Contains Non-Binding Recommendations

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Guidance for Industry

Target Animal Safety for Veterinary Pharmaceutical Products

VICH GL43

This VICH document supersedes FDA Guidance for Industry #33, "Target Animal Safety Guidelines for New Animal Drugs" dated 6/89.

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Additional copies of this guidance document may be requested from the Communications Staff (HFV-12), Center for Veterinary Medicine, Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, and may be viewed on the Internet at http://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/default.htm.

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VICH GL 43 (TARGET ANIMAL SAFETY) - PHARMACEUTICALS
July 2008
For implementation at Step 7

TARGET ANIMAL SAFETY FOR VETERINARY PHARMACEUTICAL PRODUCTS

Adopted at Step 7 of the VICH Process

by the VICH Steering Committee

in July 2008

for implementation in July 2009

THIS GUIDELINE HAS BEEN DEVELOPED BY THE APPROPRIATE VICH EXPERT WORKING GROUP AND HAS BEEN SUBJECT TO CONSULTATION BY THE PARTIES, IN ACCORDANCE WITH THE VICH PROCESS. AT STEP 7 OF THE PROCESS THE FINAL DRAFT IS RECOMMENDED FOR ADOPTION TO THE REGULATORY BODIES OF THE EUROPEAN UNION, JAPAN , AND USA.

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This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

1. INTRODUCTION

Data from target animal safety (TAS) studies are required for registration of veterinary products[1] in the regions participating in the VICH. International harmonization of standards for essential TAS studies will facilitate adequacy of data and minimize the need to perform separate studies for regulatory authorities of different countries. Appropriate international standards should reduce research and development costs by minimizing repetition of similar studies in each region. Animal welfare should benefit because fewer animals may be needed. This VICH TAS guidance has been developed as a harmonized standard to aid in development of mutually acceptable TAS studies for relevant governmental regulatory bodies. The use of this VICH guidance to support registration of a product for local distribution only is strongly encouraged but is up to the discretion of the local regulatory authority.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word "should" in Agency guidances means that something is suggested or recommended, but not required.

1.1 Objective

The purpose of this harmonized guidance is to provide recommendations regarding TAS evaluation for regulatory submission of an Investigational Veterinary Pharmaceutical Product (IVPP), which is appropriate for determining the safety of an IVPP in the target animal, including identification of target organs, where possible, and confirmation of margin of safety, using the minimum number of animals appropriate for the studies.

1.2 Background

The VICH was initiated to develop internationally harmonized guidance that outlines recommendations for meeting regulatory requirements for registration of an IVPP in the regions

[1] *See 21* U.S.C. § 360b(b)(1); 21 U.S.C. § 360b(d)(1)(A); 21 CFR 514.1(b)(8).

participating in the program. By their nature, guidance documents cannot address all possibilities. The TAS Expert Working Group has developed the general principles included in this guidance document to aid in the development and conduct of TAS studies and to support the monitoring of potential adverse events in field studies. For more specific recommendations, review of the study protocol by the relevant regulatory authorities prior to the initiation of the study is encouraged, where review is available.

If in a particular circumstance an alternative approach is deemed more fitting, preparation of a reasoned explanation by the sponsor and discussion with the regulatory authorities is encouraged before work is initiated.

1.3 Scope

This guidance document is intended to cover TAS evaluation for any IVPP used in the following species: bovine, ovine, caprine, feline, canine, porcine, equine, and poultry (chickens and turkeys). The recommendations in this guidance may not be appropriate for registration by national or regional authorities of products for use in minor species or minor uses. The guidance does not provide information for the design of TAS studies in other species, including aquatic animals. For other species and for minors uses, TAS studies should be designed following national or regional guidance.

This guidance contributes to the international harmonization of methods used for evaluation of IVPPs. The guidance is provided to aid sponsors in preparing and conducting TAS studies under laboratory conditions and in the field. All recommendations in this document may not be necessary for every IVPP. For other IVPPs, additional information not specified in this document may be important to show target animal safety.

2. MARGIN OF SAFETY STUDIES

The aim of TAS studies is to provide information on the safety of an IVPP in the intended species under the proposed conditions of use. The margin of safety study is indispensable in the approval of an IVPP.[2] Furthermore, adverse effects associated with overdoses and increased duration of administration of the IVPP should be identified, if possible. Dose confirmation and field studies conducted to confirm the effectiveness of the IVPP provide further information on safety in the target species. Depending on the known or suspected properties of the IVPP, it may be necessary to conduct additional toxicologic or specialized tests.

