Monitoring For Humane Endpoints: Developing An Appropriate Strategy

OLAW Online Seminar
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MONITORING FOR HUMANE ENDPOINTS: DEVELOPING AN APPROPRIATE STRATEGY

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OBJECTIVES

- Definition of endpoints
- Assessment of pain and chronic distress
- Development of humane endpoints
- Case studies
DEFINITION OF ENDPOINTS
STUDY ENDPOINTS

Established at the beginning of the study.

Desired experimental outcomes and expected times of data collection.
STUDY ENDPOINTS: TOXICITY TESTING

Day 0
Give test compound

Day 7-90
Weekly measure of BUN & Creatinine

Day 90
Euthanize for tissue collection
STUDY ENDPOINTS: TUMOR STUDY

Day 0: Inject tumor cells

Day 7-90: Weekly measure of tumor size

Day 90: Study ends 3 months after injection
**STUDY ENDPOINTS: BEHAVIORAL TESTING**

- **Day 0**: Start training*
  - *includes fasting

- **Day 1-15**: Continue daily behavioral assessment

- **Day 16**: Study ends
Day 0
Induce MS (experimental autoimmune encephalopathy, aka EAE)

Day 1-30
Daily treatment with proposed therapeutic agent

Day 30
Animals euthanized for tissue collection and histology
The criteria that are used to determine when to terminate the study for an individual animal (or cohort of animals) before the defined experimental endpoint for humane reasons.
HUMANE ENDPOINTS, CONTINUED

Does not always mean euthanasia – can mean terminating a painful procedure and/or giving treatment to alleviate pain and/or distress.
Refinement
- Minimize pain and/or distress

Replacement
- Non-animal models
- “Less sentient” animal models

Reduction
- Appropriate animal number use

R.L. Burch and W.M.S. Russell
**HUMANE ENDPOINTS: FIVE FREEDOMS**

- Freedom from hunger or thirst
- Freedom from discomfort
- Freedom from pain, injury or disease
- Freedom to express (most) normal behavior
- Freedom from fear and distress

Brambell Report, 1965
**Generic Humane Endpoints**

- Weight loss
- Inability to ambulate
- Labored respiration
- Dehydration
- Hunched posture
- Poor coat (piloerection)
- Wounds or hair loss
- Ocular or respiratory discharge
- Inability to access food or water
WHAT TOOLS DO WE HAVE TO BE MORE OBJECTIVE?
HOW DO WE MEASURE WELL-BEING?
BASIC HEALTH & FUNCTIONING: GROWTH

![Graph showing growth of body weight over age in weeks, comparing different genotypes.](image)

- **+/+**
- **-/- Tg**
BASIC HEALTH & FUNCTIONING: PHYSIOLOGY

Fight or Flight Response

- saliva flow decreases
- eyes pupils dilate
- skin blood vessels constrict; chills & sweating
- lungs quick, deep breathing occurs
- heart beats faster & harder
- bowel food movement slows down
- stomach output of digestive enzymes decreases
- blood vessels blood pressure increases as major vessels dilate
- muscles become more tense; trembling can occur
CLINICAL EXAM

- Temperature, pulse, respiratory rate (TPR)
  - Increase or decrease
  - Expected changes dependent upon model
- Body weight
- Bloodwork
BODY TEMPERATURE

Infrared thermometer  Telemetry transmitter  Infrared thermometer  Rectal thermometer
BODY WEIGHT

- Labor intensive
- Requires specialized equipment
- Assessment of change
  - Age dependent
  - Tumor growth can mask cachexia
BODY CONDITION SCORE: MICE

- Does not require baseline
- Does not require specialized equipment
- Age independent
- Appropriate for many tumor studies
- Available for multiple species

Ullman-Cullere & Foltz 1999
**BODY CONDITION SCORE: RAT**

**BC 1**
**Rat is emaciated**
- Segmentation of vertebral column prominent if not visible.
- Little or no flesh cover over dorsal pelvis. Pins prominent if not visible.
- Segmentation of caudal vertebrae prominent.

**BC 2**
**Rat is under conditioned**
- Segmentation of vertebral column prominent.
- Thin flesh cover over dorsal pelvis, little subcutaneous fat. Pins easily palpable.
- Thin flesh cover over caudal vertebrae, segmentation palpable with slight pressure.

**BC 3**
**Rat is well-conditioned**
- Segmentation of vertebral column easily palpable.
- Moderate subcutaneous fat store over pelvis. Pins easily palpable with slight pressure.
- Moderate fat store around tail base, caudal vertebrae may be palpable but not segmented.

**BC 4**
**Rat is overconditioned**
- Segmentation of vertebral column palpable with slight pressure.
- Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae not palpable.

