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

JAN 8 1998

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Triforine: Evaluation of two developmental toxicity studies in rabbits

Caswell No. 890AA
PC Code:. 107901
Submission No. S465983

DP Code: D216222
MRID No. 43664901 & 43664902

TO: Tom Myers /Kathy Monk, PM Team 52
Special Review and Re-registration Division (7508W)

FROM: Whang Phang. Ph.D. W Phang 1/8/98
Branch Senior Scientist
Reregistration Branch I/HED (7509C)

THROUGH: Susan Makris, M.S. Susan J Makris 1/8/98
Toxicologist
Tox. Branch I/HED (7509C)
&
Mike Metzger, Branch Chief
Reregistration Branch I/HED (7509C) W. J Hazel, for 1/8/98

The registrant, American Cyanamid, submitted 2 developmental toxicity studies on triforine. Both of these studies were conducted in the rabbits. One is a preliminary study (dose-range finding study; MRID 43664902) while the other is a definitive study (MRID 43664901). The dose-range finding study was conducted much later (1995) than the definitive study (1988). The results of the definitive study indicated that the highest dose (150 mg/kg/day) was not sufficient in testing the development toxicity of triforine. This was strongly supported by the 1995 dose-range finding study which employed doses as high as 1000 mg/kg/day which produced no significant maternal or developmental toxicities. The author of the study recommended that 1000 mg/kg/day be used as the high dose for a development toxicity study. Unfortunately, the dose-range finding study did not conduct the skeletal and visceral examinations, otherwise the results of this could have been more valuable. Both studies contained deficiencies which prevented them from satisfying the guidelines for a developmental toxicity study, and they were classified as **unacceptable** under the current evaluation system. The citation and summary of each study are presented below:

1. Muller, W. (1988) Oral (gavage) teratogenicity study in the rabbits. Unpublished Study conducted by Hazleton lab. Deutschland GmbH, Germany. Study No. 460/29. April 27, 1988. Revised final report: April 27, 1995. Submitted to US EPA by American Cyanamid Co.; MRID No. 43664901.

In a developmental toxicity study (MRID No. 43664901), groups of mated New Zealand White rabbits (18 females/group) received triforine (98.1% pure) by gavage on gestation days 6 through 18 at doses of 6, 30, and 150 mg/kg/day. A control group (18 mated females) was also included in the study. On gestation day 28, all test animals underwent cesarean section and were examined. The fetuses were removed and examined for developmental effects.

The results indicated that triforine at doses tested in this study did not produce any treatment-related maternal or developmental effects. The test animals could have tolerated higher doses. Therefore, this study is classified as **supplementary** (unacceptable), and does not meet the guideline for a developmental toxicity study (83-3).

2. Muller, W. (1988). Triforine: Preliminary oral (gavage) embryotoxicity study in the rabbit. Unpublished Study conducted by Hazleton Lab. Deutschland GmbH, Germany. Study No. 121-003. April 27, 1991. Revised final report: April 27, 1995. Submitted to US EPA by American Cyanamid Co.; MRID No. 43664902. Unpublished.

In a preliminary developmental toxicity study (MRID 43664902), groups of mated New Zealand White female rabbits (8/group) received triforine (99.6% pure) by gavage on gestation days 6 through 18 at doses of 250, 500, and 1000 mg/kg/day. A control group of 8 mated females was also included in the study. On gestation day 28, all test animals underwent cesarean section. The fetuses were removed and examined for any toxic and developmental effects.

Under the conditions of this study, triforine at doses tested did not produce any treatment-related maternal or developmental effects (NOEL $\geq$1000 mg/kg/day). There was a slight decrease in the mean body weights of the high-dose maternal animals, but the decrease did not show a statistical significance. The mean body weight gain from gestation days 6-19 was decreased, and the reduction was statistically significant. Visceral or skeletal examinations were not conducted in this preliminary study. Based on the results from this study, the study author suggested that a high dose of 1000 mg/kg/day was suitable for the definitive developmental toxicity study.

This study is a dose-range finding study. It used an inadequate number of test animals, and it lacks fetal skeletal and visceral examination data. It does not meet the Guidelines for a developmental toxicity study in rabbits (83-3b). Therefore, it is <u>unacceptable</u> as a developmental toxicity study and can not be upgraded

Reviewer: Whang Phang, Ph.D. 12/23/97
Reregistration Branch I/HED (7509C)

012445

Secondary Reviewer: Susan L. Makris, M.S. 1/5/98
Tox. Branch I/HED (7509C)

DATA EVALUATION REPORT

Study Type: Preliminary developmental toxicity study in rabbits
OPPTS 870.3700 [§83-3b]

Tox. Chem. No.	890AA	**DP Barcode.**	D216222
MRID No.	433664902	**CASE No.**	819415
PC Code	107901	**Submission**	S465983

Test Material: Triforine

Synonyms: 1,4-bis(2,2,2-trichloro-1-formamidoethyl)-piperazine; SAG 102

Sponsor: Shell International Chemical Co. Ltd.
London, England

Testing Facility: Hazleton Laboratories Deutschland GmbH
Kesselfeld 29
4400 Munster, Germany

Citation: Muller, W. (1988). Triforine: Preliminary oral (gavage) embryotoxicity study in the rabbit. Unpublished Study conducted by Hazleton Lab. Deutschland GmbH, Germany. Study No. 121-003. April 27, 1991. Revised final report: April 27, 1995. Submitted to US EPA by American Cyanamid Co.; MRID No. 43664902. Unpublished.