The margin of safety may be documented if the study includes both the recommended dose and overdoses, given for the proposed and longer treatment periods. The selection of dose and overdose levels and durations of treatment should always be justified by the sponsor, taking into account the proposed use of the product and the known pharmacologic and toxicologic properties of the active pharmaceutical ingredient (API). Where usage or formulation involves a greater risk or consequence of overdose, then a separate study, or inclusion of a higher dose level in the

[2] *See* 21 U.S.C. § 360b(b)(1); 21 U.S.C. § 360b(d)(1)(A); 21 CFR 514.1(b)(8)

margin of safety study, is recommended for the IVPP. This may include cases where a dose calculation error may be likely, such as a mistake in decimal point identification during addition to feed.

The design of the TAS evaluation and the prediction of potential adverse effects that may occur in the target species should be assisted by reference to data including published literature and preliminary studies, including pharmacokinetics, pharmacodynamics and toxicology from target and non-target laboratory animal studies. The specific information used to evaluate the safety of an IVPP depends on factors such as proposed usage regimen and dose, type of drug, chemistry and manufacturing considerations, claims, previous use history of similar products, and animal species including class and breed. Appropriate observations, physical examinations, clinical pathology tests (hematology, blood chemistry, urinalysis, fecal analysis, etc.), necropsy and histopathology should be conducted to identify possible adverse effects of the IVPP.

Margin of safety studies are generally required for new salts or formulations of an API.[3] Exceptions should be justified, for example, on the basis of known toxicology and target animal safety profiles for the API, widespread clinical use of existing products, and/or where the systemic or local exposure (as applicable) of the new product is proven to be equivalent to or less than that of the existing product.

If systemic exposure to the API is negligible, and based on pre-existing knowledge in pharmacology and toxicology there is no safety concern, then the margin of safety study may not be needed. This should, however, be justified by the sponsor, and a safety study at the site of administration (see 3.1. to 3.4.) is recommended.

2.1 Standards

Margin of safety and other laboratory safety studies must be performed in conformity with the principles of Good Laboratory Practices (GLP).[4] The concepts of current Good Manufacturing Practices (cGMP) must be applied to the IVPP as appropriate for new animal products intended for investigational use.[5]

2.2 Animals

Healthy animals, representative of the species and class in which the IVPP will be used, should generally be used in TAS studies. The age of animals should be considered carefully; if the product is intended for use in young, immature animals, then the animals in the TAS studies should generally be the youngest age for which product approval is sought. Otherwise, healthy young mature animals should be used. Additional studies may be needed for potentially sensitive subpopulations, if such groups have been identified in the intended target population.

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[3] *See* 21 U.S.C. § 360b(b)(1); 21 U.S.C. § 360b(d)(1)(A); 21 CFR 510.3(i)

[4] *See* 21 CFR Part 58.

[5] *See* 21 U.S.C. § 351(a)(2)(B); 21 CFR Parts 210 and 211.

Acclimatization of the animals to the study conditions is recommended. Treated and control animals should be managed identically and prophylactic treatments completed before the baseline period of the study, where possible. Use of concurrent therapy with other products during the study may make it more difficult to identify safety concerns due to the IVPP and is not recommended. Studies should be carefully planned to provide adequate information while minimizing the number of animals used. Housing and husbandry should be adequate for the purpose of the study, as well as conform to local animal welfare regulations. Environmental conditions, diet, and water should be controlled throughout the study as appropriate to the species, physiological state, and age. It is recommended that quality and composition of diet and water are monitored throughout the study. Reduction or elimination of suffering during the study is essential. Euthanasia and necropsy of moribund animals is recommended.

2.3 Route of Administration

The IVPP to be evaluated should be the product intended to be marketed. If the market formulation is not used, comparative (bridging) studies may be necessary, e.g., the relevance of TAS data for one formulation of an IVPP to another formulation can be demonstrated by the use of bioequivalence or other data between the two formulations. The IVPP should be evaluated by comparison to a placebo (e.g., saline) or untreated control. The formulation details, generic or trade name, and batch number should be documented. Details of preparation, handling and storage of the IVPP should be documented and the product should be used in accordance with the study protocol. The site of administration is to be identified. Dosing should follow the use conditions suggested in the proposed labeling. If food affects API bioavailability, animals should be fed or fasted before administration to provide the highest likelihood of showing adverse effects. If volume or palatability becomes a limiting factor for higher dose levels, alternative techniques (for example, multiple sites, gavage, or increased frequency of administration) may be considered. If multiple routes of administration are proposed by the sponsor, the route that is most likely to cause adverse effects should be selected as the basis for safety studies. Additional studies on local tolerance (see 3.1. or 3.2.) should be conducted, as appropriate.

