#226

Guidance for Industry

Target Animal Safety Data Presentation and Statistical Analysis

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Additional copies of this guidance document may be requested from the Policy and Regulations Staff (HFV-6), Center for Veterinary Medicine, Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, and may be viewed on the Internet at either http://www.fda.gov/AnimalVeterinary/default.htm or http://www.regulations.gov.

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Table of Contents

I. INTRODUCTION 3

II. PRESENTATION OF TARGET ANIMAL SAFETY DATA 4

A. Data Generated from Margin of Safety Studies 4
1. General Animal Data 4
2. Physical Examination Data 5
3. Health Observations 5
4. Clinical Pathology 6
5. Necropsy and Histopathology Examination Data 7

B. Data Generated from Field Effectiveness Studies 8
1. Adverse Event Data from Field Effectiveness Studies 9
2. Monitored Health Outcomes from Field Effectiveness Studies 9

C. Data Generated from Other Types of Studies 11

III. STATISTICAL ANALYSIS 12

A. Blocking 12

B. Baseline Covariates 12

C. Continuous Variables 12
1. Continuous variables measured once 13
2. Continuous variables measured repeatedly 15

D. Categorical Variables 18
1. Dichotomization of categories 18
2. Analysis methods 18
3. Presentation of frequency tables 19

E. Variables Measured but not Statistically Evaluated 19

Guidance for Industry

Target Animal Safety Data Presentation and Statistical Analysis

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA office responsible for this guidance as listed on the title page.

I. INTRODUCTION

This document provides recommendations to industry regarding the presentation and statistical analyses of target animal safety (TAS) data submitted to CVM as part of a study report to support approval of a new animal drug. These recommendations apply to TAS data generated from both TAS and field effectiveness studies conducted in companion animals (e.g., dogs, cats, and horses) and food animals (e.g., swine, ruminants, fish, and poultry).

TAS studies are usually laboratory studies conducted in the target species at one site containing multiple treatment groups under good laboratory practice (GLP) regulations.[1] Measurements are often collected for a large number of variables using a small number of animals per treatment. Field effectiveness studies also provide useful safety information because the drug is administered under proposed field conditions of use in a large number of animals.

Measurements of a large number of variables are typically collected in clinical studies. The examples in this document are based on data collected from typical margin of safety studies, such as those described in FDA Guidance for Industry (GFI) #185.[2] Although there are many ways to organize and summarize these data, we have found that some methods enable us to review the data and results more efficiently. These methods are described in this guidance. Presenting the data in the manner described in this document will facilitate our review of the study report.

Example tables are used throughout this document. Only portions of each table are presented to maintain the conciseness of this document.

Although this guidance proposes specific methods of data presentation and statistical analysis, alternative strategies may be appropriate. We recommend that you discuss your proposed methods for data presentation and analysis before submitting any study protocol. We recommend obtaining protocol concurrence before initiating any studies that you intend to use to

[1] 21 CFR part 58.
[2] CVM Guidance for Industry #185/VICH GL43, "Target Animal Safety for Veterinary Pharmaceutical Products," April 24, 2009.

support approval. If alternate study designs for evaluating safety are used, the examples in this guidance may still be useful to organize and summarize the data.

In general, FDA's guidance documents 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.

II. PRESENTATION OF TARGET ANIMAL SAFETY DATA

Safety data should be presented in a clear and organized manner. Tables serve this purpose well. Tabular data presentation enhances efficient summarization of pre-treatment and post-treatment findings and comparison of treated and control group(s) responses.

Tables should be clear and concise. They should be titled appropriately and column and row headings should accurately describe the data being presented. Terms and abbreviations not addressed elsewhere in the document should be defined. If symbols or footnotes are used, these should be referenced.

A. Data Generated from Margin of Safety Studies

Example tables are presented for various types of frequently submitted safety data. These examples serve to illustrate our recommendations but with the diversity of studies conducted, there are other possibilities. Because these are examples, only partial tables are presented, as indicated by serrated borders.

1. General Animal Data

   General animal data commonly include the following: treatment group, animal identification (ID), sex, age, and breed. Additional information (pen assignment, feeding regimen, replicate, site, etc.) should be provided as appropriate. Table 1 provides an example of a recommended presentation format, shown with data that might be collected from a typical equine safety study.

