Experimental and Humane Endpoints

I. Background

IACUCs must perform a harm-benefit analysis for studies with potential pain or distress, ensuring alignment with the Three Rs (*The Guide* (p.27, 8th ed.).

The AAALAC International FAQs specify how this weighing of study objectives against animal welfare concerns should occur, outlining expectations for IACUCs to perform a formal harm–benefit analysis consistent with the Three Rs:

> This analysis is typically already performed by IACUCs in their reviews of proposed animal studies. AAALAC International expects that IACUC's (or comparable oversight body), as part of the protocol review process, will weigh the potential adverse effects of the study against the potential benefits that are likely to accrue as a result of the research. This analysis should be performed prior to the final approval of the protocol, and should be a primary consideration in the review process.

II. Purpose:

The purpose of this document is to provide criteria for identifying and utilizing the earliest endpoints that are compatible with the scientific objective of research studies while preventing, minimizing, or alleviating any actual or potential pain, distress or discomfort to study animals. This policy applies to all researchers, staff involved in animal care and use, and to the IACUC in its protocol review and post-approval monitoring. Any exceptions or deviations from this policy will be handled on a case by case basis by the IACUC

III. Guidelines

Investigators must identify the earliest scientifically valid endpoints compatible with achieving research objectives while preventing, minimizing, or alleviating animal pain, distress, or discomfort. The IACUC expects that humane endpoints will normally precede severe morbidity (showing clinical signs), moribundity, (an irreversible condition leading to death) or death.

Whenever feasible, investigators should use pilot studies to refine and validate experimental and humane endpoints. Pilot studies may be necessary to refine endpoints, understand disease progression, and determine appropriate animal numbers. Information gained during pilot work should be incorporated into subsequent protocol submissions.

Defining Endpoints:

Endpoints may be **experimental** (when data collection is complete) or **humane** (when animal welfare considerations require removal or euthanasia). Endpoints may occur during prodromal (before the onset of specific clinical signs), morbid, or moribund phases; prodromal or early-morbidity endpoints are preferred whenever scientifically feasible. Species and strain characteristics must be considered when selecting endpoints, particularly in rodents that often mask early signs of illness and therefore require more frequent and well-documented observations.

Studies may involve either acute or chronic progression. Acute models may require intensified monitoring during narrow high-risk periods. Chronic models often rely on gradual indicators such as body condition, weight trends, or tumor progression over days to weeks. Monitoring frequency must be appropriate for the expected progression of the model and clearly described in the protocol.

Observation and Monitoring

Animals must be monitored at a frequency sufficient to detect clinical decline before humane endpoints are exceeded. Documentation of each observation—time, date, observer, and findings—is required. Research staff bear primary responsibility for study-specific monitoring, though husbandry and veterinary personnel may contribute additional observations.

Investigators must ensure adequate monitoring during critical periods, including evenings, weekends, and holidays. Study schedules should avoid initiating procedures immediately before weekends or holidays whenever possible. When after-hours monitoring, euthanasia, or tissue collection may be required, investigators must ensure that supplies, equipment, and coordination plans are in place.

Supportive and Palliative Care

Supportive care measures, such as hydration support or softened food, may be provided in consultation with the veterinary staff. If an animal fails to respond to supportive measures, euthanasia must occur at the IACUC-approved humane endpoint. Supportive care may not be used to delay euthanasia beyond an approved endpoint unless explicitly authorized by the veterinary staff and, when required, the IACUC.

Objective and Surrogate Endpoints

Objective and surrogate endpoints should prioritize measurable, model-specific indicators supported by clearly defined thresholds whenever possible. Common indicators include:

- Physical appearance and clinical signs: hunched posture, dehydration, rough coat, sunken eyes, nasal or ocular discharge.
- Body weight: loss of 10% warrants veterinary consultation; loss of 20% requires euthanasia unless otherwise approved. Weight should be compared to both baseline and age-matched controls where appropriate.
- Body Condition Score (BCS): BCS is especially useful when weight is unreliable (e.g., tumor, ascites, gestational studies). Unless otherwise approved by the IACUC, animals must be euthanized when they reach BCS 2.
- Body temperature/hypothermia: progressive reductions in temperature can serve as validated surrogate endpoints in many models. Thresholds should be supported by literature or pilot data when available.
- Behavior and function: reduced ambulation, failure to access food or water, respiratory distress, or unresponsiveness.

- Tumor progression: for subcutaneous tumors, maximum acceptable size is typically 1.5 cm in mice and 2 cm in rats. Any ulceration, interference with normal activity, or evidence of pain or distress requires euthanasia.

Investigators may propose additional surrogate or biomarker-based endpoints when justified by literature or pilot data. All proposed endpoints must be reviewed and approved by the IACUC.

