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

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DEC 3 . 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Triforine: Evaluation of developmental toxicity studies in rats and rabbits

Caswell No. 890AA
EPA Chemical No. 107901
Submission No. S447090

DP Code: D194646
MRID No. 428892-01 (rat)
428892-02 (rabbit)

TO: R. Kendall / V. Dietrich, PM Team 51
Special Review and Re-registration Division (H7508W)

FROM: Whang Phang, Ph.D. 12/1/93
Pharmacologist
Tox. Branch II/HED (H7509C)

THROUGH: James Rowe, Ph.D. James N. Rowe 12/1/93
Section Head
and
Marcia van Gemert, Ph.D. Concurred 12/1/93
Branch Chief
Tox. Branch II/HED (H7509C)

The registrant, Biologic Inc., submitted 2 developmental toxicity studies on triforine. One was conducted on rats, and the other was carried out on rabbits. These 2 studies were evaluated by Clement and approved by Tox. Branch II. The Data Evaluation Reports are attached. The citation and conclusion of each study are presented below:

1. Fuchs, A. (1993) Triforine: Oral (gavage) teratology study in rats. Unpublished Study by Hazleton, Deutschland GmbH. Study No. 121-006. Feb. 1996. Submitted to EPA by Biologic, Inc.; EPA MRID No. 428892-01.

Groups of Sprague Dawley Crl:CD(SD)BR female rats (30/dose group) were mated, and the pregnant females received triforine by gavage at doses of 0, 200, 500, or 1000 mg/kg/day on gestational days 6-15, inclusive. No maternal or developmental toxicity was seen at any dose level. The analytical chemistry data indicated that the highest dose tested (HDT) might not have been above 840 mg/kg/day; therefore, the maternal NOEL and the developmental NOEL were established as 840 mg/kg/day (HDT).

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The study was classified as supplementary because no information on the purity of the test chemical was presented in the report. This study does not meet the data requirements for a developmental toxicity study in rats (Guideline No. 83-3a). However, the study may be upgraded after receipt and satisfactory evaluation of the missing data.

2. Fuchs, A. (1993) Triforine: Oral (gavage) teratology study in rabbits. Unpublished Study by Hazleton, Deutschland GmbH. Study No. 121-004. June 14, 1996. Submitted to EPA by Biologic, Inc.; EPA MRID No. 426892-02.

Two groups of New Zealand white female rabbits (18/group) were mated, and the pregnant females received triforine by gavage at doses of 0 or 1000 mg/kg/day on gestational days 6-18, inclusive. During the dosing period, there was a significant decrease in maternal body weight gain and food consumption, and, based on these results, the maternal toxicity LOEL was 1000 mg/kg/day. No maternal toxicity NOEL could be established. The fetal data indicated a significant decrease in fetal body weights; based on this observation, the developmental toxicity LOEL was 1000 mg/kg/day. No developmental toxicity NOEL could be established.

This study was classified as supplementary, and it could not be upgraded because only one dose was used.

FINAL

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DATA EVALUATION REPORT

TRIFORINE

Study Type: Developmental Toxicity (Rat)

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: Pia Lindström, D.P.H. Date 11/23/93

Independent Reviewer: Sanju Diwan, Ph.D. Date 11/22/93

QA/QC Manager: Sharon Segal, Ph.D. Date 11/23/93

Contract Number: 68D10075
Work Assignment Number: 3-06
Element Number: 30
Project Officer: Caroline Gordon

Guideline Series 83-3: Developmental Toxicity

EPA Reviewer: Whang Phang, Ph.D.
Review Section III, Toxicology Branch II/HED
Signature: [signature]
Date: 11/20/93

EPA 2nd Reviewer: James Rowe, Ph.D.
Review Section III, Toxicology Branch II/HED
Signature: James N. Rowe
Date: 11/30/93

DATA EVALUATION REPORT

STUDY TYPE: Developmental Toxicity (Rat); Guideline Series 83-3

EPA IDENTIFICATION NUMBERS

TOX CHEM. NUMBER:

MRID NUMBER: 428692-01

TEST MATERIAL: 1,4-bis(2,2,2-trichloro-1-formamidoethyl) piperazine

SYNONYMS: Triforine; CME 74770; SAG 103

SPONSOR: Shell International Chemical Company, London, England

STUDY NUMBER: 121-006

TESTING FACILITY: Hazleton Deutschland GmbH, Münster, Germany

TITLE OF REPORT: Oral (Gavage) Teratogenicity Study in the Rat

AUTHOR: A. Fuchs

REPORT ISSUED: February 1993

CONCLUSIONS: In a developmental toxicity study, Sprague Dawley rats received triforine by gavage at dose levels of 0, 200, 500, or 1000 mg/kg/day on gestational days (GDs) 6-15, inclusive. No maternal or developmental toxicity was seen at any dose level. Analytical chemistry data, however, indicate that the highest dose tested (HDT) may not have been above 840 mg/kg/day.