2.4 Dose, Frequency, and Duration of Administration

The general design for margin of safety studies uses multiples of the proposed use dose and duration of administration of the IVPP. Specific dose, frequency, and duration combinations for use in TAS studies should be selected and justified based on the pharmacology and toxicology of the IVPP.

Unless otherwise justified by the pharmacologic-toxicologic properties of the API and the proposed use of the product, the design of the margin of safety study should include a negative control, the highest recommended dose level (1X), and two multiples of this use dose (in most cases three times (3X) and five times (5X)) for a period of time in excess of the recommended maximum duration of use. The highest recommended dose level (1X) is defined as the highest actual dose that will be stated on the proposed product label. The highest dose may be for the lowest body weight animal in a weight range for which a fixed mass of the API in a discrete dosage form (e.g., unit dose volume, tablet combination etc.) is recommended. In some regions,

alternative designs based on the pharmacology and toxicology may be acceptable, for example, the administration of the product in excess of the recommended maximum duration of treatment given only in the highest recommended dose level (1X). Regardless of design, a negative control should always be included.

In general, it is recommended that each group be treated for at least 3 times the proposed duration up to a maximum of 90 days (for example, for a proposed single use IVPP, treatments should be administered for 3 consecutive intervals as determined by the pharmacologic characteristics of the IVPP; or for a proposed daily dosing for 7 days, treatments should be given for 21 consecutive days). If short-term, intermittent use is intended, treatments should be administered 3 times at the recommended interval (for example, proposed weekly treatments should be administered for 3 consecutive weeks). Where product use is expected to exceed 3 consecutive months in individual animals then, depending on pharmacology and toxicology, longer duration studies may be recommended up to 6 months or longer if appropriate (e.g., where drug accumulation may increase over time or where duration of drug activity following a single dose exceeds 2 months).

2.5 Study Design

The most important techniques for avoiding bias in studies are randomization and masking (blinding). A randomization plan should be used to allocate animals to treatment groups. Blocking may be used to control, as far as possible, the distribution of the one or two most important factors, such as sex, age, stage of lactation or body weight to ensure balance between treatments.

Target animal safety studies typically include relatively small numbers of experimental units (generally 8 per treatment) and assess large numbers of variables. Both males (4 per treatment) and females (4 per treatment) should be included unless the product is only intended for use in one sex. Medical, animal welfare, and statistical considerations are generally used to determine the total number of animals used to evaluate potential safety concerns. When interim sacrifice or withdrawals of animals for other reasons are anticipated, the number of experimental units should be increased accordingly. Although there is strong interest in the null hypothesis of no difference between treatments, study design constraints limit the statistical power and discriminatory ability of these studies. Under these conditions, statistical analysis alone may not detect potential adverse effects and thus provide assurance of safety. Results should be evaluated and interpreted based on a combination of medical, toxicologic, and statistical principles with consideration of biological significance and plausibility.

Where group housing is needed to provide appropriate animal welfare and allow for adequate experimental conditions, certain variables, such as diarrhea, vomiting, or feed or water consumption may be difficult to measure on an individual animal basis. In addition, even measurements that can be accurately made for individual animals from the same cage or pen may be influenced by the presence of the other animals in the group. For example, presence of a dominant animal in a pen may be a contributing factor in weight loss among other animals in the pen. Potential influence of group housing should be taken into consideration in interpreting drug

effects from a study, even if statistical analyses are not used. Failure to consider this information might lead to incorrect attribution of effect to the IVPP.

The planned times for measuring each variable should be described in the study protocol. Often this schedule includes daily observations of animals throughout the study period, with more detailed measurements at several time points, including the beginning and end of the study. Pretreatment measurements should be made to identify baseline levels. Measurement at time points during the proposed label duration of IVPP use may help characterize the time course of potential safety issues. Equally-spaced measurement intervals may facilitate statistical modeling. For longer studies, data collection may be planned in designated phases, with different frequencies of data collection.

