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

JAN 31 1995

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Triforine: Evaluation of supplemental data of a 2-generation reproduction study in rats

Caswell No. 890AA DP Code: D206297
EPA Chemical No. 107901 MRID No. 433233-01
Submission No. S47137[illegible]

TO: R. Kendall/V. D[illegible]h, PM Tea 51
Special Review and re-registration Division (7508W)

FROM: Whang Phang, Ph.D. Whang Phang 1/26/95
Pharmacologist
Tox. Branch II/HED (7509C)

THROUGH: James Rowe, Ph.D. James N. Rowe 1/26/95
Section Head
and
Marcia van Gemert, Ph.D.
Branch Chief
Tox. Branch II/HED (7509C)

Introduction

In 1993, Biologic Inc. submitted a 2-generation reproduction study in rats with triforine[1]. This study was evaluated, and the NOEL and LOEL for systemic toxicity were tentatively set at 500 and 3000 ppm, respectively. The NOEL and LOEL for the reproductive toxicity were not established. The study was classified as supplementary (Tox. Doc. No. 010779) because the report did not contained the following pieces of data:

1. Individual adult body weights for the periods of premating, gestation, and lactation
2. Individual food consumption
3. Individual summary food efficiency
4. Individual pup weight

[1] McCay, C. and Hazelden, K.P. (1990) Triforine two generation study in rats. Unpublished study conducted by Inveresk Research International Ltd, Scotland. Study No. IRI 437656. Oct 12, 1990. Submitted to EPA by Biologic Inc.; EPA MRID No. 423718-01.

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3/11-30

5. Individual pup clinical signs

In addition, statistical analysis should be conducted on the data for body weight, weight gain, food efficiency, pup body weight, and reproductive indices.

Evaluation and Discussion

Currently, American Cyanamid Co., submitted a supplemental report to upgrade this study. The supplemental report contained essentially all of the missing data except the individual pup clinical signs some of which could be found in the necropsy section of the report. The summarized data containing statistical analysis for food efficiency, body weights, weight gain, pup body weight, and reproductive indices are excerpted from the report and presented in 1, 2, 3, 4, and 5.

The food efficiency data during the premating period for F0 and F1 males and F1 females were comparable between treated and the control animals (Tables 1 & 2). There was a statistically significant decrease in food efficiency in 20,000 ppm F0 females at weeks 1-5 and 6-10, and a significant decrease was also seen in 3,000 ppm F0 females at weeks 6-10 (Table 3).

For F0 male rats, there was a statistically significant decrease in body weight gains in all treated groups during week 1 and in 20,000 ppm group during weeks 0-16 ($p<0.05$). A statistically significant decrease in body weight at week 10 and in food consumption was seen in 20,000 ppm at week 1-2 (Table 3).

In F0 females, a statistically significant decrease in body weight was seen in 3,000 and 20,000 ppm groups at week 10 ($p<0.01$). The body weight gain was decreased in all treatment groups, and the decrease showed a significant difference from the controls at the test interval of weeks 0-10, and the body weight gain was also significantly dropped during gestation days 0-20 in 20,000 ppm group. A decrease in food consumption was seen at weeks 1-2 in 3,000 and 20,000 ppm groups, but this decrease in early treatment period could be attributed to the unpalability of the test diet. A statistically significant decrease in food consumption was seen in 20,000 ppm group at weeks 3-10 ($p<0.01$). Food efficiency was significantly decreased in 3,000 and 20,000 ppm groups at weeks 6-10 (Table 3).

In F1 male and female pups similar patterns of decreases in body weights, body weight gain, and food consumption were seen in 3,000 and 20,000 ppm groups as those seen in the F0 animals (table 4). It is important to note that the body weight decrease in F1 pups appeared to commence at approximately day 14 after birth in 3,000 and 20,000 ppm males and 20,000 ppm females. This effect is probably due to the increased intake of the test compound via both

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milk and food. There was also a decrease in the male fertility index as indicated by the decrease in the number of F1 males siring a litter in the 20,000 ppm group, and a slight decrease was also seen in 3,000 ppm F1 males also (Table 4). The mean testes weight of the 20,000 ppm F1 males was slightly decreased (Tox. Doc. No. 010779; attached). The individual animal data of F1 males which were mated with the F1 females were examined. A marked decrease in testicular weight of these males which did not sire a litter was not evident. In general, the data showed that the 20,000 ppm F1 males which were mated had a slight decrease in testicular weight relative to the controls.

For the F2 male and female pups, the was a statistically significant decrease in pup weights in 20,000 ppm groups at days 14 and 21 (Table 5).

Conclusion

Based on the supplemental data, and the data summarized in the DER for this study (Tox. Doc. No. 010779; Attached), the LOEL for systemic toxicity was 3000 ppm as indicated by the treatment-related decrease in body weights, body weight gains and food efficiency in the parental animals, and the NOEL was 500 ppm. The LOEL for reproductive toxicity was conservatively established at 20,000 ppm as indicated by a slight decrease in testes weight and a noticeable decrease in the fertility index in 20,000 ppm F1 males, and a consistent decrease in pup weights in F1 and F2 generations. The NOEL for reproductive toxicity was 3,000 ppm.

The study is upgraded from supplementary to minimum, and it meets the data requirements for a 2-generation reproduction study (83-4).

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Tolerance Petition Review

Page ___ is not included in this copy.