Table 1. General Animal Information

Treatment Group	Animal ID	Sex (Male Castrate [MC], Female [F])	Age (years)	Breed (Quarter Horse [QH], Thoroughbred [TB])
1 (0X)	1	F	2	QH
	4	F	3	TB
	5	F	2	QH
	13	MC	5	QH
	18	F	5	QH

2. Physical Examination Data

Although the results from all physical examinations should be collected on standardized forms, we recommend creation of a table that includes only those animals observed to have abnormal findings during physical examinations (scheduled or unscheduled). To facilitate examination of the data for any potential treatment-related effects, we recommend organizing the data by treatment groups, including information such as treatment group, animal ID, study day, area examined, description of abnormal examination finding, and medical treatment or further follow-up. If the medical treatment or further follow-up information is too extensive to be included in the table, the location of that information should be clearly identified. Table 2 presents abnormal findings from our example of a typical equine study in this format.

Table 2. Abnormal Physical Examination Findings

Treatment Group	Animal ID	Study Day	Area Examined	Abnormality and severity	Outcome
1 (0X)	2	-14	Oral mucous membranes	Dry, pale CRT[a] 3 secs[b]	An additional Physical Exam was conducted on Day 20 and the CRT was normal. The sclera was still slightly yellow; however, the horse had resumed eating and was bright and alert. Clinical pathology and necropsy findings were reviewed and no abnormalities were reported except that Total Bilirubin was elevated on Days 0, 7, and 14. An additional serum sample was taken on Day 20 and Total Bilirubin had returned to normal levels.
	2	-7	Oral mucous membranes	Dry, pale CRT 3 secs	
	2	0	Oral mucous membranes	Dry, pale CRT 3 secs	
	2	0	Eyes	Sclera yellow bilateral	
	2	7	Eyes	Sclera yellow bilateral	
	2	14	Oral mucous membranes	Yellow, dry	

[a] Capillary Refill Time
[b] seconds

3. Health Observations

In addition to raw data on all health observations, we also recommend a table including only those animals observed to have abnormal health or behavior on one or more study days. Appropriate data may include treatment group, animal ID, study day(s), description of abnormal health observation(s), action taken, and outcome of the abnormal event. Table 3 provides an example presenting abnormal observations from a typical equine safety study.

In some cases, abnormal health observations include data from physical examinations. In such cases, the information in the two tables may overlap. Also note that in Table 2, findings from each examination are presented separately (by day), while in Table 3 results are combined across days. Both methods are useful and the format should be chosen that best communicates the study findings.

Table 3. Abnormal Daily Health and Behavior Observations

Treatment Group	Animal ID	Study Day(s)	Abnormal Health Observation(s)	Description of Abnormal Observation(s)	Action/Outcome
1 (0X)	1	-7 to -3 and 5 to 25	Not eating grain	Picks at grain, paws, consumes approx. ¼ of total amount grain fed	Molasses added to oats
	4	-5 to 15	Ocular discharge, bilateral	Mucopurulent copious discharge from both eyes, rubbing eyes on stall door/walls	Triple antibiotic with hydrocortisone, ophthalmic ointment TID; Resolution by Day 15

4. Clinical Pathology

Tables showing clinical pathology results should include all animals, regardless of whether abnormalities were noted. Normal reference ranges should be provided in a convenient location including citation of the source of the range (literature, laboratory-specific, etc.). High-low flags are acceptable but the numerical reference ranges should be provided. Any abnormal or out-of-reference range values should be clearly identified. If the table spans multiple pages, column headings and reference ranges should be included on each page. Table 4a is an example of a table structure for hematology variables from an equine study with laboratory specific reference ranges. Table 4b is an example presentation of some blood chemistry results. These tables are not intended to provide guidance on the appropriate clinical pathology parameters or reference ranges for any particular safety study; they merely provide examples of data organization.

