PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION F

HAZARD EVALUATION:

HUMANS AND DOMESTIC ANIMALS

Series 83-3

Rat or Rabbit Developmental Toxicity Study

ADDENDUM 1 ON DATA REPORTING

Prepared by:

Science Mission Support Staff
Toxicology Branch
Hazard Evaluation Division

Project Manager:

Elizabeth M.K. Leovey, Ph.D
Hazard Evaluation Division

Office of Pesticide Programs
U.S. Environmental Protection Agency

Subdivision F - Rat or Rabbit Developmental Toxicity Study

Table of Contents of Addendum

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HUMANS AND DOMESTIC ANIMALS

Rat or Rabbit Developmental Toxicity Study

Subdivision F, Series 83-3

DATA REPORTING

INTRODUCTION

A. Purpose

This study is designed to determine the potential of the test substance to induce structural and/or other abnormalities to the fetus which may arise from exposure of the mother during pregnancy.

B. Objective

The objective of this Guideline is to provide an example of an acceptable format for reporting materials, methods and results of developmental toxicity studies. These recommendations are intended to show how the requirements in §80-4 and §83-3(h) of this Subdivision and requirements in the Agency's Good Laboratory Practice Standards (40 CFR Part 160) may be organized in a Final Report that can be reviewed effectively and expeditiously in accordance with "Proposed Guidelines for the Health Assessment of Suspect Developmental Toxicants and Request for Comments" (Federal Register, Vol. 49, No. 227, November 23, 1984, pages 46324-46331) and the Hazard Evaluation Division's Standard Evaluation Procedure for Teratology studies (EPA-540/9-85-018 published for the Agency in June, 1985). While following this Guideline is not mandatory, data submitters are encouraged to submit complete reports which can be efficiently reviewed by the Agency.

RESPONSE TO PUBLIC COMMENTS

Comments were received from 17 sources including testing facilities, pesticide manufacturers, a manufacturer's organization, a scientific association, and one state government agency. There were three general concerns described by most commenters. Specific comments on the Teratology Data Reporting Guideline (Addendum to §83-3 of Subdivision F of the Pesticide Assessment Guidelines) were related to those three issues.

A. Comments

The proposed Data Reporting Guideline (DRG) was described as inconsistent with guidelines published in Subdivision F (§80-4 and §83-3), 40 CFR Part 160, Good Laboratory Practice Standards, and "Proposed Guidelines for the Health Assessment of Suspect Developmental Toxicants and Request for Comments" (Federal Register, Vol. 49, No. 227, November 23, 1984, pages 46324-46331). Commenters also expressed concern that the Teratology DRGs represented an extension of previous Guidelines to include conduct of the study.

The second concern most commenters had about the proposed DRG was that it does not accomodate computerized systems developed by various laboratories for presenting and analyzing data from Developmental Toxicity studies.

Finally, commenters were concerned that the proposed format may represent a trend toward a clerical, statistical, or "checklist" approach to interpretation of results rather than one that depends upon scientific judgement.

B. Response

The Agency has reconsidered the proposed Data Reporting Guideline with respect to Subdivision F reporting requirements (§80-4 and §83-3), Good Laboratory Practice Standards (40 CFR, Part 160), and the proposed Health Assessment Guidelines for Suspect Developmental Toxicants, and appropriate revisions have been made.

In order to accomodate the variety of formats used for computerized data recording and analysis, the use of example tables has been limited to a generalization of commonly submitted formats. The examples should not be considered as required or preferred, but they represent the most frequently submitted and accepted format.

In addition, the revised Data Reporting Guideline includes citations of appropriate sections of the three Agency sources mentioned above to avoid extending or repeating existing requirements or Guidelines.

GUIDELINE

The Final Report should contain the following items.

COVER PAGE

Cover page and additional documentation requirements (ie. requirements for data submission, Good Laboratory Practice statement, and procedures for claims of confidentiality of data), if relevant to the study report, must precede the content of the study formatted below. These currently proposed requirements are described in 49 FR (188) 37596 (9/26/84).

TABLE OF CONTENTS

This item should be a concise listing of the essential elements of the Final Report including the page numbers for each. Essential elements should include a Summary, an Introduction, the Materials and Methods section, Results, Discussion, Bibliography, Tables, Figures, Appendices, and key subsections as appropriate.

BODY OF THE REPORT

This item shall include all information required in §80-4(b)(2), §80-4(c), and §83-3(h) of this subdivision. Additional recommendations are provided in paragraphs identified as III and IV of this addendum.

I. SUMMARY

As per §80-4 (b)(1) of this Subdivision, this section of the test report shall contain a summary and analysis of the data, and a statement of the conclusions drawn from the analysis. The summary should highlight any and all positive data or observations, and any deviations from control data which may be indicative of toxic effects. The summary should be presented in sufficient detail to permit independent evaluation of the results.

II. INTRODUCTION (include the objectives of the study)

III. MATERIALS/METHODS

This section of the Final Report shall include information required by §80-4 of this Subdivision, and it may also contain the following descriptions specific to developmental toxicity studies:

A. Test Animals. Include pre-test mating or insemination procedures;

B. Observations. Include descriptions of the type, frequency and duration in accordance with §83-3(g)(7) for maternal toxicity and developmental toxicity end points as required in §83-3(g) as well as descriptions of the methods for fetal examinations (e.g., Staples' technique, Wilson's technique, etc).