Executive Summary: In a preliminary developmental toxicity study (MRID 43664902), groups of mated New Zealand White female rabbits (8/group) received triforine (99.6% pure) by gavage on gestation days 6 through 18 at doses of 250, 500, and 1000 mg/kg/day. A control group of 8 mated females was also included in the study. On gestation day 28, all test animals underwent cesarean section. The fetuses were removed and examined for any toxic and developmental effects.

Under the conditions of this study, triforine at doses tested did not produce any treatment-related maternal or developmental effects (NOEL $\geq$1000 mg/kg/day). There was a slight decrease in the mean body weights of the high-dose maternal animals, but the decrease did not show a statistical significance. The mean body weight gain from gestation days 6-19 was decreased, and the reduction was statistically significant. Visceral or skeletal examinations were not conducted in this preliminary study. Based on the results from this

study, the study author suggested that a high dose of 1000 mg/kg/day was suitable for the definitive developmental toxicity study.

This study is a dose-range finding study. It used an inadequate number of test animals, and it lacks fetal skeletal and visceral examination data. It does not meet the Guidelines for a developmental toxicity study in rabbits (83-3b). Therefore, it is unacceptable as a developmental toxicity study and can not be upgraded.

Compliance: Signed and dated GLP, Quality Assurance, data confidentiality, and flagging statements were included in the report.

METHODS AND MATERIALS

Test Article: Triforine with a purity of 99.6% and a batch No. of HT08/91/1 was described as colorless to cream powder or crystals. The purity of the chemical was presented in a letter from the registrant, American Cyanamid Co. (D. Little to W. Phang, June 18, 1996).

Test Animals: Female New Zealand White rabbits were obtained from H. Fortkamp, 4540 Lengerich, W. Germany. The body weights of the test animals were ranging from 2.9 to 4.1 kg. These animals were 14 to 17 weeks old and sexually mature. The test animals were acclimated to the laboratory conditions for at least a week prior to the initiation of the study. The test rabbits were housed individually in a controlled environment and received tap water and food (pelleted rabbit diet) ad libitum.

Vehicle: Distilled water

Study Design

1. In life dates: Began on July 3, 1991 and ended on September 4, 1991.

2. Mating: The test females were mated with males. The report does not contain additional detail about mating. The report stated that females which successfully completed coitus receive an IV dose (50 I.U.) of a luteinizing hormone (HCG: Primogonyl®) to ensure ovulation. The day of mating was considered as gestation day 0.

3. Animal Assignment: As indicated in Table 1, the mated female rabbits were randomly assigned to a control and 3 treatment groups.

Table 1+: Dose groups and number of mated female rabbits/dose group.

Test Group	Dose (mg/kg/day)	Number of Females
Control	0	8
Low (LDT)	250	8
Mid (MDT)	500	8
High (HDT)	1000	8

+: Data excerpted from the report, p. 20 (MRID No. 43664902).

4. Dose selection rationale: This is a preliminary study. The report indicated that the dosages were selected based upon the results of previous developmental toxicity studies in rats and rabbits.

5. Dosage preparation and analysis: The dose formulations were prepared daily as a suspension in water. During the first week of treatment, samples of the prepared dosages for Low and High Dose groups were taken (from top, middle, and bottom) for analysis. The analysis was conducted to determine the concentration of the test material, homogeneity, and stability of the test article formulations over 4 and 24 hrs.

   Results: The results showed that the test formulation were relatively homogenous among the top, middle, and bottom sections of the suspension. The prepared dosing suspensions were acceptable for both stability and percentage of the nominal concentrations.

6. Dosing: All doses were administered by gavage once daily, on gestation days 6 through 18, in a volume of 10 ml/kg/day. Dose volume was based on the body weight on gestation days 6, 9, 12, and 15.

7. Observation
   a. Maternal observation: The animals were checked "at least once daily" for clinical signs of toxicity, behavior changes, and mortality.

8. Body weights: Body weights of each doe were measured and recorded on days 0, 6, 9, 12, 15, 19, 24, and 28 post-coitum.

9. Food consumption: Food consumption was apparently not measured.

10. Necropsy: All surviving does were sacrificed on gestation day 28 by IV injection of Euth 77®. The ovaries and uteri were removed and examined for the following parameters:
    No. of corpora lutea in each ovary
    No. and position of implantation subdivided into:
    a. live fetuses

b. early resorptions
c. late resorptions
d. dead fetuses

The uteri of non-pregnant females were examined for evidence of implantation by immersing them in a 10% ammonium sulphide solution.

b. Fetal evaluations: The sex of each fetus was determined. Each fetus was weighed and examined for external abnormalities. All fetuses were preserved in 10% neutral buffered formalin. Visceral and skeletal examinations were not carried out.