Table 4a. Hematology: Complete Blood Count

Treatment Group	Animal ID	Study Day	WBC (10^3/µL) [xx-xx][a]	RBC (10^6/µL) [xx-xx]	HGB (g/dL) [xx-xx]	HCT (%) [xx-xx]	MCV (fL) [xx-xx]	MCH (pg) [xx-xx]
1 (0X)	1	-28	14.8 (H)[b]	10.90	18.6	54.1	52	17.1
		-14	8.2	9.55	16.2	47.9	39	16.9
		-7	10.7	9.16	15.5	45.9	47	17.0
		0	4.4 (L)[c]	9.74	16.5	48.7	49	16.9
		7	7.6	8.5	14.1	42.5	51	16.5
		14	8.2	8.0	14.0	40.0	42	16.3

[a] Reference range (citation of source)
[b] Above reference range
[c] Below reference range

Table 4b. Clinical Chemistry

Treatment Group	Animal ID	Study Day	Albumin (g/dL) [xx-xx][a]	A/G Ratio [xx-xx]	AP (µ/L) [xx-xx]	AST (µ/L) [xx-xx]	ALT (µ/L) [xx-xx]	Calcium (mg/dL) [xx-xx]
1 (0X)	1	-28	2.8	0.6	327	403	6	10.6
		-14	2.6	0.5	222	326	8	9.9
		-7	3.3	0.6	212	382	5	11.2
		0	3.4	0.5	400	378	6	12.0
		7	3.5	0.6	433	357	7	10.3
		14	1.6	0.2	750	242	8	9.8
	4	-28	3.5	0.4	332	484	5	9.8
		-14	3.6	0.5	356	445	5	10.2
		-7	2.8	0.5	545	465	7	11.0
		0	3.0	0.5	678	490	9	12.2

[a]Reference range (citation of source)

5. Necropsy and Histopathology Examination Data

Gross and microscopic pathology observations should be organized by treatment to help identify treatment-related findings. Table 5a is an example for presenting a summary of gross necropsy findings for all animals, by treatment group. Additionally, another table which summarizes gross necropsy findings and corresponding histopathology for any abnormalities will help to provide insight into the primary pathologic processes underlying each finding. Table 5b is an example of how these findings might be presented.

Table 5a. Gross Necropsy: Summary of Findings by Organ/Treatment Group/Sex

Organ and Finding	Males				Females			
Treatment Group	1 (0X)	2 (1X)	3 (3X)	4 (5X)	1 (0X)	2 (1X)	3 (3X)	4 (5X)
Number of Animals	n	n	n	n	n	n	n	n
Heart - dilated lymphatic - tissue discoloration - effusion	1 (23)[a]	None	None	None	None	None	None	1 (6)
Lungs -atelectasis, right and left lobes	1 (23)	None	2 (7,2)	1(14)	None	None	None	None
Right Dorsal Colon -ulcers -erosions	None	None	1 (7)	2 (14,10)	1(43)	None	2 (22,3)	2 (6,8)
Right Ventral Colon -ulcer	None	1 (12)	None	None	None	1 (19)	1 (22)	None

[a] Number of animals affected (animal IDs in parentheses).

Table 5b. Associated Gross and Microscopic Pathology

Treatment Group	Animal ID	Sex	Gross Necropsy Finding	Description of gross findings (number, severity and/or extent)	Corresponding Microscopic Finding
1 (0X)	43	F	None	N/A	N/A
	23	M	Pericardial effusion	Clear, increased amount of pericardial fluid	No corresponding microscopic finding
	23	M	Atelectasis of right and left lung lobes	Pulmonary edema; Mucopurulent exudate in both lungs, lungs dull red in color	Compressed alveoli and bronchioles, decreased perfusion

B. Data Generated from Field Effectiveness Studies

Safety data from field effectiveness studies may be presented in tables similar to those described in the previous section. Additionally, two example tables are given for summaries of other safety data that are generally collected from field effectiveness studies. However, the design of field effectiveness studies is more varied than that of margin of safety TAS studies and therefore no particular strategy can be provided for the presentation of safety data from these studies. The study protocol should address the collection and analysis of safety data from field effectiveness studies.

1. Adverse Event Data from Field Effectiveness Studies

Field effectiveness studies are an important source of TAS data in the target population under the proposed conditions of use. Adverse events should be classified by body system (musculoskeletal, gastrointestinal, etc.) and tabulated to help identify associations between adverse events, treatment groups, and other study factors. Table 6 is an example of a way to summarize adverse events from a multi-site effectiveness study. More details on specific animals or body systems may also be presented as shown in previous tables.

