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Note: Text has been edited for clarity.

Contents:

- **Transcript**
- **Additional Questions**

Housing Laboratory Rodents According to the 8th Edition of the *Guide*

Speakers: Guy Mulder, DVM, MS, DACLAM, Charles River Laboratories; Joseph Garner, PhD, Stanford University; and Axel Wolff, MS, DVM, OLAW, NIH. Moderator: Jerry Collins, PhD, Division of Policy and Education, OLAW and Yale University Broadcast Date: July 19, 2012. A recording of the seminar can be viewed at http://grants.nih.gov/grants/olaw/2012-07-19_13.01_OLAW_Online_Seminar__Housing_Laboratory_Rodents_According_to_the_8th_Edition_of_the_Guide.wmv (Windows Media Player - 2hrs).

Slide 1 (Title Slide)
>> Dr. Collins: [Hello, and welcome to this special OLAW [Office of Laboratory Animal Welfare] webinar entitled: **Housing Laboratory Rodents According to the 8th Edition of the *Guide***. My name is Jerry Collins and I will be the moderator for today's webinar.]

Our webinar today is the last in a series of special webinars that OLAW developed to assist IACUC [Institutional Animal Care and Use Committee] members and staff, and animal program personnel at PHS Assured institutions in implementing the recommendations of the 8th Edition of the *Guide for the Care and Use of Laboratory Animals* [*Guide*]. This webinar is also special because we are joined today by participants from Iran, Japan, Mexico and South [Africa].

We are delighted to offer the expertise and experience of today's speakers who will share with you some of the ways that their organizations are implementing the recommendations of the 8th Edition of the *Guide*. Our first speaker today will be Dr. Joe Garner. Dr. Garner is an Associate Professor in the Department of Comparative Medicine at Stanford University. Dr. Garner received his doctoral degree from the University of Oxford where he studied the developmental neuroethology of stereotypies in captive animals. His postdoctoral research in animal behavior and well-being was undertaken at UC Davis. He served as an assistant and an associate professor of animal behavior and well-being in the Department of Animal Sciences at Purdue University prior to moving to Stanford. Dr. Garner's research topics include the development of refined methods in behavioral research; abnormal behaviors in animals (including barbering and ulcerative dermatitis) and their relationships with abnormal behaviors in humans; mouse well-being and enrichment; and the scientific impact of well-being problems in lab animals.

Our second speaker will be Dr. Guy Mulder. Dr. Mulder is the Director of Professional Services and he serves as the attending veterinarian for North American Research Models for Charles River. His responsibilities include regulatory, technical, and clinical support of commercial rodent and rabbit production and surgical services. Dr. Mulder is a Diplomate of the American College of Laboratory Animal Medicine. He received his Master of Science degree in Comparative Medicine from the University of Washington, Seattle; his veterinary training is from Washington State University. Guy and Joe have been working collaboratively on rodent enrichment and performance standards. We're really pleased that they agreed to share some of the results of that collaboration with us today.

Dr. Axel Wolff will join Drs. Mulder and Garner. Dr. Wolff is the Director of the Division of Compliance Oversight at the Office of Laboratory Animal Welfare at the National Institutes of Health. Joe is in California. Guy is in Massachusetts. I am in Connecticut and the OLAW staff is in Bethesda. So let's go to Bethesda. Axel, will you please get us started?

Slide 2 (*Guide* Definition of Performance Standards)
>> Dr. Wolff: Sure. Thank you, Jerry. OLAW decided to offer today's webinar, Housing Laboratory Rodents According to the 8th Edition of the *Guide*, because about 60% of respondents to OLAW'S request for comments on the *Guide*, indicated concern with changes to caging and housing specifications. OLAW concurs with the *Guide* that performance standards are to be applied to housing issues.

"Performance standard means a standard or guideline that, while describing a desired outcome, provides flexibility in achieving this outcome by granting discretion to those responsible for managing the animal care and use program, the researcher, and the IACUC. The performance approach requires professional input, sound judgment, and a team approach to achieve specific goals… Performance standards can be advantageous because they accommodate the consideration of many variables… so that implementation can be best tailored to meet the recommendations in the *Guide*." (*Guide*, pages 6-7)

Slide 3 (OLAW Performance Standard Criteria)
OLAW believes that IACUCs and institutions are better able to meet their responsibilities to ensure humane animal care and humane research with animals while advancing the quality of the scientific research through the use of performance standards. We encourage the cooperative application of the diverse expertise at Assured institutions to develop outcome-based performance standards that enhance the quality of their animal programs. We expect Assured institutions to apply appropriate professional judgment and experience to the challenges inherent in developing policies and procedures to maintain a quality program that provides humane care.

We expect performance standards to meet the criteria on this slide. And as it says, a well-established performance standard meets the following criteria:

- It supports scientific objectives;
- It supports the health and welfare of the animal;
- Includes a justified performance index; and
- Has associated outcome criteria.

OLAW believes that outcome-based performance standards are paramount when evaluating cage or pen space for housing animals. While the *Guide*'s space recommendations are accepted reference points for addressing space needs, performance standards allow flexibility to improve animal welfare and advance scientific research. An institution's animal housing practices must be species-specific, appropriate for the animals, and in compliance with all federal and local regulatory requirements.

>> Dr. Collins: Thank you, Axel. And now switch over to California, Dr. Garner.

Slide 4 (Title Slide)
>> Dr. Garner: Hello. Good morning. Well, thank you. It's an absolute pleasure to be here. It's a pleasure to be on such a wonderful panel. So what I'm going to try and do is really start off today with a 30,000 foot view, and talk about how we might practically go about assessing performance standards. And to do that – next slide, sorry – just realized my keyboard doesn't move the slides. Wonderful.

Slide 5 (Animal Wellbeing IS the ultimate Performance Standard)
So I'm going to suggest to you that certainly in terms of thinking about the aspects of the *Guide*, which have to do with animals, the ultimate performance standard is actually animal well-being. So first of all, I'm going to talk a little bit about what animal well-being is and how we might go about measuring it. I'm going to develop that to suggest to you that the central thing [which] we really need to think about is whether or not the animal has control over things in the environment that it cares about. I'm going to work through an example of an enrichment success story. Unashamedly, I'm going to be talking about mice, because they're a wonderful example, but also I think they're probably the species that most of us are concerned about on this call. And then I'm going to try and develop those concepts out a little bit and think about the lab from the mouse's point of view.

Slide 6 (Three different visions of wellbeing: different people have different values)
So the really important thing to think about, and understand when we're talking about animal well-being is that, in fact, animal well-being is a socially defined construct. And different people in society have very different things that they think make up or are central to animal well-being, and those three different things fall into three different groups. What we can call functional integrity, psychological suffering, or the *Telos* or the nature of the animal. Let's talk through that a little bit.

Slide 7 (Three different visions of wellbeing: each can be measured in different ways)
So each of these different concepts of well-being actually have associated with them objective measures that we can take in the lab or in a husbandry system. So in the case of functional integrity, for example, whether the animal is healthy, is obviously something which veterinarians for instance care about a lot. When we're thinking about producers, especially in the farm animal world, then they tend to [focus on] growth, survival and reproduction. In either case, when you see that the animal is growing or surviving, or you see that an animal is healthy, [then] people who are focused on functional integrity will say the welfare is good. And this all boils down to essentially whether or not the animal is fit for the purpose for which it's being used. So this is a very sort of industrial concept in a way, and in the case with lab animals, this is obviously whether or not they are good research [subjects].

Slide 8 (Three different vision of wellbeing: each can be measured in different ways)
So in terms of psychological suffering, then we can measure things quite easily, such as whether the animal is anxious or fearful. We can measure whether or not the animal prefers particular things in its environment, or whether it is trying to avoid particular things in its environment.

Slide 9 (Three different vision of wellbeing: each can be measured in different ways)
And in the case of *Telos*, (this mysterious word, which is essentially the nature of the animal) at the end of the day, what we try and assess in order to assess welfare is whether or not the species-specific needs of the animal are being met.

Slide 10 (*Telos* - The mouseness of mouse)
So I'd like to sort of develop this idea of *Telos* a little bit, because whilst functional integrity and psychological suffering are probably ideas you all are familiar with, *Telos* may well not be. You can think of this as being the mouseness of mouse or the pigness of pig or the rhesusness of rhesus; whatever it is that is unique and special about that animal. This involves really having a very good understanding of the animal's natural history in the wild, or at least of its ancestors in the wild. For me the most useful way of thinking about the *Telos* of an animal is to think about: What is the one thing that this animal does that no other animal in the world can do as well?

So when we think about lab animals, they all tend to be a kind of animal called a commensal. A commensal animal is one which has evolved to exploit human environments, to exploit the resources which we concentrate, and to essentially live off of us. Rhesus are commensals and Norway rats are commensals, and [house] mice not only are commensals, but they're probably the most highly evolved commensal that there is. They're certainly the only commensal species that has successfully followed us around the world, and has actually subspeciated with different major human migrations, which is what this graph is showing. In order to do this, they have to be as adaptable to new environments as we are, as we create all of these new environments in different places, and they do this without the benefits of tools or clothing or language, which we have.

They do it using something called phenotypic plasticity, which is the ability of one genome to express multiple different phenotypes depending upon the environment in which the animal is found. (We also are very phenotypically plastic species. This is actually one of the reasons why mice are such good models of humans.)

Slide 11 (Three different visions of wellbeing: each can be measured in different ways)
So going back to these three different ideas of what makes up animal well-being, obviously they overlap. There's an area of overlap between functional integrity and psychological suffering where we might talk about the Malthusian needs of the animal, the need for food, the need for air, and so on. Probably more in line with the sort of things that we worry about on a daily basis, pain and injury are obviously an overlap between functional integrity and psychological suffering.

Slide 12 (Three different visions of wellbeing: each can be measured in different ways)
So in terms of the overlap between the *Telos*, the nature of the animal, and psychological suffering, we can think about motivation. Are there highly motivated things that the animal is trying to get hold of in its world that it can't manage to get hold of? And in particular, are there abnormal behaviors which we are seeing?, because abnormal behaviors generally stem from a failure of highly motivated behaviors to achieve the animal's needs.

Slide 13 (Three different visions of wellbeing: each can be measured in different ways)
And then finally, we can think about the overlap between *Telos*, the animal's nature, and functional integrity of the animal. And the most obvious way of measuring this is by looking at normal behavior.

Slide 14 (These measures are progressively more specific to the animal, but not always harder to interpret)
So the important thing to understand about these different ways of thinking about animal well-being and the different measures that are associated with them is that as you move from thinking about functional integrity to thinking about psychological suffering to thinking about *Telos*, you have to understand the animal better and better and better. You have to know more and more of its natural history in order to figure out the right way in which to measure things. So as we move from functional integrity to psychological suffering to *Telos*, measuring these different potential performance standards requires a greater degree of knowledge and a greater degree of professional judgment.

Slide 15 (The simple measure can be the most misleading – we have selected animals to breed despite their wellbeing)
But, that doesn't mean that functional integrity is a silver bullet, and this is a very, very common mistake that you see especially in the mouse well-being literature, that people assume that a simple measure of health is easy to interpret. In fact, these measures are often the most difficult to interpret. The really obvious reason for this is that we have selected animals, and in the case of mice for several hundred generations, to breed and

reproduce and grow despite impairments to their well-being in the captive situation. [In which] case, one of the big issues we have to worry about is whether or not measures of health and function are, in fact, divorced from the animal's welfare in terms of psychological suffering or *Telos*. So that is just a warning not to rely on these easier, simple measures.

