PROTOCOL REVIEW

Protocols and the IACUC's approval and monitoring of protocols must be completely and thoroughly reviewed.

You (the inspector) are responsible for conducting a thorough inspection of:

- IACUC approved protocols and changes to protocols
- the IACUC's monitoring of protocol activity
- the protocol approval process

Detailed below are some aids to assist you in evaluating the IACUC. However, you must use the regulations and your professional judgment to determine if an IACUC or protocol is in compliance.

For the protocol review, you may decide to review:

- all of the research facility's protocols for regulated animals, or
- a representative sample of protocols, such as:
  - one or two protocols for each regulated species
  - Category E protocols
  - protocols involving invasive procedures

Ways to verify IACUC activities include, but are not limited to:

- protocols
- protocol submission forms
- written meeting minutes
- correspondence
- memos/notes
- e-mails and e-mail records
- interviews with IACUC members

PROTOCOL APPROVAL

Process

In assessing the protocol approval process, you should look for verification that:

- all protocols involving regulated animal use are submitted to the IACUC

- NO animal activity is started before the protocol has been properly approved
  NOTE: No IACUC member can approve a protocol or give permission for an animal activity to start before the protocol has gone through the proper approval process.
- the IACUC has a mechanism for distributing protocols and other pertinent information to IACUC members which is accessible to all members, i.e., if distributed by e-mail, all members have e-mail or an alternate method of distribution is used for members without e-mail
- all members are sent a list of protocols to be reviewed prior to the review in sufficient time to request a copy of the protocol or participation in the review
- if the protocol was reviewed by the full IACUC:
  - there was a quorum present
  - approval was by a majority vote of the quorum
- no IACUC member voting on the protocol had a conflicting interest
- any **significant changes to protocols** were approved using the same procedures as for a protocol review
- any IACUC requested additions or changes to protocols were made before final approval was given
- all IACUC decisions regarding protocols, or significant changes to protocols are documented in writing and available for inspection
- no official, department, or committee of the research facility overrides IACUC denials of protocols or significant changes to protocols.
  NOTE: Implementation of an IACUC approved protocol may be delayed or prohibited by another official, department or committee, for example, the Radiation Safety Committee if the protocol does not meet its requirements.

Notification

In assessing the protocol notification requirement, you should look for verification that:

- the Institutional Official is notified in writing of all protocol review decisions
- the Principal Investigator is notified in writing of the

	IACUC's decision on his/her protocol • if protocol approval was denied, the IACUC: ▸ notified the Principal Investigator of the reason for the denial ▸ gave the Principal Investigator the opportunity to respond
Annual Review	In assessing the annual review of protocols, you should look for verification that: • all active protocols are reviewed by the IACUC or a subcommittee annually • all IACUC members are informed of the annual reviews • all members are given the opportunity to participate in the annual reviews • the IACUC reviews and decisions are documented in writing and available for inspection
PROTOCOL	
General Requirements	In assessing a an IACUC's review of a protocol, you should look for verification that:: • the rationale for using animals is clearly stated, acceptable, and scientifically justified • the species of animal(s) to be used is clearly stated • the appropriateness of the species is adequately and scientifically justified • the number of animals to be used is clearly stated • how the approximate number of animals to be used was determined is clearly stated or shown, such as: ▸ required for statistically significant results (tests used or statisticians consulted should be included) ▸ based on scientific literature or past experience (references should be cited) ▸ based on results of pilot study ▸ required by FDA or other Federal agency (Federal code, regulation or standard, etc., must be cited) ▸ required by international testing requirements (code, regulation, standards, etc. must be cited)

- the proposed use of the animals is clearly and adequately detailed
- the principal investigator has provided an assurance that the proposed activity is not an unnecessary duplication of previous experiments
- medical care is provided for the animals when needed
- the animals' living conditions and care are adequate and appropriate
- personnel conducting the research or handling the animals are properly trained and qualified
- there is a description of how pain/distress/discomfort are minimized, if applicable
- the method of euthanasia:
  - is clearly stated, including drug(s) and dosages, and
  - is consistent with the current *Report of the AVMA Panel on Euthanasia,* **or**
  - is an alternative method justified in the protocol and approved by the IACUC
- disposition of animals at termination of study is stated, including harvesting of tissues or body parts
- any exemption/exception to the AWA regulations or standards is adequately justified (see page 17.5.7)

NOTE: Routine veterinary care, housing, euthanasia, etc., may be detailed in standard operating procedures (SOPs), but the protocol must refer specifically to that SOP(s).