Unexpected Outcomes

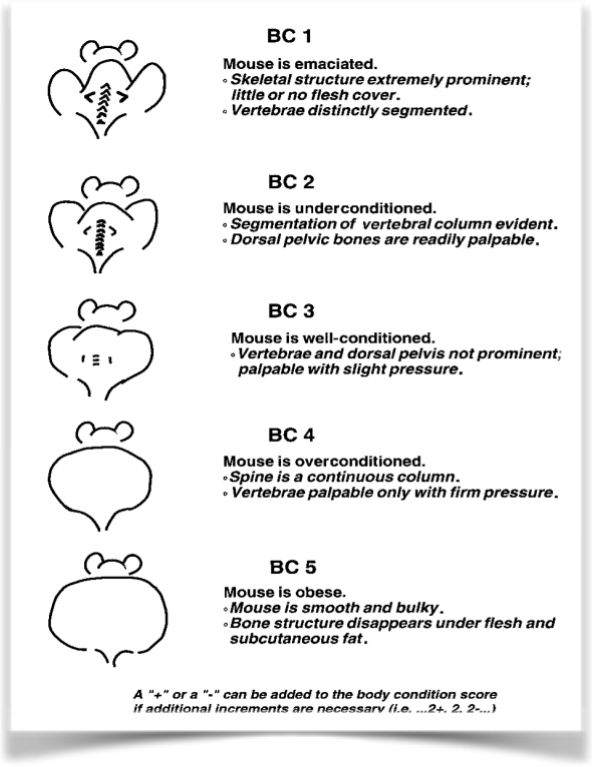
Unexpected morbidity, mortality, or adverse clinical signs must be treated as potential endpoints and prompt immediate reassessment of the study. These events must be reported to the attending veterinarian and, when required, the IACUC. Protocol modifications may be necessary if unexpected patterns emerge.

Communication and Coordination

Effective communication among investigators, veterinary staff, and animal care personnel is essential. Investigators must notify staff in advance of expected critical periods and ensure that plans for euthanasia, tissue collection, carcass identification, and storage are clear. Any deviation from approved endpoint criteria must be documented and reported according to institutional policy.

A visual of body condition score (BCS) provided in Table 1.

Table 1. Body Condition Score (BCS) Drawings and Descriptions



IV. Examples of Experimental and Humane Endpoints

The following examples illustrate commonly used experimental and humane endpoints across a range of research models. They are intended to guide investigators in selecting model-appropriate, objective, and measurable criteria that allow early identification of animals requiring removal from a study or euthanasia. Investigators must propose endpoints that are scientifically justified and supported by literature, pilot data, or veterinary consultation. Whenever an animal meets or exceeds an approved endpoint, prompt action is required.

Body Weight as an Endpoint

Body weight is frequently used as an early and objective indicator of declining health.

- A weight loss of 10% from baseline or age-matched controls requires consultation with the veterinary staff to identify and mitigate underlying causes. Weight loss may reflect both study-related and unrelated factors.
- A weight loss of 20% generally requires euthanasia unless the IACUC has specifically approved an alternative threshold.
- Weight change should be evaluated against both the animal's starting weight and age-matched reference values, particularly in long-term studies or in growing animals. This information is summarized for C57BL/6J mice in Appendix A.
- Certain models may show atypical weight patterns; for example, some sepsis models exhibit weight gain as a more reliable predictor of mortality (Nemzek et al., 2004). In such cases, investigators must provide appropriate justification and thresholds.

Body Temperature and Hypothermia

Hypothermia is a validated surrogate endpoint for a wide range of conditions including infectious disease, sepsis, aging, and tumor progression.

- Progressive decreases in body temperature provide an objective indication of irreversible decline and may precede severe morbidity or death.
- Model-specific thresholds (e.g., absolute temperatures or defined temperature drops from baseline) may be used to trigger euthanasia or intensified monitoring.
- Temperature monitoring devices may include implanted microchips, rectal probes, infrared thermometry, or other validated methods.
- Investigators may incorporate combined indices—such as temperature × body weight—which have shown predictive value in several models.

A summary of published temperature-based endpoints for specific models is provided in Table 2.

Tumor Endpoints

Tumor-bearing animals must be monitored frequently using consistent measurement techniques. Humane endpoints include:

- Tumor diameter up to 1.5 cm in mice or 2.0 cm in rats, unless otherwise justified;
- Tumor ulceration, skin disruption, or necrosis;

- Interference with normal movement, grooming, feeding, drinking, or respiration;
- Any indication that the tumor is causing significant pain, discomfort, or distress.

