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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of a Chronic Toxicity/Oncogenicity Study with Triforine Technical

TO: Ms. Barbara Brisbane/Emily Mitchell PM-51
RD (7505C)

FROM: David S. Liem, Ph.D.
Section II, Toxicology Branch II/HED (7509C)

THROUGH: K. Clark Swentzel, Section Head 10/28/93
Section II, Toxicology Branch II/HED (7509C)
and
Marcia van Gemert, Ph.D., Branch Chief 11/1/93
Toxicology Branch II/HED (7509C)

MRID#: 4241120-01 to 4241120-05 Submission#: S423122

DP Barcode#: D181370 PC#: 107901 Caswell#:890A

ACTION REQUESTED

To review a chronic toxicity/oncogenicity study in rats with Triforine technical submitted by the Shell International Company

CONCLUSIONS: Administration of Triforine technical in male and female Sprague Dawley rats via the diet at 0, 200, 2000, and 20000 ppm for a period of 104 weeks, produced the following effects:

- Decreased body weight gain in the 2000 ppm males and 20000 ppm males and females. Mean absolute body weights of these animals were lower than their respective controls ($P<0.05$ or 0.01) during the first year of the study.

- Decreased hemoglobin concentration in the 2000 ppm males and 20000 ppm males and females; decreased red blood cells in the 20000 ppm females; and decreased mean cell hemoglobin volume in the 2000 ppm females, and in the 20000 ppm males and females.

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- o Increased mean cell volume in the 20000 ppm females

- o Increased adrenal weight in the 200 ppm males and in the 20000 ppm females; increased kidney weight in the 200, 2000, and 20000 ppm females; increased liver weight in the 200 and 2000 ppm females and in the 20000 ppm males and females; increased spleen weight in the 2000 and 20000 ppm females; and increased thymus weight in the 20000 ppm males

- o Increased hemosiderin deposit in the spleen of the 200 ppm males, and in the 2000 and 20000 ppm males and females.

- o Increased bile duct hyperplasia in the 20000 ppm females; increased pale cell foci in the 20000 ppm males; increased Kupffer-cell and macrophage pigmentation in the 2000 ppm males and in the 20000 ppm males and females.

- o Increased lung focal alveolitis in the 20000 ppm males and females.

Although changes were observed in the 200 ppm animals (increased liver and kidney weights and increased hemosiderin deposit in the male spleen), they were not corroborated by other changes in these organs at this dose, the increased kidney weight was transient, and none of these changes were seen in both sexes. Therefore, the biological significance of these observations is doubtful.

Based on the results of the study, the systemic NOEL is 200 ppm. The LOEL is 2000 ppm based on decreased body weight and hemoglobin in males, and increased adrenal, kidney, liver and spleen weights in females as well as increased hemosiderin deposit in the spleen of males and females. .

No treatment-related neoplastic lesions were evident in the study. Thus Triforine Technical is judged to be not carcinogenic when administered up to 20000 ppm in the diet of Sprague-Dawley rats for 104 weeks.

The doses employed in this study were sufficient to produce a compound-related systemic effect and appear to be adequate to test the carcinogenic potential of the test material. Also, 20000 ppm is considered a limit dose for a chronic toxicity/ carcinogenicity study

Classification: Core-Minimum. This study satisfies USEPA's guideline 83-5 requirements for a combined chronic toxicity/ carcinogenicity study in rats.

cc: Whang Phang (TexIT, HED)

Primary Reviewer: David S. Liem, Ph.D.
Section II, Toxicology Branch II/HED

Secondary Reviewer: K. Clark Swentzel, Section Head
Section II, Toxicology Branch II/HED

K. Clark Swentzel 1/28

DATA EVALUATION REPORT

Study Type: Chronic Toxicity/Oncogenicity Study Guideline 82-5

Test Animal: Sprague-Dawley Rats

Ident.#: DP Barcode#: D181370 PC#: 107901 Caswell#: 890AA

Submission#: S423122 MRID#: 424120-01 to 05

Test Material: Triforine Technical (98.9% pure); N,N'-[1,4-piperazinediyl bis-(2,2,2-trichloroethylidene)] bis-formamide

Synonym: CME 102, CME 74770, Cela 50, CW 524, Saprol, Denarin, Biformylchlorazin, Funginex, Cela W-524, CA 7302, Compound W

