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Standard Practice for Detailed Clinical Observations of Test Animals[1]

This standard is issued under the fixed designation E2045; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice describes the terms used in observing and recording cutaneous, gastrointestinal, respiratory, reproductive, neuromuscular, ocular, and general clinical signs of animals undergoing toxicological testing. This practice also assists in properly observing and assessing laboratory animals for signs of disease or adverse effects of compound administration.

1.2 This practice includes codes and descriptions for a wide variety of clinical signs, anatomical locations, and other descriptive qualifiers, and a technique for scoring the extent or severity of clinical signs.

1.3 This practice assumes that the reader is knowledgeable in animal toxicology and related pertinent areas and is trained in making clinical observations.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.5 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *Federal Standards:*

Title 40, Code of Federal Regulations (CFR), Environmental Protection Agency, Subchapter E, Pesticide Programs, Part 160, Good Laboratory Practice Standards[2]

Title 40, Code of Federal Regulations (CFR), Toxic Substances Control Act, Part 792, Good Laboratory Practice Standards[2]

Title 40, Code of Federal Regulations (CFR), Environmental Protection Agency, Part 798, Health Effects Testing Guidelines[2]

3. Significance and Use

3.1 This practice pertains to all forms of toxicological testing (acute, subchronic, or chronic) performed by any route of administration (inhalation, oral, dermal, ocular, or other).

3.2 The U.S. Environmental Protection Agency, Good Laboratory Practices for Nonclinical Laboratory Studies, as listed in 40 CFR, requires that a testing facility maintain specific standard operating procedures (SOPs) including an SOP covering clinical observations in test animals.

3.3 This practice serves as a basis for consistency in clinical observations and is not meant to serve as a comprehensive list of observations that may be observed. Actual procedures and forms to be used in recording observations must be described in individual study protocols.

4. Procedure

4.1 Observe the health of an animal at a distance and of its housing environment to gain a general impression of its health. Also note environmental factors such as temperature, humidity, ventilation, air quality and hygienic conditions.

4.2 Observe each animal and note any subtle changes in animal behavior, physical appearance, posture, gait, vocalization, food and water consumption, and waste production. See Section 5 for details.

4.3 Observe animals blinded to treatment group, or at least in random order, to minimize unintentional bias.

4.4 Note any dead animals and collect necessary tissues and data to minimize the extent of tissue decomposition.

4.5 Report animals that show signs of sickness so that appropriate diagnosis, treatment, or euthanasia, if appropriate, can be performed.

5. General Clinical Signs

5.1 Note the overall activity, behavior, and condition of the animal. Determine the hydration status by examining skin turgor, position of the eyes such as normal or sunken, mucous

[1] This practice is under the jurisdiction of ASTM Committee E50 on Environmental Assessment, Risk Management and Corrective Action and is the direct responsibility of E50.47 on Biological Effects and Environmental Fate.

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[2] Available from U.S. Government Printing Office, Superintendent of Documents, Washington, DC 20402.

membrane color, and capillary refill time. Look for asymmetry or the presence of abnormal swellings, hemorrhage or signs of pain.

5.2 The following are some general conditions along with suggested codes for record keeping that do not fall into any specific organ system. Refer to Annex A1 – Annex A3 for a detailed listing of the codes and their descriptions. Other general reference material will also be helpful.[3,4,5]

5.2.1 Activity may be described as: decreased (ACD); increased (ACI); hyperexcitable (HX); hyperactive (HYP); lethargic (LE); irritable (IRR); moribund (MB), that is near death; prostate (PRO), that is, exhibiting inability or unwillingness to maintain upright posture.

5.2.2 Body condition may be described as: obese (OBS); thin (THN); decreased rectal temperature (BTD); increased rectal temperature (BTI); hypothermia that is cold to touch (HPO); hyperthermia that is warm to touch (HPR).

5.2.3 Death may be described as: accidental death (AD); euthanized (ETH); found dead (FD).

5.2.4 Examine skin for dehydration (DHY). The skin should fall back into place immediately after it is pulled out of position or tented; if the skin is pulled out of position and tends to stick together or slowly fall back into place, the animal may be dehydrated. Other signs of dehydration include sunken eyeball (SUN), pale or dry mucous membranes (MM), and a capillary refill time of >3 s (CR4). Distinguish between dehydration and various types of shock.

5.2.5 Generalized edema (EDE) may appear as swelling of the limbs, lower abdomen, head or under the mandible. When the apparently fluid-filled tissue is pressed, an indentation may persist for a short time.

