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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, FOOD AND DRUG ADMINISTRATION

Center for Drugs and Biologics
Food and Drug Administration
Department of Health and Human Services

GUIDELINE FOR THE FORMAT AND CONTENT
OF THE
NONCLINICAL PHARMACOLOGY/TOXICOLOGY
SECTION OF AN APPLICATION

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TABLE OF CONTENTS

INTRODUCTION 1

I. General Principles 2

 A. Editorial Considerations 2

 B. Abbreviations 2

 C. Availability of Data 3

 D. Tables and Graphs 3

 E. Drug Identification 4

 F. Animals 5

 G. Route and Mode of Administration 6

 H. Doses 6

 I. Determinations 7

 J. Study Reports 8

 K. Published Literature 9

 L. Contact Person 9

II. FORMAT AND CONTENT 10

 A. Table of Contents and Cross-References 10

 B. Summary Discussions 10

 C. Order of Presentation of Studies 11

 D. Format and Content of Individual Study Types 12

 1. Pharmacology Studies 12

 2. Acute Toxicity Studies 13

 3. Subchronic/Chronic/Carcinogenicity Studies 14

 4. Special Toxicity Studies 20

 5. Reproduction Studies 21

 6. Mutagenicity Studies 23

 7. Absorption, Distribution, Metabolism 23

 Excretion (ADME) Studies

III. APPENDICES

 A. Multidose Toxicity/Carcinogenicity Studies 24

 B. Format for Carcinogenicity Study Data 25

GUIDELINE FOR THE FORMAT AND CONTENT
OF THE
NONCLINICAL PHARMACOLOGY/TOXICOLOGY SECTION
OF AN APPLICATION

INTRODUCTION

This guideline describes an acceptable format for organizing and presenting the pharmacology and toxicology data required under 21 CFR 314.50(d)(2), and any related data, in the nonclinical section of the application. These recommendations pertain only to organization of existing data. This guideline is not intended to affect documentation required by the Good Laboratory Practices (GLP) reporting regulations under 21 CFR 58.185, nor does it describe the specific study requirements for particular therapeutic uses or regimens: these are addressed in other guidelines.

This guideline is issued under 21 CFR 10.90. An applicant may, but is not required to, rely upon the guideline in preparing the Nonclinical Pharmacology/Toxicology section of an application. When a different approach is chosen, the applicant is encouraged to discuss the matter in advance with FDA to prevent the expenditure of money and effort on preparing a submission that may be determined to be unacceptable.

The agency recognizes that most or all of the nonclinical data submitted to an application will have been developed over several years and submitted intermittently to an investigational drug application (IND). We recommend the reorganization of the studies to the extent feasible, for submission to an application, even though the formats of all individual studies cannot always easily be made to conform in all details to these guideline recommendations. We anticipate that this guideline will also shape future IND submissions so that relatively little revision will be needed for the application other than to rearrange study order.

I. GENERAL PRINCIPLES

The following are general principles that apply to the submission of all pharmacology and toxicology studies regardless of the characteristics of a particular study.

A. Editorial Considerations

The entire submission should receive careful editorial, as well as scientific, review. It should be proof-read to assure that all pages are in consecutive order and are distinctly copied and otherwise readable.

B. Abbreviations

Standard abbreviations acceptable to a refereed U.S. pharmacology/toxicology journal, such as Toxicology and Applied

Pharmacology, should be used wherever possible. All other abbreviations should be identified at the beginning of each section in which they are used or in footnotes to tables and graphs.

C. Availability of Data

Format recommendations in this guideline address typical circumstances of data review. However, it is not possible to anticipate all further analyses that may require display of certain data in a different format.

Data should therefore be kept available by the sponsor, properly edited and quality controlled, so that the reviewer may be rapidly supplied with requested data in the form needed. This data base should document all relevant observations for each animal (e.g., toxic signs, clinical pathology, tumor palpation, sacrifice or death, necropsy results, etc., and the dates of these events). Errors detected after submission should be brought to the attention of the FDA reviewer and tabulated separately.