Maternal NOEL = 840 mg/kg/day (HDT)

Developmental NOEL = 840 mg/kg/day (HDT)

CORE CLASSIFICATION: Core Supplementary Data. This study does not meet the minimum requirements (83-3) for a developmental toxicity study in rats. Information regarding the purity of the test compound was not reported. The study may be upgraded upon submission and satisfactory evaluation of the missing information.

A. MATERIALS

Test Compound

Purity:	Not reported
Description:	Colorless to cream powder or crystals
Batch number:	Rt 08/91/1
Receipt date:	January 1, 1992
Contaminants:	None reported
Storage:	Room temperature in the dark
Vehicle:	Distilled water

Test Animals

Species:	Rat
Strain:	Sprague Dawley Crl: CD (SD)BR
Source:	Charles River Wiga GmbH, Sulzfeld, Germany
Age:	8-12 weeks at receipt
Weight:	181-247 g on GD 0
Males used:	Not reported

B. STUDY DESIGN

This study was designed to assess the potential of trifoxine to cause developmental toxicity in rats when administered daily by gavage on GDs 6-15, inclusive.

Mating Procedure

Following at least 7 days of acclimatization, females were mated with males in a ratio of 4 females to 1 male until observation of sperm or a copulatory plug. The maximum time allowed for mating was not stated. The day a plug or sperm was observed was considered to be GD 0.

Animal Husbandry

Food (Semiff R 10) and tap water were available ad libitum throughout the study. A 12-hour light/dark cycle was maintained. Temperature and humidity were maintained at 19°-25°C and 30%-70%, respectively.

Group Arrangement

Animals were assigned to the following dose groups using a random numbers table:

Test Group	Dose Level (mg/kg/day)	Number of Dams Assigned per Group
Control	0	30
Low-dose	200	30
Mid-dose	500	30
High-dose	1000	30

Dose Administered

Doses were prepared and administered daily via gavage from GD 6 through 15 in a volume of 10 ml/kg. Individual doses were calculated based on the most recently recorded body weight. The study author did not indicate if individual doses were adjusted for purity of the test compound. Analyses for concentration, homogeneity, and stability of the test material in the vehicle were conducted twice during the study.

Dose Rationale

Concentrations were selected based upon the results of a range-finding study (MB Project No. 121-005). The results of this study were not presented.

Observations

Animals were observed twice daily for mortality and moribundity, and at least once daily for clinical signs of toxicity. Body weight data were recorded on GDs 0, 6, 9, 12, 16, and 20. Food consumption data were recorded for the following intervals: GDs 0-6, 6-9, 9-12, 12-16, and 16-20. On GD 20, all animals were sacrificed by carbon dioxide asphyxiation and litters were delivered by cesarean section. Examination of the dams at sacrifice included the following:

- Gross pathology examination
- Number of corpora lutea
- Number and position of implantation sites
- Number of resorptions (early and late) and number of live and dead fetuses

Uteri from apparently nonpregnant animals were stained with a 10% ammonium sulfide solution to detect early embryonic loss.

Examination of live fetuses included the following:

- Individual fetal weight and sex
- External examination of all fetuses

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- Visceral examination of approximately one-half of the fetuses using a modification of the Wilson-Barrow technique

- Skeletal examination of approximately one-half of the fetuses by staining with alizarin red S

Statistical Analysis

The following methods were used:

- Maternal body weight, weight gain, and food consumption--Levene's test, ANOVA, and Dunnett's test

- Litter weight; numbers of corpora lutea, implantation sites, resorptions, and live fetuses; pre- and postimplantation losses; and sex ratio--Bartlett's test, ANOVA, and Dunnett's test (homogeneous data) or Kruskal-Wallis test and Wilcoxon rank-sum test (heterogeneous data)

- Fetal body weight--Bartlett's test, ANCOVA, and Dunnett's test

Compliance

The following statements were submitted:

- A signed Statement of No Data Confidentiality Claims, dated June 28, 1993

- A signed Statement of Compliance with EPA, OECD, and Japanese MAFF GLPs, dated June 11, 1993

- A signed Quality Assurance Statement, dated June 11, 1993

C. RESULTS

Test Material Analyses

Homogeneity analyses revealed values that were 86%-98% of target. Concentration and stability analyses revealed values that were 83%-104% and 76%-99% of target after 4 and 24 hours, respectively.

Maternal Toxicity

Mortality: No compound-related mortalities were observed at any dose level. One pregnant female at 500 mg/kg/day and one nonpregnant female at 1000 mg/kg/day died on GDs 7 and 16, respectively. Necropsy revealed lung changes and red colored vagina in both animals and blood in the uregenital tract of the female at 500 mg/kg/day. These mortalities were considered to be incidental.

Abortion: No abortions were observed at any dose level.

Clinical observations: No compound-related clinical signs were observed at any dose level.