For tumors developing within body cavities, monitoring must focus on clinical signs relevant to the affected organ systems (e.g., neurological signs for intracranial tumors). Imaging modalities may be used to document progression in non-visible sites.

All tumor cell lines must be assessed for relevant pathogens and authenticated prior to use.

Pulse oximetry
Pulse oximetry provides a rapid and noninvasive measure of oxygen saturation and can serve as an endpoint in models involving respiratory compromise.

- Oxygen saturation values below 95% indicate significant pulmonary dysfunction.
- Serial measurements can provide additional insight into disease trajectory.
- Device placement and handling techniques should be standardized to reduce variability.

Imaging-Based Endpoints
Noninvasive imaging (e.g., radiography, MRI, ultrasound, bioluminescence or fluorescence imaging) can be used to:

- Identify tumor burden and metastatic spread;
- Monitor organ enlargement or dysfunction;
- Detect tissue damage without repeated invasive procedures.

Imaging can provide objective, serial measures that refine endpoints and reduce the number of animals needed.

Biomarkers
Validated biomarkers may serve as early indicators of disease progression and can improve endpoint precision.

- Biomarkers may include hematologic parameters, cytokine levels, metabolic markers, or model-specific analytes.
- Investigators should justify biomarker-based endpoints with supporting literature or pilot data.
- Biomarkers may be used alone or in combination with clinical signs to establish more reliable thresholds.

Table 2. Studies using hypothermia as an experimental or humane endpoint.

Study Type	Temperature (°C)	Device	Reference (PMID)
Aging			
Used a body weight x temperature metric	Temperatures fell by more than 1 °C the final few weeks of life	SQ Microchip	20587157
Used a body weight x temperature metric	Temperatures fell by more than 1 °C the final few weeks of life	SQ Microchip	22776049
Bacteria			
Vibrio vulnificus	≤23.5	IR Thermometer at the sternal base	28629317
P. aeruginosa *S. aureus* *S. epidermidis*	34	Rectal probe	1576081
K. pneumoniae	36	SQ/IP Microchip	9718473
Streptococcus pneumoniae	Temperature x Body Weight < 90% of baseline	SQ Microchip	22330575
Fungal			
Aspergillus fumigatus	29.0	IR Thermometer at the lower abdomen	24326222
Candida albicans	33.3	SQ Microchip	12689423
LPS/Sepsis			
—	<30 or body temperature reduction by >5	Rectal probe	30054760
—	24.3	Noncontact thermometer, perianal	29476115

Study Type	Temperature (°C)	Device	Reference (PMID)
Tumor Progression			
Used a body weight x temperature metric	Hypothermia used to intensify monitoring (1 °C) or euthanasia (>3 °C)	SQ Microchip	22776049
Viral			
Influenza	≤ 32 °C	Rectal probe	9150492