Slide 16 (Nevertheless, *Telos* is hard to find in the literature (and thus *The Guide*))
Having said that, though, if you look at the literature, then you'll see that really almost all of the papers out there that we can turn to for making evidence-based decisions really focus on functional integrity or psychological suffering, so they tend to measure gross survival and reproduction, health, the physical environment, such as ammonia levels, whether or not the animal is in pain, so on and so forth. And it's no surprise really, I think, that the overlap for these two areas is the area which is really emphasized in legislation and in the *Guide*.

Slide 17 (The 'sweet spot' is what really matters, it is measurable, and *Telos* is the key)
However, if we go back to the very first point I made, which is that animal well-being is really a social construct, then if we want to really justify the use of animals to everybody in society, then obviously the one of the key places we ought to be trying to focus on is this sort of sweet spot, where these three different ideas of what makes up animal well-being overlap. Because if we can show that we have good well-being by looking at the sweet spot, then we can convince producers, we can convince veterinarians, we can convince scientists, we can convince even the animal rights folks, perhaps.

So the rather interesting thing about this sweet spot is that it's actually pretty well-understood and it's understood in terms of coping and distress, particularly whether or not the animals are showing coping behaviors. [As a result] there are other objective measures we can use, such as stress physiology, immune function, and autonomic function. The thing that is rather interesting about these measures is although they may involve some technical complexity, they're actually very well-understood in terms of how to do them, [and] they're often a lot more straightforward to interpret than some of the other measures that I've already mentioned.

Slide 18 (Behavior and control - the *Telos* of animals)
So what I've done at this point is give you a crash course in animal well-being, if you're in an undergraduate class that would have been two lectures! So what I'm going to do now is move on to some concepts of control, and in particular what I'm going to try to suggest to you is that behavior and control is the *Telos* of animals in general. If you want to know what it is in general that makes an animal an animal, what is the most important thing for us to think about, then I would argue that it's behavior and control.

Slide 19 (Stress and Distress (Moberg 2000) Example: Mouse thermoregulation)
So I'm going to introduce this by talking about a guy called Gary Moberg, who unfortunately died a few years ago, far too early, and he's really, really, really important

in the modern ideas of how we go about measuring well-being in animals. In fact the book he wrote shortly before he died is, I think, one of the most important things ever written in the field.

In this graph what I'm showing you is how a mouse's metabolic rate increases as ambient temperature falls.

[Comfort] So first of all, there's a zone of ambient temperatures which we call the thermoneutral zone where animals don't have to burn any additional resources in order to maintain homeostasis, and this is also the zone of temperatures in which human beings feel subjectively comfortable. For mice this ranges from about 26° to about 34° centigrade [although the range for any one individual is only 1-2° centigrade].

[Stress] So once that ambient temperature falls beneath that point, the animal has to start to burn energy in order to stay warm. It has to begin to spend some of its resources to maintain homeostasis, and this is the physiological definition of stress. Stress is just part of life, right? It's no bad thing as long as we can cope with it.

[Distress] The problem is there becomes a point where you begin to run out of spare resources. At that point the animal has to make a physiological decision about which other biological functions it's going to compromise in order to maintain homeostasis, and this is the point where we say that an animal is physiologically distressed. In mice this occurs at about 18° to 20° centigrade where we begin to see a fall-off in growth rates and we begin to see changes in immune function.

[Death] And obviously, if this falls too far, then the animal will ultimately die.

Slide 20 (Animals exist to behave)
So there's a complication to this story, and the complication is actually the really important thing to understand, so what I just presented to you was a really grotesque oversimplification of what Gary Moberg was saying. Gary Moberg's real point is that, actually, distress occurs at a very subjective point and this is neatly illustrated by mice. So we know from work in the '60s that if you expose mice to ambient temperatures of minus 40° C, then they die of hypothermia in around about 15 minutes. Yet at the same time, we know from the pest control literature that mice are really significant pests in meat freezers at minus 30° centigrade, and this is because they can build nests.

So in fact as Gary Moberg and many other people have pointed out, the ambient temperature at which a mouse becomes distressed isn't a physiological set point; it actually depends entirely on the animal's perception of its ability to control it. The same is true for humans. There's a very entertaining literature when people were designing flight suits for military pilots looking at how that thermoneutral zone changes with different amounts of clothes you're wearing. This is essentially what a nest is doing: changing the

physiological range of comfort, stress and distress by altering the microenvironment. That fundamentally is what I would argue animals are about.

So the reason that plants are boring is that if a plant gets thirsty it dies, whereas if an animal gets thirsty, it walks to water. This may seem like a sophomoric point but it's actually the fundamental thing to understand about animals and about behavior. Animals exist to behave. It's what makes them animals in the first place. And behavior exists to allow the animal to control the world and to survive and thrive in environments which physiologically might well kill it (in the case of meat freezers, for instance).

Slide 21 (Behavior exists to predict and control)
So this notion of behavior and control was first really well-developed by a guy called Weiss in the '70s. He also conducted the thermal stress work that I just showed you. In the experiment [on this slide] – and I hope this animation works – the two [rats] are connected to the same electric shock machine. One of the rats either has control over when the shock arrives or has a little light that warns it when the shock is going to arrive. So the two animals have exactly the same physiological stressors imposed upon them. The animal on the right has some psychological prediction and control, and the animal on the left doesn't. The amazing thing is that despite the fact these two animals are receiving identical physiological stressors, the one on the right, which has prediction and control, is largely unaffected. And the one on the left, which does not [have any control], is severely impacted by that physiological stressor. But in fact the reality is the only difference between them is the psychological components.

Slide 22 (What is environmental enrichment?)
This leads us to a really refined definition of environmental enrichment. If animals exist to behave, and behavior exists to predict and control stressors in the world, then an animal that can't control its environment in the wild is going to die. So animals have been designed by millions of years of evolution to do everything in their power to exert control over their environment, and when they can't, they try so hard that every aspect of their biology can and will change and can and will ultimately fail if they don't gain control over stressors that they care about. This is the key fundamental piece of biology that leads us to predict that enrichment should improve animal models. Because by definition, an animal that does not have control over its environment is one where the fundamental *Telos* of all animals is denied and it is fundamentally abnormal and it will mess up your animal models.

So this also leads us to be very strict about what we consider an enrichment to be. In particular, we consider an enrichment to be biologically relevant. This means that it’s any change which we make in housing or husbandry that either removes stressors, [or gives the animal control over stressors.] [Removing stressors] is actually very hard to do because we're not mice, we can't see in the ultraviolet, we can't hear in the ultrasound. So it's hard for us to perceive the stresses that the animal perceives. It's a lot easier for

us to provide the animals with ways to control the stressors in their world, and that's what I'm going to illustrate to you next.

Slide 23 (An enrichment success story… (…the best investment you'll ever make))
What I'm going to do now is I'm going to talk about work in our lab with nesting material. And unashamedly so, because this is I think a really nice story, but it's also probably the most thorough workup of an enrichment that has ever been done in mice. And also it's work that we did in collaboration with Charles River and Guy.

Slide 24 (Too cold for comfort?)
So first of all, let's ask the question whether mouse environments are too cold for comfort. I've already shown you a slide that suggests that they're physiologically stressed at these temperatures. This is another slide basically making the same point. So this is a thermal image of two mice in a cage. I pick these two mice because they're barbered. The reason for this is I really want to make the point that mice can't vasoconstrict the skin underneath their fur. So where they've lost fur in case of these barbered mice, you can see that actually the skin temperature is very close to the core temperature. The fur provides about another 7° centigrade of insulation, and you will see, then, the tail is very close to ambient room temperature. The reason for this is that when mice are cold stressed, they shut off blood flow to their tail almost completely, and that's what you're seeing in this image.

Slide 25 (Mice prefer warmer cages… but warmer cages cause aggression)
So the first thing that we can do is to simply ask mice to vote with their feet and tell us what they think about ambient temperature. In this experiment, we gave mice the choice between three different cages which they could run between, one at 20°, one at 25°, and one at 30° C. As you can see, the mice prefer to spend their time at 30° C, and they avoided the 20° and 25° centigrade cages, which of course is the range indicated by the *Guide*. The reason, fundamentally, that we keep mice in colder conditions is because cold suppresses aggression, and that's a point which I'll come back to later.

Slide 26 (Performance standards measured)
So in terms of a little Venn diagram we can use to think about what we're talking about, in the data I've just shown you, I've shown you that there's a basic need, that the animals are cold (a basic Malthusian need); and I've shown you that there's preference for warmer temperatures and avoidance of colder temperatures, so there may be some evidence of psychological suffering.

Slide 27 (*Telos* – in nature nests keep mice warm when temperatures are low and fighting is prevented)
So let's think about the *Telos*. What is the mouseness of mouse, or even the nestness of mouse nests? Well, in nature, which is shown at the bottom, mice build these fantastically beautiful nests that involve structural components that are woven together very tightly and then thermoregulatory components that are packed into that structure in order to

insulate the nest even further. As you can see if we provide even a crude mimic of these sorts of materials to mice, a crinkly paper material to give them structure and tissues to give them something soft and thermoregulatory, then they build wonderful, wild-looking nests. These are completely naive mice, by the way, in that picture. And even if all we do is give them the crinkly paper material, then there's an enormous thermal benefit, and that's demonstrated in the thermal image which you can see in the top left. We can see that there's a wonderful conservation of heat within the nest.

Slide 28 (Biologically relevant enrichment- mice need the right kind of nesting material)
So there's not just the animalness of animal or the mouseness of mouse; there's also the appropriateness of the material that we are giving them. In this experiment, we looked at whether or not Nestlets, which were at the time the predominant nest enrichment people were using, actually allowed mice to build nests at all. The answer is, at least in the way they were being manufactured at this point in time, (they've changed since we published this work) they don't. They are, in fact, no better than providing additional bedding material. In fact, the only material that does help mice build better nests is this crinkly paper material that they can weave together. So to illustrate that, I would like to show you the video on the next slide.

Slide 29 (Video)
What we're looking at here is a thermal imaging video. The little blue cross that's bouncing around is the warmest point in the image and what we're going to do here is we're going to pull open the mouse nest, and you can see how tightly woven that material is. And as the mouse leaves the nest, you will notice that the base of its tail, and in fact its whole tail, is extremely warm. It's actually only a few degrees shy of core body temperature, and that the inside of the nest is also a nice and toasty 34° C. (I'm sorry this video has jumped around so much – some of the best parts of it were missing.) So this tells us that inside the nest the mouse is thermoneutral. It's in that zone of comfort where all [the mouse is] doing is passively thermoregulating by altering blood flow to the tail.

Slide 30 (Performance standards measured)
These data show you the species-specific needs for very particular kinds of materials to build a nest with. They show you what the normal nest-building behavior looks like in terms of these wonderfully woven nests. And although we didn't go into it in too much detail, there's abnormal nest building behavior which is generally what we see with inadequate materials like some of the older versions of the Nestlets where we don't get proper nest building.

Slide 31 (Heat loss decreases with better nests, regardless of treatment, strain or sex)
So this begs the question of whether this actually benefits the animal at all. Maybe it's just, you know, fun to build a nest. But in fact if we look at – these are a variety of different strains with a variety of different materials, we see that universally the better

built the nest, the less heat escapes from it. This benefits the animal, as you can see, by about 5 degrees centigrade.