Pages 4 through 9 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

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

FEB 10 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Triforine. Two-Generation Reproductive Toxicity Data Review.
Tox. Chem. No. 890AA
Shaugnessy No. 107901
Submission No. S422130
MRID Nos. 423718-01
Case No. 816127
DP Barcode No. D180803
Action No. 627

From: Alberto Protzel, Ph.D.
Review Section III
Toxicology Branch II
Health Effects Division (7509C)

2/8/94

To: Ron Kendall
Accelerated Reregistration Branch
Special Review and Registration Division (7508W)

Thru: James N. Rowe, Ph.D., Head
Review Section III
Toxicology Branch II
Health Effects Division (7509C)

2/8/94

and

Marcia van Gemert, Ph.D., Chief
Toxicology Branch II
Health Effects Division (7509C)

2/10/94

ACTION:
Review of the following study on the chemical TRIFORINE submitted by Shell International Chemical Company, London, England:

Triforine Two Generation Study in Rats [MRID 423718-01]

CONCLUSIONS:

In a two-generation reproduction study, Sprague-Dawley rats from Charles River (UK) Ltd., Kent, England (F_0: 28/sex/dose, F_1: 24/sex/dose) were fed triforine in the diet at dosage levels of 0, 500, 3000, or 20,000 ppm (during premating, for

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the diet at dosage levels of 0, 500, 3000, or 20,000 ppm (during premating, for males 0, 38, 226, and 1525 mg/kg/day and for females 0, 48, 288, and 1924 mg/kg/day, respectively). The animals were mated on a one to one ratio with the F_0 parental animals and were given test diets for 10 weeks before they were mated. Selection of parents for the F_1 generation was made on day 21 of lactation and the animals were treated for ca 11 weeks after weaning prior to mating. Compound-related parental toxicity was observed at 3000 and 20,000 ppm, as evidenced by decreased body weight or body weight gain (10 to 22% below controls) during the premating period and increased adjusted organ weights (for liver, kidney, spleen, and/or thyroid) in both sexes and generations. The tentative LOEL for systemic toxicity is 3000 ppm and the tentative NOEL for systemic toxicity is 500 ppm, based on decreased body weight gain and organ weight changes.

Compound-related reproductive toxicity was suggested at 20,000 ppm by decreased pup body weight in both generations and possibly by decreased F_1 corrected testes weights ($p<0.01$) and reduced F_1 male fertility. Body weights were below controls in F_1 pups on day 14 by 14-15% and on day 21 by 17-18%; and in F_2 pups, on day 14 by 13-14% and on day 21 by 19-20%. Because no statistical analysis was done on pup weights and fertility indices, it is not possible to evaluate the quantitative significance of the observed effects. Therefore, the NOEL and the LOEL cannot be determined at this time. This study is classified as Core Supplementary pending submission of individual data and statistical analysis for various parameters.

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FINAL

DATA EVALUATION REPORT

TRIFORINE

Study Type: Reproductive Toxicity

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 Date 9/28/93
Pia Lindström, DPH

Independent Reviewer: Sanjay Diwan Date 9/28/93
Sanjay Diwan, Ph.D.

QA/QC Manager: Sharon Segal Date 9/28/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-68
Clement Number: 191
Project Officer: Caroline Gordon

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See pp 4, 5, + 6

EPA Reviewer: Alberto Protzel, Ph.D.
Review Section III, Toxicology Branch II/HED
Signature: _____
Date: _____

EPA Section Head: James Rowe, Ph.D.
Review Section III, Toxicology Branch II/HED
Signature: _____
Date: _____

DATA EVALUATION REPORT

STUDY TYPE: Reproductive Toxicity (Rat); Guideline Series 83-4

EPA IDENTIFICATION NUMBERS

TOX CHEM. NUMBER: 890AA PC NUMBER: 107901

MRID NUMBER: 423718-01 CAS NUMBER: 26644-46-2

TEST MATERIAL: N,N'-[1,4-piperazinediylbis(2,2,2-trichloro-ethylidene)]-bis-[formamide]

SYNONYMS: Triforine; CME 102; CME74770; W524-XX

SPONSOR: Shell International Chemical Company, London, England

STUDY NUMBER: IRI 417656

TESTING FACILITY: Elphinestone Research Centre, Inveresk Research International Limited, Tranent, Scotland

TITLE OF REPORT: Triforine Two Generation Study in Rats

AUTHORS: C. McCay and K.P. Haselden

REPORT ISSUED: October 12, 1990

STUDY COMPLETION DATE: November 17, 1991 (based on the date of the signature by the study director)

CONCLUSIONS: In a two-generation reproduction study, Sprague-Dawley rats were fed triforine in the diet at dosage levels of 0, 500, 3000, or 20,000 ppm (during premating, for males 0, 38, 226, and 1525 mg/kg/day and for females 0, 48, 288, and 1924 mg/kg/day, respectively). Compound-related parental toxicity was observed at 3000 and 20,000 ppm as evidenced by decreased body weight or body weight gain (10%-22%) during the premating period and increased adjusted organ weights (for liver, kidney, spleen, and/or thyroid) in both sexes and generations. Based on these conclusions, the NOEL and LOEL for systemic toxicity were tentatively set at 500 and 3000 ppm, respectively.

Compound-related reproductive toxicity was suggested at 20,000 ppm by decreased pup body weight in both generations and possibly by decreased F_1 testes weights and reduced fertility. Due to the lack of statistical analyses of these data, the NOEL and LOEL for reproductive toxicity cannot be determined at this time.

