

Monitoring For Humane Endpoints:
Developing An Appropriate Strategy



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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

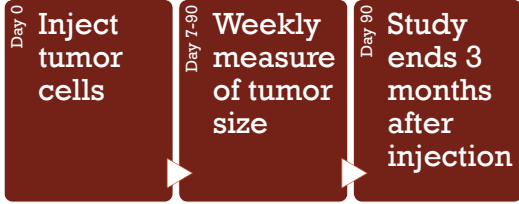
Day 0 Give test compound

Day 7-90 Weekly measure of BUN & Creatinine

Day 90 Euthanize for tissue collection



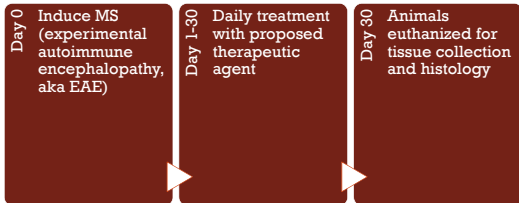
STUDY ENDPOINTS: TUMOR STUDY



STUDY ENDPOINTS: BEHAVIORAL TESTING



STUDY ENDPOINTS: MULTIPLE SCLEROSIS



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



R.L. Burch and W.M.S. Russell

- 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?

16 **HOW DO WE MEASURE WELL-BEING?**

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

DAVID FRASER

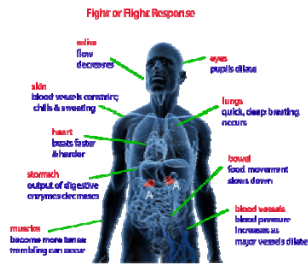
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BASIC HEALTH & FUNCTIONING: GROWTH

Age (weeks)	+/+ (g)	-/- Tg (g)
2	15	15
4	25	22
6	32	28
8	35	30
10	36	32
12	37	34

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BASIC HEALTH & FUNCTIONING: PHYSIOLOGY



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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

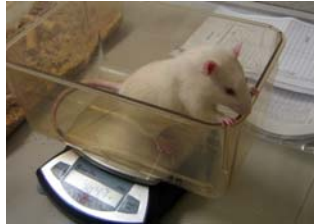


Infrared thermometer Telemetry transmitter Infrared thermometer Rectal thermometer

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BODY WEIGHT

- Labor intensive
- Requires specialized equipment
- Assessment of change
 - Age dependent
 - Tumor growth can mask cachexia



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BODY CONDITION SCORE: MICE

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

BC 1
 Mouse is emaciated.
 - Skeletal structure extremely prominent; little or no flesh cover.
 - Vertebrae distinctly segmented.

BC 2
 Mouse is underconditioned.
 - Segmentation of vertebral column evident.
 - Dorsal pelvic bones are readily palpable.

BC 3
 Mouse is well-conditioned.
 - Vertebrae and dorsal pelvis not prominent palpable with slight pressure.

BC 4
 Mouse is overconditioned.
 - Spine is a continuous column.
 - Vertebrae palpable only with firm pressure.

BC 5
 Mouse is obese.
 - Mouse is smooth and bulky.
 - Bone structure disappears under flesh and subcutaneous fat.

Ullman-Cullere & Foltz 1999

A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e., ...2+, 3, 2-...)

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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. Pits 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. Pits 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 stores over pelvis. Pits easily palpable with slight pressure.
 - Moderate fat stores 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 stores over dorsal pelvis. Pits of pelvis palpable with firm pressure.
 - Thick fat cover 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 stores over dorsal pelvis. Pits of pelvis not palpable with firm pressure.
 - Thick fat cover over tail base, caudal vertebrae not palpable.