10. Data analysis

a. Statistical analysis: For the data on maternal body weight and body weight gain, Levene's test for homogeneity of variance, one-way analysis of variance, and Dunnett's test were performed. For litter weight data, analysis of variance and by Student-Newman-Keuls test for multiple group comparisons were employed..

Analysis of Variance and Student-Newman-Keuls tests were used to analyze the number of corpora lutea, number of implantations, number of fetuses, mean fetal weights (overall and in each sex), preimplantation loss, postimplantation loss, number of intra-uterine deaths, and proportion of male fetuses.

All tests were performed using a two-sided risk and a significance level of $p \leq 0.05$.

b. Indices: The following indices were calculated from the cesarean section record of the test animals using the formula shown below:

$$\text{Preimplantation loss: } \frac{\text{No. of corpora lutea - No. of implantations}}{\text{No. of corpora lutea}} \times 100$$

$$\text{Postimplantation loss: } \frac{\text{No. of implantions - No. of live fetuses}}{\text{No. of implantations}} \times 100$$

RESULTS

A. Maternal toxicity

1. Clinical observations: Compound-related clinical signs of toxicity were not seen. One mid dose (500 mg/kg/day) group animal was shown to have low food and water consumption. On gestation day 20, this animal aborted and was sacrificed. Necropsy findings on this animal did not indicate the abortion was due to treatment.

2. Mortality: The data showed no treatment-related death.

3. Body weight: The body weight and body weight gain data are excerpted from the report and presented in Tables 2 & 3, respectively. The mean body weights of the compound-treated animals from gestation days 6 to 19 were decreased relative to the controls, but the decreases were not statistically

significant (Table 2). In addition, the mean body weights of all the treated groups were less than that of the controls at the initiation of treatment, and this pattern continued to the end of the study.

Table 2 +. Mean maternal body weight ± S.D. (kg)

Day of Gestation	0 mg/kg N=6	250 mg/kg N=8	500 mg/kg N=8;7++	1000 mg/kg N=6
0.00	3.7±0.4	3.3±0.3	3.5±0.5	3.5±0.4
6	4.0±0.5	3.6±0.4	3.8±0.4	3.8±0.4
12	4.2±0.5	3.7±0.4	3.9±0.3	3.8±0.3
19	4.4±0.5	3.8±0.3	4.0±0.3	3.9±0.4
24	4.5±0.5	4.0±0.4	4.2±0.3	4.2±0.3
28	4.6±0.5	4.1±0.3	4.3±0.2	4.3±0.4

+: Data excerpted from the report, p. 45 (MRID No. 43664902). The data were calculated from animals with live fetuses at necropsy.
++: At day 24 onward, N=7.

The body weight gain data indicated that during the interval of gestation days 6-9, there was a mean weight loss in all groups of the compound treated animals relative to the controls, and the decrease was statistically significant at the mid-dose. However, a dose-related effect was not seen. During the interval of gestation days 6-19; the mean body weight gain in the mid- and high-dose groups were decreased, and the reduction in the high dose group was statistically significant ($p < 0.01$) (Table 3).

Table 3+. Mean maternal body weight gain ± S.D.(kg)

Day of Gestation	0 mg/kg N=6	250 mg/kg N=8	500 mg/kg N=8;7++	1000 mg/kg N=6
0 - 6	0.32±0.12	0.29±0.06	0.35±0.06	0.32±0.10
6 - 9	0.09±0.05	-0.01±0.07	-0.07±0.12**	-0.03+0.07
12 - 15	0.11±0.05	0.10±0.05	0.10±0.09	0.12±0.05
19 - 24	0.13±0.05	0.19±0.04	0.17±0.05	0.27±0.15*
0 - 28	0.89±0.20	0.83±0.11	0.81±0.34	0.81±0.33
6 - 19	0.36±0.09	0.22±0.09	0.14±0.30	0.13±0.07**

+: Data excerpted from the report, p. 35 (MRID No. 43664901).
++: At day 24 onward, N=7.
Significantly different from the controls: *: $p<0.05$; **: $p<0.01$

4. Food consumption: No food consumption data were reported. However, the clinical observation data in Table 2 (p.44) of the report showed that 2/8 and 4/8 animals in the mid- and high-dose groups, respectively, had low food consumption.