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Body weight: No compound-related effects on body weight or weight gain were observed at any dose level. A summary of body weight gain data is presented in Table 1.

Food consumption: No compound-related effects on food consumption (g/animal/day) were observed at any dose level. Incidental decreases at all dose levels (93% at 200 mg/kg/day; 97% at 500 mg/kg/day; and 91% at 1000 mg/kg/day (significant)) were observed on Gds 6-9 as a result of high food intake in the control group (data not shown).

Gross pathology observations: No compound-related gross findings were observed at any dose level.

Cesarean section observations: No compound-related effects were observed. A summary of cesarean section observations is presented in Table 2.

Developmental Toxicity

No compound-related effects were observed at any dose level. Summaries of fetal external, visceral, and skeletal anomalies are presented in Tables 3 and 4.

External examinations: Multiple external malformations (Table 3) were found in one fetus in the control group and consisted of exencephaly, an-ophthalmia, microphthalmia, agnathia, and displaced ear. In addition, one fetus at 200 mg/kg/day had arthrogryposis. No variations were observed at any dose level (data not shown).

Visceral examinations: Visceral malformations (Table 3) were limited to cephalocele in one fetus in the control group. Variations occurred at all dose levels and included dilation of the lateral ventricles and increased renal pelvis cavitation (data not shown).

Skeletal examinations: No skeletal malformations (Table 3) were observed at any dose level. Variations occurred at similar incidences in all dose groups and included poorly or unossified bones (Table 4) and few incidences of extra, wavy, or kinked, or clubbed ribs and bipartite sternebrae and vertebrae (data not shown).

D. DISCUSSION/CONCLUSIONS

Acceptance Criteria

The reviewers have completed an Acceptance Criteria check list (Attachment I) which is included with the evaluation of the study. Criterion 1 (technical form of the active ingredient) was not fulfilled in that purity of the test compound was never reported. All other criteria were satisfied.

TABLE 1. Mean Body Weight Gain (g ± S.D.) of Rats Exposed to Isofenphos During Major Organogenesis[a]

Dose Group (mg/kg/day)	Pre-Exposure Period (GDs 0-6)	Exposure Period (GDs 6-16)	Post-Exposure Period (GDs 16-20)	Overall Period (GDs 0-20)
0	42.4 ± 7.8	61.1 ± 11.2	58.6 ± 14.5	162.1 ± 22.2
200	39.3 ± 11.5	65.8 ± 10.5	63.2 ± 13.1	168.3 ± 23.1
500	39.7 ± 10.8	65.1 ± 13.1	68.1 ± 14.6	172.5 ± 29.6
1000	39.6 ± 9.0	62.4 ± 11.3	59.3 ± 14.8	161.4 ± 27.7

[a]Data were extracted from Study No. 121-006, Table 4.

TABLE 2. Cesarean Section Observations in Rats Exposed to Triforine During Major Organogenesis[a]

Parameter	Observation for Each Dose Level (mg/kg/day)			
	0	200	500	1000
No. animals mated	30	30	30	30
No. animals pregnant	27	25	24	23
Pregnancy rate (%)[b]	90	83	80	77
Maternal wastage				
No. died/nonpregnant	0	0	0	1
No. died/pregnant	0	0	1	0
No. nonpregnant	3	5	6	6
No. aborted	0	0	0	0
Dams with live litters	27	25	22	22
Dams with 100% resorptions	0	0	1	1
Total corpora lutea	446	423	379 (23)[b,c]	384 (23)[b,c]
Corpora lutea/dam[d]	16.5 ± 2.4	16.9 ± 2.6	16.5 ± 1.6	16.7 ± 2.2
Total implantations	373	365	336 (23)[c]	324 (23)[c]
Implantations/dam[d]	14.8 ± 3.0	14.6 ± 2.8	14.6 ± 3.5	14.1 ± 4.2
Total live fetuses	350	340	319	305
Live fetuses/dam[d]	13.0 ± 3.5	13.6 ± 3.9	13.7 ± 4.8	13.3 ± 4.5
Total resorptions	23	18	19	16
Early resorptions	23	18	18	15
Late resorptions	0	0	1	1
Resorptions/dam[d]	0.9 ± 1.2	0.7 ± 0.8	0.9 ± 1.1[b]	0.7 ± 0.9[b]
Total dead fetuses	0	0	2	3
Dead fetuses/dam[d]	0	0	0.1 ± 0.4	0.1 ± 0.6
Fetal weight/litter (g)[d]	3.7 ± 0.3	3.7 ± 0.3	3.9 ± 0.3	3.8 ± 0.3
Preimplantation loss (%)	16	13	8 (23)[b,c]	16 (23)[b,c]
Postimplantation loss (%)	7	5	11	9
Sex ratio (% male)	50	51	56	45

[a]Data were extracted from Study No. 121-006, Tables 1, 7, 8, and 10.

