

Guidelines for Preparing USDA Annual Reports

Animal Welfare Regulations (AWRs) 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. Animal Care Policy #17, "Annual Report For Research Facilities" further explains this requirement (http://www.aphis.usda.gov/animal_welfare/downloads/policy/policy17.pdf) All Institute/Center (IC) programs must submit this report (APHIS Form 7023) to the Office of Animal Care and Use (OACU) each November. The OACU compiles the IC reports into one NIH report. A blank APHIS Form 7023, Form 7023A (Additional), and an accompanying form titled "Explanation for Column E Listing" are appended as Attachments 1, 1A, and 2 respectively. Blank forms and specific instructions will be distributed to each IC in August/September as they are received from USDA.

The Scientific Directors of all IC's that use animals must sign the form (APHIS Form 7023) as the operational Institutional Official within that IC. Their signature provides assurance that the IC is in compliance with the four assurances stated near the bottom of the first page of the form.

GENERAL GUIDELINES

The intent of these guidelines is to standardize the compilation and reporting of animal use in the NIH annual report to the 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.

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.

In this document, instructions concerning Columns B, C, D and E are **not applicable to** aquatic species; and birds, 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 AWRs and must be listed appropriately in Columns A through F.

Regulated species, for the purpose of this Guideline, includes all live warmblooded species acquired or bred specifically by/for NIH for use in the IRP except for: aquatic species; and birds, rats of the genus *Rattus* and mice of the genus *Mus* bred for use in research.

While aquatic species; and birds, rats of the genus *Rattus* and mice of the genus *Mus* bred for use in research, will be 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.

*Number count = animal census on October 1st of the reporting year + all purchased during the year (Oct 1 – Sep 30) + all generated during the year (Oct 1 – Sep 30).

Regulated species being bred, conditioned, or held for use, but not yet used as of September 30 are listed in Column B of the report. The numbers listed in this column reflect a one-day inventory (to be performed on September 30 of the reporting period) of animals being bred, conditioned, or held for use, but not yet used (i.e., not assigned to an active ASP) during the reporting year.

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 NIH annual report.

Animals (regulated species) which have been used during the reporting period are listed in Column C, D, or E of the annual report. Sentinel animals or animals used in training, other than aquatic species, rats of the genus Rattus (bred for use in research) and mice of the genus Mus (bred for use in research), 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.

Reparative surgery or medical treatments provided or prescribed by a veterinarian for an animal due to non-ASP-related illness or injury is considered normal veterinary care and **does not** determine the placement of the animal in Columns C, D or E. Likewise, 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. 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.

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.

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 (usually September 30 of the reporting period) of animals being bred, conditioned, or held for use, but not yet used for such purposes during the reporting year. This is not a tally of the animals held for use throughout the year, but a tally of animals not yet used as of September 30 of the reporting year.

EXAMPLE B1: A breeding colony of squirrels was established to provide animals for research. The animals were not subjected to any procedures except breeding, rearing of offspring, and holding for future use. On September 30, the mated pairs (animals being bred) and their weaned offspring (animals being held for future use), are counted and reported in Column B.

EXAMPLE B2: As of September 30 of the reporting period, a breeding colony of 10 hamsters had 94 weaned offspring and 27 offspring that were still nursing. None of the hamsters had been used. All of the adult hamsters and their weaned offspring are listed in Column B. The 27 offspring still nursing and not yet used to generate data for an ASP are not listed on the annual report.

EXAMPLE B3 Twenty male and 20 female cotton rats were bred and 165 offspring were weaned during the reporting period. Forty five of the offspring and the 40 breeding cotton rats were not subjected to any experimental procedures during the fiscal year and are listed in Column B. One hundred of the offspring were used in research procedures during the reporting period and are listed in the appropriate USDA column (Columns C, D, or E - see below). Twenty of the offspring were euthanized prior to September 30 of the reporting period with no experimental procedures having been performed on them. These twenty cotton rats are listed on the annual report under Column C.

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 AWRs 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."
 - 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 be painful or distressful to the animals, the ACUC will be informed, the ASP modified or halted, and those animals listed in Column E.

NIH has been advised that USDA does not consider approved primary methods of euthanasia to be experimental procedures and subject to animal "Use" classification.

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: An appropriate volume of an isotonic solution was instilled into the upper tracheas of three dogs and promptly removed. The dogs exhibited no signs of pain and only momentary or slight distress from the procedure. These dogs are listed in Column C.

EXAMPLE C6: Five hamsters were inoculated by intra-peritoneal 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.

NIH has been advised that USDA does not consider approved primary methods of euthanasia to be experimental procedures and subject to animal "Use" classification.

