions)

>>*Silk*: [Question 8] One of the questions that has come in refers to slide 47, so I hope you don't get seasick, I'm flipping back to that. The question is: Shouldn't all normal scores be zero?

>>*Hickman*: That is a great question. And as I was putting together the score chart, I actually had that same question. The way the chart is presented there is exactly how it was posted on the website. But I would agree, I would probably have scored animals – I would start my score 0 to 3 instead of 1 to 4. Excellent catch. [Correction: Scores chart range is from 0-3]

>>*Silk*: [Question 9] Now I'm going to go back to another [slide]. There we go. Would you include stereotypies as part of a humane endpoint?

>>*Hickman*: That is a very good question. Yes and no. How's that for covering my bases?

>>*Silk*: The famous "it depends."

>>*Hickman*: It depends, right, yeah. If what you are doing – study endpoints and humane endpoints are specifically around the project that the research is being done, the science hypothesis, the experimental design. Stereotypies can fall out of that. They can be a response to the manipulation that's being done in the name of the study. But more often, those are things that are coming out because of an individual animal having difficulty adjusting to the environment that it's in, or a strain difference, or a response to something that's more environmental, more husbandry related than the actual experimental study itself, which opens the door to – we have to realize that you may have your study and all of the expected complications, and then an animal gets sick for a reason that's completely not related to the study at all.

And a stereotypy is no different from an animal exhibiting clinical illness that was not expected. And this is where the veterinary team comes in to help when unexpected sequelae are identified, like stereotypies or illness, and then work with the scientist on what the next steps should be. Is it possible to alleviate that stereotypy, is it possible to treat the disease, does that behavior – does the clinical syndrome make the animal not useful as a research subject anymore. But, again, this is where the research team and the veterinary staff can work very closely together. But that is a great question.

>>*Silk*: [Question 10] What percentage of body weight is a consideration for euthanasia, and does this vary from study to study?

>>*Hickman*: It does vary from study to study. With those asterisks that I put in that a growing animal should always be growing, so if its body weight is staying stable, that's a problem. And tumor studies with their inherent problems with the bulk of the tumor versus muscle loss. In general, I think most of us are pretty comfortable saying that a loss of 20% body weight from baseline, or 20-25ish, could be considered an indicator that it might be time to euthanize the animal. I think you probably have gotten from my talking today, that I'm a much bigger fan of body condition score, because I think it gives you more information than just weight.

And the best example of that that I will give is in my experience with that EAE model, those SJL mice when they're in the relapsing and remitting stage, they can lose upwards of 20 to 25% of their body weight and yet still come back, especially if we provided them with hydration support, because they were losing most of that weight because they just simply couldn't get to the water effectively and if we fixed that issue, it went away. But when I first started working with that model, we actually set a 30% weight loss as the criteria for euthanasia because of how severe those animals could get and still recover.

So 20%, if you're working with weight and you're taking regular weights and you actually are not working with a growing animal, 20% is probably a good rule of thumb. But I would strongly – because there's so many ifs in that sentence, I would strongly recommend working with a body condition score more for your species because I think it does give you a lot more information that's more valuable than just the body weight.

>>*Silk*: [Question 11] And our next question is about body condition score. A number of the examples included a body condition score of 1 as a humane endpoint. Do you generally recommend BCS 1 as a reasonable endpoint for mice? Are there cases when a body condition score 2 would be preferred?

>>*Hickman*: That is a great question, yeah. I chose 1, because 1 in my mind, 1 definitely is the time for euthanasia. But you could also make the case that 2, especially if there's other clinical signs, if their grooming is delayed or if they're showing poor nest-building behavior or other things like that, a score of 2 might be an indicator that it's time to euthanize the animal as well. But again this is something you'd want to define up front so everybody is clear which score means what.

>>*Silk*: [Question 12] Do the body condition scores account for sexual differences such as the dewlap of rabbits?