5. Gross pathology: Compound- and dose-related gross findings were not seen in any treatment group.

6. Cesarean section data: The data on cesarean section are excerpted from the report and summarized in Table 4. The number of animals pregnant in the controls and the treated groups was not significantly different. The maternal wastage was not affected by the treatment. The total numbers of corpora lutea and implantation were slightly reduced in all treatment groups (statistically significant at low dose), but a dose-response relationship was not evident. The numbers of live or dead fetuses were comparable between the treated and the control animals. There was a slight decrease in the total number of resorptions in the treated groups in comparison to the controls. However, the resorptions consisted mainly of early resorptions, and the difference was not statistically significant. Preimplantation loss was comparable between the high dose group and the controls. The low- and mid-dose groups showed a slightly higher percentage of preimplantation loss, but the increase was not statistically significant. The post implantation loss in the controls was greater than that of any treated groups whether calculated with or without litters in which there was total fetal loss. There was a slight reduction in the mean fetal body weights in the high dose group, but the reduction did not show a statistical significance.

B. Developmental Toxicity

One fetus each from the control and the low groups showed signs of enlarged kidneys. No additional external anamolies were seen in the control or the treated animals (Table 6). No explanation was provided to indicate why 2 fetuses were examined for visceral anomalies, since neither visceral nor skeletal evaluation was performed as stated in the report (p.53).

Table 5+: Cesarean section observations.

Observations	Dose (mg/kg/day)			
	0 (Control)	250	500	1000
# Animals assigned (mated)	8	8	8	8
# Animals pregnant	7	8	8	8
Pregnancy rate (%)	88	100	100	100
# Animals nonpregnant	1	0	0	0
Maternal wastage				
# Dead	0	0	0	0
# Died pregnant	0	0	0	0
# Died nonpregnant	0	0	0	0
# Aborted	0	0	1	0
# Premature delivery	0	0	0	0
Total # corpora lutea[a]	88	81	77	72
Corpora lutea/dam	14.7±2.7	10.1±1.7*	11.0±2.2	12.0±3.8
Total # of implantions[b]	53	64	54	41
Implantation/dam	7.6±3.6	8.0±2.2	7.7±2.5	5.1±3.6
Total # litters (live)	6	8	7	6
Total # live fetuses	34	53	42	34
Live fetuses/dam	5.7±3.4	6.6±3.3	6.0±2.9	5.7±2.9
Total # dead fetuses	0	1	0	45.9±30.0 0
Dead fetuses/dam	0	0.1±0.4	0	0
Total # resorptions[b]	19	10	12	7
Early	19	10	12	5
Late	0	0	0	2
Resorption/dam[b]				
Early	2.7±2.0	1.3±2.4	1.7±2.6	0.6±0.7
Late	0	0	0	0.3±0.7
Litters with total intra-uterine death	1	0	0	2
Mean fetal weight (g)	43.8±3.1	42.3±3.8	40.6±5.0	39.8±4.2
Males	43.8±2.4	43.2±6.0	41.2±4.3	41.4±4.4
Females	44.7±3.9	41.2±3.8	39.7±5.5	38.7±5.5
Sex Ratio (% male)[c]	47	55	50	50
Preimplantation loss (%)[a]	42.2±24.2	21.7±15.3	29.8±18.2	45.9±30.0
Postimplantation loss (%)[a]	32.7±27.8	18.4±29.2	20.8±28.1	8.7±11.4
Postimplantation loss (%)[b]	42.3±36.0	18.4±29.2	20.8±28.1	31.5±43.4

+: Data excerpted from the report pp. 49-52 (MRID No. 43664902).
a: Calculated from animals with live fetuses at necropsy; excerpted from pp. 49-50 of the report.
b: Calculated from animals with live fetuses at necropsy and total intra-uterine death (p. 51 of the report).
c: Values are calculated by this reviewer based on the data presented in this table.
*: Significantly different from controls ($p<0.05$)

Table 6+: External Malformations

Observations	Dose (mg/kg)			
	0 (control)	250	500	1000
#Fetuses (litters) examined	34 (6)	53 (8)	42 (7)	34 (6)
#Fetuses (litters) affected	1 (1)++	1 (1)++	0 (0)	0 (0)

+: Data excerpted from the report (p. 53) (MRID No. 43664902).
++: The finding was enlarged kidneys, a visceral variation.

DISCUSSION

Groups of mated New Zealand White rabbits (8 females/group) received triforine (99.6% pure) by gavage on gestation days 6 through 18 at doses of 250, 500, and 1000 mg/kg/day. A control group (8 mated females) was also included in the study. On gestation day 28, all test animals underwent cesarean section. The fetuses were removed and examined for any toxic and developmental effects.

The results indicated that triforine at doses tested in this study did not produce any treatment-related maternal or developmental effects (NOEL $\geq$1000 mg/kg/day). There was a slight decrease in the mean body weights of the high-dose maternal animals, but the decrease did not show a statistical significance. The mean body weight gains from gestation days 6-19 was decreased, and the reduction was statistically significant. Triforine did not produce any effect on the developmental parameters which were examined in this study. However, the mean fetal body weight in the high dose group was slightly decreased, but it was not statistically significant. Visceral or skeletal examinations were not conducted in this preliminary study. Based on the results from this study, the study author suggested that a high dose of 1000 mg/kg/day was suitable for the definitive developmental toxicity study.