Data should be collected in a manner that minimizes bias. For example, when examinations are needed on a subgroup from each treatment, animals should be randomly designated before study initiation. Personnel collecting data, including gross *post mortem* results, should be masked to treatment. Histopathology data should be evaluated by recognized procedures (e.g., Crissman et al., Toxicologic Pathology, 32(1), 126-131, 2004).

2.6 Variables

Many variables are measured during an animal safety study. The types of observations, examinations and tests for safety depend on the nature of the IVPP, proposed use, target animal, and potential for adverse effects. In general, there are four types of variables that should be considered in margin of safety studies: physical examinations and observations, clinical pathology tests, necropsy, and histopathology examinations. In addition, other types of variables may be considered, such as toxicokinetic evaluation of drug exposure (e.g., sparse sampling around the expected peak and trough concentration times). However, care should be taken to minimize the number of additional sampling points to avoid interference with primary safety endpoints.

2.6.1 Physical Examinations and Observations

A detailed physical examination by qualified personnel (generally a veterinarian) should be conducted at several time points during the study, including the beginning and end. Baseline observations of other variables should be made by qualified personnel at the beginning of the study. Observations relating to general health and behavior by trained personnel (not generally a veterinarian) should be recorded on all animals daily, seven days a week, or at pre-determined intervals appropriate for the purpose of the study, during the entire period. Food and water consumption should be monitored at appropriate intervals. Body weights should be measured at the beginning, end, and several other appropriate times.

Generally, the following should be considered and measured depending on the nature of the IVPP and the intended population:

General Physical Examination (these generally should be done by a veterinarian)

Ocular system	Nervous system
Musculoskeletal system	Integumentary system

Cardiovascular system	Respiratory system
Reproductive system	Urinary system
Lymphatic system	Gastrointestinal system
Behavior	

Specific Examination of Injection/Application Site (semi-quantitative or quantitative assessment should be used wherever possible)

Appearance (e.g., erythema, eschar formation, hair loss, scaling, pigmentation)	Swelling
Pain	Heat

Observations (these should be done by appropriately trained staff)

Feed intake	Water intake
Weight	Behavior
Body temperature	Signs of illness
Feces (consistency, color and mucus, blood)	

2.6.2 Clinical Pathology Tests (Hematology, Blood Chemistry, Urinalysis)

Hematology, blood chemistry, and urinalysis should be conducted at several points during the study, including at the beginning and end of the study. Other specialty tests to monitor appropriate physiologic parameters may be appropriate, depending on the IVPP. A standardized feeding schedule prior to sample collection should be followed. Tests should be conducted on all animals or, where group size is greater than 8 (e.g., poultry), on subsets of animals that were selected for testing by a random process carried out at the beginning of the study. These tests are subject to influence by the conditions under which the samples are collected such as feeding or fasting, and sedation or anesthesia, and therefore it is critical that samples are collected in the same manner from concurrent negative control and treatment groups of animals. Blood samples from multiple animals should not be pooled. For animals showing adverse events, additional clinical pathology and other diagnostic tests to determine the etiology may be appropriate. Collection of clinical pathology data twice during the pre-treatment stage can be helpful in providing reliable baseline data for interpretation of study results. Measured variables, depending on the nature of the IVPP and the intended population, may include (units should be appropriate for regions):

Hematology

Erythrocytes: Total counts; and if applicable, reticulocyte count	Leukocytes: Total and differential counts
Packed cell volume (PCV)	Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC)	Hemoglobin
Prothrombin time	Platelet count
Activated partial thromboplastin time	Buccal mucosal bleeding time
Whole blood clotting time	Fibrinogen
Acute phase protein	

Blood Chemistry

Sodium	Urea nitrogen
Potassium	Creatinine
Chloride	Alanine aminotransferase (ALT)
Calcium	Aspartate aminotransferase (AST)
Phosphate	Lactate dehydrogenase (LDH)
Magnesium	Gamma-glutamyltransferase (GGT)
Total protein	Alkaline phosphatase (AP)
Albumin	Creatine kinase (CK)
Globulin	Total bile acids
Glucose	Cholesterol
Amylase	

Urinalysis

Color	Protein
pH	Ketone bodies
Specific gravity (e.g., by refractometer)	Bilirubin
Glucose	Urobilinogen
Microscopic examination of sediment (crystals, casts, RBCs, WBCs, epithelial cells)	