[b]Calculated by the reviewers including an animal with 100% intra-uterine deaths; not statistically analyzed

[c]In parenthesis is the number of litters included in the calculation.

[d]Mean ± S.D.

TABLE 3. Incidences of Malformations in Fetuses Exposed to Triforine During Major Organogenesis[a,b]

Findings[c]	Incidence at Each Dose Level (mg/kg/day)			
	0	200	500	1000
Total number of fetuses examined	350 (27)	348 (25)	317 (22)	308 (22)
Total number of fetuses with any malformation	1	1	0	0
External Malformations				
Number of fetuses examined	350 (27)	348 (25)	317 (22)	308 (22)
Exencephaly	1	0	0	0
Anophthalmia	1	0	0	0
Microphthalmia	1	0	0	0
Agnathia	1	0	0	0
Displaced ear	1	0	0	0
Arthrogryposis	0	1	0	0
Total number of fetuses with any external malformation	1	1	0	0
Visceral Malformations				
Number of fetuses examined	179 (27)	174 (25)	161 (22)	151 (22)
Omphalocele	1	0	0	0
Total number of fetuses with any visceral malformation	1	1	0	0
Skeletal Malformations				
Number of fetuses examined	170 (27)	174 (25)	156 (22)	154 (22)
Total number of fetuses with any skeletal malformation	0	0	0	0

[a]Data were extracted from Study No. 121-006, Tables 11 and 12.

[b]Numbers in parentheses indicate number of litters.

[c]More than one type of malformation may be found in one fetus.

TABLE 4. Incidences of Selected Skeletal Variations in Fetuses Exposed to Triforine During Major Organogenesis[a,b]

Findings[c]	Incidence at Each Dose Level (mg/kg/day)			
	0	200	500	1000
Number of fetuses examined	178 (27)	174 (25)	154 (22)	154 (22)
Bones with incomplete ossification				
Frontal(s)	17 (9)	15 (9)	25 (9)	15 (9)
Parietal(s)	42 (21)	17 (11)	40 (12)	31 (15)
Interparietal	128 (25)	127 (24)	121 (21)	118 (21)
Occipital	143 (25)	139 (25)	131 (22)	133 (20)
Zygomatic arch(es)	27 (14)	23 (11)	20 (10)	19 (8)
Sternebra(e) in region 1-4	94 (18)	65 (21)	44 (17)	65 (18)
Sternebra(e) 5-6	145 (25)	141 (23)	135 (22)	134 (21)
Thoracic vertebral centrum/a	24 (14)	22 (10)	11 (8)	18 (13)
Pubis/es	14 (9)	17 (10)	11 (5)	9 (6)
Bones with no ossification				
Sternebra(e) 5-6	78 (24)	77 (21)	56 (16)	60 (19)
Metacarpal(s)	109 (36)	103 (22)	93 (20)	79 (20)
Total number of fet ses with any skeletal vari.tion	169 (27)	176 (25)	134 (22)	154 (22)

[a]Data were extracted from Study No. 121-000, Tables 11 and 12.

[b]Numbers in parentheses indicate number of litters.

[c]More than one type of malformation may be found in one fetus.

Test Material Analyses

Analytical chemistry data revealed that the highest dose level did not attain 1000 mg/kg/day but rather 840-890 mg/kg/day. The test material was homogeneously distributed in the vehicle. Stability analyses revealed that the test material was probably stable in the vehicle for 24 hours at room temperature, although variation in the results exceeded ±10%.

Maternal Toxicity

Compound-related maternal toxicity was not observed in this study. Consequently, the NOEL for maternal toxicity was 840 mg/kg/day; the LOEL was not determined.

Developmental Toxicity

Compound-related developmental toxicity (anomalies, intrauterine deaths, or altered growth) was not observed in this study. The NOEL for developmental toxicity was 840 mg/kg/day (Highest Dose Tested, [HDT]).

Study Design/Reporting Deficiencies

The study author did not report the purity of the test material. No information was provided regarding the male animals used for mating. Contrary to guideline recommendation, females and males were mated in a ratio of 4:1.

E. CORE CLASSIFICATION: This study is classified as Core Supplementary Data pending submission and evaluation of the information regarding the purity of the test compound.

Maternal NOEL = 840 mg/kg/day (HDT)

Developmental Toxicity NOEL = 840 mg/kg/day (HDT)

F. RISK ASSESSMENT: Not applicable

ATTACHMENT I

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83-3 Teratology Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1. _Y/N_ 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 (three test groups and control group).
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. _Y/N_ 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.