Hickman & Swan 2010

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BODY CONDITION SCORE: RABBIT

Score	Appearance	Characteristics
1 Very Thin		Ribs, ribs and spine are very sharp to the touch. Loss of muscle and fat cover. The hump is curved in.
2 Thin		Ribs, ribs, ribs and spine are easily felt. Loss of muscle and very little fat cover. Hump is straight.
3 Ideal		Ribs, ribs, ribs and spine easily felt, but are rounded, not sharp. Ribs feel like a pillow, flat or gentle. No stomach bulge. Hump is straight.
4 Overweight		Pressure is needed to feel the ribs, spine and ribs. Some fat layers. The hump is rounded.
5 Obese		Very hard to feel the spine and ribs. Ribs can't be felt. Fat layer with obvious fat coating. Hump is bulged.

<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		Some ribs with minimal cover between the ribs and spine.
3 Ideal body weight		Ribs can be felt through light 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.

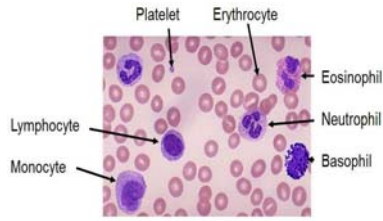
BODY CONDITION SCORE: MACAQUE

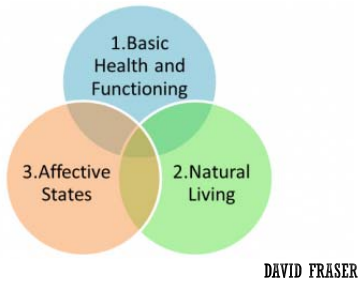
1 EMACIATED		Very emaciated. Ribs, spine and pelvic bones are very sharp to the touch. Loss of muscle and fat cover. The hump is curved in.
1.5		Ribs, ribs, ribs and spine are easily felt. Loss of muscle and very little fat cover. Hump is straight.
2		Ribs, ribs, ribs and spine easily felt, but are rounded, not sharp. Ribs feel like a pillow, flat or gentle. No stomach bulge. Hump is straight.
2.5		Pressure is needed to feel the ribs, spine and ribs. Some fat layers. The hump is rounded.
3 OPTIMAL		Very hard to feel the spine and ribs. Ribs can't be felt. Fat layer with obvious fat coating. Hump is bulged.
3.5 SLIGHTLY OVERWEIGHT		Very hard to feel the spine and ribs. Ribs can't be felt. Fat layer with obvious fat coating. Hump is bulged.
4		Very hard to feel the spine and ribs. Ribs can't be felt. Fat layer with obvious fat coating. Hump is bulged.
4.5		Very hard to feel the spine and ribs. Ribs can't be felt. Fat layer with obvious fat coating. Hump is bulged.
5		Very hard to feel the spine and ribs. Ribs can't be felt. Fat layer with obvious fat coating. Hump is bulged.

Summers I, 2012

BLOODWORK

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





EXPLORATION OF NATURAL LIVING: STRATEGIES

Natural History

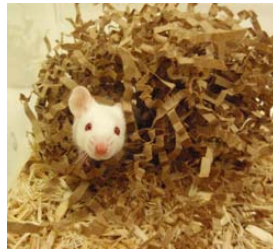
- Burrow
- Build nests
- Forage
- Gnaw
- Social groups

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
 - <https://www.jove.com/video/51012/nest-building-as-an-indicator-of-health-and-welfare-in-laboratory-mice>



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QUANTIFIABLE GROOMING

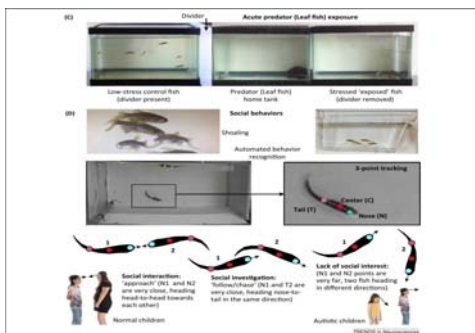
- Use of non-toxic fluorescent powder in mineral oil
- Measure and score time to groom