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CLASSIFICATION: CORE Supplementary Data. This study does not meet the minimum requirements set forth under Guideline Series 83-4 for a two-generation reproductive toxicity study in rats but may be upgraded if the missing data and analyses (listed in Reviewers' Discussion/Conclusions) are submitted and found acceptable.

A. MATERIALS

Test Compound

Purity:	99.1% ± 0.9% (prior to study commencement) 98.9% ± 1.3% (after study completion)
Description:	Colorless to cream powder or crystals
Batch number:	2764
Dates received:	October 10, 1989 and July 2, 1990
Contaminants:	Chemical structures of impurities reported on pp. 190-191 of the study report
Storage:	At room temperature, protected from light

Vehicle: None used; the test material was administered in the diet.

Test Animals

Species:	Rat
Strain:	Crl:CD (SD) BR
Source:	Charles River (UK) Limited, Kent, England
Age:	5 1/2 weeks at the start of dosing (F_0)
Weight:	68-140 g on arrival

B. STUDY DESIGN

This study was designed to assess the potential of triforine to cause reproductive toxicity when administered continuously in the diet for two successive generations.

Mating

After 11 days of acclimatization followed by 10 weeks of dietary treatment, F_0 females were mated with males from the same group in a ratio of 1:1 until a plug or sperm was detected in a vaginal smear (or for a maximum of 7 nights). If after 7 nights no evidence of mating was observed, females were rested for 2 days and then mated with a successfully mated male of the same dosage group for a maximum of 7 nights.

Following 11 weeks of treatment, F_1 animals were mated in the same fashion as the F_0 animals. Sibling matings were avoided.

Animal Husbandry

Food (Rat and Mouse Breeder Diet No. 3SQC) and tap water were provided ad libitum. Temperature and humidity were maintained at 20° ± 2°C and 55% ± 10%, respectively. There were 15-20 air changes per hour and a 12/12-hour light/dark cycle was maintained.

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Group Arrangement

F_0 animals were distributed using a computer-generated random algorithm. F_1 animals were selected based on the median body weight of each sex per group (i.e., there was no random selection). The groups were as follows:

Test Group	Dietary Level (ppm)	Number Assigned per Group F_0 Males	F_0 Females	F_1 Males	F_1 Females
Control	0	28	28	24	24
Low dose	500	28	28	24	24
Mid dose	3000	28	28	24	24
High dose	20000	28	28	24	24

Dosage Administered

The test material was administered in the diet [Rat and Mouse Breeder Diet No. 3 SQC (Special Diets Services Limited)] for two consecutive generations. Diets were prepared weekly and stored at ambient temperature. The test material was mixed with the diet in a Winkworth change drum mixer. Analyses for stability and homogeneity of the test material in the diet was conducted prior to study initiation. In addition, analyses for concentration and homogeneity were performed on diets from all test groups sampled on weeks 1, 6, 11, 15, 20, 24, 30, and 34 of treatment.

Dosage Rationale

Dosages were selected based upon a one-generation range-finding study (IRI Project No. 437614). The results of this study were not presented.

Observations

Observations for mortality were conducted daily for clinical signs; twice daily for mortality; and weekly for a detailed clinical examination. Body weight data were recorded weekly during premating for all animals and on gestation days (GDs) 0, 7, 14, and 20 and on lactation days 1, 7, 14, and 21 for females. Food consumption data were recorded weekly, with the exception of the mating period for all animals, and on GDs 0-7, 7-14, and 14-20 and lactation days 0-7, 7-14, and 14-21 for females.

The following data were recorded for each litter:

- Number of live and dead pups at birth
- Number of live and dead pups, sex, external abnormalities, and presence of milk in the stomach, on lactation days 1, 4, 7, 14, and 21

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- Collective pup weight/sex on lactation days 1, 4, 7, and 14 and individual pup weight on lactation day 21

Litters were not standardized on day 4 postpartum. Pups dying or killed before lactation day 14 were sexed and examined for external abnormalities and the presence of milk in the stomach. Pups dying or killed after lactation day 14 were sexed and examined externally, then necropsied and examined for visceral abnormalities. Twenty-four male and 24 female F_1 pups were selected as F_1 parental animals. All F_1 pups not selected for the F_1 parental group and all F_2 pups were sacrificed and examined externally. Animals showing abnormalities were subjected to a gross necropsy. All others were discarded following sacrifice.

Parental animals of both generations were sacrificed and necropsied after weaning of their litters. Representative samples of all abnormal tissues and the tissues listed below were preserved in 10% neutral phosphate buffered formalin. The reproductive tract of apparently nonpregnant females was examined at necropsy for signs of pregnancy (method not stated). The reproductive tract from nonfertile F_0 males and females were examined histologically. Tissues marked with an asterisk (*) were also weighed.

- *Liver
- *Kidneys
- *Thyroid
- *Spleen
- Bone marrow
- Ovaries
- *Prostate gland
- Pituitary gland
- Uterus
- Cervix
- Vagina
- *Testes
- *Epididymides
- *Seminal vesicles and coagulating gland
- *Adrenals

Statistical Analysis

The only parameters that were statistically analyzed were the organ weights and terminal body weights.

