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

JUL 29 1993

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Triforine: Review of a mouse carcinogenicity study

Caswell No.	~~809~~ 890AA	DP Barcode.	D185221
MRID No.	424540-01	CASE No.	816127
EPA ID No.	107901-021137	PC Code:	107901

TO: B. Briscoe/R. Kendall, PM Team 51
Re-registration Divis: (H7508C)

FROM: Whang Phang, Ph.D. 7/27/93
Pharmacologist
Tox. Branch II/HED (H7509C)

THROUGH: James Rowe, Ph.D. James N. Rowe 7/27/93
Section Head
and
Marcia van Gemert, Ph.D. 7/28/93
Branch Chief
Tox. Branch II/HED (H7509C)

The registrant, Biologic, Inc., submitted a mouse carcinogenicity study. This study has been reviewed, and the DER is attached. The citation and conclusion are presented below:

Heath, J., Mulhern, M., Perry, C. J., and Henderson, W. (1991) 105-Week dietary carcinogenicity study in mice. Unpublished study conducted by Inveresk Research International, Tranent, EH33 2NE, Scotland. Study No. 7746. Aug 14, 1991. Submitted to EPA by Biologic, Inc.; EPA MRID No. 424540-01.

Groups of Crl:CD-1 (IRC) BR mice received triforine at dietary concentrations of 0, 70, 700, and 7,000 ppm for 105 weeks. The means for the compound intake were calculated to be 0, 11.4, 117, and 1,204 mg/kg bw/day for males and 0, 15.9, 161, and 1,570 mg/kg bw/day for females. Triforine caused an increase in clinical signs in mid and high dose males; the clinical signs included firm/swollen abdomen, hunched/emaciated animals, subdued animals, pale extremities, cyanosis, and body and head tremors. An increased incidence of agitation and subdued animals was seen in mid and high dose females.

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Triforine produced a statistically significant ($p<0.05$) increase in mortality and reduction in mean body weights in mid and high males, and the decrease in body weight was approximately 11% and 16% in mid and high dose males, respectively.

Triforine caused an increase in liver masses in all treated males and lung masses in high dose females. An increased incidence of enlarged colon or rectum was seen in mid and high dose males. A statistically significant increase in absolute liver weights in high dose females was found. Histopathology results for non-neoplastic lesions indicated an increased incidence of thick and enlarged colon or rectum in mid and high dose males.

Triforine produced an increased incidence of hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas in high dose males. The increased incidence of hepatocellular adenomas and combined hepatocellular adenomas and/or carcinomas in high dose males was statistically significant and also showed a significant trend ($p<0.05$).

An increased incidence of lung alveolar/bronchiolar (ALB) adenomas, carcinomas, and combined ALB adenomas and/or carcinomas was observed in high dose females, and the increase was statistically significant ($p<0.01$) and also showed a significant trend for ALB adenomas and combined ALB adenomas and/or carcinomas.

Based upon the results presented in this study, the NOEL for systemic toxicity was 70 ppm, and the LEL was 700 ppm (increase mortality and reduced body weight). The findings of increased mortality, decreased body weight, and increased incidence of non-neoplastic lesions showed that the highest dose tested (7,000 ppm) was sufficient for testing the carcinogenic potential of triforine. In addition, 7,000 ppm is the limit dose for a mouse carcinogenicity study.

This study meets the requirements for a carcinogenicity study in mice (Guideline No. 83-2) and is classified as minimum.