Table 6. Adverse Events: Summary by Body System and Site

Body System	Site 1		Site 2		Site 3		All Sites	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Neurologic	0	0	0	0	1	0	1	0
Eyes (Discharge)	0	0	1	0	0	0	1	0
Gastrointestinal (Diarrhea)	0	0	0	0	0	1	0	3
General (Dead)	16	4	11	1	4	0	37	8
Musculoskeletal And Feet (Lameness)	4	1	2	0	0	0	6	1

2. Monitored Health Outcomes from Field Effectiveness Studies

In addition to a general listing of adverse events, monitoring of certain specific health outcomes may be planned during effectiveness trials. Data summaries should demonstrate the appropriate measures of the number, duration, and frequency of a specific health outcome.

As applicable, parameters using scoring or other numerical data should be captured by describing the values seen in each animal and each treatment group for each study phase when data was collected. Values might be described using means, ranges or other descriptive summaries. Examples would be somatic cell scores and severity scores for quarter health or milk quality, as in Tables 7a and 7b below.

Table 7a: Monitored Health Outcomes: Scored or Numerical Values

Somatic Cell Counts (SCC) in 1000 cells/mL					
Treatment Group	Animal ID	Pre-Treatment Period	Treatment Period	Withdrawal Period	Post-Treatment Period
Treated	101, range	30 – 34	43 - 58	55 - 72	42 - 54
	102, range	31 – 50	80 - 96	83 - 98	40 - 45
	103, range	59 – 62	90 - 112	78 - 100	50 - 73
	Group Mean	41	90	87	47
Control	201, range	40 – 45	44 - 70	62 – 72	64 - 70
	202, range	35 – 47	51 - 60	55 – 74	53 - 57
	203, range	60 – 71	53 - 59	55 - 71	52 - 68
	Group Mean	48	55	65	56

Animal observations, such as milk quality and quarter health, that are evaluated using presence/absence criteria could be summarized using this example format:

Table 7b: Monitored Health Outcomes: Presence/Absence Criteria

Milk Quality Abnormalities[a]					
Treatment Group	Animal ID	Pre-Treatment Period	Treatment Period	Withdrawal Period	Post-Treatment Period
Treated	101	None	flakes (Day 1 AM & PM, Day 2 AM)[b]	None	None
	102	None	None	flakes (Day 4 AM)	None
	103	None	None	None	None
	Total Number of Abnormal Observations	0	3	1	0
Control	201	None	None	None	None
	202	None	None	None	None
	203	None	None	flakes (Day 4 AM)	None
	Total Number of Abnormal Observations	0	0	1	0

[a] presence of clots, flakes, or stringy, watery, or bloody milk
[b] observed change (days/observation periods in parentheses)

A safety variable may be observed over time and in multiple animals during an effectiveness study. Table 8 displays an example showing the

incidence and type of cardiac arrhythmias seen in dogs after treatment with one of two drugs.

Table 8. Monitored Health Outcomes: Summary for Groups over Time

Number (%) of dogs for each cardiac arrhythmia category before and following treatment at 0 minutes with Drug 1 or Drug 2.									
Treatment	Category[a]	Timepoint (minutes) After Treatment[b, c]							
		-10	5	15	30	60	90	120	180
Drug 1		n=9	n=32	n=76	n=118	n=130	n=187	n=63	n=24
	AVB	0	2 (6%)	4 (5%)	0	0	0	0	0
	AVD	4 (44%)	10 (31%)	19 (25%)	17 (14%)	6 (5%)	3 (2%)	9 (14%)	2 (8%)
	BRDY	1 (11%)	15 (47%)	32 (42%)	29 (25%)	35 (27%)	52 (28%)	18 (29%)	13 (54%)
	PC	0	2 (6%)	7 (10%)	5 (4%)	10 (8%)	0	3 (5%)	1 (4%)
	TCHY	3 (33%)	7 (22%)	0	0	0	0	0	0
Drug 2		n=9	n=34	n=65	n=115	n=128	n=95	n=62	n=30
	AVB	0	4 (18%)	18 (28%)	0	0	3 (3%)	0	0
	AVD	0	2 (6%)	1 (2%)	29 (25%)	15 (12%)	12 (13%)	4 (6%)	2 (7%)
	BRDY	0	23 (68%)	13 (20%)	54 (47%)	49 (38%)	29 (31%)	7 (11%)	4 (13%)
	PC	0	0	5 (8%)	11 (10%)	20 (16%)	7 (7%)	2 (3%)	0
	TCHY	2 (22%)	0	12 (18%)	0	0	0		0

[a]AVB = atrioventricular block; AVD = atrioventricular dissociation or ventricular or junctional escape rhythm; BRDY = bradycardia; PC = supraventricular or ventricular premature complexes; TCHY = tachycardia.
[b] Treatment (Drug 1 or Drug 2) was administered at 0 minutes.
[c] Number of dogs (% of total) with available ECG recordings varied across time points and treatment group.

