Guideline for Preparing USDA Annual Reports and Assigning USDA Reporting Columns

The Animal Welfare Act and Regulations (AWARs) require each registered 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 year. All Institute/Center (IC) animal programs must submit this report to the Office of Animal Care and Use (OACU) each November. The OACU compiles the IC reports into one, overarching 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.

General Guidelines

The intent of these guidelines is to standardize the compilation and reporting of animal use in the NIH IRP for the 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 USDA 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 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 defined as: all live, warm-blooded species acquired or bred specifically by/for the NIH for use in the IRP *except for* aquatic species; 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 regulated by the AWARs and must be reported in the annual report.

Regulated species that have been used during the reporting period (October 1 through September 30) are reported in Column C, D, or E. Regulated species being held for use, but not yet used are reported in Column B. See details below regarding these four reporting columns and their classification.

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 column (C, D, or E) consistent with the greatest amount of accompanying pain or distress they experienced 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 30 will include it on their report and this IC will make a good faith effort to determine the appropriate column for reporting.

Husbandry, veterinary care, or colony management procedures should not be considered when classifying animals for reporting purposes. Only study-related procedures should be considered.

Reporting Exceptions to the AWAR

A summary of any ACUC-approved exceptions to the regulations or standards of the AWARs must be submitted to USDA as part of the annual report. At a minimum, this summary must include the following:

- Identification of the ACUC-approved exception(s) to the regulations or standards, including exemptions to the dog exercise plan and/or the nonhuman primate plan for environmental enhancement;

- Description of the ACUC-approved exception(s); and
- Identification of the species and number of animals affected in the reporting period.

Supplemental NIH Reporting

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

USDA Annual Report Columns

Column A: Animals Covered by the Animal Welfare Regulations

Include the common names of all regulated vertebrate animal species being held, bred, or used.

Column B: Number of Animals Bred, Conditioned, or Held

Include all animals present on September 30, which were:

- Bred, conditioned, or held for research, testing, teaching, or experiments, but not used in a project, including those that died without being used for a project during the reporting year;
- Used for research, testing, teaching, or experiments in prior years but were not used this reporting year; and
- Used for breeding and their offspring, even if they were not used for research this reporting year. In the NIH IRP, offspring are counted at first cage change or experimental manipulation, whichever occurs first.

Column C: Number of Animals Experiencing No Pain or Distress

Include all animals that underwent study-related procedures that involved no more than slight or momentary pain, distress, or use of pain-relieving drugs (see Attachment 2). Column C procedures include, but are not limited to:

1. Methods of euthanasia conducted in accordance with the current AVMA Guidelines for Euthanasia of Animals and Section 1.1 of the AWARs;
2. Routine procedures (e.g., injections, tattooing, blood sampling);
3. 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 nonhuman primates (NHPs), minimizing animal movement to facilitate anatomical measurements, or preventing animal movement during imaging procedures;
4. Non-surgical catheterization;
5. Manipulative procedures such as injections, palpations, skin scrapings, and radiography;
6. Intracerebral inoculations in neonatal rodents prior to cranial ossification when performed by trained personnel; and
7. Chair restraint of an adapted NHP that has been conditioned for restraint, or the training of an un-adapted 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 distress to the animals, the ACUC will be informed, the ASP modified or halted, and those animals reported in Column E.

Column D: Number of Animals Experiencing Alleviated Pain or Distress

Include all animals that underwent study-related procedures that involved pain or distress that was alleviated with anesthetics, analgesics, or tranquilizers. Column D procedures include, but are not limited to:

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

Column E: Number of Animals Experiencing Unrelieved Pain or Distress

Include all animals that experienced pain or distress that could not be relieved for study-related reasons. A Column E Justification form is required for these animals and must be attached to the ASP and the USDA annual report. Column E procedures include, but are not limited to:

1. Chair-restraint of a NHP that has not been conditioned for the time period of restraint;
2. Drug or radiation toxicity testing producing unrelieved pain and/or distress;
3. LD_{50} determinations or any other studies involving death as an endpoint;
4. The exposure of an animal to an agent which produces unrelieved pain and/or distress; and
5. The exposure of an animal to electrical shocks that are generally accepted as causing pain in humans.

Column F: Total Number of Animals

Include the sum of the animals listed in Columns C, D, and E, by species.

Assurance Statements

The Scientific Directors of all ICs that use animals must sign their IC 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 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 (PI) has considered alternatives to painful procedures.
3) This facility is adhering to the standards and regulations under the AWA, and it has required that exceptions to the standards and regulations be specified and explained by the PI and approved by the IC Animal Care and Use Committee (ACUC). A summary of all exceptions is attached to the annual report. In addition to identifying the ACUC approved exceptions, the 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 provisions of adequate veterinary care and to oversee the adequacy of the other aspects of animal care and use.

