

LD50 Animal Study Proposal Checklist

Follow this after reading the IACUC's Policy on Humane Interventions and Endpoints.

1. Create an **EXPERIMENT** specifically for the LD50 study you are proposing.

- **Question #1:** "LD50" should be part of the name of the experiment. Do not include other parts of your animal studies within the LD50 experiment.
- **Question #3** (Describe the experiment) should include the following details:
 - Animal strain, ~ age, ~weight and sex. It is okay to give age and weight ranges.
 - Experimental groups and number of animals per group.
 - Details about how increasing doses of a toxic substance or infectious agent will be used to determine the dose required to kill 50% of the animals.
 - What is the starting dose and how was this selected?
 - Explain your dosing strategy completely.
 - How often (and for what reason) would this experiment need to be conducted? For example, would you need to determine the LD50 for every strain of a particular bacteria or virus studied?
- **Question #4** (Justify the purpose of this experiment)
 - State: "Administering <<substance or infectious agent>> to determine the LD50 is a Category E Procedure".
 - State: "The research benefit of conducting an LD50 study for <<substance or agent>> is __ <<add your reasoning>> ____." This outweighs the very high research harm (animals proceeding to death as an endpoint) because __ <<add your reasoning>> ____." Your evaluation of why the harm to the animals is outweighed by the research benefit should be very clear to the IACUC. IACUC must conduct a Harm-Benefit Analysis and vote on this at a fully convened meeting in order to approve.
 - After a very exhaustive literature search utilizing the services of a UB Research Librarian, state whether an LD50 study has ever been completed for the particular toxin or infectious agent you are evaluating. If this has been done, please explain and include why you need to repeat this (what is unique about the information or circumstances of your LD50 study proposal)?
 - State: "Please see the Alternatives Section for further details on consideration of alternatives to the proposed LD50 Study."
 - State: "Please see the Animal Justification Section for the full statistical justification of the number of animals to undergo the LD50 study."

2. Create a **SUBSTANCE ADMINISTRATION PROCEDURE** specifically for the LD50 study.

Effective Date: 2/1/2025
Next Review Date: 2/2028
First Approved: 1/21/2025
Revised: --

- **Procedure Identification, Question #1:** Name the procedure “<<Toxin or infectious agent and Route of Administration>> for LD50.” A generic example is “Virus IP Injection for LD50 Study.”
- **Procedure Identification, Question #4:**
 - Answer “**YES**” to the question, “Will administering this procedure cause any more than momentary pain and/or distress?”
 - Answer the follow-up question, **4-i**, “Will it be relieved by anesthesia, analgesia, or other treatments or interventions?”
 - Answer the follow-up question, **4-ii**, “Identify **expected symptoms** from administering this procedure.” You need to describe the clinical signs you expect after administering the toxin or infectious agent to the animals. This includes the expectation of death as an endpoint for some animals, as LD50 is determined.
 - Answer the follow-up question, **4-iii**, “What **objective criteria** will be **monitored** to identify pain and/or distress, **frequency** and **duration** of monitoring?” List EACH monitored parameter which **must include**:
 - **Activity (4x daily, ~ every 6 hours, is required for LD50)**
 - **Grooming (4x daily)**
 - **Posture (4x daily)**
 - **Respiratory rate (4x daily)**
 - **Weight loss (at least 1x daily)**
 - **Other-Hydration (assess skin turgor at least 1x or whenever animals are inactive)**
 - **Body temperature is recommended**, especially for infectious disease studies where hypothermia can be used as a surrogate humane endpoint for death.
 - Other model specific parameters may be recommended.
 - **MONITORING 4x DAILY is not negotiable for LD50 studies.**
 - Answer the follow-up question, 4-iv, Based upon these criteria, when will animals be removed from research. This question typically refers to when animals will be euthanized based upon humane endpoints. Respond based upon your intent with the LD50 studies.
- **Substance Administration, Question #2** (Description of the administration procedure):
 - Describe how the toxin or infectious agent is administered (procedural details of how injection, oral gavage, etc. is conducted). Include needle size, if applicable and how the animal is restrained for the procedure.
 - Describe any **supportive care measures** that will be used. It is important to the IACUC that animals do not die from starvation or dehydration when they get too sick to access food and water.
 - Explain the placement of wet food or gel products on the cage floors.

Effective Date: 2/1/2025
Next Review Date: 2/2028
First Approved: 1/21/2025
Revised: --

- Fully explain the provision of any supplemental injectable fluid support (subcutaneous injections).
 - Add any isotonic fluids (LRS or 0.9% saline) or other medications as substances included in this procedure.
- **Procedure Documents (Supporting Documents):** Attach the **Monitoring Chart** specifically for monitoring animals in the LD50 Experiment.
 - Include in the name the Chart: “LD50 Monitoring.”
 - Baseline weight should be included (before toxin or infectious agent exposure).
 - The chart should allow space for the required number of daily monitoring timepoints (with a place to note date/time and lab staff recording).
 - The chart should allow for recording of any supportive care measures.
 - The chart should give a place to document whether an animal was euthanized (E) or found dead (FD).
 - All parameters to be monitored and any scoring systems should be explained and have a place for recording on the chart.
 - Monitoring charts are to be submitted directly to the IACUC at the completion of each cohort, and this should be written on the chart.

3. Add a paragraph to the **Animal Justification Section** that gives complete statistical justification for why the number of animals proposed in the LD50 Study is required. This should be a very robust scientific justification inclusive of current statistical approaches. Review the IACUC’s Policy on Humane Interventions and Endpoints for more guidance.

4. Record an **Alternatives Search** specifically for the LD50 Procedure.

- You **MUST** work with a UB Research Librarian when conducting this search. Refer to the IACUC’s Policy on Humane Interventions and Endpoints for more information.
- A minimum of 2 databases must be searched.
- Keywords must include:
 - Species terms (example “Mus” OR “Mouse” OR “Mice”)
 - LD50 terms
 - Toxin or infectious agent terms
 - “3Rs” Alternatives terms
- A bibliography of the search **MUST** be included and attached at the very end of the protocol in the LAST Supporting Documents Section. Please name this document “Alternatives Search Bibliography for LD50.”
- **Question #3** (If alternative procedures were identified that would result in less pain and/or distress, clarify why they cannot be used or are not appropriate for this study).
 - Respond as thoroughly as possible to this if you find alternative approaches that are unsuitable for your studies.
 - If you are unable to use an up-and-down procedure to reduce the number of animals, you must explain why in this response.

Effective Date: 2/1/2025
 Next Review Date: 2/2028
 First Approved: 1/21/2025
 Revised: --