C. Data Generated from Other Types of Studies

The examples presented in this document are from studies that are commonly used to generate animal safety data, such as a margin of safety study and field effectiveness study. Other types of studies may also be used to generate safety data. These may include injection site or administration site safety studies, reproductive safety studies, mammary gland safety studies, or others as needed. The information collected in these

specialized studies may differ from that presented above, but the general principles of data presentation described still apply.

III. Statistical Analysis

The following recommendations are intended to summarize important concepts in the statistical analysis of safety data and to help outline useful approaches for the analysis and presentation of results. They are not intended to be an all-inclusive list or to address every situation.

In the analysis of safety data, the interpretation of results will be based on both clinical relevance and statistical significance. CVM generally considers a significance level of α=0.10 useful as a conservative screen for identifying potential treatment-related safety concerns among endpoints in TAS studies. This significance level allows more safety variables to be flagged for further consideration of clinical relevance than α=0.05 or α=0.01. Pairwise mean comparisons between each treatment against the control group are also performed using an unadjusted $\alpha = 0.10$.

To facilitate the review process, the sponsor should provide: statistical programs with documentation, all statistical output (e.g., analysis results), raw data in its original form, and raw data in an electronic format. Refer to GFI #197 for guidance on adequate documentation of statistical analysis in data submissions to the agency.[3]

A. Blocking and Stratification

CVM does not generally recommend the use of blocks in the design of TAS studies unless adequate justification is provided at the protocol stage. If blocking is a component of the study design, blocks should be included in the statistical model as a random effect. If stratification was employed in the design, the stratification factor should be included as a random or fixed effect as appropriate. For example, margin of safety studies are typically stratified by sex. In this guidance, sex is used as an example of a fixed effect.

B. Baseline Covariates

Often baseline or pre-treatment data for a given variable are collected and should be included in the statistical model as a covariate. The covariate should be included as a linear effect assuming a common covariate slope. If a pre-treatment covariate is proposed in the design, it should be retained in the model regardless of its statistical significance.

C. Continuous Variables

Blood chemistry values, body weights, and organ weights are examples of continuous variables collected in TAS studies. These data are collected once or repeatedly on the same subject throughout the study. Data are usually analyzed using analysis of variance (ANOVA) or analysis of covariance (ANCOVA) where the statistical model reflects the experimental design.

[3]CVM Guidance for Industry #197, "Documenting Statistical Analysis Programs and Data Files," December 2015.

1. Continuous variables measured once

   Examples of continuous variables measured once are organ weights at necropsy. The statistical model for the ANOVA or ANCOVA should be consistent with the experimental design. If the subjects are stratified by sex, then the model should evaluate treatment, sex, and the treatment-by-sex interaction as fixed effects. Summaries of the statistical decision process and the outcomes of the statistical evaluations should be presented in tabular form. Recommended criteria for evaluating analysis results and making comparisons are detailed below.

   a. Evaluate the treatment-by-sex interaction ($\alpha = 0.10$) and present p-values and interpretation in a summary table. If significant, calculate least squares (LS) means and perform pairwise comparisons between treatments and the control within sex. If not significant, proceed to step b.

      Table 9 is an example of a way to present p-values from overall tests of treatment effects from the statistical analyses. Tables 10a and 10b are examples of ways to present least squares means (LS means) and p-values from pairwise comparisons of treatment to control.

   b. Evaluate the main effect of treatment ($\alpha = 0.10$) and present p-values and interpretation in a summary table (Table 9). If significant treatment effects are detected, calculate LS means and perform pairwise treatment comparisons with the control (Table 10b). If treatment effects are not significant, include the p-value and interpretation in the table but additional comparisons should not be performed.

   c. In addition to the model-based analysis in (a) and (b) above, you should also summarize data using descriptive statistics. Descriptive statistics should include number of subjects, arithmetic mean, standard deviation, minimum value, and maximum value. Tables of descriptive statistics by treatment and treatment by sex should be provided. Summary tables of descriptive statistics should not include results from inferential analyses such as p-values. Table 11 is an example of how to present descriptive statistics by treatment within sex. A similar table excluding the column for sex should be provided for descriptive statistics by treatment combined across sex.