D. Tables and Graphs

1. Well-constructed tables are fundamental to the reporting and evaluation of pharmacological and toxicological data. All tables should be clearly identified and captioned in

English, with symbols keyed to a footnote or conveniently accessible reference page.

2. Where possible, summary tables should permit comparison of selected results from all dosage groups and relevant controls on the same page. Separate tables may be used for males and females if direct comparison between the two sexes is not crucial.
3. For each multidose study, comprehensive data tables should present the data in order of increasing dosage within each sex, males preceding females (See I.F. and I.H.).
4. Graphs should supplement, not replace, data tables. They should be of a quality acceptable to a refereed U.S. scientific journal.

E. Drug Identification

1. When compared in tables or discussions with numbered congeners or metabolites, the drug's code number should be highlighted by underlining or other prominent marker. Metabolites or reference compounds referred to by code number should be identified by chemical name or structure in a conveniently accessible, nearby location.

2. Batch or lot numbers should be included where appropriate or needed for comparative review.

F. Animals

1. Where more than one animal species is used in a particular type of test or study, these data should be reported or tabulated in the following relative order, males preceding females:

- Mouse
- Rat
- Hamster
- Other rodent(s)
- Rabbit
- Dog
- Monkey
- Other non-rodent mammal(s)
- Non-mammals

2. Data for typical adult animals should precede that for infant, geriatric, or disease-model animals if comparative studies are done in one or more of the latter groups. Age and weight ranges should be indicated.
3. Strains and animal suppliers should be specified for each study.

G. Route and Mode of Administration

1. Studies for each species within each type of study should first represent the intended route of human use, followed by data for other routes in the following relative order:

Oral
Intravenous
Intramuscular
Interperitoneal
Subcutaneous
Inhalation
Topical
Other in vivo
In vitro

H. Doses

1. Multidose data should be displayed from the lowest to the highest dose.
2. Within each multigroup study, results should similarly be presented in all tables in order of increasing dosage:

Untreated control
Vehicle control
Low dose
Middle dose(s)
High dose
Positive or comparative control(s)

3. Dose should preferably be based on the active moiety component if the drug is a salt or other dissociable derivative. In any case, it should be clearly stated that whether the calculation of dose is based on the active

moiety component or on the entire drug substance, and the calculation should be consistent for all studies.

4. Drug doses should be expressed on a body weight basis (e.g., mg/kg, mcg/kg), except when there is a specific reason to express doses in some other way, e.g., on a body surface basis (mg/m^2) or as plasma or serum concentration.
5. When the drug is administered in diet or drinking water, the daily dose, calculated periodically from actual body weight and food or water consumption data, should be included in the report as the dose range per sex for each group at the beginning and end of the study and at intervals throughout a chronic study. Designated or average group doses may be used in tables for brevity. Doses should not be expressed solely as concentration in food or drinking water.

I. Determinations

1. All biological tests, laboratory determinations and statistical methods should be described in the study report or properly referenced to a methods appendix or literature citation.

2. Units for each determination should be specified in the results. Where appropriate, the variability or group mean values should be shown by approximately labeled standard errors, standard derivations or confidence limits.
3. Studies with a radioactive drug should indicate the molecular location of the radioisotope and its specific activity.

J. Study Reports

1. Reports of studies related to safety should contain GLP statements required by 314.50(d)(2)(v) and 21 CFR Part 58.
2. New studies or final reports not previously submitted to an IND or a previous marketing application submission should be so identified.
3. When appropriate, study reports should include description of pre-study conditioning, the method of assignment of animals to control and test groups, the basis for dose selection, pertinent information about animal husbandry, and pathologic diagnosis procedures (e.g., group review, blinding, etc.).