This study is a dose-range finding study. It used an inadequate number of maternal test animals, and it lacks skeletal and visceral examination data. It does not meet the Guidelines for a developmental toxicity study in rabbits (83-3b). It is unacceptable as a developmental toxicity study and can not be upgraded. .

Reviewer: Whang Phang, Ph.D. 7/23/96
Section III/Tox. Branch II/HED (7509C)

Secondary Reviewer: Susan L. Makris, M.S. 7/23/96
Section III/Tox. Branch II/HED (7509C)

DATA EVALUATION REPORT

Study Type: Developmental toxicity study in rabbits
OPPTS 870.3700 [§83-3b]

Tox. Chem. No.	890AA	**DP Barcode.**	D216222
MRID No.	433664901	**CASE No.**	819415
PC Code:	107901	**Submission No.**	S465983

Test Material: Triforine

Synonyms: 1,4-bis(2,2,2-trichloro-1-formamidoethyl)-piperazine; SAG 102

Sponsor: SHELL AGRAR GMBH & Co. KG
Binger Strasse 170
6507 Ingelheim am Rhein, Germany

Testing Facility: Hazleton Laboratories Deutschland GmbH
Kesselfeld 29
48163 Munster, Germany

Citation: Muller, W. (1988) Oral (gavage) teratogenicity study in the rabbits. Unpublished Study conducted by Hazleton lab. Deutschland GmbH, Germany. Study No. 460/29. April 27, 1988. Revised final report: April 27, 1995. Submitted to US EPA by American Cyanamid Co.; MRID No. 43664901.

Executive Summary: In a developmental toxicity study (MRID No. 43664901), groups of mated New Zealand White rabbits (18 females/group) received triforine (98.1% pure) by gavage on gestation days 6 through 18 at doses of 6, 30, and 150 mg/kg/day. A control group (18 mated females) was also included in the study. On gestation day 28, all test animals underwent cesarean section and were examined. The fetuses were removed and examined for developmental effects.

The results indicated that triforine at doses tested in this study did not produce any treatment-related maternal or developmental effects. The test animals could have tolerated higher doses. Therefore, this study is classified as **supplementary**.

Compliance: Signed and dated GLP, Quality Assurance, data confidentiality, and flagging statements were included in the report.

METHODS AND MATERIALS

Test Article: Triforine with a purity of 98.1% and a batch No. of 2764 was described as colorless to cream powder or crystals.

Test Animals: Male and female New Zealand White rabbits were obtained from H. Fortkamp, 4540 Lengerich, W. Germany. The males weighed from 5 to 5.5 kg while the females weighed 3.1 to 4.0 kg. Both male and female rabbits were at least 14 weeks old and were sexually mature. The test animals were acclimated to the laboratories for one to 4 weeks prior to the initiation of the study. The test rabbits were housed individually and in a controlled environment and received water and food ad libitum.

Vehicle: Distilled water

Study Design

1. In life date: Begins on Feb. 2, 1988 and ends on March 21, 1988.

2. Mating: The female and male rabbits were mated naturally, and the mating record indicated that only one male was used to inseminate each female. The report stated that does which successfully completed coitus receive an IV dose of luteinizing hormone (HCG:Primogonyl^{R}) to ensure ovulation.

3. Animal Assignment: As indicated in Table 1, the mated female rabbits were randomly assigned to 3 dose groups.

Table 1^{+}: Dose groups and number of mated female rabbits/dose group.

Test Group	Dose (mg/kg/day)	Number of Females
Control	0	18
Low (LDT)	6.0	18
Mid (MDT)	30.0	18
High (HDT)	150.0	18

+: Data excerpted from the report, p. 15 & 18 (MRID No. 43664901).

4. Dose selection rationale: The dose selection was based on the results of teratogenicity study in Himalayan rabbits (Shell Document No. 102AD-451-003). In that study,

triforine as a suspension in aqueous CMC cellulose and administered (by gavage) in doses of 0, 5, 25, and 125 mg/kg during the gestation days of 6 to 18. Cesarean section was performed on gestation day 29. The report stated that slight to significant decreases in body weights were found in the mid and the high dose maternal animals, respectively. Food consumption was also decreased in mid and high dose animals. No developmental toxicity was seen in any dose group.

5. Dosage preparation and analysis: The dose formulations were prepared daily and immediately prior to dosing. The dosages were prepared by gradually adding the vehicle (water) to the appropriate amount of the test material while stirring.

   During the first week of treatment, samples of the prepared dosages were taken (from top, middle, and bottom) and froze immediately until analysis. The analysis was conducted to determine the concentration of the test material, homogeneity, and stability of the test article formulations over 4 and 24 hrs.

   Results: The results showed that the test formulation was relative homogenous among the top, middle, and bottom sections of the solution. However, the low dose preparations appeared to be closer to the target dose (102% to 113% of the target dose) than the mid and high dose preparations (73% to 95% of the target dose). The formulated doses were not stable after the first 4 hrs after preparation (59% to 108% of the target dose). After 24 hrs, the percent of the target dose dropped markedly (48% to 80% of the target dose).

