Designation: ~~E2045 – 99 (Reapproved 2014)~~ E2045 – 22

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~~ safety, health, and ~~health~~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.

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[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.

3.3 This practice serves as a basis for consistency in clinical ~~observations.~~ 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 ~~control animals first, followed by test groups in order of increasing level of treatment. Observe positive control group, if any, last.~~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 ~~before decomposition occurs.~~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 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;~~ 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.

[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.

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.

6.2 Gastrointestinal signs are observed during external evaluations of the gastrointestinal system conducted from the oral cavity to the anal area. Visually inspect the teeth and mucous membranes of the oral cavity, palpate the abdomen and inspect the perianal area. An inspection of the animal's cage will allow evaluation of the volume, color, and consistency of the stool.

6.2.1 Oral cavity signs and codes are:

6.2.1.1 Salivation may be described as: increased salivation (SAL); or lack of salvia, that is, xerostomia (XER).

6.2.1.2 Dentition (TE) includes: missing teeth (TEM); loose teeth (TEL); discolored teeth (TEC); damaged teeth (TED); or malocculation (TEO).

6.2.1.3 For mucous membranes, note capillary refill time (CR), color, and condition, such as erosions or vesicles.

6.2.1.4 Gums may be described as: healthy, intact (GUH); or gingivitis (GUI), that is, inflamed or bleeding gums.

6.2.2 For the abdomen, look at the overall symmetry and size. The abdomen may be described as: smaller or more "tucked-in" (STA) if the animal is dehydrated; larger as distended or pendulous abdomen (OPA); or asymmetrical as if an enlargement or swelling in a focal area (SW).

6.2.3 In the perianal area, look for: abnormal anal sphincter (OEA); fecal (FEF) or urine staining (PEU); matter hair (PEH); mucous (PEM); or rectal prolapse (RPR), that is, a red to dark red tubular protrusion from the anus.

6.2.4 For feces, note: consistency, such as normal (FEN), hard or dry (FEH), soft or watery (FED), oily (FEO); amount, such as none (FEA), small (FES), normal (FEN) or large (FEL); content, such as normal (FEN), blood (FEB), mucous (FEM) or foreign material (FEF); or evidence of straining to defecate, that is, tenesmus (TEN). Hard feces may indicate constipation. Soft or watery feces may indicate diarrhea.

6.2.5 Function may be anorexia or loss of appetite (ANO), emesis or vomiting (EM).

6.3 Respiratory signs are found by evaluating the following:

6.3.1 Rate of breathing (RR) may be slow (RRS), normal (RRN), or fast (RRF).

6.3.2 Depth of breathing (RD) may be shallow (RDS), normal (RDN), or deep (RDD).

6.3.3 Difficulty in breathing is dyspnea (DYS).

6.3.4 Periodic cessation of breathing is apnea (APN).

6.3.5 Nasal discharge (ND) is either none (NDN), clear (NDC), red (NDR), yellow (NDY), green (NDG), or white (NDW).

6.3.6 Respiratory sounds include rales (RAL), coughing (COU), gasping for air (G), and sneezing (SNE).

6.3.7 Epistaxis (EPI) is the free flow of blood or hemorrhage from the nose. This is sometimes indicated by the presence of dry blood or dark material around the nose.

6.4 For reproductive or urogenital signs, carefully examine the external genitalia and look for evidence of normal or abnormal reproductive discharge. Examine the amount and color of urine and whether or not the animal is expressing signs of incontinence or difficulty in urination (difficult to distinguish from tenesmus). When applicable, note breeding behavior, pregnancy, abortion, and quality of mothering.

6.4.1 Male examination is as follows:

6.4.1.1 For external genitalia, look for the presence (TSB), absence (TSA or TSC), and condition of the testicles (TSE, TED, or TSN); paraphimosis (PM), that is, inability to retract penis into foreskin, or abnormal discharge from the penis.

6.4.1.2 Penile discharge is either none (PDA), normal (PDN), increased (PDI), bloody (PDB), serous (PDS), or mucous (PDM).

6.4.2 Female examinations include:

6.4.2.1 In external genitalia look for mucous membrane abnormalities (MM) or swellings (SW) of the vagina (VA) or vulva (VA).

6.4.2.2 In vaginal discharge types or consistency of discharge are: none (VDN), normal (VDN), increased (VDI), decreased (VDD), bloody (VDB), serus (VDS), or mucous (VDM).

6.4.3 For urine or urination note the following conditions:

6.4.3.1 Hematuria (HEU) is bloody urine. Use caution with rabbits as their urine may range from amber to light red without the presence of blood.