- Organ weight data-- ANOVA, Analysis of Covariance, F-protected least Significance Difference procedure

Compliance

The following statements were provided:

- A signed Statement of No Data Confidentiality Claim, dated March 13, 1992
- A signed Statement of Compliance with EPA GLPs dated March 13, 1992
- A signed Quality Assurance Statement, dated November 12, 1991

C. RESULTS

Test Material Analysis

Concentration analyses revealed values from 92.3% to 107.2% of target. Stability (after 14 days of storage at ambient temperature) and homogeneity analyses conducted on samples at 100, 1250, and 20,000 ppm, revealed values from 87.0% to 104.5% of target.

Systemic Toxicity

Mortality: No compound-related mortalities were observed in either sex or generation. Incidental deaths included one F_0 male (found dead during week 7) and one F_0 female (found dead on lactation day 18 during week 16) both at the 500-ppm dose level. The cause of death was not determined for either animal. One F_1 female at 20,000 ppm was sacrificed moribund during gestation on week 33 of the study. Prior to sacrifice, this animal was observed to have dystocia, emaciation, and piloerection; six dead pups were found in utero.

Clinical observations: No compound-related clinical signs were observed in either sex or generation. Incidental findings included damaged claws, encrusted eyes and nipples, scabbing, tip of tail absent, weight loss, pale extremities, mild piloerection, hunched, white incisors, subcutaneous mass, and swollen purple ears.

Body weight: Compound-related effects on body weight and/or body weight gain were observed at 3000 and/or 20,000 ppm in both generations and sexes during the first week(s) of premating. Summaries of body weight and weight gain data for selected intervals are presented in Tables 1, 2, and 3. Detailed results are discussed below.

In the F_0 generation, body weights of males and females were comparable to control during the entire treatment period with the exception of week 10 of premating and week 1 of lactation when female body weights were 10% below control at 20,000 ppm. Among males, body weight gains during the first week of dosing were 11% and 18% below control at 3000 and 20,000 ppm, respectively. Among females, body weight gains during premating were 9%, 11%, and 20% below control at 500, 3000, and 20,000 ppm, respectively. During gestation, body weight gains were 9% below control at 20,000 ppm; during lactation, body weight gains were 15%, 85%, and 100% above control at 500, 3000, and 20,000 ppm, respectively.

In the F_1 generation at 20,000 ppm, body weights were 10%-22% below control at 20,000 ppm during premating for both males and females; 10%-12% below control for females during gestation; and 6%-11% below control for females during lactation. Among males at 3000 and 20,000 ppm, body weight gains were 9% and 13%, respectively, below control during the first 2.5 weeks of premating. Among females at 20,000 ppm, body weight gains were 10% below control during premating. During gestation, body weight gains were 6% and 5% below control at 3000 and 20,000 ppm, respectively; during lactation, body weight gains were 9% and 30% below control at 500 and 3000 ppm, respectively, and 22% above control at 20,000 ppm.

Terminal body weights were significantly below control among males in both generations at 20,000 ppm and among F_1 females at 3000 and 20,000 ppm. Terminal body weights are presented with the organ weights in Tables 4 and 5.

Food consumption: Compound-related decreases in food consumption were observed in both generations and sexes at 3000 and/or 20,000 ppm during the first week of treatment as follows:

F_0 males	--	20,000 ppm	--	9% decrease
F_0 females	--	20,000 ppm	--	10% decrease
F_1 males	--	3000 ppm	--	10% decrease
F_1 males	--	20,000 ppm	--	11% decrease
F_1 females	--	3000 ppm	--	10% decrease
F_1 females	--	20,000 ppm	--	12% decrease

These decreases may have been related to problems with palatability of the treated feed and may have been responsible for decreased body weight and body weight gain during the premating period. For treated females during the gestation and lactation periods, food consumption was comparable to control in all groups for both generations.

Compound intake: All values for mean compound intake were calculated by the reviewer using the summary group mean test article intake values (individual values were not submitted).

Generation/Sex	Dietary Level 500 ppm	3000 ppm	20,000 ppm
F_0 Generation	Compound intake[a] (mg/kg/day)		
Premating[b]			
Males (n=7)	38.4	226.4	1524.9
Females (n=5)	48.4	287.6	1924.4
Gestation			
Females (n=3)	41.3	237.0	1729.0
F_1 Generation			
Premating			
Males (n=7)	40.3	278.6	2011.7
Females (n=4)	60.8	359.0	2494.0
Gestation			
Females (n=2)	40.0	230.5	1785.5

[a] Values are averages calculated by the reviewer from data in Appendix 4 of the study report (IRI 437658), pp. 84-93.

[b] Due to the presence of systemic effects in both generations, the more conservative dietary intake levels of the F_0 premating period were selected as representative of overall study dosage levels.

Gross pathology: No compound-related gross findings were observed in either sex or generation.

Organ weights: Compound-related effects on organ weights were observed at 3000 and 20,000 ppm. These changes (increases in covariate-adjusted mean weight) were most consistent across sexes and generations for liver, spleen, thyroid, and kidney. Additionally, decreases in F_1 male adjusted mean testes weights for all treated groups were suggestive of a possible compound effect. Histopathology was not performed on any of these organs. Summaries of organ weight changes are presented in Tables 4 and 5.

In the F_0 generation among males, covariance-adjusted ("adjusted") kidney and liver weights and absolute thyroid weights increased significantly at 3000 and 20,000 ppm. Among females, adjusted kidney, liver, thyroid, and spleen weights increased significantly at 3000 and 20,000 ppm. In addition, at 500 ppm, adjusted thyroid weight increased significantly.

