Determination of humane end points (HEP) in biomedical experiments
Definition of humane endpoint (and scientific endpoint)

The **earliest** indicator in an animal experiment of (potential) pain and/or distress that, **within the context** of the scientific endpoints to be met, can be used to such **actions** as humane killing or terminating or alleviating the painful or stressful procedure (oncogenicity Vs. effects of therapy)

(Hendriksen C.)

And the outcome…

promote the objective of establishing a scientifically valid answer to the experimental question with a minimum of harm to the animals
What types of humane endpoint can be expected?

• When unexpected pain and/or distress occurs (rectal prolaps, bone fracture, biting wounds, etc.)

• Pain and/or distress were foreseen at the start of the experiment, but are ultimately more severe than anticipated (noxious agent, breast tumor)

• Pain and/or distress are an inherent part of the experiment (challenge experiment, EAE, tumor metastasis)
Why applying humane endpoints?

- **Moral/Social**

- **Legislative** - All experiments shall be designed to avoid distress and unnecessary suffering to the experimental animals

- **Scientific** - circulatory collapse, organ failure, dehydration or starvation, as secondary causes rather than direct consequences of the condition under study.
What kind of humane endpoint parameters are available?

(adapted from Richmond, 1999)
Who is Responsible to apply Humane Endpoints?

• The principle investigator (PI)
• the animal ethics committee (IACUC)
• The veterinarian (animal welfare officer)
• the laboratory animal technician/care taker
• the pathologist

• Assign responsibilities (in advance)
• Contact information (weekends)
Methods to observe and supervise HEP application

• *Attitude and expertise*
  – Teaching
  – Preliminary meetings with the research group
  – Frequent controls
  – Marking cage cards

“only those who are prepared to see will see”
Methods (Cont.)

• Severity levels
1. **Collection of organs** from animals that did not go under any experimental process and were euthanised in an acceptable method for organ collection.

2. Experiments that cause **slight temporary discomfort or stress**. Examples: IV IM IP SC injections, behavioral experiments that do not cause stress (do not include water maze, predator experiments), infliction of slight pain that the animal can avoid, blood sampling from peripheral vessels up to the quantity of blood that does not require anesthesia, feeding experiments that do not cause clinical manifestations, tail tip sampling.

3. Experiments that cause **slight stress or short term pain**. Such experiments **should not cause significant changes in the animal's appearance, in physiologic parameters such as heart rate or respiratory rate or social behaviour**. During and after such experiments animals should not indicate signs of self injury, anorexia, dehydration, anxiety, excessive recumbency, vocalization, over aggressiveness or tendency for isolation. Examples: nonsurvival major surgery, canulations, small survival surgeries, blood withdrawal under anaesthesia from the retro-orbital sinus or from the heart, restraint for short periods, water or food restriction for less than 12 hours a day.

4. Experiments that cause **medium pain or distress that are alleviated by analgesics**. Examples: major survival surgeries where animals receive analgesics, local-non metastatic tumours where animals receive analgesics, restraining animals for over 60 minutes, water or food restriction for over 12 hours during the activity phase of the animal's day, significant change in environmental parameters (temperature, lighting), procedures that cause sensorial or motor damage or severe and constant anatomical and/or physiological changes, the use of CFA-Complete Freund's Adjuvant.

5. Experiments that cause **severe and lasting pain or distress that are non alleviated by analgesics**. Metastatic tumours or experiments which end point is death. In all such experiments the researcher is requested to justify the lack of using analgesics.
C57BL/6J Ola Hsd mice
FEMALE 4-5 WEEKS
10-09-2009

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46914-28999 0394240-466

EK - SPF Main Unit - 7th floor
14-16 gr only
Methods (Cont.)

- Scoring tables
Model Rhematoid Arthritis

Arthritis development can be monitored by a macroscopic scoring system for the four limbs ranging 0-4:
0- Normal;
1- swelling and redness of one joint;
2- two joints involved;
3- more than two joints involved;
4- severe arthritis in the entire paw.

The scores of the four paws were added, yielding a maximum total score of 16 for each rat. Since in most cases the hind limbs are primarily affected, score of 6-8 represent severe arthritis.
# EAE Model

<table>
<thead>
<tr>
<th>Signs/Symptoms</th>
<th>Grade</th>
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<tbody>
<tr>
<td>Normal, absence of any neurological signs</td>
<td>0</td>
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<tr>
<td>Tail weakness, tail limp and droops</td>
<td>1</td>
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<tr>
<td>Hind leg weakness &amp; paresis, wobbly walk and/or hind legs unsteady</td>
<td>2</td>
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<tr>
<td>Hind leg paralysis, when moving animal dragging its hind legs</td>
<td>3</td>
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<tr>
<td>Paraplegia with forelimb weakness</td>
<td>4</td>
</tr>
<tr>
<td>Quadriplegia - Moribund</td>
<td>5</td>
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<tr>
<td>Death</td>
<td>6</td>
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</table>
Methods (Cont.)

- Procedure follow up sheets
Monitoring animals in research – severity level 4-5

Ethic application number ______________________ Expiry date ______________________

Principal Investigator ______________________ Phone ______________________

Authorized researchers and Phone numbers
__________________________________________________________

Experiment start date ______________________ Animal species ______________________

Expected clinical signs _______________________________________

Type and frequency of monitoring _______________________________________

Humane end points ____________________________________________

Remarks ______________________________________________________

<table>
<thead>
<tr>
<th>date</th>
<th>time</th>
<th>Type of monitoring / test</th>
<th>Remarks</th>
<th>name</th>
<th>signature</th>
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Key clinical signs

- Irreversible inability to eat and/or drink
- Rapid or continuous weight loss
- Dehydration
- Generalized decrease in grooming behaviour
- Neurological disorders
- Conditions indicating severe pain, distress or suffering
- Severe or continuing respiratory distress
Conclusions

• Attitude, knowledge and competence obtained through education and training are essential for the continuous refinement of animal experimentation.

• The search for early endpoints in projects and tests must be given a high priority.

• Commitment in both scientific and technical staff is needed to search actively for ways of terminating experiments as early as possible.