>>*Hickman*: Yes, they do. I can speak specifically to the rat study. We looked at both males and females in that study, and our body condition score chart does reflect that. And

if I recall correctly, I had that same question when I looked at the rabbit chart. And the rabbit chart is much more focused on where the fat is on the abdomen and on the perianal area, and not so much on the front because we do know that the bucks and does have a significant difference in that dewlap area, but that's a good question.

>>*Silk*: [Question 13] When intervention other than euthanasia is appropriate, are non-pharmaceutical options such as supplemental heat or feed considered adequate? That would be called supportive care, right?

>>*Hickman*: Supportive care, yeah. So yes, they would. And so that becomes, again, this can be part of the study design where you determine if you see the complications, what are you going to do? And it doesn't always have to mean that the animal has to be euthanized, but we do know that you can start confounding a study pretty quickly if one animal gets treated with supportive care but the other animals don't, or other situations like that. So defining up front what you think you're going to do and when you're going to do it is good. But, yeah, definitely non-pharma methods can be very helpful, again, with that EAE model you can help survivability quite a bit by putting a food/water source on the bottom of the cage and some supplemental heat does wonders for helping those animals get through that relapsing/remitting hump. Other models that are similar to that, supportive care can be very useful too.

>>*Silk*: [Question 14] How do you evaluate nest-building time in multiple animal caging? Moving animal to a new cage will increase nest-building time.

>>*Hickman*: So the nice thing about the nest-building is you can watch it. And you can do it cage side and this is one you can just kind of keep an eye on the animal and see. So you can get a better idea – if you have group housed, which we hopefully would have, you can look to see – you should know how many animals are in the cage, and you can look to see what they – and by pulling the cage out to put the nesting material in, you're going to disturb the cage, which should get all of the mice moving around. And so you can watch to see how those individual mice in the group interface with the piece of nesting material that you put in there and if one is showing no interest that should stand out pretty obviously as compared to the others. So it is a useful test, even in group housed cages. And yeah, I agree. You would not necessarily want to do this test after moving an animal to a brand new cage, because as we know, most of the time when you move an animal to a brand new cage, they're spending a lot of their time exploring the new cage and checking out the surfaces and seeing what new and interesting different odors are in their new environment and so nesting behavior won't necessarily happen for a while until they get more comfortable with the new cage. So being cognizant of where you are in the cage-change cycle is important when you're looking at nesting integration.

>>*Silk*: [Question 15] What scientific conditions would allow for death as an endpoint? Could you give an example?

>>*Hickman*: Like I said, the best example would be an LD50 study where you're doing toxicity and you're trying to figure out what the lethal dose is and to calculate that, you have to let the animals die, in general. That's probably the best example that I have. But again, those are hopefully rare, would be the intent.

>>*Silk*: [Question 16] Can you discuss the appropriateness of having various humane endpoints specific to procedures within the overall study? That is to say overarching

humane endpoints, and then specific endpoints related to the proposed procedure. So that sounds very customized, doesn't it?

>>*Hickman*: Yeah. And that is a model that I have seen in some places where there is an expectation. And this – that can be a way to help with regulatory burden to some degree, because if 90% of the animals in your facility, say, are breeding or not being used in anything that would be considered – that would have a significant need for customized endpoints, coming up with an overarching umbrella of these are the things that we would consider criteria for euthanasia would be appropriate, because then the investigator doesn't have to come up with that list every single time.

It's something the IACUC and the veterinary staff have put together to say, hey when we see these things, it's time for the animal to be euthanized. What that does it that it covers the vast majority of the cases at the institution. But you need to make sure that you leave that approach flexible enough so that for the scientist who needs to have different endpoints so that it's customizable for them. But again, try not to fall in the trap of just hunched posture, dehydrated, weight loss. I'll tell you, at my institution, if you just write weight loss, I'll ask you how frequently you're weighing the animals and what interval, and how you're comparing to baseline, because most people will just write weight loss.