The results of this study and other relevant toxicological data will be presented to the HED Carcinogenicity Peer Review Committee which will determine the carcinogenic potential of this chemical to humans.

cc
Dr. Esther Rinde
Manager of Carcinogenicity Peer Review Process
SACB/HED (7509C)

Reviewer: Whang Phang, Ph.D. Whang Phang 7/22/93
Section III/Tox. Branch II (H7509C)

Secondary Reviewer: James Rowe, Ph.D. James N. Rowe 7/27/93
Section Head
Section III/Tox. Branch II (H7509C)

DATA EVALUATION REPORT

Study Type: Carcinogenicity study in mice

Chemical: Triforine; N,N'-1,4-piperazinedidylbis(2,2,2-trichloroethylidene)-bis-(formamide)

Caswell No.	889	DP Barcode.	D185221
MRID No.	424540-01	CASE No.	816127
EPA ID No.	107901-021137	PC Code:	107901

Sponsor: Shell International Chemical Co.
Shell Centre
York Rd., London

Testing Facility: Inveresk Research International
Tranent, EH33 2NE
Scotland

Citation: Heath, J., Mulhern, M., Perry, C. J., and Henderson, W. (1991) 105-Week dietary carcinogenicity study in mice. Unpublished study conducted by Inveresk Research International, Tranent, EH33 2NE, Scotland. Study No. 7746. Aug 14, 1991. Submitted to EPA by Biologic, Inc.; EPA MRID No. 424540-01.

Conclusion: Groups of Crl:CD-1 (IRC) BR mice received triforine at dietary concentrations of 0, 70, 700, and 7,000 ppm for 105 weeks. The means for the compound intake were calculated to be 0, 11.4, 117, and 1,204 mg/kg bw/day for males and 0, 15.9, 161, and 1,570 mg/kg bw/day for females. Triforine caused an increase in clinical signs in mid and high dose males; the clinical signs included firm/swollen abdomen, hunched/emaciated animals, subdued animals, pale extremities, cyanosis, and body and head tremors. An increased incidence of agitation and subdued animals was seen in mid and high dose females.

Triforine produced a statistically significant ($p<0.05$) increase in mortality and reduction in mean body weights in mid and high males, and the decrease in body weight was approximately 11% and 16% in mid and high dose males, respectively.

Triforine caused an increase in liver masses in all treated males and lung masses in high dose females. An increased incidence of enlarged colon or rectum was seen in mid and high dose males. A statistically significant increase in absolute liver weights in

high dose females was found. Histopathology results for non-neoplastic lesions indicated an increased incidence of thick and enlarged colon or rectum in mid and high dose males.

Triforine produced an increased incidence of hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas in high dose males. The increased incidence of hepatocellular adenomas and combined hepatocellular adenomas and/or carcinomas in high dose males was statistically significant and also showed a significant trend ($p<0.05$).

An increased incidence of lung alveolar/bronchiolar (ALB) adenomas, carcinomas, and combined ALB adenomas and/or carcinomas was observed in high dose females, and the increase was statistically significant ($p<0.01$) and also showed a significant trend for ALB adenomas and combined ALB adenomas and/or carcinomas.

Based upon the results presented in this study, the NOEL for systemic toxicity was 70 ppm, and the LEL was 700 ppm (increase mortality and reduced body weight). The findings of increased mortality, decreased body weight, and increased incidence of non-neoplastic lesions showed that the highest dose tested (7,000 ppm) was sufficient for testing the carcinogenic potential of triforine. In addition, 7,000 ppm is the limit dose for a mouse carcinogenicity study.

This study meets the requirements for a carcinogenicity study in mice (Guideline No. 83-2) and is classified as minimum.

Methods and Materials

Test Article: Triforine with a purity of 98.9% and a batch No. of 2764. The compound was described as colorless to cream powder or crystals with a weak musty odor.

Test Animals: Four week old Crl:CD-1 (IRC) BR mice weighing 21 ± 2 gm for males and 18 ± 3 gm for females were obtained from Charles River (UK) Ltd., Margate, Kent, England. The mice were acclimatized to the environment of the testing laboratory for 2 weeks prior to the initiation of the study.