6. Dosing: All doses were administered by gavage once daily, on gestation days 6 through 18, in a volume of 10 ml/kg/day. Dosing was based on the daily body weight, and it was performed at approximately the same time each day in the morning.

7. Observation

   a. Maternal observation: The animals were checked twice daily for clinical signs of toxicity, behavior changes, and mortality. Body weights of each doe were measured and recorded on days 0, 6 to 18, 24, and 28 post-coitum. Food consumption was determined for gestation days 0-6, 6-12, 12-18, 18-24, and 24-28. All surviving does were sacrificed on gestation day 28 by IV injection of Euth 77^{R}. The ovaries and uteri were removed and examined for the following parameters:

      No. of corpora lutea in each ovary
      No. and position of implantation subdivided into:

a. live fetuses
b. early resorptions
c. late resorptions
d. dead fetuses

Individual fetal weights
Sex of the fetuses

b. Fetal evaluations: All fetuses were weighed and examined for external malformations. Then they were dissected to detect visceral abnormalities. "After evisceration, the head was removed from approximately half of the fetuses from each litter (taking each second fetus in accordance with the position in the uterine horn, if possible), fixed in Bouin's fluid, and examined for visceral abnormalities using a modified Wilson's technique."

For skeletal examination, all fetuses were fixed in 95% ethanol and using the Alizarin staining technique.

c. Non-pregnant females: The uterus of each non-pregnant female was immersed in 10% solution of ammonium sulphide to reveal evidence of implantation (Salewski technique).

8. Data analysis

a. Statistical analysis: For the data on body weight, food consumption, litter weight, and mean fetal weight, analysis of variance was employed, followed by Newman-Keuls test for multiple group comparisons.

The Kruskal-Wallis test was used to analyze the number of corpora lutea, number of implantations, number of fetuses, preimplantation loss, postimplantation loss, number of intra-uterine deaths, and proportion of male fetuses.

All tests were performed using a two-sided risk and a significance level of $p \leq 0.05$.

b. Indices: The following indices were calculated from the cesarean section record of the test animals using the formula shown below:

$$\text{Preimplantation loss: } \frac{\text{No. of corpora lutea - No. of implantations}}{\text{No. of corpora lutea}} \times 100$$

$$\text{Postimplantation loss: } \frac{\text{No. of implantions - No. of live fetuses}}{\text{No. of implantations}} \times 100$$

c. Historical control data: Relevant historical control data were provided to allow comparison with concurrent control.

RESULTS

A. Maternal toxicity

1. Clinical observations: Some incidental clinical observations were reported, but no dose- or compound-related clinical signs of toxicity were seen.

2. Mortality: In the control group, 2 animals died. One died on gestation day 19, and the other died on gestation day 20. In the 6.0 mg/kg group, one animal was killed following abortion on gestation day 27, and another one died on gestation day 28. A third female in this group was sacrificed on gestation day 20 because this animal littered on this day. Apparently, this animal was pregnant when it was entered into the study, and it was excluded from analysis. These deaths, which occurred in this study, were considered as incidental deaths and not related to dosing because no deaths were seen in the mid and high dose groups.

3. Body weight: The body weight and body weight gain data are excerpted from the report and presented in Tables 2 & 3, respectively. The mean body weights of the compound-treated animals and the controls were comparable (Table 2). The body weight gain data indicated that at the measuring interval of gestation days 12-18, there was a slight decrease in mean body weight gain in the 150 mg/kg group relative to the controls, but the decrease was not statistically significant (Table 3). During the interval of days 18-24, the mean body weight gain in the 150 mg/kg group was increased, and this increase showed a statistically significant difference ($p<0.05$) from the controls.

Table 2+. Maternal body weights and the standard deviations (kg)

Day of Gestation	0 mg/kg N=15	6 mg/kg N=13	30 mg/kg N=17	150 mg/kg N=13
0	3.5±0.2	3.6±0.2	3.7±0.2	3.6±0.1
6	3.8±0.2	3.8±0.2	4.0±0.1	3.9±0.2
12	3.9±0.2	3.9±0.2	4.1±0.2	4.0±0.2
18	4.1±0.2	4.1±0.2	4.2±0.2	4.1±0.3
24	4.2±0.2	4.2±0.2	4.4±0.2	4.3±0.3
28	4.3±0.2	4.3±0.2	4.5±0.3	4.4±0.3

+: Data excerpted from the report, p. 33 (MRID No. 43664901). The data were calculated from animals with live fetuses at necropsy.

4. Food consumption: The data showed that there was a slight decrease in food consumption in the 150 mg/kg group during the measurement intervals of gestation days 6-12 and 12-18. Although the decrease during the interval of days 12-18 attained a statistical significance, it was not appreciably different from the controls. During the periods prior to initiation and after cessation of the compound treatment, the food consumption of the 150 mg/kg group was consistently greater than that of the controls (Table 4). The food consumptions in the other treatment groups were comparable to the controls throughout the during of the study.