FINAL

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DATA EVALUATION REPORT

TRIFORINE

Study Type: Developmental Toxicity (Rabbit)

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: Pia Lindström, D.P.H. Date 11/23/93

Independent Reviewer: Sanju Diwan, Ph.D. Date 11/23/93

QA/QC Manager: Sharon Segal, Ph.D. Date 11/23/93

Contract Number: 68D10075
Work Assignment Number: 3-09
Clement Number: 31
Project Officer: Caroline Gordon

Guideline Series 83-3: Developmental Toxicity

EPA Reviewer: Whang Phang, Ph.D.
Review Section III, Toxicology Branch II/HED
Signature: [signature] 11/30/93
Date:

EPA 2nd Reviewer: James Rowe, Ph.D.
Review Section III, Toxicology Branch II/HED
Signature: [signature] 11/30/93
Date:

DATA EVALUATION REPORT

STUDY TYPE: Developmental Toxicity (Rabbit); Guideline Series 83-3

EPA IDENTIFICATION NUMBERS

TOX CHEM. NUMBER:

MRID NUMBER: 428692-02

TEST MATERIAL: 1,4-bis(2,2,2-trichloro-1-formamidoethyl) piperazine

SYNONYMS: Triforine; CME 76770; SAG 102

SPONSOR: Shell International Chemical Company, London, England

STUDY NUMBER: 121-004

TESTING FACILITY: Hazleton Deutschland GmbH, Münster, Germany

TITLE OF REPORT: Oral (Gavage) Teratogenicity Study in the Rabbit

AUTHOR: A. Fuchs

REPORT ISSUED: June 14, 1993

CONCLUSIONS: In a developmental toxicity study, New Zealand White rabbits received triforine daily by gavage at doses of 0 or 1000 mg/kg/day on gestational days (GDs) 6-18, inclusive.

Maternal NOEL = not determined
Maternal LOEL = 1000 mg/kg/day based on decreased body weight gain and food consumption

Developmental NOEL = not determined
Developmental LOEL = 1000 mg/kg/day based on decreased fetal weight

CORE CLASSIFICATION: Core Supplementary Data. This study does not meet the guideline requirements (83-3) for a developmental toxicity study in rabbits. Only two dose groups (one test group) were used. If lower doses had been tested, it may have been possible to establish a NOEL. Information regarding the purity of the test compound was not reported.

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A. MATERIALS

Test Compound

Purity:	Not reported
Description:	Colorless to cream powder or crystals
Batch number:	2764
Receipt dates:	January 18, 1988, and October 16, 1991
Contaminants:	None reported
Storage:	Room temperature in the dark
Vehicle:	Distilled water

Test Animals

Species:	Rabbit
Strain:	New Zealand White
Source:	P. Rollié, Lette, Germany
Age:	Approximately 14-17 weeks at receipt
Weight:	3035-4302 g on GD 0
Males used:	Not reported

B. STUDY DESIGN

This study was designed to assess the potential of triforine to cause developmental toxicity in rabbits when administered daily by gavage on GDs 6-18, inclusive.

Mating Procedure

Following at least 7 days of acclimatization, females were fertilized by natural mating. The day of mating was considered to be GD 0. Following mating, females received 50 I.U. of human chorionic gonadotropin (Primogonyl).

Animal Husbandry

Food (Hanfit in pelleted form) and tap water were available ad libitum throughout the study. A 12-hour light/dark cycle was maintained. Temperature and humidity were maintained at 17°-23°C and 30%-70%, respectively.

Group Arrangement

Animals were randomly assigned to the following dose groups using a random numbers table:

Test Group	Dose Level (mg/kg/day)	Number of Does Assigned per Group
Control	0	18
Limit dose	1000	18

Dose Administered

Doses were prepared and administered daily via gavage from GD 6 through 18 in a volume of 10 mL/kg. Individual doses were calculated based on the most recently recorded body weight. The study author did not indicate if individual doses were adjusted for purity of the test compound. Analyses for concentration, homogeneity, and stability of the test material in the vehicle were conducted during the first and last weeks of treatment.

Dose Rationale

Doses were selected based upon the results of a range-finding study (HD Project No. 121-003). The results of this study were not presented.

Observations

Animals were observed twice daily for mortality and moribundity, and at least once daily for clinical signs of toxicity. Body weight data were recorded on GDs 0, 6, 9, 12, 15, 19, 24, and 28. Food consumption data were recorded for the following intervals: GDs 0-6, 6-9, 9-12, 12-15, 15-19, 19-24, and 24-28. On GD 28, all animals were sacrificed by intravenous injection of Suths 77 (Coopers Tierarzneimittel GmbH) and litters were delivered by cesarean section. Examination of the does at sacrifice included the following:

- Gross pathology examination
- Number of corpora lutea
- Number and position of implantation sites
- Number of resorptions (early and late) and number of live and dead fetuses

Uteri from apparently nonpregnant animals were stained with a 10% ammonium sulfide solution to detect early embryonic loss.