Slide 32 (Mice use nests to control cold they can't escape from)
And in fact if we confine animals in cages that are set at different temperatures, at 20°, 25°, or 30° C, then we see that the kinds of nests they build change. And the colder they are, the more complete and dome-like the nest is; the warmer they are, the more they open the nest up. It's a little like you being a bit too hot in bed and sticking a leg out from underneath the blankets. On the other hand if we give animals a choice and we allow them to escape to cages of other temperatures, then we don't see this change in nesting behavior. And this really illustrates to us that they're using nest-building behavior as a very adaptive response to the environment.

Slide 33 (Performance standards measured)
So this really is one of the ways in which we can demonstrate coping behavior. And so now we've hit that sweet spot as well. We've demonstrated that nests actually help the animals cope with the stressors that they care about.

Slide 34 (Commercial scale implications – Nests conserve energy, reducing food use)
So what about the issue of functional integrity? Well, to get at this we did a lot of work with Charles River, and these are very large scale experiments that we did with Charles River. So first of all because nests conserve heat, they conserve energy. And so the animal actually, as a result, doesn't have to burn energy to stay warm. It has a lot more free energy it can use for other things. One of the most obvious pieces of evidence of that is if we look at something called the feed conversion ratio, which is the grams of feed that an animal has to eat in order to produce a gram of pup. As you can see, there is an enormous [27%] decrease in the food consumption to produce the same weight of pups when you give animals nesting material.

Slide 35 (Commercial scale implications – Nests improve breeding performance)
And at the same time because the mother has more free energy available to her, she can invest it in producing more offspring. And so on average, most strains of mice produce one additional offspring at birth and successfully wean one additional offspring 21 or 23 days later, depending on when you want to do your weaning. And even in some strains, we actually see a very marked decrease in pup mortality. That is particularly true in Black 6 strains. For those of you who are dealing with, for instance, recently derived, very precious knockout line that you're trying to backcross on to a Black 6 [background] and none of the mothers are breeding, or they're killing their pups (which is often an issue in early transgenic mouse development), then I would suggest that nesting material [might be] hugely helpful there.

Slide 36 (Nesting material reduces feed cost per pup by roughly 27%)
So just to illustrate this in another way, here are two pups from this study. And the control pup is sitting on the amount of food it took to produce that one animal. And the

nesting pup is sitting on the amount of food it took to produce that one animal. One of the things you can see here, these two pups are actually essentially the same size. In fact, the pups don't differ in weaned body weight. The total litter weight is greater because there are more pups in it. So what the mother is doing, is she's repartitioning her energy resources into producing more pups, but she's not actually changing the weight of pups – which actually is quite a nice result especially when we're worried about consistency in toxicology [studies], for instance.

Slide 37 (Commercial scale implications – Return on investment)
Okay. So we can now really drill down into the economics of this, because at the end of the day, any animal system, we have to think about the economics of it. Providing nesting material costs about 60 odd cents or so per 6 month period, and the reason we chose 6 months is that is how long commercially you normally breed a pair or a trio for. And in the same time period, we can also figure out how much additional money we saved or we made in terms of the additional pups that survived and the cost of those pups. And I've chosen the two extremes from all the work that we've done at Charles River here. The smallest amount of money you save is about $200 and the largest amount of money you save is $800 to $900. And that I think is, especially in today's economic climate, an investment we would all like to make. 60 cents to make 200 bucks; I think that's fantastic.

Slide 38 (Performance standards measured)
So we've shown you some information pertaining to health here. Very importantly, we've shown you growth, survival and reproduction. But at the end of the day, we've really shown you fitness for purpose; that providing animals with nesting material, and most importantly the correct nesting material, makes them much better breeders.

Slide 39 (The lab from a mouse's point of view – Class tower blocks and Tyrannosaurs)
Hopefully this has illustrated some general principles in terms of thinking about how we can very objectively measure performance standards. What I'm going to do now is just take a couple of minutes to talk about the environment from the mouse's point of view and to think about a couple of other problems that we may be overlooking in the mouse environment.

Slide 40 (Mouse environments deny *Telos*, and expose mice to stressors they cannot control)
First of all, let's think about how realistically a typical mouse environment actually meets the *Telos*, the mouseness of mouse. I think it's pretty obvious for a variety of different reasons that there are some pretty serious issues in any mouse housing situation. This is because typical mouse housing exposes mice to stressors that they just can't control. We've already talked about cold and draft and the lack of shelter or nest site. If you think about it, mice are commensals, right? Their job is to steal food and resources from us and not get noticed by us. So we're, as far as they're concerned, we're a predator. So for you, imagine that you lived in a glass tower block and the custodian was a Tyrannosaurus Rex

and every couple of days it picked you up, promised not to eat you and moved your furniture around. I think that you would find that rather stressful.

Mouse cages obviously do not have the amount of space which allow for any kind of serious locomotion. Mice can move hundreds of meters in a day on a regular basis in the wild. There's far too much light during the day, a point we'll come to in a second. And at night when mice are most active, because the mice are nocturnal, so night is their day, so to speak, they would normally be running around under starlight or moonlight or at low level of ambient light; but we've switched lights off completely.

Most importantly we're asking mice to live in a shoe box cage when the densest that you ever find a mouse living in the wild is in two square meters, so that is about 40 odd shoe box cages. And in the wheat fields of Australia, they occupy a space of 80,000 square meters, which is several million shoebox cages. The fact that we're asking them to live in these much smaller spaces than they normally would in the wild is compounded by the fact that we disrupt social cues that are really important to them. There's a lot of ultrasound in mouse environments, especially in ventilated cages or from HVAC systems, and from water flow. But mice actually communicate in the ultrasound, and again this makes sense. They are trying to avoid us detecting them, so they use frequencies of sound that we can't hear. But males actually have to sing to females in order for females to mate to them, which they're doing in the ultrasound. And that can be drowned out or impaired by ultrasonic noise in the environment.

Probably much more importantly, though, every time we clean the cage, we disrupt the essential odor cues, what one of my students wonderfully called “pee mail” that the mice use in order to establish and maintain their social hierarchy, to avoid fighting, to avoid infanticide. And so in several strains you'll see that cage cleaning induces aggression. This is clearly an issue, for example in CD-1s. Similarly, when we put some physical enrichments in the cages, such as shelters, this may be wonderful for some strains or for females. But especially for males, it can provide cues that they need to really start fighting, [because] they like to ambush each other. And we've shown for instance that putting shelters in a cage of CD-1 males is pretty much anarchy in the mouse cage.

Slide 41 (The undesirable penthouse shoebox)
So let's take a step back and think about some of the things we do. First of all, we like to house mice in these nice, tall, brightly lit, elevated shoe box systems. In fact, the brightness of the lights are sufficient to blind most albino strains. This is something beautifully documented by Brown and Wong. We elevate the cages for efficiency, but as far as the mouse is concerned in the wild, it's trying to hide from us and it likes to hide from us by hiding in dark corners, pressed into little spaces, out of sight and out of mind. And so actually mice find brightly-lit, open, elevated spaces very frightening, and this is the basis of anxiety and fear tests that we use in neuroscience to find anti-anxiety drugs. This is why the open field test works, or the elevated plus maze, or the light-dark box. So it's rather unsurprising, then, that if we actually look at anxiety, we see that the further

off the floor (and more brightly lit as a result) the cage tends to be, the more anxious animals are. There's more abnormal behavior as you move from the bottom of the rack to the top of the rack. There's more immune suppression as you move from the bottom of the rack to the top of the rack. And in fact this immune suppression is so severe, that in Type 1 diabetes models, you actually have less conversion to Type 1 diabetes and it takes longer in the top of the rack versus the bottom of the rack.

Slide 42 (Performance standards measured)
So in terms of just our basic housing design, we, from a performance standard point of views, have some pretty serious issues. We have fear. We have preference and avoidance. We have abnormal behavior. We're clearly not meeting the species-specific needs for somewhere to hide. The health of the animals is impacted, we hit that sweet spot. We know the immune function is impacted. And in the case of, for instance, many behavioral models or many immune function-related models, we know that we're introducing a lot of variability into these animals that actually really impairs their usefulness as models.

Slide 43 (Too drafty for comfort?)
So how about cage ventilation? Ventilated cages are taking over the United States at a rate of knots and if we are honest about it, the reason that we're doing this is to reduce labor costs, to reduce bedding costs and most, importantly, to reduce footprint. In other words, [we are doing this so] that we can get more mice into the same physical space in an animal room in the lab. But if you actually look at the literature, there are very few papers that have ever asked whether or not this is good for mice, and there are several reasons to think that it wouldn't be. Most obvious being that within a typical shoe box, a typical ventilated cage system, in order to maintain the minimum required 10 air changes per hour, because of pressure changes across the plenum; you're going to wind up with cages that often are experiencing 60 air changes an hour. And if you think about what that means in terms of a typical inlet size, that represents a wind speed of three and a half meters per second, which is very, very, very fast and unpleasant. Now, it's particularly unpleasant because the last thing that a mouse normally feels before it's grabbed by a human or grabbed by a cat or pounced upon by an owl is a big puff of air. So big puffs of air are major predator cues to mice and are extremely aversive.

When we think about some of these systems that are out there where air is injected in a port around the drinker, we're actually asking mice to approach a cue which in the wild would mean certain death in order to get a nice drink of water. This is probably not a good way to design a mouse cage. If we actually ask the mice, then, sure enough, they find ventilation aversive. If we allow them to choose between ventilated and unventilated cages, they prefer [un]ventilated cages. But here, just to go back to my central point that it's all about control, if you give mice nesting material, then you see that they will choose a cage which is ventilated and stressful, but with the nesting material that they need to control that stressor, over a cage that is not. Mice in ventilated cages are more fearful. The HPA or axis, one of the branches of the stress system, is activated and they are

immune suppressed as a result; and you also see variability in reproductive performance as well.

Slide 44 (Performance standards measured)
So again we're pretty much hitting all of our different definitions of well-being, all of our different kinds of performance standards we might care about and seeing evidence that ventilated cages actually impair them.

Slide 45 (Too crowded for comfort?)
So, all right, the issue that everybody is really, really upset about. So I'm going to go through this really quickly, because Axel and Guy are going to cover this in a lot more detail. The *Guide* recommends now a minimum of 15 square inches for a breeding adult male and 51 square inches for a female with pups. I think perfectly understandably, most people, in fact I certainly did when I first read this, assumed that these numbers are additive. In other words, that you would need 66 square inches per breeding pair and 70 square inches for a trio. But if you read the *Guide* closely, it actually doesn't say this at all. It implies it, kind of, for nonbreeding cages, but it says nothing about breeding cages. I actually find the literature review of cage space in the *Guide* rather frustrating, because [the literature is] actually a lot clearer than the *Guide* makes out. The *Guide* basically says there's all of this literature, it's kind of confusing, make up your own mind. But if you read it critically, especially using some of the principles that I've hopefully illustrated to you, you will see a lot of those papers are a lot better than others. And the ones that are better than others I think tell a pretty consistent message, some points of which we'll hit in the next slide.

So OLAW interprets the *Guide* in such a way that these values are not considered to be additive. In other words, you don't need to add up 15, 51 and 51 to figure out how much space you need for a trio. I think there's actually a good biological reason for that, which I'll explain on the next slide. OLAW does emphasize, though, that you can't make decisions purely for economic reasons.

Slide 46 (This begs three questions)
So this whole situation begs three questions. Number one: Is the welfare of trios worse than pairs? Number two: If it is, would increasing floor space solve it? And number three: Is there actually any benefit to breeding animals in trios in the first place? Let's think about the first issue.