Dosages: 0, 200, 2000, and 20000 ppm

Sponsors: Shell International Chemical Co., York Rd., London

Study Number: Report #7745; Study # : 437504

Study Period: 29 June, 1989 to 17 July, 1991 (In Life Study)

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

Title of Report: Triforine: 104 Week Dietary Carcinogenicity Study in Rats Incorporating 52 Week Toxicity Study

Author: C.J. Perry, M. Mulhern, and J. Finch

Report Issued: July 22, 1991

Conclusions: Based on the results of the study, administration of technical Triforine at 200, 2000, and 20000 ppm in the diet of male and female Sprague-Dawley rats for a period of 104 weeks, did not produce notable differences in the mortality, clinical signs, food and water consumption, clinical chemistry and urinalysis data, or gross macroscopic findings. No treatment-related neoplastic lesions were evident in this study. Treatment-related findings could be summarized as follows:

PARAMETERS	200 ppm		2000 ppm		20000 ppm	
	♂	♀	♂	♀	♂	♀
Body Weight ↓			✓		✓	✓
Hemoglobin ↓			✓		✓	✓
Red Blood Cell ↓						✓
Mean Cell Hemoglobin Volume ↓				✓	✓	✓
Mean Cell Volume ↑						✓
Adrenal Weight ↑				✓		✓
Kidney Weight ↑		✓[1]		✓		✓
Liver Weight ↑		✓[2]		✓	✓	✓
Spleen Weight ↑				✓		✓
Thymus Weight ↑					✓	
Spleen - Hemosiderin deposit ↑	✓[3]		✓	✓	✓	✓
Liver +Bile duct hyperplasia ↑ +Pale cell foci ↑ +Kupffer-cell and macrophage pigmentation ↑				 ✓	 ✓ ✓	✓ ✓
Lung Focal Alveolitis ↑					✓	✓

1 = only occurred during the first year; 2 = only occurred in the second year; 3 = slightly increased throughout the study but not statistically significant.

Although changes were observed in the 200 ppm animals (increased liver and kidney weights in the females and increased hemosiderin deposit in the male spleen), they were not corroborated by other changes in these organs at this dose, the increased kidney weight was transient, and none of these changes were seen in both sexes. Therefore, the biological significance of these observations is doubtful.

Based on the results of the study, the systemic NOEL is 200 ppm. The LOEL is 2000 ppm based on decreased body weight and hemoglobin in males, and increased adrenal, kidney, liver and spleen weights in females as well as increased hemosiderin deposit in the spleen of males and females.

No treatment-related neoplastic lesions were evident in the study. Thus Triforine Technical is judged to be not carcinogenic when administered up to 20000 ppm in the diet of Sprague-Dawley rats for 104 weeks.

The doses employed in this study were sufficient to produce a compound-related systemic effect and appear to be adequate to test the carcinogenic potential of the test material. Also, the 20000 ppm is considered the limit dose for a chronic toxicity/carcinogenicity study.

This study satisfies USEPA's guideline 83-5 requirements for a combined chronic toxicity/carcinogenicity study in rats.

Classification: Core-Minimum

Study Title: Triforine: 104 Week Dietary Carcinogenicity Study in Rats Incorporating 52 Week Toxicity Study (MRID#: 424120-01 to 05)

Author: C.J. Perry, M. Mulhern, and J. Finch

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

Report Issued: July 22, 1991 Study No.: 437504

1. OBJECTIVE

To evaluate the chronic toxicity and carcinogenic potential of Triforine Technical (98.9% pure) when administered in the diet to Sprague-Dawley rats for a period of 104 weeks.

2. MATERIALS AND METHODS

The in-life, necropsy, and histopathologic phases of this study were conducted at the Elphinstone Research Centre of Inveresk Research International Limited (IRI), Tranent, Scotland.

Test Material

- Physical Description: A colorless to cream powder or crystals
- Batch #: Ch. 2764; 98.9% pure
- Source: Shell Agrar GmbH and Co, Ingelheim am Rhein, Germany
- Storage: Room temperature (pure material) in the dark.