6.4.3.2 Anuria (ANU) is the absence of urine for a prolonged period.

6.4.3.3 Dysuria (DYU) is difficulty urinating; be sure to differentiate with tenesmus.

6.4.3.4 Polyuria (PLY) is excessive volume of urine.

6.4.4 Fertility signs include the following:

6.4.4.1 Breeding efficiency may be failure to breed (FTB), conceive (FTC), or low litter size or weight (LLS).

6.4.4.2 Abortion (ABO) is premature delivery of dead offspring.

6.4.4.3 For pregnancy status, animal appears pregnant (APR).

6.4.4.4 Mothering may be described as: poor care of young (PCY); evidence of cannibalism (CAN); or poor milk production (PMP).

6.5 Neuromuscular signs include disease, trauma, and compounds which adversely affect the central or peripheral nervous system resulting in structural or functional changes being seen in the neuromuscular system, or degree of alertness or activity in animals. Evaluation of locomotion and coordinated movements may reflect the status of the animal's neuromuscular system.

6.5.1 Musculoskeletal signs may be lameness or weight bearing (LMW) or non-weight bearing (LMN), limb paralysis (LP), or enlarged appendage (ENA).

6.5.2 Posture or head carriage may be normal (HDN), tilted (HDT), raised (HDR), lowered (HDL), or hunched posture (HP); gait may be normal (GAN), exaggerated (GAE), or slow (GAS).

6.5.3 Central nervous systems signs include comatose (COM), tremors (TR), convulsions (CON), ataxia (ATX), circling (CIR), or paralysis (PAR).

6.6 For ocular signs, carefully examine the orbit and eyeball including the eyelids, conjunctiva, cornea, sclera and pupil. If necessary, use a penlight to determine the direct and consensual pupillary light reflexes. Note abnormal eye movements such as excessive squinting, nystagmus, photophobia and apparent inability to see. Note the presence, consistency and color of any tearing or discharge.

6.6.1 Squinting (SQ) ~~is blepharosis or~~ involuntary partial eye closure (see also 6.6.9).

6.6.2 Excessive blinking (BLI).

6.6.3 Conjunctivitis (CJS) is inflammed conjunctiva or reddening and swelling of membranes around the eyeball.

6.6.4 Tearing (LAC) is lacrimation or excessive secretion of clear tears.

6.6.5 Chromodacryorrhea (CHR) is brown, red, or yellowish ~~tears.~~tears due to release of porphyrins from the Harderian lacrimal glands.

6.6.6 Crusty eyes (CRE) denotes a collection of dried material around the eyeball.

6.6.7 Miosis (MIO) is the contraction of the pupil, that is, dark portion, of the eye.

6.6.8 Mydriasis (MYD) is dilation of the pupil, that is, dark portion, of the eye.

6.6.9 Photophobia (PHB) is squinting due to light intolerance.

6.6.10 Eyelid ptosis (PTO) is drooping of the upper eyelid.

6.6.11 Relaxed nictitating membrane (RNM) is protruding or prominent third eyelid.

6.6.12 Nystagmus (NYS) is involuntary, rapid eye movement.

6.6.13 Pannus (PAN) describes blood vessels or granular tissue visible on the cornea.

6.6.14 Corneal opacity (COP) is when the cornea is opaque, but does not seem ~~inflammed.~~inflamed.

6.6.15 Scleritis (SCL) is inflammation of the sclera or white part of the eyeball.

6.6.16 Lens opacity or cataract (CAT) is an opaque lens, apparent behind the pupil.

6.6.17 Exophthalmos (EXO) is a protrusion of the eyeball from the bony orbit.

6.6.18 Microphthalmia (MIC) is when the eye appears abnormally small.

6.6.19 Apparent blindness (BLD) is seeming inability to see objects.

6.6.20 Pupillary light reflex is as follows:

6.6.20.1 No direct light reflex (PLD) is when the pupil fails to constrict when stimulated with light.

6.6.20.2 No consensual light reflex (PLC) is when light is shined in one eye and the pupil of the opposite eye fails to constrict.

6.6.21 Sunken eye ball (SUN) is when the eyeball appears to be sunken into the orbit.

7. Quality Assurance

7.1 To ensure the quality and reliability of data developed using this practice, follow good laboratory practices (see Section 2).

8. Keywords

8.1 adverse reactions; animals; clinical codes; clinical signs; toxicity

ANNEXES

(Mandatory Information)

A1. CLINICAL SIGNS AND CODES

A1.1 Table A1.1 gives the codes for the clinical signs described in this practice.