So the answer to this is perhaps. Certainly, the only good data we have from this is from studies of wild mice. And in the wild, females only cooperate if they are siblings and they colonize a territory at the same point in time. Otherwise, they fight like hell and they try and compete by killing each other's pups. It is possible that we have either selected for this behavior or selected against it in lab mice. We might have selected for it if for the last 300 odd generations of lab mice, we only ever bred from the animals that produced pups. We may have selected against it if, on the other hand, we tended to breed from animals

where both of the animals in the cage produced pups. Those two breeding schemes actually have very different evolutionary consequences. This is something that we're actually looking at Charles River right now, and there does seem to be some evidence that we may have bred some of this behavior out, which is a good thing.

So it's rather unclear whether the welfare of trios is worse than pairs. Let's just suppose that it is. Would the *Guide* suggestion for solving this problem, increasing floor space, solve it? The answer is almost certainly not. And this is essentially OLAW's position, right? The reason for this is that although this has not been looked at in breeding mice, (it's only been looked at in nonbreeding mice) mice care about the number of animals in the group. They don't care about the floor space. They're not doing math. They're not dividing the area of the cage by the number of mice, and somehow that's fine. They care about the number of animals in the group. So if there is an issue in trios, the thing that is going to solve it is reducing the number of animals in the group, it's not giving them a little bit of extra space. So let's move on to the last question.

So is there any benefit to trio breeding? Well, I really think this depends upon your strain, upon many, many different aspects of husbandry. I've always felt that I got much better breeding out of animals that were in monogamous pairs than in trios, and yet my friends at Charles River do as well as I can with trios as I can with pairs. But anyway, there might not be that much of a benefit per female, but there certainly is an economic benefit per cage. But just remember, that can't be the sole basis of your decision according to OLAW.

Slide 47 (Beware! Absence of evidence is not evidence of absence.)
So what I really want to emphasize here, and again I think people are rightly frustrated about this, is that actually there is almost no evidence out there that helps us make this decision about breeding animals. But please don't fall into the trap of thinking that an absence of evidence is evidence of absence. It's actually evidence of an absence of funding. And that is the point that is worth making. Even though making these decisions about housing animals the right way is central to doing biomedical research well, we do not have a federal research budget for this kind of research, unlike Europe, and I think that is something that we seriously need to think about in our industry.

Slide 48 (In summary)

Slide 49 (Animals exist to behave, behavior exists to control. When animals cannot control their environment, wellbeing, science, and performance standards suffer)
I'll finish up by emphasizing the key points from this talk; that animals exist to behave, and that behavior exists to control the environment. And when animals can't control their environment, well-being suffers, the scientific quality of the model suffers and the performance standards that we are trying to optimize all suffer. At the end of the day, it's all about this sweet spot where these three different societal ideas about what well-being is overlap, and that is whether or not the animal is able to cope with the stressors that it cares about.

Slide 50 (Thanks to… and a shameless plug)
So if we flip to final slide, there's some credits, and I will finish and hand over. Thank you so much.

>> Dr. Collins: Joe, thank you very much, as always, for some very interesting data as well as some very challenging questions that you raise. Let's switch now from California to Massachusetts, and Dr. Mulder, your presentation.

Slide 51 (Title Slide)
>> Dr. Mulder: All right. Thank you.

Slide 52 (Overview)
All right. So it's always nice hearing Joe talk. As Joe mentioned, we've done a lot of work with Joe and his lab looking at various aspects of rodent behavior, and in particular how we can affect and ideally improve some of our production processes, and ask questions such as "What is the benefit of nest material?" and you saw some of those results. So today what I thought I would talk about is how we're approaching some of the new recommendations of the *Guide*, and in particular how we're trying to apply a performance measure or a performance sort of approach to exceptions to recommendations of the *Guide*, and specifically for today's talk, we'll be looking at how we're evaluating the rodent cage space recommendations in the *Guide*, and as Joe pointed out, this relatively new recommendation of increased space for moms with nursing pups. And I want to point out here that what I'm going to talk about today really only applies to our commercial production operations for mice and rats. Charles River does certainly more than just commercial production, but the recommendations and the efforts I'm going to show here in a couple of minutes really are centered on our commercial production operations and not other parts of that company.

And just as a foreshadowing here, the key aspects of the process is certainly that the IACUC is involved in the review, approval and the entire process as we evaluate and discuss alternatives to recommendations of the *Guide*. We use the performance-based approach, and I'll talk more about that in a moment. We've coined our performance measures as clinically relevant. They aren't all classic clinical assessments, it's not heart beats and looking for wounds on animals, but it is practical based and what we're terming clinically relevant assessment criteria that really can be performed in almost any research setting with rodents. We think it's [invariably] important that any assessments that are looking at alternatives to recommendations in the *Guide* be performed in the same setting where those animals normally live, where they normally reproduce in our case, where production occurs. So in our case, that is production settings, that is barrier production rooms, isolators, and very little of the studies are done in more sterile procedure and laboratory sort of settings.

Slide 53 (Background)
So just as a little background, we have production facilities, a number of them, across North America. We have seven production facilities in the United States. Much like many of you listening, we are AAALAC [Association for Assessment and Accreditation of Laboratory Animal Care International] accredited at those production sites. We also carry OLAW or PHS Assurance at those sites. We also have USDA license and registrations as well. Our Canadian facility is accredited by somewhat analogous process as the AAALAC process, and that is the Canadian Council on Animal Care. Just to help set the stage, we're living to the same expectations, the same regulations, and the same guidelines as the general research community.

Again, most of our effort here, and certainly what I want to talk about today, is commercial production of both barrier- and isolator-reared animals, and my examples will be barrier-maintained animals, and you see a picture on the right, just to give you some approximation or appreciation, perhaps, of perhaps the scale. We have a number of – five barrier production sites across the country, and each of those is comprised of multiple rooms, and these rooms can have upwards into the neighborhood of 10,000 cages per room, so it gives you some idea of the scale. And again, most of this is commercial production, so it's production of mice and rats. We do very little research within that production setting, and the research that we typically do perform tends to be oriented toward production efficiencies, and I'll show you some examples. Some of the nesting study results that Joe shared came out of our production rooms. So it tends to look at the environment of the animal, and questions related to production and welfare.

Slide 54 (Cage space recommendations)
So cage space recommendations. Joe already touched on this, and I think we have a very similar outlook on the recommendations of the new edition of the *Guide*, and there's a good list of, oh, I don't know, 10 plus, probably more than that, probably 15 plus citations now in the *Guide* under the section of cage space, and the message that comes out of all of that, I think, is really, the scientific basis for space recommendations in the *Guide* is really unclear. So those are my words, not the *Guide*'s words. But the *Guide* does go on to say that it's really difficult to compare these studies, due to their study design, their experimental variables that have been measured, so there's a great deal of inconsistency both in study design, and therefore it's really difficult to draw conclusions from these studies that are generalizable across lots of settings. Although the *Guide* does, I think, do a good job of highlighting perhaps some of the reasons why there're difficulties for that, in that there's just an awful lot of variables that can affect cage space and animal well-being and that can be everything from the species, strain or substrain, and the phenotype of the animal that you're housing or looking at, the age of the animal, the gender, the quality of the space, is it enriched, is it not enriched, is there vertical aspect to the space and structures within the space.

So the highlight that pops up, those circles, that is really I think what made an awful lot of the research community nervous, when the new edition of the *Guide* came out and it's

certainly from a commercial producer's standpoint, it certainly raised our eyebrow in that we now have this new recommendation of approximately 51 square inches for a female with a litter, and at first glance, and as Joe pointed out, it's a little ambiguous in the *Guide* as to: Is that additive? So if we have two females that could each have a litter simultaneously in a cage, do we need to double that space up to 102 inches? If you then add in a breeder male, again, is it additive or is it shared space, so there's some uncertainty there, and I think that's driving a lot of nervousness. I've talked to institutions – folks at institutions that are looking at ceasing all use of breeding one male to two females because it doesn't appear to be in compliance with the new recommendations of the *Guide*. We commonly breed both one to one, so one male to one female, and we have [inaudible] strains where we breed one male to two females, so it depends on the strain, it depends on the setting. But we do both, and depending on the setting, we may or may not be in compliance with these new recommendations with the cages that we're using.

If you read some of the fine print in the *Guide*, some of the footnotes at the bottom there, the *Guide* does allow, and actually recommend that the interpretation of this table, and I would extend that to applying the recommendations here, should be considered in light of performance evaluation or performance goals described in the text elsewhere, and I'll show those in a moment. So certainly this is a recommendation, recommended minimum space, but certainly I think the general approach of this from the community and certainly from Charles River has been if we're going to vary significantly from these guidelines, we need some kind of data to back up our practices and show that whatever we're doing is equivalent to the recommendations of the *Guide*.

Slide 55 (Performance standards)
So as Axel mentioned earlier, the *Guide* does endorse a performance-based approach to all sort of aspects of recommendations described in the *Guide*, not just cage standards, and when you're looking at or considering a performance approach, it actually spells out there's a few requirements that should be addressed or considered, and that is professional input, sound judgment, a team approach, and that team typically would be your veterinary staff, your scientific staff, and the IACUC. There should be clearly defined outcomes and goals, and that there should be some program of monitoring on a regular basis the performance indices or the performance measures that you've identified through this process.

Slide 56 (Performance standards)
So if you go further into the *Guide*, page 56, within the topic of space allocation, the *Guide* also goes further and actually recommends parameters that should be considered or assessed or reviewed when considering variations from space recommendations in the *Guide*, and those are listed here, so health, the health of the animal, certainly we shouldn't be compromising the health of the animals, the reproductive performance, efficiency or success of the animals, the growth, the behavior, the activity, the space utilization. As Joe pointed out earlier, a larger cage for a breeding pair of animals may not be important to those animals if you look at some of these parameters or indices. Does

more space actually equal somehow improved welfare, better behavior, different behavior, et cetera? And of course, some of our stocks and strains, particularly in rodents, may have special needs that also should be considered. If you're working with diabetic animals that produce [increased] urine, have the diabetic phenotype developed, they may have different housing considerations than animals that are not diabetic.

Slide 57 (IACUC review & approval)
So we're addressing this whole process, and this is something that started before the 8th Edition of the *Guide*. We've had what we call in our vernacular, variances to the *Guide*, for quite some number of years, and we've developed over time a standardized process and review format to address, and discuss, and ultimately, if deemed acceptable, approved from the IACUC standpoint, variances to the *Guide*. And I'll show you a picture in a moment, but the topics we hit, and we being the IACUC and the veterinary professional staff at Charles River, we try to be fairly systematic or methodical in our approach, so we outline what the specific procedure or practice for the exception that is under consideration, so in this case it's cage space. It could be changing frequency, cleaning frequency, almost any variance to the recommendations of the *Guide*. Certainly what's the species? What's the recommended guidance – or *Guide* recommendation for that particular subject, so what is the actual text in the *Guide* tell us? What is the proposed exception? What are we hoping to alter? What is the rationale for that exception?

And then I think where it becomes a little more difficult is: What are the performance measures that we're proposing or recommending to evaluate the exception to the *Guide*? And then ultimately those performance measures, we need to roll them into actual methods to investigate the exception. So what's the study design? What's the actual research proposal? And of course, we should include statistical analysis. I say "when applicable". It's hard to imagine when it would not be applicable. But certainly, this needs to be sound science. It needs to stand up to rigorous review, and in many cases now we're also hoping that as we perform these studies within Charles River, our goal is to publish the results in the scientific literature as information to the community, but perhaps helpful as well, and then of course, depending on the variance or the exception being considered: Is there post-approval requirements from the IACUC standpoint? Should this be revisited? Should data be examined at some frequency? And that's described as well in the process.

