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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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FEB 24 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Evaluation of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) for Developmental Toxicity in Both the Rat and Rabbit - Guideline Series 83-3 (MRID Nos.: 423515-01 and 423928-01)

Tox Chem No. 016E
EPA ID No. 069105
DP Barcode No. D187063
Submission No. S433883
Case No. 819070

FROM: Brian Dementi, Ph.D., D.A.B.T. Brian Dementi 9/27/93
Review Section III
Toxicology Branch I
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THRU: Karen Hamernik, Ph.D., Section Head
Review Section III
Toxicology Branch I
Health Effects Division (H7509C) K. Hamernik 2/4/94
KB 2/22/94

The Data Evaluation Reviews for the ADBAC Rat and Rabbit Developmental Toxicity Studies, submitted by the ADBAC QUAT Joint Venture/Chemical Specialties Manufacturers Association toward satisfying the Registration guideline Series 83-3 testing requirements is herewith submitted to SRRD.

Results of the studies are summarized as follows. For further details see the respective Data Evaluation Reviews.

Developmental Toxicity Study in Rats - The test material was evaluated in this study at dosage levels of 0, 10, 30 and 100 mg/kg/day as administered daily by oral gavage (days 6 through 15 of gestation) to each of 25 CD rats per dose group. The maternal LOEL = 30 mg/kg/day, based on clinical signs (perioral wetness and

audible respiration) and decreased body weight gain and food consumption; NOEL = 10 mg/kg/day. For developmental toxicity, NOEL = 100 mg/kg/day (HDT), and, hence, the LOEL was not identified as it would exceed 100 mg/kg/day. This study is rated Core Guideline and satisfies the Guideline testing requirements for Series 83-3, developmental toxicity study in rats.

Developmental Toxicity Study in Rabbits - The test material was evaluated in the study at dosage levels of 0, 1, 3 and 9 mg/kg/day as administered daily by oral gavage (days 6 through 18 of gestation) to each of 16 NZW rabbits per dose group. The maternal LOEL = 9 mg/kg/day based on clinical signs (hypoactivity and labored and/or audible respiration); NOEL = 3 mg/kg/day. For developmental toxicity, NOEL = 9 mg/kg/day (HDT) and, hence, the LOEL was not identified as it would exceed 9 mg/kg/day. The study is rated Core Guideline and satisfies the guideline testing requirements for Series 83-3, developmental toxicity study in rabbits.

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FINAL

DATA EVALUATION REPORT

ALKYL DIMETHYL BENZYL AMMONIUM CHLORIDE

Study Type: Developmental Toxicity in Rabbits

Prepared for:

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by:

Clement International Corporation
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Fairfax, VA 22031

Principal Reviewer: Sanju Diwan — Sanju Diwan, Ph.D. — Date 8/25/93

Independent Reviewer: Pia Lindström — Pia Lindström, D.P.H. — Date 8/25/93

QA/QC Manager: Sharon Q. Segal — Sharon Segal, Ph.D. — Date 8/25/93

Contract Number: 68D10075
Work Assignment Number: 2-86, 2-121
Clement Number: 226
Project Officer: Caroline Gordon

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EPA Reviewer: Ann Clevenger, Ph.D.
Review Section I, Toxicology Branch I/HED

EPA Section Head: Marion Copley, D.V.M.
Review Section IV, Toxicology Branch I/HED

Signature: [signature]
Date: 9/8/93

Signature: Marion Copley
Date: 9/8/93

DATA EVALUATION REPORT

STUDY TYPE: Developmental toxicity in rabbits; Guideline Series 83-3

EPA IDENTIFICATION NUMBERS

PC CODE: 069105

TOX CHEM. NO.: 016 E

MRID NO.: 423928-01 (Definitive study)
427344-01 (Range-finding study)

TEST MATERIAL: Alkyl dimethyl benzyl ammonium chloride

SYNONYM: ADBAC

SPONSOR: ADBAC QUAT Joint Venture/Chemical Specialties Manufacturers Association, Washington, DC

STUDY NUMBER: 91N0032

TESTING FACILITY: Bushy Run Research Center (BRRC), Export, PA

TITLE OF REPORT: Developmental Toxicity Evaluation of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) Administered by Gavage to New Zealand White Rabbits

AUTHORS: T.L. Neeper-Bradley and M.F. Kubena

REPORT ISSUED: July 8, 1992

CONCLUSIONS

Dose levels: 0, 1, 3, and 9 mg/kg/day

Administered by gavage on gestational days (GDs) 6-18, inclusively

NOEL (maternal) = 3 mg/kg/day
LOEL (maternal) = 9 mg/kg/day based on clinical signs (hypoactivity and labored and/or audible respiration)

NOEL (developmental) = 9 mg/kg/day
LOEL (developmental) = not determined > 9 mg/kg/d

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CLASSIFICATION: Core Guideline Data. This study meets the requirements set forth under EPA Guideline Series 83-3 for a developmental toxicity study in rabbits.

A. MATERIALS

Test Compound

Purity:	81.09% (Manufacturing-use product)
Composition:	C-12, 40%; C-14, 50%; C-16, 10%
Description:	Pale yellow, viscous liquid
Lot number:	7293K
Receipt date:	November 11, 1987
Contaminants:	Not reported
Storage:	At room temperature
CAS No.:	68391-01-5

Vehicle: Milli-Q® filtered water

Test Animals

Species:	Rabbit
Strain:	New Zealand White
Source:	Hazleton Research Products, Inc., Denver, PA
Age:	6-6.5 months on GD 0
Weight:	3-4.7 kg on GD 0
Males used:	Same strain from the same supplier

B. STUDY DESIGN

This study was designed to assess the potential of ADBAC to cause developmental toxicity in New Zealand White rabbits when administered daily via gavage from GDs 6-18, inclusively.

Mating: Following approximately 2 weeks of acclimation, females were mated to males (1:1) of proven fertility. The date of copulation was designated GD 0. The mating period was 3 days.