In the F_1 generation among males, adjusted testes weight decreased significantly in all dosage groups. Also in males, adjusted liver and thyroid weights increased significantly at 3000 and 20,000 ppm, while adjusted spleen and kidney weights increased significantly only at 20,000 ppm. In addition, at 500 ppm, adjusted liver weight increased significantly. Among F_1 females, adjusted spleen and liver weights increased significantly at 3000 and 20,000 ppm, while adjusted kidney weights increased significantly at 3000 ppm only.

Histopathology: Histopathological examination was only performed on the reproductive tract of nonpregnant F_0 females and F_0 males failing to induce pregnancy. There were no compound-related findings. Incidental findings consisted of three females at 3000 ppm and one female at 20,000 ppm with acute mild inflammation of the reproductive tract and two males at 0 ppm, two males at 500 ppm, and four males at 3000 ppm with slight chronic changes in prostate and seminal vesicles.

Reproductive Toxicity

Compound-related reproductive effects were observed at 20,000 ppm. Summaries of these effects are presented in Tables 6 and 7. In both generations at 20,000 ppm, pup body weights were below control from day 14 by 15% and 14% and day 21 by 17% and 18% among F_1 males and females, respectively and from day 14 by 14% and 13% and day 21 by 20% and 19% among F_2 males and females, respectively. No compound-related clinical signs or external anomalies were observed among pups.

D. REVIEWERS' DISCUSSION/CONCLUSIONS

Test Material Analyses

Concentration, homogeneity, and stability of the test material in the diet were within ±13% of target.

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Study/Reporting Deficiencies:

The following data were not included in the study report. These data should be provided:

1) individual adult body weight data for the periods of premating, gestation, and lactation
2) individual food consumption
3) individual and summary food efficiency
4) individual pup weight
5) individual pup clinical signs

Statistical analyses were only conducted on organ weights. Analyses of the following data should be provided and the study report should be revised as appropriate:

1) body weight and weight gain
2) food efficiency
3) pup body weight
4) reproductive indices

The animals in this study were too young at the start of dosing. The guidelines recommend that animals be 8 weeks old at the start of dosing; animals in this study were 5 1/2 weeks old.

Group arrangements for the F_1 generation were not carried out randomly as is required, but were based on the median body weight of each sex per group.

Systemic Toxicity

Compound-related systemic toxicity was observed at 3000 and/or 20,000 ppm in both generations and sexes. It was manifested as decreased body weight gain during the premating period, significantly decreased terminal body weights, and significantly increased organ weights (liver, kidney, spleen, and/or thyroid). Based on these results and pending further statistical analyses of the data, the NOEL and LOEL for systemic toxicity were tentatively set at 500 and 3000 ppm, respectively.

Reproductive Toxicity

Compound-related reproductive toxicity was manifested at 20,000 ppm in both generations as an apparent decreased pup body weight. In addition, there was an apparent decrease in F_1 male fertility at the high-dosage level, which may have been related to decreased testes weight. Since no statistical analyses of the data were performed, it is impossible to quantitatively evaluate effects at the low- and mid-dosage levels. Therefore, the reproductive NOEL and LOEL cannot be determined at this time.

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E. CLASSIFICATION: CORE Supplementary Data. This study may be upgraded if the missing data and analyses indicated above are provided.

Systemic toxicity NOEL = 500 ppm
Systemic toxicity LOEL = 3000 ppm (based on decreased body weight and increased organ weights)

Reproductive toxicity NOEL = (cannot be determined at this time)
Reproductive toxicity LOEL = (cannot be determined at this time; may be based on decreased pup body weight)

F. RISK ASSESSMENT: Not applicable

Table 1. Body Weight (g ± S.D.) During the Premating Period for Rats Fed Triforine for Two Successive Generations[a,b]

Week of Treatment	Dietary Level (ppm) 0	500	3000	20,000
F$_0$ Males				
0	201 ± 26	203 ± 30	205 ± 25	198 ± 24
1	267 ± 29	267 ± 30	264 ± 29	252 ± 27
4	389 ± 36	394 ± 31	387 ± 40	366 ± 31
8	479 ± 53	496 ± 41	488 ± 54	459 ± 46
10	514 ± 58	528 ± 41	517 ± 60	486 ± 48
Weight gain 0-1 (% of control)	66[c]	62 (94)	59 (89)	54 (82)
Weight gain 0-10 (% of control)	313	323 (103)	312 (100)	288 (92)
F$_0$ Females				
0	162 ± 17	167 ± 13	161 ± 15	160 ± 13
1	193 ± 23	193 ± 15	184 ± 16	184 ± 14
4	255 ± 22	251 ± 17	243 ± 17	233 ± 16
8	293 ± 24	295 ± 21	281 ± 19	267 ± 20
10	316 ± 27	303 ± 24	292 ± 19	275 ± 24
Weight gain 0-10 (% of control)	148	135 (91)	131 (89)	118 (80)
F$_1$ Males[d]				
3.5	67 ± 12	67 ± 9	59 ± 8	52 ± 9
6	236 ± 23	241 ± 26	214 ± 22	201 ± 20
8	328 ± 23	329 ± 25	308 ± 30	292 ± 26
10	396 ± 29	399 ± 29	389 ± 40	352 ± 34
15	508 ± 43	505 ± 35	486 ± 56	457 ± 48
Weight gain 3.5-6 (% of control)	171	174 (102)	155 (91)	149 (87)
Weight gain 6-15 (% of control)	270	274 (101)	272 (101)	256 (95)
F$_1$ Females				
3.5	64 ± 9	58 ± 11	56 ± 8	51 ± 10
6	183 ± 15	180 ± 13	169 ± 12	160 ± 16
8	231 ± 17	228 ± 15	216 ± 16	201 ± 19
10	284 ± 17	282 ± 16	251 ± 21	232 ± 23
15	307 ± 24	306 ± 23	296 ± 27	269 ± 28
Weight gain 3.5-6 (% of control)	97	86 (99)	79 (91)	78 (90)
Weight gain 6-15 (% of control)	154	162 (104)	159 (102)	149 (96)

[a]Data were extracted from Study No. IRI 437654, Tables 3, 4, 7, and 8, pp. 50-51 and 58-56.