Slide 58 (Form)
So this is just to give you a glimpse of the particular form we've developed over the years, and again, it hits all those topics that I just mentioned. But this is a form that we fill out and that the IACUC, the ethics committee can utilize to assure ourselves that we're following the established format that we think is important. We can track the progress of these sorts of things, and over a year's time, we can look at the history of a variance or an exception as it's moved through time, and did we decide that we were going to require post-approval monitoring at some frequency, and it helps us live up to those frequencies. So there's nothing magic about this. I think it's fairly common. It's much like having a

research protocol proposal form; we just happen to have one that we call our variance form or request for exception to the *Guide*.

Slide 59 (Performance Measures)
So, performance measures, I guess now come to the area where there starts to be a little bit of debate. What is a valid performance measure? So what we've decided is that these need to be objective measures. Again, we're calling these clinically relevant. They need to be quantifiable. Whatever the alternative or exception to the recommendation of the *Guide* is that you're considering, you still need to compare it to the *Guide*, so if we're considering an altered cage density than the *Guide* recommendation, we're going to evaluate the impact of that change of density on the animals, at the same time we need to run in parallel the *Guide* recommended practice. So you need to have a control, you need to have your experimental group.

We think it's very important, these measurements need to be practical and collected in the animal room where it would normally take place, and that such studies shouldn't be overly reliant on extensive instrumentation, for example, something like telemetry, blood pressure, body temperature, that sort of thing may be important variables, but by extensive instrumentation of the animals, you're going to materially alter aspects of the animal's behavior and just general physiology that may corrupt your ability to truly evaluate this change and what it means to the animal sitting in its home cage.

And often, typically also means removing those animals from their standard housing environment, and again, possibly inducing another confounding variable, and also significantly limits sample size. If we can set up in a barrier room evaluation of a hundred animals in a hundred different cages, and if we practically and realistically wanted to put telemetry devices in all of those hundred animals, it becomes a very expensive proposition from both the surgical standpoint and the hardware standpoint to accomplish those sorts of studies.

We also decided up front that we weren't going to rely heavily on serum and urine analysis, things like glucocorticoids circulating, or in the urine or in the feces, or complex behavioral assessments that would have to occur outside of the animal room. So again, we're looking at the animals in their setting where they normally would be raised and where the breeding take place, and stock animals would be raised without great reliance on external instrumentation. Now you can certainly supplement, and I'll show you an example in a minute with laboratory measures. For space utilization, for example, we've decided to use 24 hour, 7 day a week videotaping of the animals. That is very difficult to achieve in a functioning barrier room within our setting. So for those studies, we do need to remove the animals from the barrier room and place them in a laboratory sort of setting where we can perform those studies.

Slide 60 (Performance measures)
And of course, whatever you're measuring, the measure of the parameter should be directly related and have relevancy to the alternative or the variance that's being proposed. The further you get away from direct relationships and more toward the indirect, you run a greater and greater possibility that other uncontrolled variables and environmental factors may be impacting those results of that data, and so it's less desirable. An example of this is breeding performance or breeding success. As Joe pointed out earlier, as you start stressing an animal, they're going to start giving up normal behaviors to cope with the stressors, and breeding performance is one of those. And so breeding success, breeding performance should be a measure that is meaningful when looking at varying the environment in the cage setting for the animals. Less so in our case, for example, might be something like tumor burden at two years of age. If our traditional stock animal is provided, shipped to a customer by six to eight weeks of age, and a typical, oh, breeding rat as Joe mentioned, breeding lifetime is six to nine months, looking at a two year measure may not have much relevancy back to the animal room in a production setting. So, again, you need to take some thought and care in designing: What are we measuring and is it directly relevant to the variance that we're proposing?

Slide 61 (Assessing breeding colonies)
So finally, assessing breeding colonies. So if space-related welfare issues are going to be impacted by cage size, we should be able to detect them using quantifiable measures of some sort, and statistically analyzing those measures. And we've broken those down into, and this actually correlates I think fairly well with some of Joe's earlier material, into these different buckets or areas of parameters that would generally address animal welfare, animal well-being. So we look at reproductive indices, behavioral observations, and then some true clinical observations. We think it's important that within these three different larger groups or parameters that multiple sub parameters be measured within each category. So for example, for reproductive indices, we may look at productive indexes, sex ratio of the pups, a weanling survival, but probably multiple indices within each one of these larger categories. And again, we're performing these studies within our standard production setting, so that in this case that's barrier rooms and also some studies in isolators.

And then if equivalency is established, so that we show by changing our stocking density, our cage density, that we have equivalent results to those parameters or space requirements recommended in the *Guide*, it's post-approval monitoring, and again, that is an IACUC call, and if it is monitoring, what key performance indicators should be reviewed and at what frequency?

Slide 62 (Reproductive performance)
So within reproductive performance, there are lots of things you can measure if you have active breeding colonies. Many of these are indices that we routinely collect data on within our commercial production operation because it's important data for our own assessment of the health of the colony, the production of the colony, and it gives us indications if

there's other issues going on in the colony unrelated to the caging setup; so certainly one is production index, and that's the number of pups weaned per female per week. There's published information on different stocks and strains that identify this material. We also have our own in-house developed data from just years of raising these animals. Litter size at birth, survival to weaning, the sex ratio, the weaning weight, the inter-litter interval. So do you routinely have litters every 35 days, every 65 days, every 120 days? That may give you an idea of the reproductive performance of the colony. So those are relatively routinely collected pieces of data. Less routinely and done as true parts of the studies within the rooms now, would be time to first plug, so when are animals becoming sexually active and willing and able to breed? And another measure would be time to female pups' vaginal opening, so when is the female going to be receptive and capable of breeding? And again, those would be indices that could be affected by the environment, by the cage size, perhaps.

Slide 63 (Behavioral assessment)
So within these categories, I think many institutions could perform similar studies. You may not do each of those measures, but selecting a combination of those measures should give you a fairly broad ability to look at each of these larger categories with multiple measures. Behavioral assessment, I think, as Joe pointed out, there are many different ways assess the behavior of mice or rats. We've tried to pick here primarily measures that can be performed in our housing space, by our standard husbandry technicians with training as needed, and I think these are accomplishable in most settings. So aggression and fighting, and that can be maternal aggression toward the pups, as well as aggression between the weanlings and the adults. Hair loss, which is really reflective of barbering primarily, stereotypic behaviors and space utilization within the cage.

Space utilization is actually something that we think is fairly difficult to accomplish in an operating barrier room. And I'll show you a picture in a moment that we've pulled that portion of this evaluation out and done it in a laboratory setting. And then for each of these parameters, we've developed certain training materials to standardize so that we know our staff are each assessing similar items similarly. We've developed scoring sheets for each parameter, and this allows folks with some extra training in the rooms to actually collect the data, so it helps utilize our ability – helps improve our ability to do these studies in multiple rooms throughout our system. And again, I'll show you in a moment that space utilization is probably one that is not easily accomplished [within production rooms]. And then this allows us to quantify the data in comparison across sites or across even the same room under two different caging systems.

Slide 64 (Behavioral assessments)
So this is just an example of two of our behavioral assessments, fighting and aggression and barbering on the lower half here, and it’s a scoring system. So going along with the training on this would be pictures that would help the staff standardize what they would call a zero score, a mild, a moderate or a severe. And it's pretty self-explanatory here, so zero obviously is an animal with no observable wounds, and as you climb up the scale,

you can have slight scratching up to true bite wounds of minimal to greater size, and then that's reflective of a score. Barbering is similar. You can have no barbering, mild barbering with 30% of the pelt removed and then again more and more severe as it climbs up.

Slide 65 (Behavioral Measures)
So there are an awful lot of stereotypic behaviors that have been described in rodents, and we certainly aren't trying to score these animals for every possible stereotypic behavior that has been described. Again, our goal here is to pick some practical measures that can be accomplished within our barrier rooms so these are the four we've come up with. So bar chewing, jumping, pacing, or this repeated walking in a purposeless way in the cage. And then I think what many of us are familiar with is circle, flipping, tail carrying, and again, this would come along with training, video training as necessary so that we know we have consistent evaluation and records from our multiple sites.

Slide 66 (Behavioral measures)
So this is the one – the space utilization – we decided to remove that from the barrier room, and you can see why in this picture, it's very equipment intensive, it's expensive equipment, so we've set up a behavioral suite where we can bring animals in in different size cages and measure up to 32 animals simultaneously and record their actual use of cage space, we can [use both] smaller cages and larger cases, both below *Guide* recommendations, and [provide greater] space than the *Guide* recommends, and follow them over multiple litters, prolonged multi-month sequence of putting breeders in this setting, and we can look at their space utilization. As Joe mentioned, we don't know that simply providing a larger cage changes their behavior in a beneficial way or detrimental way, and one way to get at that is to look at their actual space utilization: How do they use that cage and all the space within that cage?

Slide 67 (Clinical measures)
And then finally, the more traditional clinical sort of measures. And so again, here are some examples, mortality, and within mortality, we break that down within breeding colonies by sex of the animal, by the breeding colony segment, so that would be moms with breeders, breeding pairs, pre-weanlings, weanlings, stock (et cetera), animals that are euthanized for cause. If necessary, we use body conditioning scoring. We're not doing that on a routine basis, but it's certainly a well described process and it has value and utility, if you think it's helpful in your area. And then finally growth curve comparison, so we're looking at growth up through 10 weeks of age. It's a fairly common standard sort of practice within commercial breeding operations, and certainly could be impacted by cage size and just resource availability, so we've included that in our parameters as well.

Slide 68 (Will all stocks, strains and genotypes be evaluated?)
So as we develop these guidelines and we've shared them with folks, some common questions come up, and one of those questions is: Will all stocks, all strains, all genotypes be evaluated? And I think this is a question I've heard from the academic world, and certainly most of us have multiple, we might have dozens to hundreds or thousands of

stocks and strains within our facilities, and do you need to evaluate every single one of them, if you're changing a variable, changing a recommendation such as cage size? We decided very early in the process that the answer is no. It's really not practical or even possible to evaluate every possible stock, strain and genotype that in our case would be commercially produced [~~and in an academic setting that's resonant within the institution~~]. So we're concentrating our efforts on common lines, and some examples down here, the Black 6, CD-1, the CD rat, and we have purposely selected stocks and strains that reflect the majority of the mice and the rats that are being both produced and utilized for research and that form the background strains for many of the genetically modified animals out there. So again, you can't do everything. So we've picked the ones that seem to be most meaningful and most common, and will proceed forward with those.

Slide 69 (Will animals be evaluated at every production site?)
Another question that we've grappled with is: Will animals be evaluated at every production site? We have five barrier sites across North America and three or four other sites that are non-barrier, and do we need to evaluate and perform the same study in the same cages with the same animals in each one of those? And again, we've decided up front that, no, we don't think that if we have a fairly tightly defined environmental and husbandry conditions that are similar across those sites, caging is similar across those sites, that we need to repeat these studies at every single site. We certainly may repeat them at one or two sites to assure ourselves that they're generalizable across more than a single site. But we're not going to repeat every study at five or six sites. But I think we also have to remember that ultimately the IACUC needs to be involved, in their judgment if they think there is an important difference in environmental conditions between the sites then a comparison study may certainly be warranted or required. Our approach now is a couple of rooms at a couple of sites, certainly not all rooms and all sites.