Animal husbandry: Food (Agway® PROLAB® Rabbit Diet) and municipal tap water were available ad libitum throughout the study and were analyzed for contaminants. A 12-hour light/dark cycle was maintained. Temperature and humidity ranges were 61-70°F and 40-60%, respectively; frequency of air changes was not reported. Females were individually housed.

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Group arrangement: Mated females were assigned to study groups based on body weight on GD 0 using a stratified randomization procedure as follows:

Test Group	Dose Level (mg/kg/day)	Number Assigned per Group
Control	0	16
Low-dose	1	16
Mid-dose	3	16
High-dose	9	16

Dose administered: Doses were administered daily via gavage from GD 6 through 18 in a volume of 2.0 mL/kg of body weight. Individual doses were calculated based on GD-6 body weight data. The doses were adjusted for percent active ingredient. Dosing solutions were prepared once, analyzed for concentrations and stored at room temperature. The homogeneity of the lowest and highest doses was determined prior to initiation of dosing; stability was determined after 7, 14, and 21 days concurrently with the dosing period.

Dose rationale: Dose levels were selected based on the results of a preliminary range-finding study (BRRC Report 54-603) in which ADBAC was administered daily via gavage on GDs 6-18, inclusively, at doses of 0, 1, 3, 10, 30 or 60 mg/kg/day to pregnant New Zealand White rabbits (5/group). Maternal toxicity included the following:

Mortality at 60 and 30 mg/kg/day (5 and 2 does, respectively)
Clinical signs at 60 mg/kg/day (paralysis, cold extremities, prostration, slow respiration, emaciation, loose feces, and perioral encrustation); at 60 and 30 mg/kg/day (hypoactivity, perioral wetness, and labored respiration); and at 60, 30, and 10 mg/kg/day (audible respiration)
Decreased body weight gain at 60, 30, and 10 mg/kg/day
Decreased food consumption at 60, 30, and 10 mg/kg/day

No developmental toxicity was observed. However, the number of litters evaluated was limited to 4, 3, 3, and 3 at 1, 3, 10, and 30 mg/kg/day, respectively.

Based on these results, the maternal NOEL and LOEL were 3 and 10 mg/kg/day, respectively, while the developmental NOEL and LOEL were 10 and 30 mg/kg/day, respectively.

Observations: Animals were observed twice daily for mortality and moribundity and at least once daily for clinical signs. In addition, detail clinical examination was performed weekly. Body weight data were recorded on GDs 0, 6, 9, 12, 18, 24, and 29. Food consumption data were recorded daily on GDs 0-29. On GD 29, does were euthanized by lethal injection of sodium pentobarbital and litters were delivered by cesarean section. Examination of the does at sacrifice included the following:

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- Gross pathology examination of thoracic and abdominal cavities and reproductive organs
- Liver and gravid uterine weights
- Number of corpora lutea and implantation sites
- Number of resorptions (early and late), and live and dead fetuses

The uteri of apparently nonpregnant does were stained with a 10% aqueous solution of ammonium sulfide to detect early embryo loss.

All fetuses were examined in the following manner:

- Individual fetal weight and sex
- External anomalies
- Craniofacial structures of one-half of the live fetuses (heads fixed in Bouin's solution) using the modified method discussed by van Julsingha and Bennett (1977)
- Visceral anomalies using the method described by Staples (1974)
- Skeletal anomalies using the Alizarin red S staining method described by Crary (1962) and Peltzer and Schardein (1966)

Statistical analysis: The following methods were used.

- Quantitative continuous variables-- Levene's test for equal variances, ANOVA, and t-tests
- Nonparametric data-- Kruskal-Wallis test and Mann-Whitney U-test
- Frequency data-- Fisher's Exact test

Compliance

- A signed Statement of No Data Confidentiality Claim, dated June 30, 1992, was provided.
- A signed Statement of Compliance with FIFRA and OECD GLPs, dated June 30 and July 1, 1992, was provided.
- A signed Quality Assurance Statement, dated July 8, 1992, was provided.

C. RESULTS

Test Material Analysis

Purity of the test compound was 81%. Concentration analyses of the dosing solutions indicated mean values ranging from 93% to 95% of target

for all three concentrations. Stability of the test compound in the low- and high-dose solutions over 21 days ranged from 93% to 100% of nominals. Homogeneity of the dosing solutions ranged from 92% to 96% of target.

Maternal Toxicity

Mortality/moribundity: No mortality was observed.

Abortions: No abortions were noted.

Clinical observations: Compound-related clinical signs were observed in 2/15 does at 9.0 mg/kg/day. A summary of clinical signs is presented in Table 1. Clinical signs included hypoactivity and labored respiration in one doe and audible respiration in another. Similar findings were noted in an earlier probe study at 10 mg/kg/day and therefore, these were considered to be compound related. At 3 mg/kg/day, 2/14 does exhibited perioral wetness, and 1/14 had nasal encrustation and discharhe, loose and/or black feces, perioral encrustation, and salivation. These signs were not observed in a dose-related manner or at the same dose level in an earlier range-finding study and therefore, they were not considered to be compound related. Alopecia was observed in 3, 4, and 1 doe(s) at 9, 1, and 0 mg/kg/day, respectively.

Body weight: No compound-related effects on body weight (data not shown) and weight gain were observed in any dose group. A summary of maternal body weight gain for selected intervals is presented in Table 2.

Body weights (data not shown) were comparable in all dose groups. Significant differences in body weight gain between treated and control groups were not dose-related and were considered to be incidental.

Food consumption: No compound-related effect on food consumption (g/animal/day) was observed at any dose level (data not shown).

Necropsy observations: No compound-related necropsy findings were observed. Incidental findings included body ulcerations, pale or scalloped liver, accessory or anomalous spleen, and ovarian cyst.

Cesarean section observations: No compound-related effects were observed in any parameter. A summary of cesarean section data is presented in Table 3.

Developmental Toxicity

No compound-related anomalies were observed at any dose level. Summaries of visceral and skeletal malformations are presented in Table 4.

External examinations: No external malformations were observed. Incidental variations, occurring in all groups, included ecchymosis of head and/or trunk and short tail (data not shown).