[b]Statistical analyses of these data were not performed.

[c]Standard deviations for weight gain were not provided.

[d]For F$_1$ animals, weeks represent weeks of age not of treatment.

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Table 2. Body Weight (g ± S.D.) During Gestation for Rats Fed Triforine for Two Successive Generations[a,b]

Gestation Day	Dietary Level (ppm) 0	500	3000	20,000
F$_0$ Generation-F$_1$ litters				
0	307 ± 22	305 ± 24	299 ± 19	292 ± 21
7	333 ± 23	331 ± 24	324 ± 20	305 ± 22
14	370 ± 25	370 ± 28	359 ± 23	338 ± 23
20	453 ± 33	458 ± 34	443 ± 28	415 ± 31
Weight gain 0-20[c]	146	153	144	133
(% of control)		(105)	(99)	(91)
F$_1$ Generation-F$_2$ litters				
0	306 ± 23	304 ± 24	299 ± 30	270 ± 38
7	338 ± 27	341 ± 24	326 ± 33	299 ± 28
14	378 ± 31	376 ± 23	361 ± 37	331 ± 27
20	456 ± 40	458 ± 29	440 ± 47	412 ± 32
Weight gain 0-20[c]	150	152	141	142
(% of control)		(101)	(94)	(95)

[a]Data were extracted from Study No. IRI 437656, Tables 4 and 8, pp. 32 and 37.

[b]Statistical analyses of these data were not performed.

[c]Standard deviations for weight gain were not provided.

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Table 3. Body Weight (g ± S.D.) During Lactation for Rats Fed Triforine for Two Successive Generations[a,b]

Lactation Day	Dietary Level (ppm) 0	500	3000	20,000
F$_1$ Generation-F$_1$ litters				
1	330 ± 32	331 ± 28	318 ± 21	298 ± 27
7	363 ± 29	367 ± 23	355 ± 20	331 ± 26
14	369 ± 26	366 ± 28	361 ± 22	336 ± 25
21	343 ± 28	346 ± 25	342 ± 24	324 ± 22
Weight gain 1-21[c]	13	15	24	26
F$_1$ Generation-F$_2$ litters				
1	333 ± 31	338 ± 30	326 ± 38	298 ± 31
7	369 ± 24	370 ± 27	334 ± 35	327 ± 27
14	378 ± 26	381 ± 28	369 ± 35	335 ± 24
21	356 ± 32	356 ± 26	342 ± 36	326 ± 26
Weight gain 1-21	23	21	16	28

[a]Data were extracted from Study No. IRI 437656, Tables 4 and 8, pp. 52 and 57.

[b]Statistical analyses of these data were not performed.

[c]Standard deviations for weight gain were not provided.

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Table 4. Organ Weights (± S.D. or S.E. for adjusted values) in the F_0 Generation for Rats Fed Triforine for Two Successive Generations[a]

Organ	Dietary Level (ppm) 0	500	3000	20,000
Males				
Kidneys				
Absolute (g)	4.27 ± 0.43	4.39 ± 0.47	4.54 ± 0.54	4.43 ± 0.44
Adjusted[b]	4.23 ± 0.06	4.35 ± 0.06	[illegible].51 ± 0.0[illegible]**	4.60 ± 0.06***
Liver				
Absolute	21.32 ± 3.[illegible]	22.73 ± 2.87	23.57 ± 3.71	24.31 ± 3.01
Adjusted	21.02 ± 0.37	21.87 ± 0.38	23.30 ± 0.37***	23.88 ± 0.38***
Spleen				
Absolute	0.93 ± 0.14	0.97 ± 0.19	0.99 ± 0.14	0.96 ± 0.[illegible]
Adjusted	0.93 ± 0.03	0.9[illegible] ± 0.03	0.99 ± 0.0[illegible]	1.00 ± 0.03
Testes				
Absolute	3.74 ± 0.30	3.7[illegible] ± 0.30	3.67 ± 0.32	3.74 ± 0.[illegible]
Adjusted	3.73 ± 0.06	3.76 ± 0.06	3.66 ± 0.06	3.79 ± 0.[illegible]
Thyroid				
Absolute	0.022 ± 0.004	0.023 ± 0.005	0.024 ± 0.004*	0.025 ± 0.005***
Adjusted	0.022 ± 0.001	0.024 ± 0.001	0.024 ± 0.001	0.026 ± 0.001
Body weight				
Terminal	583 ± 67	596 ± 5[illegible]	582 ± 77	543 ± 56*
Females				
Kidneys				
Absolute	[illegible].63 ± 0.22	2.65 ± 0.19	2.75 ± 0.19	2.75 ± 0.25
Adjusted	2.60 ± 0.06	2.66 ± 0.04	2.72 ± 0.04*	2.79 ± 0.04***
Liver				
Absolute	15.85 ± 2.25	15.42 ± 1.72	19.29 ± 3.02	21.89 ± 3.14
Adjusted	15.45 ± 0.44	15.56 ± 0.44	18.88 ± 0.45***	22.46 ± 0.42***
Spleen				
Absolute	0.63 ± 0.08	0.63 ± 0.07	0.70 ± 0.10	0.89 ± 0.18
Adjusted	0.62 ± 0.02	0.63 ± 0.02	0.69 ± 0.02*	0.91 ± 0.02***
Thyroid				
Absolute	0.016 ± 0.003	0.017 ± 0.003	0.018 ± 0.002	0.017 ± 0.003
Adjusted	0.015 ± 0.001	0.017 ± 0.001*	0.0[illegible] ± [illegible].001**	0.018 ± 0.001**
Body weight				
Terminal	311 ± 27	302 ± 19	311 ± 23	293 ± 24