2.6.3 Necropsy and Histopathology Examinations

Tissues from all dose groups should be examined grossly and preserved for microscopic evaluation. Gross and microscopic examination of tissues of animals in all dose groups is recommended for IVPPs containing a new API, due to the small number of animals used and a general lack of other safety information in the target species. For other products, at a minimum, tissues from all animals in the negative control and highest dose groups should be examined microscopically (for recommended procedures, see Crissman et al., Toxicologic Pathology, 32 (1), 126-131, 2004). If lesions are found in any tissue from the highest dose group, then samples from tissues in animals in the next lowest dose group of the IVPP should be examined until a no-observable-adverse-effect level is determined by microscopy. In addition, all animals showing systemic clinical signs or abnormal findings in clinical pathology should normally be examined

grossly and microscopically. Where the toxicity of the IVPP is anticipated to be relatively high, or where there is already information on toxicity from previous studies, different necropsy schemes may be recommended, to include gross and microscopic examinations for all animals or for subsets of animals that were selected for testing by a random process carried out at the beginning of the study. For an IVPP with a well documented broad margin of safety, including but not limited to comparative pharmacokinetic and comparative metabolism data, post-mortem examination may not be necessary in the absence of systemic clinical signs or abnormal findings in clinical pathology, based on appropriate justification and pre-specified in the study protocol.

Organ weights, where appropriate, and gross lesions should be recorded. The organs selected for gross and microscopic examination will depend upon animal species and target tissues. Histopathology should be conducted on organs/tissues, with particular attention given to organs/tissues showing macroscopic lesions, including at the injection site, where applicable. Generally, it is recommended that the following organs/tissues be considered in gross and microscopic examinations, as appropriate:

Organs/tissues considered for gross and microscopic examination:

Pituitary gland	Brain	Bone and marrow
Thyroid gland	Spinal cord	Marrow smear
Parathyroid gland	Eyes	Spleen
Adrenal gland	Lung	Stomach
Pancreas	Muscle	Duodenum
Ovaries	Mammary gland	Jejunum
Uterus	Liver	Ileum
Testes	Gall bladder	Colon
Prostate	Kidneys	Cecum
Epididymis	Urinary bladder	Thymus
Heart	Lymph nodes	Injection site: e.g., muscle, subcutaneous tissue
Crop	Proventriculus	Bursa of Fabricius
Ventriculus	Skin	

2.7 Statistical Analysis

In most studies, the safety implications are best addressed by applying descriptive statistical methods to the data. Tables and descriptive text are common methods of data summarization; however, it is also valuable to make use of graphical presentations in which patterns of adverse events are displayed both within treatments and within individual animals. Selection of the general form for a statistical model and the factors to be included in the model will depend on the nature of the response variable being analyzed and the study design. Regardless of the methods chosen, the process and steps used to conduct any statistical evaluations should be described. The outcomes of the data analysis should be clearly presented to facilitate evaluation of potential safety concerns. The terminology and methods of presentation should be chosen to clarify the results and expedite interpretation.

Tables may be used to present the data from individual animals and summary statistics from treatments. For quantitative variables, useful descriptive statistics include the number of animals in each treatment, median, mean, standard deviation, maximum, minimum, and the number and percentage of cases with values falling outside a recognized reference range. For some quantitative variables, categorization of animals with values that fall within different ranges may help to identify patterns. For qualitative variables, useful descriptive statistics include the total number of animals evaluated and the number and percentage of experimental units within each response category. Other events, such as adverse events, mortalities, and early terminations may also be tabulated.

Graphs may be very helpful in depicting the data and identifying potential safety concerns, including possible dose trends, time-related patterns, and values that fall outside reference ranges. Plots, that show responses over time, both within treatment groups and within individuals, may illustrate consistency of responses between animals, or sex, age, or dose levels. These graphs may show trends or time-related patterns in adverse effects of treatment.

Statistical models should represent the study design. The individual animal may be considered to be the experimental unit when each animal is penned individually or animals from all treatments groups are mixed in a single pen. When animals that are penned together are all assigned to the same treatment, the pen is typically the experimental unit. If housing unit, environmental conditions, sex, or pretreatment covariates differ between experimental units, these factors should be balanced in the design and accounted for in the analysis, as appropriate. A useful approach is to include the fewest number of terms that adequately represent the underlying process that generated the safety data, and to represent the longitudinal nature (repeated measurements) of the design (if applicable). Choice of model form should be driven by the nature of the response variable being analyzed. The potential impact of any missing data on the results should be considered.