K. Published Literature

1. Pertinent published methods or data should be appended to the appropriate study report sections or subsections.
2. References to published reports otherwise submitted to the application should be so identified.
3. When published literature is used to replace or augment studies bearing on application approval, reprints should be provided in the same relative study-type sequence described in II.C.2.
4. References or reprints submitted to an application supplement should also be grouped in relative sequence corresponding to II.C.2.

L. Contact Person

The name, title, address, and telephone number of the person FDA may contact concerning any issues or questions about the data submitted in the nonclinical pharmacology/toxicology section of an application should be provided.

II. Format and Content

A. Table of Contents and Cross-References

1. All nonclinical studies, with volume and page numbers, should be listed in the application's table of contents and replicated at the beginning of this technical section.
2. Each application volume should display the pertinent portion of the table of contents, expanded to include page references for all major subparts of the studies therein, e.g., study descriptions, GLP statements, tables, etc.
3. New studies or final reports, not previously submitted to the IND or to a previous NDA submission for the drug under review, should be identified in a consistent and conspicuous manner in all tables of contents.

B. Summary Discussions

1. In the technical section, each study report should include a brief narrative description of its notable findings, as well as a comprehensive discussion of notable findings in all related studies for each species and notable species differences in the various subsections detailed in II.D.

2. The overall pharmacology/toxicology summary required as part of the application (Section 314.50(c)), addressed in a separate guideline, should provide an integrated discussion of all pertinent findings, including interstudy and interspecies comparisons, with appropriate cross-references to the technical section. Data location cross-references should be included when correlations or comparisons are made among different types of data: e.g., pharmacology or drug metabolism with some aspect of toxicology, pharmacokinetics and metabolism in animals with that in humans, etc.

C. Order of Presentation of Studies

1. Chronological sequence of dates of conduct or previous submission of studies to the IND is not an acceptable basis for organizing study reports in an application.
2. The following order is recommended for submission of various types of studies, as appropriate to a particular application:

Pharmacology Studies
Acute Toxicity Studies
Multidose Toxicity Studies (Subchronic,
Chronic, Carcinogenicity)
Special Toxicity Studies
Reproduction Studies
Mutagenicity Studies
Absorption, Distribution, Metabolism,
Excretion (ADME) Studies

D. Format and Content of Individual Study Types

1. Pharmacology Studies

(a) Pharmacology studies should be presented in the following order, with pharmacodynamic ED₅₀ in dose-ranging studies preceding mechanism of action studies within each subset.

(1) Effects related to the therapeutic indication

- primary activity
- secondary activities

(2) Effects related to possible adverse reactions

(3) Interactions with other drugs (or cross-reference location of this information in any of the above subsections)

(b) Within the above categories, data should be grouped in the following order:

Neuropharmacological
Cardiovascular/respiratory
Gastrointestinal
Genitourinary
Endocrine
Anti-inflammatory
Immunoactive
Chemotherapeutic
Enzyme effects
Other (identify)

- (c) The data should be summarized in tabular form, with the various studies within each category grouped to present a coherent pharmacological profile of the drug.
- (d) To the extent possible, species and routes of administration for each category of study should follow the sequence recommended under I.F. and I.G.

2. Acute Toxicity Studies

- (a) Pretest conditioning and age of animals, dosing procedures, vehicles used, and dosage volumes should be specified for each study.
- (b) The types and severity of toxic signs and their onset and progression, or reversal in relationship to dosage and time after dosing should be described for each species.
- (c) Lethal dose data (approximate or calculated median, limit doses, etc.) should be tabulated for interstudy and/or interspecies comparison. Background information should include total numbers dosed and mortality incidence with time of death for each sex at each dose.

- (d) Order of species and routes of administration should follow what is recommended under I.F. and I.G.