Slide 70 (Post-approval monitoring)
And so ultimately, and again we're right in the middle of these studies and I'll highlight one in a moment, what kind of post-approval monitoring is required? So we have a number of what we call variances to the *Guide* in place right now, and we treat them much like I think most institutions treat their standard research protocol review from an IACUC standpoint. When a variance is approved, [~~we then have – the IACUC requires, and we comply with this~~] we have an annual review of that variance for two consecutive years, so yearly for two years, and we look at key performance indicators that are determined at the time of the approval of the variance. Then, at the three year point, we do what we call a *de novo* review, that is a more exhaustive literature review, and certainly a review of the key performance indicators, and then a decision is made: Do we need new or additional studies based on either literature or key performance indicators before proceeding further with this?

And then superimposed over that is ongoing continual review that's performed by our production management and our veterinary staff. Many of those performance indices that I talked about earlier, those are being generated weekly to monthly across our production

colonies, and they're reviewed because they're important indications of just production – health of the production system in general, and health of the animals, and so those are reviewed, and when problems arise, they're brought to the attention of the appropriate folks which could be production management, it could be veterinarians, it could be both, and depending on the issue, and the size of the issue, we communicate that back to the IACUC if we think that's necessary as well. So we're not just relying on a once a year snapshot; there's a continual review that is superimposed over the system.

Slide 71 (What happens if differences are found?)
And so certainly one of the questions you have to ask is: What happens if you do find differences? You're going to evaluate two different housing systems or housing conditions. My guess is at some point you're going to find a difference. If you look at 20 or 30 parameters, one, two or 10 of them may be altered, and then I think your response really is going to have to depend on what you're finding. Are the differences physiologically and behaviorally relevant? Are there single or multiple parameters within those buckets of behavior, clinical observation or reproductive performance that are altered?

It seems unlikely that all findings will be in agreement between *Guide* recommendations and alternatives. So certainly, we're going to see differences, and just like the rest of science, it's how we decide to now deal with those differences and are they important, are they not important? If those differences are important and the detrimental affects to animal welfare are truly substantiated, there should be differences in more than a single parameter, so hopefully you've built your study where you have additional parameters looking at that similar larger bucket. You know, reproductive performance is a good one. Or if not, you may need future studies or additional studies to try to delineate: Is it a problem that is arising due to the housing condition or is it something that is perhaps being driven by another environmental factor?

And ultimately, I think you need to somehow correlate changes that you've identified with some kind of reasonable mechanism of action. If you can't associate it with mechanism of action, it's difficult to say it's important or being driven by the variable that you modified, and I think sometimes as a community, we don't always go to that next step.

Slide 72 (Post-approval monitoring)
And of course, any significant departures need to be investigated by the appropriate folks, so just because there's a significant departure – we found a difference, doesn't necessarily mean it's due to the parameter we have under study. And you need to get the right people looking at it, so if it's a clinical issue, you get the attending veterinarian involved; the production staff if it's a production issue, or both; and certainly the IACUC. And so just like any other health issue or adverse outcome requires some degree of investigation. I don't think you stop simply by looking at the data and saying, yes, it was just as we expected, or no, there is a problem, we better stop. And a good example is decreased reproductive performance. If we see a difference between caging system or cage density, I should say, it may be due to the caging. It may also be due to other medical issues and

infectious issues. There are other reasons productive performance can drop in the animals. So we have to remember the broader scheme of things, the broader picture. These are whole animal systems that can be affected by variables outside of the one that we're attempting to study at the moment.

Slide 73 (Current studies: Cage space and breeding performance)
And this is just to give you a little snapshot of where we are [investigating] at the moment. We have a number of studies both underway and planned looking at cage space and breeding performance. The one that is the farthest along at the moment [involves] rats. [We are looking] at a number of breeding pairs. Two lines of rats, an inbred and an outbred. We're looking at four commercially available cage sizes. A small cage, smaller than our current cage used at Charles River, our current Charles River cage, the *Guide* standard, and then a larger cage. And our approach is what I just described. We're looking at three broad categories, breeding performance, behavioral performance or assessment, and clinical assessment, and multiple indices within each one of these. Within behavioral assessment, we're also doing space utilization studies with 24 hour videotapes of these animals for three months, so several litter intervals as they're breeding. The videography portion, the space utilization portion of the study has now ceased and there's someone that's going to spend much of the next month reviewing that video footage. And we've now moved to the barrier room for the next stage of this study where we're [housing breeding pairs] in these four different cage arrangements, and we will follow the similar performance measures out over the breeding life of these animals. So the study that's [furthest] underway at the moment or [maybe] halfway to completion is a study involving rats. Mouse studies are planned to start here in the very near future.

Slide 74 (Summary)
So in summary, our goal is to evaluate the equivalency of different cage densities or cage sizes utilizing clinically relevant and statistically based measures: Is one cage density, is one cage size truly more beneficial than another? We're looking at that through multiple assessment criteria that I've talked about before. We're limiting that to representative lines. We can't certainly look at every single line that is commercially produced, and our IACUC is involved from the beginning of this, the original idea and proposal and certainly will be involved with the review of the data, and any recommendations going forward.

Slide 75 (Thank you)
So with that, I think Joe and I are both available to answer questions.

>> Dr. Collins: Okay, Guy, thank you very much. You, as did Joe, have provided us with a lot of reasons to give a lot of thought to things that are happening in our facilities at this point in time. I would like to remind all of our participants that we would encourage you to submit your questions to us, using the question box in your participating box.

Slide 76 (Upcoming OLAW Online Webinars)

Slide 77 (Questions?)
We're going to start with some questions that we received prior to the session and then we will move on to questions that you send in now, so again, please do send us some. What I'll do is read the questions and then ask that one of our speakers respond, and then I'll ask to see if either of the other two speakers have any additional comments to make.

And the first question: **[1] Is negative pressure or positive pressure recommended for IVCs (individually ventilated caging) when biohazardous and/or chemical hazards are present in the animals housed in the caging?**
>> Dr. Wolff: Well, I can take that. That's really dependent on the agent that's being studied. I mean, if you're trying to keep something in the cage, you want negative pressure, but if it's toxic and building up, then you need to have it vented out. This is the kind of question that really needs to be taken to your institutional biosafety committee, work in conjunction with the experts there and the IACUC, and it's risk-based and dependent on the type of study and what the ultimate aim is as to whether you want the substance building up in there or trying to get it out or prevent it from coming out into the room.
>> Dr. Collins: Joe, or Guy, any other comments?
>> Dr. Garner: I couldn't have thought of a better answer myself.
>> Dr. Collins: Okay.
>> Dr. Mulder: I'm in full agreement.

>> Dr. Collins: Next question: **[2] What specific parameters should we consider when looking at performances standards for rodent density and cage changing frequency? If no health issues are noted, are institutions expected to monitor in-cage ammonia levels? If we should monitor ammonia levels, then at what frequency?** Sounds like it's addressing a lot of the materials that, Guy, you especially provided as far as, you know, not only when to measure, but what to measure as well.
>> Dr. Mulder: Yeah, and there's really no easy answer to that. The framework that we developed and when I say "we", I should back up a little bit, it was also developed in conjunction with a group called the Laboratory Animal Breeders Association, which represents the larger commercial breeders primarily of rodents but also other animals in the U.S., and we got together to talk about how might we all kind of head in a similar direction of complying with the recommendations of the *Guide*. And this development, this framework was really meant to apply to any variance that would be considered within our vivaria. So I think for changing frequency, one of the questions was: Is ammonia a valid measure? It certainly may be. It's going to depend much on your setting, your ventilation rates, your caging system. With density, we decided not to look at ammonia, for example, because we have open top cages. They're changed weekly or when they're excessively soiled, and we actually do perform ammonia monitoring at the room level, and so we have data to at least indicate and provide comfort to us that it doesn't seem to be an issue in our setting, with our caging, with our animals. But I think if you're looking at change frequency, what are the parameters that are affected by longer change cycles, and certainly, health is one of them, but perhaps bacterial load within the cage, and that is

difficult to assess. Ammonia monitoring may be difficult as well. I'm not sure there's one clean answer other than I think you should pull together the resources at your organization, identify what seems to be important and achievable in your setting and outline a study.

>> Dr. Collins: It sounds like you're saying performance based standards as determined by the local folks, the IACUC, and the folks working with them?
>> Dr. Mulder: Right.
>> Dr. Collins: Okay. Other comments?
>> Dr. Mulder: Sounds like the *Guide* is right in line with that.

>> Dr. Garner: Well, I'll definitely pitch in on this. So the ammonia has been something that a lot of the papers in the literature have focused on which is, I think, probably why this question is being asked, and those papers are pretty inconsistent. One that I would be very critical of is the use of the human OSHA limit of 25 parts per million, which was used in a lot of the early papers as some magic bullet number at which point we think that the animals have too much or too little ammonia, or not too little, but sorry, I really messed up explaining that. But if it goes over 25 parts per million, that the animals are automatically in a situation of bad welfare.

If you think about it, the mouse is a burrowing animal, and burrowing animals are generally designed to detect poisonous, noxious, biogenic gasses such as carbon dioxide and ammonia. And so when you really get into the biology of it, I think it's crazy to think that the level at which we would worry about ammonia for, you know, a six foot high, upright, savannah dwelling ape, (i.e., a human), would be the same as for a three inch long mouse that lives in a burrow; it just doesn't make any sense. And in fact when this work came out, I spent a lot of time talking to a friend of mine, who is a pathologist, about this. And he actually dug back into literature with me, a very long time, and it turns out that really, if you look at pretty much any study of atmospheric ammonia in rodents, it's very, very hard to find any study which found a level of ammonia of which there wasn't some kind of impairment.

So I think that is a real warning that first of all, ammonia is detrimental to animals [mice] at pretty much any concentration, it's just more detrimental at higher concentrations. And secondly that really underlies the point that Guy was trying to make, that this may not just be a very useful way of assessing the welfare of the animal. Sure, if ammonia is really high, then that's a bad thing, but there are probably much more useful things that we can be looking at. And so very obviously, you can look, for instance, for signs of injury, for signs of fighting. As Guy suggested, the bacterial load in the bedding is a very clever thing to look at, especially if you're looking at mice which may be urinating a lot. And another thing to bear in mind is that when you're ventilating a cage, it's a little like you're brewing alcohol and you're constantly pulling some of the alcohol off to try to fortify the wine. Ammonia is produced by a fermentation process, so if you're ventilating the cage, you're actually drawing off the ammonia that would otherwise be slowing down the ammonia

production of the bacteria that are in the bedding. So there's at least a couple of talks I've seen in the last couple of years which seem to suggest that actually ventilation may actually increase the total ammonia output of the cage. So this is a complicated issue and I do not think ammonia is a good way of looking at it. I hope that is a helpful answer. Not just a technical nerdy one.

