

Guidelines for Preparing USDA Annual Reports and Assigning USDA Pain & Distress Categories

Animal Welfare Act Regulations (AWARs) require each reporting facility to submit an annual report to the Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) on or before December 1 of each calendar year. All Institute/Center (IC) programs must submit this report to the Office of Animal Care and Use (OACU) each November. The OACU compiles the IC reports into one, single NIH Intramural Research Program (IRP) report. Blank forms (APHIS Form 7023) and specific instructions are distributed by OACU to each IC in early fall after receipt from USDA.

The Scientific Directors of all IC's that use animals must sign their IC's APHIS Form 7023 as the operational Institutional Official within that IC. Their signature indicates that the IC is in compliance with the following four assurances:

- 1) Professionally acceptable standards governing the care, treatment, and use of animals, including the appropriate use of anesthetics, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.
- 2) Each principal investigator has considered alternatives to painful procedures.
- 3) This facility is adhering to the standards and regulations under the Animal Welfare Act, and it has required that exceptions to the standards and regulations be specified and explained by the PI and approved by the IACUC.
- 4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of the other aspects of animal care and use.

GENERAL GUIDELINES

The intent of these guidelines is to standardize the compilation and reporting of animal use in the NIH IRP for our combined annual report to USDA. The objectives of these instructions are to 1) clarify the word "used" in the context of the annual report and 2) provide assistance in selecting the correct columns on Form 7023 for recording the numbers of animals used; examples are included below.

Only vertebrate species are reported in the annual report. In this document, the words "**use**" or "**used**" refer to the incorporation of vertebrate animals in teaching, testing, experiments, or research protocols. When animals are assigned to an active Animal Study Proposal (ASP), whether as experimental or control animals, those animals are considered "used". Animals must be reported each year they are used.

Regulated species are the only species included in this report and they are all live, warm-blooded species acquired or bred specifically by/for NIH for use in the IRP except for: aquatic species; birds; and rats of the genus *Rattus* and mice of the genus *Mus* bred for use in research.

Rats and mice of any other genera, or rats or mice not bred for use in research, are covered by the AWAR's and must be listed appropriately in Columns A through F.

Regulated species which have been used during the reporting period are listed in Column C, D, or E of the annual report. Regulated species being held for use, but not yet used are listed in Column B of the report. See details below regarding these 4 reporting columns and their classification.

For assistance in classifying various groups of animals, such as sentinels; those that were used in procedures prior to ownership; unintended experiment outcomes; offspring at various ages and weaning status; etc., refer to **Attachment 1**.

Animals that were assigned to more than one ASP during the reporting period are reported *only once for that year*, but should be listed in the columns (C, D or E) consistent with the greatest amount of accompanying pain or distress they were subjected to during the reporting period. If animals were used by more than one investigator or more than one IC within a given year, the IC that holds the animal on September 30th will include it on their report and this IC will make a good faith effort to determine which pain and distress category to which is shall be assigned.

REPORTING EXCEPTIONS TO THE AWAR

A summary of any IACUC-approved exceptions to the regulations or standards must be submitted in hard copy to USDA as part of the Annual Report. At a minimum, this summary must include the following:

- Identify IACUC-approved exception(s) to the regulations or standards, including exemptions to the dog exercise plan and/or the nonhuman primate plan for environmental enhancement.
- Describe the IACUC-approved exception(s).
- Identify the species and number of animals affected.

See **Attachment 2** for a requested reporting format.

SUPPLEMENTAL NIH IRP REPORTING

While aquatic species; and birds, rats of the genus *Rattus* and mice of the genus *Mus* bred for use in research, are specifically excluded from the USDA report, their acquisition through purchase, transfer or in-house breeding, or use by assignment to an active ASP will continue to be reported separately to OACU. The number of animals reported should consist of the following groups: animal census on September 30th of the reporting year + all purchased during the year (Oct 1 – Sep 30) + all generated during the year (Oct 1 – Sep 30).

USDA ANNUAL REPORT COLUMNS

COLUMN A

Column A of the form contains a preprinted partial list of regulated species by **COMMON NAMES** (e.g. dogs, cats, etc.) and space to enter the common names of other regulated species.

At the NIH, the common names of all regulated vertebrate animal species being held, bred, or used must be listed in Column A.

COLUMN B

Column B listings represent the number of animals (regulated species) being bred, conditioned, or held for future use. The numbers listed in this column reflect a one-day inventory, on September 30 of the reporting period.