Test Animals

- Species: CDBR Sprague-Dawley Rat
- Source: Charles River (UK), Limited, Manston Rd., Margate, Kent, UK
- Total Number Ordered: 313 ♂ and 306 ♀ (BW: ♂ =84-105g and ♀ = 55-56 g on arrival)
- Total Number of Animals in the Study: 280 ♂ and 280 ♀ (BW: ♂ = 216-225 g; ♀ = 142-147 g on day 0 of study)
- Age: Approximately 7 weeks old at start of study
- Caging: 5 ♂ or 5 ♀ per cage in suspended polypropylene cages
- Acclimation period: 20 days

Feed and Water: SDS Rat and Mouse (Modified) No. 1 Diet SQC Expanded (Fine Ground) from Special Diet Services Limited, Stepfield, Witham, Essex, CM8 3 AD and tap water were provided ad libitum. Feed was withheld one day prior to blood collection and prior to a scheduled necropsy.

Environmental Parameter: Air temperature = 20°C ± 2°C; Relative Humidity= 55% ± 10%; 12 hours dark/light cycle; 15 - 20 air changes per day

Experimental Design: Four groups of 70 male and 70 female rats were used for this study. Twenty males and twenty females per group designated for Interim Kill were sacrificed, necropsied and subjected to histopathological examination after the 52 weeks of dosing. Rats in moribund condition were sacrificed, necropsied and subjected to histopathological examination. The survivors were killed, necropsied and subjected to histopathological examination after the completion of 104 weeks of dosing.

Dose Determination: The study report noted that the dose levels of this study were determined on the basis of the results from a 13-week study with Triforine which showed slight disturbances in red blood cell parameters and increased spleen and liver weight in the 20000 ppm dose animals (IRI Project #437499). This 13-week study report was not submitted with the study report.

Group Arrangement: Animals were assigned to the study using a computer-generated randomization as follows:

Dose Group	Dosage (ppm)	# Males	# Females
Control	0	50 + 20*	50 + 20*
Low Dose	20	50 + 20*	50 + 20*
Mid Dose	2000	50 + 20*	50 + 20*
High Dose	20000	50 + 20*	50 + 20*

* = These animals were designated for the 52-week study

Diet Preparation: Triforine was mixed in the basal diet to the appropriate dose level in a Wentworth Change Drum Mixer. The concentration of Triforine in the diet remain constant throughout the duration of the study. Fresh diet were given to animals every two week.

Statistical analysis

Statistical analysis tests and methods used in this study are described in Appendix A.

3. Diet Analyses: A 100 g sample of the diet from each group/sex was collected after each diet preparation. Samples of all diets were analyzed during weeks 1, 3, 7, 12, 26, 37, 51, 64, 78, 90, and 103 to determine the concentration and homogeneity. The stability of triforine was determined at the start of the study.

Analysis of diet samples collected during weeks 1, 3, 7, 12, 26, 37, 51, 64, 78, 90, and 103 were homogeneous and were within the acceptable concentration limits (± 10%). Only once (low-dose male at week 78 interval), was the diet concentration outside the acceptable ± 10% concentration limit (see p. 274-277 and p. 556-557 of the study report). Triforine was stable for 3 weeks.

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4. Clinical Observations: The rats were checked twice daily for mortality, moribundity and signs of toxicity. Detailed clinical examinations were conducted once a week and on the day of scheduled sacrifice.

a. Mortality

A total of 176 rats died or were sacrificed in extremis. The number of mortalities in each group is as follows:

	0 ppm ♂ / ♀	200 ppm ♂ / ♀	2000 ppm ♂ / ♀	20000 ppm ♂ / ♀
Total # on Study	70/70	70/70	70/70	70/70
Total Mortality	25/22	25/20	20/21	20/23

In the first year there were only 3 premature deaths (one low-dose male, one mid-dose male, and one high-dose female). The Wilcoxon test on the mortality pattern for all animals (see table above) in the study did not show any statistically significant intergroup differences. Differences in the number of deaths are not judged to be related to treatment.

b. Clinical Observations

Clinical signs observed are summarized in Appendix B1 and B2. The tables show that there were no clinical signs that could be considered to be related to treatment.

5. Body Weights: Individual body weights were taken during the week before the start of the study, on day 0, weekly until week 13 and once every 4 weeks thereafter. Body weights were also taken at the scheduled sacrifice and at death for moribund sacrificed rats

Mean absolute body weights, body weight gain, and percent mean body weight of control are presented in Appendices C1 (Mean body Weights for the 52-Weeks study) and C2 (Mean body Weight data for the 104-weeks study).

There were no statistically significant differences in the absolute body weights observed in the treated males and females (except on week 32 for mid- and high-dose females) as compared to the controls at 52 weeks sacrifice (Appendix C1).

