Monitoring For Humane Endpoints: Developing An Appropriate Strategy

OLAW Online Seminar
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**OBJECTIVES**

- Definition of endpoints
- Assessment of pain and chronic distress
- Development of humane endpoints
- Case studies
**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

**STUDY ENDPOINTS: MULTIPLE SCLEROSIS**

- Day 0: Induce MS (experimental autoimmune encephalomyelitis, aka EAE)
- Day 1-30: Daily treatment with proposed therapeutic agent
- Day 30: Animals euthanized for tissue collection and histology
**HUMANE ENDPOINTS**

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.

**HUMANE ENDPOINTS: 3RS**

- **Refinement**
  - Minimize pain and/or distress

- **Replacement**
  - Non-animal models
  - "Less sentient" animal models

- **Reduction**
  - Appropriate animal number use
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?

1. Basic Health and Functioning
2. Natural Living
3. Affective States

BASIC HEALTH & FUNCTIONING: GROWTH

body weight (g)

age (weeks)
**BASIC HEALTH & FUNCTIONING: PHYSIOLOGY**

- Temperature, pulse, respiratory rate (TPR)
  - Increase or decrease
  - Expected changes dependent upon model

- Body weight

- Bloodwork

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**CLINICAL EXAM**

- Temperature, pulse, respiratory rate (TPR)
  - Increase or decrease
  - Expected changes dependent upon model

- Body weight

- Bloodwork

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**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-Cullen & Foltz 1999

**BODY CONDITION SCORE: RAT**

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

Hickman & Swan 2010
**BLOODWORK**

- Total number of white blood cells
- Ratio of neutrophils to lymphocytes
- Evidence of infection
- Evidence of chronic stress
- Hematocrit (HCT/PCV)
- Hemoglobin

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**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

ZEBrAfish BEHAVIOR

- AM Stewart 2014
• Assessment of pain
• Cageside “analgesia”
• Retrospective and requires specialized equipment

https://www.nc3rs.org.uk/grimacescales
**Affective State**

- Unrewarded location
- Probe nearest un.rewarded location
- Probe halfway
- Probe nearest rewarded location
- Rewarded location

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**Thou Shalt Never Perform a Well-Being Study That Evaluates Only One Component**
They can't see red, so they feel nice and secure. They are demonstrating increase in corticosteroids – it must be stressing them.

They are fighting over it – bad idea! They are in the open sleeping in field – it must cause anxiety. They seem a little hesitant. They are fighting over it – bad idea! They are in the open sleeping in field – it must cause anxiety.

He is sleeping in his nest, he must be just fine. I don't see anything that looks like the animal is in pain. He is ignoring the nesting material provided – must not be good. Am those eyes squinty or is the mouse just sleeping? There is a shift in the NE:LY ratio – animal must be stressed out.

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

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**STUDY ENDPOINTS: TOXICITY TESTING**

- **Give test compound**
- **Weekly measure of BUN & Creatinine**
- **Euthanize for tissue collection**

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**TOXICITY TESTING: HUMANE ENDPOINTS**

- Body weight/body condition score
- Hydration status
  - Skin test
  - Blood work
- Renal function
  - Blood work
- Imaging
  - Ultrasound
- Behavior
  - Nest building
  - Grooming

[Images of kidney health showing normal and disease conditions]
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

TOXICITY TESTING: ZEBRAFISH

<table>
<thead>
<tr>
<th>General Health</th>
<th>0: normal</th>
<th>1-4: moderate changes: should be monitored daily</th>
<th>&gt; 8: euthanize</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swimming</td>
<td>0: normal</td>
<td>1: intermittent loss of equilibrium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2: frequent loss of equilibrium</td>
<td>3: complete loss of equilibrium</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Body Score (Estimated)</th>
<th>0: normal</th>
<th>1: loss of 10-15% BW</th>
<th>2: loss of 15-20% BW</th>
<th>3: loss of &gt;20% BW</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Abnormal Abdominal Muscle Tone</th>
<th>0: normal</th>
<th>1: mild</th>
<th>2: moderate</th>
<th>3: severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal Distension</td>
<td>0: normal</td>
<td>1: mild</td>
<td>2: moderate</td>
<td>3: severe</td>
</tr>
<tr>
<td>Behaviour</td>
<td>0: normal</td>
<td>1-3: all fish at surface gasping for air</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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**Study Endpoints: Behavioral Testing**

- Start training*
  *includes fasting*
- Continue daily behavioral assessment
- Study ends

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**Behavioral Testing: Humane Endpoints**

- Body weight/body condition score
- Passive Behavior
  - Nesting
  - Grooming
- Active Behavior
**STUDY ENDPOINTS: MULTIPLE SCLEROSIS**

- **Day 0**: Induce MS (experimental autoimmune encephalomyelitis, aka EAE)
- **Day 1-30**: Daily treatment with proposed therapeutic agent
- **Day 30**: Animals euthanized for tissue collection and histology

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**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

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**MULTIPLE SCLEROSIS (EAE): HUMAN 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?

ANSWER 2

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?

ANSWER 4
• 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)?
Who should be involved in the establishment of species-specific and study-appropriate humane endpoints?

Scientist
Veterinarian
IACUC
Outside subject matter experts

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

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