Study Design: According to the report, 200 male and 200 female mice were selected for the study. However, the basis for selecting the test animals was not clearly stated except that "any animal that failed to performed adequately during acclimation period was replaced with a spare animal from the same batch". The selected animals were randomly assigned to different treatment groups. The number of animals in each dose group and the dose levels were presented below:

Group	Dose Levels ppm	Number of Mice Males	Females
1 (Control)	0	50	50
2 (Low)	70	50	50
3 (Mid)	700	50	50
4 (High)	7000	50	50

The report mentioned that the dose levels of this study were selected based upon the results of a previous 13-week study in mice (Project No. 437478). There was a slight disturbance in red blood cell parameters and increased spleen and liver weight at 7000 ppm.

Administration: The test diet was prepared every two weeks by mixing an appropriate amount of the test chemical with the Mouse (Modified) No. 1 Diet SQC Expanded (Fine Ground) to obtain the desired concentrations. Samples of test diet were taken for determining the concentration and homogeneity. The report stated that prior to the initiation of the study the test diet was analyzed and found to be stable for 3 weeks.

Clinical observation: The test animals were observed at least once daily for viability and any clinical signs a d received a detailed clinical examination and palpitation weekly.

Body weights: The body weights of each mouse were determined during pretest and weekly during for the first 13 weeks of the study. Subsequently, the test animals were weighed every 4 weeks.

Food consumption: Food consumptions were measured at pretest and weekly for the first 13 weeks of the study. Subsequently, the food consumption was measured one week in every 4 weeks.

Clinical pathology:

Hematology: Blood samples were collected from the controls and high dose animals at 52, 78, and 104 weeks. Blood smears were prepared from the collected samples, and differential blood counts were conducted.

Gross and Histopathology

All test animals received a postmortem examination. All macroscopic abnormalities were recorded.

Organ weights: Representative tissue samples were collected from each animal and fixed in phosphate-buffered neutral formalin where appropriate. The following organs from 10 mice/sex/dose group were weighed:

brain (with stem)	ovaries
liver	kidneys
testes	spleen

Histological examinations were conducted on the tissue samples collected at the end of the study, moribund sacrifice, or death on test (if possible). The following tissues were collected for microscopic examinations:

adrenals	lymph nodes (mesenteric & cervical)
aorta	uterus
bone (sterrum)	mammary glands
brain	pancreas
eye	pituitary
any gross lesions	stomach
esophagus	jejunum
duodenum	cecum
ileum	rectum
colon	salivary glands
prostate	gall bladder
sciatic nerve	testis with epididymis
ovaries	skin
skeletal muscle	spleen
spinal cord	thyroid/parathyroid
thymus	lungs
heart	liver
kidneys	stomach
urinary bladder	

Statistical analysis: The details of the statistical analysis are excerpted from the report and presented in Appendix A (Page 21).

A quality assurance statement and Good Laboratory Practice statement were signed and included in the report.

A statement of no claim of confidentiality and a statement that this study did not meet the criteria for flagging of studies for potential adverse effects were also submitted in the report.

RESULTS

1. Test diet analysis: The actual concentrations of the test chemical in the diet at different sample preparations varied from the nominal concentrations generally within ±10% of the targeted concentrations except one value was varied -21% of the nominal concentration.

2. Clinical signs: Based upon the data, there was an increase in the incidence of firm/swollen abdomen, hunched/emaciated/thin animals, subdued animals, pale extremities, cyanosis, and body/head tremors in mid-and high dose males relative to those of the controls (see attached Table 1). In females, an increase in the number of the agitated animals was seen in mid

and high dose groups, and a slight increase in subdued animals was also seen in mid and high dose groups.

3. Mortality: An increase in mortality was seen in all groups of treated males and in mid and high dose females relative to the mortality of the controls (Table 1). The increase in mid and high dose males was statistically significant ($p<0.05$).

Table 2*. Mortality Rates in the Control and the Triforine Treated Mice

	Dose level (ppm)			
	0	70	700	7000
Males	14/50	19/50	35/50**	28/50*
Females	27/50	27/50	32/50	30/50

**: $p<0.001$ (Wilcoxon test modified for censored survival times).

*: $P<0.05$ (Wilcoxon test modified for censored survival time).