Table 9. P-values for Evaluating Treatment Effects: Data from a Canine Safety Study

Variable	Evaluation of Treatment-by-Sex Effect	Evaluation of Treatment Effect	Decisions
Heart Weight	**0.0246** [b]	– [c]	1
Kidney Weight	0.2186	0.4215	3
Liver Weight	0.3577	**0.0456**	2

[a] Decisions:
1 = The treatment*sex interaction is significant. Follow up treatment mean comparisons will be made within sex, Table 10a.
2 = The treatment*sex interaction is not significant but the main effect of treatment is significant. Follow up treatment mean comparisons will be made averaged over sex, Table 10b.
3 = There are no significant fixed effects.
[b] P-values in bold-face indicate statistical significance ($\alpha = 0.10$).
[c] P-values of the treatment effect are excluded for variables that had a significant treatment*sex interaction.

Table 10a. LS Means and P-values from Treatment Comparisons within Sex for Variables with a Significant Treatment-by-Sex Interaction: Data from a Canine Safety Study

Variable	Treatment Group	Females		Males	
		LS Mean	P-value[a]	LS Mean	P-value[a]
Heart Weight (g)	1	91.8		72.5	
	2	92.5	0.4521	80.5	**0.0293**
	3	93.0	0.3506	73.3	0.4542
	4	90.0	**0.0892**[b]	60.0	**<0.0001**

[a] P-value for the difference between each treatment group and group 1 within sex.
[b] P-values in bold-face indicate statistical significance ($\alpha = 0.10$).

Table 10b. LS Means and P-values from Treatment Comparisons for Variables with a Significant Treatment Effect: Data from a Canine Safety Study

Variable	Treatment Group	LS Mean	P-value[a]
Liver Weight (g)	1	344.8	
	2	346.1	0.7149
	3	364.5	**0.0021** [b]
	4	373.0	**< 0.0001**

[a] P-value for the difference between each treatment group and group 1.
[b] P-values in bold-face indicate statistical significance ($\alpha = 0.10$).

Table 11. Descriptive Statistics for Raw Data

Variable	Sex	Treatment Group	Number of Subjects	Arithmetic Mean	Standard Deviation	Minimum Value	Maximum Value
Kidney Weight (g)	Females	1	4	47.6	19.2	22.3	68.9
		2	4	49.0	25.8	27.6	80.3
		3	4	78.4	42.9	29.3	132.1
		4	4	40.4	20.1	13.1	55.9
	Males	1	4	80.9	34.6	29.6	104.7
		2	4	62.8	29.1	24.2	89.1
		3	4	57.1	42.3	22.2	117.7
		4	4	74.4	40.3	33.01	122.2

2. Continuous variables measured repeatedly

Several measurements of the same variable may be taken repeatedly on the same experimental unit (animal, pen, or herd) over time. For repeatedly measured variables, ANOVA or ANCOVA should also be used, using a statistical model that is consistent with the experimental design. If the subjects are stratified by sex, then the statistical model should evaluate treatment, sex, and time as fixed effects and the two-way and three-way interactions. Repeated measurements from a single subject tend to be correlated across time and the correlation structure can be incorporated into the repeated measures analysis. CVM recommends investigating the less complicated covariance structures and choosing the least complicated structure that is appropriate for the data. If there are a large number of variables to analyze, *a priori* the variables may be divided into meaningful groups and the most clinically relevant variable selected to determine the covariance matrix structure for all the variables in the same group. The covariance matrix should be selected from the candidates investigated using pre-specified criteria, such as the Akaike Information Criterion (AIC) score, corrected AIC (AICC) or Bayesian Information Criterion (BIC) as appropriate.[4]

Criteria for evaluating analysis results and recommended comparisons are detailed below. All fixed model effects are tested at α=0.10 except the three-way treatment-by-sex-by-time interaction, which is tested at α=0.05. Examples of tables for presenting results and summary statistics are also provided.