So it can be good, but still definitely keep it objective, like body condition score of 2, and things like that. And make sure you leave the room for the individual study to customize to their expectations as well.

>>*Silk*: [Question 17] Here, someone sent us a comment about IACUC protocol applications. I assume you recommend protocol applications be reworded by replacing the word endpoints with humane endpoints. Do you have a comment on that?

>>*Hickman*: Yeah, I think that that's probably fair because I think sometimes our scientists are trying to figure out what it is that we're asking with that and by specifically calling them humane endpoints, because in the scientist's mind an endpoint is the end of the study. It's when I'm done with data collection. So, when you ask a scientist what's your endpoints, they're going to say well at 90 days I'm euthanizing the animal so I can collect the tissues from them. What you're asking is, no, when are we going to euthanize before your actual endpoints. So your protocol form needs to ask for the timeline of the study, which will get to the study endpoint in an experimental design, but you do need to ask them about their humane endpoints and I think you might help them understand what you're looking for if you call them a humane endpoint.

>>*Silk*: [Question 18] And here's one that the listener asks: Is physical injury considered a humane endpoint?

>>*Hickman*: It depends. If the study is inducing a physical injury then the sequelae to that physical injury would be a study-defined humane endpoint, right, an endpoint defined within the protocol of the study. So, if you're inducing a traumatic spinal injury, then as part of the protocol development, you do need to have a discussion of what potential sequelae will happen there and then when will the animal be euthanized based on different criteria. But we also know that injury can happen outside of the protocol. So again, that's where the veterinarian – so that situation where an injury happens outside of the experimental manipulation would not be captured by the protocol, but that goes back

to the judgment of the attending vet and any of their clinical staff working with the scientist about what needs to be done with that animal.

>>*Silk*: [Question 19] And the last question we have asks something rather specific, so I saved it for the end. Can you please let me know if any humane endpoints for the liver cirrhosis animal models, chronic or acute? Do you happen to know of any? They didn't really specify what animal model they're talking about.

>>*Hickman*: I would say that a liver cirrhosis model would be similar to my example about the kidney toxicity. So, you're looking at what complications you would expect with that model. In this case, body condition score may not be a bad idea. Even if they're starting to retain weight in the abdomen because they're not able to process fluid correctly, you're going to see the muscle wasting and that would be more meaningful than body weight, for example. You know that you've got a problem with the liver. And so looking at what – you can [search] the literature and look at what's normal for that species and then make your judgment calls with the scientist about how high, how far outside of normal is acceptable as they're looking at those liver parameters. Depending on the species that you're looking at, the imaging may be useful so you can assess, if you're able to do ultrasound and assess the amount of damage that's been done and how extensive the cirrhosis is, those can all be things that can help you.

But again trying to come up with objective criteria with the scientist, balancing having that dialogue between the veterinarian and scientist about what it is that the scientist needs to get out of [the study] and what criteria are going to signal that it's time for the animal to be euthanized, even though we're not quite to the study endpoint, is important. But I think if you look at the slides for the kidney toxicity, I would set up something relatively similar to that for a cirrhotic liver study.

>>*Silk*: Well, we've come to the end of the questions. These are really interesting questions, and such an interesting topic. If you listeners think of additional questions in the next week or two as you reflect on this webinar, please send them in to us and we will impose on Deb to answer them, and then amend them to the end of the transcript, which we'll be posting on the OLAW website. So, now I would like to thank you, Dr. Hickman. You have been incredibly generous with your time. I'd like to thank Indiana University for loaning you to us, and I want to thank all of you listeners for participating in our webinar, with special thanks to those who sent in questions.

Slide 70 (OLAW Online Seminar)

The next OLAW Online Seminar will be on December 13th, when Dawn O'Conner and Bill Greer from the University of Michigan will talk to us about Semiannual Program Review. I wish everyone a good fall, and look forward to having all of you join us for our next webinar in December. Good-bye.

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