[a]Data were extracted from Study No. (R) 437656, Tables 21-24, pp. 70-73.

[b]Covariate-adjusted mean organ weight

*Significantly different from control (p≤0.05)

**Significantly different from control (p≤0.01)

***Significantly different from control (p≤0.[illegible]

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Table 5. Organ Weights (± S.D. or S.E. for adjusted values) in the F_1 Generation for Rats Fed Triforin for Two Successive Generations[a]

Organ	Dietary Level (ppm) 0	500	5000	20,000
		Males		
Kidneys				
Absolute (g)	4.28 ± 0.53	4.15 ± 0.47	4.11 ± 0.40	4.26 ± 0.52
Adjusted[b]	4.18 ± 0.06	4.09 ± 0.08	4.11 ± 0.08	4.42 ± 0.08*
Liver				
Absolute	20.34 ± 2.84	21.29 ± 2.69	22.10 ± 3.42	24.42 ± 3.33
Adjusted	19.5* ± 0.44	20.62 ± 0.43*	22.11 ± 0.43***	25.66 ± 0.45***
Spleen				
Absolute	0.91 ± 0.14	0.93 ± 0.09	0.93 ± 0.12	1.00 ± 0.15
Adjusted	0.89 ± 0.02	0.91 ± 0.02	0.93 ± 0.02	1.0* ± 0.02***
Testes				
Absolute	3.90 ± 0.32	3.65 ± 0.33	3.68 ± 0.28	3.57 ± 0.22
Adjusted	3.68 ± 0.06	3.64 ± 0.06**	3.68 ± 0.06*	3.60 ± 0.06**
Thyroid				
Absolute	0.025 ± 0.005	0.025 ± 0.005	0.029 ± 0.005	0.028 ± 0.002
Adjusted	0.024 ± 0.001	0.025 ± 0.001	0.029 ± 0.001**	0.029 ± 0.001**
Body weight Terminal	376 ± 53	369 ± 41	** ± 72	326 ± 38**
		Females		
Kidneys				
Absolute	2.77 ± 0.24	2.69 ± 0.18	2.69 ± 0.24	2.73 ± 0.29
Adjusted	2.73 ± 0.04	2.68 ± 0.05	2.71 ± 0.05*	2.79 ± 0.05
Liver				
Absolute	16. ± 2.19	17.26 ± 2.16	19.18 ± 3.68	22.63 ± 2.20
Adjusted	16.14 ± 0.44	16.89 ± 0.44	19.52 ± 0.45***	23.36 ± 0.44***
Spleen				
Absolute	0.68 ± 0.10	0.69 ± 0.09	0.78 ± 0.16	0.91 ± 0.18
Adjusted	0.69 ± 0.02	0.68 ± 0.03	0.79 ± 0.03**	0.94 ± 0.03***
Thyroid				
Absolute	0.019 ± 0.003	0.019 ± 0.003	0.020 ± 0.004	0.022 ± 0.004
Adjusted	0.019 ± 0.001	0.019 ± 0.001	0.021 ± 0.001	0.022 ± 0.001
Body weight Terminal	327 ± 30	321 ± 22	309 ± 3**	303 ± 23**

[a]Data were extracted from Study No. IRI 437656, Tables 25-28, pp. 74-77.

[b]Covariate-adjusted mean organ weights

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

**Significantly different from control ($p \leq 0.01$)

***Significantly different from control ($p \leq 0.001$)

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Table 6. Effects of Dietary Administration of Triforine on F_0 Reproductive Parameters and F_1 Offspring Survival and Body Weight[a,b]