a. Evaluate the treatment-by-sex-by-time interaction (α =0.05). If significant, simply provide summary statistics for each treatment group at each time point within each sex. If not significant, proceed to step b.

b. Evaluate the treatment-by-sex and the treatment-by-time interactions (α =0.10). If the treatment-by-sex interaction is significant, perform treatment mean

[4]R.C. Littell, G.A. Milliken, W.W. Stroup, R.D. Wolfinger and O. Schabenberger. *SAS for Mixed Models*, Second Edition (Cary, NC: SAS Institute, Inc., 2006), 183-184.

comparisons for each sex. If the treatment-by-time interaction is significant, perform treatment mean comparisons for each time point. Mean comparison results may be presented similar to Table 10a. If neither interaction is significant, proceed to step c.

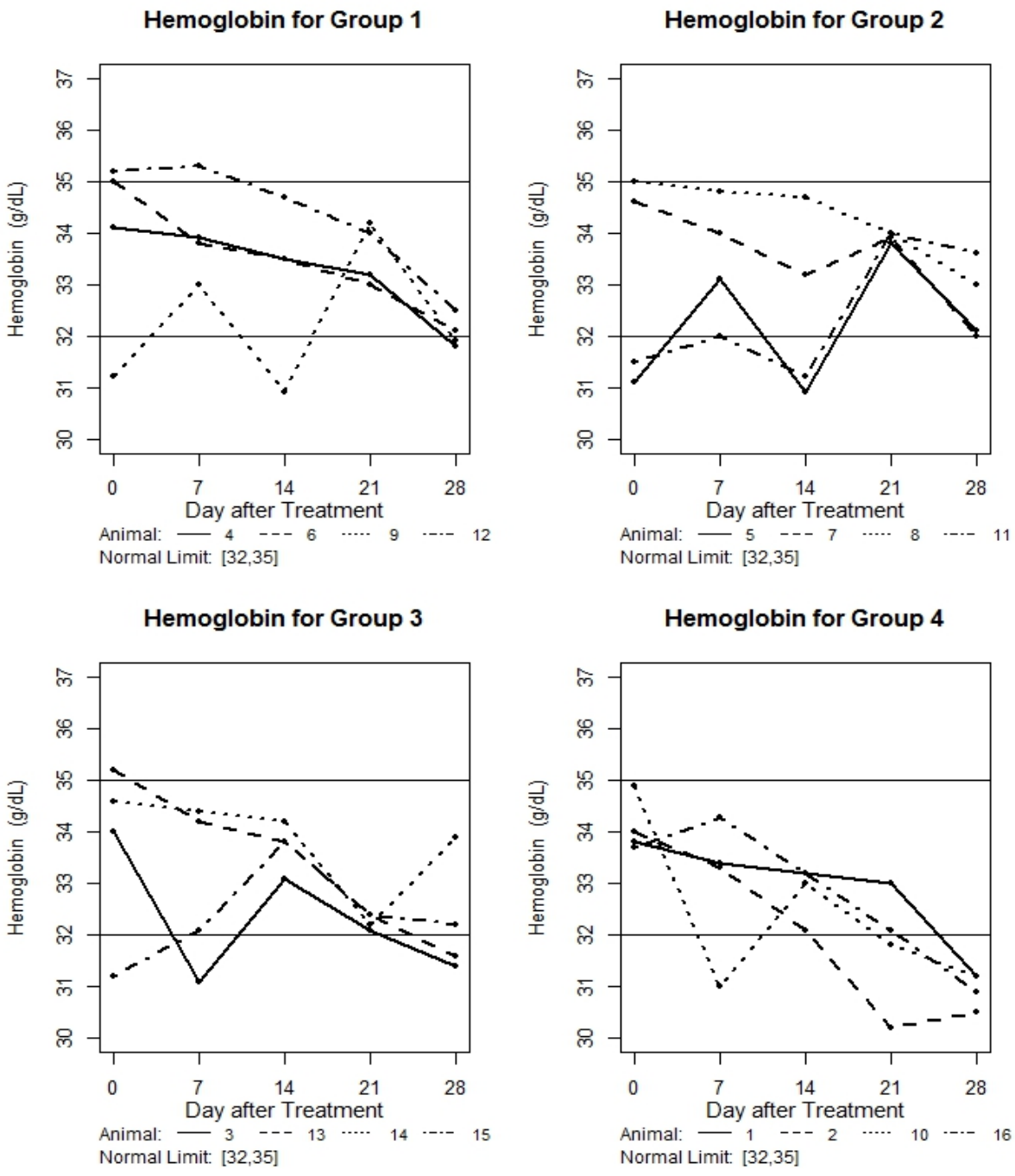
c. Evaluate the main effect of dose ($\alpha = 0.10$). If significant, perform treatment mean comparisons. Results may be presented as in Table 10b. If not significant, conclude there is not enough evidence to reject the hypothesis of no difference in treatment group means.

