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**UC Davis
Institutional Animal Care and Use Committee (IACUC)**

Title: *Use of Non-Pharmaceutical Grade Sodium Pentobarbital for Anesthesia of Laboratory Animals*

I. Purpose:

The purpose of this document is to describe the necessary information to obtain IACUC approval for the use of non-pharmaceutical grade sodium pentobarbital. This document also provides guidance on acceptable methods for preparation and use.

II. Background:

Sodium pentobarbital is a barbiturate injectable anesthetic sometimes used in laboratory rodents. Many investigators prefer not to use the pharmaceutical grade version due to evidence of reported hemolysis. The availability of pharmaceutical grade pentobarbital has been unreliable and inconsistent and, due to recent changes in Drug Enforcement Administration (DEA) regulations, compounded versions can no longer be produced for general use.

III. Policy:

A. IACUC Approval:

If the use of non-pharmaceutical grade pentobarbital is required, it must be approved by the IACUC through an Animal Care and Use Protocol or amendment. Scientific justification must be provided for the inability to use other pharmaceutical grade anesthetics. The policy [Use of Non-Pharmaceutical-Grade Compounds in Animals](#) must be followed.

B. Preparation:

In order to use non-pharmaceutical grade sodium pentobarbital, it must be prepared in a sterile manner and used within 24 hours of preparation. Both the powder and diluted solutions must be stored and documented as controlled substances. All preparations, use, and disposal must be documented according

to the [UCOP Controlled Substances Policy](#) and the [UC Davis Controlled Substances Policy](#).

IV. **Procedure:**

The following is the standard method of preparation. Other methods may be proposed to the IACUC on a case-by-case basis. Sodium pentobarbital powder can be purchased from Sigma-Aldrich or other vendors as long as the purity is greater than or equivalent to 98%. It is recommended to use 10% ethanol and sterile water for dilution. Injectable solutions can be prepared in various concentrations; however, concentrations of 50-65 mg/ml are most commonly used. For example, a 50 mg/ml solution can be made by placing 102.04 mg of powder (98% active sodium pentobarbital salt) into a sterile vial under aseptic conditions and adding sufficient quantity of 10% *undenatured* ethanol in sterile water to bring the volume to 2 ml. It is important to remember that the powder adds to the volume of the final solution. Add only enough ethanol and sterile water to attain the final volume needed.

Drug powder must be weighed into a sterile vial using a sterile spatula and preferably in a clean biosafety cabinet using aseptic technique including sterile gloves. The appropriate volume of 10% ethanol in sterile water should then be added and mixed to dissolve the powder, then drawn into a sterile syringe with a sterile needle attached (e.g., 22 gauge). The sterile needle is then removed and a sterile 0.22 µm filter attached to the sterile syringe (all under aseptic conditions and in the biosafety cabinet with sterile gloves) with another new sterile needle attached and the solution transferred into a new sterile empty vial. The vial must be clearly labeled "Pentobarbital" with the concentration, the date the compound was prepared, and the expiration date (24 hours after mixing), with the initials of the person that prepared the solution.

V. **Resources:**

1. IACUC-09 Use of Non-Pharmaceutical-Grade Compounds in Animals
<https://research.ucdavis.edu/wp-content/uploads/IACUC-09.pdf>
2. UCOP "Controlled Substances"
<https://policy.ucop.edu/doc/3520503/BFB-BUS-50>
3. UC Davis "Controlled Substances"
<https://safetyservices.ucdavis.edu/units/ehs/research/chemical/controlled-substances>