Parameter	Dietary Level (ppm) 0	500	3000	30,000
No. matings (F_0 parents)	28	28	28	28
Fertility index, males (%)[c]	82	96	75	82
Fertility index, females (%)[d]	86	96	82	96
Gestation index (%)[e]	100	96	100	100
Gestation length (days)	21.8	21.9	22.1	22.0
No. females with liveborn pups	24	26	23	27
Total no. live pups[f]				
Day 0	332	393	344	368
Day 4	305	367	294	346
Day 21	292	341 (25)[g]	289	325 (25)[g]
Mean no. live pups/litter				
Day 0	13.8	15.1	15.0	13.6
Day 4	12.7	14.1	12.8	12.8
Day 21	12.2	13.6 (25)[g,h]	12.6	12.5 (26)[g,h]
Live birth index (%)[i]	96	98	99	99
Viability index (%)[j]	92	94	86	94
Lactation index (%)[k]	96	98	98	99
Mean pup body weight, males (g)				
Day 1	6.7 ± 0.7	6.6 ± 0.8	6.7 ± 0.9	6.7 ± 0.7
Day 7	14.7 ± 2.5	14.3 ± 2.2	14.0 ± 2.3	13.6 ± 2.6
Day 14	29.3 ± 5.9	27.4 ± 5.8	26.4 ± 5.7	24.9 ± 6.7
Day 21	47.9 ± 7.6	45.6 ± 7.3	43.8 ± 8.9	39.6 ± 7.6
Mean pup body weight, females (g)				
Day 1	6.3 ± 0.7	6.3 ± 0.8	6.3 ± 0.8	6.2 ± 0.6
Day 7	13.9 ± 2.2	13.5 ± 2.2	13.4 ± 2.0	12.8 ± 2.0
Day 14	27.6 ± 4.1	26.2 ± 3.7	25.7 ± 3.2	23.4 ± 3.3
Day 21	45.7 ± 7.1	43.6 ± 7.2	42.0 ± 5.6	37.4 ± 6.2
Sex ratio (% males, day 0)	..[m]	..[m]	..[m]	..[m]

[a] Data were extracted from Study No. IBT 437636, Tables 11, 13, 14, and 16 and Appendices 5 and 6, pp. 60, 62, 63, 65, and 96-103.
[b] Statistical analyses of these data were not performed.
[c] Male fertility index: No. of siring males expressed as % of no. of paired males
[d] Female fertility index: No. of pregnant females expressed as % of no. of paired females
[e] Gestation index: No. of females delivering a live litter expressed as % of no. of pregnant females
[f] Calculated by the reviewer; not analyzed
[g] Number within parenthesis represents number of litters included in calculation; reduced litters are due to mortality or moribund sacrifice of dams and or pups.
[h] Excludes the litter in which dam died
[i] One pup killed accidentally
[j] Live birth index: Percentage of pups born alive based on no. of total pups born
[k] Viability index: Percentage of pups surviving 4 days based on no. of live pups on day 0
[l] Lactation index: Percentage of pups surviving 21 days based on no. of pups on day 4 postnatal
[m] Cannot be determined due to lack of individual data

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Table 7. Effects of Dietary Administration of Triforine on F_1 Reproductive Parameters and F_2 Offspring Survival and Body Weight[a,b]

Parameter	Dietary Level (ppm) 0	500	3000	20,000
No. matings (F, parents)	24	24	24	24
Fertility index, males (%)[c]	92	88	83	79
Fertility index, females (%)[d]	96	92	92	100
Gestation index (%)[e]	100	100	100	100
Gestation length (days)	22.0	22.0	22.0	22.1
No. females with liveborn pups	23	22	22	24
Total no. live pups[f]				
Day 0	313	304	297	328 (23)[g]
Day 4	271	274	268 (21)[g]	284 (22)[g]
Day 21	259 (22)[g]	270	262 (21)[g]	261 (21)[g]
Mean no. live pups/litter				
Day 0	13.6	13.8	13.5[f]	14.3 (23)[f]
Day 4	11.8[f]	12.5	12.8 (21)[g]	12.9 (22)[g]
Day 21	11.8 (22)[g]	12.3	12.5 (21)[g]	12.4 (21)[g]
Live birth index (%)[h]	97	98	98	99
Viability index (%)[i]	89	92	91	86
Lactation index (%)[j]	94	99	98	97
Mean pup body weight, males (g)				
Day 1	6.8 ± 1.1	6.5 ± 0.6	6.8 ± 0.6	6.6 ± 1.1
Day 7	14.9 ± 3.0	15.1 ± 1.7	14.7 ± 1.9	13.3 ± 4.5
Day 14	28.2 ± 4.6	28.6 ± 3.5	27.5 ± 3.8	24.2 ± 4.9
Day 21	46.5 ± 7.9	46.8 ± 6.9	44.4 ± 7.7	37.1 ± 9.5
Mean pup body weight, females (g)				
Day 1	6.4 ± 1.2	6.2 ± 0.7	6.3 ± 0.8	6.1 ± 1.0
Day 7	13.9 ± 2.9	14.0 ± 1.5	13.9 ± 1.9	12.6 ± 2.2
Day 14	26.8 ± 4.4	26.7 ± 2.9	26.1 ± 3.8	23.3 ± 4.2
Day 21	44.0 ± 7.5	43.2 ± 5.5	41.9 ± 6.7	35.8 ± 8.6
Sex ratio (% males day 0)	..[k]	..[k]	..[k]	..[k]

[a] Data were extracted from Study No. IBI 437656, Tables 12, 17, 18, and 20 and Appendices 9 and 10, pp. 61, 66, 67, 69, and 112-119.
[b] Statistical analyses of these data were not performed.
[c] Male fertility index: No. of siring males expressed as % of no. of paired males
[d] Female fertility index: No. of pregnant females expressed as % of no. of paired females
[e] Gestation index: No. of females delivering a live litter expressed as % of no. of pregnant females
[f] Calculated by the reviewers; not analyzed
[g] Number within parenthesis represents number of litters included in calculation; reduced litters are due to mortality or moribund sacrifice of dams and/or pups.
[h] Live birth index: Percentage of pups born alive based on no. of total pups born
[i] Viability index: Percentage of pups surviving 4 days based on no. of live pups on day 0
[j] Lactation index: Percentage of pups surviving 21 days based on no. of pups on day 4 postcull
[k] Cannot be determined due to lack of individual data