C. Rationale for deviations from standard protocol. A description of the rationale for deviations from the protocol recommended in §83-3 should be provided along with rationale for such changes;

D. Evaluation Procedures. The Final Report should describe:

1. Definitions used by the investigators for terms such as malformation, variation, alterations, etc., which are used to classify fetal changes;

2. Observations used to calculate percentages, weight changes, or other indices (e.g., number of affected litters = no. with resorptions + no. with dead fetuses + no. with malformed fetuses; see paragraph (d) of this addendum for additional examples);

3. Methods for adjusting data (censoring, pooling, or transforming results) prior to statistical analysis; and

4. Statistical procedures shall be described according to the recommendations of §80-4(c) of this Subdivision.

IV. RESULTS

Example formats in this section are generalized from those commonly submitted to the Agency. They do not represent required or preferred formats, but they are provided as a guide.

A. Summary Tables. Paragraph 83-3(h) and §80-4 require results to be summarized in tabular form. Example Formats 1-4 show common presentations of summary data that are accepted by the Agency.

1. Section §83-3 of this Subdivision does not mention counting implantation sites, but that parameter is described in the Example Formats and in the proposed Health Assessment Guidelines mentioned in the Introduction §B to this addendum. Reports received by the Agency routinely include that parameter, and it is necessary for calculation of such indices as pre-implantation loss or percent pre-implantation loss.

2. The proposed Health Assessment Guidelines cited in the Introduction §B to this addendum indicate the observations and indices to be used in the Agency's assessment of results from developmental toxicity studies. With the addition of implantation data to the observations required under §83-3, any or all of the end points described in the proposed assessment guidelines may be calculated from the required observations and included in a Final Report as shown in Example Formats 1 through 4.

B. Individual Animal Data. These data may be presented as in Example Formats 5 through 8 and appended in the Final Report.

V. DISCUSSION

VI. BIBLIOGRAPHY

This item should contain a list of references cited in the body of the report.

VII. VERIFICATION

This item shall contain information required by §80-4(b)(2). Each test report shall be:

A. Signed by each of the senior scientific personnel, including the laboratory director, responsible for performing and supervising the testing, preparing, reviewing, and approval of the test report; and

B. Certified by the applicant or an authorized agent of the applicant as a complete and unaltered copy of the report provided by the testing laboratory whether independent or owned, operated, or controlled by the applicant.

VIII. ARCHIVES

This section of the Final Report shall contain all information required in 40 CFR Part 160. 185.

IX. TABLES/FIGURES

X. APPENDIX(ES)

These should include individual animal data, historical control data, pathology report, analytical method and results of analyses on the test substance and test diet (if the test material is administered via the diet), details of statistical analyses, protocol, and other information as appropriate.

Example Format 1

Example of frequently submitted summary table presentations of some maternal and developmental toxicity end points

Observation	Dose (mg/kg/day) 0	Y	5Y	10Y
Clinical sign A	X	X	X	X
Clinical sign B	.	.	.	.
.....	.	.	.	.
Clinical sign Z	X	X	X	X
Number of animals	N	N	N	N
Number pregnant; number died before, during, and after dosing; totally resorbed litters; abortions; litters with dead fetuses; number not pregnant; live litters at cesarean section:	X	X	X	X
Number of litters with resorptions; Corpora lutea/dam; implantations/dam; pre-implantation losses/litter*; resorptions/litter (early, late, and total)*; mean % resorptions or dead conceptuses/litter*: live fetuses/litter*; live male fetuses/litter*; number live female fetuses/litter*; sex ratio (males/females)/litter*; dead fetuses/litter*; uterine weight (g); mean live fetal weight (g);	Mean S. D. N	Mean S. D. N	Mean S. D. N	Mean S. D. N

*These parameters should also be presented on a total per group basis

Example Format 2

Example of a frequently submitted summary table format for maternal body weight results in a Developmental Toxicity Study

Observation	Dose (mg/kg/day) 0	Y	5Y	10Y
Body weight (g) at				
Day 0	Mean S. D. N	Mean S. D. N	Mean S. D. N	Mean[b] S. D. N
Each treatment day when weights are obtained	Mean S. D. N	Mean S. D. N	Mean S. D. N	Mean[b] S. D. N
Day of sacrifice	Mean S. D. N	Mean S. D. N	Mean S. D. N	Mean[b] S. D. N
Gravid uterine weight (g)	Mean S. D. N	Mean S. D. N	Mean S. D. N	Mean[b] S. D. N
Corrected body weight (subtract gravid uterine weight from day of sacrifice weight)	Mean S. D. N	Mean S. D. N	Mean S. D. N	Mean[b] S. D. N
Body weight change, food and water consumption	Mean S. D. N	Mean S. D. N	Mean S. D. N	Mean S. D. N

[a]Throughout gestation, during treatment (increments of time between measurements), post-treatment to sacrifice, corrected body weight change throughout gestation (minus gravid uterine weight or litter weight at sacrifice).