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TABLE 1. Incidence of Maternal Clinical Observations[a]

Findings[b]	Dose Level (mg/kg/day) 0	1.0	3.0	9.0
Number of animals evaluated	16	16	16	16
Hypoactivity	0	0	0	1
Labored respiration	0	0	0	1
Audible respiration	0	0	0	1
Nasal discharge	0	0	1	0
Perinasal encrustation	0	0	1	0
Loose feces	0	0	1	0
Black ("tarry") feces	0	0	1	0
Perioral wetness	0	0	2	0
Perioral encrustation	0	0	1	0
Salivation	0	0	1	0
Alopecia	1	4	0	3

[a]Data were extracted from study number 91N0031, Table 2.

[b]More than one clinical signs may be found in one animal.

TABLE 2. Mean Body Weight Gain (g ± S.D.)[a]

Dose Group (mg/kg/day)	Prior to Dosing Period (GDs 0-6)	Dosing Period (GDs 6-18)	Post Dosing Period (GDs 18-29)	Entire Gestation Period (GDs 0-29)	Corrected Body Weight Change[b] (GDs 0-29)
0	158.6 ± 50.5	32.2 ± 94.6	112.9 ± 135.9	303.8 ± 118.4	-250.9 ± 115.6
1.0	170.8 ± 87.3	148.4 ± 89.1	154.9 ± 83.3	474.1 ± 96.7**	-86.6 ± 158.0**
3.0	129.6 ± 72.6	104.3 ± 209.5	153.1 ± 106.4	386.9 ± 184.8	-115.1 ± 161.2*
9.0	162.2 ± 71.7	67.4 ± 86.6	121.5 ± 131.4	351.2 ± 192.5	-180.4 ± 160.2

[a]Data were extracted from study number 91N0032, Tables 3 and 6.

[b]Corrected body weight change = (Body weight on GD 29 - body weight on GD 0) - gravid uterus weight

*Significantly different than control ($p<0.05$)

**Significantly different than control ($p<0.01$)

TABLE 3. Cesarean Section Observations[a]

Parameter	Dose Level (mg/kg/day) 0	1.0	3.0	9.0
No. animals assigned	16	16	16	16
No. animals pregnant	13	15	14	15
Pregnancy rate (%)	81	94	88	94
Maternal wastage				
No. died/nonpregnant	0	0	0	0
No. died/pregnant	0	0	0	0
No. nonpregnant	3	1	2	1
No. aborted	0	0	0	0
Gravid uterine weight (g)	554.7	560.7	502.1	531.5
Litters w/live fetuses	13	15	14	15
Total corpora lutea	123	150	131	140
Corpora lutea/doe	9.5 ± 1.7[b]	10.0 ± 2.2	9.4 ± 2.4	9.3 ± 1.4
Total implantations	120	137	114	133
Implantations/doe	9.2 ± 1.2	9.1 ± 2.3	8.1 ± 2.5	8.9 ± 1.5
Total live fetuses	112	126	110	124
Live fetuses/doe	8.6 ± 1.6	8.4 ± 2.2	7.9 ± 2.5	8.3 ± 0.9
Total resorptions	5	3	0	3
Early	5	3	0	2
Late	0	0	0	1
Resorptions/doe	0.4 ± 0.9	0.2 ± 0.6	0	0.1[c] ± 0.3
Total dead fetuses	3	8	4	6
Dead fetuses/doe	0.2 ± 0.4	0.5 ± 0.9	0.3 ± 0.6	0.4 ± 0.6
Mean fetal weight (g)	41.7 ± 4.4	43.6 ± 3.8	41.9 ± 5.5	41.8 ± 3.8
Preimplantation loss (%)[c]	3	8	14	5
Postimplantation loss (%)[d]	7	8	4	7
Sex ratio (% male)[c]	39	53	56	54

[a]Data were extracted from study number 91N0032, Tables 1, 6, 7, and pp. 90 and 91.

[b]Mean ± S.D.

[c]Discrepancy exists between summary and individual data; values were taken from summary table.

[d]Calculated by the reviewers using individual data

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Visceral examinations: Two fetuses (1 litter) at 9 mg/kg/day and 2 fetuses (2 litters) at 3 mg/kg/day had dilated lateral ventricles (with tissue depression). Diaphragm was herniated in 1 fetus at 1 mg/kg/day; azygous lung lobe was missing in 4 (2 litters), 6 (4 litters), 4 (4 litters), and 6 fetuses (3 litters) at 9, 3, 1, and 0 mg/kg/day, respectively (Table 4).

Variations observed in one or more dose groups, included dilated lateral ventricle (no tissue depression), heart indented at apex, dilated nasal passages, fetal atelectasis and small and/or pale gall bladder (data not shown).

Skeletal examinations: Skeletal malformations (Table 4), occurring as single events, included fused thoracic centra or ribs, unilateral extra rib between rib numbers 10 and 11 (9 mg/kg/day), missing #6 lumbar centrum and #6 lumbar arches (0 mg/kg/day).

Variations occurring in all dose groups were noted in the cervical centra, first lumbar arch, thoracic arch #13, maxillary bones, hyoid, sternebra, and some inter phalanges (data not shown).

D. REVIEWERS' DISCUSSION/CONCLUSIONS

Acceptance Criteria

The reviewers have completed an Acceptance Criteria check list (Attachment I) to be included with the evaluation of the study. All criteria were satisfied.

Test Material Analyses

The purity of the test compound was confirmed. Concentration, homogeneity and stability analyses of the test compound in the vehicle indicated values within the acceptable range of ±10% of target.

Maternal Toxicity

Compound-related maternal toxicity was observed at 9 mg/kg/day. It was manifested as clinical signs including hypoactivity, labored respiration and/or audible respiration. Similar findings were noted at 10 mg/kg/day in an earlier range-finding study (BRRC Report 54-603), therefore, the clinical signs observed at 9 mg/kg/day in this study were considered to be compound related, although they occurred in only two animals.

Based on these results, the NOEL and LOEL for maternal toxicity were 3 and 9 mg/kg/day, respectively.