If animals were held during the reporting cycle, but died or were euthanized without being used for research purposes they should also be reported in this column.

Facilities with breeding colonies should report their breeding animals and any *weaned* offspring which are *not* being used for research purposes in this column. Viable offspring that are born as a result of an ASP requiring breeding are counted at weaning or when subjected to experimental manipulation if that manipulation occurs earlier than weaning (USDA, 2009).

Animals present in the facility which were used for research in *previous* years, but were not used in the current year (i.e. retired animals, recycle pool animals, etc.) should also be reported in this column.

EXAMPLE B1: As of September 30 of the reporting period, a breeding colony of 20 (10 males-10 females) hamsters had 100 weaned offspring and 30 offspring that were still nursing. None of the hamsters had been used for experimental purposes. All of the adult breeding hamsters (20) and their weaned offspring (100) are listed in Column B. The 30 offspring still nursing and not yet used to generate data for an ASP are not listed on the annual report.

EXAMPLE B3: A breeding colony of 20 transgenic guinea pigs was established to provide animals for research. By September 30th, 80 offspring had been generated, 60 of which did not meet the desired genotype and were euthanized prior to weaning. The additional 20 offspring were weaned and being held for future experiments. All 100 guinea pigs were counted and reported in Column B.

COLUMN C

Animals that are used in procedures which do not involve pain and/or distress, and for which the use of anesthetics, analgesics or tranquilizers was not indicated are listed in Column C. The AWARs state that routine procedures producing no or only momentary or slight pain should be reported in this column (see examples below); this shall include animals used only for tissue collection. The numbers reflect a *retrospective summation* of animals on ASPs that were not subjected to pain or distress. Animals promptly euthanized when first signs of morbidity are first observed are also listed in Column C.

TYPES OF PROCEDURES LISTED IN COLUMN C

The following procedures are examples of Column C research procedures when performed by trained individuals. The list is not meant to be definitive:

1. Administration of:

- a. Electrolytes and other fluids.
 - b. Immunizations, including the proper use of Complete Freund's Adjuvant (CFA) see Animal Research Advisory Committee (ARAC) "Guidelines for the Research Use of Adjuvants: <http://oacu.od.nih.gov/ARAC/documents/Adjuvants.pdf>."
 - c. Oral medications.
2. Most blood collection procedures.
 3. Gastric gavage.
 4. The administration of an anesthetic, analgesic or tranquilizing drug to an animal for restraint purposes to perform a procedure that involves no pain or distress. Examples include but are not limited to: TB testing NHPs, minimizing animal movement to facilitate anatomical measurements, or preventing animal movement during imaging procedures.
 5. Non-surgical catheterization.
 6. Certain manipulative procedures such as injections, palpations, skin scrapings, and radiography.
 7. Intracerebral inoculations in neonatal rodents prior to cranial ossification when performed by trained personnel.
 8. Chair restraint of an adapted nonhuman primate (NHP) that has been conditioned for the time period of restraint up to 12 hours, or the training of an unadapted NHP to chairing utilizing an ACUC-approved plan that results in only slight or momentary distress.

If the result of any of the above procedures is observed to cause more than momentary or slight pain or distressful to the animals, the ACUC will be informed, the ASP modified or halted, and those animals listed in Column E.

EXAMPLE C1: Two hundred weaned hamsters received a drug subcutaneously that produced only momentary or slight pain. Two weeks later they were euthanized by CO₂ inhalation. These hamsters are listed in Column C.

EXAMPLE C2: Two rabbits were immunized with a mixture of an antigen and Complete Freund's Adjuvant following the ARAC "Guidelines for the Research Use of Adjuvants." This guideline reflects NIH's opinion that adjuvants can be used in a responsible and humane manner while avoiding more than slight or momentary pain and distress. No inflammatory lesions or tissue necrosis developed in the rabbits at the site of immunization throughout the reporting period and pain was not evident. These two rabbits are listed in Column C.

EXAMPLE C3: Twenty euthymic female guinea pigs were inoculated intra-vaginally with the *Herpes simplex* virus. The guinea pigs were examined twice-a-day for genital lesions and euthanized immediately when herpetic vesicles were observed. These guinea pigs are listed in Column C.

EXAMPLE C4: A macaque was chemically restrained with ketamine and an appropriate volume of blood was obtained from the femoral vein for an investigator. This animal is listed in Column C.