Examination of live fetuses included the following:

- Individual fetal weight and sex
- External examination of all fetuses

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- Visceral examination of all fetuses by microdissection
- Examination of the heads of approximately one-half of the fetuses using a modification of the method described by Wilson (1973)
- Skeletal examination of all fetuses using alizarin red S

Statistical Analysis

The following methods were used:

- Maternal body weight, weight gain, and food consumption--Levene's test, ANOVA, and Dunnett's test
- Litter weight; numbers of corpora lutea, implantation sites, resorptions, and live fetuses; pre- and postimplantation losses; and sex ratio--Bartlett's test, ANOVA, and Dunnett's test (homogeneous data) or Kruskal-Wallis test and Wilcoxon rank-sum test (heterogeneous data)
- Fetal weight--Bartlett's test, ANCOVA, and Dunnett's test

Compliance

The following statements were submitted:

- A signed Statement of No Data Confidentiality Claims, dated June 28, 1993
- A signed Statement of Compliance with EPA, OECD, and Japanese MAFF GLPs, dated June 14, 1993
- A signed Quality Assurance Statement, dated June 14, 1993

C. RESULTS

Test Material Analysis

Analysis for concentration revealed values ranging from 94% to 126% of target. Homogeneity analysis revealed values ranging from 87% to 126% of target. Stability analysis revealed values ranging from 73% to 93% of target after 24 hours at room temperature.

Maternal Toxicity

Mortality: No compound-related mortalities were observed. Three females from the treatment group died during the study. One of these females died on GD 8 as a result of an intubation error. Necropsy of the other two animals, which were found dead on GDs 10 and 20, revealed signs of pneumonia.

Abortion: No abortions were observed in either the control group or the treatment group.

Clinical observations: No compound-related clinical signs were observed in either group. The incidence of animals having few feces in the treatment group was greater than control (17 to 7). This finding was attributable to the reduced food consumption in this group and was not considered to be a direct effect of compound administration.

Although not a true clinical sign, the occurrence of low water consumption was observed in more animals in the treatment group (13 to 7) than in the control group. In 12/13 treated animals, however, reduced water consumption was also noted before the dosing period. Therefore, this finding was considered to be incidental.

Body weight: Compound-related effects on body weight gain were observed in the treatment group. A summary of body weight gain data is presented in Table 1. Body weight gain decreased during the dosing period (GDs 6-19) to 51% of control (significant) and during the entire gestation period (GDs 0-28) to 78% of control (nonsignificant). This effect is partly explained by the lower (87%) weight gain in the treatment group during the predosing period (GDs 0-6). However, weight loss (33 g) was observed in treated animals on GDs 6-9, while controls gained 122 g (data not shown). Similarly, on GDs 12-15 treated animals gained only 29 g, while controls gained 121 g (data not shown). Body weights were similar to control throughout the study (data not shown).

Food consumption: Compound-related effects on food consumption were observed in the treatment group. A summary of food consumption data (g/animal/day) is presented in Table 2. Food consumption decreased significantly during the dosing period on GDs 6-9 (61%), 9-12 (70%), 12-15 (73%), and 15-19 (78%) (data not shown). When determined for the entire dosing period (GDs 6-19) and the gestation period (GDs 0-28), it also decreased significantly (72% and 86%, respectively, Table 2).

Gross pathology observations: No compound-related gross findings were observed in the treatment group.

Caesarean section observations: Compound-related effects on fetal body weight were observed in the treatment group. A summary of caesarean section observations is presented in Table 3. Fetal weight decreased significantly for males alone (data not shown) and for males and females combined (Table 3).

Developmental Toxicity

No compound-related effects were observed in the treatment group with respect to fetal external, visceral, and skeletal malformations (Table 4). However, a statistically significant decrease in fetal weight was found in the 1000-mg/kg group (Table 3).

External examinations: External malformations in the control group consisted of one fetus with multiple malformations (displaced ears and nares not patent); one fetus with oligodactyly and another fetus from the same litter with kinked tail; and two fetuses (one litter) with

TABLE 1. Mean Body Weight Gain (g ± S.D.) of Rabbits Receiving Triforine by Gavage During Major Organogenesis[a]

Dose Group (mg/kg/day)	Pre-Dosing Period (GDs 0-6)	Dosing Period (GDs 6-19)	Post-Dosing Period (GDs 19-24)	Post-Dosing Period (GDs 24-28)	Entire Gestation Period (GDs 0-28)
0	302 ± 114	460 ± 163	147 ± 63	105 ± 56	1015 ± 281
1000	264 ± 126	234 ± 188**	219 ± 188	76 ± 167	793 ± 312

[a]Data were extracted from Study No. 121-004, Table 4.

**Significantly different from control (p≤0.01)

TABLE 2. Mean Food Consumption (g/animal/day ± S.D.) of Rabbits Receiving Triforine by Gavage During Major Organogenesis[a]

Dose Group (mg/kg/day)	Pre-Dosing Period (GDs 0-6)	Dosing Period (GDs 6-19)	Post-Dosing Period (GDs 19-24)	Post-Dosing Period (GDs 24-28)	Entire Gestation Period (GDs 0-28)
0	221 ± 24	233 ± 36	218 ± 39	173 ± 69	213 ± 34
1000	217 ± 30	167 ± 29**	203 ± 56	138 ± 42	182 ± 29*

[a]Data were extracted from Study No. 121-004, Table 5.