Developmental Toxicity

No compound-related deaths/resorptions, developmental anomalies or altered growth were observed in any dose group. Consequently, the NOEL for developmental toxicity was 9 mg/kg/day; the highest dose tested.

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TABLE 4. Summary of Fetal Malformations[a]

Findings[b]	Dose Level (mg/kg/day) 0	1.0	3.0	9.0
No. fetuses (litters) examined	112 (13)	126 (15)	110 (14)	124 (15)
Visceral Examination				
Lateral ventricle dilated, -Tissue depressed	0	0	2 (2)	2 (1)
Azygous lung lobe, missing	6 (3)	4 (4)	6 (4)	4 (2)
Herniated diaphragm	0	1	0	0
Total no. fetuses (litters) with any visceral malformation	6 (3)	4 (4)	8 (6)	6 (3)
Skeletal Examination				
Thoracic centra - Fused	0	0	0	1
Lumbar centrum #6 missing	1	0	0	0
Lumbar arches #6 missing - bilateral	1	0	0	0
Ribs - fused	0	0	0	1
Extra rib between rib #10 and #11 -Unilateral	0	0	0	1
Total no. fetuses (litters) with any skeletal malformation	1	0	0	2 (1)
Total no. fetuses (litters) with any malformation	7 (4)	4 (4)	8 (6)	8 (4)

[a]Data were extracted from study number 91N0032, Tables 8 and 9.

[b]More than one type of anomalies may be found in one fetus.

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Discussion

ADBAC is a quaternary ammonia compound, which acts as a cationic surfactant-type detergent. Quaternary ammonia compounds can be caustic at concentrations of 10%-15% and mucosal irritants at concentrations as low as 0.1%-0.5% (Ellenhorn and Barceloux, 1988). The dose concentrations of ADBAC used in this study ranged from 0.05% to 0.45%. The maternal clinical signs may have been related to mucosal irritation and surfactant effects. Consequently, caution should be used in extrapolating the maternal LOEL (obtained from repeated bolus dosing with relatively high concentrations) to other exposure scenarios (e.g., dietary or drinking water exposure).

The highest dose (9 mg/kg/day) was sufficient to evaluate the developmental toxicity of ADBAC in rabbits. Higher doses (10, 30, and 60 mg/kg/day) were used in the dose range-finding study and produced clinical signs, reduced food intake, and body weight loss early in the treatment period, gastrointestinal lesions (30 and 60 mg/kg/day), and mortality (30 and 60 mg/kg/day).

E. CORE CLASSIFICATION: Core Guideline Data.

NOEL (maternal) = 3 mg/kg/day
LOEL (maternal) = 9 mg/kg/day based on clinical signs of hypoactivity, audible and/or labored respiration

NOEL (developmental) = 9 mg/kg/day
LOEL (developmental) = not determined

F. RISK ASSESSMENT: Not applicable

G. REFERENCE: Ellenhorn, M.J. and D.G. Barceloux. 1988. In: Medical Toxicology. Diagnosis and Treatment of Human Poisoning. pp. 899-901. New York: Elsevier.

ATTACHMENT I

83-3 Teratology Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.	YES	Technical form of the active ingredient tested.
2.	YES	At least 20 pregnant animals/dose group for mice, rats, or hamsters are available. At least 12 pregnant animals/dose group for rabbits are available.
3.	YES	At the high dose, overt maternal effects such as slight weight loss are reported (or a limit dose is given, 1,000 mg/kg).
4.*	YES	At the low dose, no developmental toxicity is reported.
5.	YES	Dosing duration is at least during the period of major organogenesis, but may extend up to one day prior to term.
6.*	YES	Analysis for test material stability, homogeneity, and concentration in dosing medium.
7.	YES.	Individual daily observations.
8.	YES	Individual body weights.
9.	YES	Individual food consumption.
10.	YES	Necropsy on all animals.
11.	YES	Individual uterine examination, including numbers of fetal deaths, early and late resorptions, and viable fetuses per sex.
12.	YES	All ovaries examined to determine number of corpora lutea.
13.	YES	Individual litter weights and/or individual fetal weights/sex/litter.
14.	YES	Individual fetal external examination.
15.	YES	Individual fetal skeletal examination for 1/3 to 1/2 of each litter for rodents and all for rabbits.
16.	YES	Individual fetal soft tissue examination.

Criteria marked with an * are supplemental, may not be required for every study.

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ATTACHMENT I

83-3 Teratology Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.	YES	Technical form of the active ingredient tested.
2.	YES	At least 20 pregnant animals/dose group for mice, rats, or hamsters are available. At least 12 pregnant animals/dose group for rabbits are available.
3.	YES	At the high dose, overt maternal effects such as slight weight loss are reported (or a limit dose is given, 1,000 mg/kg).
4.	YES	At the low dose, no developmental toxicity is reported.
5.	YES	Dosing duration is at least during the period of major organogenesis, but may extend up to one day prior to term.
6.	YES	Analysis for test material stability, homogeneity, and concentration in dosing medium.
7.	YES	Individual daily observations.
8.	YES	Individual body weights.
9.	YES	Individual food consumption.
10.	YES	Necropsy on all animals.
11.	YES	Individual uterine examination, including numbers of fetal deaths, early and late resorptions, and viable fetuses per sex.
12.	YES	All ovaries examined to determine number of corpora lutea.
13.	YES	Individual litter weights and/or individual fetal weights/sex/litter.
14.	YES	Individual fetal external examination.
15.	YES	Individual fetal skeletal examination for 1/3 to 1/2 of each litter for rodents and all for rabbits.
16.	YES	Individual fetal soft tissue examination.

Criteria marked with an asterisk (*) are supplemental, may not be required for every study.