Score	Description	Example Image
1	A strong fluorescent signal is present at the application site on the forehead between the ears	
2	Fluorescence present at the application site as well as the front and/or ear nails	
3	Fluorescence present at the application site and the ears. Front and/or ear nails may also fluoresce	
4	Fluorescence is absent from the nails and ears but remains present in trace amounts at the application site	
5	Fluorescence is no longer detected	

Oliver et al 2018

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ZEBRAFISH BEHAVIOR

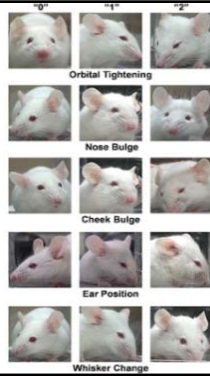


AM Stewart 2014

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GRIMACE SCALE: MICE

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

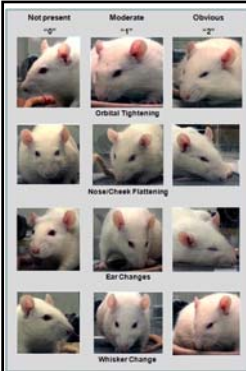


<https://www.nc3rs.org.uk/grimacescales>

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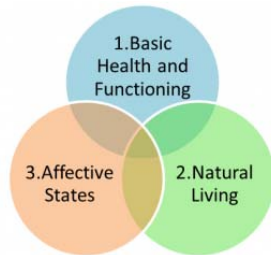
GRIMACE SCALE: RAT, RABBIT

Rabbit Pain Face
• Keating et al, 2012.



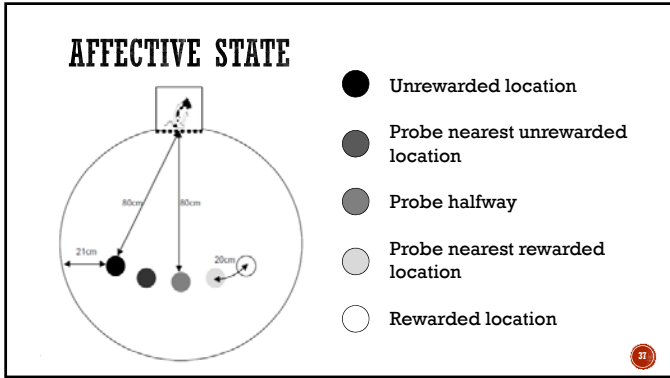
<https://www.nc3rs.org.uk/grimacescales>

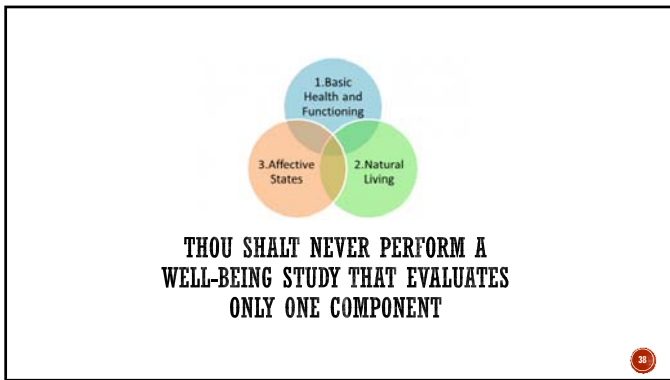
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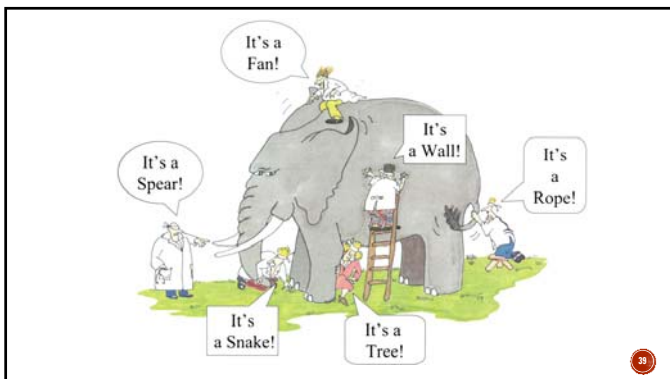


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They can't see red, so they feel nice and secure

They are sleeping in it - they love it!

