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

004328

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Triforine, Replacement IBT mutagenicity data

To: Henry Jacoby, PM21
Registration Division (TS-767)

From: Stephanie P. April, Ph.D.
Review Section III
Toxicology Branch
HED (TS-769)
Stephanie P. April 2/19/85

Through: Clint Skinner, Ph.D. Section Head C.S.
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Theodore M. Farber, Ph.D., Chief
Toxicology Branch
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Compound: Triforine Caswell No.: 890A

Registration No. 21137-6 Registratrant: E. M. Industries

Accession No.: 253815

Act on Requested: Review studies (number RCC 026256 and RCC 026257) : Triforine : Mouse Micronucleus Assay as a replacement for the dominant lethal assay (IBT 622-5459) which was invalid as verified by Mitre Corporation. (EPL).

Conclusion: These studies are inconclusive and not acceptable as a replacement for IBT mutagenicity data previously submitted.

Toxicology Review

Compound: Triforine

Compound Numbers: Caswell Number 890A

Citation: Mouse Micronucleus Assay with Triforine RCC 026256, Celamerk, Research and Consulting Company (RCC), Ettingen, Switzerland, Document No. 102AD-457-03, Dr. G. Leber, January 11, 1984.

Clinical examination was conducted for signs.

Results:

No treatment related signs were observed in any test group. No treatment related deaths or toxic effects were observed during the observation period.

There was no significant nor compound related increase in micronucleated polychromatic erythrocytes (PCE) observed PCEs from in either male or female treated groups at 24 and 72 hours post application when compared to the negative controls. At 48 hours post application there was a significant increase in PCEs in the treated females in contrast to the males or pooled males and females when compared to the negative controls.

The positive control group exhibited a toxic effect by a reduced PCE/NCE ratio and an increase in micronucleated polychromatic erythrocytes relative to the negative control group mirocnuclei.

Discussion: This study can not be evaluated as a mutagenicity assay because no evidence has been presented showing the test material reached the target tissue (e.g., by changing PCE/NCE ratios.)

Toxicology Review

Compound: Triforine

Compound Number: Caswell No. 890A

Citation: Mouse Micronucleus Assay with Triforine, FII 031037, Celamerck Document No. 102AD-457-004. Dr, Wilhelm. June 6, 1984

Reviewed By: Stephanie P. April, Ph.D. SPA
Section III
Toxicology Branch, HED

Secondary Review: Irving Mauer, Ph.D., Geneticist
Review Section — VI
Toxicology Branch
HED(TS769) IM 3-4-?

Core Classification: Inconclusive

Conclusion: As in the previous experiment as there were no effects on the target tissue the as evidenced by changes in PCE/NCE ratio. Thus transport of the test material to the bone marrow was not shown. Hence this study cannot be evaluated as a mutagenicity assay.

Materials:

(1) Test Material: Triforine (N,N' - 1,4 piperazinediyl - bis - (2,2,2 - trichloroethylidene) - bis (formamide), batch lot number [illegible], 98.8 pure powder.

Reviewed by: Stephanie April, Ph.D.
Section III
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Secondary Review: Irving Mauer, PH.D., Geneticist
Review Section ~~IV~~ VI
Toxicology Branch
HED(TS769)
3-4-85

Core Classification: Inconclusive

Conclusion: There was no evidence of chromosome mutations by damage to the chromosomes or to the mitotic apparatus (as manifested by induced number of micronuclei) at the 24 or the 72 hour levels after acute dosing. The increase in micronuclei found in the females 48 hours after dosing was repeated for verification.

Materials:
(1) The test material used was Triforine [N,N'-[1,4 - piperazinediyl - bis - (2,2,2 - trichloroethylidene) - bis - (formamide)]], Lot number 1990, 98.8% pure (analysis number ALH 2319312 v. 20.07.83) powder dissoved in 2% Carboxylmethylcellulose in distilled water at MTD of 5000 mg/kg body weight.
(2) The negative control group received the test material vehicle 2% carboxymethylcellulose sodium salt in distilled water.
The positive control group received 50 mg/kg body weight of cyclophosphamide (reference mutagen) dissolved in 0.9% saline solution.
(3) Mice NMRI KFM (outbred, SPF quality from Kleintierfarm Madoerin AG4414 Fuellinsdorf/Schweiz (54 males and 54 females), seven weeks old at initiation of experiment, weighing 23-40 grams were used as the species and strain. NMRI KFM, of choice for this assay.

Methods:
The mice were randomly assigned to three groups consisting of 18 males and 18 females each to be treated with a single oral dose by gavage of negative control material, positive control material or test material. Six mice per sex per group were sacrificed at 24, 48, and 72 hours after treatment. Five animals per sex per group were evaluated microscopically. The remaining animal per group was evaluated macroscopically if death or imperfections precluded evaluation of the first five per group. The dosing volume of all materials was 20 ml/Kg animal body weight.
The body marrows from both femurs were prepared for evaluation. The evaluation (Schmid, W.: The micronucleus test, Mutatin Res 31: 9-15.1975) was based upon scoring 1000 erythrocytes polychromatic (PCE) and normochromatic (NCE) per animal for micronuclei incidence.

(2) As the International standard for micronucleus assay, 30 female 7 week old mice "NMRI" weighing 26-31 gms. were used. (~~Smid~~ Schmid., 1975)

Methods: The mice received a single oral dose of 200, 1000, or 5000 mg/Kg test material in 0.1 ml CMC. CMC was used as the negative control. Cyclophosphamide was used as a positive control substance.

All groups were sampled 48 hours after dosing.

Conclusion:

Triforine dosed animals were apparently unaffected by treatment with the test compound and there were no mortalities. There was no effect on the PCE/MCE ratio at ~~any~~ dose level, or any direct or indirect evidence that triforine reached the target tissue.

The positive control group had a significant increase in the number of micronucleated polychromatic erythrocytes validating the test as well as an altered PCE/NCE ratios.

Background

There are no acceptable mutagenicty studies with Triflorine at this time in the Agency. The Mouse Micronucleus Assay was submitted to replace an invalid IBT Dominent Lethal Study. The Agency should be consulted for guidance prior to submitting future mutagenicty assays to fill this data gap.