3. Subchronic/Chronic/Carcinogenicity Studies

- (a) At the beginning of this subsection, all studies should be listed and briefly described in a table for quick reference to the various studies in each species. Studies should be grouped by species, in order of increasing duration and/or route of administration in the sequence recommended in the General Principles in section I of this guideline. Table headings should include animal species and strain, initial group size per sex, dosing, route and mode (e.g., gavage, diet), designated group doses, duration of study in weeks, week of any scheduled interim sacrifices, the name of the laboratory performing the study, and the report number. (See Appendix A for example.)
- (b) Detailed study reports should be grouped by species in the same sequence as in the table described in the preceding paragraph. Summary discussions of notable findings in all studies in each species should emphasize relationships to dose and duration of treatment.

- (c) The description of each study should include the following information:

Species, strain, source, age at initiation of dosing
Males, number per group at the beginning and end of the study, then females, number per group at the beginning and end of the study
Route and mode of administration (See I.G.)
Calculated dosage levels and ranges for each sex in each group, (See I.H.)
Basis or rationale for dose selection
Administration vehicle and control treatment
Drug batch or lot number
Duration of treatment (weeks)
Duration of study (weeks)
Interim sacrifice, if any (number per sex at _____ weeks)

- (d) Each individual study report should include group mean tabulations and individual animal tabulations.

- (e) Within the individual study reports and tabulations, data from all dose levels in the sequence recommended in I.H. should be presented, as appropriate for each study, in the following relative order:

Observed effects
Mortality
Body weight
Food/water consumption
Physical examinations (ECG, ophthalmic exam, etc.)
Hematology/bone marrow/coagulation
Blood chemistry/urinalysis/ADME data
Organ weights
Gross pathology
Histopathology

- (f) Individual animals should be listed in the same sequential order in all tables in which they appear. If animals are listed by random number assignment, a consecutive numbering system should be added to ease tracking of individual animals among tables in the NDA submission.
- (g) Tables should be well constructed to permit comparison among individuals, among several related determinations, or among patterns of responses over time. Mean values and standard deviations should be included where appropriate for inter-column or inter-group comparison.
- (h) This guideline recognizes that a variety of automated data systems currently exist for recording histopathology data. Nevertheless, we recommend presentation of histopathology data in a logical and consistent manner for all studies within any one application. To the extent possible, the pathologic nomenclature used should be consistent among all studies and histopathologic lesions should be listed by physiologic/anatomic systems in a manner similar to what is used by the National Toxicology Program.

- (i) The pathology table should:
- (1) Identify the organs and tissues examined and all lesions identified in each animal, as well as the comparative group incidence of these lesions and, as appropriate, their relative severity.
 - (2) Identify or appropriately tabulate the incidence of pathologic findings among animals that died, or were prematurely sacrificed, for comparison with findings observed at scheduled sacrifice.
 - (3) Maintain consistency of order of presentation of non-neoplastic and neoplastic pathology, if tabulated separately, for convenience of comparison.
 - (4) To facilitate statistical review, present tumor data for male and female animals separately as follows:
 - (a) A chronological listing showing the period (e.g., week) in which each tumor was discovered, the dose group of the animal, the animal number, whether the animal was sacrificed or died, the site of the tumor, the tumor type, and an assessment of the

malignancy of the tumor. In this table, the number of lines for each animal should be equal to the number of tumors identified.

(See example 1 in Appendix B.)

(b) A summary table showing for each period (e.g., week) in which a death or sacrifice occurred and for each dose group the number of animals entering the period, the number dying, the number sacrificed, the number of these animals necropsied completely, and the number necropsied to any extent. (See example 2 in Appendix B.)

(c) A summary table of tumor occurrences with deaths and sacrifices combined, organized by body system, organ, tumor type, and dose level (including historical and positive controls, if any). The body of the table should contain the total number of animals with tumors of the stated type, disregarding time of discovery. The table should facilitate comparisons across dose groups. (See example 3 in Appendix B.)