5.2.6 Evidence of hemorrhage (HE) may appear on the haircoat (HEH) or underlying skin or nails (HES), in urine (HEU) or feces (HEF), from the mouth (HEM), nose or epistaxis (EPI), eyes (HEO), ears (HEE), genitalia (HEG), or anus (HEA).

5.2.7 Jaundice (JAU) is an overall slight yellow to pale orange tinge to the skin and mucous membranes.

5.2.8 Mucous membrane condition (MM) is noted by the color and condition of the mucous membranes of the eye, nose, mouth, or external genitalia.

5.2.9 Swelling (SW) is noted by the size, location and probable cause, such as edema (SWE) from: a solid tissue or tumor (SWT); blood (SWH); air (SWA); or pus (SWB).

6. Specific Clinical Signs

6.1 Inspect the entire haircoat and underlying skin for integumentary signs. Some common clinical signs and their suggested codes are as follows:

6.1.1 Alopecia, that is, hair loss (ALO), includes hair thinning, patchy/focal hair loss or balding.

6.1.2 Haircoat condition (HC) may be described as: oily (HCO); rough (HCR); wet (HCW); soiled (HCS); dry (D); bloody (HEH); or piloerection (HCP), that is, distinctly raised fur, excluding the vibrissae, giving a bristled or porcupine-like appearance.

6.1.3 Swelling (SW) is an increase in tissue size or increased abnormal shape of the skin or other organs from abnormal presence of: air, that is, emphysema (SWA); fluid or water, that is, edema (SWE); solid tissue or tumor (SWT); blood, that is, hematoma (SWH); pus, that is, abscess (SWB).

6.1.4 Skin condition (SK) may be described as: thickened (SKT); thinned (SKH); scaly (SKS); dry (SKD); or red (SKY) (see 6.1.5).

6.1.5 Erythema (ERY) is an increased pink or red color on smooth skin.

6.1.6 Rash (RAS) is small red, pink or white dots or pustules on the skin; petechiae (PET) are red dots formed from blood.

6.1.7 Blisters (BLS) are fluid-filled vesicles. The fluid is usually clear, but can be pink (blood-tinged) or red/brown (filled with blood). White-filled vesicles are either pustules (RAS) (< 5 mm) or abscesses (SW) (> 5 mm).

6.1.8 Color change (CC) may be other than pink or red, for example, bluish-black as in brushing, green as in bruising or severe infection, brown as in increased pigmentation, or white as in blanching.

6.1.9 Abrasions (ABR) are denuded skin or mucous membrane.

6.1.10 Lacerations (LCN) are cuts in the skin from mechanical injury, that is, bite, scratch, foreign object, and so forth.

6.1.11 Ulceration (ULC) is an open sore accompanied by the disintegration of tissue, usually with necrosis, that is, death of tissue.

6.1.12 Scab (SCB) is an eschar formed from sloughed skin.

6.1.13 Pruritis (PRU) is itching evidenced by scratching, with or without a rash or abrasion.

6.1.14 Urticaria (URT) is a transient appearance of smooth, slightly elevated bumps which are redder or paler than the surrounding skin and often accompanied by severe itching. Uricaria often appears as localized, discrete or confluent areas of edema.

6.1.15 Purpura (PUR) is confluent petechiae, that is, pinpoint hemorrhages, which form ecchymoses, that is, blotchy hemorrhages over any part of the body.

6.1.16 Common manifestations of dermal sensitivity reactions are:

6.1.16.1 Contact dermatitis (COD) is pruritis, erythema and vesiculation that may be followed by pustulation and necrosis and that has a pattern consistent with the touch of a foreign object or substance.

6.1.16.2 Exanthema (EXA) is macular or papular redness in discrete areas.

6.1.16.3 Exfoliation (EXF) is loss of superficial skin layers with redness, swelling, and presence of free blood.

6.1.16.4 Bullous eruption (BUL) is the presence of discrete serous or seropustular areas.

6.1.16.5 Erythema multiform (EMF) is the presence of multiple types of macules, papules and nodules.

[3] Taylor, E.J., ed., *Dorland's Illustrated Medical Dictionary*, W.B. Saunders, Philadelphia, PA, 27th edition, 1988.

[4] *Stedman's Medical Dictionary*, Williams and Wilkins, Baltimore, MD, 25th edition, 1990 .

[5] Thomas, C.L., ed., *Tabor's Cyclopedic Medical Dictionary*, F. A. Davis Co., Philadelphia, PA, 17th edition, 1993.