Table 3+. Maternal body weight gain ± S.D.(kg)

Day of Gestation	0 mg/kg N=15	6 mg/kg N=13	30 mg/kg N=17	150 mg/kg N=13
0 - 6	0.3±0.1	0.2±0.1	0.3±0.1	0.3±0.1
6 - 12	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1
12 - 18	0.2±0.1	0.2±0.1	0.1±0.1	0.1±0.1
18 - 24	0.1±0.1	0.1±0.1	0.2±0.1	0.2±0.1*
24 - 28	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1
0 - 28	0.8±0.2	0.7±0.1	0.8±0.2	0.8±0.2
6 - 18	0.3±0.1	0.3±0.1	0.2±0.1	0.2±0.1

+: Data excerpted from the report, p. 35 (MRID No. 43664901).
*: Significantly different from the controls ($p<0.05$)

Table 4+. Group mean food consumption ± S.D. (g/day)

Day of Gestation	0 mg/kg N=15	6 mg/kg N=13	30 mg/kg N=17	150 mg/kg N=13
0 - 6	194.5±17.7	205.3±23.4	211.0±19.4	215.1±30.6
6 - 12	198.2±25.5	202.3±23.1	199.0±30.0	185.9±32.4
12 - 18	202.4±30.9	203.2±27.6	192.0±26.3	169.7±37.9*
18 - 24	180.1±32.8	182.2±33.5	197.5±32.2	196.5±42.2
24 - 28	165.8±21.0	161.8±32.5	173.3±33.6	179.9±28.1
0 - 28	188.7±21.5	193.0±21.0	196.1±22.1	191.1±26.5
6 - 18	200.6±27.2	202.7±21.9	195.5±26.7	177.8±25.5

+: Data excerpted from the report, p. 36 (MRID No. 43664901).
*: Significantly different from the controls ($p<0.05$)

5. Gross pathology: Compound- and dose-related gross findings were not found in any treatment group. However, at cesarean section, incidental findings of enlarged spleen, yellowish kidneys, and/or granulated liver were seen in 4, 4, and 1 animals of 6, 30, and 150 mg/kg groups, respectively.

6. Cesarean section data: The data on cesarean section are excerpted from the report and summarized in Table 5. The number of animals pregnant in the controls and the treated groups are not significantly different. The maternal wastage was not affected by treatment. The total of numbers of corpora lutea and implantation were comparable between the treated and the control groups. The numbers of live or dead fetuses did not show a dose-related effect. There was a slight increase in the total number of resorptions in the treated groups. However, the resorptions consisted mainly of early resorptions, and this increase did not showed a dose-related response. The fetal weights were comparable among the control and the treated groups. The preimplantation loss was comparable between the treated and the control animals. There was an increase in the post implantation loss in all treatment groups relative to the controls, but this increase did not show a statistical significance or a dose-related effect.

B. Developmental Toxicity

1. External examination: External malformations were seen in the control and treated animals, but the incidences were low. These findings were not considered to be compound- or dose-related effects. No external variations were seen.

2. Visceral examinations: The malformations seen at visceral examination were comparable between the treated and the control animals (Table 6b). In addition, one visceral variation, dilatation of lateral ventricles of the brain, was observed in 0, 1, 4, and 1 fetuses in the control through high-dose groups, respectively.

3. Skeletal examinations

a. Malformation: The incidence of skeletal malformations in the control and the treated animals did not show a significant difference, as indicated in Table 6c.

b. Variations: The skeletal variation findings did not indicated a treatment-related effect in the test groups relative to the controls (Table 6c).

Table 5+: Cesarean section observations.

Observations	Dose (mg/kg/day)			
	0 (Control)	6	30	150
# Animals assigned (mated)	18	18*	18	18**
# Animals pregnant	17	16	18	14
Pregnancy rate (%)	94.4	94.1	100	82.4
# Animals nonpregnant	1	1	0	3
Maternal wastage				
# Dead	2	1	0	0
# Died pregnant	2	1	0	0
# Died nonpregnant	0	0	0	0
# Aborted	0	1	0	0
# Premature delivery	0	0	0	0
Total # corpora lutea	165	156	199	151
Corpora lutea/dam	11.0±2.4	12.0±2.8	11.7±1.5	11.6±3.1
Total # of implantations***	118	91	154	116
Implantation/dam	7.9±2.3	6.5±3.0	8.6±2.3	8.3±3.4
Total # litters	15	13	17	13
Total # live fetuses	115	81	122	100
Live fetuses/dam	7.7±2.1	6.2±3.1	7.2±2.5	7.7±2.8
Total # dead fetuses	0	0	8	2
Dead fetuses/dam			0.5±1.5	0.2±0.4
Total # resorptions	3	9a	20	13
Early	3	9	19	11
Late	0	0	1	2
Resorption/dam				
Early	0.2±0.4	0.6±0.6	1.1±1.7	0.8±1.4
Late			0.1±0.2	0.2±0.6
Litters with total intra-uterine death	0	1	1	1
Mean fetal weight (g)	40.1±4.3	39.2±5.3	39.7±4.6	39.4±5.8
Males	40.7±4.2	39.6±5.9	39.3±5.8	39.2±5.6
Females	38.9±4.5	38.1±4.4	38.2±3.6	40.2±6.4
Sex Ratio (% male)	48.7	57.5	49.2	60.6
Preimplantation loss (%)	26.9±21.1	39.1±28.4	23.8±18.2	21.9±20.5
Postimplantation loss (%)	2.2±4.7	12.9±20.7	18.0±24.2	13.2±15.8