Analysis results from statistical modeling include statements of significance levels for terms included in the model. The calculation of p-values is sometimes useful either as an aid to evaluating a specific difference of interest, or as a ‘flagging’ device applied to a large number of safety variables to highlight differences worth further attention. This is particularly useful for clinical pathology data, which otherwise can be difficult to summarize appropriately. It is recommended that clinical pathology data be subjected to both a quantitative analysis, e.g., evaluation of treatment means, and a qualitative analysis where counts of numbers above or below certain thresholds are calculated. While p-values are one indication of a substantial difference that should receive clinical appraisal, the small size of safety studies makes it crucial that clinical judgment be used to evaluate all differences, irrespective of the p-value observed. A statistically significant test does not necessarily indicate the presence of a safety concern. Similarly, a non-significant test does not necessarily indicate the absence of a safety concern. Statistical adjustments for multiplicity can be counterproductive for considerations of safety; the importance and plausibility of results will depend on prior knowledge of the pharmacology of the IVPP and this evaluation should be made by clinicians or scientists with appropriate experience and training in interpreting the biological relevance of the results.

2.8 Study Reports

A study report is a document describing the objectives, material and methods, any amendments or deviations from the protocol, results (including individual animal data, data summaries, and any analyses), and conclusions of a TAS study. In some regions, additional provision of raw data may be required.[6]

3. OTHER LABORATORY SAFETY STUDY DESIGNS

Additional safety studies may be appropriate for a particular IVPP, depending on the conditions of use and the characteristics of the IVPP. Such studies may be combined with the margin of safety evaluation and, in food producing animals, residue studies. These specialized studies should be designed according to the general principles outlined below and follow any official local guidance. It is recommended that the specific study plans be determined by communication between the sponsor and the local regulatory agency.

3.1 Injection Site Safety Studies

The basic study design should consider dose (1X), duration, route(s), vehicle, and maximum volume of the injection, generally using 8 animals per group. The study should include a saline control of the same volume as the complete and final formulation of the IVPP. In the case of non-liquid IVPP, an alternative suitable negative control should be used. The location and timing of each injection should be specifically noted to facilitate determination of time to resolution. The study should consider site lesions that may be produced by administration by syringe or other applicator by intravascular, intradermal, intramuscular and/or subcutaneous routes. If intravascular administration is the only route proposed, consideration should be given to the effects of extravascular administration of the IVPP. For formulations where intravascular use is not intended and there is a potential risk associated with unintended intravascular injection (e.g., certain subcutaneous injections in the ear), the safety in the event of intravascular injection should be considered.

Evaluation of safety data from injection site studies may include the following:

- Clinical signs including changes in behavior or locomotion.
- Appearance, inflammation, edema, or other changes at the injection site.
- Measuring Creatine kinase and aspartate transaminase levels.
- Gross pathology and histopathology of lesions at appropriate times.

If there is inflammation at the injection site that has not resolved on visual examination or by palpation by the end of the planned study, then the time required for return to clinically acceptable resolution at the injection site should be determined. Where clinical signs indicative of injection site effects are evident, it may be necessary to conduct histopathology of the lesions.

3.2 Administration Site Safety Studies for Dermally Applied Topical Product

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[6] *See* 21 CFR 514.1(b)(8).

Local adverse reactions to a topically applied IVPP should be evaluated, generally in 8 animals per group, at the dosage proposed on the label unless the pharmacology and toxicology of the product warrants multiples of dose and/or duration. For a systemically absorbed topical IVPP, it is recommended that evaluation of topical administration sites be included in studies of systemic TAS outcomes. In general, the site should be examined for swelling, pain, heat, erythema, and other clinical signs. Changes in animal movement or behavior should be noted. If there is inflammation or other clinical signs at the topical application site that have not resolved on visual examination or by palpation by the end of the planned study, then the time required for return to clinically acceptable resolution at the topical application site should be determined. Where clinical signs indicative of administration site effects are evident, it may be necessary to conduct histopathology of the lesions.

Oral dosing, generally at the maximum proposed dose of a topical formulation, is recommended to examine the safety of the IVPP if accidental ingestion is likely to occur after treatment (e.g., by licking). If, based on pharmacology and toxicology, there are no safety concerns regarding oral exposure, it may be appropriate to dispense with this study.