Examples

EXAMPLE B1: As of September 30 of the reporting period, a breeding colony of 20 (10 male and 10 female) 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), their weaned offspring (100), and the nursing offspring (30) are listed in Column B. 20 + 100 + 30 = 150 hamsters are reported in Column B.

EXAMPLE B2: 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. 20 + 80 = 100 guinea pigs are reported in Column B.

EXAMPLE C1: Two hundred weaned hamsters received a drug subcutaneously that produced only momentary or slight pain. Two weeks later they were euthanized by CO_2 inhalation. 200 hamsters are reported 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 Use of Adjuvants in Research. 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. 2 rabbits are reported 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. 20 guinea pigs are reported 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. 1 macaque is reported 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. 5 hamsters are reported in Column C.

EXAMPLE C6: Ten guinea pigs were anesthetized with ketamine-xylazine, their vena cavas 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-acceptable adjunctive method of euthanasia. As such, the use of this adjunctive method of euthanasia is reported in Column C.

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-acceptable adjunctive method of euthanasia. As such, the use of this adjunctive method of euthanasia is reported in Column C.

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. 12 hamsters are reported 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 54 gerbils (10 breeders and the 44 offspring) are reported 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 30, all 17 rabbits are reported in Column C 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. 30 hamsters are reported in Column C.

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. 2 control dogs are reported in Column C and the other 18 dogs are reported in 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 scapulae. 12 macaques are reported in Column D.

EXAMPLE E: Twenty guinea pigs were involved in a pain control study. 2 guinea pigs were untreated controls and experienced more than minimal pain or distress because the use of anesthetics, analgesics, or other palliative efforts would compromise the experimental design. 2 untreated guinea pigs are reported in Column E and 18 treated guinea pigs are reported in Column D.

References

1. Lab Animal, 2006; 35(1) 15-16. A Word from OLAW. Commentary on Protocol Review Column regarding documenting number of animals acquired for research.
2. Submitting Research Facility Annual Reports - USDA, APHIS
3. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition

Approved: 03/08/1982
Reapproved: 05/08/1996
Revised: 10/02/1985, 06/11/1986, 08/13/1986, 09/12/1990, 09/30/1992, 09/13/1995, 04/09/1997, 03/27/2002, 05/11/2005, 04/12/2006, 05/16/2007, 12/09/2009, 10/06/2011, 11/14/2012, 12/11/2013, 10/14/2015, 9/27/2017, 09/23/2020, 12/13/2023

Attachment 1: <u>USDA Column Classification - Additional Information</u>

Euthanasia procedures: When euthanasia is performed in accordance with AVMA Guidelines, these procedures are, by definition, painless, and therefore are classified as Column C procedures. This classification applies to AVMA acceptable methods and methods acceptable with conditions, provided that all criteria for application are met.

Vendor procedures: Surgical modifications performed by the vendor prior to delivery to the NIH are not considered when classifying animals into USDA reporting columns. 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 annual report.

Sentinel or Training Animals: These animals are considered to have been "used" and are counted and reported in the appropriate column.

Offspring of Regulated Species: These animals are reported in Column C, D, or E if they have been used on an ASP, or in Column B if they have not yet been assigned to an 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 reported in the appropriate column(s) of the USDA annual report. 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 a breeding ASP are counted at first cage change or manipulation, whichever comes first.

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 classification 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 classification 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 classification of an animal in Columns C, D, or E.

Unexpected Pain or Distress: Animals experiencing unexpected pain or distress, and animal incidents unrelated to ongoing research should be brought to the attention of the IC ACUC for purposes of ASP and program oversight. The following examples should be considered:

- An animal is accidently 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 postmortem signs of pain or distress. The animal had not experienced pain as part of the study prior to its death.
  - *This animal should be reported in 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.
  - *This animal should be reported in 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.
  - *This animal should be reported in 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.
  - *This animal should be reported in 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. Husbandry methods assist in controlling, but do not substantively mitigate, the pain.
  - *Because the research activity did not cause the pain/distress (i.e., caused by an unrelated ear infection), the animal should be reported in the pain category appropriate to its experiences in the study.*

Attachment 2: **Pain and Distress Definitions**

Pain: The International Association for the Study of Pain (IASP; http://www.iasp-pain.org/) defines pain in humans as, "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" (IASP 2020). 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.

Painful Procedure: As applied to any animal means any procedure that would reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure was applied, that is, pain in excess of that caused by injections or other minor procedures (AWA, Part 1, Definitions).

Pain Descriptors:

- Momentary pain: short-lasting, brief, transient (i.e., seconds) and usually with low intensity.
- Post-procedural/post-surgical pain: lasting longer than momentary (i.e., hours to days to weeks), a consequence of tissue injury due to surgery or other procedures.
- Persistent pain: lasts for days to weeks such as encountered in studies that investigate pain (and caused by mechanisms other than post-procedural pain).
- 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: 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.