+: Data excerpted from the report pp. 32, and 38-41 (MRID No. 43664901).
*: One animal of this group littered, prior to cesarean section, and discarded from study.
**: One of these animals was not included in this evaluation due to pneumonia.
***: Values calculated from animals with live fetuses at necropsy and total intra-uterine deaths.
a: This number is different from that reported in Table 7 of the report (p.40), and it is derived from the individual animal data by this reviewer.

Table 6a+. External Malformations

Observations	Dose (mg/kg)			
	0 (control)	6	30	150
#Fetuses (litters) examined	115 (15)	81 (13)	122 (17)	100 (13)
#Fetuses (litters) affected	0 (0)	2 (2)	2 (2)	3 (3)
Acrania	0	1*	0	0
Cranioschisis occulta	0	0	0	1
Gnathoschisis	0	0	1	0
Macromelia	0	1	0	0
Tail reduced/rudimentary	0	0	1	0
Omphalocoele	0	1*	0	1
Arthrogryposis	0	0	0	1

+: Data excerpted from the report (p. 42 & 43) (MRID No. 43664901).
*: The same fetus had acrania and omphalocoele.

Table 6b+. Visceral Malformations

Observations	Dose (mg/kg)			
	0 (Control)	6	30	150
#Fetuses (litters) examined	115 (15)	80 (13)	122 (17)	99 (13)
#Fetuses (litters) affected	1 (1)	0	1 (1)	1 (1)
Hemorrhagic eye	0	0	0	1 (1)
Retinal dysplasia	1 (1)	0	0	0
Gnathoschisis	0	0	1 (1)	0

+: Data excerpted from the report (p. 42 & 43) (MRID No. 43664901).
Value in the parenthesis represents # of litters.

Table 6c+. Skeletal examinations

Observations	Dose (mg/kg)			
	0 (Control)	6	30	150
#Fetuses (litters) examined	115 (15)	80 (13)	122 (17)	99 (13)
#Fetuses (litters) with skeletal malformations	2 (2)	1 (1)	4 (4)	0
#Fetuses (litters) with skeletal variations	114 (15)	80 (13)	122 (17)	99 (13)
Examples of skeletal malformation				
Scoliosis	2 (2)	0	3 (3)	0
Ribs proximally fused	0	0	1 (1)	0
Examples of skeletal variations				
Interparietal incompletely ossified	1 (1)	1 (1)	1 (1)	2 (2)
Extra thorac-lumber rib(s)	76 (14)	67 (13)	103 (17)	85 (13)

+: Data excerpted from the report (p. 43-47 and 76-95) (MRID No. 43664901).
Value in the parenthesis represents # of litters.

DISCUSSION

Groups of mated New Zealand White rabbits (18 females/group) received triforine (98.1% pure) by gavage on gestation days 6 through 18 at doses of 6, 30, and 150 mg/kg/day. A control group (18 mated females) was also included in the study. On gestation day 28, all test animals underwent cesarean section and were examined. The fetuses were removed and examined for developmental effects.

The results indicated that triforine at doses tested in this study did not produce any treatment-related maternal or developmental effects. The test animals could have tolerated higher doses. Although there was a slight decrease in food consumption in 150 mg/kg animals, the decrease not appreciably different from the controls. The body weights or any other parameters were not affected. A slight decrease in food consumption in a rabbit developmental study, without supporting evidence of toxicity, such as significant effects on body weight gain, could not be considered a treatment-related effect. There was information concerning the rationale for the dosages employed in this study; however, the rabbits used in the study, upon which the dose selection was based, were Himalayan rabbits. It is apparent that Himalayan and New Zealand White rabbits responded differently to the treatment of this chemical. However, there was a prelimary developmentat toxicity study in New Zealand rabbits (MRID 43664902). This study was

conducted between July 3, 1991 and Sept. 4, 1991 and employed the dose levels of 250, 500, and 1000 mg/kg/day. The highest dose did not produced a significant maternal or developmental toxicity, and the study author suggested that a high dose of 1000 mg/kg/day was suitable for the definitive developmental toxicity study. The results of this plreliminary study clearly indicated that the test animals in the 1988 study could have tolerated much higher doses.

Therefore, this study is classified as **supplementary** because the highest dose tested (150 mg/kg/day) did not produce any maternal or developmental toxicity.