3.3 Reproductive Safety Studies

Reproductive safety studies are required for systemically absorbed API intended for use in breeding animals.[7] The goal of reproductive safety studies is to identify any adverse effects of the IVPP on male or female reproduction or on offspring viability. These studies generally focus on reproductive variables, although safety data on other body systems may be collected. These studies do not usually extend to considering viability of offspring beyond the post-natal period, unless there is specific evidence of possible effects on, for example, sexual maturity, based on pharmacology and toxicology of the API.

Healthy, intact, reproductively-sound males and females should be selected that are representative of the species, age, and class in which the drug will be used. In general, it is recommended that 8 animals per sex be included per treatment. Males and females may be evaluated in the same or separate studies. Dose, route, frequency, and duration of administration should be selected and justified based on the pharmacology and toxicology of the IVPP and intended use, and should ensure continuous exposure throughout the study interval. Generally, males should be treated with a negative control and 3X doses of the IVPP throughout at least one spermatogenic cycle. Generally, females should be treated with a negative control and 3X doses of the IVPP prior to breeding (covering the follicular phase until conception), throughout the gestation period (including embryonic phase, fetal phase, and natal phase), and after parturition for an appropriate time covering the post-natal period that is sufficient to assess the initial development and locomotor function of the offspring. It is not necessary to administer the IVPP at or around parturition, unless the IVPP is specifically indicated for use at that time.

Reproductive safety studies should evaluate, as appropriate:

- In the male: spermatogenesis, semen quality and mating behavior.

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[7] *See* 21 U.S.C. § 360b(b)(1); 21 U.S.C. § 360b(d)(1)(A); 21 CFR 514.1(b)(8)

- In the female: estrous cycle, mating behavior, conception rates, length of gestation, parturition, and lactation.
- In the offspring from treated males and/or females: developmental toxicology (including teratogenicity, fetotoxicity), fetal development, number of offspring, viability and growth, health and development to weaning.
- In poultry: also, egg weight, shell thickness, number of eggs laid by a hen, egg fertility, hatchability, and chick viability.

Ideally, reproductive safety studies are conducted in the target species; however, data obtained from reproductive studies in laboratory animals may be considered, provided that the pharmacokinetic profiles of the API are comparable in laboratory animals and in all species in which the IVPP is intended for use. Depending on the results of such evaluation, appropriate information should be included on the labeling. However, if reproductive safety studies have not been conducted in the target species, labeling should reflect this and state that safety has not been determined in breeding, pregnant, or lactating animals or their offspring.

3.4 Mammary Gland Safety Studies

Mammary gland safety studies should be conducted to evaluate the safety of IVPPs intended for intramammary use in lactating or non-lactating animals. For these studies, animals should be free of subclinical or clinical mastitis. The IVPP should be administered as one dose to each teat. The conditions of use, dose (1X), and frequency of administration should be those proposed on the label. Alternatives should be justified by the sponsor.

It is recommended that safety evaluation of an IVPP intended for use in lactating females include objective evaluation of acute inflammatory effects in early to mid-lactation animals (data collected in conformity with the principles of GLP[8]). It is recommended that safety evaluation of an IVPP intended for use in non-lactating animals include both an objective evaluation of acute inflammatory effects in lactating animals (data collected in conformity with the principles of GLP[9]) and clinical evaluation of chronic inflammatory effects in non-lactating animals (data collected in conformity with the principles of GCP or GLP[10]).

For both lactating and non-lactating claims, it is preferred that a one-group, comparative design be used to evaluate similarity of values from pre-treatment and post-treatment periods within each animal. A two-group design may also be used, with treated animals compared to negative control animals. In general, 8 lactating animals, including 4 in their first lactation, should be assigned to each treatment in either study design for both lactating and non-lactating claims.

For all study animals, physical examination, including palpation, should be done to determine swelling, erythema, pain, or heat. For either study design in lactating animals (both lactating and non-lactating claims), data on all relevant variables associated with tissue irritation and milk

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[8] *See* 21 CFR Part 58.

[9] Id.

