

I. Purpose

This policy provides standards to ensure that animals undergoing procedures involving neuromuscular blocking agents receive proper anesthesia to preserve their welfare. Neuromuscular blocking drugs present a risk to animal welfare by eliminating normal signs of pain or distress. This policy has been developed to ensure that UCSF complies with the Guide for the Care and Use of Animals, 8th Edition, and the Animal Welfare Act.

II. Regulatory or Accreditation Authority

Animal Welfare Act and Regulations, 9 CFR 2.31(d)(1)(iv)(C), Institutional Animal Care and Use Committee - "Procedures that may cause more than momentary or slight pain or distress to the animals will ... not include the use of paralytics without anesthesia."

Guide for the Care and Use of Laboratory Animals, Eighth Edition, p. 123. November, 2013 - "because this paralysis eliminates many signs and reflexes used to assess anesthetic depth, autonomic nervous system changes (e.g., sudden changes in heart rate and blood pressure) can be indicators of pain related to an inadequate depth of anesthesia. It is imperative that any proposed use of neuromuscular blocking drugs be carefully evaluated by the veterinarian and IACUC to ensure the well-being of the animal."

III. Scope

This policy applies to all animals cared for at UCSF, both in and outside of centralized LARC care space.

IV. Definitions

Neuromuscular Blocking Agent: Any compound, drug that blocks the neuromuscular transmission causing paralysis of the skeletal muscles. NMBAs are also referred to as paralytic agents.

V. Policy

NMBA can be administered only when the animal is adequately anesthetized and an appropriate means of mechanical ventilation is provided. The level of anesthesia must be sufficient to induce unconsciousness and to provide adequate pain relief.

When using NMBA it is more difficult to determine if the level of anesthesia is adequate. In the absence of NMBA, changes in skeletal muscle tone (especially jaw tone), changes in the rate and effort of respiration, and gross purposeful movement are all valuable tools used clinically to

determine the depth of anesthesia. When NMBA are administered to animals, these useful signs of adequate depth of anesthesia are lost.

NMBA must therefore be used only in conjunction with an anesthetic protocol known from clinical veterinary experience to produce a stable and adequate level of general anesthesia for the entire period the animal will be under chemical paralysis. The investigator must take appropriate measures to insure that the correct amount of anesthetic agent(s) is being administered at all times.

Requirements for Submission of an Animal Use Protocol that Proposes to use NMBA

1. The need for NMBA must be scientifically justified.
2. The anesthetic protocol, NMBA regimen, and method of ventilation must be specified.
3. A description of the method(s) that will be used to monitor anesthetic depth during NMBA paralysis must be included. A thorough anesthetic monitoring record must be kept for all animals. Physiological parameters appropriate for the species being studied must be monitored and documented periodically. Details of the specific parameters to be monitored and frequency of documentation must be included as part of the Animal Use Protocol.

Some examples of adequate anesthetic and monitoring procedures to be used in conjunction with NMBA are included in the Appendix.

IACUC Appendices for Neuromuscular Blocking Agents

Appendices are provided as IACUC suggestions or recommendations. Deviation from the attached may require additional IACUC approval.

Appendix A: Adequate Anesthesia Monitoring and Ensuring Adequate Anesthetic Depth IACUC Approved: September 2015

There are several methods used to determine that the level of anesthesia is appropriate for a protocol that utilizes NMBA. One method is to perform either a sham procedure or to carry out the entire procedure in a subset of animals without administering NMBA, thereby determining the anesthetic requirement. Subsequent animals (same weight, strain, etc) would all receive that predetermined anesthetic dose (or a higher dose if indicated by clinical signs in an individual animal). A second method is to determine the anesthetic requirement for each animal, prior to administering the NMBA. With this method, once NMBA are administered, the amount of anesthesia is never decreased and the level of noxious stimulation (from pain-inducing portions of the surgery) not escalated. With a third method, the NMBA is periodically withdrawn and the anesthetic requirement determined when skeletal muscle function has returned. The most suitable method used to determine that an appropriate level of anesthesia is maintained during NMBA administration may depend on the type of investigation. These methods are described in more detail below. In animals receiving NMBA, the following parameters may be considered for monitoring: electrocardiogram, electroencephalogram, body temperature, respiratory rate, respiratory volume, oxygen saturation, end-tidal CO₂. If a volatile anesthetic agent is used, the inspired and endtidal O₂ concentration, N₂O concentration, and agent concentration should be continuously monitored. Regardless of which method(s) will be used for a study, it must be described in detail in the IACUC protocol.