010796

FINAL

DATA EVALUATION REPORT

ALKYL DIMETHYL BENZYL AMMONIUM CHLORIDE

Study Type: Developmental Toxicity in Rats

Prepared for:

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031

Principal Reviewer: Sanju Diwan — Sanju Diwan, Ph.D. — Date 8/16/93

Independent Reviewer: Pia Lindström — Pia Lindström, D.P.H. — Date 8/16/93

QA/QC Manager: William L McLellan for — Sharon Segal, Ph.D. — Date 8/16/93

Contract Number: 68D10075
Work Assignment Number: 2-86
Clement Number: 224, 225
Project Officer: Caroline Gordon

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EPA Reviewer: Ann Clevenger, Ph.D.
Review Section I, Toxicology Branch I/HED

Signature: [signature] for
Date: 8/23/93

EPA Section Head: Marion Copley, D.V.M.
Review Section IV, Toxicology Branch I/HED

Signature: Marion Copley
Date: 8/26/93

DATA EVALUATION REPORT

STUDY TYPE: Developmental toxicity in rats; Guideline Series 83-3

EPA IDENTIFICATION NUMBERS

PC CODE: 069105

TOX CHEM. NO.: 016 E

MRID NOS.: 423515-01 (Definitive study)
426451-01 (Range-finding study)

TEST MATERIAL: Alkyl Dimethyl Benzyl Ammonium Chloride

SYNONYM: ADBAC

SPONSOR: ADBAC QUAT Joint Venture/Chemical Specialties Manufacturers Association, Washington, DC

STUDY NUMBER: 91N0031

TESTING FACILITY: Bushy Run Research Center (BRRC), Export, PA

TITLE OF REPORT: Developmental Toxicity Evaluation II of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) Administered by Gavage to CD® Rats

AUTHOR: T.L. Neeper-Bradley

REPORT ISSUED: June 8, 1992

CONCLUSIONS

Dose levels: 0, 10, 30, and 100 mg/kg/day

Administered by gavage on gestational days (GDs) 6-15, inclusively

NOEL (maternal) = 10 mg/kg/day
LOEL (maternal) = 30 mg/kg/day based on clinical signs (perioral wetness and audible respiration) and decreased body weight gain and food consumption

NOEL (developmental) = 100 mg/kg/day
LOEL (developmental) = not determined
> 100 mg/kg/d

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CLASSIFICATION: Core Guideline Data. This study meets the requirements set forth under EPA Guideline Series 83-3 for a developmental toxicity study in rats.

A. MATERIALS

Test Compound

Purity:	81.09% (Manufacturing-use product)
Compositions:	C-12, 40%; C-14, 50%; C-16, 10%
Description:	Pale yellow, viscous liquid
Lot number:	7293K
Receipt date:	November 11, 1987
Contaminants:	Not reported
Storage:	At room temperature
CAS No.:	68391-01-5
Vehicle:	Milli-Q® filtered water

Test Animals

Species:	Rat
Strain:	CD® [Crl:CD® BR]
Source:	Charles River Breeding Laboratories, Inc., Portage, MI
Age:	Approximately 70 days (females) on GD 0
Weight:	222-274 g on GD 0
Males used:	Same strain from the same supplier

B. STUDY DESIGN

This study was designed to assess the potential of ADBAC to cause developmental toxicity in CD® rats when administered daily via gavage on GDs 6-15, inclusively.

Mating: Following approximately 2 weeks of acclimation, females were mated to males (1:1). The day a copulation plug was observed was designated GD 0. The mating period was no more than 5 days.

Animal husbandry: Food (Ground Certified Rodent Chow® #5002) and municipal tap water were available ad libitum throughout the study and were analyzed for contaminants. A 12-hour light/dark cycle was maintained. Temperature and humidity ranges were 66-77°F and 40-70%, respectively; frequency of air changes was not reported. Females were housed individually after mating.

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Group arrangement: Sperm-positive females were assigned to study groups based on body weight on GD 0 using a stratified randomization procedure as follows:

Test Group	Dose Level (mg/kg/day)	Number Assigned per Group
Control	0	25
Low-dose	10	25
Mid-dose	30	25
High-dose	100	25

Dose administered: Doses were administered daily via gavage from GD 6 through 15 in a volume of 5.0 mL/kg of body weight. Individual doses were calculated based on GD-6 body weight data. The doses were adjusted for percent active ingredient. Dosing solutions were prepared once and stored at room temperature; concentrations were verified prior to dosing. The homogeneity and stability (after 14 days) at low- and high-doses were determined prior to initiation of dosing using HPLC.

Dose rationale: Dose levels were selected based on the results of a preliminary range-finding study (BRRC Report 54-613; MRID no. 426451-01) in which ADBAC was administered daily via gavage at doses of 0, 25, 50, 100, 200 or 400 mg/kg/day to pregnant CD® rats (5/group) during GDs 6-15, inclusively. Doses of ≥200 mg/kg/day resulted in 100% mortality; necropsy findings revealed change in the color of liver and stomach, and distended intestines filled with mucoid fluid. These dams also exhibited clinical signs including loose feces, perioral wetness and perioral encrustation, ataxia, hypoactivity, urogenital area wetness, and audible respiration. Maternal toxicity, observed at 100 mg/kg/day, was manifested as significantly increased incidence of perioral wetness. Based on these findings, the NOEL and LOEL for maternal toxicity were 50 100 mg/kg/day, respectively. The NOEL for developmental toxicity was 100 mg/kg/day based on no survival of dams at 200 and 400 mg/kg/day.

Observations: Animals were observed twice daily for mortality and moribundity, and at least once daily for clinical signs. Body weight data were recorded on GDs 0, 6, 9, 12, 15, 18, and 21. Food consumption data were recorded at 3-day intervals throughout the study. On GD 21, dams were euthanized by carbon dioxide asphyxiation and litters were delivered by cesarean section. Examination of the dams at sacrifice included the following:

- Gross pathology examination of abdominal, thoracic, and peritoneal cavities and reproductive organs
- Liver and gravid uterine weight
- Number of corpora lutea and implantation sites

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- Number of resorptions (early and late) and live and dead fetuses

The uteri of apparently nonpregnant dams were stained with a 10% aqueous solution of ammonium sulfide to detect early embryo loss.