- (d) For each tumor found to be statistically significant at the $p = .05$ level (one-sided), by use of a statistical test of dose response over the entire study that is adjusted for mortality as appropriate, and that is not adjusted for multiple comparisons or multiple testing:

Report the estimated incidence rate of fatal tumors in each time period (number of tumor-bearing animals divided by number of animals entering the period) and the prevalence rate of nonfatal tumors in each time period (number of tumor-bearing animals divided by the number of animals dying in the period).

State which statistical test is used and provide the calculated p-value for that test (not simply that the p-value is less than .05).

Provide results for both statistically significant positive and for statistically negative dose-response findings.

Further guidance on the display and analysis of tumor data and the formatting of a computer-readable database, if desired, is available on request.

4. Special Toxicity Studies

- (a) These include studies appropriate to a particular formulation or route of administration, e.g., parenteral or topical irritation studies, in vitro hemolysis, etc., or studies with a particular animal model relevant to the human disease or age. The study description will usually direct the format of the results, with order of species and dosage as described under I.F. and I.H.
- (b) In vivo results should be tabulated to show group comparison and time-related or progressive effects within each group.
- (c) In vitro results should be tabulated to indicate the type of test or test system, dose range in increasing order, and effects related to dose.

5. Reproduction Studies

(a) All reproduction studies should be summarized at the beginning of this section, in a table patterned after that recommended for multidose toxicity studies (II.D.3.a. and Appendix A) in the following order of presentation.

(b) Studies should be presented in the following sequence, with order of species and dosage within each type of study as recommended in I.F. and I.H.:

- Segment I — Fertility and Reproductive Performance
- Segment II — Teratology
- Segment III — Perinatal-Postnatal
- Other Studies — Multigeneration, etc.

(c) Study descriptions should include dosing regimens and procedural details. Any procedural differences from FDA reproduction study guidelines should be identified.

(d) As appropriate, observations and their incidence in relationship to dosage and/or time should be presented in the following relative order:

Maternal effects and day of parturition/necropsy (and paternal effects in Segment I study)

Maternal necropsy:

Corpora lutea
Uterine contents
Implantations
Dead fetuses

Fetuses (grouped by litter):

Sex ratio
Weight
Viability
Gross observations
Visceral abnormalities
Skeletal abnormalities

Neonates to weaning:

Sex ratio
Viability
Growth
Behavior and performance
Anatomical abnormalities

6. Mutagenicity Studies

(a) The results of available studies should be tabulated to indicate type of study, methods used, dose range in increasing order, and effect at each dose.

(b) Studies should be presented in the following order (note difference from I.F. and I.G.):

In vitro non-mammalian cell system
In vitro mammalian cell system
In vivo mammalian system
In vivo non-mammalian system

7. Absorption, Distribution, Metabolism, Excretion (ADME)

Studies

(a) Organize available data for each species/strain, in order recommended in I.F., in the following sequence in summary tables and detailed reports:

Absorption, pharmacokinetics, serum
half-life, etc.
Protein binding
Tissue distribution/accumulation
Enzyme induction or inhibition
Metabolism characteristics and metabolites
Excretion pattern

(b) Study descriptions should clearly state the dose(s) used in each study.

SUMMARY

MULTIDOSE TOXICITY/CARCINOGENICITY STUDIES

Species	Strain	Initial Group	Mode of Administration	Doses Mg/kg/Da	Duration (wks)	Interim Sacrifice (wks)	Laboratory	Report No.
Mouse	C57B1/6	20M + 20F	Diet	0,10,50,100	6	-	PDQ Labs	xxx
	C57B1/6	50M + 50F	Diet	0,5,10,50	96	-	PDQ Labs	xxx
Rat	Wistar	10M + 10F	Gavage	0,10,50,100	2	-	New Drug Co.	YYY
	Wistar	20M + 20F	Gavage	0,10,30,60	13	-	New Drug Co.	YYY
	Wistar	35M + 35F	Diet	0,10,30,60	52	-	New Drug Co.	YYY
	Fisher344	70M + 70F	Diet	0,5,20,50	104	52	PDQ Labs	YYY
Dog	Beagle	2M + 2F	Capsule	0,2,5,10	2	-	New Drug Co.	YYY
	Beagle	4M + 4F	Capsule	0,1,3,6	13	-	New Drug Co.	YYY
	Beagle	5M + 6F	Capsule	0,1,3,6	52	26	New Drug Co. (EZI Labs-Path)	YYY ZZZ
Monkey	Rhesus	3M + 3F	Gavage	0,2,5,10	13	-	New Drug Co.	xxx