[b]Means that are statistically significantly different from control group means should be highlighted.

Example Format 3

Example of a frequently submitted summary table presentations of fetal effects (ie., malformations, variations, alterations or other designations used by various investigators)*

Observation	Dose (mg/kg/day) 0	Y	5Y	10Y
External Alterations				
No. fetuses or litters examined	N	N	N	N
Alteration A	X	X	X	X
Alteration B	X	X	X	X
.	.	.	.	.
.	.	.	.	.
Alteration Z	X	X	X	X
Soft Tissue Alterations				
No. fetuses or litters examined	N	N	N	N
Alteration A	X	X	X	X
Alteration B	X	X	X	X
.	.	.	.	.
.	.	.	.	.
Alteration Z	X	X	X	X
Skeletal Alterations				
No. fetuses or litters examined	N	N	N	N
Alteration A	X	X	X	X
Alteration B	X	X	X	X
.	.	.	.	.
.	.	.	.	.
Alteration Z	X	X	X	X

*These results are presented on a per litter and/or a per group basis. They are also expressed as percentages in some reports.

Example Format 4

Presentation of some optional maternal toxicity end points such as liver weight[a] and weight ratios (% body weight)

Dosee (mg/kg/day)	Body weight (g) at Day 0	Body weight (g) at Sacrifice	Liver wt as % body wt on Day 0	Liver wt as % body wt on Sacrifice
0	Mean S. D. N	Mean S. D. N	Mean S. D. N	Mean[b] S. D. N
Y	Mean S. D. N	Mean S. D. N	Mean S. D. N	Mean[b] S. D. N
5Y	Mean S. D. N	Mean S. D. N	Mean S. D. N	Mean[b] S. D. N
10Y	Mean S. D. N	Mean S. D. N	Mean S. D. N	Mean[b] S. D. N

[a]May include other organs as appropriate.

[b]Means that are statistically significantly different from control group means should be highlighted.

Example Format 5

Example of a format for reporting individual survival and toxic signs results

Dose group	Animal no.	Day of death or sacrifice	Toxic signs: Onset day	Toxic signs: Duration	Toxic signs: Description
Y	1	X	X	X	Brief description of changes in behavior, appearance, or other clinical signs.
	2	X	X	X	
	3	X	X	X	
	.	.	.	.	
	.	.	.	.	
	.	.	.	.	

Example Format 6

An example of a format for reporting individual maternal body weight data

Dose group	Animal no.	Body weight (g) on Gestation Day* 0	6**	9***	...	15†	20††
Y	1	X	X	X	...	X	X
	2	X	X	X	...	X	X
	3	X	X	X	...	X	X
	.	.	.	.	...	.	.
	.	.	.	.	...	.	.
	.	.	.	.	...	.	.
	N	X	X	X	...	X	X
		Mean	Mean	Mean	...	Mean	Mean
		S. D.	S. D.	S. D.	...	S. D.	S. D.
		N	N	N	..,	N	N

*For food consumption data these would be increments such as Days 0-6 (prior to treatment); 6-9, 9-12, 6-16, etc (during treatment); 6-16 (during treatment); 0-20 (throughout gestation); etc.

**First treatment day ***Weighing days during treatment
†Last treatment day ††Day of sacrifice

Example Format 7

Example format for presentation of individual animal data for pregnancy status and litter results

Dose Group	Animal no.	Corpora lutea	implan-tations	Pre-implanta-tion losses	Resorptions Early	Late	Total
Y	1	X	X	X	X	X	X
	2	X	X	X	X	X	X
	3	X	X	X	X	X	X
	.	.	.	.	.	.	.
	.	.	.	.	.	.	.
	.	.	.	.	.	.	.
	n	X	X	X	X	X	X

Dose Group	Animal no.	Dead fetuses	Live fetuses Males	Females	Total	Average fetal weight (g)
Y	1	X	X	X	X	X
	2	X	X	X	X	X
	3	X	X	X	X	X
	.	.	.	.	.	.
	.	.	.	.	.	.
	.	.	.	.	.	.
	n	X	X	X	X	X

Example Format 8

Example format for presentation of individual animal data for external, soft tissue, and skeletal fetal defects

Dose group	Animal no.	Fetus	Description of defect(s)
Y	1	A	External alterations Skeletal alterations (if skeleton was examined Soft tissue alterations (if fetus examined for soft tissue effects)
		B	External alterations Skeletal alterations (if skeleton was examined Soft tissue alterations (if fetus examined for soft tissue effects)
		-	—
		-	—
		K	External alterations Skeletal alterations (if skeleton was examined Soft tissue alterations (if fetus examined for soft tissue effects)
	—	—	—
	n	A	External alterations Skeletal alterations (if skeleton was examined Soft tissue alterations (if fetus examined for soft tissue effects)
		B	External alterations Skeletal alterations (if skeleton was examined Soft tissue alterations (if fetus examined for soft tissue effects)
		-	—
		-	—
		K	External alterations Skeletal alterations (if skeleton was examined Soft tissue alterations (if fetus examined for soft tissue effects)