They are fighting over it - bad idea!

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

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

[insert rat language to describe their opinion]

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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

Are those eyes squinty or is the mouse just sleeping?

[insert mouse language to describe their opinion]

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

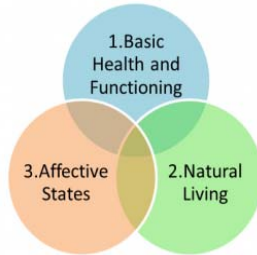
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DEVELOPMENT OF HUMANE ENDPOINTS

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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

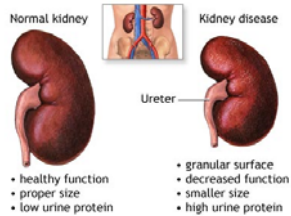


STUDY ENDPOINTS: TOXICITY TESTING



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



TOXICITY TESTING: ZEBRAFISH

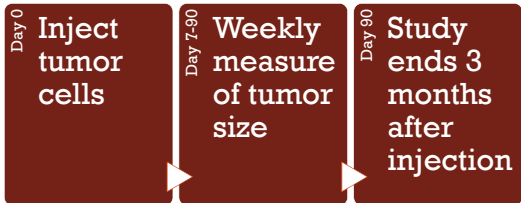
General Health Swimming	0. normal 1. intermittent loss of equilibrium 2. frequent loss of equilibrium 3. complete loss of equilibrium
Body Score (Estimated)	0. normal 1. loss of 10-15% BW 2. loss of 15-20% BW 3. loss of >20% BW
Abnormal abdominal muscle tone	0. normal 1. mild 2. moderate 3. severe
Abdominal Distension	0. normal 1. mild 2. moderate 3. severe
Behaviour	0. normal 1-3. all fish at surface gasping for air

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

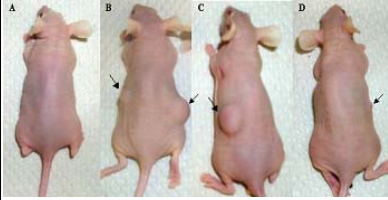
<https://www.humane-endpoints.info>



STUDY ENDPOINTS: TUMOR STUDY



TUMOR STUDY: HUMANE ENDPOINTS



- Body condition score
 - *Not body weight!*
- Tumor size/ulceration
- Mouse behavior
 - Nesting score

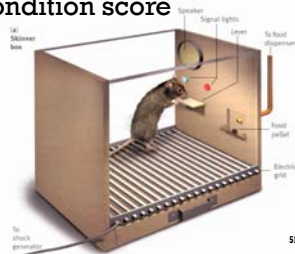


STUDY ENDPOINTS: BEHAVIORAL TESTING

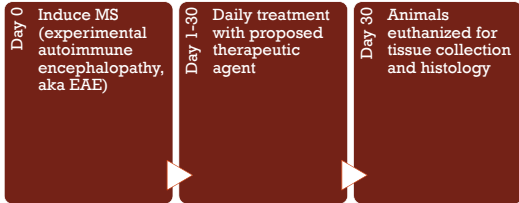


BEHAVIORAL TESTING: HUMANE ENDPOINTS

- Body weight/body condition score
- Passive Behavior
 - Nesting
 - Grooming
- Active Behavior



STUDY ENDPOINTS: MULTIPLE SCLEROSIS

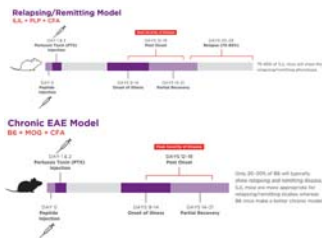


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MULTIPLE SCLEROSIS (EAE): EXPECTED OUTCOMES

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): HUMANE ENDPOINTS

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

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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)?



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?



ANSWER 6

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