**BC 5**
**Rat is obese**
- Segmentation of vertebral column palpable with firm pressure; may be a continuous column.
- Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis not palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae not palpable.

Hickman & Swan 2010
BODY CONDITION SCORE: RABBIT

Size-O-Meter Score: 1  2  3  4  5

Very Thin
- Hip bones, ribs and spine are very sharp to the touch
- Loss of muscle and no fat cover
- The rump area curves in

Thin
- Hip bones, ribs and spine are easily felt
- Loss of muscle and very little fat cover
- Rump area is flat

Ideal
- Hip bones, ribs and spine easily felt but are rounded, not sharp
- Ribs feel like a pocket. Pull of penis
- No abdominal bulge
- Rump area is flat

Overweight
- Pressure is needed to feel the ribs, spine and hip bones
- Some fat layers
- The rump is rounded

Obese
- Very hard to feel the spine and hip bones. Ribs can't be felt!
- Tummy bags with obvious fat padding
- Rump bulges out

https://rabbitsrequirerights.com/health/
BODY CONDITION SCORE: COMPANION ANIMALS

1. 20% below ideal body weight
   - Ribs easily felt with no fat cover

2. 10% below ideal body weight
   - Bones raised with minimal tissue between the skin and bone

3. Ideal body weight
   - Ribs can be felt through slight fat cover

4. 10% above ideal body weight
   - Difficult to feel ribs through moderate fat cover. A slightly sagging abdominal fat pad may be seen in cats

5. 20% above ideal body weight
   - Ribs are difficult to feel under thick fat. Cats have a prominent sagging abdominal fat pad
**BODY CONDITION SCORE: MACAQUE**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>EMACIATED</strong> – Very prominent hip bones (easily palpable and likely visible), prominent facial bones, spinous processes and ribs. Minimal to no muscle mass palpable over fat or skin. Areas may be received between spinous processes. Body is angular, no subcutaneous fat layer to smooth out prominences.</td>
</tr>
<tr>
<td>1.5</td>
<td><strong>VERY THIN</strong> – Hips, spinous processes, and ribs are prominent. Facial bones may be prominent. There is very little muscle present over the hips and back. Areas may be received between spinous processes. Body is angular, no subcutaneous fat to smooth out prominences.</td>
</tr>
<tr>
<td>2</td>
<td><strong>THIN</strong> – Very minimal fat reserves, prominent hip bones and spinous processes. Hips, spinous processes and ribs are easily palpable with only a small amount of muscle mass over hips and lumbar region.</td>
</tr>
<tr>
<td>2.5</td>
<td><strong>LEAN</strong> – Overlying muscle gives hips and spine a more firm feel. Hip bones and spinous processes are readily palpable, but not prominent. Body is less angular because there is a thin layer of subcutaneous fat.</td>
</tr>
<tr>
<td>3</td>
<td><strong>OPTIMUM</strong> – Hip bones, ribs and spinous processes are palpable with gentle pressure but generally not visible. Well developed muscle mass and subcutaneous fat layer gives spine and hips smooth but firm feel. No abdominal, subcutaneous or inguinal fat pads.</td>
</tr>
<tr>
<td>3.5</td>
<td><strong>SLIGHTLY OVERWEIGHT</strong> – Hip bones and spinous processes palpable with firm pressure but are not visible. Bone prominences smooth. Rib contours are smooth and only palpable with firm pressure. Small abdominal fat pads may be present.</td>
</tr>
<tr>
<td>4</td>
<td><strong>HEAVY</strong> – Bony contours are smooth and less well defined. Hip bones, spinous processes and ribs may be difficult to palpate due to more abundant subcutaneous fat layer. May have fat deposits starting to accumulate in the axillary, inguinal or abdominal areas.</td>
</tr>
<tr>
<td>4.5</td>
<td><strong>OBESE</strong> – This animal will often have prominent fat pads in the inguinal, axillary or abdominal region. Abdomens will be pendulous when animal sitting or ambulating. Hip bones and spinous processes difficult to palpate. Bony contours smooth and poorly defined.</td>
</tr>
<tr>
<td>5</td>
<td><strong>GROSSLY OBESE</strong> – Obvious, large fat deposits in the abdominal, inguinal and axillary regions. Abdominal palpation is very difficult due to large amount of mesenteric fat. Pronounced fat deposits may alter posture/ambulation. Hip bones, rib contours and spinous processes only palpable with deep palpation.</td>
</tr>
</tbody>
</table>

Summers L, 2012
**BLOODWORK**

- Total number of white blood cells
- Ratio of neutrophils to lymphocytes
  - Evidence of infection
  - Evidence of chronic stress
- Hematocrit (HCT/PCV)
- Hemoglobin
1. Basic Health and Functioning
2. Natural Living
3. Affective States