All live fetuses were examined in the following manner:

- Individual fetal weight and sex
- External anomalies
- Craniofacial structures of one-half of the fetuses (heads fixed in Bouin's solution) using the modified method described by Wilson (1965 and 1973)
- Visceral anomalies of the decapitated fetuses using the method described by Staples (1974)
- Skeletal anomalies of one half of the fetuses using the Alizarin red S staining method described by Crary (1962) and Peltzer and Schardein (1966)

Statistical analysis: The following methods were used:

- Quantitative continuous variables-- Levene's test for equal variances, ANOVA, and t-tests
- Nonparametric data-- Kruskal-Wallis test and Mann-Whitney U-test
- Frequency data-- Fisher's Exact test

Compliance

- A signed Statement of No Data Confidentiality Claim, dated June 1, 1992, was provided.
- A signed Statement of Compliance with FIFRA and OECD GLPs, dated May 29 and June 1, 1992, was provided.
- A signed Quality Assurance Statement, dated June 8, 1992, was provided.

C. RESULTS

Test Material Analysis

Purity of the test compound was 81%. Concentration analyses of the dosing solutions revealed mean values ranging from 95% to 105% of target for all three concentrations. Stability of the test compound in the low- and high-dose solutions over 14 days ranged from 91% to 100% of nominals. Homogeneity of the dosing solutions ranged from 91% to 93% of target.

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Maternal Toxicity

Mortality/moribundity: No mortality was observed.

Abortions: No abortions were noted. One dam at 30 mg/kg/day delivered early (day not reported) and was consequently removed from the study.

Clinical observations: Compound-related clinical signs were observed at 100 mg/kg/day. A summary of clinical signs is presented in Table 1. At 100 mg/kg/day, a significant increase (67%) in the incidence of perioral wetness was observed in 14/21 dams; one of these dams (#24372) exhibited multiple clinical signs including dehydration, unkempt appearance, loose feces, urine stains, and perioral wetness. Audible respiration was observed in 3/21 and 2/23 dams at 100 and 30 mg/kg/day, respectively. Multiple clinical signs were noted in one dam (#24360) at 30 mg/kg/day and consisted of perioral wetness, gasping, perinasal encrustations, loose feces, and urine stains. Perioral wetness, audible respiration, and loose feces were prominent clinical signs in the range-finding study at doses of 100 mg/kg/day or higher (200 and 400 mg/kg/day).

Body weight: Compound-related effects on body weight gain were observed at 100 and 30 mg/kg/day. A summary of maternal body weight gain for selected intervals is presented in Table 2.

Group mean body weights and body weight gains were not statistically significantly different from controls; however, biologically relevant decreases were observed at 100 and 30 mg/kg/day. The following decreases in body weight gain were observed during the dosing period:

GDs 6- 9,	100 and 30 mg/kg/day,	26% (data not shown)
GDs 12-15,	100 mg/kg/day,	20% (data not shown)
GDs 12-15,	30 mg/kg/day,	12% (data not shown)
GDs 6-15,	100 mg/kg/day,	11% (Table 2)
GDs 6-15,	30 mg/kg/day,	13% (Table 2)

The decreases at 100 and 30 mg/kg/day were due primarily to body weight loss (35 to 64 g on GDs 6-9) by the two animals (#24360 and 24372) showing pronounced clinical signs of toxicity.

Food consumption: A compound-related effect on food consumption was observed at 100 mg/kg/day. A summary of food consumption data is presented in Table 3.

Significant decreases (≥9%; $p<0.05$) in food consumption were noted at 100 mg/kg/day on GDs 6-9 and at 30 mg/kg/day on GDs 12-15. The two animals showing poor health and body weight loss (GDs 6-9) also had substantially reduced (50%-90%) food consumption on GDs 6-9 (and GDs 9-12 for #24372).

Necropsy observations: No compound-related necropsy findings were observed (data not shown). Incidental findings included ulcerated stomach, swollen or changed color of the liver, small spleen, and

TABLE 1. Incidence of Selected Maternal Clinical Observations During Dosing and Postdosing Periods[a]

Findings[b]	Dose Level (mg/kg/day) 0	10	30	100
Number of animals evaluated	25	25	25	25
Dehydration	0	0	0	1
Unkempt appearance	0	0	0	1
Urine stains	0	0	1	1
Audible respiration[c]	0	0	2	3
Gasping	0	0	1	1
Periocular encrustation (eye-both, eye-left)	1	0	0	1
Perinasal encrustation	0	0	1	0
Loose feces	0	0	1	1
Perioral wetness[c]	0	0	1	14*
Crust around mouth	0	1	0	0

[a]Data were extracted from study number. 91N0031, Table 2 and Appendix 2.

[b]More than one clinical signs may be found in one animal.

[c]Discrepancy in the summary and individual data; values reported are from summary table.

*Significantly different from control ($p<0.05$)

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TABLE 2. Mean Body Weight Gain (g ± S.D.)[a]

Dose Group (mg/kg/day)	Prior to Dosing Period (GDs 0-6)	Dosing Period (GDs 6-15)	Post Dosing Period (GDs 15-21)	Entire Gestation Period (GDs 0-21)	Corrected Body Weight Change[b] (GDs 0-21)
0	34.7 ± 6.4	50.6 ± 12.2	97.8 ± 22.9	183.1 ± 32.5	75.3 ± 15.4
10	35.3 ± 7.3	53.1 ± 6.4	106.1 ± 12.8	194.5 ± 20.5	78.3 ± 16.4
30	34.2 ± 4.9	44.0 ± 15.6	101.9 ± 11.2	180.2 ± 22.4	68.6 ± 19.2
100	34.3 ± 6.9	45.1 ± 15.5	96.0 ± 32.6	175.5 ± 38.7	64.3 ± 28.6

[a]Data were extracted from study number 91N0031, Tables 3 and 6.

