

	UCSD INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE POLICY MANUAL	POLICY #10.04 Originally Issued: 1/16/95 Revised: 7/16/01 Revised: 11/17/04 Revised: 8/19/15
	Policy on the Use of Neuromuscular Blocking Drugs in Animal Research	

I. Background and Purpose

The University of California is committed to the improvement of human and animal health and the advancement of science. When these endeavors involve the use of neuromuscular blocking drugs, UCSD is committed to their humane care and use as specified in the Guide for the Care and Use of Laboratory Animals, the PHS Policy and the Animal Welfare Act. When neuromuscular blocking (NMB) drugs are used on animals in experimental research, it is critical that the animals be adequately anesthetized and ventilated. NMB drugs paralyze muscles, thereby making it possible for animals to experience pain or discomfort without being able to move or withdraw from a painful stimulus. They can also no longer breathe effectively. This policy is designed to allow the investigator to paralyze muscles for the shortest time possible once adequate dose or depth of anesthesia has been assured.

II. Who Should Read This Policy

All personnel engaged in or responsible for the use of neuromuscular blocking drugs in animals.

III. Definitions

Term	Definition
Neuromuscular Blocking Drug (NMB)	Drugs that block neuromuscular transmission at the neuromuscular junction, causing paralysis of the affected skeletal muscles. NMB drugs are sometimes used for specific indications, such as preventing animal motion during surgery which is technically delicate, or for procuring physiological recordings where muscle activity would interfere.

IV. Policy

1. NMB drugs may not be used in animals without rigorous scientific justification as described and approved in a UCSD animal protocol. The IACUC does not consider general restraint or ease of routine procedures (e.g. endotracheal intubation, positive pressure ventilation) as adequate justification for NMB use.
2. NMB drugs may not be used alone without anesthesia.

3. Anesthetic regimen (dose and depth) must be determined to be adequate for the procedure in the absence of the NMB drug. Anesthesia must be initiated before the administration of the NMB drug and maintained throughout the procedure.
4. Since animals will not be able to move in response to pain, their blood pressure (BP) and heart rate (HR) must be carefully monitored and the anesthetic dose adjusted appropriately. Both the upper and lower range of acceptable BP and HR values need to be specified in the protocol. The parameters of any other signs (e.g., pupil size) which might be used for depth assessment also need to be specified. These values must be determined before NMB drug administration; e.g., during preparatory surgery, pilot studies, or from previous experimental procedures not using NMB drugs.
5. A detailed anesthesia record must be completed for each investigative or surgical procedure utilizing NMB drugs.
The anesthesia record must document:
 - a. Timing and dose of anesthetic and NMB drug administration, and
 - b. Vital signs and depth of anesthesia signs obtained during the procedure
 - c. A copy of each anesthetic record must be maintained and readily available for review or inspection at all times.
6. As the animal will not be able to breathe on its own, it must be intubated and mechanically ventilated.

V. Related Documents

The Guide for the Care and Use of Laboratory Animals	http://www.nap.edu/catalog.php?record_id=12910
Anesthesia Policy 34.03	http://iacuc.ucsd.edu/policies/Policy34.03.pdf

VI. Additional information

When it is unclear if a NMB drug is necessary for a project, the investigator should consult with the UCSD ACP veterinarians. In some instances a pilot procedure done without the use of an NMB drug may be required by the IACUC to determine 1) whether these drugs are even necessary for the study and 2) what an adequate depth of anesthesia would be. An alternative to the pilot procedure is a modification in the experimental design, where surgery/instrumentation is performed without NMB drugs, and data collecting post-instrumentation is performed under an adequate level of anesthesia with NMB drugs.

DETERMINATION OF ADEQUATE ANESTHESIA:

One complication of using NMB drugs is that it is more difficult to determine if the level of anesthesia is adequate. 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 level of anesthesia. When NMB drugs are administered to animals, these useful parameters are lost. NMB drugs 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.

Monitoring the depth of anesthesia during the use of NMB drugs needs to include:

- Heart and respiratory rate
- Blood pressure
- Body temperature
- Peripheral capillary oxygenation saturation (SpO₂) and End Tidal carbon dioxide (ETCO₂)
- End Tidal anesthetic concentration
- Periodic withdrawal of the NMB drug, especially for studies of long duration

There are several methods used to determine that the level of anesthesia is appropriate for a protocol that utilizes NMB drugs. One method is to either perform a sham procedure or carry out the entire procedure in a subset of animals without administering NMB drugs, thereby determining the anesthetic requirement. Subsequent animals 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 NMB drug. With this method, once NMB drugs are administered, the amount of anesthesia is never decreased. With a third method, the NMB drug 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 NMB drug administration may depend on the type of investigation. These methods are described in more detail below. In animals receiving NMB drugs, 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 end tidal 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.

Performing sham procedures or complete experiments where the NMB drug is withheld 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 ACP veterinarians.

A similar type of determination could be carried out where all procedures of a study are performed; however, the NMB drug is not administered. 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. The specific details of conditions and number of animals studied should be determined in consultation with ACP veterinarians.

If animals are anesthetized for several days, the anesthetic regimen may require adjustment during this period. To do so, NMB drugs 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 (MiniStim®) that stimulates skeletal muscles to contract. The frequency of NMB drug interruptions and anesthetic adjustments must be specified and justified in the protocol for approval. An alternative to NMB drug 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 protocol for approval.

Neuromuscular function should be monitored whenever a muscle relaxant is administered. Appropriate monitoring will facilitate proper dosing of both the muscle relaxant and its antagonist. To prevent residual paralysis and muscle weakness in the recovery period it is critical that monitoring be continued until the function is fully restored. Evoked motor responses to peripheral nerve stimulation are used to evaluate the degree of neuromuscular blockade.

If animals are to recover from the experimental procedures, clinical signs of neuromuscular recovery should complement the nerve stimulation tests (e.g., normal breathing pattern, sustained head lift, or ability to stand). In some instances the use of a reversal agent such as edrophonium, neostigmine, or pyridostigmine might be indicated. It is recommended that an anticholinergic drug, either atropine or glycopyrrolate, be administered immediately prior to reversal of these anticholinesterase drugs, especially with the use of either neostigmine or pyridostigmine.