V. References

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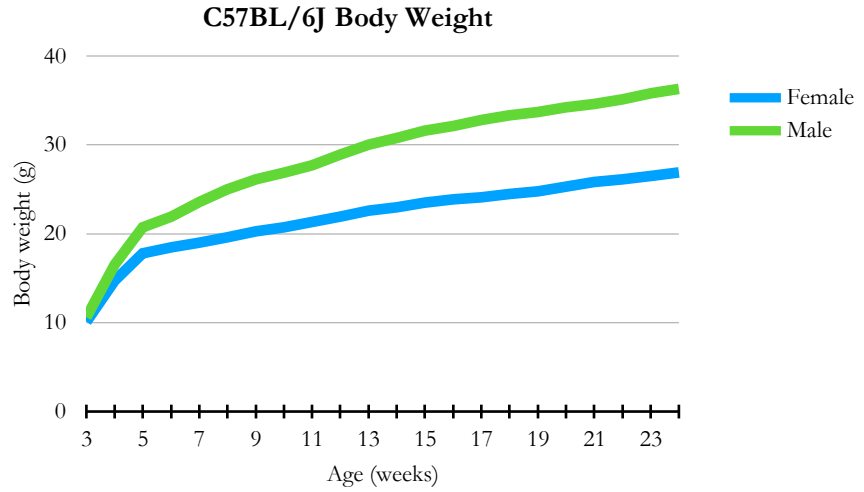
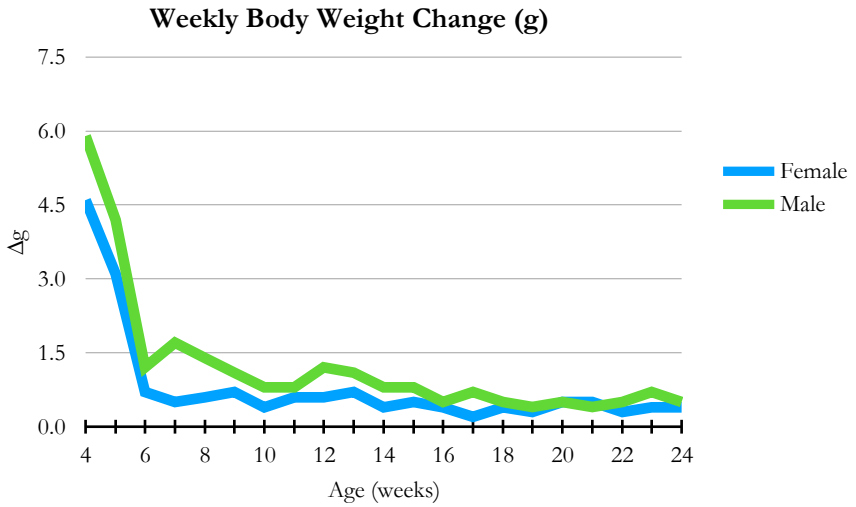
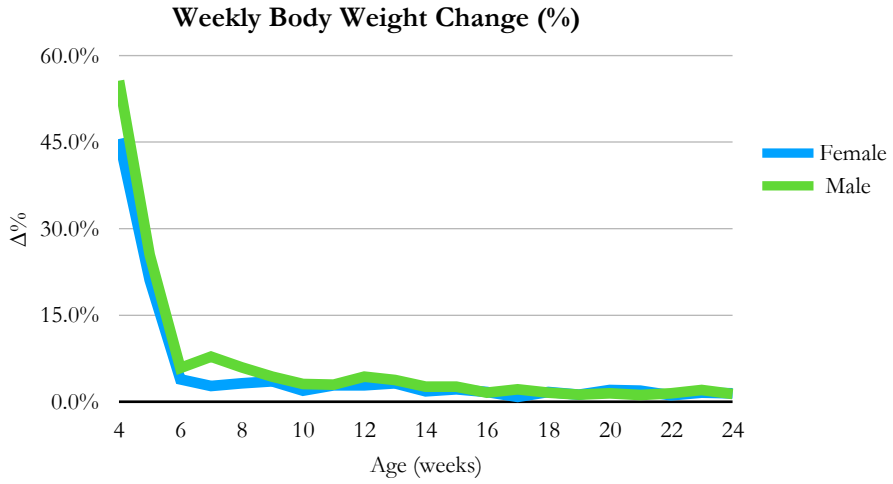

C57BL/6J Body Weight
40
30
20
10
0
Body weight (g)
3 5 7 9 11 13 15 17 19 21 23
Age (weeks)
Female
Male


Weekly Body Weight Change (g)
7.5
6.0
4.5
3.0
1.5
0.0
Δg
4 6 8 10 12 14 16 18 20 22 24
Age (weeks)
Female
Male


Weekly Body Weight Change (%)
60.0%
45.0%
30.0%
15.0%
0.0%
Δ%
4 6 8 10 12 14 16 18 20 22 24
Age (weeks)
Female
Male

Weekly Body Weight Information for C57BL/6J Mice.

Age (w)	Body Weight (g)		Weekly Δg		Weekly Δ%	
	♀	♂	Δg ♀	Δg ♂	Δ% ♀	Δ% ♂
3	10.1	10.6	—	—	—	—
4	14.7	16.5	4.6	5.9	45.5%	55.7%
5	17.8	20.7	3.1	4.2	21.1%	25.5%
6	18.5	21.9	0.7	1.2	3.9%	5.8%
7	19	23.6	0.5	1.7	2.7%	7.8%
8	19.6	25	0.6	1.4	3.2%	5.9%
9	20.3	26.1	0.7	1.1	3.6%	4.4%
10	20.7	26.9	0.4	0.8	2.0%	3.1%
11	21.3	27.7	0.6	0.8	2.9%	3.0%
12	21.9	28.9	0.6	1.2	2.8%	4.3%
13	22.6	30	0.7	1.1	3.2%	3.8%
14	23	30.8	0.4	0.8	1.8%	2.7%
15	23.5	31.6	0.5	0.8	2.2%	2.6%
16	23.9	32.1	0.4	0.5	1.7%	1.6%
17	24.1	32.8	0.2	0.7	0.8%	2.2%
18	24.5	33.3	0.4	0.5	1.7%	1.5%
19	24.8	33.7	0.3	0.4	1.2%	1.2%
20	25.3	34.2	0.5	0.5	2.0%	1.5%
21	25.8	34.6	0.5	0.4	2.0%	1.2%
22	26.1	35.1	0.3	0.5	1.2%	1.4%
23	26.5	35.8	0.4	0.7	1.5%	2.0%
24	26.9	36.3	0.4	0.5	1.5%	1.4%
Avg.			0.8	1.2		