[b]Corrected body weight change = (Body weight on GD 29 - body weight on GD 0) - gravid uterus weight

TABLE 3. Mean Food Consumption (g/animal/day)[a]

Dose Group (mg/kg/day)	Prior to Dosing Period (GDs 0-6)	Dosing Period (GDs 6-9)	Dosing Period (GDs 9-12)	Dosing Period (GDs 12-15)	Post Dosing Period (GDs 15-21)
0	23.5 ± 1.7	24.3 ± 1.4	25.0 ± 2.1	26.7 ± 2.4	28.6 ± 3.0
10	24.4 ± 3.0	26.4 ± 3.8*	27.1 ± 2.7*	27.1 ± 2.7	29.5 ± 2.5
30	23.8 ± 1.9	22.9 ± 5.0	24.9 ± 2.6	24.4 ± 3.2**	27.5 ± 1.8
100	23.7 ± 2.4	21.9 ± 3.9*	24.1 ± 3.9	26.0 ± 2.8	28.1 ± 6.2

[a]Data were extracted from study number 91N0031, Table 4.

*Significantly different from control ($p<0.05$)

**Significantly different from control ($p<0.01$)

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changes in the color and size of the lymph node(s). The two animals showing poor health (#24360 and 24372) had no gross lesions at necropsy.

Cesarean section observations: No compound-related effects were observed in any parameter. A summary of cesarean section data is presented in Table 4.

Developmental Toxicity

No compound-related anomalies were observed at any dose level. Summaries of external and visceral malformations and selected visceral and skeletal variations are presented in Tables 5 and 6, respectively.

External examinations: External malformations (Table 5) were observed in two fetuses (2 litters) at 100 mg/kg/day and consisted of gastroschisis, imperforated anus and a thread-like tail. This 10% increase in the litter incidence was considered incidental based on the following reasons: the increase was not significant; no increase was noted in visceral or skeletal malformations; and no similar malformations had been noted in the range-finding study in the four litters evaluated.

Variations occurred in all dose groups and consisted of ecchymosis of head, trunk, and or extremities (data not shown).

Visceral examinations: Visceral malformations (Table 5) were noted in fetuses (litters) of all dose groups and consisted of bilateral hydroureter (all dose groups); unilateral hydroureter (30 mg/kg/day); bilateral hydronephrosis (100, 30, and 10 mg/kg/day), unilateral hydronephrosis (30 and 0 mg/kg/day); and missing innominate artery (10 mg/kg/day).

Among variations (see Table 6 for selected variations), fetal atelectasis was noted in all dose groups; the incidences of partial fetal atelectasis and dilated renal pelvis were significantly higher on a litter basis only at 10 and 30 mg/kg/day, respectively. In addition, variations occurring at low frequency in one or more dose groups were noted in the ventricle, brain, and liver.

Skeletal examinations: No skeletal malformations were observed.

Variations (see Table 6 for selected variations) were noted in all dose groups and consisted mainly of poorly ossified or unossified bones (cervical and thoracic centra, atlas, proximal phalanges of the fore and hind limbs, frontals, lumbar arches, ribs and sternebrae). Among these, only the incidences of poorly ossified proximal phalanges of hind limbs (100 and 30 mg/kg/day) and split cervical centrum #1 (30 mg/kg/day) were significantly higher on a litter basis compared to control; however these findings occurred in a nondose-related manner. The incidences of poorly ossified cervical centrum #6 (100 mg/kg/day) and thoracic centrum #10 (30 mg/kg/day), and unossification of the proximal phalanges of the forelimb (30 mg/kg/day) were significantly lower than control.

TABLE 4. Cesarean Section Observations[a]

Parameter	Dose Level (mg/kg/day) 0	10	30	100
No. animals assigned	25	25	25	25
No. animals pregnant	25	25	24	21
Pregnancy rate (%)	100	100	96	84
Maternal wastage				
No. died/nonpregnant	0	0	0	0
No. died/pregnant	0	0	0	0
No. nonpregnant	0	0	1	4
No. aborted/early delivery	0	0	1	0
Gravid uterine weight (g)	107.8	116.2	111.6	111.2
Litters w/live fetuses	24	25	23	21
Total corpora lutea	393[b]	449	406	359
Corpora lutea/dam	16.3 ± 2.3[c]	18.0 ± 2.1	17.7 ± 2.7	17.1 ± 1.7
Total implantations	375	412	366	337
Implantations/dam	15.0 ± 2.8	16.5 ± 1.3	15.9 ± 1.9	16.0 ± 1.5
Total live fetuses	357	389	350	324
Live fetuses/dam	14.3 ± 3.5	15.6 ± 1.5	15.2 ± 2.2	15.4 ± 1.4
Total resorptions	18	22	15	13
Early	18	22	15	13
Late	0	0	0	0
Resorptions/dam	0.7 ± 1	0.9 ± 0.9	0.7 ± 0.9	0.6 ± 0.8
Total dead fetuses	0	1	1	0
Dead fetuses/dam	0	0.0 ± 0.2	0.0 ± 0.2	0
Mean fetal weight (g)	5.5 ± 0.2	5.4 ± 0.3	5.4 ± 0.3	5.3 ± 0.7
Preimplantation loss (%)	6[d]	8	10	6
Postimplantation loss (%)[e]	5	6	4	4
Sex ratio (% male)	53	57	51	46

[a]Data were extracted from study number 91N0031, Tables 1, 6, 7, and pp. 20-23.

[b]One female had no corpora lutea

[c]Mean ± S.D.

[d]Discrepancy exists between summary and individual data; value were taken from summary table.

[e]Calculated by the reviewers using individual data

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TABLE 5. Incidence of Fetal Malformations[a]

Findings[b]	Dose Level (mg/kg/day) 0	10	30	100
External Malformations				
No. fetuses (litters) examined	357 (24)	389 (25)	350 (23)	324 (21)
Gastroschisis	0	0	0	1
Thread-like tail	0	0	0	1
Imperforate anus	0	0	0	1
Total no. fetuses (litters) with any external malformations	0	0	0	2 (2)
Visceral Malformations				
No. fetuses (litters) examined	185 (42)	200 (25)	182 (23)	169 (21)
Missing innominate artery	0	1	0	0
Hydronephrosis				
Bilateral	0	2 (2)	3 (1)	2 (1)
Unilateral	1	0	3 (2)	0
Hydroureter				
Bilateral	1	2 (2)	6 (3)	5 (2)
Unilateral	0	0	2 (1)	0
Total no. fetuses (litters) with any visceral malformations	1	3 (3)	8 (3)	5 (2)
Total no. fetuses (litters) with any malformations	1	3 (3)	8 (3)	7 (4)

[a]Data were extracted from study number 91N0031, Table 8.

