



Grading of Pain and Stress in Research Using Animals

Level

Examples and Comments

Level 1

Experiments on vertebrate animals that are expected to produce little or no discomfort.

Simple procedures such as injections and blood sampling; observational field behavior; physical examinations; experiments on anesthetized animals that do not regain consciousness; food/water deprivation for short periods and methods of euthanasia that induce rapid unconsciousness.

Level 2

Experiments that involve some minor stress or short-duration pain to vertebrate animals.

With anesthesia, "cut downs" or implantation of catheters; behavioral experiments on conscious animals that involve restraint; immunization employing Freund's adjuvant; noxious stimuli from which escape is possible; surgical procedures under anesthesia that may result in postsurgical discomfort.

Level 3

Experiments that involve significant unavoidable stress or pain to vertebrate animals.

Deliberate induction of behavioral stress; major surgical procedures under anesthesia that result in significant postoperative discomfort or an anatomic or physiologic deficit that will result in pain or distress; noxious stimuli from which escape is impossible; prolonged periods of physical restraint; procedures that produce pain in which anesthetics are not used (toxicity testing, radiation sickness, certain injections, and stress and shock research, experimental infection producing systemic disease or death). Level 3 mandates responsibility on the part of the investigator to explore alternative designs.

Level 4

Procedures that involve inflicting severe pain on unanesthetized, conscious animals.

Use of muscle relaxants or paralytic drugs without the use of anesthetics; surgery, severe burn or trauma infliction on unanesthetized animals; attempts to induce psychotic-like behavior or severe stress or terminal stress. Many of these procedures are specifically prohibited and therefore may result in withdrawal of federal funds and/or institutional USDA registration.

**Note: The preceding levels correspond to the following animal use categories on the APHIS annual report form:
Level 1 = Category C or D; Level 2 = Category D; Levels 3 and 4 = Category E.**