FORMAT FOR CARCINOGENICITY STUDY DATA

(1) Example of chronology listing

Males

<u>Week</u>	<u>Dose Group</u>	<u>Animal Number</u>	<u>Death Status</u>	<u>Organ</u>	<u>Tumor Type</u>	<u>Malignancy</u>
35	C	010	D	Liver	hepatoma	N
52	C	024	S	Liver	adenoma	N
52	C	024	S	Pituitary	adenoma	N
53	M	018	D	Pituitary	adenoma	N

(continue to the end of the study)

Term	H	046	S	Testis	fibroadenoma	N
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(2) example showing summary of animal deaths and sacrifices

Males																				
Week	Controls					Low Dose					Medium Dose					High Dose				
	E	D	S	N	NP	E	D	S	N	NP	E	D	S	N	NP	E	D	S	N	NP
34	70	--	--	--	--	70	--	--	--	--	70	--	--	--	--	70	--	--	--	--
35	70	1	1	1	1	70	--	--	--	--	70	--	--	--	--	70	--	--	--	--
36	68	--	--	--	--	70	1	--	--	1	70	--	--	--	--	70	--	--	--	--
39	68	--	--	--	--	69	--	--	--	--	70	--	--	--	--	70	1	--	1	--
41	68	--	--	--	--	69	--	--	--	--	70	1	--	--	1	69	--	--	--	--
43	68	--	--	--	--	69	--	--	--	--	69	1	--	1	--	69	--	--	--	--
49	68	--	--	--	--	69	--	--	--	--	68	--	--	--	--	69	1	--	--	--
52*	68	--	10	10	--	69	--	10	10	--	68	--	10	10	--	68	--	10	10	--
53	58	1	--	1	--	59	--	--	--	--	58	--	--	--	--	58	--	--	--	--
58	57	--	--	--	--	59	--	--	--	--	58	3	--	3	--	58	--	--	--	--
62	57	1	--	1	--	59	--	--	--	--	55	--	--	--	--	58	1	--	1	--
65	56	--	--	--	--	59	--	--	--	--	55	--	--	--	--	57	1	--	1	--
70	56	--	--	--	--	59	--	--	--	--	55	3	--	3	--	56	1	--	1	--
71	56	--	1	1	--	59	1	1	2	--	52	1	--	1	--	55	--	--	--	--
74	55	1	--	1	--	57	--	--	--	--	51	2	--	2	--	55	--	--	--	--
75	54	1	--	1	--	57	--	--	--	--	49	--	--	--	--	55	--	--	--	--
(continue to the end of the study)																				
Term*	41	2	39	41	--	40	--	40	40	--	36	--	36	36	--	38	1	37	36	2

Note: E = Number entering period
D = Deaths; S = Sacrificed moribund
N = Necropsied completely; NP = necropsied to some extent
* = Scheduled and terminal sacrifices

(3) Number of Tumor-bearing Animals

Males

Body system Organ Tumor type	Historical Controls (n = 500)	Untreated Controls (n = 50)	Low (n = 50)	Medium (n = 50)	High (n = 50)	Positive Controls (n = 50)
Digestive						
Liver	71	6	8	10	8	15
adenoma	32	4	9	9	.13	22
fibroadenoma						