At 104 weeks (Appendix C2), there was a slight absolute body weight reduction in the mid- and high-dose males as compared to the control. Statistically significant reductions were observed between weeks 1 and 28 ($P<0.05$ - $P<0.001$).

There was a slight body weight gain reductions in the [illegible]-dose males (-7%), and moderate body weight gain reductions in the treated females (-11%, -13%, and -21% for the [illegible], and high-dose, respectively) at the 52 weeks sacrifice.

For weeks 0 - 104, slight body weight gain reduction was noted in both the mid- and high-dose males (both with a -7% reduction). In the females, absolute body weight increases (with scattered statistically significant values) were observed during the study in the low-dose group.

There was a 15% moderate body weight gain increase (weeks 1-104) in the low-dose females as compared to the control. Since the body weight gain increase in the low-dose females was not observed in the mid- and high-dose groups, this is not judged to be related to treatment. Scattered statistically significant ($P < 0.05$-$P < 0.01$) body weight decreases (weeks 3 to 36) were observed in the high-dose females. The body weight gain decrease in these high-dose females was -3% of the control.

Based on the above observations, the depressed absolute body weight and body weight gains in the mid- and high-dose males and in the high-dose females are judged to be related to treatment.

5. Food Consumption: Food consumed per cage was recorded during the week prior to study initiation, on day 0, weekly until week 13 and once every 4 weeks thereafter.

There were no food and water consumption differences between the treated groups as compared to the controls (p. 32 and 429 of the study report).

7. Compound Intake: Compound intakes were calculated from food consumption data.

The mean compound intake for the 52-Weeks and 104-Weeks studies (derived from p.49-50 and 439) are as follows:

STUDY	Compound Intake in Mg/Kg/Day (Mean ± S.D.)					
	Treated Males			Treated Females		
	200ppm	2000ppm	20000ppm	200ppm	2000ppm	20000ppm
52 Week Study	12±3	110±20	1228±305	15±3	158±32	1671±344
104Week Study	10±3	101±28	1038±307	13±3	136±34	1436±376

As seen from the above Table, group mean compound intake for both studies are within the expected concentration ranges.

8. Clinical Pathology Evaluation: A blood smear for blood count differential was prepared using a blood sample taken via a tailnip without anesthesia from all animals conducted during weeks 51, 78 and 102 of the study. Blood samples were collected from the orbital sinus of 10 rats/sex/group under light ether anesthesia from each sex/group during weeks 26 and 51 for the 52-Weeks animals, and at week 102 for the 104-Weeks animals.

a. Hematology

The following hematological parameters (✓ = required for this study) were evaluated:

✓ hemoglobin	✓ leukocyte count (total & differential)
✓ hematocrit	✓ platelet count
✓ erythrocyte count	reticulocyte count
✓ Hematocrit	mean cell volume
Hepato Quick test	mean cell haemoglobin concentration

The hematology measurements were conducted at weeks 26 (males rebled at weeks 26) and 51 for the 52-Weeks animals, and at week 102 for the 104-Weeks animals. Summary of selected hematological values are presented in Appendix D. This Appendix shows that hemoglobin values were slightly reduced in the mid- and high-dose males at week 26 (-4% at P<0.05 and -5% at P<0.01, respectively) and at week 51 intervals (-4% at P<0.05 and -4% at P<0.01, respectively). At week 26 interval, MCHC was also reduced in the high-dose males (-2% at P<0.01). As for the females, there was a slight reduction of hemoglobin (-5% but not significant), red blood cell count (-7% at P<0.01), and MCHC (-2% at P<0.001) in the high-dose group at the week 26 interval. MCV of the mid- and high-dose females were elevated at all intervals, but only the high-dose females (5%, P<0.01) at week 26 was it statistically significant. At week 51, there was an increase of white blood cells (29% at P<0.01) and lymphocyte (22% at P<0.05) in the mid-dose females, but because of an absence of an increase in the high-dose females (no dose-related trend), this increase is not judged to be a dose-related effect. A moderate lymphocyte count reduction was noted and in the low- (-25% at P < 0.01) and mid-dose (-36% at P < 0.001) males, at week 103 interval. The absence of a similar reduction in the high-dose males at week 26 and week 51 intervals, suggests that the lymphocyte value reduction in the low- and mid-dose males was not a treatment-related effect. Hematology testing was not done at the week 78 interval.