EXAMPLE C5: Five hamsters were inoculated by intraperitoneal injection with a hybridoma cell line and the ascitic fluid was removed from the peritoneal cavity three times. All hamsters remained alert, active, and eating and drinking normally. The hamsters were euthanized and the remaining ascitic fluid removed postmortem. These hamsters are listed in Column C.

EXAMPLE C6: Ten guinea pigs were anesthetized with ketamine-xylazine, their vena cavae were cannulated, and the animals subsequently perfused with 4% paraformaldehyde. The resulting exsanguination (replacement of the blood volume with perfusate), while the animals are under deep anesthesia, is an AVMA-approved adjunctive method of euthanasia. As such, the use of this adjunctive method of euthanasia is not considered a research procedure and does not otherwise influence the pain categorization of these animals.

EXAMPLE C7: Eight rabbits used for polyclonal antibody production were deeply anesthetized with ketamine and xylazine and subsequently exsanguinated by cannulation of the abdominal aorta. The resulting exsanguination, while the animals are under deep anesthesia, is an AVMA-approved adjunctive method of euthanasia. As such, the use of this adjunctive method of euthanasia is not considered a research procedure and does not otherwise influence the pain categorization of these animals.

EXAMPLE C8: Twelve hamsters were received and acclimated in their home cages for two weeks. The hamsters were then euthanized in a CO₂ chamber and various tissues and blood harvested for in vitro use. These hamsters are listed in Column C.

EXAMPLE C9: Ten gerbils were assigned to a Column C immunology ASP incorporating breeding and research use of the young adult gerbils. Seven of the females produced 44 pups; 41 of those pups were weaned and used in subsequent research procedures. The remaining three pups died of natural causes prior to weaning. A total of 51 animals (the 10 breeders and the 41 weaned gerbils) are listed in Column C.

EXAMPLE C10: Five pregnant rabbits were fed a high fat diet through-out their pregnancy and then a few days after the 12 kits were born (prior to weaning) the does and kits were euthanized to compare the effects of the diet on the adults and offspring. On September 30th, all 17 animals were counted since the experimental procedures involved both adults and neonates.

EXAMPLE C11: Thirty hamsters were acquired during the year for use in a research project. The principal investigator was unexpectedly transferred from the NIH and the protocol cancelled by the ACUC. The animals were subsequently euthanized, prior to September 30. These animals should be counted in Column C.

COLUMN D

Animals that are used in procedures which would involve more than slight or momentary accompanying pain or distress, and for which appropriate anesthetic, analgesic, tranquilizing drugs, were used must be listed in Column D. USDA Animal Care Policy #11, Painful Procedures, provides detailed guidance on painful procedures [http://www.aphis.usda.gov/animal_welfare/policy.php?policy=11]. Listings in this column

represent a *retrospective* summation of animals that were used during the year under the conditions described in the heading of Column D.

TYPES OF PROCEDURES LISTED IN COLUMN D

Examples of procedures that may produce pain or distress as defined in Attachment 3, but which are performed using anesthetics, analgesics or tranquilizers appropriate to prevent or alleviate pain or distress are:

1. Surgery, including biopsy, gonadectomy, neurophysiological manipulations or preparations such as the implantation of electrodes and recording devices.
2. Terminal surgical procedures in which the animal(s) are euthanized before recovering from anesthesia.
3. Periorbital collection of blood in species without a true orbital sinus, such as rats and guinea pigs.
4. Intracardiac blood collection.

EXAMPLE D1: Twenty dogs were assigned to an ASP involving a major survival surgical procedure, but two were actually used as non-surgical controls and experienced no pain or distress. The two control animals are listed under Column C and the other 18 dogs under Column D.

EXAMPLE D2: Twelve macaques were anesthetized, for placement of in-dwelling catheters and flow probes in and around the animals' major abdominal vessels. The catheters were exteriorized between the scapulas. These animals are listed under Column D.

EXAMPLE D3: Ten pregnant guinea pigs were anesthetized and the fetuses harvested for tissue culture. On September 30th, only the 10 females were reported.

COLUMN E

Animals (regulated species) must be listed in Column E if they are subjected to procedures involving more than slight or momentary accompanying pain or distress in which appropriate anesthetics, analgesics, or tranquilizing drugs are withheld because their use would have adversely affected the teaching, testing, or experiments. Animals must be listed in Column E if they are subjected to painful procedures and the anesthetics, analgesics, tranquilizing drugs, or other palliative treatment did not adequately preclude more than slight or momentary pain or distress.