David Fraser
EXPLORATION OF NATURAL LIVING: STRATEGIES

Natural History
- Burrow
- Build nests
- Forage
- Gnaw
- Social groups

Enrichment Strategies
- Deep bedding
- Nesting materials
- Supplementary diets
- Chewing toys
- Social housing
NESTING MATERIAL INTEGRATION

- Can provide information about mouse behavior

References
- Rock et al 2014
- Yuan et al 2018
- Corder et al 2018
- Oliver et al 2018
QUANTIFIABLE GROOMING

- Use of non-toxic fluorescent powder in mineral oil
- Measure and score time to groom

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
<th>Example Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A strong fluorescent signal is present at the application site on the forehead between the ears</td>
<td>CD1</td>
</tr>
<tr>
<td>2</td>
<td>Fluorescence present at the application site as well as the front and/or rear nails</td>
<td>C57BL6</td>
</tr>
<tr>
<td>3</td>
<td>Fluorescence present at the application site and the ears. Front and/or rear nails may also fluoresce</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Fluorescence is absent from the nails and ears but remains present in trace amounts at the application site</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Fluorescence is no longer detected</td>
<td></td>
</tr>
</tbody>
</table>

Oliver et al 2018
Low-stress control fish (divider present)  
Predator (leaf fish) home tank  
Stressed 'exposed' fish (divider removed)

Social behaviors
Shoaling
Automated behavior recognition

3-point tracking
Tail (T)
Center (C)
Nose (N)

Social interaction: 'approach' (N1 and N2 are very close, heading head-to-head towards each other)
Social investigation: 'follow/chase' (N1 and T2 are very close, heading nose-to-tail in the same direction)
Lack of social interest: (N1 and N2 points are very far, two fish heading in different directions)

Normal children  Autistic children
GRIMACE SCALE: MICE

- Assessment of pain
- Cageside “analgesia”
- Retrospective and requires specialized equipment

https://www.nc3rs.org.uk/grimacescales
GRIMACE SCALE: RAT, RABBIT

Rabbit Pain Face


https://www.nc3rs.org.uk/grimacescales
1. Basic Health and Functioning
2. Natural Living
3. Affective States

DAVID FRASER
AFFECTIVE STATE

- Unrewarded location
- Probe nearest unrewarded location
- Probe halfway
- Probe nearest rewarded location
- Rewarded location
THOU SHALT NEVER PERFORM A WELL-BEING STUDY THAT EVALUATES ONLY ONE COMPONENT
They are sleeping in it – they love it!

They can’t see red, so they feel nice and secure

They seem a little hesitant in the open field – it must cause anxiety

They are demonstrating increase in corticosteroids – it must be stressing them.

They are fighting over it – bad idea!

[insert rat language to describe their opinion]
I don’t see anything that looks like the animal is in pain.

He is sleeping in his nest, he must be just fine.

He is ignoring the nesting material provided – must not be good

Are those eyes squinty or is the mouse just sleeping?

There is a shift in the NE:LY ratio – animal must be stressed out.

[insert mouse language to describe their opinion]
DEVELOPMENT OF HUMANE ENDPOINTS
THEORY OF DEVELOPING HUMANE ENDPOINTS

- What is happening to the animal?
- What is the expected response?
- What kinds of complications can be predicted?
- What specific criteria will be used to determine that it is time to treat?
- What specific criteria will be used to determine that it is time to remove from study (including euthanasia)?

Very study dependent
STUDY ENDPOINTS: TOXICITY TESTING

Day 0: Give test compound
Day 7-90: Weekly measure of BUN & Creatinine
Day 90: Euthanize for tissue collection
TOXICITY TESTING: HUMANE ENDPOINTS

- Body weight/body condition score
- Hydration status
  - Skin tent
  - Blood work
- Renal function
  - Blood work
- Imaging
  - Ultrasound
- Behavior
  - Nest building
  - Grooming
TOXICITY TESTING: OBJECTIVE ENDPOINTS

- Mouse model criteria for euthanasia
  - BCS of 1
  - BUN >45 mg/dL
  - Creatinine >1.2 mg/dL
  - Time to integrate nesting material >15 minutes
### General Health

#### Swimming

<table>
<thead>
<tr>
<th>Level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>normal</td>
</tr>
<tr>
<td>1</td>
<td>intermittent loss of equilibrium</td>
</tr>
<tr>
<td>2</td>
<td>frequent loss of equilibrium</td>
</tr>
<tr>
<td>3</td>
<td>complete loss of equilibrium</td>
</tr>
</tbody>
</table>