*Significantly different from control (p≤0.05)

**Significantly different from control (p≤0.01)

reduced or rudimentary tail. Malformations in the treatment group were limited to one fetus with a filamentous tail. Variations consisted of slightly bent tail and which occurred with similar incidences in both the control and treated groups (data not shown).

Visceral examinations: Visceral malformations in the control group consisted of one fetus with multiple malformations (abnormal brain shape, brain degenerate, aphakia, agnathia, and microstomia); two fetuses (two litters) with abnormal brain shape and internal hydrocephaly (one of these fetuses also had a cleft palate); and one fetus with abnormal brain shape. No visceral malformations were observed in the treatment group. Variations, occurring in both the control and treatment groups, consisted of dilation of lateral cerebral ventricles and retinal folds (data not shown).

Skeletal examinations: Skeletal malformations in the control group consisted of eight fetuses (four litters) with multiple malformations (see Table 4) and one fetus with fused caudal vertebrae. In the treatment group, three fetuses (two litters) had multiple malformations (see Table 4); one fetus had fused cervical vertebral centrum to arches; one fetus had major fusion of the sternebrae; and one fetus had fused thoracic vertebral arches. The most frequent variations (Table 5), occurring at similar rates in both groups, included speculated, extra thoraco-lumbar, and/or thickened rib(s). Incomplete, asymmetric, and/or no ossification were most frequently noted in sternebra(e), forelimbs, pubes, hindlimbs, and astragali and, in general, occurred at increased incidences at 1000 mg/kg/day as an effect of decreased fetal body weight.

D. Discussion/Conclusions

Acceptance Criteria

The reviewers have completed an Acceptance Criteria check list (Attachment I) which is included with the evaluation of the study. Several criteria were not satisfied. This study used only one treatment group. The study author might have attempted to establish a limit dose. However, developmental toxicity (reduced fetal weight) was observed at 1000 mg/kg/day. EPA guidelines also state that no developmental toxicity should be observed in the low-dose group. Because there was only one dose group and developmental toxicity was observed in this group, this criterion was not satisfied either.

Test Material Analyses

Analytical chemistry data indicate that there may have been a problem with the dosing solutions as indicated by the large variation in those results. Since this study is classified as Core Supplementary Data and cannot be upgraded, the reviewers will only suggest that more care should be taken in the future when dissolving this test compound in water.

TABLE 3. Cesarean Section Observations in Rabbits Receiving Triforine by Gavage During Major Organogenesis[a,b]

Parameter	Observation for Each Dose Level (mg/kg/day)	
	0	1000
No. animals mated	18	19
No. animals pregnant	15	18
Pregnancy rate (%)[c]	83	95
Maternal wastage[c]		
No. died/nonpregnant	0	1
No. died/pregnant	0	2
No. nonpregnant	3	1
No. aborted	0	0
Does with live litters	13	16
Does with 100% resorptions	2	0
Total corpora lutea	161 (14)[c]	212 (16)
Corpora lutea/doe	11.5 ± 4.4[c,d]	13.3 ± 1.9
Total implantations	103 (15)	138 (16)
Implantations/doe	6.9 ± 3.8	8.6 ± 2.8
Total live fetuses	65 (13)	119 (16)
Live fetuses/doe	4.4 ± 3.8	7.4 ± 3.4
Total resorptions	20	19
Early resorptions	20	17
Late resorptions	0	2
Resorptions/doe	1.3 ± 1.3	1.2 ± 1.3
Total dead fetuses	0	0
Dead fetuses/doe	0	0
Fetal weight/litter (g)	42.5 ± 5.6	37.2 ± 4.4*
Preimplantation loss (%)	37 (13)[c]	35
Postimplantation loss (%)	14	16
Sex ratio (% male)	49	50

[a]Data were extracted from Study No. 121-004, Tables 1, 9, and 10.

[b]Numbers in parentheses represent number of litters included in calculation

[c]Calculated by the reviewer; not statistically analyzed

[d]Mean ± S.D.