No intergroup Differential Blood Count differences in either sex were noted in the study (see p. 51-56 of the study report).

In conclusion, treatment-related effects were observed in the following parameters: hemoglobin depression in the mid- and high-dose males and in the high-dose females; Mean Cell

Hemoglobin Concentration (MCHC) reduction in the mid-dose females and in the high-dose males and females; and the total red blood cell count (RBC) reduction in the high-dose females; and an increased of Mean Cell Volume (MCV) in the high-dose females. All values, except the hemoglobin depression in the high-dose female were statistically significant.

b. Clinical Chemistry

Clinical chemistry tests were conducted at weeks 26, 51, and 102 of the study. At each interval, blood serum samples were collected from 10 rats selected at random. All parameters required by the guideline for this study were examined, as follows: Alkaline phosphatase, blood urea nitrogen (BUN), lactic acid dehydrogenase, alanine aminotransferase, aspartate aminotransferase, glucose, total protein, albumin, globulin, albumin/globulin (A/G) ratio, inorganic phosphate, calcium, sodium, potassium, chloride, creatinine, creatine phosphokinase, total bilirubin and total cholesterol.

The results of the selected clinical chemistry parameters conducted at weeks 26 (week 28 for males) and 51 for the 52-Weeks study and at week 103 for the 104-Weeks study are presented in Appendix E. Appendix E shows great variations in clinical chemistry values among the groups, and compound related patterns were not evident. There was a statistically significant increase in aspartate aminotransferase (AST) level (26%, P<0.01) in the low-dose males at week 28 interval, but since no similar changes were noted the mid- and high-dose groups, it is not considered a treatment-related effect. AST reduction was noted in the mid- and high-dose females at week 26 and in the high-dose males and females at week 103 intervals as compared to the controls. These reductions are not biologically significant, because AST increase is generally associated with a toxic response. Similar statistically significant reductions were noted in the following: alanine aminotransferase (ALT) levels in the mid- and high-dose females at week 26 and in the high-dose males at week 51 intervals; lactate dehydrogenase (LDH) in the high-dose females at week 26; total bilirubin (T.Bi.) in the low-, mid-, and high-dose females at week 103 interval; and creatine phosphokinase in the high-dose females at weeks 28 interval. These reductions are not biologically significant, because the increase of these parameters are generally associated with a toxic response. Statistically significant increases were also noted in the total protein in high-dose males, cholesterol in high-dose females, and the calcium in the mid- and high-dose females, all at the week 103 interval. No clinical chemistry test was done at week 78.

All clinical chemistry changes discussed above are not judged to be related to treatment, because either they are not clinically significant or considered to be chance occurrences rather than reproducible treatment-related effects.

c. Urinalysis

Urine was collected overnight from ten randomly selected rats of each sex/group at weeks 26, 51, and 102 of the study (same rats used for hematologic and clinical chemistry evaluations). Parameters evaluated were the volume, specific gravity, occult blood, protein, pH, bilirubin, ketones, glucose, nitrites, urobilinogen and sediment.

There were no notable intergroup differences in the urinalysis data for both sexes and at all intervals tested (p. 51-62 and p. 448-452 of the study report).

9. Gross Macroscopic Examinations

All rats which died or were sacrificed in extremis and all rats sacrificed at the scheduled sacrifices were subjected to gross macroscopic examination. At twelve month of the study, all surviving rats assigned to the 52-Weeks study were sacrificed and necropsied. Terminal necropsy of all surviving rats was conducted during week 104. Rats sacrificed at scheduled necropsy, were fasted overnight, killed by carbon dioxide asphyxiation followed by exsanguination, and then necropsied. Tissues harvested from all rats were fixed in 10% neutral buffered formalin (except eyes which were preserved in Davidron's fluid). The contracted bladders were distended with fixative and the epithelial surface was examined at trimming. The lungs were inflated prior to fixation. Liver and the kidneys were cut before fixation. All tissues required for this study (✓) were harvested as follows:

✓ Adrenal
✓ Aortic Arch
✓ Urinary Bladder
✓ Bone (Sternum and Rib)
✓ Brain
✓ Eyes
✓ Heart
✓ Intestine: duodenum
jejunum
ileum
caecum
colon
rectum
✓ Kidney
✓ Liver
✓ Lung
✓ Mammary glands
✓ Mesenteric lymph node
✓ Muscle (thigh)
✓ Nasal cavity
✓ Oesophagus
✓ Ovaries
✓ Pancreas
✓ Pituitary
✓ Prostate glands
✓ Sciatic nerve
✓ Seminal Vesical
✓ Skin
✓ Spinal Cord
✓ Spleen
✓ Stomach (grandular & nongrandular)
✓ Submaxillary gland
✓ Testes (plus epididymides)
✓ Thymus
✓ Thyroid and parathyroid
✓ Tongue
✓ Trachea
✓ Uterus
✓ Vagina
✓ Gross lesions

Pertinent gross necropsy findings are tabulated in Appendix G1 for the 52-Weeks rats and in Appendix G2 for the 104-Weeks rats.

a. 52-Weeks Rats

None of the gross necropsy findings for the 52-Weeks rats is judged to be related to treatment (p.463-468 of the study report)

b. 104-Weeks Rats (Appendix F)

As expected, a number of gross macroscopic findings related to degenerative and neoplastic disease were found in the 104-Weeks rats, but there is no evidence of a dose-related trend. Thus, none of the gross macroscopic findings is judged to be related to treatment.

10. Organ Weights

The adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes and epididymides, thymus and uterus from 10 males and 10 females animals chosen at random were weighed at the interim (week 52 for 52-Weeks rats) and terminal (at week 104 for 104-Weeks rats) necropsies. All these organ weights are required by the guideline.

Selected absolute and the computed organ weights after correction for final body weight (covariance analysis shown in shaded rows) are presented in Appendix G.

a. Week 52 Sacrifice of the 52-Weeks Rats

As seen from Appendix G, based on the covariance analysis (values in shaded rows), there was a statistically significant adrenal weight reduction in the low- (-15%, $P<0.01$), mid- (-13%, $P<0.05$), and high-dose (-15%, $P<0.05$) males and a liver weight increase (11%, $P<0.01$) in the high-dose males.

As for the females statistically significant changes included: an increase in adrenal weight in the mid- (35%, $P<0.05$), and high-dose (40%, $P<0.01$) groups; an increase in kidney weight in all treated groups (9%, $P<0.01$: 12%, $P<0.001$; and 17%, $P<0.001$, respectively); and an increase in spleen weight in the mid- and high-dose groups (17%, $P<0.01$ and 41%, $P<0.001$, respectively).

b. Terminal Sacrifice of the 104-Weeks Rats

Appendix G shows, that only the absolute liver weight in the low- and mid-dose males was statistically increased (31%, $P<0.01$ and 23%, $P<0.05$, respectively). After correction for final body weight (analysis of covariance), the thymus weight for the high-dose male was significantly increased (70%, $P<0.01$).

The absolute liver weight increase was statistically significant in low- (31%, P<0.01), mid- (26%, P<0.05), and high-dose (43%, P<0.001) females. The liver weight values after correction for final body weight were also statistically significant in the low- (18%, P<0.05), mid- (24%, P<0.01), high-dose (43%, P<0.001) females. After correction for final body weight, the high-dose female spleen weight was also statistically significantly increased (21%, P<0.05).

In summary, there were treatment-related increases in liver weight in the high-dose males and in all treated females, as well as the spleen weight in the mid- and high-dose females. A thymus weight increase was also noted in the high-dose males in the second year. Treatment-related kidney weight increase in all treated females was evident in the first year but not in the second year. A transient (observed in the first year only) adrenal weight increase was noted in the mid- and high-dose females. The statistically significant adrenal weight decrease in all treated males observed in the first year was not considered to be related to treatment, because a dose related trend was not evident, and the biological significance of this decrease was not corroborated by gross necropsy and histo-pathological findings.

11. Histopathological Evaluation

All fixed tissues (listed on p. 10 of this DER) from all control and high dose groups and from animals that died in extremis, in addition to the kidneys, liver, lungs and spleen from all other dose groups were trimmed, embedded in paraffin, sectioned, stained with hematoxylin-eosin, and were subjected to histo-pathological evaluation. All other tissues harvested were fixed and stored in fixative.