[10] Id.

production should be collected pre-treatment, at treatment, and post-treatment. The post-treatment monitoring period should be defined *a priori* based on the anticipated time for values to return to pre-treatment values. Samples for quantitative somatic cell count (SCC) and bacterial culture should be collected from each teat prior to milking. Daily milk yield, composition (e.g., fat, protein, lactose, and non-fat solids), and appearance should be recorded. Key variables for safety assessment generally include signs of mammary gland irritation, elevated SCC, and changed milk production. The presence of very high post-treatment SCC or prolonged SCC elevation following treatment may not be acceptable and should be explained by the sponsor.

4. TARGET ANIMAL SAFETY DATA FROM FIELD STUDIES

Field studies intended to evaluate effectiveness of an IVPP also provide essential TAS data under conditions of intended use. These studies should be conducted in accordance with the principles of GCP.

Field studies are typically conducted under conditions representative of the target population and provide an evaluation of potential adverse effects at the intended use dosage in a much larger number of animals. Field studies use the target population which, if applicable, includes diseased animals. Where disease and husbandry are similar between regions, international data may be used for field studies, as long as a minimum proportion of the data acceptable to the region is generated within the region where approval is being sought. Including a relatively large number of animals in the study improves the ability to detect relatively low frequency adverse events. Animals should be representative of the age range, class, breed, and sex for which the IVPP is intended. The study should be designed with an appropriate control group. In each study, health observations should be performed by appropriate masked (blinded) personnel before, during, and after treatment, with specific evaluation of potential adverse effects (e.g., physical examination and clinical pathology tests). The appropriate variables for evaluation may be based on results of pharmacodynamic studies in laboratory animals or studies in the target species. Adverse events should be reported and determination of causality for the adverse event attempted.

5. RISK ASSESSMENT IN ANIMAL SAFETY EVALUATION

For some IVPPs, laboratory and field safety data may not alone provide sufficient information to determine if an acceptable safety profile exists in relation to IVPP benefits. In these instances, risk assessment methodologies may provide a means to supplement or augment TAS evaluation. Risk assessment uses the available body of evidence to weigh the severity of an adverse effect (harm), the potential of reversibility, and the probability that it will occur.

6. GLOSSARY

Active Pharmaceutical Ingredient (API): Any substance or mixture of substances intended to be used in the manufacture of a drug (medicinal) product and that, when used in the production of a drug, becomes an active ingredient of the drug product. Such substances are intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure and function of the body.

Adverse Effect: Adverse event suspected to be related to an IVPP.

Adverse Event: Any observation in animals that is unfavorable and unintended and occurs after the use of an IVPP, whether or not considered to be product related.

Baseline Data: Information collected after the acclimatization period and before the administration of an IVPP.

Breeding Animal: Any animal that is actively breeding, intended for breeding, or pregnant.

Class: Subset of target animal species which is characterized by factors such as reproductive status and/or use (dairy vs. beef, broiler vs. layer).

Current Good Manufacturing Practices (cGMP): The part of a quality system which ensures that products are consistently produced and controlled to the quality standards which they are represented to possess.

Experimental Unit: The smallest independent grouping of animals that could receive a different treatment during the study, given the methods of allocation and treatment administration.

Good Clinical Practices (GCP): A standard for the design, conduct, monitoring, recording, auditing, analysis, and reporting of clinical studies. Adherence to the standard provides assurance that the data and reported results are complete, correct and accurate, that welfare of the study animals and the safety of the study personnel involved in the study are ensured, and that the environment and the human and animal food chains are protected.

Good Laboratory Practices (GLP): A standard for the design, conduct, monitoring, recording, auditing, analysis, and reporting of non-clinical studies. Adherence to the standard provides assurance that the data and reported results are complete, correct and accurate, that welfare of the study animals and the safety of the study personnel involved in the study are ensured, and that the environment and the human and animal food chains are protected.

Investigational Veterinary Pharmaceutical Product (IVPP): Any pharmaceutical form of, or any animal feed containing one or more active pharmaceutical ingredients (API) being evaluated in a clinical or non-clinical study, to investigate any protective, therapeutic, diagnostic, or physiological effect when administered or applied to an animal.

Margin of Safety Study: Well-controlled study designed to show if an IVPP is safe for the intended species.

Masking/Blinding: A procedure to reduce potential study bias in which designated study personnel are kept uninformed of the treatment assignment(s).

Negative Control: Study animals that either receive a placebo or are untreated.

Reference range (clinical pathology or blood chemistry): The range of usual values found in healthy animals of a given class.

Target Animal: The specific animal species, class, and breed identified as the animal for which the IVPP is intended for use.