*Significantly different from control ($p \leq 0.05$)

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TABLE 4. Incidences of Malformations in Fetuses Exposed to Triforine During Major Organogenesis[a,b]

Findings[c]	Incidence at Each Dose Level (mg/kg/day) 0	1000
Total number of fetuses examined	89 (13)	119 (16)
Total number of fetuses with any malformation	13 (4)	6 (4)
External Malformations		
Displaced ear	1	0
Nares nonpatent	1	0
Oligodactyly	1	0
Kinked tail	1	
Tail reduced/rudimentary	2 (1)	
Filamentous tail	0	1
Total number of fetuses with any external malformation	5 (3)	1
Visceral Malformations		
Internal hydrocephaly	2 (2)	0
Brain abnormal shape	2 (2)	0
Brain-degenerate	1	0
Aphakia	1	0
Aganthia	1	0
Microstomia	1	0
Cleft palate	1	0
Total number of fetuses with any visceral malformation	4 (3)	0
Skeletal Malformations		
Scoliosis	1	0
Frontals fused	1	0
Major fusion of sternebrae	1	2 (2)
Sternum bent inward (marked)	0	1
Sternebrae malformed	1	0
Rib(s) absent	3 (2)	2 (1)
Rib(s) branched	3 (2)	0
Ribs proximally fused	5 (4)	1
Sacral centrum malformed	1	0
Thoracic hemivertebrae	2 (2)	0
Sacral vertebrae absent	2 (1)	0
Caudal vertebrae fused	1	0
Caudal vertebrae absent	2 (1)	1
Cervical vertebral centrum fused to arches	0	1
Thoracic vertebral centra fused	1	0
Thoracic vertebral centra absent	1	2 (1)
Thoracic vertebral arches fused	3 (3)	1
Last lumbar arch fused to 1st sacral vertebral arch	1	0
Thoracic vertebral arch absent	1	1
Sacral vertebral centrum fused to arches	1	0
Total number of fetuses with any skeletal malformation	9 (4)	6 (4)

[a]Data were extracted from Study No. 31-004 Table 13.

[b]Numbers in parentheses, ( ) indicate number of litters.

[c]More than one type of malformation may be found in a fetus.

TABLE 5. Incidences of Selected Skeletal Variations in Fetuses Exposed to Tritorine During Major Organogenesis[a,b]

Findings[c]	Incidence at Each Dose Level (mg/kg/day) 0	1000
Total number of fetuses examined	83 (13)	119 (16)
Sternebrae		
Fused	4 (2)	4 (4)
Misshapen	2 (2)	5 (3)
Ribs		
Clubbed	1	6 (4)
Spatulate	25 (10)	30 (11)
Thickened	12 (6)	13 (7)
Extra thoraco-lumbar rib(s)	40 (12)	88 (15)
Vertebrae		
Misaligned	4 (4)	7 (4)
Bones with incomplete ossification		
Frontal(s)	5 (4)	4 (3)
Parietal(s)	6 (6)	9 (7)
Interparietals	1	6 (4)
Sternebra(e) in region 1-4	6 (6)	11 (9)
Sternebra(e) 5-6	37 (10)	63 (13)
Caudal vertebra(e)	2 (2)	10 (6)
Pubis/es	7 (4)	31 (13)
Forelimb phalanx/ges	16 (7)	40 (14)
Hindlimb phalanx/ges	2 (2)	10 (4)
Astragalus/i	9 (7)	42 (12)
Bones with no ossification		
Sternebra(e) 5-6	38 (12)	65 (11)
Pubis/es	3 (2)	7 (5)
Forelimb phalanx/ges	48 (11)	76 (16)
Metacarpals	6 (5)	16 (8)
Hindlimb phalanx/ges	8 (4)	21 (10)
Astragalus/i	8 (4)	16 (8)
Bones with asymmetric ossification		
Sternebra(e) in region 1-4	10 (7)	20 (12)
Sternebra(e) 5-6	0	5 (3)
Total number of fetuses with any skeletal variation	83 (13)	119 (16)

[a]Data were extracted from Study No. 161-084 Table 12.

[b]Numbers in parentheses indicate number of litters.

[c]More than one type of malformation may be found in one fetus.

Maternal Toxicity

Compound-related maternal toxicity was observed at 1000 mg/kg/day. It was manifested as significantly decreased maternal body weight gain and food consumption during the dosing period. Based on these results, the LOEL for maternal toxicity was 1000 mg/kg/day; the NOEL was not determined.

Developmental Toxicity

Compound-related developmental toxicity was observed at 1000 mg/kg/day. It was manifested as significantly decreased fetal body weight. Consequently, the LOEL for developmental toxicity was 1000 mg/kg/day; the NOEL was not determined.

Study/Reporting Deficiencies

Information regarding the purity of the test compound was not provided. No information was submitted regarding the males used for insemination. Only one treatment group was used. Results of the range-finding study were not provided. These results would have been useful in determining the NOELs for maternal and developmental toxicity.

E. CORE CLASSIFICATION: Core Supplementary Data. This study may not be upgraded since only one dose was used.

Maternal NOEL = not determined
Maternal LOEL = 1000 mg/kg/day based on decreased body weight gain and food consumption

Developmental Toxicity NOEL = not determined
Developmental Toxicity LOEL = 1000 mg/kg/day based on decreased fetal body weight

F. RISK ASSESSMENT: Not applicable

ATTACHMENT I

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83-3 Teratology Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.	Y/N	Technical form of the active ingredient tested.
2.	NO	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 (three test groups and control group).
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.*	NO	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.