d. Summarize the results of the statistical comparisons. Summaries of the statistical decision process and the outcomes of the statistical evaluations should be presented in tabular form. A table similar to Table 9, adding columns for the treatment-by-time and treatment-by-sex-by-time interactions, should be provided. Tables for summarizing mean comparisons are described for each of steps a, b, and c above with examples of presenting the results shown in Tables 10a and 10b.

e. In addition to the model-based summaries described above, you should also summarize data using descriptive statistics. Tables of descriptive statistics by treatment, similar to those described for continuous variables measured once, should be provided. For variables measured repeatedly, treatment summaries by time and by sex and time should be provided in addition to those described for continuous variables measured once. Table 11 can be modified for all descriptive statistics tables.

f. Provide profile plots. For continuous variables measured repeatedly, profile plots display changes across time for each animal. These plots aid in exploring response differences across time between sexes and among treatments as well as values outside the normal range. For a single variable, all dose groups should be displayed on one page in ascending or descending dose order with all animals from a given dose group depicted in one plot. The same scale should be used on all axes (vertical and horizontal) for all plots for a given variable. Line segments should connect the values between observations for each animal, and different line types should be used for each sex. Horizontal reference lines signifying the upper and lower boundaries for the normal range of the variable should be included. An example of a profile plot is provided in Figure 1. It may also be appropriate to generate other graphical displays that explore relevant trends in the data.

Figure 1: Hemoglobin (mg/dL) over time by subject in each treatment group.



D. Categorical Variables

Clinical observations are examples of categorical data collected in TAS studies. Clinical variables often rate the severity of a clinical condition such as diarrhea or vomiting and are considered ordered categorical variables. Other categorical data may be unordered or nominal in nature, e.g., presence/absence or normal/abnormal. The data may be collected once or repeatedly on the same subject throughout the study.

Dichotomization of categories, analysis methods, and presentation of frequency tables are discussed in general for both singly and repeatedly measured variables. Evaluation criteria are discussed separately for singly and repeatedly measured variables.

1. Dichotomization of categories

   For analysis, CVM recommends that the ordered categorical data be dichotomized using some clinically meaningful way. For example, categories could be dichotomized into normal versus abnormal or normal/mild response versus moderate/severe response.

2. Analysis methods

   Because of the difficulties in analyzing categorical data when the sample size is small, CVM recommends that categorical data be analyzed across sex. Several methods for analysis of categorical variables are available and appropriate but no single analysis approach is recommended. Many chi-squared based methods such as the Cochran-Mantel-Haenszel test and generalized linear mixed models (GLMM) methods are available. CVM recommends statistical analyses only when there are sufficient numbers of observations to make statistical comparisons meaningful.

   For categorical variables measured once, the criteria for evaluating the treatment effect and pair-wise comparisons to the control are the same as described for continuous variables measured once.

   Categorical variables measured repeatedly may be analyzed using GLMM methods. For example, if the sponsor chooses to use these methods on a dichotomous variable, the statistical model should assume a binomial distribution, include treatment, time, and the treatment-by-time interaction as fixed effects, and include random effects as appropriate for the experimental design. The criteria for evaluating the fixed effects and pair-wise comparisons to the control are the same as described for continuous variables repeatedly measured excluding interactions with sex. Dichotomized data could be plotted over time with frequency of the predominant response on the y-axis.

3. Presentation of frequency tables

   Frequency or percent frequency tables should be prepared for the raw categorical data as well as the dichotomized data. The raw data and dichotomized data should be summarized for each treatment by sex and combined over sex. Repeatedly measured variables should be summarized similarly at each time point.

E. Variables Measured but not Statistically Evaluated

There may be additional information on other variables for which statistical analysis is not necessary. These variables may be summarized using the examples described previously in Section II of this document.