+: Data excerpted from the report (MRID No. 424540-01).

4. Body weights: The group mean body weight data indicated that there was a reduction in mean body weight in mid and high dose males, and the decrease was statistically significant in both mid and high dose groups from approximately week 5 to the end of the study (see attached Table 3). The decrease in body weight was slightly less in the mid dose group toward the end of the study in comparison to earlier parts of the study. The body weight gain from the initiation of treatment to the end of the study was decreased in mid and high dose males relative to that of the controls, and the decrease was approximately 11% and 16% in mid and high dose groups, respectively, relative to that of the controls (Table 3). The mean body weight and body weight gain of the low dose males were comparable to those of the controls.

In females, there was a slight decrease in group mean body weight values in low and high dose groups at different measuring times relative to those of the controls; however, the decrease did not show a statistical significance. Accordingly, a decrease in body weight gain was seen in the low dose (10%) and high group (9%) females (Table 3). The values for the mean body weight and the body weight gain for the mid dose females were comparable to those of the controls. The lack of statistical significance in the slight reduction in low and high dose males might not be a treatment related-effect. The author of the report attributed this reduction in low dose group females to a reflection of the mortality patterns during the last few weeks of the study wh[illegible] most of

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the decrease was seen (Table 3).

5. Food consumption: The food consumption data did not indicate any obvious difference between the controls and the treated animals of any dose groups of either male or female mice (see attached Table 4).

6. Compound intake: Based upon the food consumption values, the mean compound intake from week 0 to the termination of the study was calculated as follows:

	Mean Compound Intake (mg/kg bw/day		
	Low Dose	Mid Dose	High Dose
Males	11.4 ± 2.7	117 ± 27	1204 ± 304
Females	15.9 ± 4.3	161 ± 43	1570 ± 419

7. Hematology: The differential blood count data showed comparable values between the high dose and the control mice.

8. Gross examinations: The gross pathology data of animals which died prior to final sacrifice and of the terminal sacrifice data indicated an increase in the total incidence of liver masses in all dose groups of male rats relative to that of the controls (see attached Table 5). The increase in the incidence of liver focal discoloration, raised foci, enlargement, was seen in mid and high dose males that died on study. An increase in the incidence of liver focal discoloration was also seen in high dose males at terminal sacrifice. In females, the liver gross observations were comparable between the treated and the control animals (Table 5).

There was a substantial increase in the total incidence of lung masses in high dose females, which died prior to final sacrifice and which survived to the end of the study, relative to the controls (Controls, 4/50; high dose, 20/50). An increase in the total incidence of discoloration of the lungs was seen in females of all dose groups prior to the final sacrifice or at final sacrifice relative the controls (Control, 5/50; low dose, 13/50; mid dose, 14/50; high dose, 15/50) (Table 5).

In male mice that died before the end of the study, there was increase in the incidence of enlarged colon or rectum in mid and high dose male mice as compared to that of the controls (For colon: Control, 0/14; low dose, 0/19; mid dose, 3/35; high dose, 4/28. For rectum: Control, 0/14; low dose, 0/19; mid dose, 3/35; high dose, 6/28).

Other slight increases in some gross pathology findings were seen, but they were not compound-related.

9. Organ weights: There was a slight increase in the [illegible] absolute liver weights of high dose males and females, and the increase in high dose females was statistically significant ($p<0.01$) (see attached Table 6). Other organ weights were comparable between the treated and the control animals.

10. Histopathology

Non-neoplastic lesions: An increase in the incidence of thickened and enlarged colon or rectum was seen in mid and high males, and this increase was statistically significant and was seen predominantly in animals which died prior to the final sacrifice. However, a clear dose-related response was not seen in this finding (see attached Table 7).