Retrospectively, list only those animals that were used and experienced pain or distress during the reporting year, *rather than listing the number of animals that were approved in the ASP*. All animals approved for use in painful or distressful procedures without appropriate and adequate anesthetics, analgesics or tranquilizers may not have been determined to have experienced pain or distress. Animals assigned to the ASP, but not actually used in Column E conditions, are listed in the appropriate column (Column C or D).

If regulated species are used under Column E conditions, the Column E justification form (**Attachment 4**) must be attached to the annual report and a copy appended as an attachment to the ASP.

TYPES OF PROCEDURES LISTED UNDER COLUMN E

Examples of procedures that must be listed in Column E when performed without the use of anesthetics, analgesics or tranquilizing drugs include:

1. The chairing of a NHP which has not been conditioned for the time period of restraint.
2. Drug or radiation toxicity testing producing unrelieved pain and/or distress.
3. LD₅₀ determinations or any other studies involving death as an endpoint.
4. The exposure of an animal to an agent which produces unrelieved pain or distress.
5. The exposure of an animal to electrical shocks that are generally accepted as causing pain in humans.

EXAMPLE: Twenty nonhuman primates (NHP) were inoculated intravenously with a splenic-derived cell suspension infected with the simian immunodeficiency virus. During the reporting period, two of the NHPs had debilitating chronic diarrhea with weight loss and/or dehydration and one NHP had clinically evident pneumonia as a result of their infection with SIV in spite of appropriate veterinary support. These three animals must be listed in Column E because they experienced unrelieved pain or distress during the reporting period.

COLUMN F

Column F listings are the sum of the animals listed in Columns C, D, and E, by species.

REFERENCES

1. USDA Animal Care Policy #11, Painful and Distressful Procedures, March 25, 2011. (http://www.aphis.usda.gov/animal_welfare/policy.php?policy=11)
2. Lab Animal, 2006; 35(1) 15-16. A Word from OLAW. Commentary on Protocol Review Column regarding documenting number of animals acquired for research.
3. Annual Report guidance documents, USDA, APHIS, Animal Care; September 11, 2009.

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Attachment 1 – Column Classification

Euthanasia procedures: NIH has been advised that USDA does not consider approved primary methods of euthanasia to be experimental procedures and subject to animal “Use” classification. See examples provided under ‘Column C’ above.

Vendor procedures: Animals (regulated species) which have undergone surgical modification by the vendor prior to delivery to the NIH will have been ‘counted’ by the vendor. Only those activities performed while the animals were in use by NIH should be considered for assignment to Column C, D or E in the USDA Report.

Sentinel animals or Training animals: are considered to have been used, and should be counted and entered in the appropriate column.

Offspring of regulated species: are reported in Column C, D, or E if they have been used on an Animal Study Proposal (ASP) or in Column B if they have been weaned but not used (i.e. not yet assigned to an active ASP) and are present as of September 30 of the reporting year. If a pregnant animal of a regulated species is administered a substance to study its effect on the offspring (i.e. the offspring are used to generate data), the offspring and their mother are listed in the appropriate column(s) of the annual report (Columns C, D or E). When fetuses are collected for tissue harvest, only the pregnant female is reported as having been used. Viable offspring that are born as a result of an ASP requiring breeding are counted at weaning or when subjected to experimental manipulation if that manipulation occurs earlier than weaning. Offspring not meeting genetic requirements of the research are counted at the time of euthanasia. (USDA, 2009)

Veterinary Care Procedures:

- Reparative surgery or medical treatments provided or prescribed by a veterinarian for an animal due to non-ASP-related illness or injury are considered normal veterinary care and **do not** determine the placement of the animal in Columns C, D or E. However these procedures must be performed using appropriate analgesics, anesthetics or tranquilizers for alleviation of pain or distress.
- Routine veterinary procedures such as castrations, dehorning and diagnostic procedures performed or prescribed by a veterinarian are considered normal veterinary care and do not determine the placement of the animals in Columns C, D or E. However these procedures must be performed using appropriate analgesics, anesthetics or tranquilizers for alleviation of pain or distress.
- Veterinary interventions to treat or correct medical/surgical conditions resulting from research procedures **are** used to determine the placement of an animal in Columns C, D or E.