>> Dr. Collins: Okay. Moving on to our next one. And Joe, this probably fits into your statements about realizing that in fact the recommendations for size in the *Guide* are not to be considered to be additive. The question is: **[3] The tables in Chapter 3 of the *Guide* list the "recommended minimum space". If an institution can demonstrate that greater than five 25 g mice can inhabit comfortably in a "standard" mouse cage of 75 sq. in, would this be allowed? Such a demonstration could include assuring ammonia levels are below the level known to cause irritation,** back to your comments just now, **maintaining breeding success compared with lower densities, and lack of an increase in adverse clinical conditions compared with lower densities.**
>> Dr. Garner: So I'm very thankful this question was asked because I could not have built a better strawman myself. This absolutely illustrates beautifully and perfectly the fundamental flaw in logic that is in so many of these papers. And that is if I don't see a difference, no difference exists. It may be that you just didn't ask the mice a question that they care about. So remember that a shoe box cage is, at the absolute most generous we can consider it, 40 times less than the smallest amount of space a mouse would occupy in the wild. And in that situation, you would have one mouse that was a territory holder and it would beat the living daylights out of anybody [mouse] that came near it. So to think that small changes in cage density are really going to have a large effect doesn't make any sense. If you look at the farm animal literature where this has been much better worked out, and the standard approach is to actually increase space until you see a benefit and that gives you a yardstick.

So if you wanted me to design a study that would find no effect of halving the amount of space that we give mice, I could do that for you very easily. I would use the wrong measures, as is very clear in many of the earlier studies, and I would use a underpowered study with a poor experimental design and far too few animals, and I wouldn't find a significant difference. So again, this question beautifully illustrates the statistical point that Guy made that you've got to know what you're doing statistically, and the problem is that failing to find the difference statistically doesn't mean that no difference exists; it just means you didn't have enough power to detect it. And so whoever asked this question, I'm not picking on you, I'm saying thank you for illustrating a whole bunch of very connected points and this is just a word of caution.

I think that the thing that comes out of the much better literature that's come out in the last few years, than the stuff that came out in the early to mid '90s, is that if we do decrease space allowances beyond what's in the *Guide* right now, we pretty consistently see impairments. So I think we've got good evidence that we shouldn't decrease it

anymore. I don't think we have good evidence as to whether or not we should increase it to something else. And I think Guy probably can say something to this as well.

>> Dr. Collins: Guy? Okay, Axel, do you have any comments?
>> Dr. Mulder: I muted and forgot to unmute.
>> Dr. Collins: Go ahead, Guy.
>> Dr. Mulder: What I liked about that question, in addition to Joe's comments, was they made a point of saying right up front that we can't just look at our current practices and say because we see no harm, it's fine. You need to compare it against the *Guide* recommendation or against whatever you're evaluating against, and there are groups out there that are relying on historical data, and I don't think we should do that. I think we need to look at what we're doing or what we're proposing to do, and what is recommended, so you really do need a well controlled study, and as Joe points out, statistically valid in the rest of it.

>> Dr. Wolff: And as you had mentioned in your talk, those other items in the *Guide* that are supposed to be taken into account when determining the cage densities, are health, reproduction, growth, behavior, activity and use of space. And other things to consider are the litter sizes, whether multiple litters, I mean, I know they're talking about single mice here but when you're talking about putting more and more in, you have to determine that based on the strain, the size, the difference, and the age of the pups of different litters, growth rate, need for cross fostering and all these other factors as well. You can't just put it on just numbers as such.

>> Dr. Garner: And I think another important point here, especially thinking about things that this questioner seems to be looking at, is to bear in mind that the more mice you put in a cage, the more urine and feces is produced per day. So the more mice in a cage, the more frequent your cage changes occur. And so you actually have two, even if all you care about is economics, you have two different competing effects here. Sure, I can put more mice in a cage and get more mice for my *per diem*, but actually my *per diem* becomes more costly to the institution because I'm having to change the cage more often. And so that I think – and again if we look at the better literature or we look critically at some of this older literature, then we do see that there is somewhat of a linear effect on these more environmental parameters that are probably related to urine and fecal output, which then obviously directly affects how often you can clean the cage. So I'm very resistant to the idea of trying to put more than five mice in a cage. And I'm resistant to it as well because my bet is that once you start doing that – in the wild, mice live in small groups and only a small population of the mice lives in small groups. The rest don't have any space to live in and they live in this sort of no man's land – between all the others – the mice actually hold real territories, and there's an enormous physiological difference between those individuals, which are very immune suppressed, extremely stressed, distressed animals, versus the ones that actually hold a territory. And my concern would be if that group size gets too big, the mice are going to begin to think that they are sort of these no man's land mice and it's going to impact them. So again, we don't have any

good data on that, but my gut feeling would be quite resistant to moving to larger group sizes in a standard shoe box.

>> Dr. Collins: Okay. It looks like we have maybe about 10 or 11 questions left, so your comments are all great, but if we can maybe cut them a little bit shorter, we can maybe get to more of the questions here. The next one: **[4] Our IACUC has decided that adult male mice are not social animals and allows them to be singly housed routinely. Is that okay?**
>> Dr. Garner: Well, it's wrong. [laughter] I mean it's factually wrong, I mean – I'm not sure. Adult male mice are social in many, many, many different ways. There's an enormous literature on it. I suggest that their IACUC is introduced to the work of Peter Crowcroft. There's a wonderful book of his documenting ten years of work watching mice. He's the first person to look at wild mouse behavior. It's called “Mice All Over”. That book should be given to the IACUC and they should change that. [laughter] Now, whether or not it's okay, the issue again comes down to performance standards, right? If the real issue is, wow, this is a facility where they have mice that tend to fight lots as males, such as CD-1s, then it might make sense to have a standard operating procedure where you have a lot of enrichment to make up for the lack of the social complexity. And obviously by the way, you know, if you have singly housed mice, you can give them shelters because there's no other mice to fight with, then that would be great. But by and large, I would instead try and troubleshoot what's going on in the production system that is leading to whatever issues are making people think that they should be housed singly, which is probably aggression and something that Guy and I are both working on a lot right now.

>> Dr. Collins: Okay. Our next question: **[5] Our IACUC and veterinary staff don't want to document performance standards for rodent housing and prefer to simply use the *Guide* space recommendations. Is that okay?** Axel, maybe that's for you.
>> Dr. Wolff: The basic answer is yes. As far as space is concerned, yes, of course if the *Guide* says this is what the expectation is, then going along with that expectation is acceptable.

>> Dr. Collins: Okay, our next one: **[6] Our animal program has documented acceptable performance standards for rodent housing that are different than the minimum space requirements in the *Guide*. How often does the IACUC have to review those standards and reapprove them?**
>> Dr. Wolff: Well, I would agree with what Guy had said the way they're doing it. Any established performance standards or standard operating procedures should, as a rule of thumb, be reassessed at least once every three years. I know they were doing some annual evaluation, but a *de novo* review of these types of established standards at the three year interval is what we usually find acceptable.

>> Dr. Collins: Okay, and our next one, again, is sort of a question of if things are okay: **[7] Our animal program has an IACUC approved exception for cage sanitation intervals for rodents with newborn litters that sometimes means cages are not changed out for as long as 10 days, instead of the usual once weekly. Is that okay?**
>> Dr. Garner: I'll jump in. I think it's not only okay; I think it's commendable. We know from a large, large literature that handling mice early in life has a lot of very detrimental effects. Actually, believe it or not, maternal behavior turns on and turns off particular genes in the animal that changes their personality and their stress physiology for the rest of their lives. So if you want to have a normal animal, you really want to leave those pups alone for as long as possible. And certainly, I spent a lot of time early in my career breeding exotic rodents that were wild-caught in the lab, and if you're dealing with a wild-caught [mouse], or an exotic [species], or if you're dealing with a fragile or a nervous strain, you simply cannot handle them, even beyond ten days without the parents just killing the pups if you change the cage. So I actually think this is a very commendable exception, obviously with all the caveats of which have been properly answered.

>> Dr. Collins: And Joe, another question that just came in while the broadcast was going on, which is relevant, so let me get to it: **[8] Mice with adequate nests are difficult to observe for health checks. With daily health checks, how stressful is it for the mouse if you disturb its nest to visualize each mouse twice a day. Suggestions to minimize this stress?**
>> Dr. Garner: That is almost a plant, that question. Actually one of the main really cool things that came out of doing the work at Charles River with the nesting material is how into the nesting material the technicians got, because once you learn to read mouse, nests you can actually see when animals are about to give birth, you can see when they're sick, from changes in the nest. It's actually easier to see health problems by looking at the nest than it is by looking at the mice. And we're actually producing hard data on this right now, but basically if you think about it, I've made this wonderful lovely, like beautiful home, and you know, I'm going to kick out a really sick or dead individual, all right? So pretty obviously also it's a safe haven, so as you just walk by the cage, you'll see they'll stick their little noses out. So if you want to count the number of animals, you can just count their noses sticking out of the nest. So actually we found we don't need to disturb the animals at all. It actually leads to a reduction in handling, reduction in disturbance of the animals and an improvement in our ability to detect things like pregnancy and birth, but the technicians just need to sort of tune in to what those nests look like but they will get it.

>> Dr. Collins: I'm going to add my own question on top of that, because both you and Guy have provided some I think very interesting information and data that is a value to the community. **[9] Will there be an opportunity for that information, at least portions of the information, to be shared with the community in a way that it's sort of readily available, they know it's there, and they can start implementing some of the practices that you folks are substantiating with your research?**

>> Dr. Garner: Well, for me, that's been the real joy of working with Charles River. I can pootle away and do my research in my lab, but the minute that we're doing it with a real breeder under real breeding conditions in a real breeding facility, it suddenly becomes so much more relevant. And things like this [webinar] as well let us get things out. But the other thing is that Charles River has been incredibly kind in terms of saying, “yeah, the work we do is stuff that we're going to publish in real journals”, and we definitely make a very conscious decision to try and get stuff out in the journals that the community reads, like JAALAS [Journal of the American Association for Laboratory Animal Science] and journals like that.

>> Dr. Mulder: And from Charles River's standpoint, over the years we've amassed a great deal of in house data to support our many, many various practices, and more of that probably should have been published and shared with the research community. So it's been a change of our focus in the last year or two, and our work with Joe is one way that we're illustrating that, that yes, we think this is important data, it's important to us, and I think it's got good, strong relevancy to the research community, so our goal is to try to publish it.

>> Dr. Collins: That is great news. Our next question is a very short question, but I have a feeling it may take up the remainder of our time, because it does sound as if it's something that needs at least some discussion. And it is this: **[10] Is there scientific evidence that trio breeding supports cross fostering to the benefit of pups?**
[laughter]
>> Dr. Mulder: Joe, you want to answer that?
>> Dr. Garner: Actually, we really thought the worst when we started working on this – “we” being me and my collaborators at Charles River. And the answer is: Actually, no, there isn't. There's evidence that they don't in the wild, and so we're actually doing an experiment right now, which is one of the most fun experiments we've ever done, where we're actually looking at individual cross-fostering, and whether trios really do do it. And the answer is: I was wrong, and my friend and collaborator, Kate Pritchett, was also wrong. Kate swore that every single mouse would do it, and I swore that every single mouse wouldn't. And what we're seeing is that rather bizarrely, there are mice that always do it and there are mice that always don't.

So now we're actually trying to figure out what it is about those particular individuals that makes them good cross-fostering moms, and ones that make them antisocial and not good, friendly breeders with a pair mate. So there's a little bit of truth in it, but there's, I think, a lot more to the story. And watch this space; it's actually one of the things we're excited about right now.

>> Dr. Collins: Okay. Any other comments on that? Okay. Moving on to the next question. And let's see, this, Axel, may be one more directed to you: **[11] Is there a specific point in the current OLAW guidance where they clarify that they are not considering the housing space per animal calculations to be additive?**

>> Dr. Wolff: I'll have to go back to one of our prior webinars where we had Dr. Garber, who was on the committee, and she stated that the *Guide* intentionally does not suggest that the minimum space recommendations for other breeding groups are additive. Or that the recommendations for like size group housed rodents be used in determining the space needed for other breeding configurations, so they purposefully did that.
>> Dr. Collins: Okay. So, then, I guess what we're basically saying is that, as we've heard several times today, these numbers should not be considered to be additive; it is performance-based standards that are essential in making a determination as to how to best work with your animals.
>> Dr. Wolff: Exactly.