Sham procedure or withholding NMBA

Performing sham procedures or complete experiments without NMBA are sometimes used to determine the anesthetic requirement for a particular study. With sham studies, the animals are anesthetized using the proposed anesthetic technique; however, the entire study is not performed. A noxious stimulus is applied (e.g., toe-pinch or tail-pinch) to determine if the depth of anesthesia is adequate. Once the anesthetic dose requirement is determined, all subsequent animals will receive at least that dose of anesthetic (higher doses should be administered if clinical signs of inadequate anesthesia are observed in individual animals). **If the noxious stimulus is greater in the actual experiment** (e.g., a thoracotomy has higher anesthetic requirements than a toe-pinch), this would not be the best method of determining the anesthetic requirement. In addition, if the dose range of anesthetic is not consistent between multiple sham animals, a good measure of anesthetic requirements for all subsequent animals is not determined. Due to biologic variation between individuals, the more sham animals that are used to determine the anesthetic dose requirement, the more accurate the minimum anesthetic determination will be. The specific details of conditions and number of "shams" necessary should be determined in consultation with LARC veterinarians.

A similar type of determination could be carried out where all procedures of a study are performed without administering NMBA. Similar to above, if the dose of anesthetic is not consistent between multiple animals undergoing the procedures, this would also not be a good measure of the anesthetic requirement for all subsequent animals. Again, the more animals that are used to determine the anesthetic dose requirement, the more accurate the minimum anesthetic determination will be. This method of anesthetic determination is not applicable to investigations where paralysis is a requirement of the study or required during the most painful

aspects of the procedure. The specific details of conditions and number of animals studied should be determined in consultation with LARC veterinarians.

Anesthetic requirement determined in each patient

This method determines the anesthetic requirement for each animal prior to administering the NMBA. A portion of the study is performed without paralysis when skeletal muscle tone and movement can be monitored (along with other clinical signs of anesthetic depth, such as toe-pinch) and the anesthetic requirement defined. This method accounts for normal biologic diversity in anesthetic dose requirements since each animal serves as its own control. With this method of anesthetic requirement determination, it is imperative that the maximum noxious stimulus that will be encountered during the study occurs before NMBA are administered. This method of anesthetic dose determination is particularly useful for protocols that include an initial surgical phase that is followed by a data collection phase.

Many investigators find it most useful to perform the surgical/instrumentation portion of the study without using NMBA. Except in certain types of procedures, the use of NMBA is not justified during the surgical phase. Without NMBA paralysis during this phase of the study, the anesthetist can monitor skeletal muscle tone, a valuable indicator of anesthetic depth. Many investigators find that inhalation anesthetics such as isoflurane are the safest and most easily controlled anesthetic for the surgical phase of the study. For certain protocols (e.g., many neuroscience investigations) inhalation anesthetics depress the responses being studied. Therefore, following the surgical/instrumentation phase, the anesthetic technique is often changed from isoflurane to another method for the data collection phase. In this case, a physiological steady state under the new anesthetic must be achieved before NMBA are administered. Following paralysis, the anesthetist will be dependent upon the sympathetic nervous system responses to indicate the level of anesthesia, thus it is important that the animal subject also be physiologically stable before NMBA are administered. The time required to achieve steady-state anesthesia with stable physiological variables can vary dramatically, depending upon the method of anesthesia. Once adequate steady-state anesthesia is achieved, NMBA can be administered. The rule of thumb is that the anesthetic level should not be decreased after the NMBA has been administered; however, it can be increased if clinical signs of an inadequate level of anesthesia are observed.

NMBA periodically withheld

If animals are anesthetized for several days, the anesthetic regimen may require adjustment during this period. To do so, NMBA should be withheld from the animal for a period adequate to allow for return of normal skeletal muscle responses prior to the anesthetic adjustment. Normal muscle responses can be assessed using an electronic twitch monitoring device (e.g. MiniStim® monitor) that stimulates skeletal muscles to contract. The frequency of NMBA interruptions and anesthetic adjustments must be specified and justified in the IACUC protocol for approval. An alternative to NMBA interruptions when using a volatile anesthetic is to monitor end-tidal anesthetic concentration and to maintain an appropriate level throughout the procedure. Again, the end-tidal anesthetic concentration must be defined in the IACUC protocol and documented in the anesthesia record for approval.

References:

1. Preparation and maintenance of higher mammals during neuroscience experiments. Report of a National Institute of Health workshop. 1991 Van Sluyters RC, Oberdorfer MD, eds. NIH Publication No. 91-3207.

2. Drummond JC, 1996. Use of neuromuscular blocking drugs in scientific investigations involving animal subjects. *Anesthesiology*, 85(4):607-699
3. Guidelines for the use of neuromuscular blocking drugs in anesthetized animals. Animal Care and Use Committee, University of California, Berkeley. Appendix K: Home Office guidelines on the use of neuromuscular blocking agents. Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. (UK)