An increase in the incidence of liver inflammatory and/or necrotic changes was seen in mid dose males. An increased incidence of secondary tumor in the liver was seen in all treated males, and that in the mid dose males showed a statistical significance (see attached Table 8). In females, there was a slight increase in the incidence of increased extramedullary hemopoiesis in all treated groups relative to the controls, but no statistical significance was seen in any dose groups.

In lungs, an increased incidence of increased alveolar macrophages was seen in females of all dose groups relative to that of the controls, and that in low and high dose groups showed a statistical significance. However, this observation did not show a dose-related response (see attached Table 8).

Neoplastic lesions:

Liver: There was an increased incidence of hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas in high dose males (Table 9). The increased incidence of hepatocellular adenomas and combined hepatocellular adenomas and/or carcinomas in high dose males was statistically significant and also showed a significant trend ($p<0.05$). Majority of the incidence of hepatocellular carcinomas in high dose males was found in animals which died prior to terminal sacrifice. Half of the incidence of hepatocellular adenomas in high dose males was found in animals which died prior to the end of the study while the other half was seen at terminal sacrifice (see attached Table 10). It should be noted that the values for the tumor incidence summarized in Tables 9 and 10 appeared to be different because the incidence of metastatic liver carcinomas was not included in Table 10 which was presented in the DER solely to show that the tumor incidence was not all found at the end of the study.

An increase in the incidence of liver tumors was not seen in the treated female mice (Table 9).

Lung: An increased incidence of lung alveolar/ bronchiolar (ALB) adenomas, carcinomas, and combined ALB adenomas and/or carcinomas was observed in high dose females, and the increase was statistically significant ($p<0.01$) and also showed a significant trend for ALB adenomas and combined ALB adenomas and/or carcinomas (Table 9). A slight increase in the incidence of combined lung ALB adenomas and/or carcinomas was also seen in high dose males, but this increase did not show a statistical significance. Like the incidence of liver tumors, half the incidence of lung ALB adenomas was seen in the animals which died during the study, and the other half was found at the terminal sacrifice. The majority of the incidence of lung ALB carcinomas was seen in animals which died prior to the terminal sacrifice (Table 10).

Discussion

Groups of Crl:CD-1 (ICR) BR mice received triforine at dietary concentrations of 0, 70, 700, and 7,000 ppm for 105 weeks. The means for the compound intake were calculated to be 0, 11.4, 117, and 1,204 mg/kg bw/day for males and 0, 15.9, 161, and 1,570 mg/kg bw/day for females. Triforine caused an increase in clinical signs in mid and high dose males; the clinical signs included firm/swollen abdomen, hunched/emaciated animals, subdued animals, pale extremities, cyanosis, and body and head tremors. An increased incidence of agitation and subdued animals was seen in mid and high dose females.

Triforine produced a statistically significant ($p<0.05$) increase in mortality and reduction in mean body weights in mid and high males, and the decrease in body weight was approximately 11% and 16% in mid and high dose males, respectively. However, triforine did not affect food consumption or differential blood counts in either male or female mice.

The necropsy results showed that triforine produced an increase in liver masses in all treated males and lung masses in high dose females. An increased incidence of enlarged colon or rectum was seen in mid and high dose males. A statistically significant increase in absolute liver weights in high dose females was found.

Histopathology results for non-neoplastic lesions indicated that triforine caused an increased incidence of thickened and enlarged colon or rectum in mid and high dose males. An increased incidence in liver inflammatory and/or necrotic changes was seen in mid dose males. A statistically significant increase in the

incidence of alveolar macrophages was seen in low and high dose females, but it did not show a dose-related response.

The results of the neoplastic lesions showed that triforine produced an increased incidence of hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas in high dose males The increased incidence of hepatocellular adenomas and combined hepatocellular adenomas and/or carcinomas in high dose males was statistically significant and also showed a significant trend ($p<0.05$).