Unexpected Pain or Distress: unexpected pain or distress and animal incidents unrelated to ongoing research should be brought to the attention of the IC ACUC for purposes of adequate protocol and program review. The following examples were offered by USDA in 2011 and should be considered:

- An animal accidentally becomes caught in a cage and experiences pain and distress which is completely unrelated to the study. The injuries are treated and appropriate analgesia is provided.
 - *This animal should be reported in the pain category appropriate to its experiences in the study. The accident does not affect the reporting category.*
- An animal is unexpectedly found dead in the cage during the course of a study. The animal had been monitored appropriately and there were no pre or post mortem signs of pain or distress. The animal had not experienced pain as part of the study prior to its death.
 - Column C
- An animal experiences unexpected pain due to the research procedures during the course of the study. The pain is recognized and treated with appropriate analgesics in a timely manner.
 - Column D
- An animal experiences unexpected pain due to the research procedures during the course of the study. The pain is recognized and the animal is euthanized in a timely manner.
 - Column D
- An animal experiences unexpected pain due to the research procedures during the course of the study, but when the pain is recognized, the PI determines that the use of analgesics, anesthetics or tranquilizers would adversely affect the study.
 - Column E
- An animal develops an ear infection and experiences pain or distress entirely unrelated to the research study. Analgesics, anesthetics or tranquilizers would adversely affect the study so the animal is treated with palliative husbandry methods.

(USDA statement) This is challenging and does not easily fit into any of the classifications. Because the pain relief must be withheld due to the study, even though the pain is not caused by a research procedure, it should still be reported as Column E and a justification should be provided for withholding analgesics.

 - Column E

Attachment 2

REQUESTED FORMAT FOR REPORTING EXCEPTIONS TO THE AWAR

51-F-0016

Exceptions to the Animal Welfare Act Regulations and Standards

1. Title and Section of Animal Welfare Act Regulations (9CFR, Subchapter A) for which an IC ACUC-approved exception was granted:
2. Description of and rationale for IC ACUC-approved exception:
3. Species and number of animals affected by this exception:

NOTE: These do not have to be reported 'by protocol', so please consolidate by the title and sections of the AWAR.

Attachment 3

DEFINITIONS AND BEHAVIORAL/CLINICAL SIGNS OF PAIN AND DISTRESS

PAIN

Pain: The International Association for the Study of Pain (IASP; www.iasppain.org) defines pain in humans as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (IASP 1979). Pain typically involves a noxious stimulus or event that activates nociceptors located in the body’s tissues that convey signals to the central nervous system, where they are processed and generate multiple responses, including the ‘unpleasant sensory and emotional experience’ central to the IASP definition.

Pain descriptors

1. Momentary pain: short-lasting, brief, transient (e.g., seconds) and usually with low intensity.
2. Post-procedural/post-surgical pain: longer-lasting than momentary (hours to days to weeks), a consequence of tissue injury due to surgery or other procedures.
3. Persistent pain: lasts for days to weeks such as encountered in studies that investigate pain (and caused by mechanisms other than postprocedural pain).
4. Chronic pain: pain of long duration (i.e., days to weeks to months), typically associated with degenerative diseases, without relief, difficult to manage clinically.

Reference: National Research Council (NRC), 2009. Recognition and Alleviation of Pain in Laboratory Animals

DISTRESS

Distress: Most definitions characterize distress as an aversive, negative state in which coping and adaptation processes fail to return an organism to physiological and/or psychological homeostasis (Carstens and Moberg 2000; Moberg 1987; NRC 1992). Progression into the maladaptive state may be due to a severe or prolonged stressor or multiple cumulative stressful insults with deleterious effects on the animal's welfare. Distress can follow both acute and chronic stress, provided that the body's biological functions are sufficiently altered and its coping mechanisms overwhelmed (Moberg 2000).

The transition of stress to distress depends on several factors. Of clear importance are stressor duration and intensity, either of which is likely to produce behavioral or physical signs of distress. In addition, predictability and controllability, i.e. the ability of the animal to control its environment, are important determinants in the transition of stress to distress.

Reference: National Research Council (NRC), 2008. Recognition and Alleviation of Distress in Laboratory Animals

Attachment 4

Column E Explanation Form for Regulated Species

This form is intended as an aid to completing the Column E explanation. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

- 1. Registration Number: 51-F-0016**
- 2. Number of animals used under Column E conditions in this study. _____**
- 3. Species (common name) of animals used in this study. _____**
- 4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.**

- 5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.**