The results of selected non-neoplastic histopathological data for the 52-Weeks rats covering the first year of the study are presented in Appendix H1. There was an increase of hemosiderin deposit (identity confirmed by Prussian Blue stain) in the spleen of all treated males, but only the mid- and high-dose males were statistically significant. Notable findings affecting the liver included an increase of Kupffer cell pigment and pigmented macrophages in the low- and high-dose males as well as in the mid- and high-dose (statistically significant) females, pale cell foci in high-dose (statistically significant) males, and the bile duct hyperplasia in the high-dose females (not statistically significant). Other findings were not judged to be treatment related effects.

The results of selected non-neoplastic histopathological data for the 104-Weeks rats covering the entire duration of the study are presented in Appendix H2. The increase of hemosiderin deposit (identity confirmed by Prussian Blue stain) in the spleen were noted in the mid- and high-dose males and females (except for the high-dose males, all values were statistically significant). Incidence of foci alveolitis of the lungs (characterized by accumulations of alveolar macrophages, thickening of alveolar wall and chronic inflammatory cell infiltrates) was statistically increased in both sexes at the high-dose.

Although there were scattered statistically significant or non-significant changes noted in different tissues at different dose levels (e.g. a decrease of basophilic foci in liver of the high-dose females, an increase of angiectasis in the liver of low-dose males, or an increase of pale cell foci in the liver of mid-dose males), none of them appeared to be treatment-related effects. These findings are not judged to be of toxicological significance.

A summary of the results of selected neoplastic findings is presented in Appendix I. No notable differences and any dose related effects in neoplastic lesion incidence were evident among the groups in both sexes. Triforine does not appear to be carcinogenic when administered up to 20000 ppm in the diet of rats for 104 weeks.

12. Ophthalmologic Examination

Ophthalmologic examination was conducted on all rats by a Veterinary ophthalmologist. No compound related ophthalmologic effects were observed.

13. Compliance Statements

A signed Statement of Confidentiality Claim, of Compliance with EPA GLP's and Quality Assurance Statement were provided.

DISCUSSIONS AND CONCLUSIONS

Based on the results of the study, administration of technical Triforine at 200, 2000, and 20000 ppm in the diet of Sprague-Dawley rats for a period of 104 weeks, did not produce notable differences in the mortality, clinical observations, food and water consumption, clinical chemistry and urinalysis data, or gross macroscopic findings. No treatment-related neoplastic lesions were evident in this study.

Treatment-related systemic toxicity changes are summarized in a table presented on the next page. The summary table shows that the decrease in the red blood cells in the mid- and high-dose groups was corroborated by the decrease in hemoglobin, mean cell

hemoglobin volume as well as the increase of hemosiderin deposit in the spleen and Kupffer-cell pigment and macrophage pigmentation of the liver. The liver and spleen weight increase may be a direct response to increased hemoglobin destruction indicated above. The kidney and liver weight increases in the low-dose females were transient, the former only occurred in the first year and the latter only occurred in the second year. The increase of hemosiderin deposit in the low-dose male spleen was not statistically significant. In the low-dose females, there was no clear correlation between the increase of the low-dose liver and kidney weights with the observed red blood parameters and the spleen conditions. In the low-dose males, there was also no clear correlation of the increased hemosiderin in the low-dose spleen with the observed red blood parameters and liver weight conditions. Findings in the low-dose groups are considered to be equivocal. The spleen weight increase in the mid- and high-dose females at weeks 52 and in the high-dose female at week 104 were correlated with other findings. Treatment-related findings could be summarized as follows:

PARAMETERS	200 ppm		2000 ppm		20000 ppm	
	♂	♀	♂	♀	♂	♀
Body Weight ↓			✓		✓	✓
Hemoglobin ↓			✓		✓	✓
Red Blood Cell ↓						✓
Mean Cell Hemoglobin Volume ↓				✓	✓	✓
Mean Cell Volume ↑						✓
Adrenal Weight ↑				✓		✓
Kidney Weight ↑		✓[1]		✓		✓
Liver Weight ↑		✓[2]		✓	✓	✓
Spleen Weight ↑				✓		✓
Thymus Weight ↑					✓	
Spleen - Hemosiderin deposit ↑	✓[3]		✓	✓	✓	✓
Liver +Bile duct hyperplasia ↑ +Pale cell foci ↑ +Kupffer-cell and macrophage pigmentation ↑				✓	✓ ✓	✓ ✓
Lung Focal Alveolitis ↑					✓	✓

1 = only occurred during the first year; 2 = only occurred in the second year; 3 = slightly increased throughout the study but not statistically significant.