EXAMPLE C7: 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 C8: Eight rabbits used for polyclonal antibody production were deeply anesthetized with buprenorphine and ketoprofen 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 C9: 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 C10: 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 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. Animal Care Policy #11, Painful Procedures, provides detailed guidance on painful procedures (http://www.aphis.usda.gov/animal_welfare/downloads/policy/policy11.pdf). 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. Intra-cardiac 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. List the two control animals 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.

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 2) must be attached to the annual report and a copy appended as an attachment to the ASP. The Column E justification forms **must be signed and dated at the end of the reporting period.**

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.

COMMENTS AND QUESTIONS RELATING TO THESE GUIDELINES:

Address comments or questions to the NIH Animal Research Advisory Committee through the Office of Animal Care and Use. Telephone 301-496-5424 or FAX 301-480-8298.

REFERENCES

1. USDA Animal Care Policy # 17, Annual Report for Research Facilities, August 25, 2006. (http://www.aphis.usda.gov/animal_welfare/downloads/policy/policy17.pdf)
2. USDA Animal Care Policy #11, Painful Procedures, April 14, 1997. (http://www.aphis.usda.gov/animal_welfare/downloads/policy/policy11.pdf)
3. Lab Animal, 2006; 35(1) 15-16. A Word from OLAW. Commentary on Protocol Review Column regarding documenting number of animals acquired for research.

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UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. REGISTRATION NO. CUSTOMER NO.

FORM APPROVED
OMB NO. 0579-0036

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

2. HEADQUARTERS RESEARCH FACILITY (Name and Address, as registered with USDA, include Zip Code)

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LOCATIONS(sites)

See Attached Listing

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS FORM 7023A)

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report)	F. TOTAL NO. OF ANIMALS (Cols. C + D + E)
4. Dogs					
5. Cats					
6. Guinea Pigs					
7. Hamsters					
8. Rabbits					
9. Non-Human Primates					
10. Sheep					
11. Pigs					
12. Other Farm Animals					
13. Other Animals					

ASSURANCE STATEMENTS

- 1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, 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 Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). A summary of all the exceptions is attached to this annual report. In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.
- 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 other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL

(Chief Executive Officer or Legally Responsible Institutional official)

I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

SIGNATURE OF C.E.O. OR INSTITUTIONAL OFFICIAL	NAME & TITLE OF C.E.O. OR INSTITUTIONAL OFFICIAL (Type or Print)	DATE SIGNED
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Attachment 2

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. (from ASP, Section F)**

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. (from ASP, Section F)**

Information below will NOT be forwarded to USDA as part of the Annual Report

IC _____ ASP Number _____ ASP Title _____

Attachment 3

DEFINITIONS AND BEHAVIORAL/CLINICAL SIGNS OF PAIN AND DISTRESS

PAIN *

Pain, as defined by the International Association for the Study of Pain is “an unpleasant sensory or emotional experience associated with actual or potential tissue damage”. Pain can be considered a potent source of stress, that is a stressor. It can also be considered a state of stress itself and lead to distress and maladaptive behaviors. Thus, whether pain is considered as a kind of stress or as a stressor depends on the point of reference.

Acute Pain is abrupt in onset and relatively short in duration. It can result from an inflammatory process that originated in damaged tissue, traumatic injury, surgery, or exposure to metabolic, bacterial, or viral disease or toxins. Such pain produces a stress response, but usually does not lead to distress, because the pain is short-lived. The pain is generally alleviated by analgesics and associated distress may be responsive to tranquilizers.

Chronic (Persistent) Pain is slow in onset, its intensity is likely not constant, and it is not necessarily associated with an obvious pathologic condition. It is more likely to lead to distress and maladaptive behavior. Chronic pain generally is not totally alleviated by analgesics but associated distress may be alleviated by tranquilizers.

DISTRESS

“Distress is an aversive state in which an animal is unable to adapt completely to stressors and the resulting stress and shows maladaptive behavior.”

The origin of these stressors can be categorized generally as physiologic, psychologic, or environmental.

Potential causes for physiologic stress are pain (resulting from injury, surgery, or disease), starvation, and dehydration. Potential causes for psychologic stress are fear, anxiety, boredom, loneliness, and separation. Environmental causes of stress include restraint, noise, odors, habitat, people, chemicals, and other animals. The appropriate intervention for alleviating distress depends on an accurate identification of the stressor(s) causing the distress.

*National Research Council (NRC). 1992. Recognition and Alleviation of Pain and Distress in Laboratory Animals. A report of the Institute of Laboratory Animal Resources Committee on Pain and Distress in Laboratory Animals, National Research Council, National Academy of Sciences. Washington, D.C., National Academy Press, 137 pp.