#### Body Score (Estimated)

<table>
<thead>
<tr>
<th>Level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>normal</td>
</tr>
<tr>
<td>1</td>
<td>loss of 10-15% BW</td>
</tr>
<tr>
<td>2</td>
<td>loss of 15-20% BW</td>
</tr>
<tr>
<td>3</td>
<td>loss of &gt;20% BW</td>
</tr>
</tbody>
</table>

#### Abnormal abdominal muscle tone

<table>
<thead>
<tr>
<th>Level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>normal</td>
</tr>
<tr>
<td>1</td>
<td>mild</td>
</tr>
<tr>
<td>2</td>
<td>moderate</td>
</tr>
<tr>
<td>3</td>
<td>severe</td>
</tr>
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</table>

#### Abdominal Distension

<table>
<thead>
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<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>normal</td>
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<tr>
<td>1</td>
<td>mild</td>
</tr>
<tr>
<td>2</td>
<td>moderate</td>
</tr>
<tr>
<td>3</td>
<td>severe</td>
</tr>
</tbody>
</table>

### Behaviour

<table>
<thead>
<tr>
<th>Level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>normal</td>
</tr>
<tr>
<td>1-3</td>
<td>all fish at surface gasping for air</td>
</tr>
</tbody>
</table>

0 = normal: no action  
1-4 = moderate changes: should be monitored daily  
5-8 = significant changes: monitor twice daily  
>8 = euthanize
STUDY ENDPOINTS: TUMOR STUDY

Day 0
Inject tumor cells

Day 7-90
Weekly measure of tumor size

Day 90
Study ends 3 months after injection
TUMOR STUDY: HUMANE ENDPOINTS

- Body condition score
  - *Not body weight!*
- Tumor size/ulceration
- Mouse behavior
- Nesting score
STUDY ENDPOINTS: BEHAVIORAL TESTING

Day 0
Start training*
*includes fasting

Day 1-15
Continue daily behavioral assessment

Day 16
Study ends
BEHAVIORAL TESTING: HUMANE ENDPOINTS

- Body weight/body condition score
- Passive Behavior
  - Nesting
  - Grooming
- Active Behavior
Day 0  Induce MS (experimental autoimmune encephalopathy, aka EAE)

Day 1-30  Daily treatment with proposed therapeutic agent

Day 30  Animals euthanized for tissue collection and histology
MULTIPLE SCLEROSIS (EAE): EXPECTED OUTCOMES

Know your model!
- Relapsing/Remitting Model
  - SJL mice
  - Will get very sick, then will improve
- Chronic Model
  - B6 mice
  - Progressively worse over time
**MULTIPLE SCLEROSIS (EAE): HUMANE ENDPOINTS**

- Body weight/body condition score
- Hydration status
  - Skin tent
  - Blood work
- Passive Behavior
  - Nesting
  - Grooming
CONCLUSION

- What is happening to the animal?
- What is the expected response?
- What kinds of complications can be predicted?
- Look at available assessments of well-being to construct appropriate humane endpoints

Very study dependent
QUESTIONS
QUESTION 1

Where can you find guidelines and regulations on humane endpoints?
ANSWER 1

https://www.humane-endpoints.info/en#
QUESTION 2

Are there set humane endpoints like those you described in your talk or can humane endpoints be “customized” depending on the research and animal model?
Customization is necessary and encouraged!
QUESTION 3

I am particularly interested in hearing thoughts on assessing endpoints for monkeys engaged in neuroscience (electrophysiological and behavioral) experiments.

There is a delicate balance between maximizing the information gleaned from any one animal given the extensive behavioral training and preparation that goes into preparing each animal and specific experiments. I am interested in hearing about guidelines for these determinations.
QUESTION 4

What are the principal considerations in developing humane endpoints in any study?
What is happening to the animal?

What is the expected response?

What kinds of complications can be predicted?

What specific criteria will be used to determine that it is time to treat?

What specific criteria will be used to determine that it is time to remove from study (including euthanasia)?
QUESTION 5

Who should be involved in the establishment of species-specific and study-appropriate humane endpoints?
ANSWER 5

- Scientist
- Veterinarian
- IACUC
- Outside subject matter experts
QUESTION 6

At what phase of the study should humane endpoints be clearly defined?
Prior to the start of the study.
QUESTION 7

What are your thoughts about death as an endpoint?
QUESTIONS

Now: Type your questions into the chat box on GoToMeeting dashboard.

Later: email your questions to OLAWDPE@mail.nih.gov
Semiannual Program Review

OLAW Online Seminar
December 13, 2018

Dawn O’Conner and Bill Greer
University of Michigan