>> Dr. Collins: Our next question: **[12] With many of the IVC cage racks, the cages tend to be darker in the back of the cages, as they're not receiving light from the sides. Is this beneficial for the dark, back corners?**
>> Dr. Garner: Okay. So if you are lucky enough to have both IVC's and nonventilated static systems, I suggest you go and look at a static room and then you go and run and look at an IVC room. And the thing that you will notice is that in the static room, the mice are doing exactly as you expect, that they are nesting and sleeping at the back of the cage as far away from the lights as they possibly can. If you go to an IVC system, what you will see is that they are nesting as far away from the air inlet as they possibly can. And for a lot of these systems where the air is injected in the back of the cage, that means you're pushing the animals toward the front of the cage. I don't think that's a good thing, right? You're making them choose between light which they don't like, and air and this ventilation which they don't like. And it's really, really obvious – as we have here in our facility, both kinds of caging – you move from one room to the other, the distribution of the animals in the cages will be completely different.

>> Dr. Collins: Okay. Our next question. Joe, this is probably for you: **[13] If we agree that lab mice may have selected against certain traits, isn't there a danger in comparing lab mice *Telos* to wild mouse *Telos*, ie how can you compare a lab mouse's response to area to an animal in Australian grasslands?** [laughter]
>> Dr. Garner: No, I think that is a very valid point, but the problem is we don't know if they have or they haven't, and so that's why we're doing the research. I mean, I'm always delighted when I'm wrong, and it's part of the joy of being a scientist. And I'm especially delighted when I'm wrong and the welfare of the animals is better than I thought it was. But in the absence of any evidence, then the best that we have to go off is the data that we have from the wild animals, but with obviously the huge caveat that we may well have bred some of these behaviors out of them. But as I said, depending, very subtly on how you bred your animals, you may actually have intensified that behavior too.

And the other thing to remember is that this fundamental point I said about mice being extremely phenotypically plastic. And that means that they can have essentially silent areas of the genome, areas of the genome that are never switched on, that are never used for hundreds of generations, because the environment doesn't require it, and a great

example of that is actually nesting material. So when we started working on nesting material, I read like these crazy papers by German behavior people who sat in fields in the '50s and watched mice, and they're describing all of these behaviors I had never seen in ten years of working with mice. The minute we gave naive animals that crinkly paper material, we suddenly saw these behaviors that were described in the '50s and have not been seen or described in the literature since.

And so that's a beautiful example of how sometimes you give the animal the right environmental conditions, and suddenly these behaviors or these pieces of physiology pop out. Just because they've been silent for a really, really long time doesn't mean they're gone. So I think it's a very good point that was raised but it's a point that can only be answered empirically; it's not one that we can answer by sitting in our arm chair and thinking really hard about it.

>> Dr. Collins: Joe, here is probably a follow-on question, although it certainly wasn't designed that way. **[14] Our IACUC has approved environmental enrichment procedures for mice that do not include any "devices that animals climb on or through, perch on, or nest in". It only includes nesting material. Is that okay?**
>> Dr. Garner: I think that if you are operating a very large facility and you have limited labor and you have to pick one thing that you are going to do, then I would absolutely pick nesting material and I would absolutely pick a shredded paper material. There's many different kinds out there. I'm not tied to any one. In fact, you know, as a professor at Stanford, I can't advocate for a particular product and I'm not. And the reason for that is that we sort of see this universal benefit. We see that if animals don't need it, they don't use it. And we don't see any of the problems that sometimes crop up with other enrichments, so the shelter or climbing enrichments provide ambush sites, and some mice use them and don't become aggressive. And some mice become incredibly aggressive, so it requires you to add an additional level of monitoring that you wouldn't need if you were just using nesting materials. So I think this is fine, I think it's very understandable and I think if you're going to do one thing, then you should be getting nesting materials.

>> Dr. Wolff: Although some strains I think people have reported irritation with some nesting materials in some of the nudes, right?
>> Dr. Garner: That is correct. That is actually something that we've looked for with this shredded paper material and we look for it in conjunction with Charles River and a very large number of animals, we never saw it. The irritation with nesting material is the cotton products.
>> Dr. Wolff: Okay.
>> Dr. Garner: Another reason why I don't recommend them.
>> Dr. Wolff: Uh-huh.
>> Dr. Mulder: And that brings up a good point: If you're going to introduce a common material to lots of your cages or many stocks and strains, test it first. So as Joe pointed out, before we started investigating, or as part of our investigation of nesting material for nude mice or hairless mice, that was one of our concerns. We don't want to cause ocular

irritation, and the finer particulate cotton sort of nestlets lead to ocular irritation, and this crinkly paper material they handle just fine.

>> Dr. Collins: Okay. As always we have more questions than time. We're going to try to get to one or two more at least, and I'm trying to pick through the list here to find ones that may be of particular interest to the largest community. First of all, this one says: **[15] Fantastic presentation**, and I will concur, you guys are doing a great job here. And then, Guy: **Do you think it's important to transfer most or all of the nest at each cage change or let the mice start over?** I guess this is both really Guy and Joe.
>> Dr. Mulder: Well, how about I answer and then we'll let Joe answer? We're debating that right now, I think there's probably value to it. We're wondering if maybe animals that are more prone to aggression, and certainly aggression following cage change is fairly frequent or fairly common in some strains as you remove all their – all their cues, all their scent cues in a cage, and we're wondering if you do transfer a portion of that nest into the next cage can you abate some of that post cage cleaning aggression. We actually have that identified as one thing we want to evaluate coming into the fall, so I don't think we know the clear answer yet. I like the idea, a lot of that nest is not wet, it's not overly soiled, and I think there's probably benefit to the animal, but I don't know that we've quantified that benefit and I'm sure Joe has an opinion.

>> Dr. Garner: Well, very, very quickly, there's a lovely study out of Vera Baumans' lab in the early '90s, I believe Pascalle van Loo is the first author, where they did exactly that and what they found is actually exactly what you would expect, which is that there's no benefit to transferring bedding from outside of the nest site, but if you transfer bedding from the nest site where nice leave friendlier, essentially, chemical cues, rather than competitive chemical cues which they leave in the rest of the cage, then you massively reduce aggression in high aggression male strains. The caveat to that is it's one very small study. It definitely needs to be replicated on an industrial scale. And the other important caveat to that is that with this sort of crinkly paper nesting material, we see that after about a week to ten days it begins to break down. The mice have used it so much it's not as effective anymore, so you would want to transfer a portion of it and then supplement it with new material.

>> Dr. Collins: Well, I unfortunately do have to call an end to this. I want to thank all three of you for I think a phenomenal presentation or series of presentations, ending with the question: Is "pee mail" really as effective as it could be? [laughter] I want to think all of the folks that participated. We had a very large audience today, as I mentioned, including some folks from out of the country. As always, we are most grateful to you for what you do for the animals at your facilities in order to help to ensure that the animals are well cared for and that good science can continue.

As you see on the last slide, [Slide 76 (Upcoming OLAW Online Webinars)] we have two upcoming webinars, September 13th and December 13th. The topic for the 13th of September is Departures from the *Guide*. The topic for December has not yet been chosen,

so again, we would encourage you to send us your suggestions, and finally, any questions that weren't addressed during the webinar today will be amended to the transcript that will be posted on the Educational Resources page. Thank you all very much. I hope you all have a very pleasant summer.

Additional Submitted Questions Not Addressed During the Webinar

[16] What clinical assessment would you use to assess the effect of cage height on rabbits welfare? If rabbits with erect ears, exhibiting normal posture, do not have the ears fold over due to touching the cage ceiling, the height is acceptable. If the ears fold over, a taller cage is needed.

[17] Please explain why mice "exist to behave" rather than "mice behave to exist". When I say animals “exist to behave” I mean that behavior is what makes an animal an animal, it is fundamental to, and unremovable from, the nature of animals. It’s a philosophical and biological point – an animal could not exist that did not behave. The converse is true too – animals do “behave to exist” – when animals cannot perform essential behaviors they die.

[18] Is nest structure modified as extremes of "stress" zone are reached? Yes, as the ambient temperature changes the nest structure changes. **At what temperature is affiliative behavior enhanced?** There’s a couple of papers coming out on this right now. The bottom line is that at 25°C or above you really see a lot of fighting – which is not quite an answer to the question, I realize, but it’s the best we have right now! This is also strain dependent. We’re seeing some evidence that C57BL/6 may have a rather different thermal set point than other strains.

[19] Because we work in LPS research, we noticed measurable differences in endotoxin levels in various nesting materials, with nestlets being very high. Endotoxin could confound our research. Has any consideration been given to this issue? This is obviously a concern. What I would say is that every lab will have different concerns, so it really does fall on individual labs to watch out for confounds like this – but please make sure you publish this kind of information. I’d also really stress that I don’t think it is fair to criticize enrichment for issues like this, without being honest about the huge changes we make in husbandry on a regular basis (like implementing IVC systems) without batting an eyelid. As a community I wish we were as hyper vigilant and critical of things like cage ventilation and diet formulation as we are about enrichment.

[20] We've always single-housed breeder males to avoid fighting. Is this not standard elsewhere? It’s received wisdom that once males have bred they are more territorial. This certainly makes sense, but I’m not sure if anyone has shown it. In response to the question, I’d suggested permanently housing males with females. There’s a lot of benefits to this (like conceiving on the induced ovulation at birth, which halves your inter-litter interval).

[21] If you consider that restricted enrichment and nesting type, ventilated high volume housing, etc., have become the norm, how can you consider changes to these conditions at a research institution where the investigators have been collecting data for years under the current housing conditions? The simple answer is because we owe it to the animals to do the research right. We owe it to the human patients to do the research right. We owe it to the funding agencies to do the research right. This “historical control” counter argument is a standard “talking point” response, and it drives me crazy, because it is fundamentally unscientific, and antithetical to the whole justification for working with animals. I’m sorry to be so blunt, I wrote a much longer more measured answer initially, but this point needs to be made very strongly, and very bluntly – because it is not bad science it is junk science to choose reliability over validity. Phrenology (predicting personality from skull shape) is junk science, but it is very reliable (the bumps on my head are always in the same place). If a model only works in a highly artificial environment where the animal’s physiology is fundamentally abnormal, then it is very unlikely that it is of any real use to understanding the human illness.

Let me say this another way – if we know that the environment in a past experiment lead to a false positive (for example that an anti-obesity drug only actually works in animals that are cold and laying down extra fat), why on earth would we want to continue that mistake just for the sake of a consistent result? At the end of the day this is the ultimate position the “historical control” argument lead to, and it is clearly a terrible idea.

Let’s be honest – even if the model does hold up across variable environments (which would be a good thing) this kind of argument is a complete fiction. There’s rarely a historical control in real biological science. As a journal editor and a reviewer I can tell you that no decent publication would ever accept a historical control (i.e., data from control animals from a couple of years ago).

Remember that these changes (e.g., from static to IVC systems) were made in the first place, so there’s no real historical control anyway. At the end of the day if you want to make this argument, then you are saying that somehow economically driven changes enjoy a special status where their impact on scientific quality is irrelevant. This is clearly unacceptable.