Painful/Distressful Procedures

In assessing requirements for procedures that cause more than momentary or slight pain/distress/discomfort, you should look for verification that:

- the procedure is properly classified
- the principal investigator has considered alternatives to the painful/distressful procedure
- there is a narrative describing the methods and sources used to determine that no alternatives to the painful/distressful procedure are available (see page 17.5.3 for electronic and non-electronic search requirements)

- measures used to alleviate the pain/distress are clearly stated, including, if appropriate:
  - drugs, dosages, and frequency of administration
    NOTE: A "PRN or as needed" frequency of administration may not be acceptable.
  - other methods, such as:
    - hydrotherapy
    - hot/cold packs
- measures used to relieve pain/distress are adequate, i.e., correct drug, dose, frequency, etc.
- if pain/distress relief is not to be used, there is an adequate justification (see page 17.5.3)
- the principal investigator has consulted and involved the attending veterinarian or his/her designee in the planning of the procedure and pain/distress relief
- if a paralytic is used, it is used with anesthesia
- animals experiencing severe or chronic pain/distress that cannot be relieved will be humanely euthanized

NOTE: If the research facility has a standard operating procedure(s) (SOP) for pain/distress relief, the protocol must reference that SOP.

Surgical Procedures

In assessing requirements for surgical procedures, you should look for verification that:

- the pre-procedural care and surgical preparation of the animals are clearly stated
- drugs given prior to and during the procedure, such as analgesics, tranquilizers or anesthetics, are appropriate and at the correct dosage for the species
- the surgical procedure is stated clearly and in detail
- all survival surgeries are performed using aseptic technique
- major operative survival surgeries on non-rodents are performed in a dedicated surgical facility
- no animal is being used in more that one major operative survival surgery UNLESS appropriately approved (see page 17.5.6 for requirements)

- post-surgical procedures are stated clearly and in detail, such as:
  - observation and monitoring of recovery
  - any special recovery environment requirements
- pain/discomfort relief measures are stated clearly and in detail, including but not limited to: (see page 17.5.4)
  - when drugs are to be administered
  - when or which drugs are **not** to be administered, if applicable, with an explanation
  - drug, dose, route, and frequency of administration
  - signs of pain/distress
  - contact person(s)
  - other or additional methods of pain/distress relief

NOTE: If the research facility has a standard operating procedure(s) (SOP) for surgical procedures or pain/distress relief, the protocol must reference that SOP(s).

INSPECTION PROCEDURES

Listed below are some additional aids to assist you in determining if the procedures outlined in the protocols are being followed:

- if protocol numbers are not listed on the cages, ask for the protocol numbers of random animals.
  NOTE: Animals may be held but cannot be used without being on a protocol.
- choose random protocol numbers from cage cards or animal charts/records and check in IACUC records that these protocols were approved
- ask how the research facility keeps track of the number of animals approved by the IACUC and the number of animals used by the principal investigator, such as, through:
  - computer records
  - acquisition and disposition records
  - dead animal records
  - inventory cards
- ask for exemption/exceptions to the regulations or standards, then check the protocol to determine that the exemption/exception was approved

- determine if the animal care staff is familiar with the protocol procedures, especially pre- and post-painful/ distressful procedure care, such as:
  - asking the staff
  - checking the availability of protocols
  - checking the availability of standard operating procedures
  - looking in medical records
- watch the animal care staff, principal investigators or laboratory personnel handle the animals (or ask them to handle the animals)
- review medical records to determine that animals with painful/distressful procedures received the proper pain/distress relieving drugs, if applicable
- observe animals for signs of unrelieved pain (see page 6.3.8)
- determine if the medical or emergency contact people's numbers are readily available, such as:
  - on bulletin boards
  - in the animal rooms
  - in medical records/charts
  - in protocols
- observe surgeries to determine that they are being conducted using aseptic technique and in dedicated surgical facilities, if required
- ask how the research facility tracks animals to ensure that they are not used for another survival surgery (unless approved by the IACUC or APHIS), such as, through:
  - health records
  - individual animal records
  - cage cards
  - surgery records

SPECIES-TYPICAL SIGNS OF PAIN*

SPECIES	POSSIBLE SIGNS OF PAIN**
DOGS	quiet, unwilling to move, lack of alertness, whimpering or howling, loss of appetite, increased respiration, growl or exhibit apprehension when approached
CATS	quiet, apprehensive facial expression, loss of appetite, crying, hissing, hiding, crouching or hunching, ungroomed appearance
GUINEA PIGS & HAMSTERS	decreased activity, piloerection, ungroomed appearance, excessive licking and scratching, rapid/shallow respiration, loss of appetite, grunting or chattering, do not try to escape when handled
RABBITS	inactive, appear apprehensive or anxious, hunched appearance, hide, squeal or cry, possible aggressive behavior with excessive scratching and licking, grinding of teeth, excessive salivation
NONHUMAN PRIMATES	huddling or crouching in corner, stops eating/drinking, sad expression, moaning, screaming, stops grooming, clenching of teeth
CATTLE, SHEEP, GOATS	dull, depressed appearance, heads bowed, lack of alertness, loss of appetite, rapid/shallow breathing, rigid posture, vocalization
PIGS	changes in social behavior, gait and posture, squealing when handled, unwilling to move, hiding

******These are possible signs of pain and do not necessarily mean the animal is in pain. A lack of these signs also does not mean that the animal is not in pain.

*****excerpted from: National Research Council: Recognition and Alleviation of Pain and Distress in Laboratory Animals, Washington, D.C., National Academy Press, 1992.