[b]More than one type of anomalies may be found in one fetus.

TABLE 6. Incidence of Selected Fetal Visceral and Skeletal Variations[a]

Findings[b]	Dose Level (mg/kg/day) 0	10	30	100
Visceral Variations				
No. fetuses (litters) examined	185 (24)	200 (25)	182 (23)	169 (21)
Partial fetal atelectasis	62 (17)	67 (22)	85 (23)**	64 (19)
Dilated renal pelvis- bilateral	37 (14)	13 (7)*	19 (10)	18 (8)
Dilated ureter- unilateral	16 (13)	8 (7)	7 (5)*	8 (6)
Total no. fetuses (litters) with any visceral variations	141 (24)	142 (25)	137 (23)	123 (21)
Skeletal Variations				
No. fetuses (litters) examined	172 (24)	189 (25)	168 (23)	155 (21)
Cervical centrum #6 poorly ossified	18 (16)	14 (10)	16 (11)	11 (7)*
Anterior arch of the atlas split	0	7 (6)*	7 (3)	2 (2)
Cervical centrum #1 split	3 (3)	5 (5)	15 (9)*	13 (8)
Thoracic centrum #10 poorly ossified	7 (7)	9 (5)	1 (1)*	8 (7)
Majority proximal phalanges (forelimb) unossified	13 (8)	8 (7)	1 (1)*	6 (4)
Majority proximal phalanges (hindlimb) poorly ossified	0	2 (2)	5 (5)*	4 (4)*
Total no. fetuses (litters) with any skeletal variations	172 (24)	189 (25)	168 (23)	155 (21)

[a]Data were extracted from study number 91N0031, Table 9.

[b]More than one type of anomalies may be found in one fetus.

*Significantly different from control ($p<0.05$)

**Significantly different from control ($p<0.01$)

D. REVIEWERS' DISCUSSION/CONCLUSIONS

Acceptance Criteria

The reviewers have completed an Acceptance Criteria check list (Attachment I) to be included with the evaluation of the study. All criteria were satisfied.

Test Material Analyses

The purity of the test compound was confirmed. Concentrations, homogeneity, and stability analyses demonstrated values within the acceptable range of ±10% of target.

Maternal Toxicity

Compound-related maternal toxicity was observed at 100 and 30 mg/kg/day. It was manifested at both these doses as clinical signs of toxicity (i.e., perioral wetness and audible respiration); decreased body weight gain during dosing; and decreased food consumption on GDs 6-9. Based on these results, the NOEL and LOEL for maternal toxicity were 10 and 30 mg/kg/day, respectively.

Developmental Toxicity

No compound-related deaths/resorptions, developmental anomalies or altered growth were observed. Consequently, the NOEL for developmental toxicity was 100 mg/kg/day; the highest dose tested.

Reporting Deficiencies

Since there was an increase in external malformations but the study author does not consider this to be treatment-related, the historical control data should be submitted to further strengthen this conclusion.

Several discrepancies were noted between the individual data and the summary data.

Discussion

ADBAC is a quaternary ammonia compound, which acts as a cationic surfactant-type detergent. Quaternary ammonia compounds can be caustic at concentrations of 10%-15% and mucosal irritants at concentrations as low as 0.1%-0.5% (Ellenhorn and Barceloux, 1988). The concentrations of ADBAC used in this study ranged from 0.2% to 2%. The clinical signs and effects on food consumption and body weight may have been related to mucosal irritation and surfactant effects. Consequently, caution should be used in extrapolating the maternal LOEL (obtained from repeated bolus dosing with relatively high concentrations) to other exposure scenarios (e.g., dietary or drinking water exposure).

The highest dose (100 mg/kg/day) is sufficient to evaluate the developmental toxicity of ADBAC in rats. Higher doses (200 and

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400 mg/kg/day) were used in the dose range-finding study and produced 100% mortality after 2-4 days of treatment.

E. CORE CLASSIFICATION: Core Guideline Data.

NOEL (maternal) = 10 mg/kg/day
LOEL (maternal) = 30 mg/kg/day based on clinical signs and decreased body weight and food consumption

NOEL (developmental) = 100 mg/kg/day
LOEL (developmental) = not determined

F. RISK ASSESSMENT: Not applicable

G. REFERENCE: Ellenhorn, M.J. and D.G. Barceloux. 1988. In: Medical Toxicology. Diagnosis and Treatment of Human Poisoning. pp. 899-901. New York: Elsevier.

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ATTACHMENT I

83-3 Teratology Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.	YES	Technical form of the active ingredient tested.
2.	YES	At least 20 pregnant animals/dose group for mice, rats, or hamsters are available. At least 12 pregnant animals/dose group for rabbits are available.
3.	YES	At the high dose, overt maternal effects such as slight weight loss are reported (or a limit dose is given, 1,000 mg/kg).
4.	YES	At the low dose, no developmental toxicity is reported.
5.	YES	Dosing duration is at least during the period of major organogenesis, but may extend up to one day prior to term.
6.	YES	Analysis for test material stability, homogeneity, and concentration in dosing medium.
7.	YES	Individual daily observations.
8.	YES	Individual body weights.
9.	YES	Individual food consumption.
10.	YES	Necropsy on all animals.
11.	YES	Individual uterine examination, including numbers of fetal deaths, early and late resorptions, and viable fetuses per sex.
12.	YES	All ovaries examined to determine number of corpora lutea.
13.	YES	Individual litter weights and/or individual fetal weights/sex/litter.
14.	YES	Individual fetal external examination.
15.	YES	Individual fetal skeletal examination for 1/3 to 1/2 of each litter for rodents and all for rabbits.
16.	YES	Individual fetal soft tissue examination.

Criteria marked with an asterisk (*) are supplemental, may not be required for every study.

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