In analyzing the significance of the tumor incidence with respect to the historical control data, this reviewer believes that it is more appropriate to compare the current finding to the mean of the historical control data than to compare to the range of the historical control data. Following this line of thinking, when the increase in the incidence of hepatocellular adenomas in high dose males (38%) was compared to the range of the historical control data (20%-32%) (Table 11), the increase was only 6% above the upper range (32%), whereas in comparing to the mean of the historical control data (≈19%), the increase is 19% above the mean. The historical control data were taken from 5 studies which were conducted "recently", but the author did not specify what time frame these studies were conducted relative to the mouse carcinogenicity study. In addition, the title of the historical control data presented a bit of confusion because the first line of the title said "105 Week Dietary Carcinogenicity Study in Rats", and followed by "Incidence of Neoplastic and Focal Hyperplastic lesions in Control CD-1 Mice in the 104 Week Carcinogenicity Studies". Do these data pertain to the rats or mice? However, the results of this study unambiguously showed that triforine at 7000 ppm caused an increased incidence of liver and lung tumors in male and female mice, respectively, and the historical control data did not substantially add or diminish the significance of these findings.

An increased incidence of lung alveolar/ bronchiolar (ALB) adenomas, carcinomas, and combined ALB adenomas and/or carcinomas was observed in high dose females, and the increase was statistically significant ($p<0.01$) and also showed a significant trend for ALB adenomas and combined ALB adenomas and/or carcinomas (Table 9). A slight increase in the incidence of combined lung ALB adenomas and/or carcinomas was also seen in high dose males, but this increase did not show a statistical significance.

In comparison to either the historical control means (12.6%) or range (4%-18%), the increased incidence of ALB adenomas in the high dose females (45%) was greater. In this comparison, the increased incidence of ALB carcinomas in high dose males (14%) was also greater than either the historical control data mean (8.6%) or range (4%-12%).

Based upon the results presented in this study, the NOEL for systemic toxicity was 70 ppm, and the LEL was 700 ppm (increase mortality and reduced body weight). The findings of increased mortality, decreased body weight, and increased incidence of non-neoplastic lesions showed that the highest dose tested (7,000 ppm) was sufficient for testing the carcinogenic potential of triforine. In addition, 7,000 ppm is the recommended limit dose for a mouse carcinogenicity study.

Table 9*. Incidence of Neoplasms in Triforine Treated Mice

	Dietary Concentrations (ppm)							
	Males				Females			
	0	70	700	7000	0	70	700	7000
Liver								
Hepatocellular adenomas (HA)	9/50* (18)	14/50 (28)	9/50 (18)	19/50* (38)	3/49 (6)	2/50 (4)	1/50 (2)	2/50 (4)
Hepatocellular carcinomas (HC)	5/50 (10)	7/50 (14)	8/50 (16)	10/50 (20)	1/49 (2)	0/50	0/50	0/50
Combined HA and/or HC	14/50* (28)	19/50 (38)	16/50 (32)	25/50* (50)	4/49 (8)	2/50 (4)	1/50 (2)	2/50 (4)
Lung								
Alveolar/bronchiolar (ALB) adenomas	17/50 (36)	13/50 (26)	8/50 (16)	18/50 (36)	5/50** (10)	7/50 (14)	7/50 (14)	22/49** (45)
ALB carcinomas	5/50* (10)	2/50 (2)	4/50 (8)	7/50 (14)	1/50** (2)	2/50 (4)	1/50 (2)	6/49 (12)
Combined ALB adenomas and/or carcinomas	20/50* (40)	14/50 (28)	12/50 (24)	24/50 (48)	6/50** (12)	8/50 (16)	8/50 (16)	27/49** (55)

+: The values of this table were derived from the individual animal histopathology data, and the statistical analysis did not include survival analysis.

Note: Significance of trend denoted at control (Cochran-Armitage trend test), and significance of trend denoted at dose level (Fisher's Exact Test).

*: P<0.05

**: P<0.01

(): percentage

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