Although changes were observed in the 200 ppm animals (increased liver and kidney weights in the females and increased hemosiderin deposit in the male spleen), they were not corroborated by other changes in these organs at this dose, the increased kidney weight was transient, and none of these changes were seen in both sexes. Therefore, the biological significance of these observations is doubtful.

From the results of the study as presented in the study report, the systemic NOEL is less than 200 ppm. The LOEL is determined to be 2000 ppm based on decreased body weight and hemoglobin in males, and increased adrenal, kidney, liver and spleen weights in females as well as increased hemosiderin deposit in the spleen of males and females.

No treatment-related neoplastic lesions were evident in the study, thus, Triforine Technical does not appear to be carcinogenic when administered up to 20000 ppm in the diet of Sprague-Dawley rats for 104 weeks.

The doses employed in this study were sufficient to produce a compound-related systemic effect and were adequate to test the carcinogenic potential of the test material, since the highest dietary level (20000 ppm) is the limit dose for a chronic/carcinogenicity study.

CLASSIFICATION: Core-Minimum. This study satisfies USEPA's guideline 83-5 requirements for a combined chronic toxicity/carcinogenicity study in rats.

APPENDICES

APPENDIX A: Statistical Evaluation of Data (copied from p. 29 of the study report)

APPENDIX B1: Clinical Signs Observation of the 52-Weeks Rats (copied from p. 436 of the study report)

APPENDIX B2: Clinical Signs Observation of the 104-Weeks Rats (copied from p. 44 of the study report)

APPENDIX C1: Mean Body Weight, Body Weight Gain and Percent Body Weight of Control for the 52-Weeks Rats (copied from p. 437 of the study report)

APPENDIX C2: Mean Body Weight, Body Weight Gain and Percent Body Weight of Control for the 104-Weeks Rats (copied from p. 45 of the study report)

APPENDIX D: Summary of Selected Hematology Parameters for the 52-Weeks and 104-Weeks Rats (derived from p. 51-58 and p. 440-443 of the study report)

APPENDIX E: Summary of Selected Clinical Chemistry Parameters for the 52-Weeks and 104-Weeks Rats (derived from p. 444-447 and p. 59-60 of the study report).

APPENDIX F: Summary Incidence of Necropsy Findings for the Combined Premature Deaths and the terminal Kill for the 104-Weeks Rats (extracted from p. 89-103 of the study report).

APPENDIX G: Absolute and adjusted (in shaded rows) Organ Weights for the 52-Weeks and 104-Weeks Rats (copied from p. 89-103 and 452-454 of the study report)

APPENDIX H1: Summary of Selected Non-neoplastic Histopathological Findings for the Combined Premature Deaths and Terminal Kill of the 52-Weeks Rats (derived from p. 404-511 of the study report)

APPENDIX H2: Summary of Selected Non-neoplastic Histopathological Findings for the Combined Premature Deaths and Terminal Kill of the 104-Weeks Rats (derived from p. 163-197 of the study report)

APPENDIX I: Summary of Selected Neoplastic Histopathological Findings for the Combined Premature Deaths and the Terminal Kills of all Rats (derived from p. 201-218 of the study report)

APPENDIX J: Summary of All Neoplastic Histopathological Findings Reported in the Study Report (copied from p. 198 - 218 of the study report)

Reference Tox Review

Page ___ is not included in this copy.

Pages 1 through 53 are not included in this copy.

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ONELINERS

TOXCHEM NO.: Triforine Technical

CITATION	MATERIAL	ACCESSION/ MRID NUMBER	RESULTS	TOX. CAT.	CORE GRADE/ DOCUMENT #
Guidelines 83-5 Chronic/Oncogenicity study Species: Sprague-Dawley Rat Inveresk Research International, Scotland St. #: [illegible] Date: July 22, 1991	Triforine Tech. (purity = 98.9%)	424120-01 to 05	Dosages 0, 200, 2000, and 20000 ppm Systemic NOEL is 200 ppm. The LOEL is 2000 ppm based on decreased body weight and hemoglobin in males, and increased adrenal, kidney, liver and spleen weights in females as well as increased hemosiderin deposit in the spleen of males and females. No treatment-related neoplastic lesions were evident in the study. Thus Triforine Technical is judged to be not carcinogenic when administered up to 20000 ppm in the diet of Sprague-Dawley rats for 104 weeks.		Minimum

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